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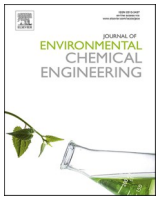

Supplementation strategies to control propionic acid accumulation resulting from ammonia inhibition in dry anaerobic digestion: Osmoprotectants, activated carbon and trace elements

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ABSTRACT

Propionic acid accumulation in anaerobic digestion is a common sign of inhibition at high ammonia levels. To mitigate accumulation three supplementations were tested: osmoprotectants, trace elements and activated carbon. Activated carbon and osmoprotectants ($MgCl_2$) achieved a 28 % increase in methane yield and a 3-fold reduction in hydrogen partial pressure compared with the control. Trace elements supplementation increased methane formation by 18 % without preventing instability. No supplementation avoided propionic accumulation, although $MgCl_2$ delayed it. Activated carbon and $MgCl_2$ supported proliferation of strict hydrogenotrophs, increasing microbial redundance with expected positive impacts on process resilience. Evidence beyond previous studies on the role of retention time as a control parameter of versatile archaea's methanogenic pathway is also provided. As retention time is reduced, syntrophic acetate oxidising bacteria are washed out of the system, likely resulting from an increase in their doubling time with inhibitors accumulation, preventing hydrogenotrophic methanogenesis and supporting previous observations of *Methanosarcina* being forced to conduct acetoclastic methanogenesis. Longer retention times to accommodate longer doubling times or alleviation of inhibition with activated carbon and $MgCl_2$ supported retention of syntrophic acetate oxidising bacteria, enabling strict hydrogenotrophic archaea to proliferate. These supplementations would allow operation of industrial scale ADs at shorter retention times and higher throughputs. Results suggest that osmoprotectants and activated carbon addition were linked to a reduction in archaea's osmotic pressure and enhanced direct interspecies transfer, respectively, leading to increased methane formation despite propionic levels.

1. Introduction

Dry anaerobic digestion (AD) is a feasible solution to divert the organic fraction of municipal solid waste (OFMSW) from landfill, although further optimisation is necessary due to operation at high total solids (TS) over 20 % [1–3]. Operating at reduced water levels increases degradation time, lag phases and accumulation of inhibitors like volatile fatty acids (VFA), total (TAN) and free ammonia (FA) [1]. FA levels over 200 mg/L are considered inhibitory for strict acetoclastic methanogens, while hydrogenotrophic methanogens can tolerate up to 1000 mg/L [4–7], making of hydrogenotrophic methanogenesis the most reported pathway in high ammonia environments. Although significantly less reported in literature, acetoclastic methanogenesis can occur at high ammonia levels if mediated by versatile archaea, which have the ability to use both the acetoclastic and hydrogenotrophic pathways of methane generation [8]. Rocamora et al. [9] reported acetoclastic

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methanogenesis at TAN over 8 g/L, conducted by the versatile archaea *Methanosarcina*. On this case, hydraulic retention time (HRT) was reported as controlling for the pathway of versatile archaea, hypothesising that inhibitors accumulation negatively impacted doubling times of syntrophic acetate oxidising bacteria (SAOB), resulting in wash out of syntrophic communities needed for hydrogenotrophic methanogenesis. The syntrophic relation between SAOB and hydrogenotrophic archaea is needed to maintain intermediates like hydrogen [10,11] and propionic acid [9,12,13] at low levels.

Addition of activated carbon (AC) or trace elements (TE) have been tested successfully in wet AD and dry AD up to 20 % TS, where the digester's broth composition is expected to be mostly homogenous, effectively reducing ammonia inhibition and preventing propionic accumulation [14]. Conductive materials such as AC or biochar have been reported to increase direct interspecies electron transfer (DIET) between syntrophic bacteria and hydrogenotrophic archaea relieving ammonia inhibition, helping growth and attachment of microorganisms [15], improving VFA degradation and reducing hydrogen accumulation [16]. Pan et al. [17] showed a 49 % increase in methane yields when adding 0.5 g of AC per gram of VS in dry batch AD of sewage sludge at 36 % TS. Zhou et al. [15] further showed a 24-fold increase in methane yields when adding AC to an inhibited AD of chicken manure with TS of 22 %. Dosing of TE has been used to reduce accumulation of propionic acid and other VFA in AD with high TAN concentrations [12,18], as TE are required to synthesize enzymes needed in syntrophic hydrogenotrophic methane production. Selenium (Se), molybdenum (Mo) and tungsten (W) are known requirements for the formate dehydrogenase enzyme, which triggers a feedback inhibition of propionic acid if not sufficiently present [19]. Other studies highlighted Se as key in food waste digestion [20], with cobalt (Co) limitation at high organic loading rates (OLR) [12]. Osmoprotectants (OP) use in AD is very limited to date, and mainly linked to high salinity digesters [21,22]. The natural accumulation of OP like betaine [23] or potassium in case of osmotic stress has been reported in archaea, hypothesising OP synthesis as a driver for archaea and bacteria adaptation to high ammonia environments [24]. OP help maintaining osmotic pressure in the cytoplasm of cells at higher levels than in the environment and sustain cell turgor (osmoadaptation) provoked by salinity or ionic accumulation, such as ammonia [23,25]. Yan et al. [25] used magnesium chloride, potassium chloride and betaine to recover a wet AD after an ammonia shock, achieving a 54.2 %, 24.9 % and 36.4 % methane recovery.

Dosing of AC, TE or OP have been reported to reduce ammonia inhibition and propionic accumulation in wet AD [12,25,26]. However, it remains unclear whether these strategies are effective in dry AD systems operating at high TS of over 40 %, where the limited dispersion of supplements and the impracticality of mechanical mixing create localized conditions within the digesting broth, often described as 'pockets of inhibition'. The aim of this study is to evaluate, for the first time, the effect of TE, AC and OP addition in dry AD to reduce ammonia inhibition and its effect on propionic acid accumulation at extreme high TS of over 40 %, where literature is inexistent. The significance of the work is on providing a science-based optimisation strategy that can foster sustainable growth of dry AD as a waste management and energy generation technology, in line with sustainable development goals 7 and 12. First, batch experiments with a digestate showing ammonia and propionic acid accumulation were used to monitor propionic acid degradation and choose the appropriate dosing. Semi-continuous ADs fed with OFMSW were then supplemented with the best performing strategies from the batch trials, to inform benefits achievable in full-scale continuous units. Microbial communities and carbon isotopic signature were analysed periodically to get a deeper understanding of the effect of the different strategies in the predominant methanogenic pathways.

2. Materials and methods

2.1. Feedstock and inoculum

Batch sacrificial reactors used to assess the effect of different supplements on propionic acid degradation were inoculated with digestate from a semi-continuous pilot-scale digester fed with the OFMSW for 145 days in a previous experiment [9] (Table 1). Digestate had a TAN concentration over 8 g/L and a propionic acid concentration of 2.2 g/L. For the semi-continuous experiments conducted to assess the effect of the different supplements on propionic acid accumulation, inoculum (propionic acid concentration of 50 mg/l) and OFMSW (Table 1) were collected from a mesophilic semi-continuous dry AD facility in North-East England, United Kingdom, treating up to 40,000 tonnes of OFMSW per year. Different inoculums with different starting propionic acid concentrations were used as the batch experiments were focussed on assessing the impact of supplements on propionic acid degradation while efforts on the semicontinuous trials were on preventing its accumulation. The OFMSW is mechanically separated from the non-organics and reduced to a particle size < 40 mm using a shredder. Both materials were collected at the same time and stored at 4°C before analysis and use in the experiments. The OFMSW was also characterised for biomethane potential (BMP), equal to 195.9 ± 13.9 l/kg VS, using the methodology recommended by Holliger et al. [27].

2.2. Propionic degradation trials in batch sacrificial reactors

Sacrificial reactors containing only digestate were operated with seven different supplementations informed from wet AD literature on TE [12], AC [28] and OP [25], and expanding the ranges to test additional concentrations (Table 2) to evaluate their effect on propionic acid degradation. Each additive (Table 2), 50 g of digestate and water (dilution to 20 % TS) were added for a final volume of 100 ml and placed in 125 ml flasks. The reduction in TS in the batch experiments was deemed necessary to facilitate the mixing of the additives, improve diffusion of the supplements and reduce 'pockets of inhibition', hence being able to test the impact of different supplements and concentrations. The reactors were continuously shaken in an incubator at 38°C for 15 days. Four reactors were set for each condition and four extra used as control units. Biogas was vented daily but not monitored, as focus was on the evolution of propionic acid in the digesting broth. One reactor was sacrificed for each condition on days 3, 6, 10 and 15 to analyse propionic acid concentration.

Addition of Se was undertaken as solid sodium selenite (Merk, New Jersey, USA), Co as a 99.99 % pure powder (Merk, New Jersey, USA), and the commercial mix T5 with 10 g Co/L, 10 g Ni/L, 2 g Se/L, 2 g Mo/L and 2 g W/L (Celtic Chemicals, Port Talbot, UK) was used for the addition of the different mixes of TE (TE mix). $MgCl_2$ was used as a hexahydrate salt (Fischer Scientific, New Jersey, USA), KCl as a 99.99 % pure solid reagent (Merk, New Jersey, USA), and betaine as > 98 % pure solid reagent (Merk, New Jersey, USA). Granular AC was used from DARCO®, with a 20–40 mesh particle size (Merk, New Jersey, USA).

The metric used to compare propionic acid degradation in the different sacrificial batch reactors was the Degradation Index (DI). The DI, expressed as a concentration • time product with units of $mg{\cdot}L^{-1}{\cdot}d$, was obtained by calculating the numerical integrals of the VFA degradation curves using the trapezoidal rule, as shown in Eq. 1 [20].

$$DI \int_0^{\infty} f(t) \bullet dt \approx \sum_{i=0}^{n} \frac{(t_{i+1} - t_i) \bullet (C_{i+1} + C_i)}{2} \quad (1)$$

Where C is the concentration of propionic acid in mg/L and t the time in days. DI ($mg{\cdot}L^{-1}{\cdot}d$) quantifies propionic acid degradation efficiency based on the degradation curve, where a smaller DI number indicates a more efficient degradation [20].

Table 1
Digestate and OFMSW characteristics[a].

Experiment	Material	TS (%)	VS (%)	VS/TS (%)	C/N
Batch	Digestate	37.5 ± 4.2	19.9 ± 1.4	30.1 ± 3.9	16.5 ± 4.1
Semi-continuous	Digestate	44.8 ± 2.1	13.5 ± 2.1	30.1 ± 3.9	15.9 ± 3.6
	OFMSW	50.8 ± 2.5	26.6 ± 1.0	52.6 ± 4.4	16.2 ± 1.1

[a] Samples were analysed in triplicate.

Table 2
Supplementations tested in the sacrificial reactors.

		Tested concentration				
	Cobalt	0.25	0.5	1.0	1.5	
	Se	0.05	0.1	0.2	0.3	0.4
		Mix 1	Mix 2	Mix 3	Mix 4	
TE[a] Mix (mg/kg digestate)	**Co**	0.25	0.5	1	1.5	
	Ni	0.25	0.5	1	1.5	
	Se	0.05	0.1	0.2	0.3	
	Mo	0.05	0.1	0.2	0.3	
	W	0.05	0.1	0.2	0.3	
OP[2] (mg/kg digestate)	**$MgCl_2$**	20	40	80		
	KCl	20	40	80		
	Betaine	50	100	150		
AC[3] (g/kg digestate)		5	7.5	10		

[a] TE: Trace elements, [2]OP: Osmoprotectants; [1]AC: Activated carbon. Concentrations are normalised to digestate fresh weight.

2.3. Semi-continuous anaerobic digesters and operating conditions

Semi-continuous experiments were performed in three 20 L digesters with no internal mixing, which were initially loaded with 15 kg of fresh digestate from a full-scale semicontinuous dry AD plant (50 mg/l of propionic acid). Biogas flow was measured with a CJC-125 gas counter and a CJC-034 data acquisition system (CJC Labs, Cumbria, UK). The digestion took place at mesophilic conditions (38°C) in a Binder FP720 incubator (Binder GmbH, Tuttlingen, Germany). The OFMSW was mixed with digestate extracted from the digester in a 1 to 3 ratio in mass before each feeding, which was done three times a week. The discarded digestate was used for analysis.

Two OLRs (3.0 and 4.0 kg VS/m^3/day) were tested to understand the operational limit of the inhibition mitigation strategies tested. All digesters started up with an OLR of 3.0 ± 0.3 kg VS/m^3/day (RT of 78 days) until the exponential increase in propionic acid accumulation ceased, which was used as a criterion for changing OLR. The OLR was then increased stepwise to 3.5 ± 0.4 kg VS/m^3/day (RT of 67 days) for a week as an intermediate step to avoid shock-loading the process before reaching the final OLR of 4.0 ± 0.7 kg VS/m^3/day (RT of 59 days). The intermediate step was done temporarily to reduce overloading and support acclimatation of microbial communities. The supplements that achieved the highest propionic acid degradation in the sacrificial reactors were used in the semicontinuous trials (Fig. 1). AD1 was the control reactor and started without any supplementation until day 34, when Se and Co were added (Table 3). Most of the TE present in the OFMSW and digestate of the control unit were in particulate form and hence likely not bioavailable, with a particular deficiency on Se and Co when compared with recommended wet AD levels (supplementary Table 1). AD2 was supplemented with TE mix until day 39, when addition of AC was initiated and TE supplementation stopped. AD3 was fed with $MgCl_2$ during its whole operation time.

2.4. Analytical methods

Analyses were done 3 times a week in triplicates for the digesting broth (digestate) and for biogas, which was sampled before each feeding. Weekly moving averages were used to eliminate the impact of the feeding pattern. Feedstock and digestate were analysed for TS and VS using standard methods [29]. Following a similar methodology to Guendouz et al. [30], digestate was diluted in water five times and mixed in an orbital shaker (Cole-Palmer, St. Neots, UK) at room temperature for 30 minutes before centrifugation in a Megafuge 16 R centrifuge (Thermo Scientific, Massachusetts, USA) for 20 minutes at 4696 *g* and 4 °C. The supernatant was then used for all the wet chemical analysis. A HQ440D Hatch multi-meter (Hach Lange ltd, Manchester, UK) was used for pH analysis, and Ripley ratio (RR) was obtained by manual titration to pH 5.7 and 4. Samples for TAN and VFA analyses were obtained after the supernatant was filtered through a 0.45 μm retention membrane filter (Whatman, Kent, UK). VFA were analysed in a Shimadzu VP Series unit (Milton Keynes, UK) following the methodology described in Rocamora et al. [31]. TAN was analysed by ion chromatography in an ICS900 column (Dionex, California, USA) with an IonPac CS12A as precolumn. Solutions of 20 mM of methanesulphonic

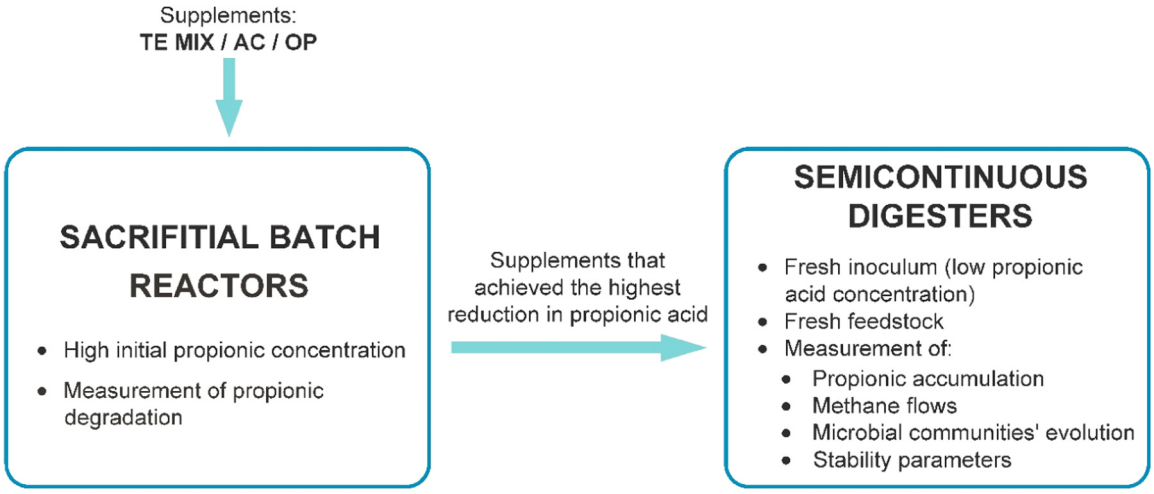

Fig. 1. Experimental design flowchart.

Table 3
Operational conditions and supplementations for each of the semi-continuous ADs.

Digester	Days	RT (days)	OLR[a] (kg VS/m^3/day)	Supplementation	Quantity (per kg OFMSW)
AD1	1–34	78	3.0	None (control period)	0
	35–60	78	3.0	Co + Se	1.5 + 0.3 mg
	61–69	68	3.5	Co + Se	1.5 + 0.3 mg
	70–82	59	4.0	Co + Se	1.5 + 0.3 mg
AD2	1–27	78	3.0	Co+Ni+Se+Mo+W	1.5 + 1.5 + 0.3 + 0.3 + 0.3 mg
	28–39	78	3.0	Co+Ni+Se+Mo+W	3.0 + 3.0 + 0.6 + 0.6 + 0.6 mg
	40–60	78	3.0	AC	7.5 g
	61–69	68	3.5	AC	7.5 g
	70–82	59	4.0	AC	7.5 g
AD3	1–37	78	3.0	$MgCl_2$	40 mg
	38–51	78	3.0	$MgCl_2$	60 mg
	52–55	68	3.5	$MgCl_2$	60 mg
	56–60	68	3.5	$MgCl_2$	80 mg
	61–82	59	4.0	$MgCl_2$	80 mg

[a] OLR: Organic loading rate

acid and 100 mM of tetrabutylammonium hydroxide were used as eluent and regenerant. 20 µl injection volume, a 1 ml/min flow and ambient temperature were used for the analysis. Davies equation was used to calculate FA as function of temperature, pH and ionic strength [7].

Methane and hydrogen concentrations in biogas were analysed in a 990 Micro GC System (Agilent, California, USA) with 2 columns operated at 110°C and 30 psi. Helium was used as carrier gas for hydrogen detection in a MS5A SS column and argon for methane detection in a PoraPLOT Q UM. 50 ms injection time and 110 seconds retention time were used. Methane flows were calculated as litres per kilogram of VS fed and day (kg VS/m^3/day) to facilitate comparison between digesters and with literature. Hydrogen partial pressure in the media is expected to be higher than measured values in the headspace, as supersaturation is expected due to the reduced diffusion linked to the high TS [32].

Carbon isotope ratios of methane ($\delta^{13}C_{CH4}$) and carbon dioxide ($\delta^{13}C_{CO2}$) in the biogas were tested by continuous flow-isotope ratio mass spectrometry in a HP 6890 N gas chromatograph (Agilent, Santa Clara, CA), as described by Keppler et al. [33]. The apparent fractionation factor (α_c) was used to analyse the dominant methanogenic pathway using Eq. 2 [34,35]:

$$\alpha_c = \frac{\delta^{13}C_{CO_2} + 1000}{\delta^{13}C_{CH_4} + 1000} \quad (2)$$

Where $\alpha_c < 1.055$ means acetate dependent (acetogenic) methanogenesis and $\alpha_c > 1.065$ CO_2 dependent (hydrogenotrophic) methanogenesis. Contribution from both methanogenic pathways occurs when α_c is between the mentioned values. All digestate and biogas samples were analysed in triplicates, and ANOVA test with 5 % significance level was used to compare results.

2.5. Microbial analysis

Samples without replicates from the three semi-continuous ADs (AD1, AD2 and AD3) were taken approximately every 10 days to analyse microbial communities' evolution and stored at −20°C until the day of the analysis. DNA extraction was done with a DNeasy PowerSoil Pro (Qiagen, UK) according to manufacturer's protocol. Universal primers 515 F – 806 R targeting both bacteria and archaea were used for amplification of the V4 region of the 16S gene [36]. Illumina MiSeq platform (Illumina, USA) was used for 16S rRNA amplicon sequencing. Raw data was analysed with RStudio version 4.0.3 as described in Rocamora et al. [37].

3. Results and discussion

3.1. Effect of supplements on propionic acid degradation in sacrificial reactors

The effect of different additions of TE, OP and AC on degradation of propionic acid was tested by comparing the propionic acid DI of all the doses (supplementary Figure 1). Propionic acid concentration profiles and DI of the best concentrations for each supplementation strategy were then compared (Fig. 2a and b) to find the best strategies to be used in the semi-continuous AD. Maximum doses of Co, Se, TE mix and betaine achieved the lowest DI (supplementary Figure 1), suggesting that a higher dose than those tested could have resulted in further reduction of propionic acid DI. Addition of KCl at 40 and 80 mg/L achieved almost identical DI, suggesting that a further dose increase would not improve propionic degradation.

The control experiment (blank), without any supplement, resulted in the poorest DI values (Fig. 2b) and the highest propionic acid concentration (Fig. 2a) for most of the experiment. Additions of Co, Se and TE mix resulted in DI values 47 %, 35 % and 55 % lower, respectively, than the control after 15 days (Fig. 2b). All the TE additions resulted in lower DI values than the rest of supplements at day 15 (Fig. 2b), showing a better effect on the reduction of the propionic acid peak concentrations on day 6 (Fig. 2a) compared to the control and the other supplements. TE mix 4 (1.5 mg Co/kg, 1.5 mg Ni/kg, 0.3 mg Se/kg, 0.3 mg Mo/kg, 0.3 mg W/kg) resulted in the lowest DI of all, probably due to an improved synthesis of the enzymes needed in syntrophic hydrogenotrophic methane production [12]. For this reason, TE mix 4 was selected as one of the conditions for the semi-continuous digesters. The co-addition of Co and Se was tested as additional strategy to recover any of the digesters in case of early failure, as its combination has been regarded as beneficial for propionic degradation in literature [12].

Addition of $MgCl_2$ at 40 mg/kg was the OP resulting in the lowest DI with a value 26 % lower than the control. The highest doses for betaine and KCl supplementations achieved a slightly lower DI reduction of 20 % and 24 % respectively. When OP additions were used, propionic acid levels were substantially lower than in the control reactors at the end of the experiment (Fig. 2a), however, all reactors showed increased values on day 6, similar to those measured in the control. Supplementing with $MgCl_2$ achieved the lowest DI out of the OP, and it was hypothesised that $MgCl_2$ helped maintaining the osmotic pressure and reduced ammonia inhibition inside the cells. For this reason, $MgCl_2$ at 40 mg/kg was selected as a propionic-control strategy in one of the semi-continuous digesters.

AC addition at 7.5 g/kg resulted in a DI reduction of 23 % compared to the control, and a lower impact on propionic degradation than the TE mix or $MgCl_2$. For this reason, AC addition was kept as a recovery option for the semicontinuous experiment. Interestingly, the effect of AC on the

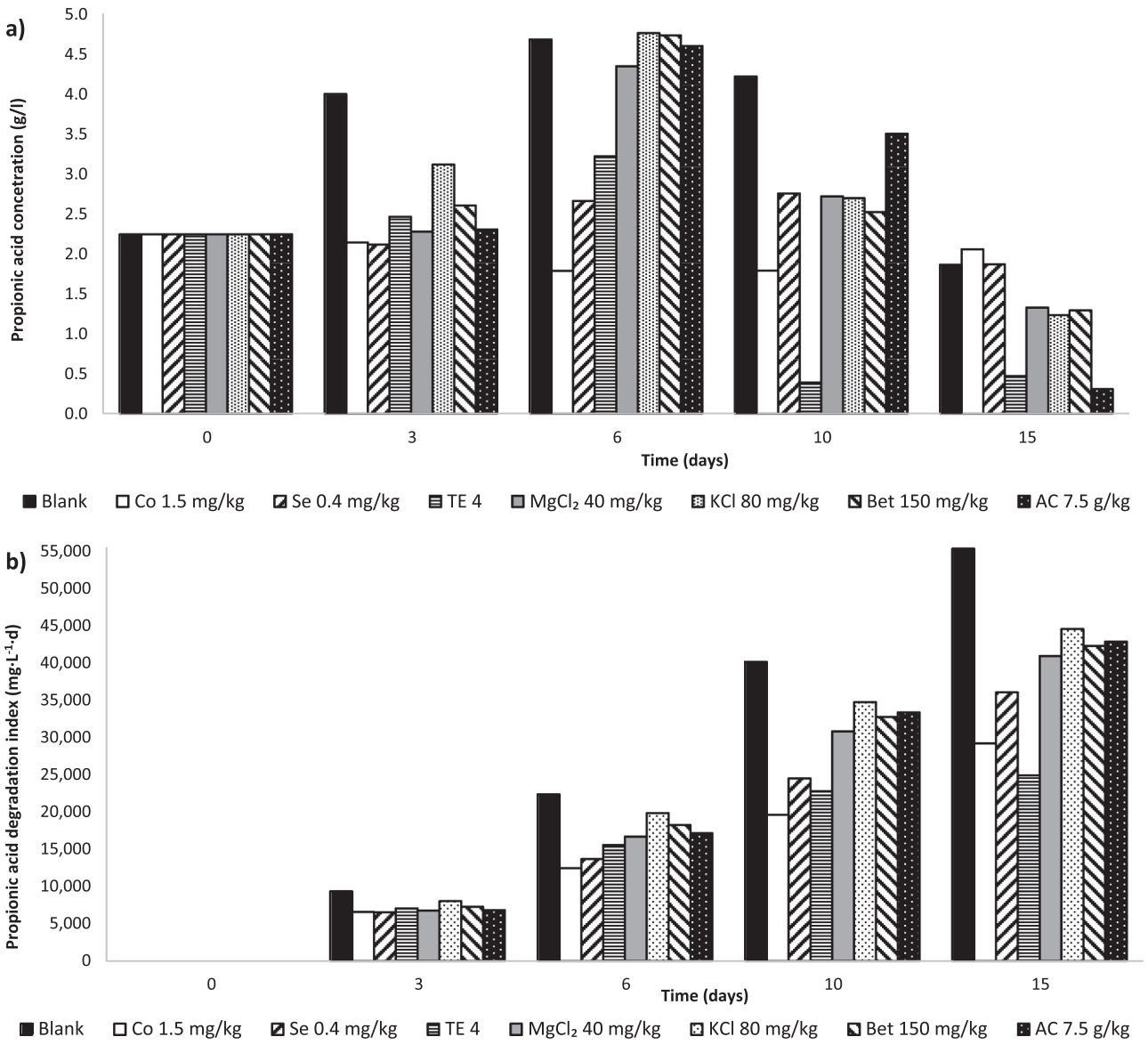

Fig. 2. a) Propionic acid concentration and b) DI profile for the best supplementation dose of each of the strategies tested in sacrificial reactors. Smaller DI numbers indicate a more efficient degradation. All additions are per kg of digestate and only one sample was analysed per day.

peak of propionic acid at day 6 was negligible when compared to the control (Fig. 2a).

3.2. *Impact of supplements on the inhibitory pathways in semi-continuous dry AD*

3.2.1. *AD1 as control reactor (without supplements addition) and Co and Se dosing from day 34*

Until day 35 of operation no supplements were added to AD1, so that performance until then would be used as a control. Methane production increased steadily until day 18, reaching a value of 82.5 ± 0.5 L CH_4/kg VS/day (Fig. 3a) before decreasing to 65.1 ± 1.2 L CH_4/kg VS/day on day 35. Hydrogen partial pressure was over 10^{-4} bar since the beginning of the digestion trial (Fig. 3a), reported as the thermodynamic limit for propionic acid accumulation in wet AD [38]. Values remained over this limit until day 35, with a maximum of 1.8×10^{-4} bar on day 10. Propionic acid accumulation in the digesting broth started on day 18 (Fig. 3c), increasing from 78 to 372 mg/L over a 4-days period co-occurring with a hydrogen partial pressure of 1.6×10^{-4} bar. Propionic acid concentration increased until day 35, reaching 3.3 g/L. TAN and FA started around 6.5 and 1.9 g/L respectively, which was significantly over the 300 mg/L to 800 mg/L of FA considered inhibitory for acetoclastic methanogens [5,39] and over the 1000 mg/L of FA reported as inhibitory for strict hydrogenotrophic methanogens [5]. TAN increased to values over 8 g/L on day 5 and remained between 8 and 9 g/L until day 35, while FA values increased to 2.5 g/L on day 5, then oscillating between 2.2 and 2.9 g/L (Fig. 3d). Clear signs of inhibition were recorded at this stage, as accumulation of VFA and propionic acid reached over 5 g/L (supplementary Figure 3) and 3.5 g/L (Fig. 3d), respectively, hydrogen partial pressure increased and methane flow decreased.

In an effort to reduce propionic acid accumulation, Co and Se at 1.5 and 0.3 mg/kg OFMSW fed were supplemented until the end of the trial, as one of the possible strategies identified with the sacrificial experiment. Following Co and Se addition, methane production increased up to 75.4 ± 1.7 L/kg VS/day on day 42 ($p < 0.05$), then decreasing until the end of the experiment as a result of propionic inhibition (Fig. 3a). A more pronounced drop on methane production occurred when OLR was increased, suggesting a further organic overload. Hydrogen partial pressure reached 4.4×10^{-4} bar on day 52 and then dropped

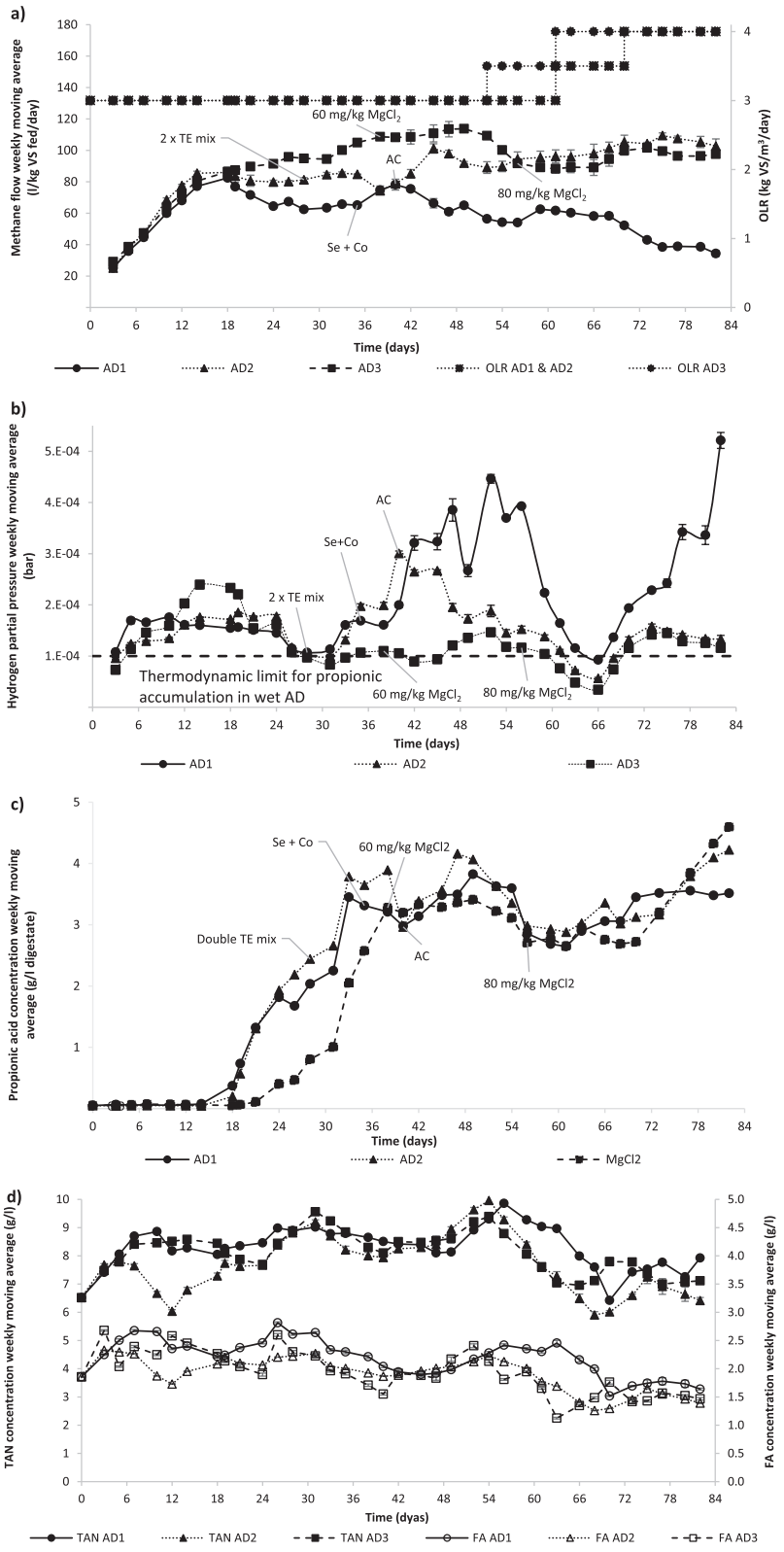

Fig. 3. AD1, AD2 and AD3 weekly moving average profiles for a) methane flow, b) hydrogen partial pressure, c) propionic acid concentration and d) TAN and FA levels. Triplicates were analysed for each point in graphs a, b and d. Only one sample per point was analysed for graph c.

continuously to values just under the thermodynamic limit for wet AD on day 66, finally rising exponentially up to 5.2×10^{-4} bar on day 82 when AD1 was stopped. Propionic acid concentration followed a similar pattern, reaching a maximum concentration of 3.9 g/L on day 49, reducing to 2.7 g/L on day 59 and steadily increasing to 3.5 g/L as the OLR was increased to 3.5 and 4 kg VS/m^3/day. TAN decreased throughout the experiment to values around 8 g/L by the end of the trial, with a peak of 9.8 g/L on day 56. FA levels increased to 2.4 g/L on day

63 and decreased since then to values close to 1.5 g/L. Methane production in AD1 was notably the lowest throughout the experiment compared with AD2 and AD3 (Fig. 3a). This result is linked to the higher inhibition in the system with no supplements addition, intensified by the high ammonia level from the start of the experiment. Addition of Se and Co was unable to recover the digester and improve the decreasing methane rates in AD1. This contradicts existing literature for food waste reactors run at lower solids levels, 20 % TS [12], suggesting a lower efficacy of Se and Co added to systems at very high TS levels (e.g. dry AD).

3.2.2. AD2 with initial TE mix addition and dosing of AC from day 18

Methane production in AD2 was similar to AD1 during the start-up period (Fig. 3a) ($p > 0.05$) and became 18 % higher than for the control AD from day 18. Hydrogen partial pressure followed a similar trend to AD1 but with lower values until day 10 ($p < 0.05$), exceeding the 10^{-4} bar thermodynamic threshold from day 5. No significant difference ($p > 0.05$) with AD1 was found on hydrogen partial pressure between day 12 and day 28 (Fig. 3b). Dosing of TE was not sufficient to prevent propionic acid accumulation, which followed a similar trend as in AD1 ($p > 0.05$) with an increase from 45 mg/L on day 18 to 200 mg/L on day 19, when hydrogen partial pressure was 1.9×10^{-4} bar (Figs. 3b and 3c). TAN values oscillated until day 12 (Fig. 3d), then increasing to similar values ($p < 0.05$) as in AD1 on day 28. FA levels increased for the first seven days, oscillating until day 26 between 2.3 and 2.8 g/L, all values over the reported inhibitory limits for both acetoclastic and hydrogenotrophic methanogens [5].

From day 28 until day 40 the TE mix addition was doubled in AD2 (3.0 mg/kg Co, 3.0 mg/kg Ni, 0.6 mg/kg Se, 0.6 mg/kg Mo, 0.6 mg/kg W) to buffer the rapid increase in VFA accumulation (supplementary Figure 2), in particular of propionic acid (Fig. 3c). Methane flow remained at similar levels after the increase in supplement dose until day 40, when 78.7 ± 0.4 L/kg VS/day were achieved ($p < 0.05$). Similar to the control reactor ($p < 0.05$), hydrogen partial pressure and propionic acid accumulation started to increase rapidly, the latter maintained close to 3 g/L until day 40, highlighting again inhibition. TAN concentration followed a similar pattern to the control until day 40 ($p < 0.05$) whereas FA levels decreased steadily from 2.2 g/L on day 28 until 1.9 g/L on day 40.

Contrarily to the control reactor, the TE mix addition managed to keep a relatively constant methane flow. Cumulative methane production in AD2 was 15 % higher by day 40, likely due to improvement of the syntrophic hydrogenotrophic route resulting from TE dosing [19,20]. However, the process indicated early signs of instability with increasing accumulation of propionic acid and rise in hydrogen partial pressure. Hence, a change of supplementation was decided with AC dosed from day 40 at 7.5 g AC per kg of OFMSW fed. From that point methane production increased, with values over 100 L/kg VS/day from day 68 until the end of the experiment. This increase was sustained even when the OLR was increased to 3.5 (Day 61) and 4 kg VS/m^3/day (Day 70). This is opposite to the trend for AD1 where the methane formation reduced at a faster pace after the OLR reached 4 kg VS/m^3/day. A 22 % increase in methane flow was recorded between the period of TE mix addition (Day 28–39) and the AC addition (Day 40–82) in AD2. These values were 15 % (TE mix) and more than 28 % (AC) higher than the values recorded for AD1 in the same periods (Day 28–39 and Day 40–82 respectively). Hydrogen partial pressure decreased when AC was added, with a 4-fold reduction compared to AD1 by the end of the experiment, regardless of OLR increases (Fig. 3b). Propionic acid accumulation remained similar to AD1 until day 70 (Fig. 3c), when the OLR was further increased to 4 kg VS/m^3/day. From that point AD2 values increased up to 4.2 g/L, while AD1 values remained close to 3.5 g/L until the end of the experiment. TAN and FA levels oscillated, with maximum around day 54, and decreasing afterwards regardless of the change in OLR, with values of 6.4 g/L and 1.5 g/L, respectively, by the end of the experiment (Fig. 3d).

Dosing of AC did not reduce the existing propionic acid accumulation, but allowed an increase in methane production, probably linked to reduction in the hydrogen partial pressure (day 40 onwards). It is therefore reasonable to postulate that hydrogen was converted to methane more efficiently, and the use of AC would have increased the DIET between syntrophic bacteria and hydrogenotrophic methanogens, improving methane flows by reducing the energy necessary for electron exchange and hydrogen consumption.

3.2.3. AD3 with $MgCl_2$ addition

$MgCl_2$ was added to AD3 with an initial dose of 40 mg/kg of OFMSW and then increased to 60 and 80 mg/kg of OFMSW on days 38 and 56 respectively. With the initial dose, cumulative methane formation in AD3 was 28 % higher than the control reactor, reaching 108.6 ± 1.1 L/kg VS/day (Fig. 3a). Hydrogen partial pressure was similar to the control ($p < 0.05$) until day 14, being over the thermodynamic limit for wet AD from day 5. After a short period with levels higher than the control (day 14 to 19), the hydrogen partial pressure was reduced to the lowest values for the three reactors (AD1, AD2 and AD3), oscillating between 3.4×10^{-5} bar and 1.5×10^{-4} bar until the end of the trial, regardless of the $MgCl_2$ dosing or the OLR increase. Propionic acid accumulation was delayed compared to AD1 and AD2 and started with a lower concentration of 108 mg/L on day 21, when hydrogen partial pressure was 1.5×10^{-4} bar, although it had been at higher values over the previous days. From day 21 its concentration increased exponentially to 3.3 g/L on day 38, reaching similar values to AD1 and AD2 ($p < 0.05$) (Fig. 3c). TAN and FA levels were consistent with AD1 ($p > 0.05$) until day 38 and remained over the inhibitory limits for methanogenic activity [5].

From day 38, $MgCl_2$ dosing was increased to 60 mg/kg to test if a higher dose could increase bacterial and archaeal osmoprotection that, in turn, could reduce hydrogen partial pressure and increase propionic acid degradation by SPOB. Methane yield continued to increase until day 52 to 109.3 ± 0.5 L/kg VS/day (Fig. 3a). OLR was increased to 3.5 kg VS/m^3/day from day 52 and methane flow dropped continuously to 91.5 ± 1.5 L/kg VS/day on day 56, remaining constant until the next OLR increase and indicating a preliminary ability of $MgCl_2$ to enable an inhibited steady state to be maintained even at extremely high inhibitors concentrations (Fig. 3c and d). Propionic accumulation was similar to AD1 until day 49 ($p > 0.05$), dropping by more than 10 % ($p > 0.05$) until day 56 despite the OLR increase to 3.5 kg VS/m^3/day from day 52. TAN and FA were comparable to AD1 until day 54 ($p < 0.05$) but both dropped around 10 % compared to AD1 on day 56. The higher dose of $MgCl_2$ (60 mg/kg) stopped the propionic exponential increase and even reduced propionic acid accumulation slightly. Notwithstanding this, supplementing $MgCl_2$ was not enough to maintain methane production at levels before the OLR increase and to avoid propionic accumulation.

From day 56 and until the end of the trial $MgCl_2$ addition was further increased to 80 mg/L $MgCl_2$. Methane production remained constant ($p > 0.05$), even when OLR was increased to 4 kg VS/m^3/day, until day 70 when it increased ($p < 0.05$) to 100 L/kg VS/day which was sustained until the end of the trial. Propionic acid concentration remained below 3 g/L until day 70, 10 days after OLR was increased. Similarly to AD2, values increased rapidly from this point reaching 4.6 g/L by the end of the experiment. The period when propionic level was below 3 g/L co-occurred with a hydrogen partial pressure under 10^{-4} bar, while propionic acid concentration increased when hydrogen went above 10^{-4} bar. This again suggests a correlation between hydrogen and propionic accumulation and the levels of H_2 supersaturation on the digesting broth for high TS digestion merits further investigation. TAN dropped to 7 g/L by day 66 and oscillated between 7 and 8 g/L until the end of the trial, similarly to AD1. FA followed a similar pattern, dropping to 1.1 g/L on day 63 and remaining around 1.5 g/L until the end, like in AD1.

Addition of $MgCl_2$ did not prevent propionic acid accumulation, but was able to delay its occurrence. It also positively impacted hydrogen levels, a known inhibitor of the propionic acid conversion to acetate, with a reduction in its partial pressure compared to AD1 throughout the

experiment. Both results could be attributed to the higher concentration of magnesium in the media, which diffuses easier from the media to the microorganisms and is essential to regulate internal osmotic pressure unbalances created by FA inhibition, reducing the osmotic stress [23]. It is hence hypothesized that $MgCl_2$ dosing helps maintaining cells' turgor pressure through intracellular accumulation of inorganic ions, hence compensating for the potassium loss resulting from potassium pump activation when FA permeating methanogens' wall converts to ammonium [25].

3.2.4. Comparison of different supplements

Overall, when compared to AD1, the different supplements showed little effect on propionic accumulation (Fig. 3c). Only $MgCl_2$ had an initial positive effect delaying its accumulation, although all reactors exhibited similar concentrations (around 3.3 g/L) from day 38. Additions of TE mix from the start of the trial in AD2 resulted on a stable

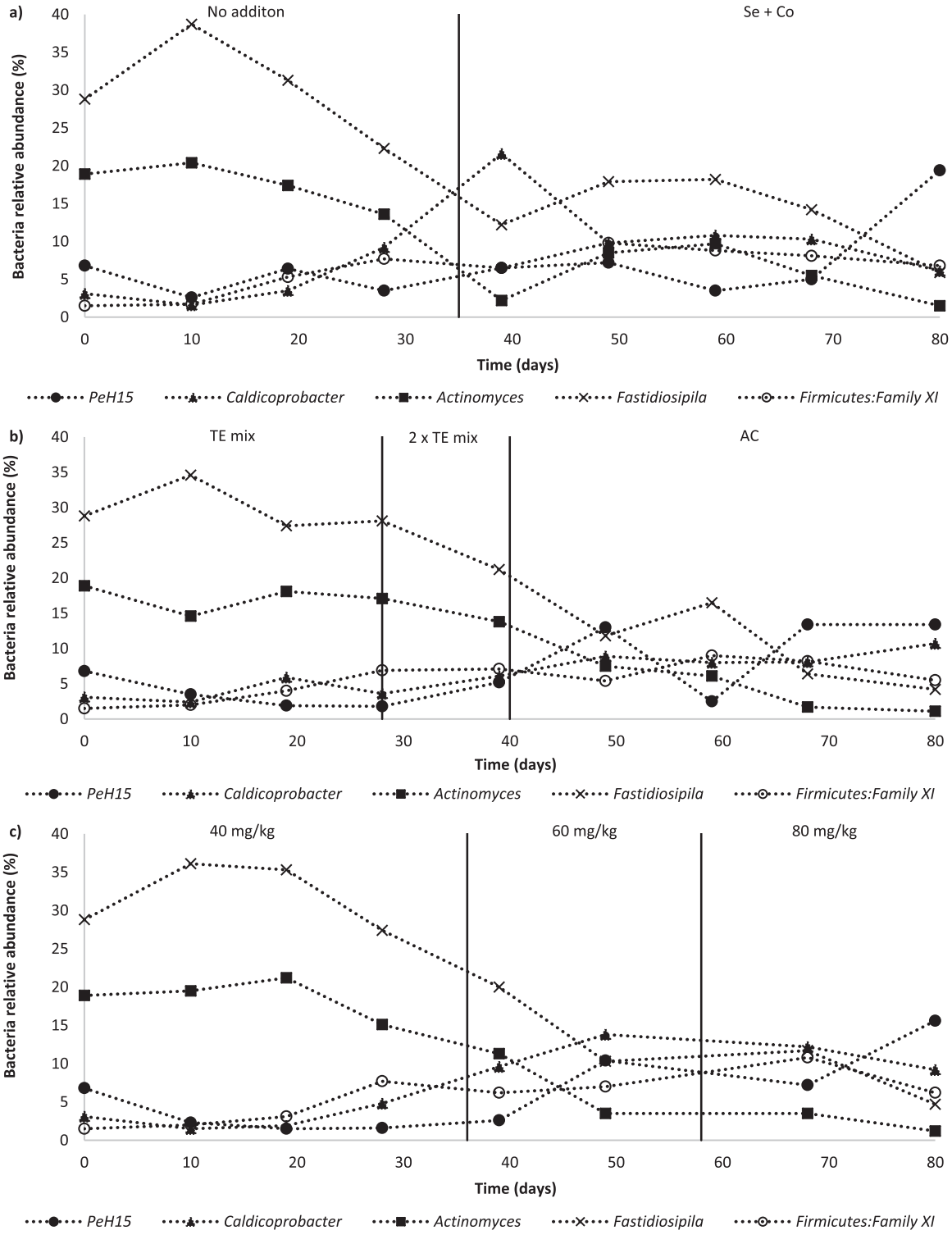

Fig. 4. Time-series of the 5 most abundant Bacteria for a) AD1, b) AD2 and c) AD3 and the 5 most abundant Methanogens for d) AD1, e) AD2 and f) AD3. Only one sample per point was analysed.

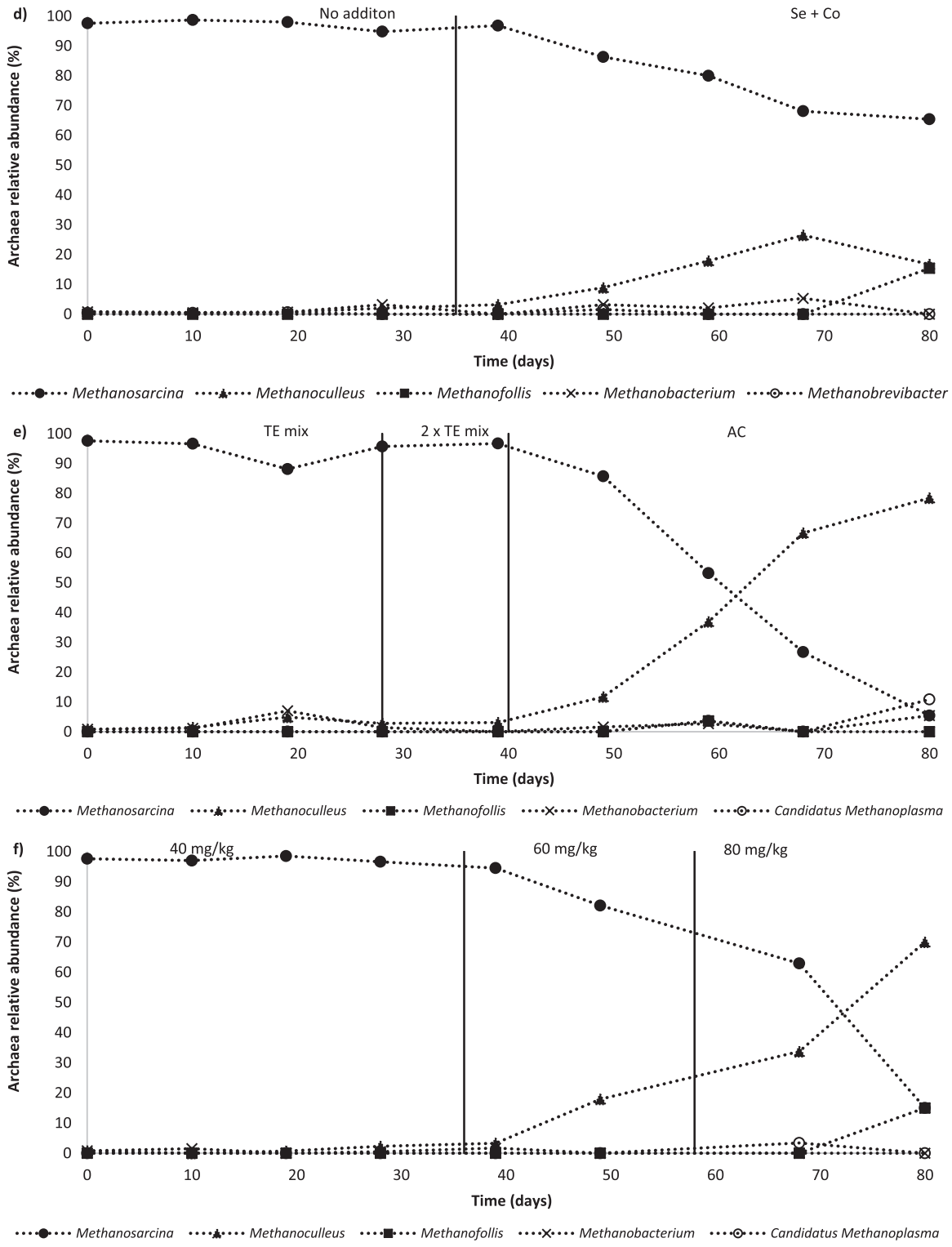

Fig. 4. (*continued*).

methane production but did not avoid accumulation of propionic acid. Combined addition of Se and Co was not able to recover AD1 from the high propionic levels. These results differed from the sacrificial reactors, where TE dosing had the highest impact on propionic acid degradation. The discrepancy could result from the lower TS used in the sacrificial experiment (20 %) and from the difficulty of translating results from batch to continuous reactors, where inhibition can exacerbate. Previous literature has also reported TE dosing as successful in reducing propionic acid accumulation for similar feedstocks [12,40]. However, those trials were typically up to 20 % TS, and reduced diffusion at higher TS could affect bioavailability [41], meaning that the TE present would be unavailable for microorganisms (supplementary Table 1) and improved mixing or a higher dosing might be necessary to trigger a positive effect in AD1. Further testing on the impact of TS in the digesting broth on TE speciation and fractionation would help further understand this aspect. Longer term trials would add value to fully elucidate whether the

positive impact of TEs observed in the sacrificial experiments can be translated into continuous dry ADs. Dosing of AC and $MgCl_2$ improved methane formation, with both ADs obtaining a 20 % higher production than the period with TE mix addition in AD2 (Day 0–40). Cumulative methane flows from days 40 to 82 were 99.6 ± 11.8 and 98.9 ± 9.7 L/kg VS ($p < 0.05$) in AD2 and AD3 respectively, accounting for roughly 50 % of the maximum methane production obtained in the BMP test of the OFMSW. Although there are limitations on comparing data from batch and continuous reactors, this gap in efficiencies indicates a reduced production despite the stable daily production. Compared to AD1, AC reduced hydrogen level by 3-fold in AD2, with similar values to AD3 by the end of the trial. Improved methane production due to higher hydrogen conversion into methane with AC and $MgCl_2$ was linked to different mechanisms, like the increased DIET between microorganisms due to the AC's conductivity or the magnesium's ability to increase osmotic pressure when is absorbed by the microorganisms. However, the reduction of hydrogen levels observed in both cases seemed to be insufficient to avoid the inhibition of propionic acid degradation, which occurred regardless of the methane increase. Further experiments and extended operation times are necessary to find the maximum OLR possible and to assess long-term stability with AC or $MgCl_2$ addition, as this will allow definition of the operation limits for a stable methane production in dry AD. Once this information is available, the use of these supplements at full scale can be evaluated economically and operationally. The potential risk of $MgCl_2$ dosing to increase struvite crystallisation also merits further investigation.

3.3. Microbial analysis and isotopic fractionation

The genus *Fastidiosipila* was the bacteria with the highest relative abundance at the beginning of the experiment in all the reactors (Fig. 4a, b, c & supplementary Figure 4). These bacteria have been linked to the decomposition of cellulosic materials [42]. Its concentration, however, decreased with time at a similar pace in all digesters. *Actinomyces* was the second genus in relative abundance at the start in all ADs and also reduced in its abundance with time (Fig. 4a, b, c & supplementary Figure 4). This genus is regarded as a fibre-degrading specialist [43], which provides circumstantial evidence of recalcitrant substrate degradation. All ADs appeared to be enriched in the family *PeH15* (Fig. 4a, b, c & supplementary Figure 4), being the most abundant by the end of the digestion. *PeH15* belongs to the phyla *Bacteroidales* and genus *HN-HF0106*, family *Hungateiclostridiaceae*. This genus is related to the degradation of complex hydrocarbons, with *Bacteroidales* being common in digestion of lignocellulosic compounds [44]. The increase in relative abundance of *PeH15* could be related to the reduction of *Fastidiosipila* and *Bacteroidales*, as all of them have similar functions and *PeH15* could be outcompeting and therefore displacing the other bacteria.

Within SAOBs, *Syntrophaceticus* [45] showed different trends depending on the type of supplement added (supplementary Figure 4). *Syntrophaceticus* spp. decreased at the end of the trial in AD1 and AD2, whereas AD3 recorded an increase in its relative abundance, probably due to the role of $MgCl_2$ against ammonia inhibition. At the end of the trial, *Syntrophomonodaceae* was found to have increased in relative abundance for AD2 and AD3 (supplementary Figure 4). These are commonly found in ADs with high ammonia concentrations [45], which would explain their increase in the digesters.

Within methanogens, *Methanosarcina* showed the highest relative abundance at the start in all ADs (Fig. 4d, e, f & supplementary Figure 4). This prominence has been observed before in dry AD digestates with high ammonia levels and could be linked to an unstable digester [37]. *Methanosarcina* forms clusters and is able to tolerate high ammonia and VFA concentrations [46], which could adapt better than strict hydrogenotrophic methanogens to the harsh conditions in the digesting broth. *Methanosarcina's* relative abundance decreased over time in all the ADs in favour of *Methanoculleus*, a strict hydrogenotrophic methanogen (Fig. 4d, e, f & supplementary Figure 4). *Methanoculleus* has been found in ADs with over 4 g/L of TAN [47,48], and grew in all ADs over time. The addition of AC and $MgCl_2$ accelerated the increase of *Methanoculleus* relative abundance, becoming dominant at the end of the digestion in AD2 and AD3. The increase was probably related to a reduction on the energy requirements to maintain osmotic pressure for methane production. Similar results were found by Zhou et al. [49], where the addition of AC increased *Methanoculleus* relative abundance due to the improvement of the SAOB pathway. *Methanosarcina* remained dominant in AD1 (Fig. 4d), likely due to the higher effect of inhibitors in the reactor. *Methanofollis,* which is another strict hydrogenotrophic methanogen [50], increased to similar relative abundances in AD1 and AD3 by the end of the trial (Fig. 4d, e, f & supplementary Figure 4). The addition of $MgCl_2$, and Se and Co seemed to benefit its increase in relative abundance, however the addition of AC did not, and it was not detectable in AD2.

AC addition resulted on a faster increase on *Methanoculleus,* becoming dominant on reactor AD2 at day 68 compared to day 80 on AD3. AC also increased methanogens diversity with the appearance of *Candidatus Methanoplasma* and *Methanobacterium*, both hydrogen-dependent methanogens [51]. Increased DIET in AD2 may be responsible for the growth of diverse hydrogenotrophic methanogens.

Both AC and $MgCl_2$ dosing increased abundance of SAOBs and hydrogenotrophic archaea, suggesting an increase in the hydrogenotrophic methanogenesis pathway. It is hypothesized that these results were linked to the improved stability and higher methane flows in AD2 and AD3 compared to AD1, where abundance of SAOBs and hydrogenotrophic archaea was reduced, and versatile *Methanosarcina* was dominant. These supplements would improve industrial scale operations, allowing the use of shorter HRT and higher throughputs leading to improved methane flows.

All the isotopic signatures (Fig. 5) remained at hydrogenotrophic values. Versatile *Methanosarcina* used the hydrogenotrophic pathway at the beginning of the digestion trial and was replaced by strict methanogens over time, at least for AD2 and AD3 where *Methanoculleus* became dominant. These results are different to others reported for semi-continuous dry AD [9,37], where *Methanosarcina* relative abundance increased to become the dominant methanogen. In the present study the inoculum started with a predominance of versatile *Methanosarcina*, probably due to a high instable period in the sampled full-scale facility, and its presence was reduced in favour of strict hydrogenotrophic methanogens, particularly for the AD and $MgCl_2$ dosed reactors but also to a certain extent at the deteriorated conditions of AD1 following Se and Co dosing (Fig. 4a). As conditions like FA and propionic accumulation were similar in both experiments [9], it is postulated that the increased HRT in this trial (59–78 days) compared to the 33 and 40 days HRT tested previously would have helped SAOBs retention. In this case the longer HRT, combined with a reduced inhibition following supplementation, could be enough to allow replication of SAOBs and maintain its presence in the ADs (supplementary Figure 4) even for the most unstable reactor (AD1). This would enable maintaining the hydrogenotrophic pathway throughout the trials either through strict methanogens or versatile archaea. The hypothesis that FA impacts the doubling times of SAOBs and hence makes of HRT a control parameter for their wash out and for selecting methanogenic pathway of versatile archaea, was already postulated by the same authors [9], with the present study adding circumstantial evidence of its validity.

4. Conclusions

Propionic accumulation occurred independently of all the supplementations at the conditions tested, but AC and $MgCl_2$ dosing resulted in increased methane production and reduction of hydrogen partial pressure. Adding AC was hypothesised to promote DIET, while $MgCl_2$ would have improved osmotic pressure preservation, reducing methanogens' energy requirements. Additionally, AC and $MgCl_2$ resulted on a shift of archaeal communities from versatile *Methanosarcina* to strict

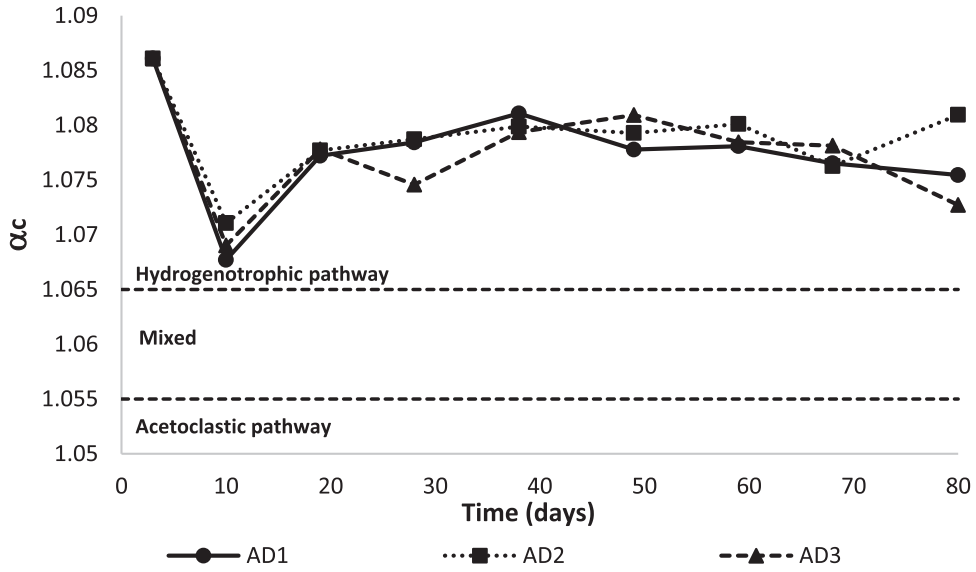

Fig. 5. Isotopic fractionation factor for AD1, AD2 and AD3.

hydrogenotrophs like *Methanoculleus,* supporting microbial redundancy with expected positive links to process resilience. TE supplementation did not avoid or allow recovery from propionic accumulation in dry AD, probably due to the high TS (> 40 %) of the experiments reducing diffusion.

CRediT authorship contribution statement

Simpson Edmon W.: Writing – review & editing, Methodology, Funding acquisition. **Bajón Fernández Yadira:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Fernández Oliver:** Writing – review & editing, Methodology, Funding acquisition. **Wagland Stuart T.:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Rocamora Ildefonso:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Villa Raffaella:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Hassard Francis:** Writing – review & editing, Formal analysis. **Fotidis Ioannis A.:** Writing – review & editing, Methodology. **Peces Miriam:** Writing – review & editing, Visualization, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ildefonso Rocamora reports financial support was provided by Engineering & Physical Sciences Research Council (EPSRC). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.116015.

Data availability

There is a data availability statement on the manuscript with information on data

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