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# Ammonia tolerant enriched methanogenic cultures as bioaugmentation inocula to alleviate ammonia inhibition in continuous anaerobic reactors



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## Introduction

Ammonia-rich substrates are known to inhibit anaerobic digestion (AD) process and it is estimated that many full-scale biogas reactors are seriously affected by ammonia toxicity leaving up to 1/3 of their methane potential unutilized. Bioaugmentation of ammonia tolerant methanogenic consortia could provide a new solution to alleviate ammonia inhibition in AD process.

Previous studies have shown that it is possible to acclimatize mixed methanogenic cultures to high ammonia levels. These complex communities could have a greater robustness and impact on the biomethanation process than a single strain. However, acclimatized mixed cultures were never tested as bioaugmentation inocula in continuous anaerobic reactors.

## Aim

The aim of the current study was to use a mixed (enriched) ammonia tolerant methanogenic culture as potential bioaugmentation inocula in a continuous stirred tank reactor (CSTR) operating under "inhibited steady-state", caused by high ammonia levels.

## Materials & Methods

The hydrogenotrophic methanogenic enriched culture (MEC, Fig. 1) used in the bioaugmentation process derived from a mesophilic manure based full-scale reactor. MEC culture was acclimatized in stepwise increasing ammonia levels (up to 5 g NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup>) in batch reactors.

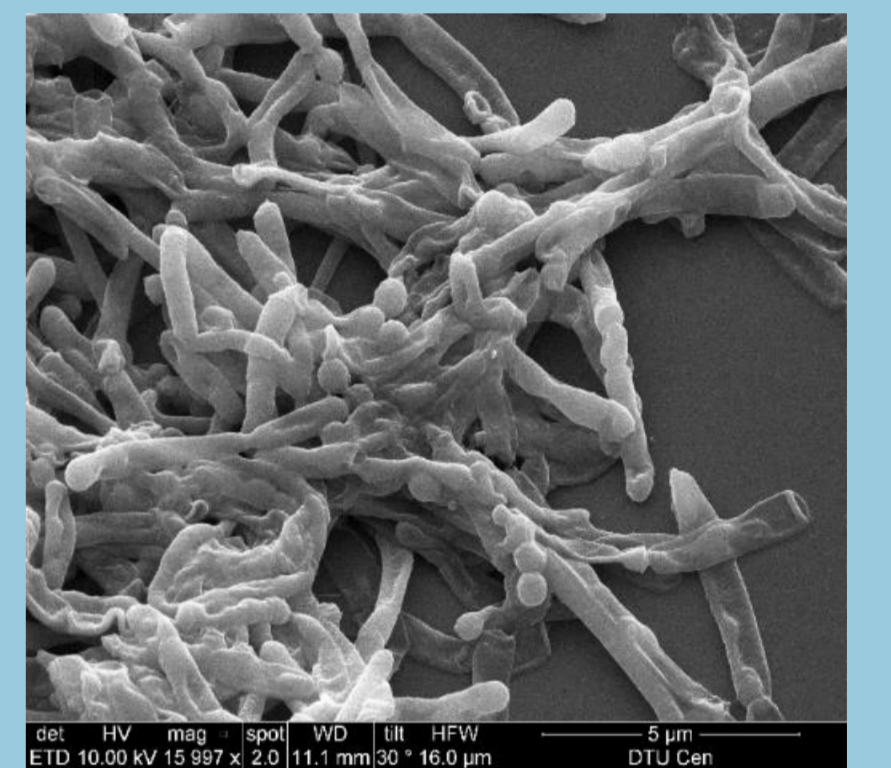


Fig. 1. SEM microscopy of MEC

## Materials & methods

The bioaugmentation was carried out in two CSTR reactors (R<sub>MEC</sub>: MEC culture bioaugmentation and R<sub>Control</sub>: abiotic augmentation) with 2.3 L and 1.8 L total and working volume, respectively. One HRT after ammonia concentration in the reactors reached 5 g NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup>, an induced "inhibited steady-state" was established (Period-I) for both reactors. The **bioaugmentation of MEC took place twice in Period-II with a total of 200 mL of the MEC culture introduced into R<sub>MEC</sub>** while 200 mL of sterile medium were also introduced in reactor R<sub>Control</sub> (Fig. 2).

Both reactors were operated continuously and the ammonia concentration, the HRT (24 days) and the OLR (1.74 g VS L<sup>-1</sup> d<sup>-1</sup>) were kept stable.

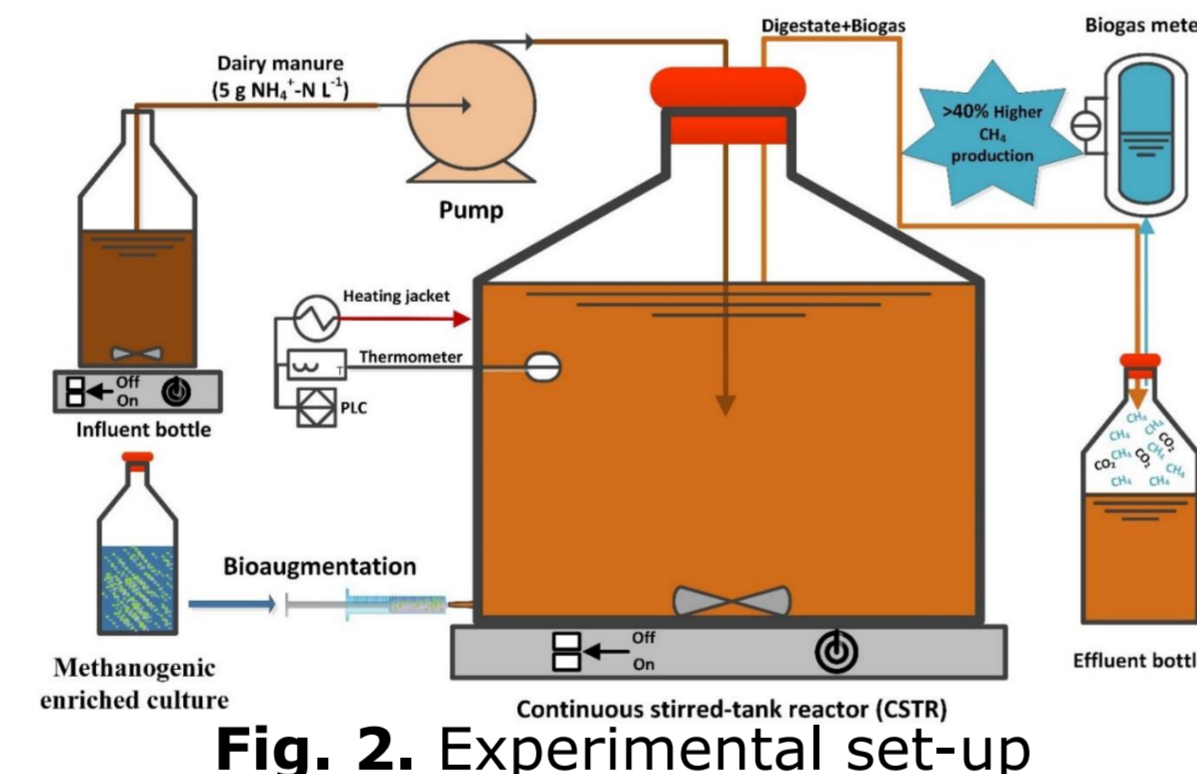


Fig. 2. Experimental set-up

## Results

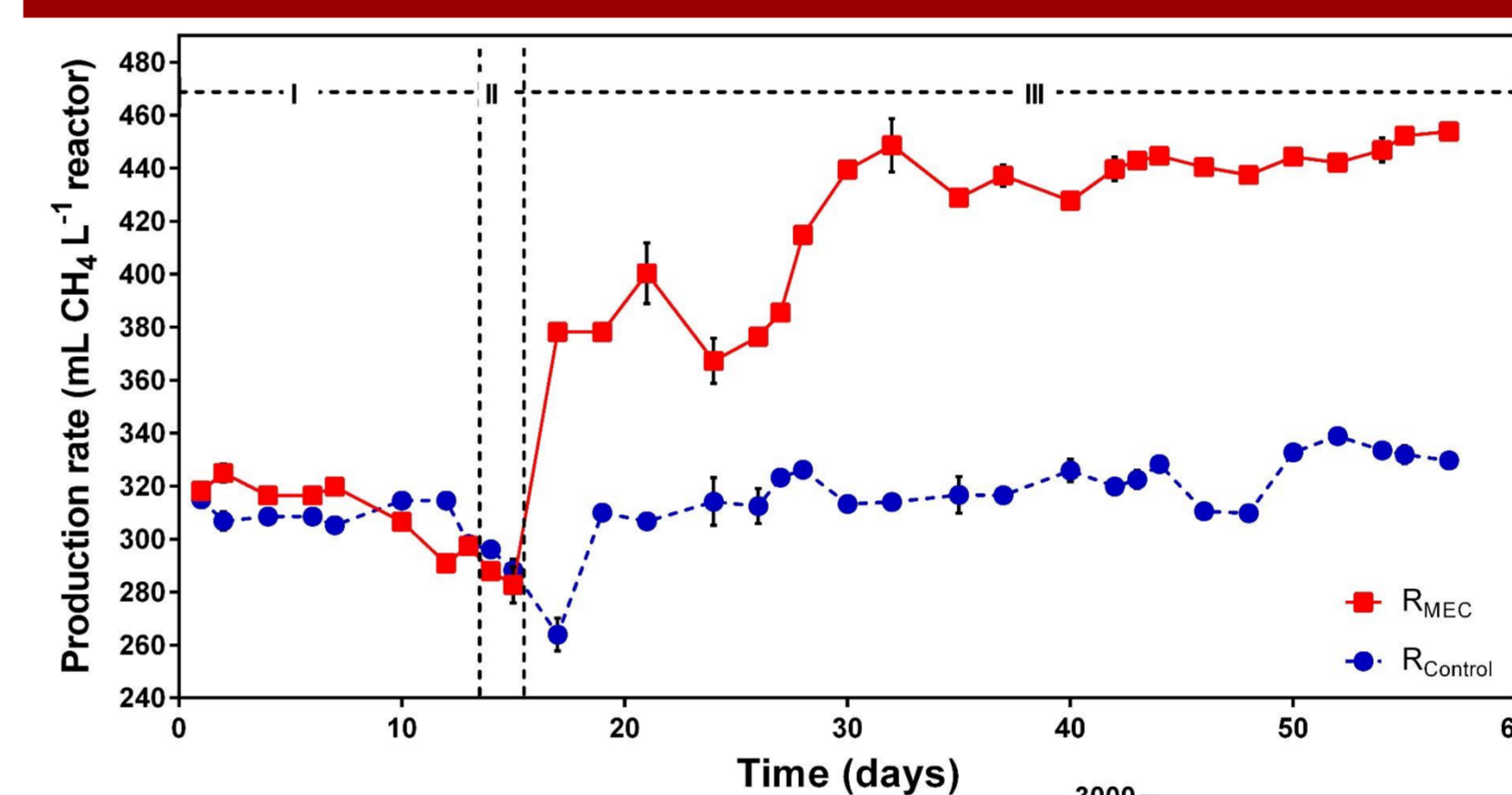


Fig. 3. Methane production rate of the CSTR reactors

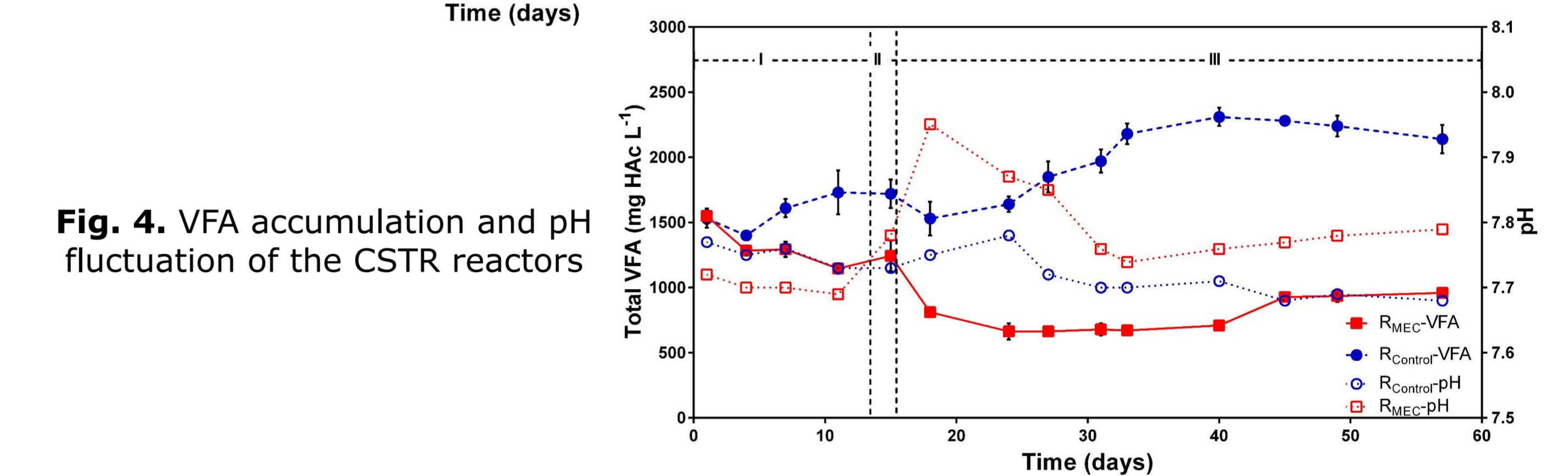


Fig. 4. VFA accumulation and pH fluctuation of the CSTR reactors

## Results

After bioaugmentation (Period-III), R<sub>MEC</sub> reactor demonstrated a significant improvement in methane production which led to an uninhibited steady-state (Fig. 3). In this steady-state, **R<sub>MEC</sub> reactor was operating continuously for more than one HRT with 40% higher methane production compared to the initial "inhibited steady-state"**. R<sub>MEC</sub> reactor regained the same methane production rate it had before the introduction of the ammonia to the feedstock, alleviating completely the ammonia inhibitory effect. VFA accumulation and pH fluctuation after bioaugmentation process of R<sub>MEC</sub> reactor, were kept within the normal limits (Fig. 4).

## Conclusions

In the current study was established that **an enriched ammonia tolerant methanogenic culture was successfully bioaugmented in an ammonia inhibited CSTR reactor and could completely alleviate ammonia toxicity**. This new method, is very promising, for the development of an efficient and cost-effective biomethanation process of ammonia-rich organic waste in full-scale reactors.

## Acknowledgments

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