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

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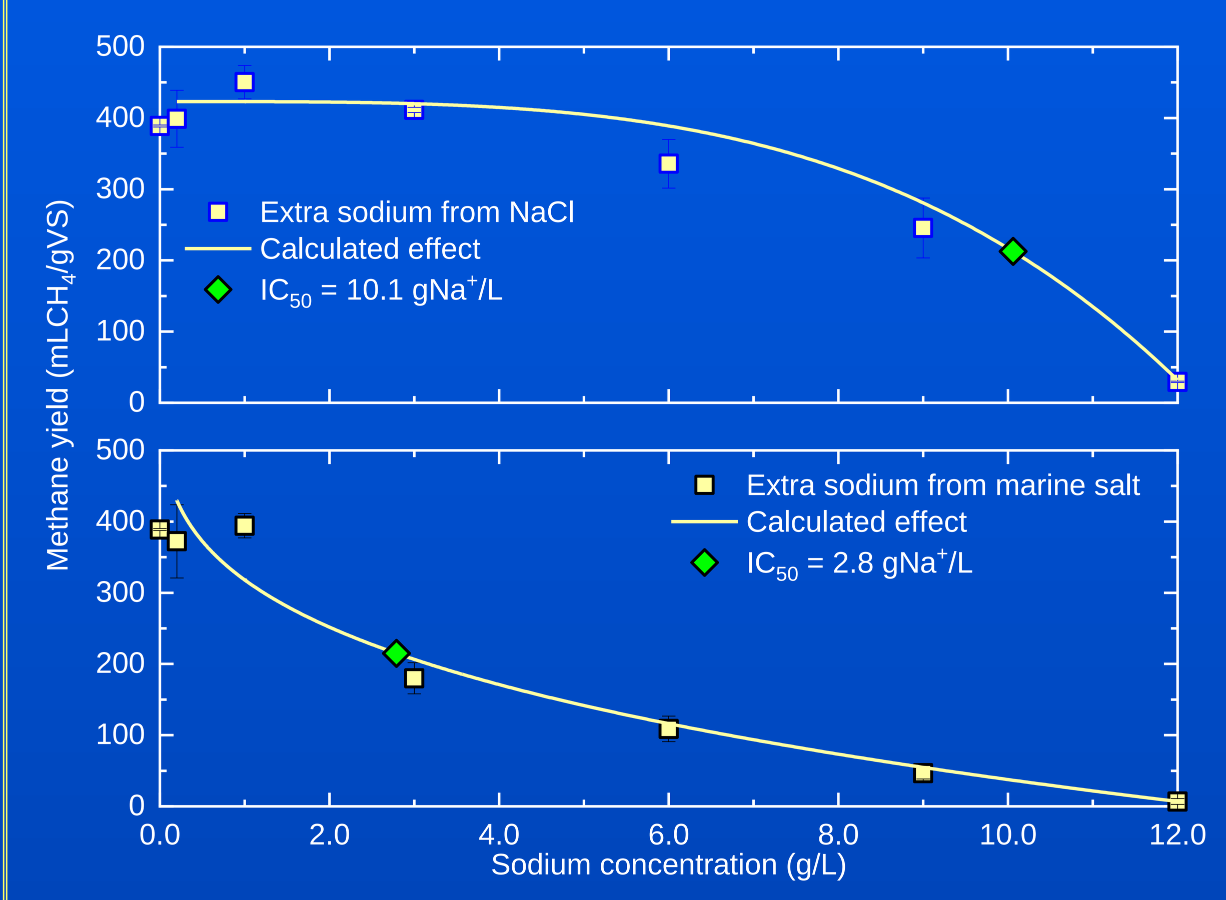
## Introduction

A comprehensive study of sodium chloride and marine salt as potential inhibitors of the anaerobic digestion process is hereby presented. The hypothesis was tested in three major steps. In the first step, batch toxicity assays showed that marine salt is already inhibitory to the anaerobic digestion microbiome, at a half-maximal inhibitory concentration of 2.8 g-Na L-1, compared to synthetic sodium that was inhibitory only at a concentration of 10.1 g-Na L-1. Next, experiments conducted in two continuous reactors R1 and R2, showed that the gradual addition of salt to reactor R1 leads to irreversible inhibition, while R2 also spiked with salt might be recoverable to some extent. Finally, a bioconversion model, which for the first time was extended with a dynamic salt inhibition module, produced simulations that agreed well with the results of the continuous experiments, suggesting the presence of agile methanogenic archaea that can temporarily buffer the negative effect of increasing salt concentrations. The findings of this work may provide inputs to future anaerobic fermentation experiments with salts and could benefit from detailed microbial analyses.

## Results & Discussion

### Batch assays – IC50




The highest methane yield (450 ± 23 $NmLCH_4/gVS$, $p < 0.05$) was attained with the addition of 1.0 g-$Na^+$/L in the form of NaCl.

Likewise, when 1.0 g-$Na^+$/L was added in the form of marine salt, the highest methane (394 ± 17 $NmLCH_4/gVS$, $p < 0.05$) yield was obtained.

IC50 adding Na+ in the form of NaCl was found at 10.1 g-$Na^+$/L which is significantly higher than the IC50 for marine salt, *e.g.* 2.8 g-$Na^+$/L.

The difference in toxicity tolerance between the two sodium sources is attributed to the high minerals content in seawater, *i.e.* $Mg^{2+}$ and $K^+$ were the most dominant minerals in sea salt.

### Sodium addition effect – modelling approach

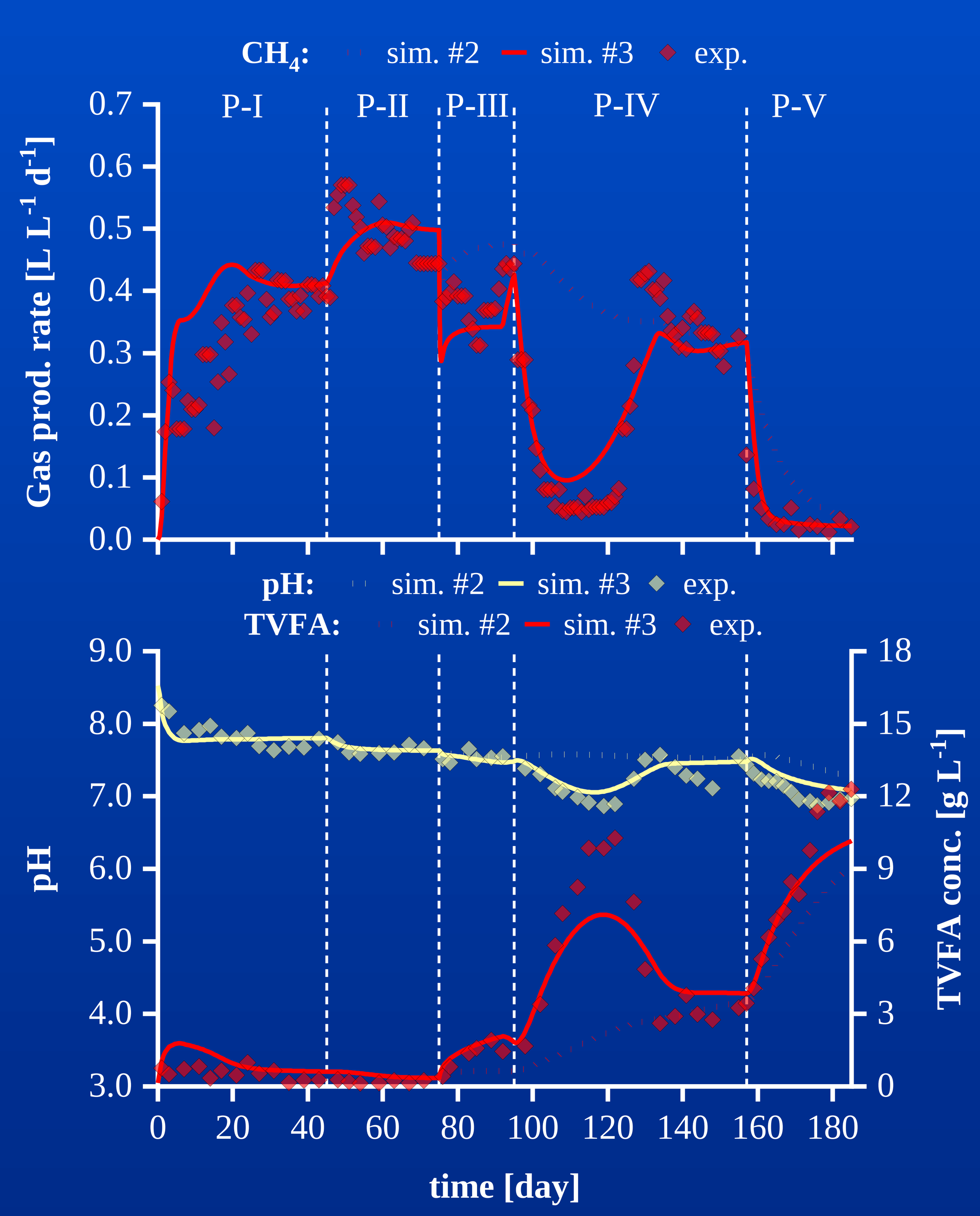
$$\frac{\Delta S_{Na,a}}{\Delta t} = \left(S_{Na,e}(t) - S_{Na,a}(t)\right) \times \left(1 - e^{-\frac{\Delta t}{\tau_a}}\right)$$

$$F_{KI,SS}(S_{Na,a}) = \begin{cases} b_{lower}, & S_{Na,a} \leq b_{lower} \\ S_{Na,a}, & b_{lower} < S_{Na,a} \leq b_{upper} \\ b_{upper}, & b_{upper} < S_{Na,a} \end{cases}$$

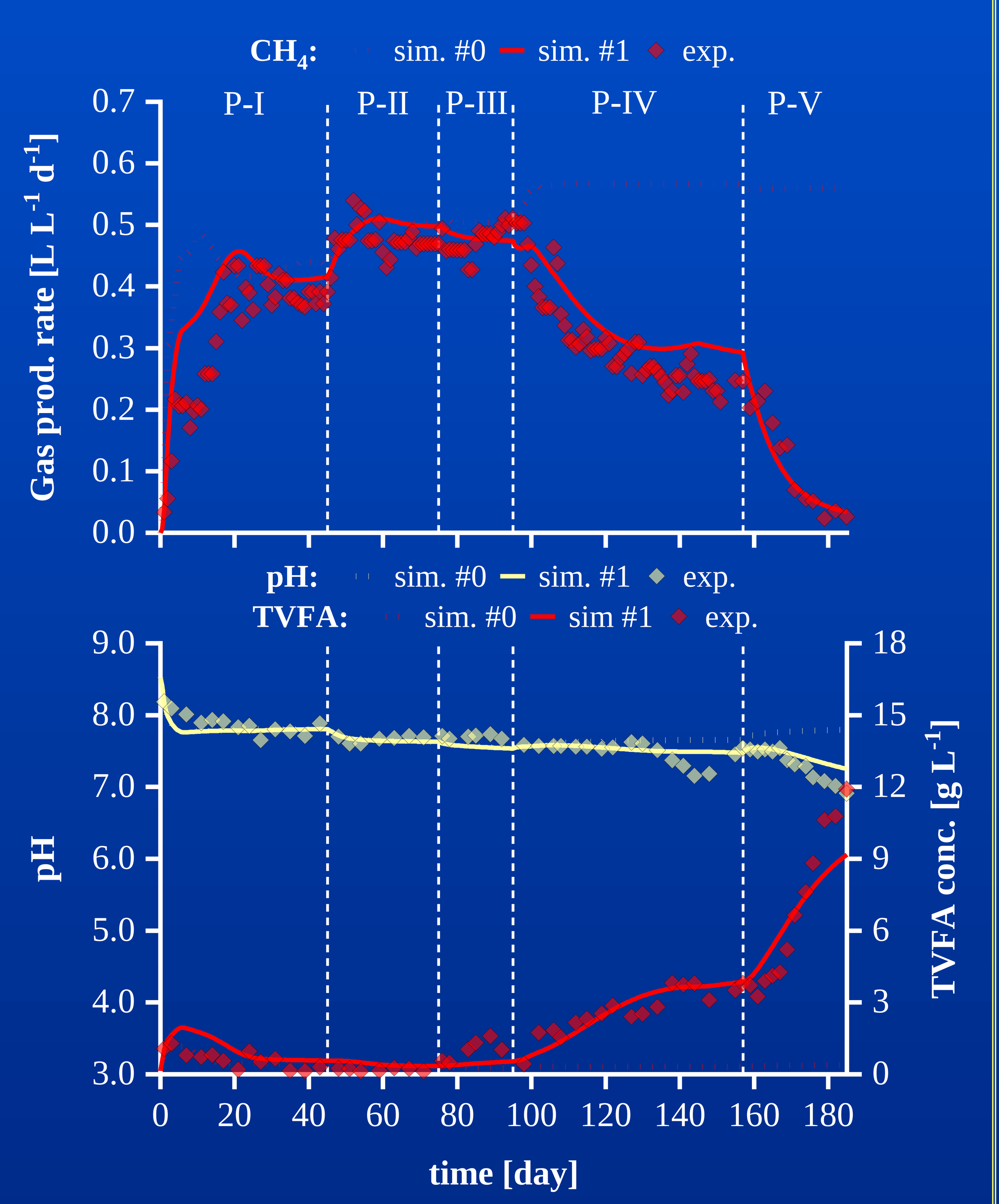
$$F_{KI,DYN}(S_{Na,e}, S_{Na,a}) = \begin{cases} 1, & S_{Na,e} \leq F_{KI,SS} \\ e^{-\frac{(S_{Na,e} - F_{KI,SS})^2}{2 \times \sigma^2}}, & F_{KI,SS} < S_{Na,e} \end{cases}$$

$$\sigma = \left(-\frac{{c_{Na}}^2}{2 \times ln\, 0.5}\right)^{0.5}$$

### Continuous reactor – R1




### Continuous reactor – R2




- ❑ The initial experimental period P-I was associated with similar performance for both reactors (R1, R2). Mono-digestion of manure did not have any operational problems, as indicated by the efficient VFA degradation and stable pH (P-I)
- ❑ During P-II gas production was significantly increased and, pH and VFA values remained relatively unchanged compared to P-I, indicating that the addition of marine biomass did not provoke any stress to the AD community.
- ❑ In response to the spiking of R1 with high amounts of salt, the microbial community could tolerate and operate under higher salt concentrations. This phenomenon was especially well represented by the bioconversion dynamics seen from days 95 to 157 (P-IV), where an initial process inhibition was followed by gradual process recovery. Nevertheless, the exceedingly high salt concentrations reached during P-V eventually led to the complete inhibition of the process
- ❑ A fundamentally different behavior was observed for R2, the progressive salt-supplementation strategy gradually led to process inhibition: first by reducing the methane output to about half of its P-III levels during P-IV, and finally reaching an irreversible state of inhibition during P-V.

## Conclusions

The above results can be highly valuable for the design and operation of full-scale, algae-based digesters. Given such putative information about the tolerance of anaerobic digestion microbiomes to salinity, decision-makers can avoid process inhibition that stems from excess sodium concentrations, by choosing suitable algae pretreatment methods and substrate mixing ratios.

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