Bioaugmentation of immobilised inocula coupled with biochar to solve the ammonia problem in continuous biogas reactors

I.A. Fotidis[1,2], Y. Yan[2], D. Fu[2]

[1]Department of Environment, Ionian University, P.C. 29100 Zakynthos, Greece
[2]School of Civil Engineering Southeast University, 210096, Nanjing, China
Presenting author email: ifotidis@ionio.gr

Anaerobic digestion (AD) process instability with suboptimal methane production usually occurs due to excess ammonia from the substrate degradation. Bioaugmentation with ammonia-tolerant methanogens into inhibited AD systems was proposed as a successful remediation strategy to alleviate ammonia toxicity (Fotidis et al., 2014). Additionally, it is proposed that biochar has the potential to assist direct interspecies electron transfer between syntrophic acetogenic and methanogenic communities (Rotaru et al., 2014). The combined effect of the bioaugmentation with ready-to-use inocula and biochar addition in continuous reactors were assessed in an earlier study (Yan et al., 2022). The commercial AD reactors may suffer from additional ammonia toxicity events but the long-term stability of bioaugmented AD systems, without further bioaugmentation, has never been assessed. The current study aims to evaluate the long-term performance of the biochar enhanced novel bioaugmentation strategy in continuous stirred tank (CSTR) reactors under increasing ammonia levels and the changes of the reactors' microbial community through the increasing ammonia toxicity events and their contribution to potential AD process stability.

Food waste was pre-treated into organic fraction by pulping and used as feedstock. The influent contained volatile solids (VS) of 45.34 ± 0.22 g L^{-1}, total ammonium nitrogen of 0.84 ± 0.02 g NH_4^+-N L^{-1} and its pH being 4.31 ± 0.04. The biochemical methane potential was measured to be 474.5 ± 18.7 mL CH_4 g^{-1} VS. Two CSTR reactors (i.e., R_1 and R_2), were operated at thermophilic conditions (55°C) with hydraulic retention time of 15 days. The total and working volume of the reactors were 2.3 and 1.8 L, respectively. Gel-immobilized inoculum and biochar were bioaugmented into R_2, while R_1 was the control reactor. To evaluate the long-term resistance to ammonia toxicity, three further ammonia shocks from 5.5, 6.5 and 7.5 g NH_4^+-N L^{-1}, were applied in the reactors (Phases 1-4, Table 1).

Table 1. The experimental periods' parameters of the CSTR reactors

Parameter	P1	P2	P3	P4
Days	1-20	21-60	61-89	89-110
Organic loading rate (VS L^{-1} d^{-1})	3	3	3	3
Total ammonium nitrogen-TAN (g NH_4^+-N L^{-1})	4.5	5.5	6.5	7.5
Biochar in R_2 (g L^{-1})	2	2	2	2

Total solids (TS), VS and TAN were analysed according to APHA (2005); Gas-chromatographs TRACE 1300 and Trace 1310 GC-TCD, were used to determine volatile fatty acids (VFA) and methane content, respectively. Microbial community were analysed with 16S rRNA gene technique (Tian et al., 2019). In the beginning of P2, R_1 and R_2 suffered 31.3% and 17.1% methane production loss due to the 5.5 g NH_4^+-N L^{-1} ammonia shock, respectively. However, after 15 days, R_2 overcame the ammonia toxicity shock and completely recovered its methane production rate to 1166.1±36.6 mL L^{-1} d^{-1} (90.7% compared to maximum production rate). In P3, R_2 kept 834.3±25.2 mL L^{-1} d^{-1} methane production rate, which was 71.7% compared to P1.

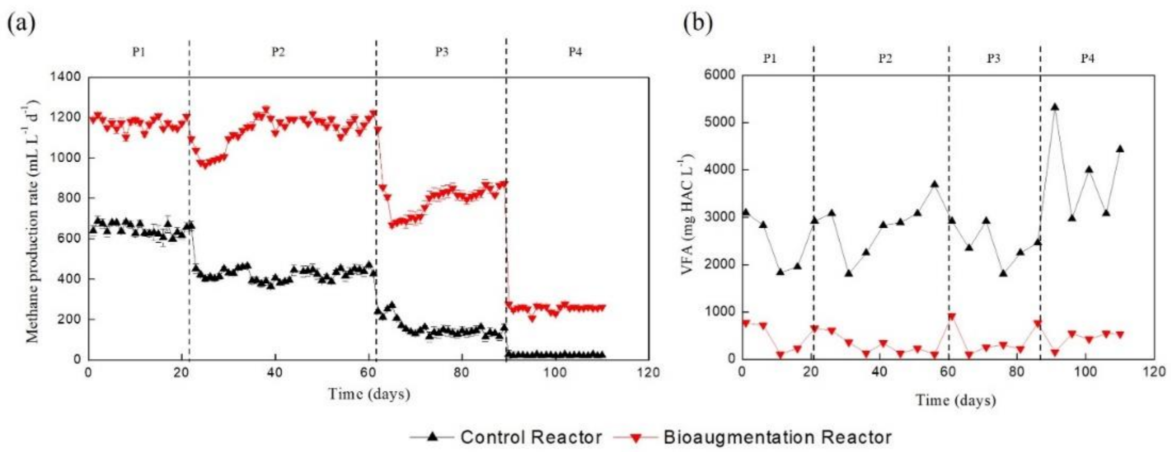

Figure 1. a) Methane yield and b) VFA variation.

Biomethanation process was significantly inhibited in R_1 with less than 40% methane production compared to P1. Finally, the R_1 suffered a complete process failure at 7.5 g NH_4^+-N L^{-1} (P4), while R_2 still managed to have a limited methane production (less than 300 mL L^{-1} d^{-1}). Hence, it was clearly demonstrated that bioaugmentation enhanced with biochar, can offer a long-term stability in continuous anaerobic reactors suffering from extreme ammonia toxicity events, without performing another bioaugmentation and without changing the ammonia-rich feedstock or adjusting any other operational parameters.

The microbial community showed significant changes after ammonia shocks (Fig. 2). *Methanoculleus thermophilus* sp. was the most tolerant methanogenic species in R_2 under the increasing ammonia stress, which was identified as the "crucial species". In R_1, the relative abundance of hydrogenotrophic methanogens (e.g., *thermophilus* sp. and *thermautotrophicus* sp.) decreased and the dominant species was replaced by *Methanosarcina* sp. Moreover, *Syntrophomonas* sp., *Tepidanaerobacter syntrophicus* sp., *Syntrophaceticus schinkii* sp. and *Bacteroidetes* sp. increased their relative abundance in R_2 compared to significant reduction in R_1, which were demonstrated as syntrophic partner in biomethanation process (Lü et al., 2016).

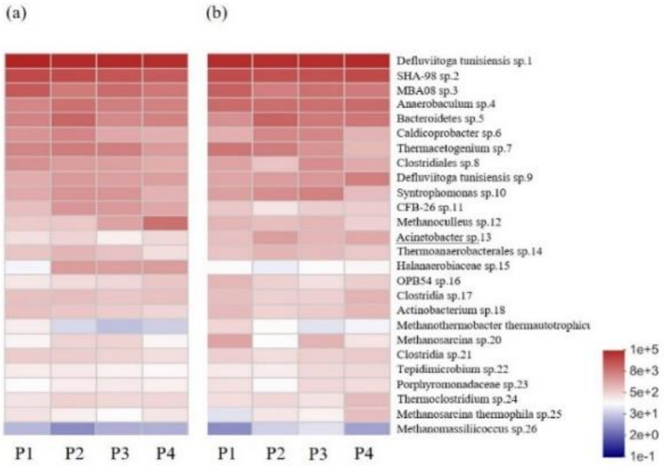

Figure 2. Relative abundance of species: a) R_1, b) R_2

Biochar addition promoted long-term stability of microbial community by protecting the introduced *Methanoculleus* sp. and syntrophic VFA-oxidizing bacteria under increasing ammonia stress. This novel bioaugmentation strategy can efficiently alleviate repetitive ammonia shocks and its results are expected to last for many HRTs.

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