

Inhibitory effect of long chain fatty acid on biogas production via a single pulse: changes in microbial community dynamics and simulation aspects

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Anaerobic digestion (AD) is a biological process in which different groups of microorganisms, mainly bacteria, and archaea, convert organic matter into biogas by exploiting syntrophic relationships and following diverse metabolic pathways. It is well known that different parameters affect the AD process, including operational conditions, biochemical substrate composition, and microbial community (Kougias and Angelidaki, 2018). Linking all these parameters and the actual experimental data with the development of mathematical models could be extremely beneficial for the improvement of the AD process. Nowadays, complex bioconversion models can handle a large number of numerical data and include features regarding microbial process kinetics, physicochemical kinetics, mass transfer kinetics, and substrate conversion (Wu et al., 2019, Lovato et al., 2017). In addition, -omic techniques are a powerful tool that provides a thorough understanding of the microbial composition, functional roles, microbial networks, and metabolite exchanges of the microbial consortia found in the biogas reactors.

The present study aimed to investigate the effect of a single inhibitory shock pulse of long chain fatty acids (LCFA) on the anaerobic digestion process. A bioconversion model (Lovato et al., 2017) was used to simulate the LCFA pulse and analyze process performance, while the microbial community shifts were monitored by applying genome-centric metagenomic analysis.

A set-up of three identical (represent biological replicates) continuous stirred tank reactors (CSTR) was settled with a total and working volumes of 2.0 and 1.5 L, respectively. The reactors were operated under mesophilic (37°C) conditions, with a hydraulic retention time (HRT) of 25 days and feeding exclusively with cattle manure at an organic loading rate (OLR) of 3 gVS L⁻¹ d⁻¹. When the reactors reached steady-state conditions a single inhibitory shock load of 3g Na-Oleate/L_R was injected. The biogas production was daily quantified as described in Tsapekos et al, (2019), the process indicators pH, volatile fatty acids (VFA), and biogas composition were measured for the entire experimental period, and the genomic DNA was extracted at three-time points: i) at steady-state conditions before the shock, ii) 14 hours after the shock and iii) at steady-state conditions long after the shock.

The process monitoring indicated that the reactors needed 3 HRT to reach steady-state conditions and afterward the operation was smooth and stable with average biogas composition of 55% methane and 45% carbon dioxide, methane yield of 200 mL CH₄/g VS, and high VFA degradation combined with stable pH. Upon the shock injection, a mild deterioration of the process was observed determined by a slight decrease in biogas production and an increase in VFA concentration followed by a boost in the biogas production due to the increase of the organic matter inside the reactors. However, the methanation process was recovered shortly after the induced perturbation and at the final period of the experiment, the methane production was at similar levels as in the primary steady-state phase. Model simulation trends (Fig. 1) were overall in good agreement with the experimental data and the model managed to predict the effect of the LCFA pulse correctly. However, biogas production showed higher levels than predicted by the simulation, which is an indicator that in the experimental operation the dynamics in the microbial composition changed due to the shock, and thus the slightly higher biogas production after the pulse. This gap was fulfilled by the genome-centric metagenomic analysis that provided information about the changes in the abundance of the microorganisms (Fig. 2). Additionally, the metagenomic analysis allowed the identification of 437 MAGs, of which 214 High Quality (HQ) and 102 Medium-High Quality (MHQ). Furthermore, the analysis revealed the information about the methanogenic population inside the reactors, unveiling that hydrogenotrophic methanogens were the most abundant suggesting that the hydrogenotrophic pathway was the dominant mechanism for methane production. The genus *Syntrophomonas* contained the key members for the LCFA degradation through the β-oxidation pathway. Of great interest, was the significant increase after the shock injection of the novel bacterium *Nigerium* sp. WMB168, the presence of which may be related to the increase of the CO₂ inside the reactors, according to its genomic content.

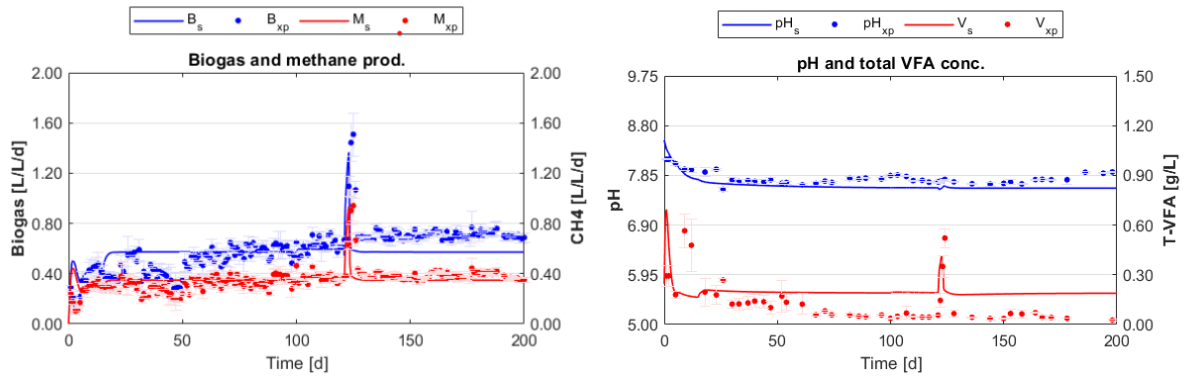


Fig 1. a) Reactors' performance regarding the biogas and methane production rates. The blue color stands for biogas production rate, while the red color stands for methane production rate. b) Reactors' performance regarding the pH variation and total VFA concentration. The blue color stands for pH, while the red color stands for VFA concentration.

Note: Each point is the average value of the three reactors, for this reason, there is a standard deviation. Points represent experimental data, while straight line the model predictions.

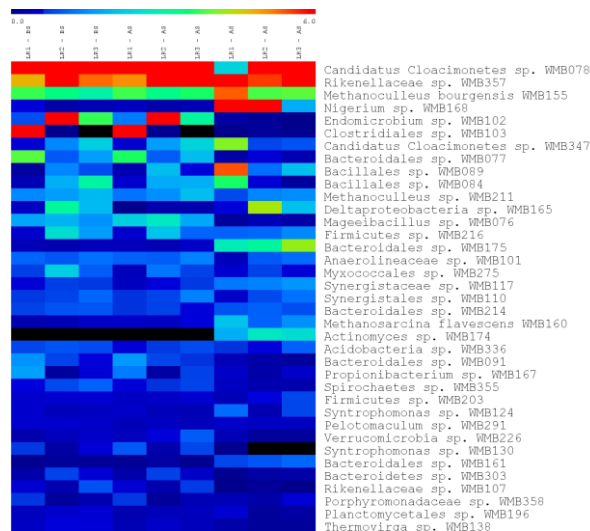


Fig. 2. Behavior of the 37 HQ most abundant MAGs in the nine samples is represented as a heatmap.

The investigation of a single inhibitory shock load of LCFA on the anaerobic digestion was successfully predicted by the mathematical model, however, prediction of biogas composition remained a challenge. The metagenomic analysis explained the reasons for the change in biogas production, by shedding light on the changes of the microbial dynamics -favoring the abundance of genus *Syntrophomonas*, -whose mechanisms were not included in the model.

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