

# Thermal hydrolysis pre-treatment has no positive influence on VFA production from sewage sludge

A. Castro-Fernández<sup>1,2</sup>, S. Balboa<sup>2</sup>, V. Paramá<sup>1</sup>, B. Álvarez<sup>1</sup>, C. Castro-Barros<sup>1</sup>, J.M. Lema<sup>2</sup>, A. Taboada-Santos<sup>1</sup>

<sup>1</sup>Water Technology Centre, AGBAR group, Santiago de Compostela, 15706, Santiago de Compostela, Spain

<sup>2</sup>CRETUS Institute, University of Santiago de Compostela, Santiago de Compostela, 15706, Spain

Keywords: sewage sludge, volatile fatty acids, thermal hydrolysis pre-treatment, microbial populations

Presenting author email: [ander.castro@cetaqua.com](mailto:ander.castro@cetaqua.com)

**Abstract:** Fermentation of sewage sludge to produce VFA appears as an alternative of greater value than generating biogas. In order to improve the hydrolytic stage rate, that has been identified as the limiting one, alternatives such as thermal hydrolysis pre-treatment (THP) have been proposed. This work studies the influence of THP on VFA production in batch and continuous operations. In batch mode, the impact of THP was detrimental (maximum VFA yields of about 0.3 g COD-VFA/COD versus 0.4 with raw sludge without pre-treatment). These results are aligned with those obtained in Bio Methane Potential (BMP) tests, in which THP also led to worse results. In continuous operation, THP had proved not exerting noticeable influence on the yield nor on the transformation rate of COD on VFA. Besides it appears that microbiome structure is very much affected by THP resulting in a lower biodiversity. However, the main key-players on VFA production seems to remain after THP.

## Introduction

The most critical stage of fermentation process to produce VFA is the initial hydrolysis, thus affecting the subsequent steps of acidification. To speed-up the process, a wide variety of pre-treatment technologies, such as thermal hydrolysis pre-treatment (THP), have emerged aiming at solubilizing organic matter. Its effect on biogas production has been well documented; in contrast, its influence on VFA production has been less studied.

The objectives of this work are: i) to evaluate the effect of the THP on the VFA production from sewage sludge in batch and continuous mode; ii) to identify changes on microbiome after THP and the main key-players.

## Material and methods

Two inocula were used: one collected from the anaerobic digester of a WWTP without THP (Inoculum 1), and the other collected from a WWTP with THP (165°C, 30 min) (Inoculum 2). The substrates used (raw and pre-treated sludge) were a mix of primary and biological sludge from the first WWTP (Table 1). The effect of THP on the higher proportion of soluble COD is clearly observed in pre-treated sludge as well as in Inoculum 2.

Batch acidification experiments were carried out in an adapted AMPTS II device, using bottles with a working volume of 0.5 L (in triplicate) at a temperature of  $37 \pm 1$  °C. Inoculum and substrate amounts were added inside the bottles to achieve a food/microorganism ratio of 2 g COD substrate/g VS inoculum. 2-Bromoethanesulphonic acid sodium salt (BES, 3 g L<sup>-1</sup>) was also added as inhibitor of methanogenesis. pH was initially adjusted to 8 adding NaOH and blanks were used to study the endogenous VFA production from inocula. BMP tests were carried out applying the protocol described by Holliger et al., (2016) with an ISR (inoculum to substrate ratio in terms of VS) of 2.

Continuous operation was carried out in two CSTR with a working volume of 5 L, which were maintained at  $37 \text{ °C} \pm 1 \text{ °C}$ . The reactors were initially inoculated with 5 L of each inoculum (Inoculum 1 and Inoculum 2), and then were fed with raw (reactor A) and pre-treated sludge (reactor B) diluted to 60 g COD/L. Both reactors were operated at a controlled pH of 8.5 at a hydraulic retention time of 10 days during the whole operation period (80 days), with an OLR in the range of 4.8-6.4 g COD L<sup>-1</sup>d<sup>-1</sup>.

A Hach D43900 spectrophotometer and associated cuvette kits were utilized to monitor the batch test as well as lab-scale reactors and associated experiments. VFA were determined by gas chromatography (Agilent Technologies, model 6850 Series II), equipped with a flame ionization detector (FID). Total genomic DNA was extracted by triplicate using Nucleospin Microbial DNA extraction kit (Macherey Nagel) that were pulled together. The V3V4 hypervariable region for *Bacteria* was sequenced in AllGenetics & Biology SL using an Illumina PE150 platform. The bioinformatic analysis was performed using the Microbial Genomics module workflow (v21.1).

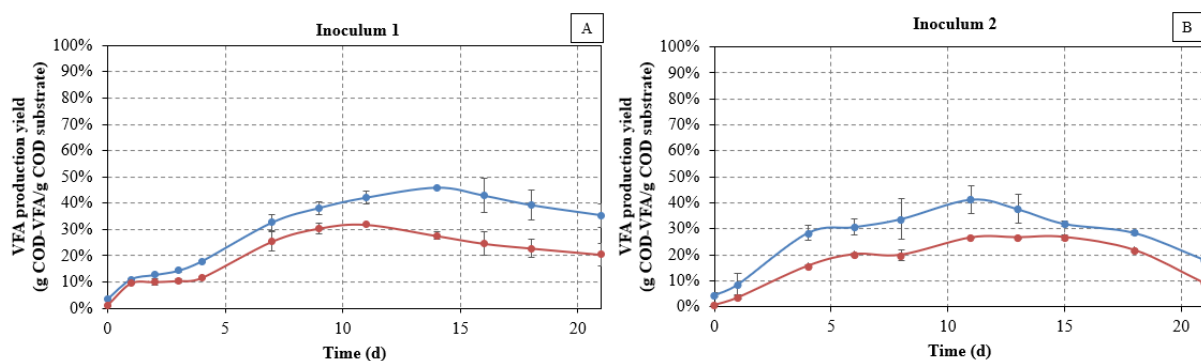
**Table 1** Characterization of both substrates and inoculums used

	Pre-treated sludge	Raw sludge	Inoculum 1	Inoculum 2
pH	6.12 ± 0.01	6.31 ± 0.00	7.88 ± 0.01	8.02 ± 0.00
tCOD (g L <sup>-1</sup> )	162.5 ± 1.1	200.8 ± 2.0	36.5 ± 0.9	58.6 ± 1.0
sCOD (g L <sup>-1</sup> )	24.9 ± 0.4	9.6 ± 0.2	0.9 ± 0.1	5.4 ± 0.2

TS (g L <sup>-1</sup> )	91.0 ± 1.5	114.2 ± 1.1	42,5 ± 0.5	46.7 ± 0.3
TAN (g N L <sup>-1</sup> )	0.26 ± 0.02	0.68 ± 0.03	1.41 ± 0.02	1.52 ± 0.04

## Results and conclusions

Firstly, an acidification test was carried out at an initial pH of 8 with an inoculum not adapted to THP (Figure 1A), noting that the highest VFA production yields were 0.46 and 0.32 g COD-VFA/g COD substrate, for raw and pre-treated sludge, respectively. The same behaviour was observed with Inoculum 2, (Figure 1B), being the VFA production yields slightly lower, namely 0.41 and 0.27 g COD-VFA/g COD for raw and pre-treated sludge, respectively, this being possibly explained by the generation of acidogenesis inhibitory compounds (Dwyer et al., 2008). VFA's distribution was practically identical in all cases, being acetic acid the most abundant one with more than 70% presence in the mix on COD basis.



**Figure 1** Acidification yield of the pre-treated (—●—) and raw sludge (—●—) at pH 8. A) With Inoculum 1 (non-adapted to THP). B) With Inoculum 2 (adapted to THP).

BMPs, in which the target product was methane, were carried out to analyse the influence of THP on biogas production. Although the kinetics of the process depend on the inoculum used, the final results are very comparable. Surprisingly, lower methane production was achieved from the pre-treated than from the raw sludge regardless the inoculum used (around 350 and 230 L(N) CH<sub>4</sub>/kg VS, for raw and pretreated sludge, respectively).

Additionally, two CSTR, reactor A (fed with raw sludge) and reactor B (fed with pre-treated sludge) were operated. In a first period, the not adapted inoculum to THP was used, and it was found that the conversion yields were much lower than in the batch tests (0.15-0.17 g COD-VFA/g COD substrate). The operation with the inoculum adapted to THP, led to comparable results in both reactors, showing again a lower yield in comparison with the attained in batch tests. As in the batch tests, the VFA percent composition obtained was practically the same with the two substrates and independently of the inoculum used, with acetic acid always having a presence greater than 65% in terms of COD.

A microbiological study of the populations present in both the inlet and outlet streams of the continuous reactors has been carried out by taking samples on four different days. Feeding to reactor A (without pre-treatment) has a greater diversity of populations than feeding to reactor B. However, both substrates were able to develop the same predominant populations, with in the order Clostridiales, taxon involved in the hydrolysis of complex organic matter, presenting an abundance higher than 50%. In any case, as indicated, this fact didn't affect VFA production yields, which are comparable with both substrates.

The results of this work show that THP has a negative influence on VFA production yield in batch test, being that effect also visible in BMP tests with methane as final product. Conversely THP has no influence on VFA production in continuous mode, which resulted substantially lower than in batch conditions regardless the inoculum used. The analysis of the microbial populations in continuous mode showed that although the influence of THP implies a lower diversity of populations, those with higher relative abundance are coincident in both reactors.

## References

- Dwyer, J., Starrenburg, D., Tait, S., Barr, K., Batstone, D. J., & Lant, P. (2008). Decreasing activated sludge thermal hydrolysis temperature reduces product colour, without decreasing degradability. *Water Research*, 42(18), 4699–4709. <https://doi.org/10.1016/j.watres.2008.08.019>
- Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffière, P., Carballa, M., De Wilde, V., Ebertseder, F., Fernández, B., Ficara, E., Fotidis, I., Frigon, J. C., De Laclós, H. F., Ghasimi, D. S. M., Hack, G., Hartel, M., ... Wierinck, I. (2016). Towards a standardization of biomethane potential tests. *Water Science and Technology*, 74(11), 2515–2522. <https://doi.org/10.2166/wst.2016.336>