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Abstract: Inhibition issues associated to high-solid food waste (FW) Anaerobic Digestion (AD) are commonly related to organic acids build-up, trace element (TE) deficiency and high NH_3 concentration. For these stakes, the role of biochar and TE supplementation have been widely studied. Most papers though have performed a single to a few batches and relatively low Organic Loading Rate (OLR). In this study, sequencing fed-batch reactors (250 mL) were operated for up to 30 weeks under different conditions: mesophilic/thermophilic, with/without biochar (2 % w/w) and with/without TE supplementation. Thermophilic microbial consortia were able to efficiently treat FW at high loads (6.0 $gVS.L^{-1}.d^{-1}$) and relatively high TAN concentration (3 $g.L^{-1}$). Mesophilic consortia were not able to express the BMP at a more moderate OLR (4.5 $gVS.L^{-1}.d^{-1}$) unless biochar was added. Despite common belief, the thermophilic population was able to handle a further increase of TAN to 5 $g.L^{-1}$ while expressing more than 75% of the BMP.

Keywords: biogas; biowaste; inhibition.

Introduction

Food waste (FW) is a trending Anaerobic Digestion (AD) feedstock as policies worldwide are evolving towards law-enforced source-separation and valorization technologies over elimination. However, FW can be a challenging AD input due notably to its high and fast biodegradability, protein content (leading to the generation of NH_3 – an inhibitory compound) and overall poor trace element (TE) content necessary for co-enzymes (Zhang et al., 2020). Straightforward process engineering strategies to overcome these challenges are dilution and co-digestion (e.g., sludge, green waste, manure). The first greatly increases costs while co-substrates are not always available/authorized. Microbiome engineering comes thus into play.

Several studies reported the importance of TE supplementation and the use of biochar as an additive enhancing syntrophic propionate oxidation, an AD metabolic pathway that is critical at high NH_3 concentrations. Moreover, AD temperature is a critical parameter as it can both impact the NH_3 concentration (i.e., NH_3/NH_4^+ equilibrium) while driving microbial selection towards microorganisms that are more/less sensible to NH_3 inhibition. For instance, Capson-Tojo et al. (2019) suggested with a meta-analytical study that thermophilic archaea should be less impacted by NH_3 than mesophilic, despite the higher free ammonia nitrogen (FAN, *i.e.*, N-NH_3) concentrations under the same total ammoniacal nitrogen level (TAN, *i.e.*, N-NH_3 plus N-NH_4^+). Most of FW AD studies testing these solutions seem to be performed at single to a few batches while, other than allowing microbiological acclimation, the full replacement of inoculum content by digested material requires a long-term operation (*e.g.*, three retention times for 95 % replacement in a continuous stirred-tank reactor).

The aim of this study is to investigate different scalable microbiome engineering strategies for stabilizing AD of FW as a single feedstock: thermophilic operation, biochar addition and trace elements supplementation. This series of experiments aim ultimately to set the operational conditions of an industrial pilot treating 50 tons of food waste per year.

Material and Methods

Household FW was collected, ground to 2 mm, homogenized and stored in frozen aliquots. Before grinding, they were visually verified to be exempt from lignocellulosic green waste and gross plastic particles. The feedstock, inocula and biochar used in this study were characterized for total solids, volatile solids, total Kjeldahl nitrogen and metals forms by ICP-AES (P, K, S, Mg, Ca, Na, B, Co, Cu, Fe, Mn, Mo, Zn, Al, As, Cd, Cr, Hg, Ni, Pb, Se. Data under request).

The experiments were carried out in 0.5 L bottles and 0.25 L working volume, in AMPTS® II systems (BPC, Sweden) where methane is measured volumetrically after biogas scrubbing in a NaOH 3 M solution. The fed-batch strategy was designed to simulate a plug flow reactor with digestate recirculation. A batch duration (7 days) represents a plug-flow 'passing time'. In the end of each batch, 5:6 (w:w) of the digestate is recirculated so, at steady-state, the mean retention time is about 40 days. The biochar used in this study was a commercial hardwood biochar obtained by fast pyrolysis. Biochar doses are expressed in weight/weight. During startup, biochar was added to achieve the target dose, then added with the feedstock in order to keep a constant concentration. The TE solution was formulated as proposed by Angelidaki et al. (2009) for BMP tests, added at a ratio of 150 µL/gVS of input.

A total of 6 conditions were performed in triplicate, which are summarized in Table 1.1. The thermophilic bottles were inoculated with a digestate from a full-scale thermophilic Dry-AD plant treating the fine organic fraction of municipal solid waste (OFMSW), which was acclimated to a TAN concentration of around 3 $g.L^{-1}$ (FAN estimated at 1 $g.L^{-1}$). The mesophilic bottles were inoculated from a full-scale mesophilic Wet-AD plant treating mostly biowaste and industrial sludge, which was acclimated to a TAN concentration of around 4 $g.L^{-1}$ (FAN estimated at 0.5 $g.L^{-1}$).

Cumulative methane production was measured constantly as described previously. Volatile fatty acids (VFA), TAN, pH, total (TS) and volatile solids (VS) and soluble chemical oxygen demand (COD) were analyzed at the end of the batches. The Organic Loading Rate (OLR) was gradually incremented during start-up, starting from 4.0 up to a value of 4.5 and 6.0 $gVS.L^{-1}d^{-1}$ for the mesophilic and thermophilic AD, respectively. In Batch 11, when all conditions seemed sufficiently stable, every thermophilic condition was supplemented with ammonium bicarbonate (NH_4HCO_3), in order to increase TAN concentration to an even more stressing level (from about 3 to a target of 5 $gN.L^{-1}$).

Table 1.1 Experimental design.

Temperature	TE supply from batch 1	TE supply from batch 27	Biochar dose (% w:w)	Ammonium bicarbonate addition at batch 11
Thermophilic	No	Yes	0	Yes
Thermophilic	Yes	Yes	0	Yes
Thermophilic	No	Yes	2	Yes
Thermophilic	Yes	Yes	2	Yes
Mesophilic	Yes	Yes	0	No
Mesophilic	Yes	Yes	2	No

Results and Conclusions

The methane yields and the total VFA concentrations at the end of each batch are presented in Figure 1.1 for the whole experiment. At the initial OLR of 4.0 gVS.L^{-1}.d^{-1}, thermophilic AD was unexpectedly capable of expressing 100 % of the BMP within the batch duration of 7 days (plug flow "passing time"). A slight VFA build-up was observed in the first two batches (up to about 5 gCOD.L^{-1}, mostly propionate) in each condition, and VFA were totally re-consumed between batches 3-4. No VFA accumulation was observed afterwards when OLR was increased to 4.5 and later to 6.0 gVS.L^{-1}.d^{-1} (interventions a. and b., Figure 1.1). No significant effect of biochar was observed.

The mesophilic conditions started with a more moderate kinetic (75 % of BMP within the first batch). As expected, the CH_4 yield increased during the following batches but increasing VFA concentrations (mostly propionate) were observed. As a consequence, the OLR was increased to 4.5 gVS.L^{-1}.d^{-1} but no further. At the end of batch 4, all mesophilic conditions had accumulated 10-15 gCOD.L^{-1} of propionate. Starting from batch 5, only the conditions with biochar started to consume propionate, achieving negligible VFA concentrations between batches 8-10, which can be testified by full BMP expression.

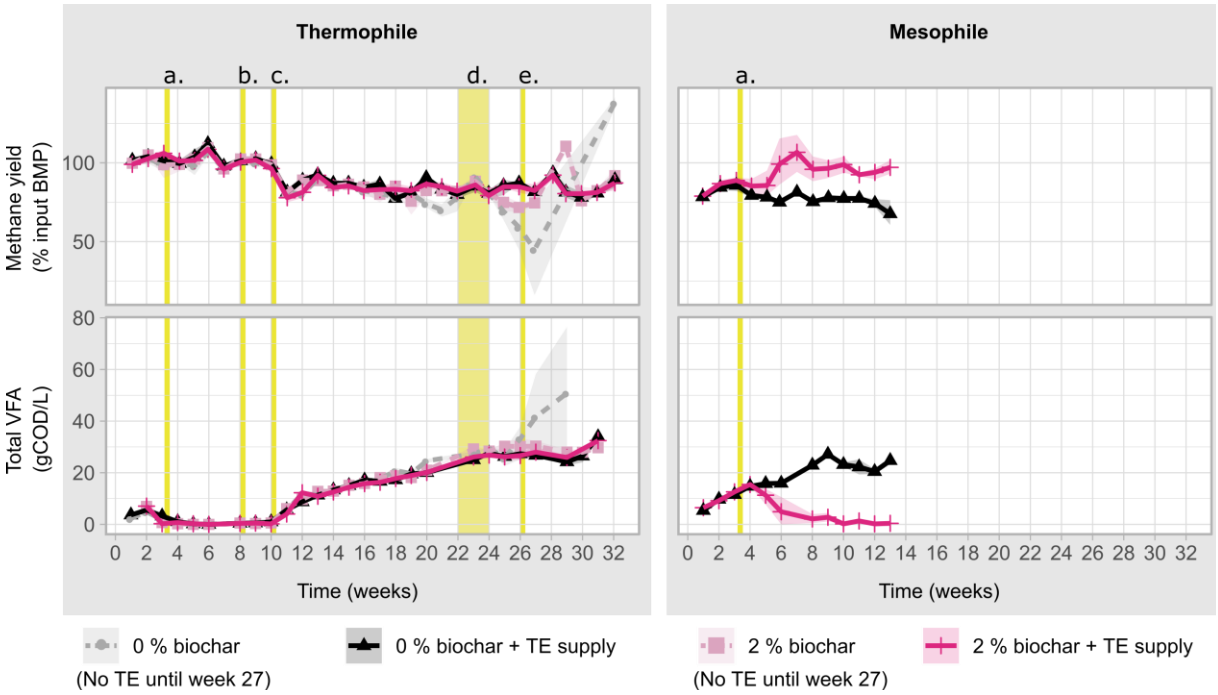

Figure 1.1 Methane yield and total VFA concentration at the end of each batch under thermophilic and mesophilic conditions. The points represent the average value of triplicate bottles, connected by lines. Shadows represent min. and max. values from triplicates. Vertical yellow lines/areas represent the following events: a. OLR increased from 4.0 to 4.5 gVS.L^{-1}.d^{-1}. b. OLR increased from 4.5 to 6.0 gVS.L^{-1}.d^{-1}. c. induced NH_3 stress to all thermophilic conditions by the addition of ammonium bicarbonate targeting 5 gTAN.L^{-1}. d. temperature control failure (temperatures reached 43°C during this phase). e. TE supplied to the yet non-supplemented thermophilic conditions.

Whatever the conditions, at batch 10, the TAN concentrations were clearly converging to 2.5-3.0 g.L^{-1} (results not presented). Given the stable and secure operation in all thermophilic conditions, an ammonia stress was induced by adding ammonium bicarbonate for achieving a TAN of 5 g.L^{-1} (intervention c., Figure 1.1).

Following the TAN shock, all the thermophilic conditions started to steadily increase VFA concentration, converging to a semi-inhibited steady-state with a total VFA concentration of 25-30 $gCOD.L^{-1}$ (> 2/3 as propionate). After a theoretical complete replacement of the initial inoculum by digested food waste, from batch 20, the condition without TE nor biochar addition started to demonstrate further inhibition. However, an unnoticed operational issue with temperature control during batches 22 and 23 (incident d., Figure 1.1), which resulted in a temperature drop, promoted an interesting re-consumption of VFA (*e.g.*, up to 5 g $COD.L^{-1}$ of less propionate at the end of batch 24 compared to 22). This result may represent opportunities for further research on fine temperature tuning, which was outside the experiment's scope. The temperature was adjusted back from 43 to 50 °C and the inhibition effect was again observed two batches later. A more moderate inhibition of the condition without TE supply but 2 % biochar might indicate that biochar contributed to TE supply. From batch 27, the inhibition trend of the bottles without TE supply could be reversed by adjusting the TE level to the same as the TE-supplied conditions (intervention e., Figure 1.1).

This whole series of long-term experiments demonstrate that food waste thermophilic AD can perform better than mesophilic AD at higher loads (6.0 $gVS.L^{-1}.d^{-1}$) and at a relatively high TAN concentration (3 $g.L^{-1}$). In thermophilic tests, no positive effect of biochar was noticed until almost 5 months of operation, with relevant evidence of the effect being associated with TE supply by the biochar. At a more moderate load (4.5 $gVS.L^{-1}.d^{-1}$), mesophilic AD coupled with biochar and TE supply was stable while adding no biochar resulted in inhibition. Ongoing genomic analyses to be presented in the conference could provide evidence of the role of biochar on promoting a microbial community shift, as suggested by the literature (Lim et al., 2020). Under the same research project, these results will be used to define the operational strategy of an industrial pilot treating 50 tons of FW per year.

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