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Humic acid inhibition of hydrolysis and methanogenesis with different anaerobic inocula

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ABSTRACT

There is increasing evidence that humic acid (HA) is hampering the performance of anaerobic digesters treating animal manures and thermally-hydrolysed waste activated sludge. In the present study, HA inhibition and inhibition resilience was examined for hydrolysis (carbohydrate and protein) and acetotrophic methanogenesis with four distinct full-scale anaerobic inocula. The aim was to further understand HA inhibition and to explore potential relationships between microbial factors and inhibition resilience. For two of the four tested inocula, cellulose degradation showed a start-up delay that lengthened as HA concentration increased from 0 to 2 g L^{-1}. This inhibition was reversible because, after the initial delay, subsequent hydrolysis rates and methane yields were not significantly influenced by HA concentration. Cellulose hydrolysis results at HA concentrations below 2 g L^{-1} support a threshold inhibition mechanism, i.e. HA complexes with hydrolytic enzymes preventing them from binding with cellulose, but once all the HA had been complexed, enzymes subsequently released are free to bind with cellulose. Inocula with higher cellulose hydrolytic activity were less affected by HA inhibition, suggesting a potential link between HA inhibition resilience and microbial activity. However, above 5 gHA L^{-1}, cellulose hydrolysis rates decreased with increasing HA concentration; indicating that the mechanisms of inhibition may change depending on some threshold HA concentration. Protein hydrolysis and acetotrophic methanogenesis were less susceptible to HA inhibition than cellulose hydrolysis, since signs of inhibition were only observed above 5 gHA L^{-1}. Acetotrophic methanogenesis was partially inhibited at 10 gHA L^{-1} and completely inhibited at 20 gHA L^{-1}. These results further support that HA inhibition is selective towards particular enzymes.

1. Introduction

Anaerobic digestion (AD) is an established treatment option for organic residues, providing renewable biogas energy and mobilising nutrients for subsequent recovery. AD involves various biological reactions requiring complex syntrophic microbial communities. When treating particulate residues (e.g. animal manure, crops, sewage sludge), the overall AD process rate is typically limited by hydrolysis (Vavilin et al., 2008). Methanogenesis can also be rate limiting, due to sensitivity of methanogens to chemical inhibition (e.g. ammonia and cations) (Chen et al., 2014). However, some chemical inhibitors such as humic and fulvic acid can have a greater impact on hydrolysis than on methanogenesis (Ghasimi et al., 2016; Khadem et al., 2017).

Humic acid (HA) is a product of the degradation and/or polymerisation of organic matter (Veeken and Hamelers, 1999). Therefore, HA inhibition is particularly relevant when considering AD and associated pre-treatment of high-solids wastes, where HA progressively accumulates up to 10 g L^{-1} as wastes degrade (Dwyer et al., 2008; Fernandes et al., 2015; Yap et al., 2017). Previous studies have reported hydrolysis inhibition by humic substances within the concentration range 0.5–5.0 g L^{-1} (Fernandes et al., 2015; Ghasimi et al., 2016). Mechanistic hypotheses have been proposed for HA inhibition of hydrolysis. These include (a) threshold-type inhibition where HA binds to active sites of relevant hydrolytic enzymes, thereby preventing access to substrates (Brons et al., 1985; Fernandes et al., 2015), and (b) that humic substances bind to hydrolytic bacterial cell walls, disrupting cell membrane integrity and/or essential cellular transport processes (Smith

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et al., 2005). In terms of threshold-style inhibition, Fernandes et al. (2015) indicated that humic compounds have a stronger affinity for enzymes hydrolysing cellulose (carbohydrate) than enzymes hydrolysing tributyrin (lipid). Accordingly, HA inhibition may be selective towards particular enzymes and/or substrates. Mechanisms of HA inhibition is a subject of ongoing research.

Variations in AD operating conditions and substrate type can be selectors for microbial composition and diversity within anaerobic digesters (Lu et al., 2018; McHugh et al., 2003; Regueiro et al., 2012; Vanwonterghem et al., 2014; Zhang et al., 2014). Differences in microbial composition could also have an impact on resilience towards inhibitors. For example, a review by Smith et al. (2005) suggested that bacteria in rumen overcome HA inhibition by producing altered enzymes and/or through membrane modification and repair. Therefore, inhibition could be mitigated by intrinsic resilience in AD microbial communities. However, prior studies have focussed mainly on physico-chemical methods to mitigate HA inhibition, such as precipitation or complexing with metal salts (Azman et al., 2015; Brons et al., 1985), or by removing HA via ion exchange (Boyer and Singer, 2006; Fearing et al., 2004). Nonetheless, for many full-scale applications, such physico-chemical methods may not be economically feasible.

The present study investigated HA inhibition and inhibition resilience for four distinct full-scale anaerobic inocula. Inocula were collected from anaerobic digesters treating different feedstocks and operating at different conditions. Inocula origin was used as selectors for different microbial composition, diversity and activity. The aim was to better understand HA inhibition and to explore relationships between microbial factors and inhibition resilience.

2. Materials and methods

2.1. Chemicals and inocula origin

All substrates were analytical reagent grade, purchased from Sigma-Aldrich. Anhydrous sodium acetate, gelatine and α-cellulose were added as model substrates to study acetate, protein and carbohydrate degradation, respectively. The inhibitor HA was added as a sodium salt (lot number 16308-048). The four inocula studied were:

(1) DSS: digestate from a 5500 m^3 mesophilic digester (35 °C) at a domestic wastewater treatment plant (WWTP) in South East Queensland (Australia), treating a mixture of primary and waste activated sludge at a hydraulic retention time (HRT) of 23–24 days.
(2) THD: digestate from a 2250 m^3 mesophilic (37 °C) anaerobic digester at a centralised municipal biosolids processing facility in South East Queensland (Australia), fed with thermally hydrolysed waste activated sludge from a CAMBI® process (155 °C, 4.5 bar).
(3) PLS: anaerobic sludge extracted from the base of a covered anaerobic pond treating coarse-screened flush manure from grower-finisher pig sheds in Victoria (Australia) at 20–25 °C. The sludge was extracted using a vacuum tanker connected to sludge extraction ports through the side banks of the covered pond near the inlet side.
(4) PPDS: digestate from a completely mixed tank digester located at a piggery in Queensland (Australia). This digester treated a mixture of piggery flush manure and macerated paunch from a nearby meat processing facility at 25 °C and about 15 days HRT (first-stage digester). Paunch (cattle stomach content) is a lignocellulosic-rich waste containing partially digested cattle feed (grass and grain), water and stomach fluids. The digester produces approximately 130 kWe and 70 kWe from digestion of the manure fraction and paunch fraction, respectively.

The inocula were characterised for pH, total solids (TS), volatile solids (VS), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), volatile fatty acids (VFAs), total ammoniacal nitrogen (TAN) and colour as described in Section 2.4. Inocula properties are summarised in Table 1. Microbial community composition of each inoculum was characterised as described in Section 2.3.

2.2. Inhibition testing

Two sets of batch inhibition testing were conducted for hydrolytic activity (see Section 2.2.1) and acetotrophic methanogenic activity (see Section 2.2.2). In Set 1, all four inocula were tested with HA concentrations of 0–2 gHA L^{-1} (added). In Set 2, only DSS was tested for a broader range of HA concentrations (0–20 gHA L^{-1}) since (i) it was not affected for HA concentrations up to 2 gHA L^{-1} and (ii) it is obtained from a well-characterised stable digester in terms of operation and performance.

2.2.1. Hydrolytic activity test

Hydrolytic inhibition tests were performed in 160 mL glass serum bottles (working volume 100 mL) at 37 ± 1 °C. This included pre-dilution of the inoculum to 10 gVS L^{-1} using deionised water to minimise mass transfer issues and reduce inhibitor background concentration (Astals et al., 2015). Cellulose or gelatine was added at an inoculum-to-substrate ratio of 5 on a VS basis. Prior to the test, the inoculum was stored at 37 ± 1 °C for 5 days to de-gas.

Six replicate bottles were run for each HA concentration and for substrate-free blanks: three bottles to measure the methane production and three bottles to measure the soluble COD and VFA concentration. The latter accounts for the hydrolysed compounds that had not yet been methanised. Blanks methane production and blanks sCOD were used to correct the inoculum background contribution. Test bottles were mixed by swirling before each sampling event. Biogas volume was measured using a water displacement

Table 1
Physico-chemical composition of the inocula under study.

Parameter	DSS	THD	PPDS	PLS
pH (–)	7.00 ± 0.03	6.98 ± 0.03	7.92 ± 0.01	6.99 ± 0.04
TS (g kg^{-1})	30 ± 2	49 ± 3	28 ± 2	52 ± 3
VS (g kg^{-1})	21 ± 2	31 ± 3	19 ± 2	40 ± 2
tCOD (g L^{-1})	33 ± 3	53 ± 4	41 ± 4	64 ± 4
sCOD (g L^{-1})	0.2 ± 0.1	5.2 ± 0.4	2.8 ± 0.1	0.6 ± 0.1
tVFA (mg L^{-1})	61 ± 10	97 ± 22	445 ± 111	87 ± 18
TAN (mgN L^{-1})	210 ± 12	2665 ± 70	1648 ± 12	612 ± 36
Colour (mgPtCO L^{-1})	2667 ± 5	13581 ± 10	2558 ± 3	10490 ± 9