Effect of high salinity conditions on anaerobic co-digestion of macroalgal biomass with cattle manure

M. Alvarado-Morales[1], P. Tsapekos[1], A. Kovalovszki[1], P.G. Kougias[2], A. Rudatis[3], R. Raga[3], I. Angelidaki[1]

[1]Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark
[2]Solid and Water Resources Institute, Hellenic Agricultural Organisation- DEMETER, Thermi, Thessaloniki, 60458, Greece
[3]Department of Industrial Engineering, University of Padova, via Marzolo n 9, 35131 Padova, Italy
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Presenting author email: meal@env.dtu.k

Livestock manures can be effectively treated *via* anaerobic digestion (AD) to produce bioenergy. In an industrial perspective, the feasibility of full-scale AD using liquid slurries as sole substrate is limited due to the high content of recalcitrant lignocellulosic fibers which results in low volumetric biogas productivity (Tsapekos et al., 2016). Hence, there is a hunt to find easily degradable substrates to enhance the volumetric methane production of manure-based AD plants. Macroalgal biomass possess unique characteristics to address this challenge such as high content of easily degradable sugars, negligible presence of recalcitrant lignin, and no need of farmland to produce them (Alvarado-Morales et al., 2013). However, detrimental results can be detected at biogas reactors fed with marine biomass due to their high mineral content (Zhang et al., 2017). Thus, the inhibition effect of cation should be carefully investigated before this biomass is used in real-scale AD plants. The aim of this study was to evaluate the anaerobic co-digestion of macroalgal biomass (i.e. *Laminaria digitata*) with livestock slurry (i.e. cattle manure) under high salinity levels. Batch toxicity tests were performed to determine the IC50 using two different sodium sources (i.e. synthetic sodium chloride and natural sea salt). In addition, continuous AD tests at IC50 and 2IC50 were conducted. Subsequently, anaerobic digestion BioModel (Kovalovszki et al., 2017) was used to simulate the continuous process and predict reactor's performance at increased salinity levels.

Inoculum, substrates and sea salt. Thermophilic inoculum was collected from a lab-scale reactor co-digesting municipal waste and cattle manure. Cattle manure was collected from Hashøj biogas plant (Sealand, Denmark) and *Laminaria digitata* was collected from Hanstholm (Jutland, Denmark).

Batch toxicity assays. The effect of two different sodium sources (i.e. NaCl and sea salt) on the co-digestion of *L. digitata* with cattle manure was tested at six concentrations (0.2, 1.0, 3.0, 6.0, 9.0 and 12.0 g-Na/L) in batch assays. Assays were performed at thermophilic conditions based on Tsapekos et al, (2019). Accumulated methane was monitored twice a week until cease of production was detected.

Continuous reactors. Mono-digestion of cattle manure and co-digestion with *L. digitata* were tested in two identical CSTRs with total and working volume of 2.3 and 1.8 L, respectively. CSTRs were operated at 54 ± 1 °C and HRT = 15 d. In period I (0-45 days) CSTRs were fed only with cattle manure at OLR of 2.3 gVS/L/d. In period II (45-75 d) marine biomass was added in the feedstock corresponding to 20% on VS basis and the OLR was 2.9 gVS/L/d. In periods, III (75-95 d) and IV (95-151 d) the effect of sea salt was examined at the IC50 and 2IC50 based on toxicity assays, respectively. Two strategies were tested: 1) for R1 spiking simultaneously both reactor and substrate at the IC50 and 2) for R2 adding the sodium source only in the feedstock. For the entire experiment, biogas production was daily quantified as described in Tsapekos et al, (2019). Gas and liquid phase samples of the CSTRs were taken twice a week for pH-VFA and methane content determination, respectively.

Batch toxicity assays. The highest methane yield (450 ± 23 mLCH_4/gVS, $p < 0.05$) was achieved when adding 1.0 g-Na/L as NaCl (Fig. 1a). Likewise, when 1.0 g-Na/L was added as sea salt, the highest methane yield (394 ± 17 mLCH_4/gVS) was obtained for the second experimental set (Fig. 1b). However, the boost was statistically no significant ($p > 0.05$) compared to the untreated sample. As sodium concentration increased from 1.0 to 3.0 g-Na/L, the methane yield was not affected using NaCl (Fig. 1a). In contrast the IC50 for sea salt occurred at 2.8 g-Na/L (Fig. 1b). A slight accumulation of VFA was observed at this point indicating that the methanogenic community could not use the produced intermediates (data not shown). In contrast, pH and COD remained in the same values as the untreated operation (data not shown). The IC50 adding the light metal as NaCl was found at 10.1 g-Na/L, which is extremely higher than the IC50 of the sea salt. Overall, the inhibitory impacts of sea salt were more severe than NaCl.

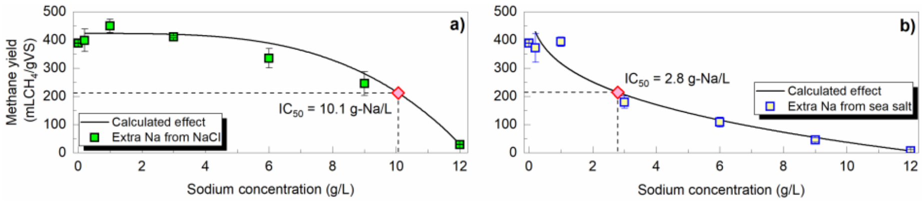

Fig. 1 Practical methane yields at different Na concentrations supplemented by (a) NaCl and (b) sea salt. IC50 is calculated fitting the experimental values to the Hill model.

Continuous operation. Similar performance was observed for both reactors in period I (0-45 d). Mono-digestion of manure did not have any operational problems as indicated by the high VFA degradation and stable pH (Fig. 2a-c). In period II (45-75 d), gas production was significantly increased ($p < 0.05$) by 20% (0.73 ± 0.03 and 0.47 ± 0.02 L/L/d) (Fig. 2a and b) which was attributed to the easy degradable sugars in *L. digitata*. The pH and VFA values remained relatively unchanged compared to mono-digestion (Fig. 2a-d), indicating that the addition of marine biomass did not provoke any stress to the AD community. In periods III and IV, performance of both CSTRs was compromised due to salt addition equivalent to IC50 and 2IC50, respectively. Strategy 1 implemented in R1 resulted in an immediate adverse impact on methanation with a slight decreased in pH as a consequence of VFA accumulation in period III (IC50) (Fig. 2b). The decreased methanogenic activity was clearly observed in period IV (2IC50) resulting in a lag phase of 15 days at which biogas and methane production were totally inhibited (Fig. 2a). However, a gradually recovery of R1 started after day 120 as observed in gas production and due to the gradual consumption of the accumulated VFAs. This suggest a shift in AD microbiome dynamics to adapt to high salinity conditions. On the contrary, a completely different behaviour was observed for R2 where the progressive increment of salt was tested. In period III no significant fluctuations were observed in gas production, pH and VFAs profiles (Fig c-d), indicating that microbiome was able to tolerate the IC50 level. However, a slight drop of gas productivity was detected at the start of period VI (Fig. 2c) at which the IC50 was doubled, so that at the end of period IV the bioenergy output was decreased ($p < 0.05$) by 35% compared to period III.

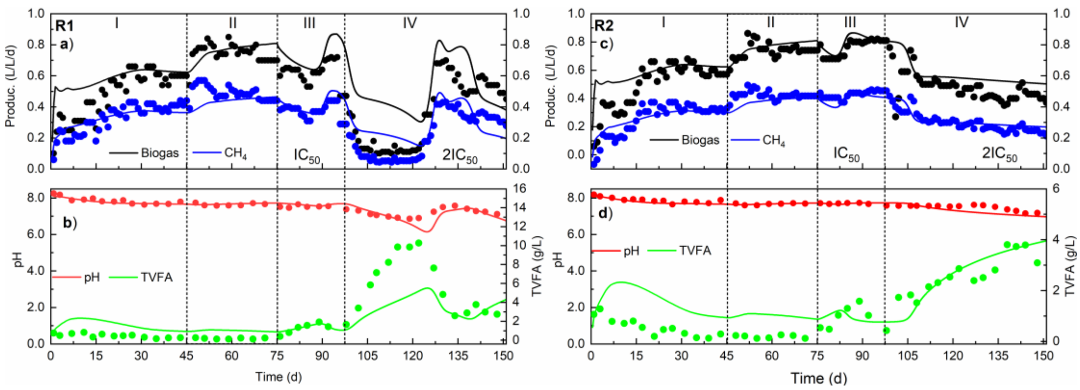

Fig. 2 CSTRs performance a-b) R1: spiking simultaneously both reactor and substrate, c-d) R2: adding the sodium source only in the feedstock. Symbols – experimental data, solid lines - BioModel simulations.

Model simulations using the BioModel were in good agreement with the experimental trend of R1 and R2. Microbiome was able to overcome severe process inhibition in R1 while in R2 a better-adapted microbiome was developed due to the gradual salt supplementation. The impact of increased salinity conditions on the AD process was elucidated using both experimental and modelling aspects.

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