

CRANFIELD UNIVERSITY

OLIVIA BERZAL DE FRUTOS

MAXIMISING THE VALUE OF TRADE WASTE

SCHOOL OF WATER ENVIRONMENT AND ENERGY
PhD Full Time Water

PhD
Academic Year: 2016 - 2020

Supervisor: Yadira Bajón Fernández
Associate Supervisor: Marc Pidou
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This thesis is submitted in partial fulfilment of the requirements for
the degree of PhD in Water

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based solely on examination of the thesis)***

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ABSTRACT

Anaerobic digestion (AD) of sewage sludge is a typical treatment method used before its disposal or land application as it has been shown to reduce pathogens, stabilise the sewage sludge and reduce sludge volumes, generating at the same time biogas for energy generation. Anaerobic co-digestion (Aco-D) is used to balance the characteristics of two or more waste in AD to improve performance and increase biogas yield. Aco-D of sewage sludge and trade wastes (TWs) has been seen to have a beneficial effect by balancing nutrients and toxicity. Currently, TWs typically with a lower chemical oxygen demand (COD) concentration of below 50g/L are accepted on large wastewater treatment for treatment with the municipal wastewater in the biological nutrients removal process (BNR). This may have the benefit of balancing the organics/nutrients ratio in the systems but the increased load in both organics and nutrients generally leads to an increase in the cost of treatment which is expected to be compensated by the charge (gate fee) applied to take these TWs. Alternatively, TWs, especially with greater COD content (> 50 g/L) could be considered for Aco-D with sewage sludge on those same sites but this approach requires careful control of TWs loads as they are known to contain toxic compounds that could affect the AD process. Furthermore, the lower strength TWs could also be considered for Aco-D, if concentrated to reach suitable organics content for addition to the AD reactor. However, there is very limited literature on the Aco-D of sewage sludge and TWs, especially when considering the wide range of wastes accepted on sites. Therefore, the potential to use Aco-D on high and low strength TWs was considered in this study. The aim of this project was to understand the effect of TWs in Aco-D with sewage sludge to maximise the benefits and reduce the negative impact of TWs.

To achieve this goal, batch and semi-continuous AD experiments with sewage sludge were carried out to investigate the impact of addition of key components of TWs, identified through the study of a data containing the characteristics of 160 TWs and ultimately identify concentrations range in which the compounds may be beneficial or inhibitory. In the batch experiments, methane yield and lag phase inhibitions were observed from 1764 mg TAN/L, 592 mg Zn/L, 22 mg Cu/L, 464 mg Al/L, 4535 mg Cl/L, 162 mg SO_4 /L, 92 mg NO_3 /L, 25 mg Hg/L and 17 mg As/L using Ammonia sulphate, Zinc chloride, Copper chloride, Aluminium sulphate, Sodium chloride, Sodium sulphate, Sodium nitrate, Mercury sulphate and Sodium arsenate, all highlighting the importance of managing loads on the AD plant. Zinc sulphate and sodium nitrate were further studied in semi-continuous experiments to understand the possible long-term impacts of the compounds in the reactor over long-term operation. Inhibitory values were identified for Zn from 700 mg Zn/L and from nitrate from 2000 mg NO_3 /L decreasing biogas production to 0 and 20-38 mL/% VS red/d compared with the value for control reactors with only sewage sludge of 80-100 mL/% VS red/d. Accumulation in the solid fraction was observed in the zinc experiments, while nitrate showed an inhibition of the methanogenesis because of the generation of by-products from the denitrification process. The concept of pre-concentrating low organic content TWs in a dead-end filtration cell using microfiltration (MF) and ultrafiltration (UF) membranes was studied, with the concentrates produced and then tested in a batch AD test as 10% of the feed with sewage sludge. The results showed that most of the TWs studied, including wastes from the food, chemical, leather, personal care products

and paper industries showed non-effect or beneficial in the Aco-D with sewage sludge except for a resin TWs. Finally, it was demonstrated that an economic benefit can be expected from the co-digestion of the concentrates with sewage sludge from most of those TWs based on the avoided cost of treating the TWs in the BNR and the increase in methane production. Overall, this work demonstrated that it is possible to maximise the value of trade wastes through co-digestion with sewage sludge, but careful addition will be required to ensure optimum operation of the AD.

Keywords:

Anaerobic co-digestion; Sewage sludge; Anaerobic digestion; Biogas; Methane; Inhibition

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LIST OF ABBREVIATIONS

7 Days Moving Average	MA7
Acetate Equivalent	Acetate _{eq}
Aluminium	Al
Anaerobic Co-Digestion	Aco-D
Anaerobic Digestion	AD
Arsenic	As
Biological Methane Potential	BMP
Biological Nutrients Removal	BNR
Cadmium	Cd
Carbon	C
Chemical Oxygen Demand	COD
Chloride	Cl
Chrome	Cr
Concentration Factor	CF
Concentration Factor For Volume	CFV
Continue Stir Reactor	CSTR
Control	C
Copper	Cu
Cyanide	CN
Data Acquisition System	DAS
Expanded Granular Sludge Bed	EGSB
Fats, Oils And Greases	FOG
F-Critical Value	Fcrit
Fluor	F
Free Ammonia Nitrogen	FAN
F-Value	F
Grass, Fats, Oils And Greases	FOG
High Strength Tanks	HST
High-Performance Liquid Chromatography	HPLC
Hydraulic Retention Time	HRT
Hydrochloric Acid	HCl
Hydrolysis Constant	K _h
Inductively Coupled Plasma	ICP
Intermediate Alkalinity	IA
Ion Chromatography	IC
Lag Phase	λ
Lead	Pb
Long-Chain Fatty Acids	LCFAs
Low Organic Loading Rate	OLR
Mercury	Hg

Microfiltration	MF
Molecular Weight Cut-Off	MWCO
Nanofiltration	NF
Nickel	Ni
Nitrate	NO ₃
Organic Fraction Of Municipal Solid Waste	OFMSW
Organic Loading Rate	OLR
Partial Alkalinity	PA
Primary Sludge	PS
Reverse Osmosis	RO
Root Mean Square Error	RMSE _n
Secondary Activated Sludge	SAS
Selenate	SeO ₄
Selenite	SeO ₃
Selenium	Se
Sodium Hydroxide	NaOH
Specific Methanogenic Activity	SMA
Specific Rate Constant	R _m
Sulphate	SO ₄ ²⁻
Total Ammonia Nitrogen	TAN
Total Kjeldahl Nitrogen	TKN
Total Nitrogen	TN
Total Solids	TS
Trade Wastes	TWs
Ultrafiltration	UF
Up-Flow Anaerobic Sludge Blanket	UASB
Volatile Fatty Acids	VFAs
Volatile Fatty Acids	TVFA
Volatile Solids	VS
Volatile Solids Reduced	VS red
Volatile Suspended Solids	VSS
Wastewater Treatment Plants	WWTP
Zinc	Zn

CHAPTER 1: Introduction

1.1. Background

Anaerobic digestion (AD) is commonly applied to treat sewage sludge to obtain methane for energy production. Additionally, some of the benefits of treating sewage sludge in AD are the use of the digestate as fertiliser on agricultural lands, decrease in pathogen levels and odour nuisance in sewage sludge, and the reduced transportation costs linked to the volume reduction of sewage sludge [1–3]. Nonetheless, the efficiency and economic benefit of AD of sewage sludge are affected by the low conversion of organic compounds to methane, which varies from 40 to 50% [2,4] and by the limited solids loading rate which is usually maintained between 1.6 and 4.8 Kg volatile suspended solids (VSS)/m³/d to avoid increases in free ammonia nitrogen (FAN) and fatty acids concentrations that could negatively affect the AD process [5,6]. Moreover, digesters are normally not operated at their maximum treatment capacity as their design on municipal wastewater treatment plants typically includes spare treatment capacity to accommodate for future increases in sludge production [7]. Anaerobic co-digestion (Aco-D) of sewage sludge with other organic substrates can support higher AD performances, with concomitant benefits of balancing the carbon to nitrogen ratio to optimum value for AD, between 20 and 30 [1,7–9], and providing micro-nutrients that can be beneficial for anaerobic microorganisms, such as heavy metals [9,10].

Aco-D is the use of two or more substrates at the same time in an AD reactor, aiming for a beneficial effect in methane production and compensating the disadvantages of each substrate [11]. For example, Nielfa *et al.* studied the effect of adding the organic fraction of municipal solid waste (OFMSW) to sewage sludge. Methane production was increased from 164 mL/g volatile solids (VS) for sewage sludge and 201 mL/g VS for OFMSW, up to 221 mL/g VS when OFMSW was added as 80% of weight [12]. Cardona *et al.* achieved a 1.7 times increase in methane production with 79.3 mg carbon (C)/g chemical oxygen demand (COD) using 75% weight of grass compared to a mono-digestion value of 68.4 mg/g COD for grass and 46.62 mg/g COD for sewage sludge [2]. Fats, oils and greases (FOG) have also been used in Aco-D with sewage sludge leading to an increase in methane production up to 492 mL/g VS when FOG comprised 60% VS of the treated feedstock from 316 mL/g VS for sludge alone [13]. Food waste has been used in co-digestion with sludge with an optimum VS ratio of 1:1, which increased the methane yield 2.8 and 1.2 times from sewage sludge and food waste, respectively, as reported by Wang *et al.* [10]. Bai *et al.* studied the combined treatment of manure with sewage sludge with an increase of 10% of the methane yield over that of sludge mono-digestion of 239 mL/g VS when a VS ratio of 7:1 sewage sludge to pig manure (182 mL/g VS) was used [14]. Finally, trade wastes (TWs) have been co-digested with sewage sludge because of their high concentration of nutrients necessary for the AD [15].

Aco-D of sewage sludge with TWs has previously been reported to be beneficial for biogas and methane production. In a study by Fountoulakis *et al.*, an increase over sewage sludge biogas production rate of 1253 mL/d was achieved by adding 1% v/v of glycerol [3]. A small volume of biodiesel waste glycerine (1.25% v/v) improved biogas production by 48% and methane yield

by 56% compared with sludge mono-digestion. A further increase of the biodiesel waste (1.35% v/v) increased the biogas production by 100% and the methane yield by 121% [16]. Aco-D of 50 mL of sewage sludge with 2.5 mL of olive mill effluent and 50 mL of sewage sludge with 1.3 mg of olive mill solid waste achieved a methane accumulated yield of 374 and 443 mL CH₄/g VS, respectively. This increase in production was used as evidence of a synergy of Aco-D as yields were higher than the methane accumulated yield from sewage sludge, olive mill effluent and olive mill solid waste individually, which were 333, 373, and 132 mL CH₄/g VS, respectively [17]. Those previous studies have evidenced benefits of Aco-D of TWs and sewage sludge, but only focussed on treatment of a specific type of TWs with the sewage sludge. The potential benefits and limitations of simultaneously dosing several TWs in sewage sludge digesters have not been thoroughly reported, which limits diversion of TWs to ADs by waste collection companies, such as water utilities, that receive hundreds of TWs daily.

The previously described benefits of the Aco-D of sewage sludge with TWs needs also to be balanced with the high concentrations of inhibitors present in TWs, which, if not carefully managed, can compromise process stability. To illustrate, wastes generated from the agro-food industry can be amenable to anaerobic digestion, especially the most bio-degradable substrates such as the ones from sugar industries [18]. Nonetheless, there are limitations or inhibitors that might negatively affect the AD process. Highly bio-degradable material produces a high amount of VFAs that could overload the methanogens and inhibit the methanogenesis phase [19]. FOG is commonly found in wastewater from slaughterhouse, dairy industry and restaurants. FOG could degrade in the AD and produce long chain fatty acids (LCFA) that are inhibitors for anaerobic microorganisms [13]. Other agro-food wastewaters that present limitations for their use in AD are olive mill and wine distillery wastewaters. Those wastes contain high concentration of organics such as polyphenols and pH values below 5.2 [20]. A high concentration of salt can also be a limitation for treatment of wastes from industries, such as stationary material production industry, canning, dairying, and processing wastewaters from for example the mussel industry [21,22].

There are other TWs that might be used as co-substrate for AD, such as by-products from the textile industry, biodiesel industry, metallurgy and electrical industries. Textile wastewater has a wide range of COD between 50 and 5000 mg/L, suspended solids between 50 and 500 mg/L and contain dyes salts, surfactants and caustic soda [23,24]. A waste from the biodiesel industry that has shown potential benefits when treated with AD is glycerol because of its highly biodegradable organics [3,16]. Glycerol waste is a mixture of glycerol, alcohol, water, salts, heavy metals, free fatty acids, mono-glycerides, di-glycerides, tri-glycerides and methyl ethers [11]. There is a potential risk to overload AD and the lack of nutrients such as nitrogen may also limit the process [3,16]. Wastes from the steel industry might contain phenolic compounds that could reduce performance in AD [25]. Also wastes from the electrical and mechanical industries can contain detergent, and oil and water emulsions in their washing water [26]. Detergents contain anionic surfactants that have poor degradability and are inhibitory as they can disrupt microbial processes related to transportation of materials into the cell [27] resulting in microbial growth reduction [28]. Oily wastewater could have a negative effect on anaerobic microorganism because of LCFA accumulation [29,30].

Even though landfill leachate is not an industrial wastewater, it is considered a TW as it is often tankered to water utilities for treatment. Landfill leachates contain toxic constituents that might negatively affect the AD process, for instance, heavy metals, xenobiotic compounds and other toxic organics and inorganics [1]. Landfill leachates are generally divided in three types depending on their biodegradability: young leachates, which are the most biodegradable one, intermediate leachate and mature leachates, which typically contains refractory and non-biodegradable organics [1].

Furthermore, nitrogen compounds can be inhibitory besides being a nutrient for anaerobic microorganisms. Industries such as food, coal-gasification, pectin factories, distilleries, cassava and fibre chemical, contain high concentrations of nitrogen compounds [31–38]. Total ammonia nitrogen (TAN) levels between 3000 to 9000 mg/L have been found in TWs from coal-gasification [39], which are higher than the inhibitory range established by McCarty of 1500 to 3000 mg TAN/L if pH was above 7.5 (Table 1.2) [34]. In a crab industry wastewater, FAN was 50% inhibitory to methane production when it reached values of 85 mg/L in a toxicity test (Table 1.2) [32]. Cassava industry wastewater, which contains cyanide (CN) in a range between 0.01 and 10000mg/L, was found to be 70% inhibitory to methane production at 5 mg/L of cyanide without a previous acclimation of the inoculum (Table 1.2) [37]. The wastewater produced from fibre chemical industries, also high in cyanide, inhibited 50% of the methane production with 4.5 mg/L of cyanide, with a complete loss of methanogenic activity at cyanide concentrations of 25 mg/L (Table 1.2) [38]. Nitrate (NO_3) is present in TWs such as those from pectin factories and distilleries, with concentration ranges of 1280-2990 mg-N/L and 18-126 mg-N/L, respectively [35,36]. Those ranges could be inhibitory as a study by Tugtas and Pavlostathis demonstrated a reduction of 52% and 90% in the methane production rate at concentrations of nitrate of 150 mg-N/L in batch tests and 300 mg-N/L in continuous experiments (Table 1.2) [40]. Besides resulting in process inhibition, the addition of nitrate could lead to proliferation of populations of microorganisms that compete with methanogenesis, such as denitrifiers that convert nitrate to nitrogen gas [28,29]. Nitrate addition in a ratio of 8 g COD/g $\text{NO}_3\text{-N}$ was observed by Yi *et al.* to decrease methanogenesis activity by 86% and 65.2% in batch and continuous tests, respectively (Table 1.2) [41]. Nonetheless, nitrate has been seen to also improve methanogenesis if concentrations are carefully managed [42].

Sulphate (SO_4^{2-}), frequently present in TWs, has also been reported to shift the population of methanogens and increase concentrations of other microorganisms such as sulphate reducing bacteria [41,43]. In an experiment by Hu *et al.*, sulphate addition from a COD/ SO_4^{2-} ratio of 20 to 0.5 reduced methane production rate by 27% using a synthetic chemical industry wastewater (Table 1.2) [43]. In another study using process wastewater from the mussel industry, Soto *et al.* found that there was a competition between the sulphate reducing microorganisms and methanogens at 2.2 kg $\text{SO}_4/\text{m}^3/\text{d}$. The sulphate reducing microorganisms degraded 15 and 2% of propionate and acetate, respectively, from the volatile fatty acids (VFAs) that were no longer available for the methanogens [22].

Some heavy metals are known to have a positive or negative impact on the AD process depending on their concentrations, acting as micro-nutrients for the digestion process until an inhibitory effect is reached at high concentrations [44]. To illustrate, iron has been reported to be inhibitory at 56 and 200 mg/L in pure cultures and sludge, respectively (Table 1.2) [45,46]. Zinc (Zn) could affect the AD process over a wide range of concentrations from 4.5 to 1500 mg/L, inhibiting 50% of hydrogen production (Table 1.2) [47,48]. Cadmium (Cd) was 50% inhibitory for methanogens in a range of 15-170 mg/L at different conditions (Table 1.2) [49,50]. A wide range of inhibition can also be found for nickel (Ni) and chromium (Cr) ranging from 35 to 1300 mg/L, and from 27 to 3000 mg/L, respectively, in methanogens and hydrogen-producing microorganisms (Table 1.2) [47,51]. The inhibition of copper (Cu) has been reported to be within a range from 2.4 to 50 mg/L for 50% to 69% inhibition of methanogenesis (Table 1.2) [50,52]. Mercury (Hg) was found to be inhibitory when continuously fed at 125 mg/L or pulse fed at 500 mg/L, reducing the biogas production by 60% and 92%, respectively (Table 1.2) [49]. Aluminium (Al) was reported to be inhibitory in a range between 1000 to 2500 mg/L with a decrease of 72% in methane activity and 85% in biogas production, respectively (Table 1.2) [53,54]. Another metal that could affect the AD process is lead (Pb) that has been reported to be 50% inhibitory to hydrogen producers above 5000 mg/L (Table 1.2) [51]. Arsenic (As) inhibition varies depending on its oxidation state, with As III as the most toxic with 50% inhibition in a range from 0.54 mg/L to 12.3 mg/L and As V as the less toxic with complete inhibition at a much higher concentration of 69.5 mg/L (Table 1.2) [55]. Selenium (Se) was found to inhibit the process by 96% at concentrations between 0.13 g/L and 1.27 g/L in the form of selenite (SeO_3) and 0.143 g/L in the form of selenate (SeO_4) (Table 1.2) [56]. Additionally, heavy metals such as zinc, lead, nickel, cadmium, copper, mercury and chromium have been seen to accumulate in sewage sludge with potential to reach inhibitory values when fed continuously to an AD reactor [57].

Fluorine (F) inhibited 50% of methanogenesis when it was added between 18.1 and 433 mg F/L (Table 1.2) [58]. Chloride (Cl) resulted in a reduction of methane when the concentration increased further than 5500 mg/L (Table 1.2) [59]. Moreover, phenol, an organic pollutant that is quite common in chemical industries, inhibited biogas production between 80% to 60% when the concentration of phenol ranged between 200 and 300 mg/L (Table 1.2) [60,61]. The previous constituents reviewed showed that inhibition of key components of TWs has been studied. Nevertheless, these constituents are common in TWs but not in sewage sludge and they have not been studied using sewage sludge as feed. The conditions in these studies differ between them and some of the experiments were not studied in the conditions in which water utilities operate AD for sewage sludge. Moreover, mercury, aluminium, arsenic, selenium and fluoride effects have not been fully studied in AD.

Table 1.2. Concentrations and impact of AD inhibitors reported in the literature.

Inhibitor	Concentration (mg/L)	Substrate	Conditions	Performance reduction	Ref.
TAN	4000	Cattle manure	CSTR 55 °C	25% reduction in methane yield	[62]
TAN	5 and 7 g-N/L TAN	Cattle manure	UASB	25% reduction in methane yield	[63]
TAN	4 and 7.5 g-N/L TAN	Cattle manure	SMA test	50% reduced growth rate for methanogens	[63]
TAN	1500-3000	-	pH>7.4-7.6	Inhibitory	[34]
TAN	10000 (1200mg/L FAN)	Cattle manure	CSTR 55 °C	Inhibition of the methanogenesis	[64]
FAN	55 at 40 d 32 at 15 and 25 d	Acetate & Propionate	CSTR	Maximum tolerable concentration	[65]
FAN	61	Crab cooker wastewater	UASB 37 °C	40% inhibition of methanogenic activity	[32]
FAN	85	Crab cooker wastewater	ATA test 37 °C	50% inhibition	[32]
FAN	>250	Chicken manure	Batch test	Methane production ceased	[66]
NO ₃ ⁻	150 mg-N/L	Dextrin peptone	Batch test 35 °C	52% reduction in methane production rate	[40]
NO ₃ ⁻	300 mg-N/L	Dextrin peptone	CSTR 35 °C	90% reduction in methane production rate 95% reduction in methane production rate (after recover of reactor)	[40]
NO ₃ ⁻	156 mg-N/L	Acetate	Batch test	Methanogenesis inhibition	[67]
NO ₃ ⁻	10 mM	Soil	Soil test	Stopped methanogenesis, recovery 70% methane production	[68]
NO ₃ ⁻	5mM	H ₂ /CO ₂ and/or Acetate	<i>M. bakery</i> Batch test	65% reduction in methane production	[68]
NO ₃ ⁻	30 mM	H ₂ /CO ₂ and/or Acetate	<i>M. bryantii</i> Batch test	59% reduction in methane production	[68]
NO ₃ ⁻	8 COD/NO ₃ -N	Glucose	Batch test 35 °C	86% reduction in methane production	[41]
NO ₃ ⁻	8 COD/NO ₃ -N	Glucose	CSTR 35 °C	34.8% reduction of % of methane in biogas	[41]
NO ₃ ⁻	14.3 mM	Acetate or methanol	Batch test	46% inhibition on <i>M. mazey</i> by competition	[69]
NO ₂ ⁻	50 mg-N/L	Dextrin peptone	Batch test 35 °C	20% reduction in methane production rate	[69]
NO ₂ ⁻	315 mg-N/L	Glucose	UASB 30 °C	12% reduction	[70]
NO ₂ ⁻	50	Glucose	Batch test Non-acclimated dispersed	50% reduction SMA (Specific methanogenic activity)	[70]
NO ₂ ⁻	100	Glucose	Batch test Non-acclimated granular	50% reduction SMA	[70]
NO ₂ ⁻	100	Glucose	Batch test Acclimated dispersed	50% reduction SMA	[70]
NO ₂ ⁻	0.18mM 0.32mM NO _x	Acetate or methanol	Batch test	96-97% inhibition <i>M. mazey</i> 95% inhibition <i>M. mazey</i>	[69]
NO ₂ ⁻	50uM		<i>M. bakery</i> Batch test	50% reduction in methane production	[68]

NO₂⁻	1mM	H ₂ /CO ₂ and/or Acetate	<i>M. bryantii</i> Batch test	50% reduction in methane production	[68]
CN⁻	4.5, possible recovery 50, no recovery	Fibber chemical plant wastewater	Batch test	50% IC (toxic to the sludge's methane-producing activity)	[38]
CN⁻	5 mg/L	Cassava derived starch and VFA	UASB 33 °C	70% reduction in methane production	[37]
CN⁻	125 mg/L	Cassava derived starch and VFA	UASB 33 °C	96% methane reduction with acclimation	[37]
SO₄²⁻	150	Glucose for semi-batch, VFAs for SMA	Granular sludge, Semi- batch, SMA to the outlet	65% SMA decrease using butyrate at 59 d, recovering at 114 d to 8% SMA decrease	[53]
SO₄²⁻	Change of COD/SO ₄ from 20 to 0.5 (6000 mg/L)	Synthetic organic chemical industry wastewater	UASB 35 °C, Batch tests to the outlet	27% reduction in methane production rate	[43]
SO₄²⁻	2.2 Kg SO ₄ /m ³ /d	Mussels process wastewater	Anaerobic filter 37 °C	15% and 2% of Propionate and acetate was converted by sulphate reducing microorganism instead of the methanogens	[22]
SO₄²⁻	1500 (6.7 COD/SO ₄ ratio)	Sewage sludge	CSTR 35 °C	Decrease of 33% of methane production	[71]
Cd	170 Pulse feed:680	Sludge	Fixed film bed reactor Semi-continuous 22 °C	55% biogas reduction Pulse feed: 84.8% decrease	[49]
Cd	15-23 11-34 (with Nickel)	Acetate solution	Pure culture 60 °C	50% inhibition of thermophilic methanogens	[50]
Cd	3300	Sucrose	Batch Hydrogen generation Granular sludge 26 °C	50% reduction of hydrogen potential	[51]
Cd	36	Glucose	Batch 5 d Granular sludge 35 °C	50% reduction of methane production	[47]
Fe	200	Sludge	Batch 55 °C	50% biogas production using Fe NO ₃	[45]
Fe (III)	1 mM	Acetate Carbon dioxide and hydrogen	Batch, Pure culture, <i>M. hungatei</i> 30 °C	Almost completely inhibited methane production	[46]
Fe (III)	5-10 mM	Acetate Carbon dioxide and hydrogen	Batch, Pure culture, <i>M. barkeri</i> 30 °C	(H ₂ /CO ₂) significantly decreased methane production (acetate) methane production was also significantly reduced but less	[46]
Zn	1500	Sucrose	Batch, Hydrogen generation, Granular	50% reduction of hydrogen potential	[51]

			sludge 26 °C		
Zn	4.5	Sucrose	Batch 72 h, Hydrogen generation 35 °C	50% reduction of hydrogen production	[49]
Zn	7.5	Glucose	Batch 5 d, Granular sludge 35 °C	50% reduction in methane production	[47]
Ni	1300	Sucrose	Batch, Hydrogen generation, Granular sludge 26 °C	50% reduction of hydrogen potential	[51]
Ni	50	Ethanol and acetate	EGSB	18% inhibition methanogen	[52]
Ni	14.5-130	Acetate solution	Pure culture 60 °C	50% inhibition of thermophilic methanogens	[50]
Ni	35	Glucose	Batch 5 d, Granular sludge 35 °C	50% reduction of methane production	[47]
Cr	3000	Sucrose	Batch, Hydrogen generation, Granular sludge 26 °C	50% reduction of hydrogen potential	[51]
Cr	60	Sucrose	Batch 72 h Hydrogen generation 35 °C	50% reduction of hydrogen production	[48]
Cr	50	Ethanol and acetate	EGSB	11% inhibition methanogens	[52]
Cr (III)	775 Pulse feed: 3100	Sludge	Fixed film bed reactor Semi-continuous 22 °C	52% biogas reduction Pulse feed: 83% decrease	[49]
Cr	27	Glucose	Batch 5 d, Granular sludge 35 °C	50% reduction in methane production	[47]
Al	1000	Glucose for semi-batch, VFAs for SMA	Granular sludge Semi-batch SMA to the outlet	72% SMA decrease using butyrate at 59 d, recovering at 114 d to 21% SMA decrease and 36% SMA when 150 mg/L of sulphate was added	[53]
Al	2500	Synthetic waste	UASB semi-continuous	Digester failure (85% decrease of biogas volume approximately)	[54]
Cu	6.5	Sucrose	Batch 72 h, Hydrogen generation 35 °C	50% reduction of hydrogen production	[48]
Cu	30	Sucrose	Batch, Hydrogen generation, Granular sludge 26 °C	50% reduction of hydrogen potential	[51]
Cu	2.4-14, 2.2-28 (with Ni)	Acetate solution	Pure culture 60 °C	50% inhibition of thermophilic methanogens	[50]
Cu	50	Ethanol and acetate	EGSB	21% methanogen inhibition with the presence of accumulated Cr	[52]

69% methanogen inhibition					
Pb	>5000	Sucrose	Batch, Hydrogen generation, Granular sludge 26 °C	50% reduction of hydrogen potential	[51]
As III	15.5 uM 27.1 uM 4.4 uM	Acetate Hydrogen Lactate	Batch test 1 d, Granular sludge 30 °C	50% inhibition of methanogens	[55]
As III	23.5	Acetate	Batch test for 1 week Granular sludge 30 °C	36.9% inhibition of methanogens	[55]
As III	100uM	Acetate	Batch test 1 d, Flocculent sludge 30 °C	50% inhibition of methanogens	[55]
As V	>500 uM	Acetate or Hydrogen or Lactate	Batch test 1 d, Granular and flocculent sludge 30 °C	Non-inhibition	[55]
As V	500 uM	Acetate	Batch test for 4 d, flocculent sludge 30 °C	Complete inhibition	[55]
SeO₃	10 ⁻³ M 10 ⁻² M	H ₂ /CO ₂ Acetate	Granular sludge Semi-batch	>96% inhibition	[56]
SeO₄	10 ⁻³ M	Acetate H ₂ /CO ₂	Granular sludge Semi-batch	>96% inhibition	[56]
Hg	125 Pulse feed: 500	Sludge	Fixed film bed reactor Semi-continuous 22 °C	60% biogas reduction Pulse feed: 92% decrease after 2 days	[49]
F⁻	325	Glucose	Batch tests, Granular fermenters 30 °C	50% reduction of control activity	[58]
F⁻	36.5 25.5	Propionate Butyrate	Batch test, acetogenic bacteria 30 °C	50% reduction of control activity	[58]
F⁻	34.5 82	Acetate Hydrogen	Batch test, Flocculent sludge 30 °C	50% reduction of methanogenesis	[58]
F⁻	18.1 433	Acetate Hydrogen	Batch test, Flocculent sludge 55 °C	50% reduction of methanogenesis	[58]
F⁻	160	Acetate	Batch test, Granular 30 °C	50% reduction of methanogenesis	[58]
Cl⁻	>5500	Tannery wastewater	Contact filter reactor	Decreasing trend	[59]
Phenol	300	Phenol, polyphenols, acetate and propionate	ATA and BMP 37 °C	Reduction of expected ratio, 2.03, to 1.62	[60]
Phenol	250	Phenol	2 stage AD 35 °C	62% biogas production reduction	[61]

Continue stir reactor: CSTR; Up-flow anaerobic sludge blanket: UASB; Specific methanogenic activity: SMA; Expanded granular sludge bed: EGSB

Acclimation to inhibitors has been recorded in previous literature. Ammonia is an example of an inhibitor that the inoculum can acclimatise to. In a study by Hansen *et al.*, the prior acclimation of the inoculum to a concentration of 3000 g-N/L of TAN allowed for an increase of the inhibitory FAN concentration to 1100 mg-N/L [72]. Acclimation has also been observed with nickel and chromium in a study by Colussi *et al.*, where the methane production in Expanded Granular Sludge Blanket (EGSB) at 50 mg/L of nickel and chromium recovered after an initial reduction [52]. Furthermore, an experiment by Leighton and Foster observed acclimation to zinc in a two-stage system after 10 to 24 h from the start of the zinc addition [73]. Acclimation can only be observed in continuous systems, which requires further study of the continuous effect of TWs constituents on biogas and methane production in order to complete the information from batch experiments available in existing literature.

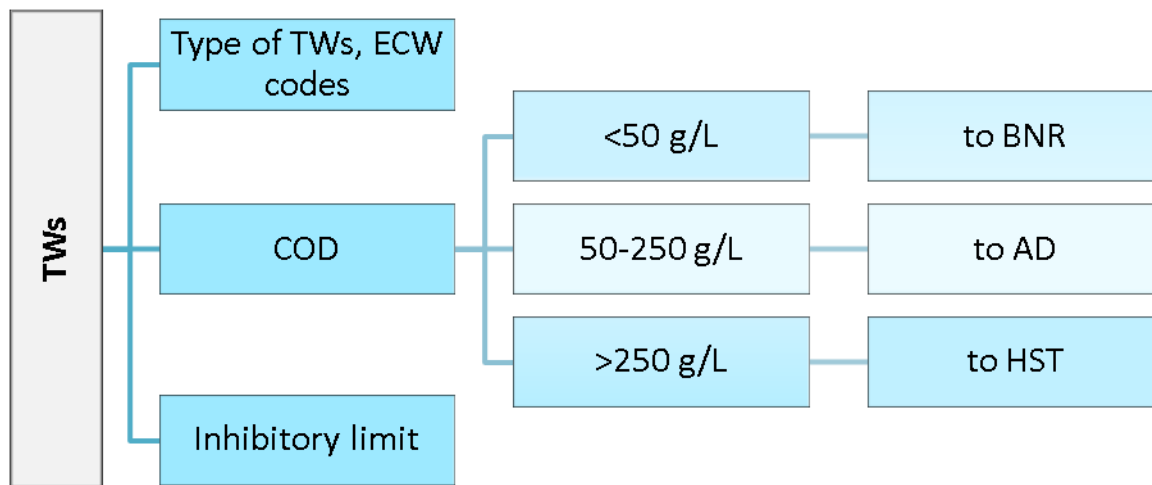


Figure 1.1. Different approaches to characterising trade wastes.

Current practice for industries without dedicated wastewater treatment is to send TWs to water utilities, where they are either treated in conventional systems typically based on a biological nutrients removal (BNR) process together with the municipal wastewater or, in limited cases, diverted for direct treatment in sewage sludge ADs. The TWs that are received in the wastewater treatment facilities are defined by the source of the TWs based on the European Waste Catalogue (EWC) [74] (Figure 1.1). As introduced above, there are inhibitors that could be associated to each type of TWs according to their source. However, their composition may vary significantly depending on the location and over time so other means of differentiating the wastes is needed. TWs are then frequently characterised according to their COD content as a representation of their potential contribution towards methane production. When received at a wastewater treatment plant, TWs below 50 g/L of COD go through the BNR process to balance the wastewater C/N ratio [75] and TWs above 50 g/L go to the AD (Figure 1.1). Nevertheless, the division by COD lacks a measurement of organic biodegradability which can be misleading regarding their impact on the treatment process.

Finally, the selection of TWs can be based on their actual composition, considering inhibitory values for each constituent (Figure 1.1). This requires that the characteristics of the TWs for key inhibitory components are provided and assessed before accepting the TWs on site. The potential inhibition to the digestion process would then be determined by considering typical inhibitory levels for the compounds and elements of concern, the TWs loads and the characteristics of the AD reactor. If a TW is found to have a potential inhibitory effect to the AD, it can be directed to the BNR for treatment with the municipal wastewater or if deemed too much of a risk it can be rejected from entering the site.

Directly treating TWs in ADs can result in benefits of greater biogas formation, as well as reducing energy demand in the BNR process that can result from TWs increasing the nutrients and organic loads to be treated [76]. Therefore, diversion of some of the low strength TWs (<50 g/L) for treatment in AD assets could mitigate the negative impact on the wastewater treatment line. This diversion of low strength TWs to ADs is currently limited by their low organic content, but could be enabled if a feasible pre-concentration step was developed.

There are several technologies known to concentrate wastewater, for example, evaporation, centrifugation and membranes [77,78]. Evaporation has been applied for high salinity wastewaters to reduce disposal volumes [79]. The use of solar energy to evaporate the wastewater is a low-cost method, but an optimization of the system to improve efficiency and reduce losses is still required [80]. Centrifugation has been used in wastewater treatment for the separation of solid and liquid phases in the wastewater based on differences in density. This technology has as advantages its easy operation, fast processing times and high separation efficiency [81]. Membrane treatment is a promising technology in wastewater treatment [82], having been applied for the recovery of chemicals in wastewaters [78,83]. Nonetheless, the pre-concentration of TWs and use of its concentrate in Aco-D of sewage sludge has not been considered so far. Therefore, the effect of co-digesting concentrated TWs with sewage sludge and the benefits of diverting TWs from the BNR treatment route needs further understanding.

1.2. Aim and Objectives

The overall aim of this project is to understand the effects of TWs addition in Aco-D with sewage sludge. The knowledge gained will be used to develop an approach to support TWs selection for maximising the benefits and reducing the risks of process inhibition.

- Objective 1: to identify the components of frequently occurring TWs that can present a risk of inhibition to the AD process if co-digested with sewage sludge (Chapter 2).
- Objective 2: to determine the impact of TWs Aco-D with sewage sludge by defining their beneficial, non-effect, inhibitory and toxic effects depending on their constituents' concentration (Chapter 2, 3 and 4).
- Objective 3: to further the knowledge of zinc and nitrate inhibition in AD of sewage sludge and elucidate their long-term impact in continuously operated units (Chapter 3).
- Objective 4: to demonstrate the potential of pre-concentration of 'low organic content' TWs to enhance sewage sludge AD (Chapter 4).

1.3. Thesis plan

This thesis is presented as a series of chapters formatted as journal papers. All papers were written by the primary author, Olivia Berzal de Frutos, and edited by Dr Marc Pidou and Dr Yadira Bajón Fernández. All experimental work was designed, coordinated and completed by Olivia Berzal de Frutos at Cranfield University with contributions from visiting students as follows: Martin Gotze who collected part of the data from Chapter 2 and Marta García who collected part of the data from Chapter 4. Furthermore, all metal analyses with inductively coupled plasma (ICP) mass spectrometer were carried out by Richard Andrews and some of the VFA analyses using high-performance liquid chromatography (HPLC) and anion analysis using ion chromatography (IC) for Chapter 3 were carried out by Monika Jodkowska.

In Chapter 2 with the title “Anaerobic Co-digestion of Sewage Sludge and Trade Wastes: Impact of Individual Compounds”, TWs Aco-D with sewage sludge was evaluated using characterisation data and batch experiments to discuss the optimal load of TWs to maximise the biogas and methane obtained from Aco-D of TWs and sewage sludge. The characterisation data was used to obtain key compounds in TWs and to choose the salts added to sewage sludge in batch experiments to understand the limits of TWs addition. The work suggested load limits of TWs to avoid inhibition of sewage sludge AD when TWs are added in full-scale sites.

Chapter 3 with the title “Long-Term Effects of Zinc and Nitrate in the Anaerobic Co-Digestion of Sewage Sludge” is a continuation of the work from Chapter 2. Two key components of TWs were selected for a semi-continuous experiment to understand the long-term impact of continuous addition during Aco-D of TWs and sewage sludge.

In Chapter 4 with the title “Membrane Concentration of Low Strength Trade Wastes for Anaerobic Co-digestion of Sludge”, real TWs are concentrated using MF and UF membranes collecting the concentrates to mix them with sewage sludge and co-digest in anaerobic digestion batch experiments. The permeability during filtration and the digestion performance were used to analyse the profitability of this pre-treatment before Aco-D.

In Chapter 5, the results from the previous chapters are discussed to evaluate their implication for water utilities.

Finally, Chapter 6 gives an overall conclusion for the thesis.

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CHAPTER 2: Anaerobic Co-digestion of Sewage Sludge and Trade Wastes: Impact of Individual Compounds

Olivia Berzal de Frutos ^a, Martin Gotze ^a, Marc Pidou ^a, Yadira Bajón Fernández ^a

^a Cranfield Water Science Institute (CWSI), School of Water, Energy and Environment (SWEE), Cranfield University, Bedford MK43 0AL, United Kingdom

Abstract

Sewage sludge is widely recognised as a potential renewable energy source by using anaerobic digestion (AD) to obtain biogas. Biogas production from sewage sludge can be improved by the use of anaerobic co-digestion (Aco-D) as the additional substrate can provide favourable nutrients and toxicity dilution. Trade wastes (TWs) have been proved as possible co-substrate for sewage sludge digestion, although careful consideration of TWs loads is paramount as treatment performance of the AD process can be affected by the high concentrations of inhibitors expected to be present in TWs. The aim of this study was to understand the impact of key components of TWs on the AD process during co-digestion with sewage sludge, in order to identify safe TW loads to be used as feedstock. For this, 160 TWs were characterised and individual toxicity of their key constituents was investigated in bio-methane potential (BMP) tests. Finally, volumetric loads of TWs that can be safely co-digested with sewage sludge were proposed.

The compounds studied in BMP tests included ammonia, zinc, copper, aluminium, mercury, arsenic, chloride, sulphate and nitrate. A performance equal or better than the control units without chemicals addition was recorded for concentrations between 635 and 1602 mg-N/L TAN, 586 and 1958 mg-N/L TAN, 0.2 and 746 mg Zn/L, 3 and 87 mg/L Zn, 6 and 167 mg Cu/L, 0 to 309 mg Al/L, 210 and 3666 mg Cl/L, 16 and 162 mg SO₄/L, 0.027 and 25 mg Hg/L, and 0.02 to 16 mg As/L when Ammonium sulphate, Ammonium chloride, Zinc sulphate, Zinc chloride, Copper sulphate, Aluminium sulphate, Sodium chloride, Sodium sulphate, Mercury sulphate and Sodium arsenate salts were used, respectively. On the contrary, Copper chloride exhibited a negative impact in biogas and methane yields. Sodium nitrate resulted in a reduced performance when dosed at low concentrations and increased up to similar gas productions than the control reactors at 427 mg NO₃/L. Considering the TWs characterised and the results of the BMP inhibition study, a volumetric ratio of 10/90 of TWs and sewage sludge was proposed as a safe feedstock for Aco-D. The potential inhibitory TWs were reduced to a 13.7% with that ratio where most of the TWs were from chemical industries and glycerol/beverage/alcohol industries.

2.1. Introduction

Sewage sludge is widely recognised as a valuable resource because of its potential use as a renewable energy source and organic fertiliser on agricultural lands. Its organic material can be converted to biogas through anaerobic digestion (AD), with additional benefits of reducing its pathogen content, odour nuisance, and volumes; with the latter contributing to reducing transportation cost for the final sludge disposal or application to land [1,2]. Despite these benefits, only 40 to 50% of organic matter from sewage sludge is converted to methane during AD, which affects the efficiency of the process and its economic profitability [2–5]. Optimum biogas production from sewage sludge digestion is also frequently limited by the organic loading rate (OLR) at which

sludge digesters are operated (1.6 to 4.8 Kg VSS/m³/d), with OLR generally limited by the risk of accumulating inhibitors such as free ammonia [6] or fatty acids [7] or in occasions determined by oversized assets designed to accommodate future increase in sludge volumes [8]. Furthermore, the carbon to nitrogen ratio associated with sewage sludge digestion is typically low, between 6 to 16, compared to the optimum value of 20 to 30 [1,8–10], and there is a deficiency of heavy metals that are micronutrients for the microorganisms in the AD of sewage sludge [10,11]. To solve these bottlenecks and increase process efficiency, anaerobic co-digestion (Aco-D) can be used.

Aco-D consists of treating several substrates in the same digester to achieve a synergistic benefit that balances their disadvantages and maximises process yield. A range of substrates has been used to increase the performance of sewage sludge AD, for example, organic fraction of municipal solid waste (OFMSW) [12], grass [2], fats, oils and greases (FOG) [13], food waste [11], manure [14] and trade wastes (TWs). TWs, which typically contain nutrients favourable for the AD of sewage sludge [15], have often been considered for co-digestion. The co-digestion with sewage sludge also allows for organic wastes that could not be treated alone to be digested, as indeed TWs are reported to frequently contain high levels of toxic constituents and insufficient buffer capacity for the digestion process [1,8]. To illustrate, Hagelqvist co-digested 50% VS of paper-mill sludge, a substrate with low biodegradability, and 50% VS of sewage sludge. This resulted in an enhancement of the methane yield from 53 mL/g VS for paper-mill sludge mono-digestion up to 84 mL/g VS, which is similar to the sewage sludge mono-digestion, and an improvement of VS degradation from 5% to above 10% [16].

Previous studies have highlighted the benefit in biogas production of sewage sludge Aco-D with industrial wastewaters. Berenjkari *et al.* recorded that the use of 10% volume of landfill leachate as co-digestate with sewage sludge increased methane production from 0.75 to 1.01 CH₄ m³/Kg VS [1]. The addition of 50% weight of petrochemical effluent to sludge improved bio-methane production by 18-32% [17]. Biodiesel wastes such as glycerol improved the production of biogas by 82% and methane when 25% of OLR was added as glycerol to sludge [4]. In another study, 5% v/v of olive mill wastewater, cheese whey and glycerol addition to sewage sludge increased biogas production by up to 220%, 350% and 86%, respectively [15].

Although the performance benefits of co-digesting TWs with sewage sludge have been widely reported, careful consideration of TWs loads is paramount as the AD process can be inhibited by the high concentrations of inhibitors expected to be present in TWs. To illustrate, TWs from food processing industries can contain between 1500 to 3500 mg/L of total Kjeldahl nitrogen (TKN), that could degrade to total ammonia nitrogen (TAN) in the digester [18–20]. Other TWs such as coal-gasification wastewater can range between 3000 to 9000 mg/L of TAN being a potential for inhibition to AD [21] (Table 1.2). Furthermore, free ammonia nitrogen (FAN) in crab industry wastewater was 50% inhibitory to methane production when it reached values of 85 mg/L in a toxicity test [19] (Table 1.2). Other nitrogen compounds that could affect AD stability are nitrate (NO₃) and cyanide (CN). Nitrate is present in TWs such as those from pectin factories and distilleries, with concentration ranges of 1280-2990 mg-N/L and 18-126 mg-N/L, respectively, which could be inhibitory to AD (Table 1.2) [22,23]. Cyanide can be found in the cassava industry, among others, varying in a range between 0.01 to 10000 mg/L, which can be inhibitory to AD [24] (Table 1.2).

Sulphate (SO_4) can be found in TWs from the chemical industry and saline food industry [25,26]. Sulphate has been reported to generate competition between the sulphate reducing microorganisms and methanogens [26]. Other constituents commonly found in TWs are heavy metals, metals, metalloids and halogens [27]. Some heavy metals are known to be micro-nutrients in AD, such as iron (Fe) and nickel (Ni) [28] (Table 1.2). Nevertheless, they could be inhibitory if present at high concentrations [29,30] (Table 1.2). Other heavy metals and metalloids commonly found in TWs that could negatively affect AD are zinc (Zn), cadmium (Cd), nickel (Ni), chromium (Cr), copper (Cu), mercury (Hg), aluminium (Al), lead (Pb), arsenic (As) and selenium (Se) (Table 1.2). Halogens can also be inhibitory to AD, such as fluorine (F) and chloride (Cl) (Table 1.2). Finally, organic pollutants common in chemical industries, such as phenol, could also pose a risk of inhibition (Table 1.2).

Previous studies on Aco-D of TWs and sludge only covered one specific type of TWs at a time. This is useful for the producer industry, but not for waste collection companies such as water utilities receiving hundreds of TWs daily. Water utilities are allowed to treat these wastes with the municipal wastewater in the main treatment train, on large sites often consist of a biological nutrients removal (BNR) process or other types of treatments removing COD and nitrogen, such as conventional activated sludge systems and oxidation ditches.. Nevertheless, there is potential to use some of the TWs received on-site directly in the sludge line by direct addition into the AD process, potentially increasing renewable energy generation by Aco-D with sewage sludge. There is then a necessity for a more systematic and holistic approach to control the addition of those TWs and avoid any inhibitory events. To achieve this, it is critical to understand the impact that TWs' characteristics have on the AD of sewage sludge. A significant number of studies are available in the literature on inhibitory constituents for AD that are commonly found in TWs (Table 1.1). However, these studies use different reactor types and experimental conditions including inoculum, feed and temperature; making a comparison of the results obtained and drawing general conclusions applicable to the Aco-D of sewage sludge with TWs difficult. Therefore, there is a need to understand the types and concentrations of constituents found in TWs and evaluate their impact on the AD of sewage sludge using the same conditions as in full-scale assets, and with concentrations that can be found in TWs.

The aim of this study is then to understand the impact of key components of TWs on the AD process during co-digestion with sewage sludge. The associated objectives of this research are to identify the key components of TWs that might affect AD, determine the impact of those constituents in Aco-D with sewage sludge, and establish the loads of each constituent that can be added to sludge digesters without hindering methane and biogas production. Through characterisation of real TWs commonly sent to wastewater treatment sites and the evaluation of the key components expected to have an impact on Aco-D of sewage sludge, this work will address the gaps in knowledge identified above and provide insights into selection of TWs for Aco-D with sludge.

2.2. Material and Methods

2.2.1. Feedstock and inoculum

The sewage sludge used as a substrate for the AD trials consisted of a mixture of primary sludge (PS) and secondary activated sludge (SAS) from two wastewater treatment plants in the West Midlands, England. The inoculum was the digestate from the same sites. The substrate and inoculum were stored at 4 °C on the day of sampling. The samples were allowed to reach room temperature for 24 hours before the start of the experiments. The average organic content in the feed was about 50 g COD/L with 34% being soluble (Table 2.1).

Table 2.1. Characteristics of the feed and inoculum used for the BMP experiments

	Feed	Inoculum
pH	6.9±0.7	7.5±0.4
COD total (mg COD/L)	49657±16717	17125±6029
COD soluble (mg COD/L)	6346±4438	480±266
TAN (mg-N/L)	1376±1216	825±415
FAN (mg-N/L)	17±36	44±50
TA (mg CaCO ₃ /L)	2785±989	5389±1699
PA (mg CaCO ₃ /L)	199±165	3611±754
IA (mg CaCO ₃ /L)	2105±814	1504±949
Total solids (TS) (% over sample)	4.38±1.30	2.43±0.66
VS (% over TS)	77.50±2.39	64.88±2.22
Cl (mg/L)	441±375	179±229
NO ₃ (mg/L)	134±230	4±6
SO ₄ (mg/L)	49±47	4±8
Na (mg/L)	2337±3415	1119±1345
Al (mg/L)	9±16	3±4
Cu (mg/L)	2±5	4±5
Zn (mg/L)	31±60	8±6
As (mg/L)	0.3±0.6	0.1±0.1
Hg (mg/L)	1.1±1.7	0.3±0.5

2.2.2. TWs data analysis

A data set containing the characterisation of TWs that were requested to be treated in a treatment plant between 2010 and 2017 was obtained from a water utility. A total of 1122 TWs were characterised, but not all wastes would have been treated at the site as some wastes or specific batches of waste may have been rejected based on an existing selection process at the utility. To account for the variability in TWs, only those with a minimum of 5 samples characterised in the data set were selected. This resulted in a total of 160 TWs characterised for 25 parameters including pH, COD, suspended solids, ammonia, chrome, copper, lead, nickel, zinc, sulphate, phenol, cyanide, fluoride, sulphide, chloride, silver, arsenic, mercury, iron, aluminium, cadmium, antimony, bromide, nitrate and nitrite. The average values for each parameter of each TW were used to evaluate and categorise TWs.

2.2.3. Batch tests

Bio-methane potential (BMP) tests were used to understand the impact of TWs addition in sewage sludge AD. To elucidate the impact of individual constituents of TWs, reactors were spiked with targeted chemicals informed from the data set characterisation, as opposed to real TWs where multiple inhibitory components can be present simultaneously. The BMP tests were carried out in 125 mL serum glass bottles consisting of a blank with only inoculum, a control with inoculum and substrate, and test reactors containing inoculum, sewage sludge as 90% mass of the feed and solutions spiked with a compound of interest as 10% mass of the feed. The constituents studied included ammonia, zinc, copper, aluminium, mercury, arsenic, chloride, sulphate and nitrate which were added using salts (Table 2.2). Ammonia, zinc, copper and aluminium were studied using both chloride and sulphate salts to understand the effect of the counter-ion dosed. The pH change resulting from aluminium salts dosing was further studied by controlling the pH with sodium hydroxide (NaOH) and through the evaluation of the impact of pH alone by reducing it with hydrochloric acid (HCl). Arsenic, chloride, sulphate and nitrate were all used with sodium as counter-ion, assumed to be inert (Table 2.2). Impact of each constituent was assessed by testing for at least 5 different concentrations and each reactor was operated in triplicates. The total concentration is reported as the background concentration in the control of the experiment plus the concentration added with the salt. All the digesters were operated with an inoculum to feedstock VS ratio of 1:1 assuming the salt solution added had no VS. The temperature of the reactors was kept at 38 °C using water baths and incubators with continuous shaking. The tests were ended when the daily biogas production variation was less than 1% [12,31].

Table 2.2. Salts added to the biological methane potential tests

Parameter studied	Salt/chemical added
Ammonia	Ammonium chloride
	Ammonium sulphate
Zinc	Zinc chloride
	Zinc sulphate
Copper	Copper chloride
	Copper sulphate
Aluminium	Aluminium chloride
	Aluminium sulphate
	Aluminium sulphate + NaOH
pH	HCl
Mercury	Mercury sulphate
Arsenic	Sodium arsenate
Chloride	Sodium chloride
Sulphate	Sodium sulphate
Nitrate	Sodium nitrate

The BMP experiments were carried out over a year; therefore, some seasonality was observed in the characteristics of the sewage sludge used as feedstock and inoculum (Table 2.1). Performance of all test reactors was normalised to their respective blanks to compensate for those variations and allow relevant comparison of the results obtained.

Biogas (Y_{Bg} , mL/g) and methane (Y_M , mL/g) yields were calculated from their accumulated production in mL ($AccV_S$) subtracted by the accumulated biogas production from the blank in mL ($AccV_B$) and normalising over the mass of VS from sewage sludge fed to the reactor (g VS_f). Yield ratio (Y_i/Y_c) was calculated dividing the sample yield over the control (Equations 2.1 and 2.2).

$$Y_{iBg}/Y_{cBg} = \frac{AccV_S - AccV_B}{g VS_f} / Y_c \quad (2.1)$$

$$Y_{iM}/Y_{cM} = \frac{AccV_S - AccV_B}{g VS_f} / Y_c \quad (2.2)$$

The biogas and methane literature data I reported refers to mL/g VS added unless stated otherwise. VS added is a measure of the organics fed to the AD reactor. However, the use of VS added assumes that all the VS in the feed is biodegradable and that methane production is the same regardless of the nature of specific organic compounds. Lately, there is an increase of literature reporting AD performance as mL/g VS removed, accounting for the microorganisms' consumption of the organics and considering substrate biodegradability. Therefore, VS removed gives a better estimation of the biogas production in a continuous system. Nonetheless, VS added is more useful in batch experiments, like BMP tests, that are generally a test of biodegradability by themselves, making redundant the use of VS removed. Moreover, in a batch test, most of the organics will be consumed by the end of the test due to the extended retention times used, which are not realistic for continuous systems.

Biogas production kinetics were estimated using First-order kinetics and Gompertz modified models [12,32], as represented by equations 2.3 and 2.4, respectively.

$$\text{1st order kinetics} \quad P(t) = P_{max} \cdot (1 - e^{(-k_h \cdot t)}) \quad (2.3)$$

$$\text{Gompertz modified model} \quad P(t) = P_{max} \cdot e^{\left(-e^{\left(\frac{R_m \cdot (n-t)}{P_{max}} + 1\right)}\right)} \quad (2.4)$$

Where $P(t)$ is the production of biogas at time t (d); P_{max} is the maximum biogas production or biogas yield (mL biogas/g VS); k_h is the hydrolysis constant (d^{-1}); R_m is the specific rate constant (mL biogas/g VS/d); λ is the lag phase time constant (d).

The differences between both models and the real data were calculated using normalised root mean square error ($RMSE_n$) and r^2 (equations 2.5 and 2.6) [33,34].

$$RMSE_n = \left(\frac{1}{n} \sum_{i=1}^n \left(\frac{X_i - Y_i}{Y_i} \right)^2 \right)^{1/2} \quad (2.5)$$

$$r^2 = \left(\frac{m(\sum_{i=1}^n X_i Y_i) - (\sum_{i=1}^n X_i)(\sum_{i=1}^n Y_i)}{[\{m(\sum_{i=1}^n X_i^2) - (\sum_{i=1}^n X_i)^2\} \{m(\sum_{i=1}^n Y_i^2) - (\sum_{i=1}^n Y_i)^2\}]^{1/2}} \right)^2 \quad (2.6)$$

Where X_i is the predicted value of biogas (mL biogas/g VS), and Y_i is the measured value of biogas (mL biogas/g VS).

The impact of each salt and their concentration tested on biogas and methane yield and formation kinetics was classified as beneficial, non-effect, inhibitory and toxic. Beneficial is defined as the samples that had a biogas or methane yield above the value of the control sample. Non-effect samples have values close to the control. Inhibition was divided into two types, which are *yield inhibition* if there was a reduction on the maximum yield of biogas or methane and *kinetic inhibition* if a condition resulted in a lag phase. Toxic refers to samples that had less biogas production than the blank and hence resulted in negative yields.

2.2.4. Analytical methods

Sludge samples were analysed before and after the BMP tests. Alkalinity, TS and VS were measured using standard methods [35,36]. The measurement of pH was carried out with a Mettler Toledo MA235 pH/ion analyser and a portable Hanna HI 991003 pH meter. Total and soluble COD, TAN, and total nitrogen (TN) were analysed using cell test kits from Millipore (VWR, UK) and a spectrometer Spectroquant NOVA 60 from the same company. Samples for analyses of soluble COD, TAN, VFA, ions and metals were obtained after centrifuging at 5000 rpm for 8 hours and filtering the supernatant through 0.45 μ m Millipore syringe filters. FAN was calculated from the TAN concentration and pH value using equation 2.7 [37].

$$[FAN] = \frac{[TAN]}{1 + \frac{10^{pH}}{K_a}} \quad (2.7)$$

Where [FAN] is the FAN concentration (mg/L), [TAN] is TAN concentration (mg/L), and K_a is the dissociation constant with a value of $1.29 \cdot 10^{-9}$ for 37 °C [37].

A Shimadzu prominence high-performance liquid chromatography (HPLC) was used to analyse VFA from 1 mL of the acidified sample that was stored in a freezer at -20 °C. The mobile phase for the HPLC consisted of a 0.5 mM sulphuric acid solution with an injection flow rate of 1 mL/h, and a Rerex 150 column [38]. The acidification consisted of the addition of 10 μ L of 96% sulphuric acid to 9 mL of sample. A Dionex ICS-1600 ion chromatography (IC) was used for the determination of Nitrate, Nitrite, Sulphate, Phosphate, Fluoride, Chloride, and Bromide with a mobile phase of 9 mM Sodium carbonate and an AS 9HC column. Finally, a NexION 350D inductively coupled plasma (ICP) mass spectrometer was used to measure cations (Calcium, Magnesium, Potassium, Sodium, Manganese, Zinc, Cobalt, Molybdenum, Nickel, Copper, Iron, Selenium, Chromium, and Tungsten, Lithium, Aluminium, Silica, Titanium, Arsenic, Cadmium, Lead, Mercury, and Antimony). ICP samples were acidified with 100 μ L of >68% nitric acid in 10 mL of diluted soluble sample.

Gas samples were characterised for biogas and methane. Biogas was measured using a 1 M hydrochloric acid trap with an inverted cylinder to measure liquid displacement. The biogas was collected in a 10 mL syringe from the measuring cylinder after filtering it through a 0.45 µm Millipore syringe filter to avoid humidity intrusion for methane analysis with a Servomex 1440 gas analyser (Surrey, England, UK).

2.2.5. TWs load calculation

The last part of the study aimed to establish the loads of each TW that could be safely co-digested in sewage sludge ADs. To estimate the impact that TWs could have on the Aco-D process, the characterization data of TWs previously analysed was compared against the different effect ranges for individual constituents found in the BMP tests. For some of the ions of interest, two counter-ions (salts) were studied. The range selected when two ranges were obtained in BMP tests was the one that showed higher inhibition, to account for the worst-case scenario. Scenarios corresponding to TWs/sewage sludge volume ratios of 0/100, 10/90, 30/70, 50/50, 70/30, 90/10 and 100/0 were considered to calculate the total concentration of the ions of interest in the digester. The percentage out of the 160 TWs investigated that constitute an inhibitory risk for those scenarios was then calculated.

A more detailed investigation was conducted for scenarios with TW as 10% and 30% of the feedstock (10/90 and 30/70), for which potential impact as beneficial, non-effect, yield inhibition for methane yields, kinetic inhibition and toxic were investigated for the individual constituents present in the TWs. Another division called *unknown* was added to refer to conditions above the maximum concentration studied where no toxicity was observed. The 30/70 scenario was selected because it is reported to be the average spare capacity in municipal wastewater treatment plants digesters in Europe [8]. The 10/90 scenario was selected as 10% is commonly accepted by wastewater utilities to be the maximum spare capacity that could be allocated to TWs digestion without hindering sewage sludge treatment.

2.2.6. Statistics

ANOVA analysis was applied to determine if the differences or similarities between the biogas and methane yields between the control and test reactors were statistically significant. ANOVA uses the F-test to analyse the similarity between groups by comparing the variances between the groups. The similarity between control and test occurred when the F-value (F) was below the F-critical value (F_{crit}). On the contrary, when F was above F_{crit} , the control and test had statistically different yields (Table A1).

2.3. Results and Discussion

2.3.1. TWs characterisation

TWs are known to be variable in their composition depending on factors such as industrial activity, material processed, region, seasonality and production cycles [39]. The data set studied in this paper showed a high variability, in particular, for concentrations of chloride (0-120000 mg/L), sulphate (0-20000 mg/L), ammonia (0-4000 mg/L), aluminium (0-1400 mg/L), and zinc (0-1500 mg/L) (Figure 2.1). The most common division of TWs is by their source. In the current research the data was divided in landfill leachates, glycol/alcohol/beverage industry, food industry, chemical industry, oil containing waste, soap containing wastes, sludge and other (Figure 2.2). Landfill leachates and sludges are not actual industrial wastewaters but, as they are often tankered to water utilities for treatment in the same way as TWs, they were considered as TWs in this work. There was a great variation of COD in the glycol, beverage and alcohol group, food group and chemical group with values as high as 700 g/L of COD down to 1 g/L of COD (Figure 2.2). These 3 groups are the most predominant ones after landfill leachate with 20 to 30 different TWs from a total of 156 TWs evaluated. Oily and soapy wastewaters that were not fitting in the other sections showed a lower variation from 400 g/L of COD to 1 g/L of COD (Figure 2.2). Finally, the lowest variation and the lowest maximum COD level were observed in the groups of landfill leachate, sludge, and other TWs that were below 100 g/L of COD (Figure 2.2). Only landfill leachates could be distinguished with a direct correlation when plotting COD and TAN concentrations against each other (Figure 2.3). Hence, grouping the TWs by source type did not show any correlation with the characteristics of those TWs, due to the variability in their characteristics within each group. It was therefore decided to evaluate the TWs based on their composition.

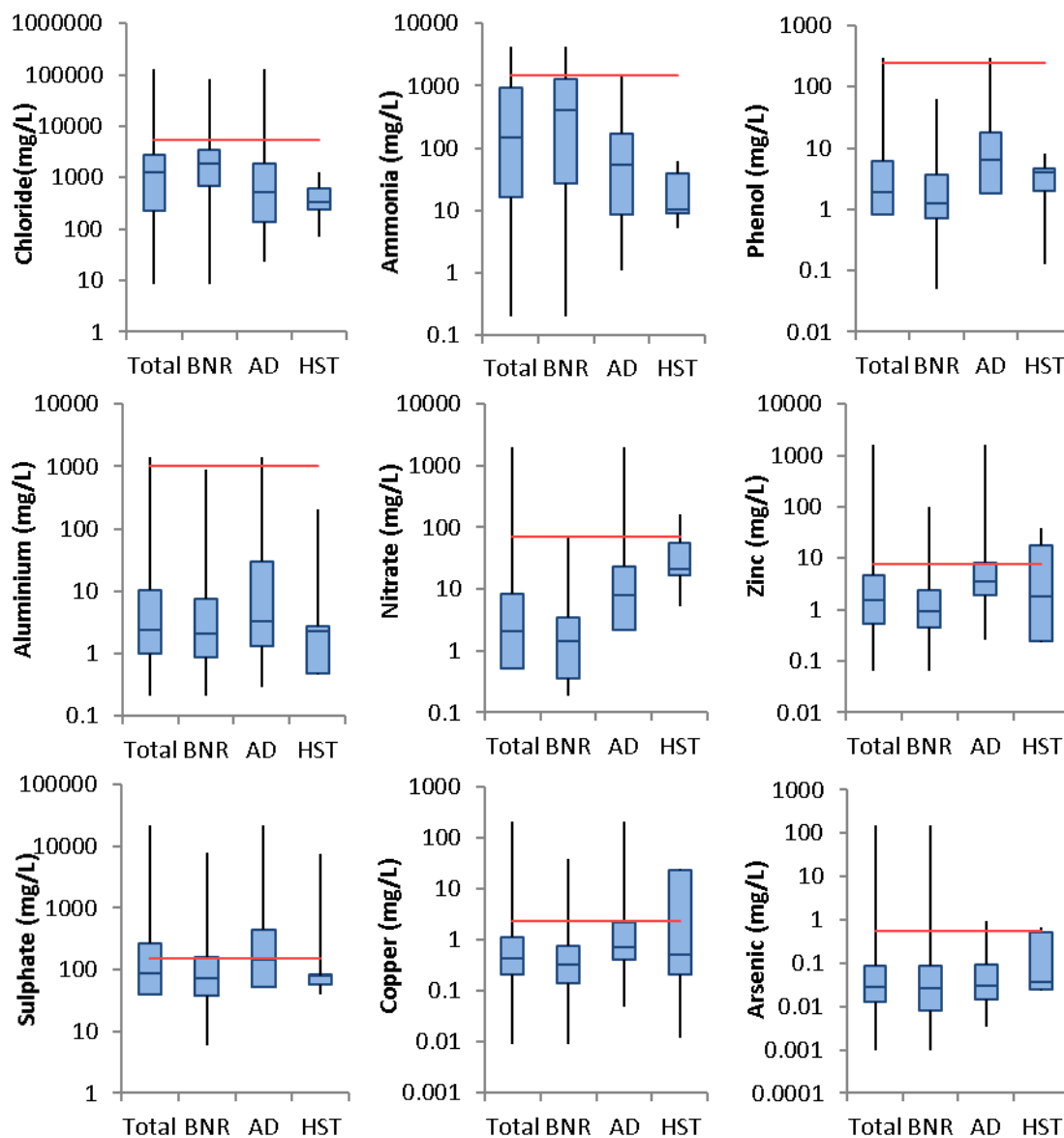


Figure 2.1. Box and whiskers plots values representing TWs composition depending on the treatment route generally chosen by water utilities. BNR represent TWs with COD below 50 g/L that are typically fed at the head of wastewater treatment plants, AD represent TWs with a COD between 50 and 250 g/L that go directly to the AD, and HST represent TWs with COD above 250 g/L that normally go to high strength tanks. The lowest inhibitory value for AD reported in the literature is represented by the red lines. The lowest point in the whisker represents the minimum value of the data, the higher point on the whisker represents the maximum value, the bottom of the box is the 25 percentile, the top of the box is the 75 percentile, and the line dividing the box is the median.

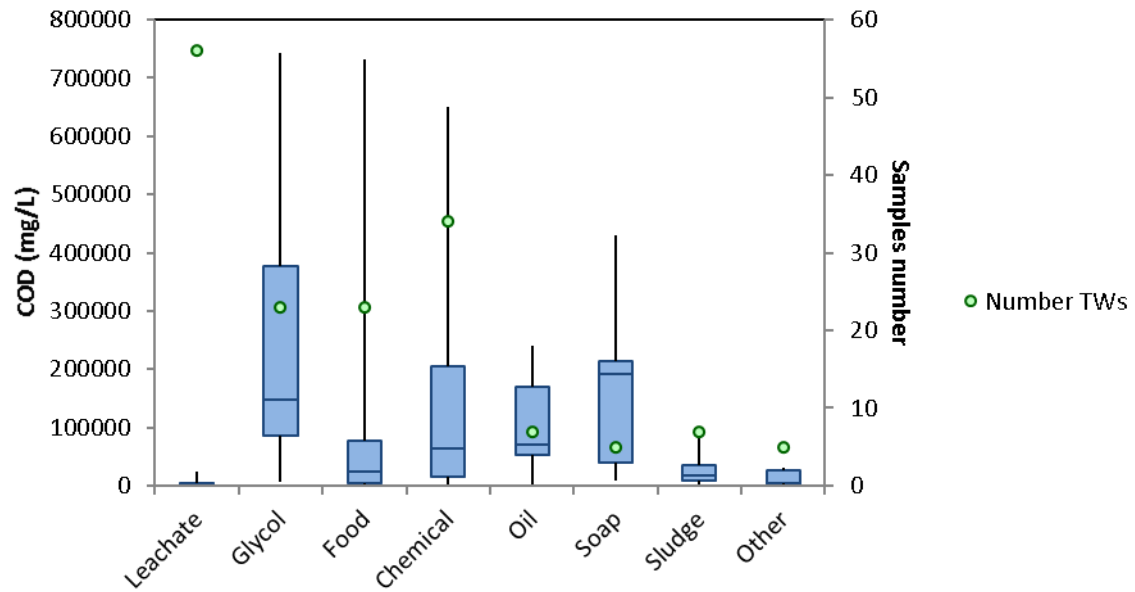


Figure 2.2. TWs divided by the type of industry where they generate from with their typical COD content.

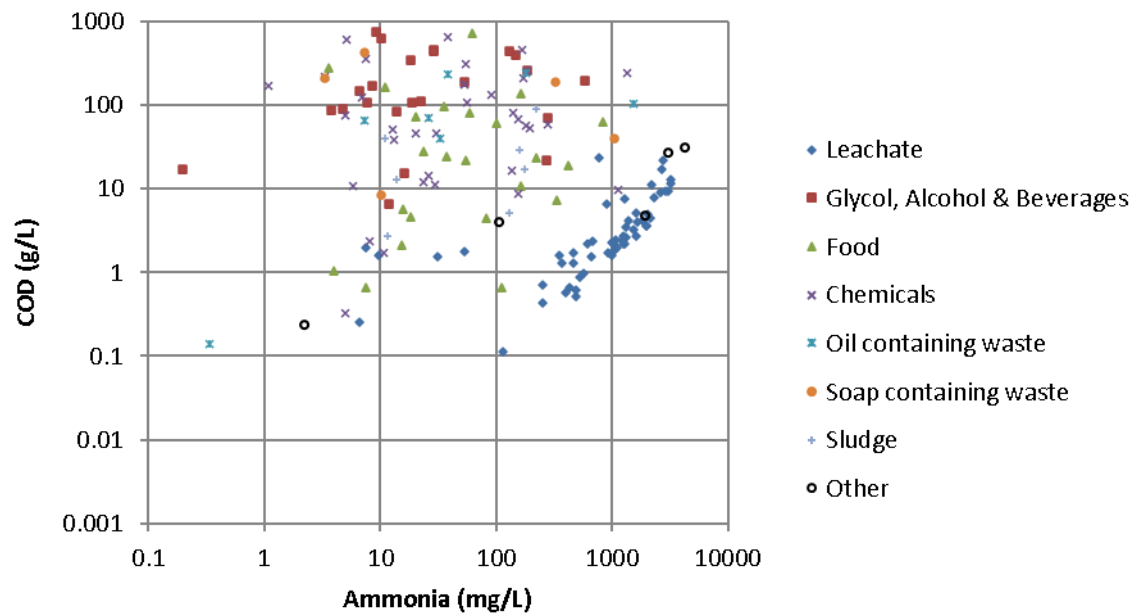


Figure 2.3. COD versus ammonia concentrations of TWs grouped by their source.

According to current practice on the full-scale site where the TWs dataset was obtained from, the TWs were first divided based on their COD content (Figure 2.4). Low strength TWs with a COD below 50 g/L are typically fed at the head of wastewater treatment plants, undertaking full treatment and serving as an additional carbon source in BNR processes [40]. TWs with a COD between 50 and 250 g/L go directly to the AD, and high strength wastes with COD above 250 g/L go to a high strength tank (HST) from where they are dosed slowly into the AD. The TWs divided into those groups were then further categorised according to concentrations of all parameters available and compared to inhibitory values reported in literature. TWs show high variability in all the compounds and elements

considered. The group that was more constant in its composition were the TWs that go to the HST (Figure 2.1). This type of TWs was found to have lower chloride, ammonia, phenol and aluminium levels than the lowest inhibitory values to AD reported in the literature (Table 1.2). Considering that TWs going to HST are highly concentrated in organics (COD ranged between 600 and 750 g/L), hence dosed slowly to AD reactors, the negative effect of the other compounds and elements present will not be as important as in TWs going directly to AD due to the dilution effect. For the wastes directly dosed into the AD, several were found to be above the lowest inhibitory value for all constituents evaluated, demonstrating a risk for the digestion process. The highest concentrations observed for chloride, ammonia, phenol, aluminium, nitrate, zinc, sulphate, copper, and arsenic were all above the lowest inhibitory value for AD reported in previous literature (Table 1.2 and red line Figure 2.1). Therefore, there is a clear risk that those TWs will negatively affect the sludge digestion if their loads to the reactor are not carefully selected. The TWs going to BNR were low in phenol, aluminium, and nitrate. To maximise biogas production, when spare capacity is available in the AD, all TWs going to the three different routes previously mentioned have been considered. The TWs going to BNR are low in organics compared with the TWs going to AD. Therefore, in another chapter of the thesis (Chapter 2), a study of pre-concentrating TWs before adding them to AD reactors with sewage sludge will be carried out and we will consider that possibility for the selection of the constituents of concern. Thus, several constituents of concern were observed such as chloride, ammonia, zinc, sulphate, copper and arsenic.

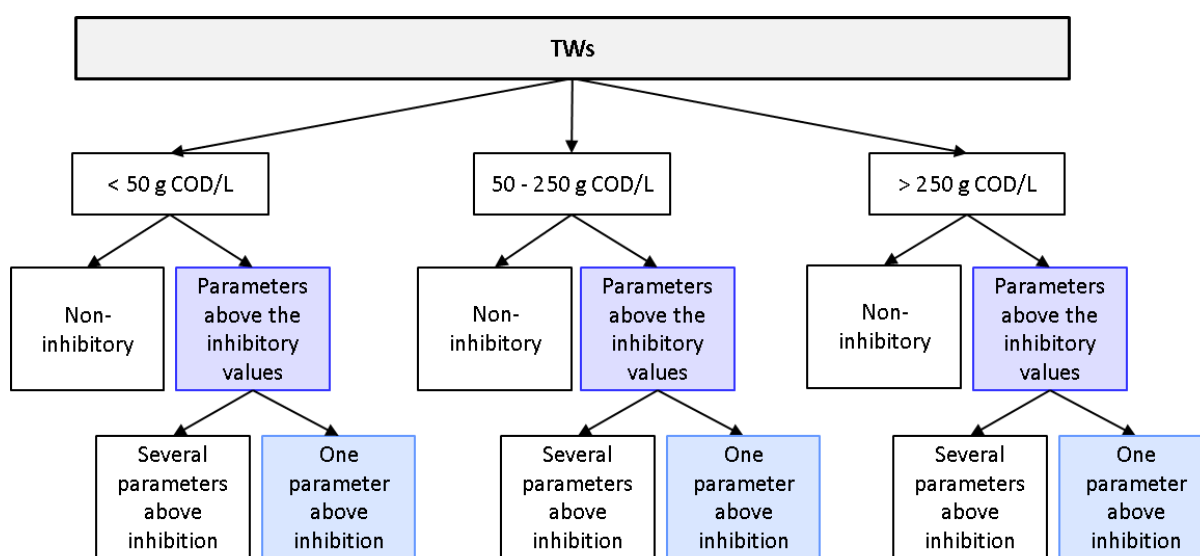


Figure 2.4. TWs division for the data set analysed.

The TWs were further separated into two groups: the first group comprised samples with one or more constituents in potentially inhibitory concentrations, and the second group contained TWs without any parameter in inhibitory levels (Figure 2.4). The first group had a sub-group with TWs with only one inhibitory constituent (Figure 2.4 and Figure 2.5). The six most common constituents in the TWs analysed that could present a risk of inhibition in AD were zinc, TAN, chloride, sulphate, iron and copper. Therefore, all these pollutants, except for iron, were considered for further study in the batch experiments. Arsenic, nitrate, and aluminium were also selected as they were found to be present above the inhibitory value, albeit at a lower end. Finally, although generally not found at

levels above the reported inhibitory value, mercury was selected due to its importance for sludge to land application.

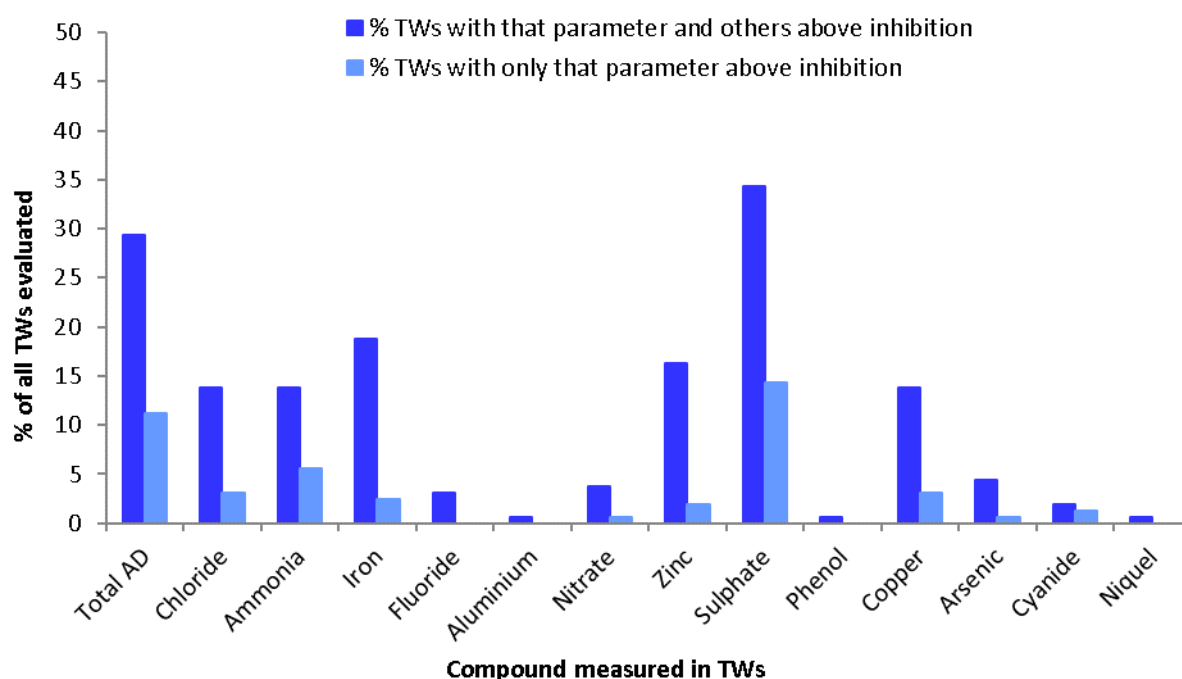


Figure 2.5. Representation of the proportion of TWs (%) with the potential to be inhibitory to AD for one or more measured compounds (dark blue) and only the compound considered (light blue). "Total AD" represents the total fraction of TWs that can go to AD based on organic content (COD > 50 g/L) that can have a potential to be inhibitory for one or more compounds (dark blue) and only one compound (light blue).

2.3.2. BMP tests

2.3.2.1. Yield

2.3.2.1.1. Ammonia

Similar trends and values in biogas and methane formation were observed when dosing ammonium chloride and ammonium sulphate in the digesters (Figure 2.6.a). The biogas and methane yields decreased from 631 to 521 mL/g VS with a 0.8 yield ratio in relation to the control and 415 to 354 mL/g VS with a 0.8 yield ratio over the control, respectively, when the TAN concentration was increased between 635 and 3405 mg/L with ammonium sulphate (Figure 2.6.a). Furthermore, addition of TAN between 586 and 5162 mg/L with ammonium chloride decreased biogas and methane yields from 624 to 431 mL/g VS with a 0.7 yield ratio in relation to the control and 377 to 257 mL/g VS with a 0.7 yield ratio over the control, respectively (Figure 2.6.a). This demonstrates increased inhibition as the TAN concentration increased and suggests that the chloride and sulphate counter-ions had similar or no effects on the results. Nevertheless, the statistical analysis suggested that the ammonium chloride trend was not statistically different from the control except for the measurement at 5162 mg TAN/L, but the ammonium sulphate was statistically different from the control for all the concentrations tested. For the highest concentration tested using ammonium sulphate, 3405 mg/L TAN, the biogas and methane yields were 18% and 32%, respectively, lower

than for the control (Figure 2.6.a). A further increase in TAN concentration as ammonium chloride, 5162 mg/L, led to 31% biogas and 15% methane inhibition (Figure 2.6.a), being the only one statistically different result from the control ($F > F_{crit}$) (Table A1). A similar concentration of TAN, 5000 mg/L, was reported as 25% inhibitory for methane yield by Borja *et al.* in cattle manure digesters [41].

As it has been previously reported, FAN is the ammonia form most toxic to the AD process as it permeates through the cell membrane more easily than ammonium, inhibiting enzymes or leading to proton imbalance [42]. In the current study, there was an increase from 7 to 255 mg/L of FAN using ammonium chloride that decreased the methane yield by 32% (Figure 2.6.b). Also, a decrease of 15% in methane formation was observed at 691 mg/L of FAN using ammonium sulphate (Figure 2.6.b). Ammonium chloride addition led to a similar reduction of the methane yield against the control (11%), to the one reported by Bujoczek *et al.* with a FAN concentration of 173 mg/L when food waste and sewage sludge were co-digested in a ratio 40%/60% [43]. There have been other studies that showed inhibition of the AD affecting the methanogens at high concentrations of FAN. Hansen *et al.* reported a decrease in the growth rate by 36% at FAN concentrations of 1400 mg-N/L [44]. A much lower concentration of FAN inhibited methanogens growth in two studies of Angelidaki and Ahring. One of the studies reduced the growth rate of methanogens by 50% at FAN concentrations of 200 and 500 mg-N/L [45]. The other study showed a negative effect of AD in a continuous AD operated at 55 °C when FAN reached 600 and 800 mg-N/L [46]. An even lower range of 280 and 520 mg-N/L of FAN was found to reduce by 50% the growth in acetate consumers and hydrogen consumers methanogens, respectively, by Borja *et al.* [41].

2.3.2.1.2. Zinc

An increment of Zn from 0.2 mg Zn/L to 2 mg Zn/L improved the biogas and methane yields reaching a ratio of 4 and a ratio of 3.7 over the control yield, respectively, for the experiments using zinc sulphate (Figure 2.6.c). This could be associated with zinc being capable of stimulating the growth of methanogens as it is needed in RNA polymerase and other biosynthetic enzymes [47]. From 2 mg Zn/L to 760 mg Zn/L there was a steady decrease of approximately 40% in both biogas (42%) and methane (37%) yields (Figure 2.6.c). Zinc sulphate in a range between 7 mg Zn/L and 112 mg Zn/L still showed an increase of performance over the control, similarly to the increase in yield observed with a zinc concentration of 8 mg/L in the study by Altaş [48]. At 1676 mg Zn/L, there was a toxic effect in the reactors as the biogas and methane productions recorded were lower than the ones of the blank units (i.e. negative yield value) (Figure 2.6.c). Using zinc chloride the biogas and methane yields were statistically similar to the control for the concentrations between 2.8 mg Zn/L and 87 mg Zn/L, indicating stable conditions as the biogas production only varied between 630 and 664 mL/g VS. Complete inhibition of biogas and methane production was reached at a concentration of zinc of 592 mg/L using zinc chloride (Figure 2.6.c). Zinc has been shown to have an inhibitory effect interfering with enzymes required for methanogenesis [47]. The accumulation of VFA in this study at 760 and 1676 mg Zn/L agrees with an effect of zinc in the methanogenesis phase of the digestion process. A concentration of 760 mg Zn/L increased iso-butyric concentration up to 147 mg/L, compared with the control concentration of 55 mg/L. A further increase up to 1676 mg Zn/L led to an increase in concentrations of all VFAs except valeric and iso-valeric above the control. The VFA

concentrations at 1676 mg Zn/L were 1085 mg/L of acetic, 2109 mg/L of propionate, 160 mg/L of iso-butyric, and 301 mg/L of butyric, while in the control were under the detection limit except for iso-butyric previously mentioned.

2.3.2.1.3. Copper

Biogas and methane yields remained in a similar range with 604-559 mL biogas/g VS and 431-388 mL methane/g VS when the concentration of copper sulphate was increased from 6 to 167 mg Cu/L (Figure 2.6.d). The similarity in biogas and methane yields for the range studied differs from previous studies that found inhibition between 2.2 and 50 mg Cu/L (Table 1.2). This difference could be linked to the zinc concentration in the system, as it has been reported to reduce copper inhibition by competition to enter the microorganism cell [47]. Nevertheless, the background zinc concentration in the current study was between 17 and 46 mg Zn/L in comparison to 65 mg Zn/L for example in the work reported by Abdel Azim *et al.* [47]. Therefore, other mechanisms such as copper polysulphide precipitation that was reported to be the most common form in sewage sludge at pH 7-7.5 at 37 °C might be responsible for the reduction of soluble copper [49]. Therefore, this decrease of soluble copper would have reduced the effect of copper on the current experiments. Moreover, attachment to the organic matter could reduce the available copper concentration for anaerobic microorganisms [50].

Alternatively, the use of copper chloride reduced the biogas and methane yields by up to 62% and 66%, respectively, with an increase in Cu concentration from 0.15 to 22 mg/L (Figure 2.6.d). This value is similar to a study with thermophile methanogen *Methanobacterium thermoautotrophicum* that was 50% inhibited at 14 mg Cu/L [51]. The biogas and methane yields further decreased with increasing copper chloride doses, resulting in 86% inhibition and toxic effect at 501 mg Cu/L and 804 mg Cu/L, respectively (Figure 2.6.d). An accumulation of acetate at concentrations of 613 mg Acetate /L was observed at the end of the BMP test when copper reached 811 mg Cu/L, compared with the control test that had 4 mg Acetate/L. Therefore, despite the methanogens being affected by the high concentration of copper, acetogens were able to grow, which agrees with previous studies on copper inhibition that have reported methanogens to be more affected than acidogens [52,53].

2.3.2.1.4. Aluminium

With aluminium sulphate, the biogas and methane yields remained constant at 574 mL biogas/g VS and 454 mL methane/g VS between 3 mg Al/L and 210 mg Al/L, with a small 13% reduction of methane at 210 mg Al/L. When increasing the aluminium concentration to 771 mg Al/L, the biogas and methane yield were reduced by 30% and 51%, respectively. The pH at 771 mg Al/L remained at 6.5, which is within the limit for AD operation; hence suggesting that the impact on process yield was attributable to the aluminium toxicity. Toxic effect for both biogas and methane production was reached at 1350 mg/L Al, although the low pH value of 5.3 recorded (Figure 2.6.e), prevents a clear understanding of whether inhibition was related with aluminium toxicity, acidification of the reactors or a combination of both.

In the case of aluminium chloride, there was a 9% increase in biogas yield and a 9% reduction of methane yield with concentrations between 1 and 203 mg Al/L. A toxic effect was then observed for

both biogas and methane production as the concentration was further increased to 780 mg Al/L and pH reached 5.8 (Figure 2.6.e and Figure 2.6.f). For both salts, aluminium chloride and aluminium sulphate, dosed reactors toxic effects occurred at a pH of 5.8 or below (Figure 2.6.f). To dissociate the impacts of the pH and aluminium on the system, an experiment with pH control for ADs dosed with aluminium sulphate between 262 and 608 mg Al/L was performed and other reactors, where pH was reduced from 6.55 to 4.5 without aluminium addition, were also operated.

The addition of aluminium even with pH control reduced biogas production by 18%, 29%, and 42% at concentrations of 262 mg Al/L, 464 mg Al/L, and 608 mg Al/L, confirming that at least part of the toxicity observed was due to aluminium. Aluminium can negatively affect microorganisms by disrupting glucose degradation by attaching to ATP and substitution of divalent ions, as it forms stronger complexes [54]. Additionally, aluminium cationic form (Al^{3+}) can compete with hydrogen cationic form (H^+) at low pH [54]. An accumulation of VFA was also observed with an increase in acetate concentration from 833 mg/L in the control to 1193 mg/L at 608 mg Al/L addition. VFA accumulation due to aluminium addition has been previously reported [55]. Nevertheless, the experiment with pH control resulted in 42% less biogas at 608 mg Al/L than the control unit, suggesting a combined inhibition due to aluminium and acidification in ADs dosed with aluminium sulphate and without pH correction. This synergistic effect could be confirmed when looking at the 84% and 86% reduction in biogas yield in ADs operated at pH 5 and 4.5 without aluminium addition, as its impact is lower than what was observed in aluminium dosed units with pH of 5.3, where toxicity was complete and resulted in negative biogas yields. Nevertheless, the methane at those pH values of 5 and 4.5 was below the blank yield, similarly to what was observed in reactors with aluminium addition and pH decrease.

2.3.2.1.5. Nitrate

Increasing levels of nitrate by dosing sodium nitrate resulted in an initial decrease in both biogas and methane performance, followed by an increase in yields reaching similar production to that of the control reactors at the maximum concentration studied of 427 mg NO_3/L (Figure 2.6.i). The 15% methane inhibition observed at 332 mg NO_3/L was similar to the reported value of 14% at that concentration by Tugtas *et al.* [56]. The lowest point was at 174 mg NO_3/L with 20% inhibition in biogas yield with 606 mL/g VS and 24% inhibition of methane yield with 411 mL/g VS (Figure 2.6.i). The change of yield performance with nitrate addition might be related to the accumulation of butyrate at the end of the experiment, >0.1 mg butyrate/L in the control against 113 mg butyrate/L at 254 mg NO_3/L , 118 mg butyrate/L at 332 mg NO_3/L , and 135 mg butyrate/L at 427 mg NO_3/L . The accumulation of butyrate might be caused by the accumulation of hydrogen caused by the inhibition of hydrogen consumers by nitrate, as hydrogen has been seen to inhibit butyrate degradation [57–59]. Kluber and Conrad found a 50% inhibition at 186 mg NO_3/L (3 mM) using *Methanosarcina barkeri*, which is double the inhibition at 174 mg NO_3/L observed in the current study, and at 1550 mg NO_3/L (25 mM) using *Methanobacterium bryantii* fed by hydrogen [59].

2.3.2.1.6. Arsenic and mercury

With Mercury, the biogas yield was mostly similar to the control units except for an increase of 17% and 13% in biogas yield above the control value of 754 mL/g VS at 239 mg Hg/L and 495 mg Hg/L.

However, the methane yield was constant at about 570 mL/g VS between 0.027 mg Hg/L and 920 mg Hg/L (Figure 2.6.j). Comparison with previous literature shows that 500 mg/L of mercury decreased by 90% the biogas production when a continuously fed system was spiked once with mercury [60]. The lower inhibition observed in the current research as compared with values reported in literature could result from the low solubility of mercury sulphate that could precipitate in the reactor or adsorb on the sludge reducing its bio-availability [61] as its concentration was reduced below 0.1 mg Hg/L at the end of the current experiment for all the concentrations studied in Figure 2.6.j.

Arsenate experiments had similar biogas and methane yields throughout the experiments from 0.02 to 16 mg As/L by adding sodium arsenate (Figure 2.6.k). Field *et al.* observed a competition for the organics between arsenate reducers and methanogens at a concentration lower than 150 mg As/L of arsenate [62]. A mechanism that might reduce the toxicity of arsenate is the precipitation with sulphur compounds [61,62]. Nonetheless, in previous experiments, a value of 960 mg SO₄/L (10 mM SO₄) which is higher than the concentration in this experiment of 34 mg SO₄/L did not show precipitation with sulphate when pH was close to neutral [62].

2.3.2.1.7. Counter-ion effect

As commented in the previous sections, there are differences between the experiments using chloride or sulphate as counter-ions. The TAN experiments showed a higher methane inhibition, 32%, using ammonium sulphate at 3405 mg-N/L than for ammonium chloride at 5162 mg-N/L, 15%. Nevertheless, biogas inhibition at 3405 mg-N/L ammonium sulphate and 5162 mg-N/L ammonium chloride was 18% and 31%, respectively. On the contrary, the FAN for those experiments showed a similar inhibition with ammonium sulphate and ammonium chloride at 255 mg-N/L, decreasing methane yield around 12% (Figure 2.6.a and Figure 2.6.b).

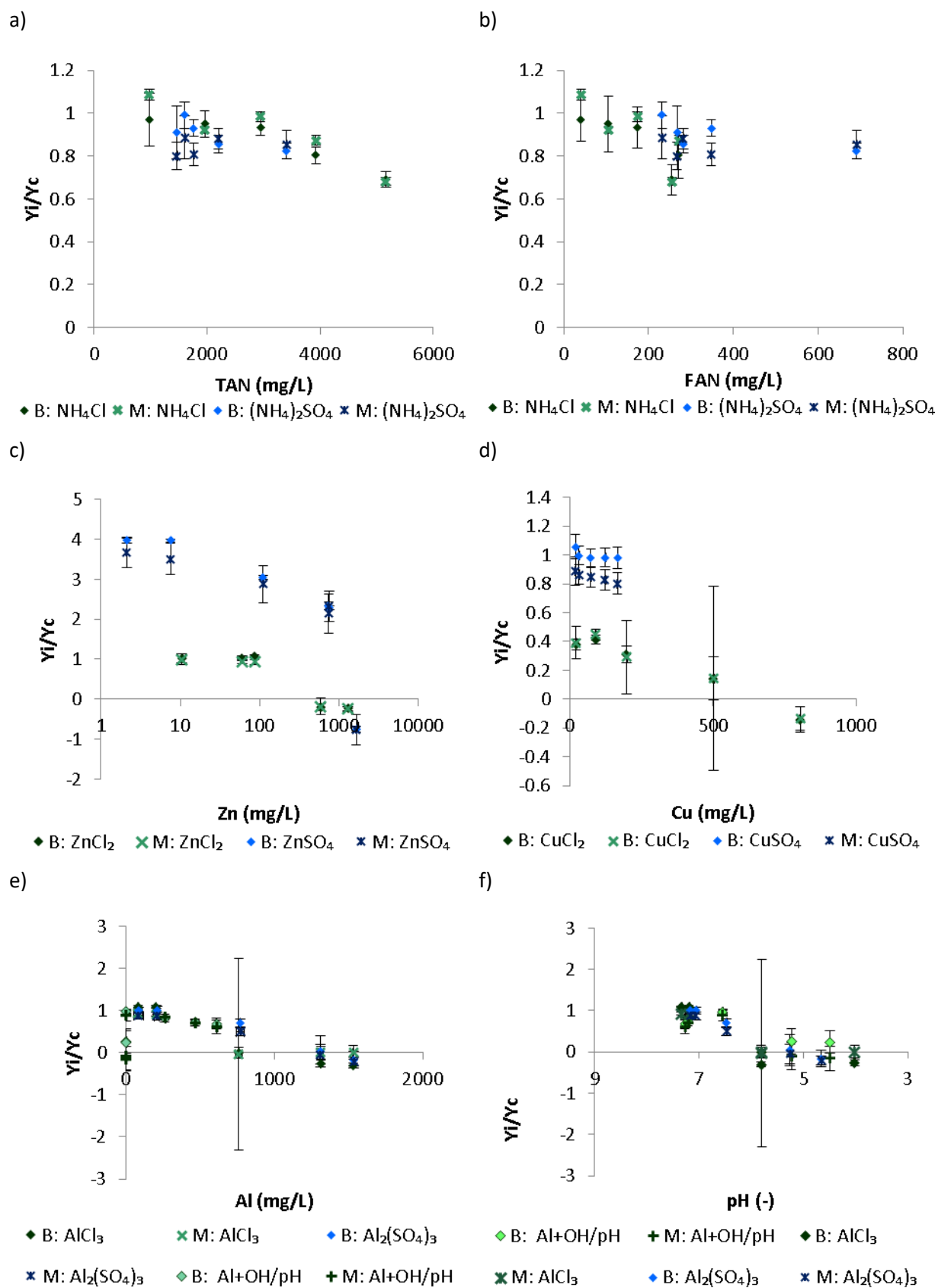
Zinc chloride presented a constant production of biogas and methane between 3 mg Zn/L and 87 mg/L, in contrast with the decreasing trend resulting from zinc sulphate from 2 mg Zn/L to 112mg Zn/L. But, further addition of zinc chloride showed a sharper decrease of biogas and methane yields than with zinc sulphate (Figure 2.6.c). This change of trend between both salts is observable at 592 mg Zn/L which is toxic using zinc chloride; but a higher concentration of 746 mg Zn/L was not toxic using zinc sulphate with a biogas yield of 507 mL/g VS and a methane yield of 315 mL/g VS (Figure 2.6.c).

Copper salts also differ in the trend of biogas and methane yields. Biogas and methane yields with copper chloride addition were down by 69% and 71%, respectively, at 198 mg/L of copper. Copper sulphate at a similar concentration of 167 mg/L did not show any significant decrease in biogas or methane yield (Figure 2.6.d). The differences in solubility between these salts could be the reason for the differences observed. Copper sulphate, which is less soluble with 22.2% in weight as compared to the solubility of copper chloride of 44.8% in weight at 40 °C [63], could have precipitated decreasing the amount of soluble copper affecting the bacteria and archaea. Additionally, the higher content of zinc in the experiment using copper sulphate could have competed with copper for transport into the cells, reducing its toxicity [47].

Both experiments with aluminium decreased the pH when the salts were added above 760 mg Al/L (Figure 2.6.f). The decrease of pH when aluminium salts are dissociated in water is produced because of the formation of $\text{Al}(\text{OH})_3$, which is the most common form in waters at pH 8 to 5 [64]. Aluminium chloride addition decreased pH more than similar concentrations using aluminium sulphate because of the formation of hydrochloric acid when aluminium chloride is mixed in water [64] (Figure 2.6.e and Figure 2.6.f). At 771 mg Al/L, aluminium sulphate showed a better performance of biogas and methane yields, 402 mL biogas/g VS with a ratio of 0.7 over the control and 241 mL methane/g VS with a ratio of 0.5 over the control; while aluminium chloride at 760 mg Al/L showed a toxic effect (Figure 2.6.e and Figure 2.6.f).

The last experiment carried out to compare the effect of the counter-ion was the one using sodium, which has been seen to be less inhibitory than other cations [65]. The experiments that were carried out using sodium chloride and sodium sulphate showed different impacts on gas yields (Figure 2.6.g and Figure 2.6.h). For the range of concentrations over which they coincide, 3360 and 4054 mg Na/L, there is an increase in biogas yield of 14% and similar methane yield than the control at 3915 mg Na/L using sodium chloride, while sodium sulphate decreased the biogas and methane yields by 8% and 16%, respectively, at 4054 mg Na/L. Hu *et al.* found that an increase of sulphate changing the $\text{COD}/\text{SO}_4^{2-}$ ratio from 20 to 0.5 decreased methane production rate by 27% [25]. In the current study, there was a decrease from 15 to 1.7 $\text{COD}/\text{SO}_4^{2-}$ ratios and a 16% reduction in methane yield. Jeong *et al.* observed 33% reduction in methane production at 1500 mg $\text{SO}_4^{2-}/\text{L}$ [66], while at similar values of 1466 mg $\text{SO}_4^{2-}/\text{L}$ in the current study led to a decrease of 16% in methane yield. Chloride addition to the experiments did not impact methane yield until 3666 mg Cl/L. A further addition to 4535 mg Cl/L decreased methane yield by only 6%. Vijayaraghavan *et al.* found a decreasing trend from the optimum when chloride was added above 5500 mg Cl/L [67].

Therefore, there was an effect of the counter-ion in the experiments. Ammonia and sodium had a synergistic effect with sulphate, while zinc, copper and aluminium had a synergistic effect with chloride. Previous studies have found a different effect for chloride salts than sulphate salts in soil bacteria because of different inhibitory mechanisms of chloride and sulphate. Chloride can affect some enzymes, such as phosphoenolpyruvate carboxylase, glutathione reductase and ribulose biphosphate carboxylase, and it can inhibit protein synthesis by preventing the binding of ribosomes to mRNA [68,69]. Meanwhile, sulphate can be metabolised by some bacteria competing with the methanogens for the substrate [26]. Sulphate addition has an effect on sulphate reducing bacteria that can be inhibited by some of the elements studied. Therefore, the negative impact on sulphate reducing species will have a beneficial effect on methanogens as they are competitors. For these reasons, the use of similar counter-ions is important when various cations are compared, and the concentration of chloride and sulphate might be an important parameter in the limit set for a cation in AD reactors.



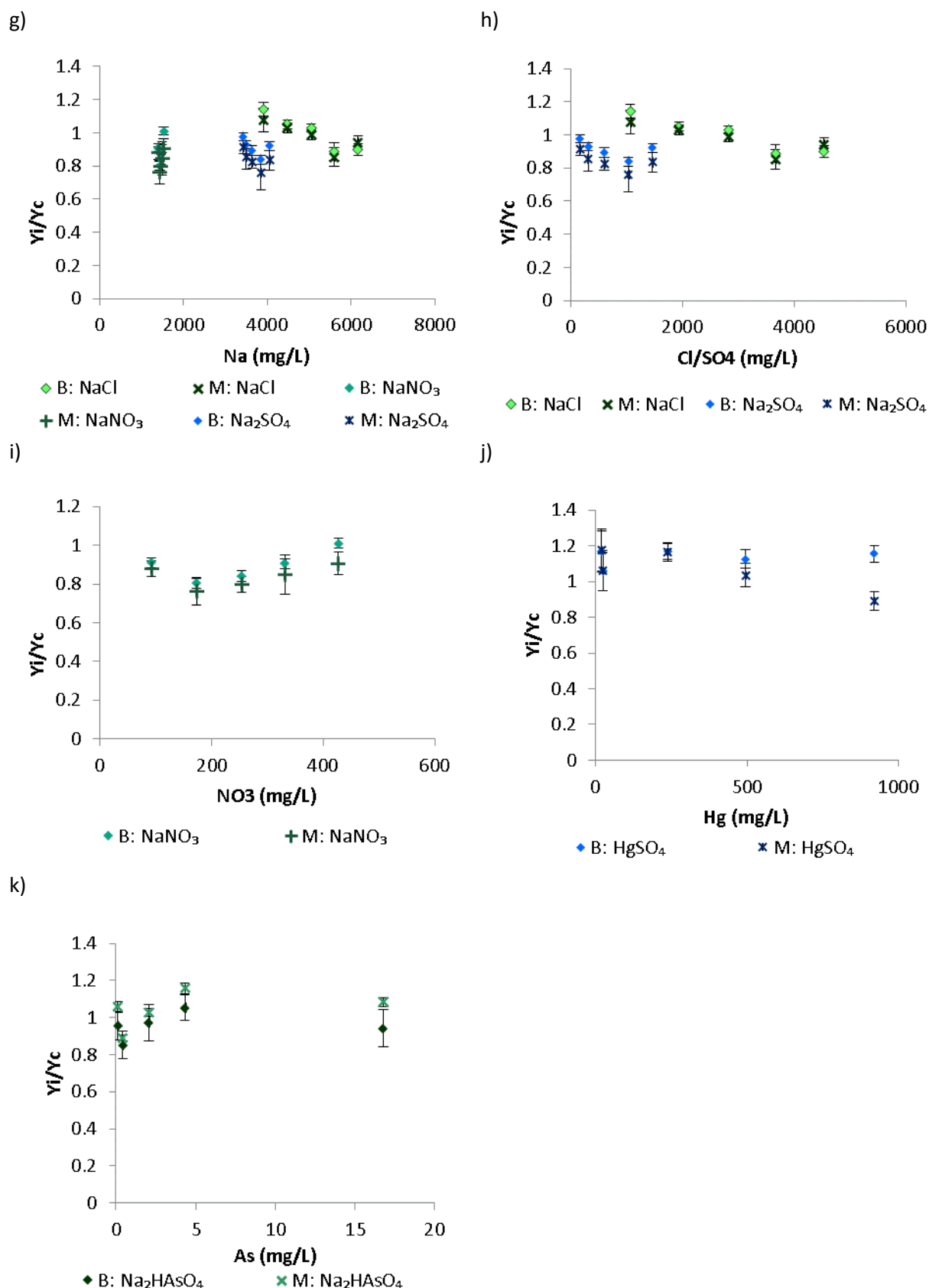


Figure 2.6. Biogas (B) and methane (M) yield ratio over the control when TAN (a) and FAN (b) were added using Ammonia sulphate and Ammonium chloride, zinc was added as Zinc sulphate and Zinc chloride (c), copper was added as Copper sulphate and Copper chloride (d), aluminium (e) was added and pH (f) was changed using Aluminium sulphate, Aluminium

chloride, Aluminium sulphate plus Sodium hydroxide and hydrochloric acid, sodium (g), sulphate and chloride (h) were added as Sodium sulphate and Sodium chloride, nitrate was added as Sodium nitrate (i), mercury was added as Mercury sulphate (j), and arsenic was added as Sodium arsenate (k).

2.3.2.2. Kinetics

A standard first-order model and the Gompertz model were utilised to analyse the kinetic data of biogas formation in the BMPs previously described. The first-order model gives a good approximation for substrates that contain easily hydrolysable organic content without inhibition in the form of a lag phase [70]. First-order kinetics were selected as they provide a good estimate of the hydrolysis constant, k_h , and give a good estimate of biogas yield if the biogas formation does not have a lag phase [13]. First-order model assumes that biogas accumulation data follows an exponential increase. This model has been shown to provide better results in sewage sludge AD fitting 99% of the results in a study by Nielfa *et al.* [12]. Instead, Gompertz accounts for any lag phase leading to a better fit of the data if samples are inhibitory for the process. The Gompertz model assumes that biogas and microbial activity are proportional variables. This model is typically used to describe the growth of bacteria in batch experiments [12]. Moreover, the Gompertz model is the kinetic model typically used in the literature for the degradation of organic substrates with a delayed degradation [70,71]. Therefore, the use of both models will give a good fitting for different cumulative biogas formation graphs and estimates for different kinetic parameters. In general, the Gompertz model showed a better fit for the data in this study than First-order kinetics as confirmed by r^2 and $RMSE_n$ values. The exception was in r^2 when 308 mg SO_4/L , 1032 mg SO_4/L and 1466 mg SO_4/L sodium sulphate were added, and in $RMSE_n$ at 1308 mg Al/L when aluminium sulphate was added, 308 mg SO_4/L and 1032 mg SO_4/L when sodium sulphate was added, 82 mg Al/L when aluminium chloride was added, and 1460 mg TAN/L , 1602 mg TAN/L and 1764 mg TAN/L when ammonium sulphate was added (Table 2.3).

The faster biogas formation kinetics of ammonium sulphate dosed reactors over ammonium chloride dosed units is noticeable with the Gompertz model results. The lag phase was found to increase from 2 up to 9 d when TAN was between 2946 and 5162 mg-N/L using ammonium chloride. The maximum value of λ using ammonium sulphate was 0.1 d at 3405 mg-N/L of TAN. Also, the concentration of ammonium sulphate for a maximum R_m was 1602 mg-N/L of TAN with 150 mL biogas/g VS/d. Control test R_m , 98 mL biogas/g VS/d, was the maximum one that was obtained in the ammonium chloride dosing experiment (Table 2.3). First-order kinetics differed from ammonium sulphate and ammonium chloride not only in the kinetic parameters but in the fit of the model. The fitting for ammonium sulphate ranges between 0.939 to 0.824 r^2 and 0.10 to 0.17 $RMSE_n$, while the fitting for ammonium chloride was 0.841 to 0.654 r^2 and 0.92 to 0.21 $RMSE_n$ (Table 2.3). The higher r^2 and lower $RMSE_n$ in ammonium sulphate than in ammonium chloride indicates a better fitting to First-order for ammonium sulphate and suggests a lag phase in ammonium chloride as it has been mentioned above. Focusing on the data from First-order kinetic, ammonium sulphate and ammonium chloride had a maximum k_h of 0.62 and 0.36 d^{-1} at 1602 and 976 mg-N/L of TAN, respectively. The increase of k_h for ammonium sulphate with respect to the control by 35% was higher than for ammonium chloride by 6%. Therefore, despite the biogas yield being similar to the control for most of the concentrations of ammonium chloride, except for 5162 mg-N/L, there was a kinetic inhibition in the shape of a delayed biogas formation between 2946 and 5162 mg-N/L (Table

2.3). On the contrary, ammonium sulphate resulted in a decrease in biogas yield from a concentration of 1764 mg-N/L, with kinetic inhibition resulting in a lag phase noticeable only at 3405 mg-N/L. Therefore, the inhibition of kinetic parameters for ammonium starts at values close to or above 3000 mg-N/L for both salts. This data showed that ammonia might not be such important on kinetic inhibition as it was on yield inhibition. But, the counter-ion, in this case chloride was responsible for the observed lag phase. Nevertheless, there could be another option where ammonium and chloride had a synergistic effect that increased kinetic inhibition.

Zinc exerted a different behaviour with different counter-ions that differs from what was observed with the ammonium salts. The lag phase in the Gompertz model observed in the control reactors, 4.5 d, decreased down to 1.4 d and 2 d when zinc sulphate was added between 2 and 112 mg Zn/L, respectively, with a later increase reaching a value of 21.4 d at a concentration of 760 mg Zn/L (Table 2.3). This contrasts with the results reported by Atılgöz that showed an increase of the length of the lag phase when zinc was increased from 4 mg Zn/L to 62 mg Zn/L [48], while in this study the zinc had to be increased up to 476 mg Zn/L to observe a longer lag phase than the control value of 7.9 d. R_m from the Gompertz model increased up to 80 mL biogas/g VS/d when zinc sulphate was added up to 7 mg Zn/L (Table 2.3). Zinc chloride did not result in any lag phase between 10 and 87 mg Zn/L, which is in agreement with the better fit of zinc chloride to the first kinetics model than zinc sulphate, though the fitting using Gompertz model is still better for both of them. In contradiction with the yield results, kinetics were faster in the experiments using Zinc chloride rather than for Zinc sulphate. Moreover, the effect of chloride and sulphate is reversed to what was found for ammonia. Therefore, the cause of the inhibition might not be one ion or the other, but the synergistic effect of both ions on the kinetic parameters. Zinc kinetic inhibition is enhanced by sulphate. Additionally, there might be an interaction with other components of the sewage sludge performance as the sewage sludge has some seasonal variation on the composition that could affect the experiments from zinc sulphate and zinc chloride. However, the lag phase in the control was reduced when zinc sulphate was added. Therefore, a synergistic effect seems to be the more plausible answer.

Copper sulphate at 32 mg Cu/L improved substrate degradation rate, as evidenced by a 7% increase in k_h from the First-order and 38% in R_m from the Gompertz model (Table 2.3). No lag phase was observed within the concentrations studied, which combined with the lack of impact on biogas and methane yields, suggest that copper within 7 and 167 mg Cu/L using sulphate is not inhibitory for the digestion of sewage sludge and can indeed accelerate gas formation. The control from the experiment with copper chloride was faster than when copper chloride was dosed as k_h from the First-order and R_m from the Gompertz model were the highest values in the experiment, 0.41 d^{-1} and 131 mL biogas/g VS/d, respectively (Table 2.3). Additionally, copper chloride led to a lag phase equal to 3 d at 198 mg Cu/L, while copper sulphate did not show any lag phase at a similar concentration. It was hence concluded that any inhibition in copper chloride reactors was attributable to the chloride counter-ion and not the copper itself.

Aluminium sulphate dosing resulted in a greater positive impact at a dose of 85 mg Al/L, as the Gompertz model showed a 23% increase on R_m up to 106 mL biogas/g VS/d (Table 2.3). A further increase of aluminium, up to 771 mg Al/L, using aluminium sulphate increased the lag phase to 0.7 d and reduced the fitting of this data with the First-order model, suggesting a small impact on

substrate degradation rate. The aluminium chloride maximum k_h from the first kinetic model was 0.49 d^{-1} at 82 mg Al/L with an increase of 19% from the control, and a maximum R_m from Gompertz model at 204 mg Al/L increasing by 17% from the control (Table 2.3). The experiments using aluminium sulphate with sodium hydroxide addition and the experiments decreasing the pH showed a better fit using Gompertz model than First-order kinetics as Gompertz model had a lower error with high r^2 and low RMSE_n , with a maximum R_m for the control test. Aluminium addition using aluminium sulphate with sodium hydroxide increased λ from 0.2 d in the control to 1.2 d at 607 mg Al/L , increasing the inhibition in the shape of an extended lag phase with aluminium addition (Table 2.3). A decrease in pH alone increased λ from 0.2 d to 25.1 d , evidencing the need for the microbial communities to recover from the initial acidic conditions (Table 2.3). Both salts alone triggered a decrease in pH, but aluminium chloride did not show any λ despite the lag phase observed when decreasing the pH alone. Therefore, there could be an interaction between pH, aluminium and chloride that shortens the lag phase observed at the acidic pH recorded in the aluminium chloride experiments. The similarities between the lag phase data at 771 mg Al/L in the experiments of aluminium sulphate without pH control and 607 mg Al/L in the experiment of aluminium sulphate with adjusted pH, suggest a higher effect of aluminium in those experiments and reduced effect of pH in kinetics when aluminium sulphate is added. Furthermore, the similarity of lag phase trend between the controls of both experiments shows that the variation of the sludge is not likely to be the cause of the differences between experiments using different counter-ions. It can be concluded that the difference in synergistic and antagonistic effect between the salt ions and the pH decrease because of the salt addition is the cause of the different behaviour between aluminium sulphate and aluminium chloride and the reason for the lag phase.

Sodium nitrate impacted process kinetics when dosed, resulting in a λ of 4 d at 1463 mg Na/L and $254 \text{ mg NO}_3/\text{L}$ (Table 2.3), which also matched a decrease of 15% in biogas yield (Figure 2.6.i). On the contrary to what was observed in the yield, the lag phase did not reduce to the values of the control, reaching 3 d at 1527 mg Na/L and $427 \text{ mg NO}_3/\text{L}$. Therefore, nitrate concentrations above $92 \text{ mg NO}_3/\text{L}$ already require time to acclimatize, which could be associated with a shift in the microbial population as commented in the previous section.

Sodium experiments using chloride and sulphate as counter-ions achieved a faster biogas production at 3915 mg Na/L and 3846 mg Na/L , and 1066 mg Cl/L and $1032 \text{ mg SO}_4/\text{L}$, respectively. First-order kinetics for sodium chloride and sodium sulphate showed an increase of k_h of 24% and 48% from the control, respectively (Table 2.3). This suggested better substrate degradation when sulphate was used as counter-ion. Contrary, the Gompertz model parameter R_m increased by 30% and 14% respectively from the control (Table 2.3). There was not an observable lag phase for any of the two experiments despite the 8% reduction of biogas yield at 4054 mg Na/L and $1466 \text{ mg SO}_4/\text{L}$ compared with the control (Table 2.3). Nevertheless, sodium sulphate data fitted better on the First-order kinetics between 3499 and 4054 mg Na/L than the Gompertz model, suggesting a lack of lag phase, while sodium chloride fitted better on the Gompertz model. Despite the small differences, there were no clear differences between the use of chloride and sulphate as counter-ion using sodium. Therefore, it seems that the kinetics are affected by a synergistic/antagonistic effect between the ions composing the salt used rather than one of them being the single cause of that negative effect.

Mercury addition improved the speed of biogas conversion when it was added up to 920 mg/L increasing R_m from the Gompertz model by 77% from the control. An increase of λ occurred at 495 mg/L up to 0.8 d showing a slight inhibition. Sodium arsenate affected more the kinetic parameters resulting in a λ of 2d when arsenic was added up to 17 mg/L, contrarily to what was observed in the yield which remained similar to the control for every concentration tested (Table 2.3). This could mean that at the concentration of 17 mg As/L there was a slight inhibition and an acclimation of the microorganisms, or a competition between the arsenate reducers and the methanogens for the organic substrate [62]. Therefore, despite no inhibitory effect on the yield, there might be an inhibitory effect on the kinetic of AD due to the competition between arsenate reducers and methanogens [61,62].

In general, there was a difference in speed of biogas formation between chloride and sulphate which differs from the results obtained in the previous section for yields. Ammonium chloride and sodium arsenate exhibited a lag phase despite having similar yields to the control. Zinc sulphate and sodium nitrate showed a slower biogas formation than the control for concentrations that resulted in similar or greater yields than the control reactors. Aluminium sulphate, copper sulphate and ammonium sulphate showed a decreasing trend on the yield and kinetics. Therefore, if a TW contain these constituents will require dilution with less concentrated TWs to avoid biogas reduction in AD of sewage sludge. There is a difference between the impact on gas formation kinetics of the ion studied depending on the counter-ion used, as it was observed with sulphate and chloride. The difference in kinetics parameters might be caused by an interaction of the two ions in the salt rather than the separate effect of one of them.

Table 2.3. Results from First-order and Gompertz model for the biogas formation kinetics on the BMP experiments (B=biogas; Conc= concentration on the reactor; C=control).

Salt	Ion	Conc (mg/L)	1st order				Gompertz model				
			k_h (d ⁻¹)	P_{max} (mL B/ g VS)	RMSE _n	r^2	R_m (mL B/g VS/d)	λ (d)	P_{max} (mL B/ g VS)	RMSE _n	r^2
(NH ₄) ₂ SO ₄	NH ₄	635 (C)	0.46	598	0.15	0.91	111	0.0	611	0.11	0.98
(NH ₄) ₂ SO ₄	NH ₄	1460	0.59	553	0.11	0.928	132	0.0	566	0.12	0.992
(NH ₄) ₂ SO ₄	NH ₄	1602	0.62	604	0.10	0.939	150	0.0	617	0.13	0.983
(NH ₄) ₂ SO ₄	NH ₄	1764	0.58	565	0.11	0.932	132	0.0	578	0.12	0.990
(NH ₄) ₂ SO ₄	NH ₄	2201	0.43	566	0.12	0.897	113	0.0	556	0.03	0.998
(NH ₄) ₂ SO ₄	NH ₄	3405	0.32	567	0.17	0.824	96	0.1	536	0.02	0.996
NH ₄ Cl	NH ₄	586 (C)	0.34	635	0.24	0.793	98	0.0	570	0.10	0.962
NH ₄ Cl	NH ₄	976	0.36	598	0.21	0.835	90	0.0	535	0.13	0.931
NH ₄ Cl	NH ₄	1958	0.19	642	0.30	0.841	57	0.0	556	0.06	0.989
NH ₄ Cl	NH ₄	2946	0.03	889	0.50	0.672	32	2.4	543	0.16	0.977
NH ₄ Cl	NH ₄	3923	0.02	889	0.47	0.682	22	3.3	505	0.17	0.993
NH ₄ Cl	NH ₄	5162	0.00	79	0.92	0.654	24	9.2	426	0.65	0.991
ZnSO ₄	Zn	0.26(C)	0.01	2202	0.55	0.278	4	4.5	261	0.17	0.935
ZnSO ₄	Zn	2	0.04	1662	0.41	0.767	69	1.4	830	0.07	0.992
ZnSO ₄	Zn	7	0.03	1988	0.45	0.753	80	1.4	821	0.11	0.983

ZnSO ₄	Zn	112	0.00	30016	0.53	0.310	28	2.0	655	0.04	0.998
ZnSO ₄	Zn	746	0.00	20620	0.63	0.308	12	7.9	522	0.07	0.994
ZnSO ₄	Zn	760	0.00	1725	0.75	0.530	18	21.4	477	0.03	0.998
ZnSO ₄	Zn	1676	-	-	-	-	-	-	-	-	-
ZnCl ₂	Zn	3 (C)	0.46	598	0.15	0.911	112	0.0	611	0.11	0.984
ZnCl ₂	Zn	10	0.49	615	0.15	0.909	117	0.0	638	0.12	0.992
ZnCl ₂	Zn	61	0.41	628	0.14	0.910	111	0.0	627	0.07	0.990
ZnCl ₂	Zn	87	0.35	659	0.16	0.895	102	0.0	640	0.05	0.984
ZnCl ₂	Zn	592	-	-	-	-	-	-	-	-	-
ZnCl ₂	Zn	1307	-	-	-	-	-	-	-	-	-
CuSO ₄	Cu	7 (C)	0.22	621	0.14	0.801	86	0.0	546	0.04	0.977
CuSO ₄	Cu	20	0.27	655	0.10	0.878	117	0.0	566	0.06	0.955
CuSO ₄	Cu	32	0.29	611	0.10	0.871	119	0.0	538	0.04	0.969
CuSO ₄	Cu	73	0.26	555	0.07	0.921	112	0.0	556	0.06	0.958
CuSO ₄	Cu	123	0.23	624	0.13	0.853	98	0.0	524	0.06	0.957
CuSO ₄	Cu	167	0.22	639	0.15	0.825	94	0.0	535	0.04	0.982
CuCl ₂	Cu	0.1 (C)	0.41	890	0.12	0.890	131	0.0	908	0.05	0.994
CuCl ₂	Cu	22	0.37	351	0.12	0.897	51	0.0	345	0.02	0.996
CuCl ₂	Cu	91	0.14	481	0.24	0.662	45	0.8	373	0.02	0.996
CuCl ₂	Cu	198	0.00	5679	0.65	0.230	23	2.9	256	0.11	0.955
CuCl ₂	Cu	501	-	-	-	-	-	-	-	-	-
CuCl ₂	Cu	804	-	-	-	-	-	-	-	-	-
Al ₂ (SO ₄) ₃	Al	4 (C)	0.22	621	0.14	0.801	86	0.0	546	0.04	0.977
Al ₂ (SO ₄) ₃	Al	85	0.27	611	0.10	0.884	106	0.0	544	0.05	0.955
Al ₂ (SO ₄) ₃	Al	210	0.24	614	0.11	0.884	94	0.0	532	0.07	0.929
Al ₂ (SO ₄) ₃	Al	771	0.10	572	0.28	0.668	60	0.7	395	0.04	0.989
Al ₂ (SO ₄) ₃	Al	1309	4.00	21	0.09	0.000	15	50.0	19	0.82	0.001
Al ₂ (SO ₄) ₃	Al	1534	-	-	-	-	-	-	-	-	-
AlCl ₃	Al	1 (C)	0.41	890	0.12	0.89	131	0.0	908	0.05	0.99
AlCl ₃	Al	82	0.49	941	0.08	0.954	148	0.0	974	0.44	0.996
AlCl ₃	Al	204	0.43	961	0.09	0.950	153	0.0	1008	0.07	0.996
AlCl ₃	Al	760	-	-	-	-	-	-	-	-	-
AlCl ₃	Al	1312	-	-	-	-	-	-	-	-	-
AlCl ₃	Al	1531	-	-	-	-	-	-	-	-	-
Al ₂ (SO ₄) ₃ +OH	Al	0 (C)	1.00	546	0.46	0.014	154	0.2	524	0.03	0.957
Al ₂ (SO ₄) ₃ +OH	Al	309	28.00	130	0.76	0.011	118	0.8	436	0.02	0.987
Al ₂ (SO ₄) ₃ +OH	Al	511	0.03	2602	0.77	0.025	95	0.9	398	0.02	0.997
Al ₂ (SO ₄) ₃ +OH	Al	607	23.21	41	0.89	0.012	80	1.2	373	0.02	0.994
HCl	pH	7.3 (C)	1.00	546	0.46	0.014	154	0.2	524	0.03	0.957
HCl	pH	6.6	1.00	536	0.45	0.019	131	0.1	516	0.03	0.974
HCl	pH	5.2	0.02	144	0.88	0.200	48	15.7	111	0.63	0.957
HCl	pH	4.5	0.03	31	0.92	0.135	25	25.1	118	0.34	0.992
NaCl	Na	3360 (C)	0.21	636	0.10	0.881	103	0.0	560	0.04	0.982

NaCl	Na	3915	0.26	700	0.09	0.895	134	0.0	643	0.04	0.978
NaCl	Na	4483	0.23	704	0.06	0.939	119	0.0	574	0.04	0.974
NaCl	Na	5054	0.22	705	0.08	0.921	112	0.0	579	0.04	0.977
NaCl	Na	5601	0.19	626	0.09	0.911	88	0.0	494	0.05	0.971
NaCl	Na	6165	0.09	913	0.18	0.774	56	0.0	512	0.02	0.995
Na ₂ SO ₄	Na	3360 (C)	0.21	636	0.10	0.881	103	0.0	560	0.04	0.982
Na ₂ SO ₄	Na	3430	0.25	637	0.05	0.952	115	0.0	538	0.05	0.958
Na ₂ SO ₄	Na	3499	0.27	595	0.05	0.962	116	0.0	512	0.05	0.951
Na ₂ SO ₄	Na	3639	0.27	575	0.05	0.956	114	0.0	499	0.05	0.959
Na ₂ SO ₄	Na	3846	0.31	523	0.03	0.978	118	0.0	466	0.05	0.947
Na ₂ SO ₄	Na	4054	0.27	593	0.05	0.954	115	0.0	511	0.05	0.952
NaNO ₃	Na	1370 (C)	0.19	784	0.41	0.775	64	0.0	748	0.04	0.995
NaNO ₃	Na	1403	0.05	1073	0.39	0.722	32	1.6	694	0.09	0.997
NaNO ₃	Na	1433	0.00	12884	0.47	0.658	23	3.3	657	0.10	0.988
NaNO ₃	Na	1463	0.00	9507	0.39	0.677	25	4.0	652	0.15	0.996
NaNO ₃	Na	1491	0.02	1689	0.39	0.704	26	2.9	713	0.13	0.993
NaNO ₃	Na	1527	0.01	4685	0.42	0.702	30	3.0	780	0.10	0.997
HgSO ₄	Hg	0.03 (C)	0.19	784	0.41	0.775	64	0.0	748	0.04	0.995
HgSO ₄	Hg	25	0.19	826	0.29	0.796	75	0.2	760	0.03	0.994
HgSO ₄	Hg	20	0.32	855	0.35	0.761	107	0.0	840	0.07	0.987
HgSO ₄	Hg	920	0.36	824	0.34	0.750	113	0.0	824	0.10	0.982
HgSO ₄	Hg	239	0.23	889	0.38	0.781	90	0.2	820	0.03	0.992
HgSO ₄	Hg	495	0.12	948	0.49	0.706	82	0.8	800	0.08	0.991
Na ₂ HAsO ₄	As	0.03 (C)	0.34	635	0.24	0.793	98	0.0	570	0.10	0.962
Na ₂ HAsO ₄	As	0.1	0.32	612	0.25	0.787	87	0.0	554	0.10	0.975
Na ₂ HAsO ₄	As	0.4	0.23	562	0.24	0.869	53	0.0	507	0.12	0.984
Na ₂ HAsO ₄	As	2	0.24	594	0.22	0.901	56	0.0	511	0.16	0.920
Na ₂ HAsO ₄	As	4	0.15	679	0.26	0.924	41	0.0	552	0.14	0.932
Na ₂ HAsO ₄	As	17	0.03	883	0.48	0.761	25	2.2	536	0.18	0.982

2.3.3. Potential for Co-digestion of TWs

One of the objectives was to establish the loads of each type of TWs that can be added to sludge anaerobic digesters without hindering methane production. A preliminary understanding of the maximum TW loads that can be safely co-digested in sewage sludge ADs can be obtained with results from the inhibition tests in BMPs and information on the characterisation of TWs obtained from the database (section 2.3.1). The BMP tests results show the limits of key components and the characterisation data relates those components with the TWs addition to Aco-D with sewage sludge. Nevertheless, it should be noted that the assessment for co-digestion in this study is limited as batch tests do not account for acclimation to or accumulation of inhibitors, but the results obtained are still relevant and reliable to address the objectives of this study. To understand the impact of adding TWs in different loads, scenarios considering different volume ratios of TWs to sewage sludge were evaluated: 0/100, 10/90, 30/70, 50/50, 70/30, 90/10 and 100/0. The concentrations of individual constituents calculated for those scenarios were then compared with the BMP inhibition and toxic values to obtain Figure 2.7 (Table 2.4). Complete toxicity was only achieved by zinc and copper for the concentrations tested in the current study (Table 2.4). Comparing these results with Table 1.1 in Chapter 1, there are differences in the inhibitory concentrations for some elements. Chloride shows in this study inhibition at a lower concentration than previous studies that reported a decreasing trend at 5500 mg/L of chloride using tannery wastewater in a continuous reactor [67]. Aluminium inhibition was observed in a lower concentration compared with previous literature that measured inhibition between the values of 1000 and 2500 mg/L of aluminium [55,72]. Ammonia, nitrate, zinc, sulphate and copper showed inhibitory values in this study within the limits of the literature (Table 1.1 in Chapter 1 and Table 2.4).

Table 2.4. Summary of concentration ranges impacting AD of sewage sludge obtained from BMP tests.

	Beneficial	Non-effect	Yield inhibition	Kinetic inhibition	Toxic	Unknown
Chloride (mg/L)	-	<3666	3666-4535	-	-	>4535
Ammonia (mg/L)	635-1460	<635 1460-1602	1602-3405	1602-3405	-	>3405
Aluminium (mg/L)	-	<309	309-607	309-607	-	>607
Nitrate (mg/L)	-	<92 332-427	92-332	92-427	-	>427
Zinc (mg/L)	-	<87	87-498	-	>498	-
Sulphate (mg/L)	-	<162	162-1466	-	-	>1466
Copper (mg/L)	-	<7	7-501	91-198	501-804	-
Arsenic (mg/L)	-	<17	-	17	-	>17
Mercury (mg/L)	-	<25 495-920	25-495	25-495	-	>920

Note: As specified in section 2.2, “beneficial” was defined as the samples with a biogas yield above the control yield; “non-effect” samples have yields similar to the control; “yield inhibition” showed a reduction on the maximum yield of biogas respect the control one; “kinetic inhibition” showed an increase of a lag phase; “toxic” referred to yield values below the blank production; and, “unknown” referred to conditions above the maximum concentration studied in the BMP tests, where no toxicity was observed. When two salts were tested for the same inhibitor, the range presented is the one that

showed higher yield inhibition at the smallest concentration to account for the worst-case scenario, with the exception of chloride and sulphate salts which had sodium as counter-ion.

Ammonia, aluminium, zinc, copper, chloride, nitrate, sulphate and arsenic showed a high number of TWs with a potentially negative effect to sewage sludge AD, with the number of potentially inhibitory TWs increasing when TWs loads to the reactor were increased. In a scenario with only TWs fed to the ADs (TWs/sludge volume ratio of 100/0), 12%, 4%, 1%, 7%, 16%, 4%, 32% and 1% of the TWs studied were regarded as potentially inhibitory when considering calculated concentrations of ammonia, aluminium, zinc, copper, chloride, nitrate, sulphate and arsenic, respectively (Figure 2.7). The only element that did not show any effect at any volume ratio was mercury (Figure 2.7).

Sulphate and chloride constituted the largest risk of inhibition, with 7% and 4% of the TWs resulting in inhibitory calculated concentrations in a 10/90 scenario and 32% and 16% in a 100/0 scenario for sulphate and chloride, respectively (Figure 2.7). These two constituents showed potential to inhibit methane yield by up to 25% at the studied concentrations (section 2.3.2.1). Ammonia, aluminium, zinc, and copper all led to a less pronounced increase in the proportion of TWs with potential to have a negative effect by those constituents as the TW/sludge volume ratio was increased (Figure 2.7). They also showed a higher methane yield inhibition at the concentrations studied with potential for 32%, 39%, 100% and 100% methane reduction, respectively (section 2.3.2.1).

Overall, a volume ratio of 10/90 TWs and sewage sludge will be the best volume ratio for co-digestion considering all the ions studied and typical concentrations encountered in real TWs (Figure 2.7). Most of the constituents in TWs remain below the inhibitory levels obtained in BMP test at this volume ratio, with only 13.7% of all the TWs studied presenting a potential for inhibition. Chemical TWs showed inhibition in 7 cases for sulphate, 3 for chloride, 3 for copper and 2 for nitrate. Glycerol/beverage/alcohol wastes showed inhibition from sulphate, chloride and nitrate for 7, 1 and 1 of them, respectively. An oil containing TW showed inhibition for zinc and sulphate. Chloride inhibition was also found in one of the food TWs. Finally, chloride and sulphate inhibitory effects were observed in, respectively, 1 and 2 landfill leachates. Moreover, there are constituents such as ammonia, aluminium, arsenic and mercury that do not show any inhibitory effect at that volume ratio. Therefore, the volume ratio of 10/90 TWs and sewage sludge will be further discussed in the next paragraphs. Also, the average spare capacity in wastewater treatment plants digesters has been reported as 30%, which could be an opportunity for Aco-D with TWs up to this volume ratio [8]. Therefore, further investigation in continuous experiments of the impact of the preferred TW/sludge volume ratio (10/90) and the volume ratio representing site capacity (30/70) is paramount to understand whether acclimation of microbial communities not observable in batch ADs could occur.

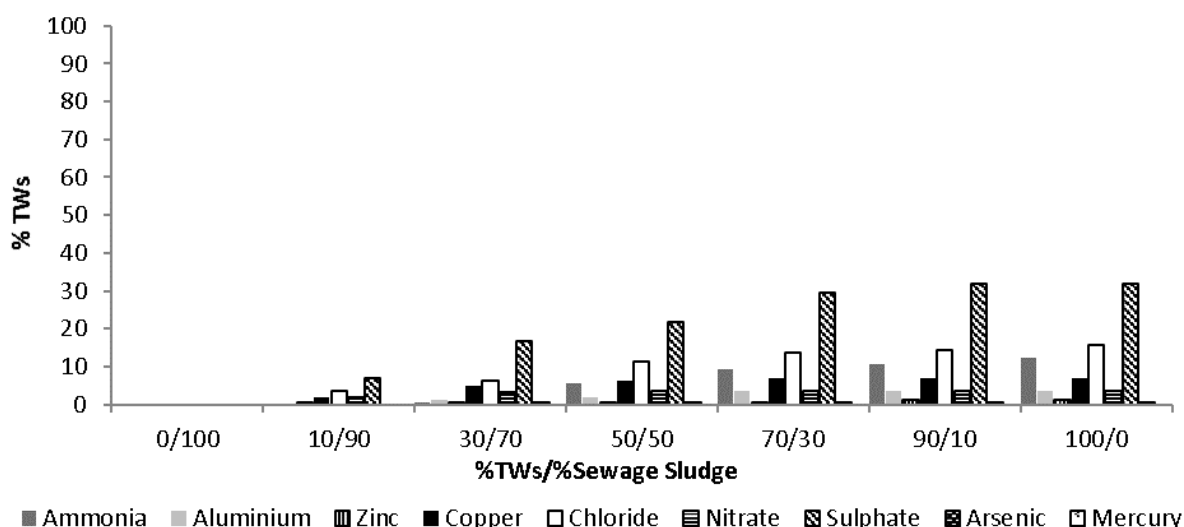
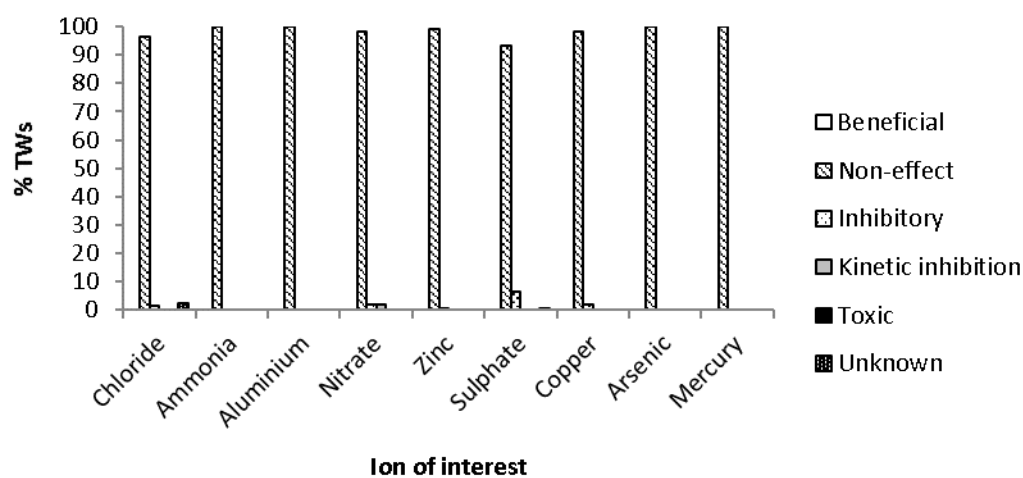


Figure 2.7 Percentage of TWs within those considered from the database (total of 160) that could result in a negative effect (inhibition or toxicity) if co-digested with sewage sludge, depending on the TWs/Sewage sludge volume ratio.

Almost all the TWs will show non-effect if added at a maximum proportion of the feedstock of 10% and 30% for zinc, chloride, ammonia, aluminium, nitrate, copper, arsenic and mercury (Figure 2.8.a and Figure 2.8.b). From a biogas yield perspective, if 10% of the AD feedstock is TW, zinc, chloride, ammonia, aluminium, nitrate, sulphate, copper, arsenic and mercury will have mostly non-effect for above 90% of the TWs characterised (Figure 2.8.a). If 30% of TWs is added to the feed, there will be below 90% of TWs that show non-effect when sulphate concentration is considered. If the kinetic inhibition is considered, nitrate will be inhibitory for 2% and 2.5% of the TWs with a proportion of 10% and 30% TWs in the feed (Figure 2.8.a and Figure 2.8.b). The chloride, nitrate, sulphate and copper showed methane yield inhibition in 1%, 2%, 6% and 2% of the TWs if added as 10%, respectively, and 1%, 2.5%, 12.5% and 5% TWs if added as 30%, respectively (Figure 2.8.a and Figure 2.8.b). For a scenario with a 10% of TWs in the feedstock, 1% of the TWs investigated constitute a risk of zinc inhibition, but a scenario with 30% of TWs showed 1% of the TWs considered could be completely toxic for the digestion process (Figure 2.8.a and Figure 2.8.b). Inhibition by aluminium and arsenic of 1% of the TWs characterised was observed at 30% addition of TWs to feed (Figure 2.8.a and Figure 2.8.b). The increase of TWs addition up to 30% of the feed showed a possible increase of inhibitory TWs from 6% and 2% to 12.5% and 5% because of their level of sulphate and copper, respectively (Figure 2.8.b).

In summary, addition of TWs up to 10% of the AD feedstock showed the least potential for inhibition of all the cases studied, including the 30/70 scenario that represents common site spare capacity. Therefore, supplementing up to 10% of the ADs feed with TWs could be used to fill part the spare capacity on municipal wastewater treatment plants while remaining safe to the operation of the reactor. Still, the TWs that are potentially inhibitory should be carefully monitored in a wastewater treatment plant treating sludge, as those TWs could be mixed with TWs containing lower concentrations of inhibitors to achieve a TW mixture within a non-effect range (Table 2.4).

a)



b)

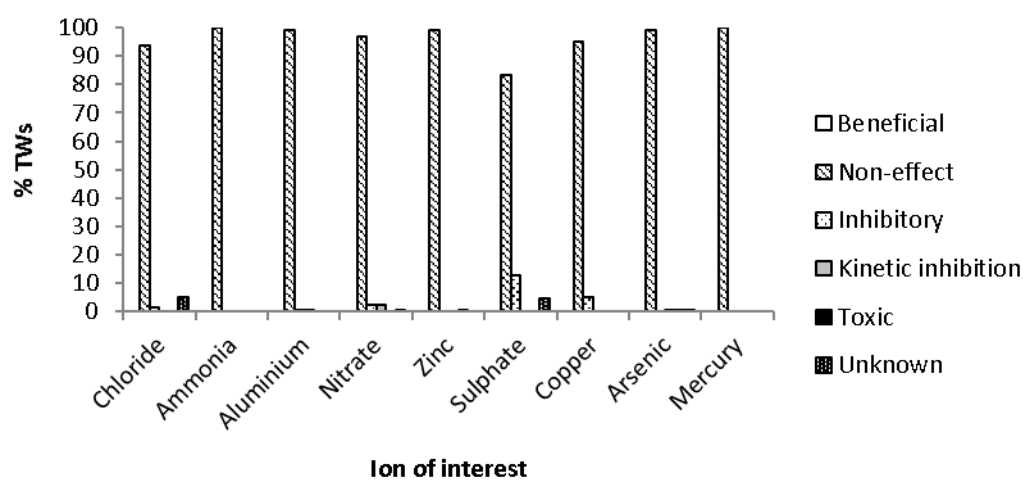


Figure 2.8. Percentage of TWs within the 160 studied that will incur in beneficial, non-effect, yield inhibition, kinetic inhibition, toxic or unknown impact if dosed to sewage sludge anaerobic digesters as 10% of the feedstock (a) or 30% of the feedstock (%).

2.4. Conclusions

The characterisation of TWs and the inhibition study carried out in BMP tests give an insight into the opportunities for Aco-D of sludge and TWs. Within the 160 TWs studied the key constituents that were identified as potential to yield inhibition or toxicity were ammonia, chloride, nitrate, sulphate, aluminium, copper, zinc, arsenic and mercury. Inhibition was observed in two ways, with some TWs constituents resulting in a reduction in biogas or methane yields (ammonia and sodium with sulphate, and zinc, copper and aluminium with chloride) while others led to a lag phase in gas formation (zinc, aluminium, copper and ammonium with sulphate, ammonium with chloride and arsenate with sodium). A synergistic negative impact on biogas and methane yields was recorded between ammonia and sodium with sulphate, while zinc, copper and aluminium had a synergistic effect with chloride. Overall, the BMP test results obtained in the current study provide a detailed understanding of the impact of the compounds and elements tested on the AD process including actual concentration ranges for beneficiary and inhibitory conditions in real conditions applied on wastewater treatment sites as well as the basis for selection of TWs for Aco-D with sewage sludge. From the characterisation of 160 TWs and results of the BMP tests it was concluded that a TW dose of up to 10% the feedstock volume in sewage sludge digesters will only be inhibitory for 13.7% of the TWs investigated, with potential for microbial acclimation that needs confirmation in continuous experiments. From that 13.7% of TWs, 15 TWs belonged to the chemical group, 9 to the glycerol/beverage/alcohol group, 3 to the landfill leachate group, 1 to the oil containing group, and 1 to the food group.

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CHAPTER 3: Long-Term Effects of Zinc and Nitrate in the Anaerobic Co-digestion of Trade Wastes and Sewage Sludge

Olivia Berzal de Frutos ^a, Marc Pidou ^a, Yadira Bajón Fernández ^a

^a Cranfield Water Science Institute (CWSI), School of Water, Energy and Environment (SWEE), Cranfield University, Bedford MK43 0AL, United Kingdom

Abstract

Anaerobic co-digestion (Aco-D) of sewage sludge with trade wastes (TWs) has been observed to improve anaerobic digestion (AD) performance. However, TWs can contain toxic constituents to the bacteria and archaea in the anaerobic digesters that require careful selection of wastes and loads to treat. Previous studies (Chapter 2) identified key components of TWs and studied their impact in batch anaerobic digesters, highlighting the need for deeper understanding of the possible long-term impacts of these compounds, with a particular interest in zinc and nitrate; which are commonly found in TWs from food, chemical and metallurgical industries.

In this study semi-continuous experiments using 5-L ADs fed with sewage sludge and an increasing concentration of zinc and nitrate were carried out, with an average organic loading rate (OLR) of 2.35 Kg VS/m³/d. There was an observable biogas and methane decrease during the periods in which the reactors were dosed with zinc concentrations of 700 and 1000 mg Zn/L with a decreasing biogas rate of -4.20 mL/% VS red/d and -5.40 mL/% VS red/d, respectively, and a percentage of methane of 0-10%. Between trials at 700 and 1000 mg/L, sewage sludge not spiked with metal was fed to the reactor, which sufficed for recovery of the system. Nitrate addition also resulted in inhibition at 2000 mg NO₃/L in the reactor with a reduction on biogas production from 80-100 mL/% VS red/d in the controls to 20-38 mL/% VS red/d in the tests reactors and a decrease of methane percentage of 37% when compared to the period without nitrate spiking. The reduction in biogas production in zinc-dosed units could be attributed to the accumulation of zinc and sulphate in the reactor, while the biogas drop in nitrate-dosed units was attributed to denitrification processes producing toxic by-products for the methanogens.

3.1. Introduction

Anaerobic co-digestion (Aco-D) of sewage sludge with other substrates has been used to improve the conversion of organic compounds to methane, fill the unused spare capacity of the anaerobic digestion (AD) plants on sewage treatment works and balance the carbon to nitrogen ratio and heavy metals content, all improving the efficiency of sewage sludge AD and its economic feasibility [1–8]. Some of the substrates previously used to increase the performance of sewage sludge digestion units include the organic fraction of municipal solid waste (OFMSW), grass, fats, oils and greases (FOG), food waste, pig manure and trade wastes (TWs) [3,8–12]. The co-digestion with sewage sludge also allows for treatment of organic wastes that could not be anaerobically digested alone because of possible toxic effects, as is the example of TWs, which are reported to have high levels of toxic constituents and insufficient buffer capacity for the digestion process [5,6].

A previous study investigated the impact of typical compounds found in TWs on sewage sludge batch anaerobic reactors and identified concentration ranges for each compound tested that resulted in beneficial, non-effect, inhibition and toxic effects (Chapter 2). Ammonia, zinc (Zn), copper, aluminium, mercury, arsenic, chloride, sulphate and nitrate (NO_3) were identified as compounds frequently present in TWs (Chapter 2) and all were tested for their impact on batch ADs. The study, however, recognised the limitations of batch trials in elucidating long-term process impacts resulting from transformation and accumulation of compounds in the reactor. Zinc and nitrate were particularly identified as meriting further investigation due to the possible inhibitory effects reported in previous studies and their highly frequent presence in TWs at critical concentrations.

Nitrate and zinc are commonly present in TWs resulting, for example, from galvanization, electroplating, manufacturing of batteries and other metallurgical industries that result in wastewaters rich in zinc [13,14], and food processing, coal-gasification, pectin factories, distilleries and fibre chemical industry lead to nitrate-rich wastewaters [15–21]. Heavy metals such as zinc have been known to accumulate in sewage sludge in values that can result in AD inhibition [22], although possible acclimation of microbial communities to long-term zinc exposure is not yet fully understood. Zinc has been reported to accumulate in reactors by adsorption to organic functional groups of the sludge, which might decrease its toxicity [23]. Zinc can be found as well in exchangeable positions in the sewage sludge and precipitates of carbonate or sulphide [24]. On the other hand, nitrogen-derived compounds such as nitrate have been seen to produce competition between denitrifiers and methanogens for carbon source [25], leading to the transformation of nitrate to nitrite in AD [26]. Therefore, to inform safe Aco-D of TWs rich in zinc and nitrate with sewage sludge, their long-term impact on the digestion process should be investigated in continuous reactors.

Zinc toxicity depends on the speciation of zinc, concentration, pH and type of compound formed with zinc [14]. In batch experiments, zinc was found to reduce methane production by 10% with concentrations as low as 1 mg Zn/L when using granular sludge [27]. Acclimation of acidogens to zinc has been reported in continuous two-stage systems, as there was a recovery of the chemical oxygen demand (COD) removal in the acidogenic reactor between 10 to 24 h from the addition of zinc between 0.5 mM (32.69 mg/L) to 3 mM (196.14 mg/L) [28]. In the same study by Leighton and Foster, COD removal was reduced when 0.5 mM (32.69 mg/L) to 3 mM (196.14 mg/L) of zinc were added, with a recovery of the COD removal once clean feed was added [28]. Zinc improved volatile fatty acids (VFAs) production, especially butyrate, at 0.5 mM (32.69 mg/L) and 1 mM (65.38 mg/L) Zn addition and negatively affected the acidogenesis at 2 mM (130.76 mg/L) and 3 mM (196.14 mg/L) [28]. The same authors investigated in another study the impact of zinc in methanogenesis in up-flow anaerobic sludge blanket (UASB) reactors, observing a 14% inhibition in COD degradation and 53% reduction in methane production at zinc concentrations of 3 mM (196.14 mg/L) [24], with further efficiency recovery when clean feed was resumed [24]. Nevertheless, this system differed from the continuously stirred tank reactors (CSTR) commonly used for sewage sludge treatment and hence further research on the long-term impact of zinc in CSTR is needed.

An example of highly concentrated waste in heavy metal is the pulp and paper-mill sludge, in which most abundant heavy metals have been found to be aluminium and zinc with a concentration of 10200 mg Al/L and 250 mg Zn/L, respectively, in a study by Hagelqvist [29]. In the same study, Hagelqvist co-digested 50% volatile solids (VS) of paper-mill sludge, a substrate with low biodegradability, and 50% VS sewage sludge to enhance the methane yield of the paper-mill sludge from 53 mL/g VS up to 80 mL/g VS and improve VS degradation from 5% to above 10% [29]. As an example of heavy metals effect on AD, zinc could affect the AD process over a wide range of concentrations from 4.5 to 1500 mg/L, inhibiting 50% of the methane and hydrogen production [30,31]. There have been other successful Aco-D with wastes containing a high concentration of zinc. Pahl *et al.* [22], achieved an improved biogas production when sewage sludge and 12.5% to 20% of municipal solid waste were co-digested. However, the methane production decreased and VFAs accumulated because of the increased concentration of heavy metals, such as zinc with a concentration of 558 mg Zn/kg when 25% of municipal solid waste and 75% of sewage sludge were co-digested [22].

Nitrate in AD is generally converted to nitrite and further to nitrogen [26,32]. Nitrate has been seen to improve methanogenesis using a synthetic solution of sucrose and proteins as feed, but the competition between denitrifiers and methanogens could result in nitrate addition negatively affecting AD [32]. Tugtas and Pavlostathis studied nitrate inhibition in both batch and continuous systems feeding a mixture of dextrin and peptone, suggesting that nitrate was inhibitory as a reduction of 52% and 90% in the methane production rate were recorded at concentrations of nitrate of 150 mg-N/L in a batch test and 300 mg-N/L in continuous experiments [21]. Similarly, Yi *et al.* found a 86% and 65.2% reduction of methanogenic activity in batch and continuous units treating synthetic wastewater, respectively, due to addition of nitrate in a COD/NO₃ ratio of 8 [26]. There have also been studies focussed on anaerobically digesting TWs containing nitrate. Austermann-Haun *et al.* studied the performance of several TWs, for instance, wastewater derived from the pectin industry. That TW was high in organics, between 22000 and 15000 mg COD/L, and in nitrates, between 2990 and 1280 mg NO₃/L. The study described a reduction in COD removal because of the nitrate in the influent, which was solved by pre-treating the influent in two denitrifying reactors and adding a separator to improve the COD reduction [19]. Nevertheless, nitrate inhibition in AD dealing with sewage sludge in a continuous system has not been studied and therefore further research is needed to fully inform sewage sludge-TWs Aco-D opportunities.

The long-term impacts on process performance and stability in sewage sludge AD when dealing with compounds commonly found in TWs has to be studied to fully understand the mechanisms involved and inform safe Aco-D practices. The aim of this study was then to further develop the understanding of the impact of zinc and nitrate, as frequently present constituents of TWs, in semi-continuous reactors. Ultimately, this work contributes to address the gap in knowledge identified in relation to impact of TW in ADs performance and stability, by studying the long-term effects in semi-continuous reactors fed with incremental doses of zinc and nitrate.

3.2. Material and Methods

3.2.1. Feedstock and inoculum

The sludge used as the substrate for the AD trials consisted of a mixture of primary sludge (PS) screened through a 5 mm screen and secondary activated sludge (SAS) from a treatment plant in the West Midlands, England. The inoculum was the digestate from the same site. The substrate and inoculum were stored at 4 °C on the day of sampling. The samples were allowed to reach room temperature for 4 to 8 hours before the start of the experiments. The percentage of SAS and PS (in a range from 20% to 80%) was adjusted to keep an OLR of 2.35 Kg VS/m³/d and to maintain the hydraulic retention time (HRT) in the reactors as close as possible to 14 days.

3.2.2. Semi-continuous anaerobic digesters

The experimental rig consisted of nine 5-L glass Pyrex reactors maintained at mesophilic conditions by means of a water bath at 38 °C (Figure 3.1). The nine reactors consisted of three control ADs, and two sets of test reactors with nitrate and zinc addition operated in triplicates. The lid of the reactors had several connections including one outlet/inlet for the sludge and one gas vent. Reactors were fed three times per week on alternate days and a similar amount of digestate was extracted before each feeding, using a peristaltic pump 521VI/R2C (Watson Marlow), at a rate of 220 rpm for digestate extraction and 143 rpm for feeding. On Fridays, the feeding amount was 50% more than the other two weekly feedings to account for the longer period without feeding over the weekend. The biogas exit ports were fitted with non-return valves and were connected to automatic gas counters from CJC labs. A sampling port was placed after each gas counter and biogas samples for analysis of methane content were taken before each feeding. Biogas volume for each reactor was continuously recorded in a Data Acquisition System (DAS) unit. The mixing of the reactors was manual before and after the feeding events.

The test reactors were spiked with the compound of interest by addition of zinc sulphate or sodium nitrate salts with the feed. The concentration in the feed was calculated to match the zinc and nitrate concentrations inside the reactors at the time of the feeding ($t=0$) (Table 3.1) to those studied in Chapter 2. Higher concentrations than the ones studied in Chapter 2 for nitrate were also studied to find inhibition on the semi-continuous reactor. Test reactors were fed with the same amount of sludge as the control units, with previous addition of the relevant salt to the sludge mixture. The pH was measured each time that the salts were added to monitor acidification, and it was adjusted with sodium hydroxide up to the pH measured in the controls on that day.

Table 3.1. The concentration of the ions of interest (Zinc and nitrate) in the test reactors, expressed as concentration in the feed and concentration calculated in the reactor contents after the first feeding ($t=0$).

<i>Zinc inhibition trials</i>						
Days	0-25	28-53	56-95	98-126	128-163	165-186
Zn in the feed (mg/L)	0	100	380	4700	0	7250
Zn in the reactor $t=0$ (mg/L)	0	15	50	700	0	1000
<i>Nitrate inhibition trials</i>						
Days	0-25	28-53	56-95	98-144	147-186	189-214
NO ₃ in the feed (mg/L)	0	100	680	2100	5450	14700
NO ₃ in the reactor $t=0$ (mg/L)	0	15	100	300	700	2000



Figure 3.1. Semi-continuous anaerobic digesters picture.

3.2.3. Analytical methods

Feed and outlet samples were analysed during the experiments. Total solids (TS) and VS were measured using standard methods [33,34]. The measurement of pH was carried out with a Titralab AT 1000 series from Hach. Soluble COD was analysed using cell test kits from Millipore (VWR, UK) and a spectrometer Spectroquant NOVA 60 from the same company. Total ammonia nitrogen (TAN) was measured using an ion-selective electrode with the addition of ammonium ionic strength adjustor pillows from Hach. Free ammonia nitrogen (FAN) was calculated from the TAN concentration and pH value using equation 3.1 [35].

$$[FAN] = \frac{[TAN]}{1 + \frac{10^{pH}}{K_a}} \quad (3.1)$$

Where [FAN] is FAN concentration (mg/L), [TAN] is TAN concentration (mg/L), and K_a is the dissociation constant.

Samples for analyses of the soluble fraction of COD, VFAs measured in high-performance liquid chromatography (HPLC), ions measured in ion chromatography (IC) and metals measured in inductively coupled plasma (ICP) were obtained after centrifuging at 5000 rpm for 8 hours and filtering the supernatant through 0.45 μm Millipore syringe filters. A Shimadzu prominence HPLC was used to analyse VFAs from 1 mL of the acidified sample that was stored in a freezer at -20°C . The mobile phase for the HPLC consisted of a 0.5 mM sulphuric acid solution with an injection flow rate of 1 mL/h, and a Rerex 150 column [36]. The acidification consisted of the addition of 10 μL of 96% sulphuric acid to 4 mL of sample. The different VFAs were converted to Acetate equivalent ($\text{Acetate}_{\text{eq}}$) and added to obtain total volatile fatty acids (TVFA). A Dionex ICS-1600 IC was used for the determination of nitrate, nitrite, sulphate, phosphate, fluoride, chloride, and bromide with a mobile phase of 9 mM sodium carbonate and an AS 9HC column. Finally, a NexION 350D ICP mass spectrometer was used to measure cations (calcium, magnesium, potassium, sodium, manganese, zinc, cobalt, molybdenum, nickel, copper, iron, selenium, chromium, and tungsten, lithium, aluminium, silica, titanium, arsenic, cadmium, lead, mercury, and antimony). ICP samples were acidified with 100 μL of >68% nitric acid in 10 mL of diluted soluble sample. A volume of 15 mL of biogas was collected using a syringe from a sampling port after the gas counter to measure methane. The analysis was carried out by a Servomex 1440 gas analyser.

3.2.4. Data analysis

Biogas production (P) was represented as biogas produced (mL) between each of the feeding days (B) over time (t) and normalised by the VS reduced (VS_{red}) (Equation 3.2).

$$P = \frac{B}{\text{VS}_{\text{red}} * t} \quad (3.2)$$

The percentage of VS_{red} was calculated using Van Kleeck equation (Equation 3.3 and Equation 3.4), where VSF_o is the solid fraction in the outlet, and VSF_f is the solid fraction in the feed.

$$\text{VSF} = \frac{\text{VS}}{\text{TS}} \quad (3.3)$$

$$\text{VS}_{\text{red}} = \frac{\text{VSF}_f - \text{VSF}_o}{\text{VSF}_f * (\text{VSF}_f - \text{VSF}_o)} * 100 \quad (3.4)$$

Biogas production and % methane data were further processed using a 7 days moving average (MA7) to avoid noise from the feeding pattern (Equation 3.5).

$$\text{MA}_7 = \frac{\sum \text{data during one week}}{\text{n of data on that week}} \quad (3.5)$$

Rates and ranges reported in the result section were calculated applying the SLOPE, MAX and MIN function in Microsoft Excel to the period of interest.

3.2.5. Statistics

The criteria to observe statistically significant differences between the biogas production of control and test reactors was the normalised difference in root mean square error (RMSE_n). When RMSE_n was equal or similar to the first period of the experiment with non-addition of salt, the difference was not significant. When the RMSE_n was higher than the one in the period of non-addition at the beginning of the experiment, then the difference was significant. RMSE_n was calculated using equation equations 3.6 [37,38].

$$RMSE_n = \left(\frac{1}{n} \sum_{i=1}^n \left(\frac{X_i - Y_i}{Y_i} \right)^2 \right)^{1/2} \quad (3.6)$$

Where X_i is the control value of biogas (mL biogas/% VS red/d), and Y_i is the value of biogas in zinc or nitrate tests (mL biogas/% VS red/d).

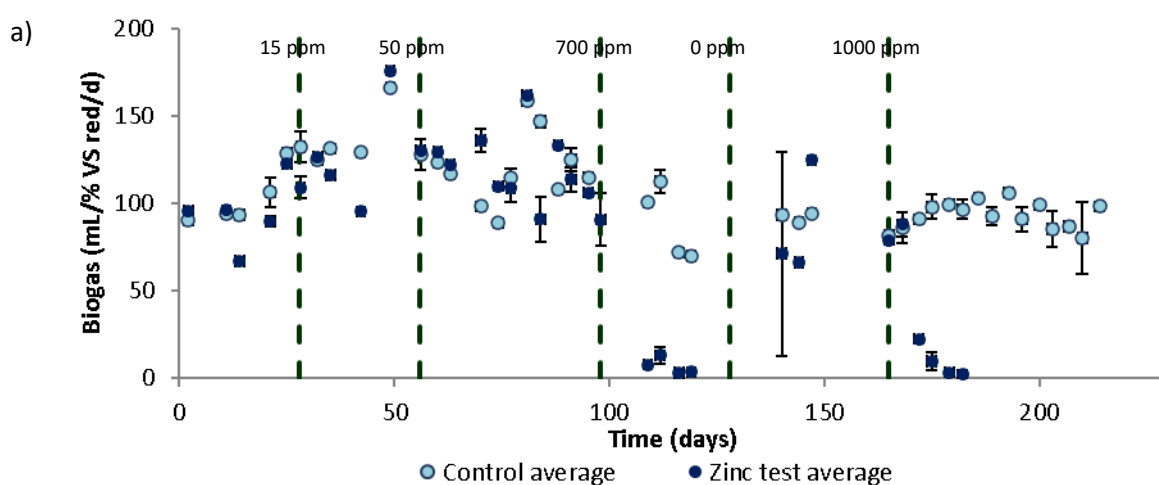
3.3. Results and discussion

3.3.1. Impact of zinc concentration in semi-continuous ADs

Before the addition of any salt, the reactors increased the biogas production from about 95 mL/% VS red/d up to 128 mL/% VS red/d on day 25. Addition of zinc to reach a 15 mg Zn/L concentration inside the test reactors resulted in higher variability in biogas production, 95-175 mL/% VS red/d, than in the control units without salt addition, 129-166 mL/% VS red/d (Figure 3.2.a). Nonetheless, the RMSE_n between test and control reactors for that period was similar to that of the initial period of non-addition, indicating that a concentration of Zn of 15 ppm did not affect biogas production significantly (Table B1 in appendix). Similarly, when Zn was increased to reach 50 mg Zn/L in the test digesters, both control and test reactors had similar biogas productions, with a decreasing trend from 130 mL/% VS red/d to 90 mL/% VS red/d and a biogas reduction rate of -0.42 mL/% VS red/d/d between days 56 and 98, likely resulting from variability in the feedstock (Figure 3.2.a) (Table 3.2). Further increases in zinc levels had a significant detrimental impact and a biogas decrease rate of -4.20 mL/% VS red/d/d, with no quantifiable biogas production (0 mL/% VS red/d) when a concentration of 700 mg Zn/L was achieved in the zinc reactors at day 98 (Figure 3.2.a) (Table 3.2). Biogas production continued to be severely inhibited during the period when a level of 700 mg Zn/L was maintained in the reactors, with only 7 mL biogas/% VS red/d recorded on day 109 and suggesting no adaptation of the microbial communities during a period of over 2 HRTs when this dose was maintained (Figure 3.2.a). Stopping exogenous zinc addition on day 128 led to reactors recovery, with similar productions recorded in both test and control units 37 days after zinc spiking stopped (Figure 3.2.a). Therefore, complete toxicity to microorganisms did not occur at a concentration of 700 mg Zn/L in the reactor, as recovery of microbial activity was possible. Further addition of zinc to reach 1000 mg Zn/L on day 165 resulted in a reduction rate of biogas formation of

-5.40 mL/% VS red/d/d, which decreased biogas production to 22 mL/%VS red/d on day 172 and resulted in full toxicity with null production from day 179 (Figure 3.2.a) (Table 3.2).

Each reactor reached different methane contents in the biogas, with marked differences also observed among replicates. The reactors were observed to be stable during the non-addition period (up to day 25), with the normal variability in daily biogas production rates expected from a biological process. The initial start-up period to ensure stable performance, allowed a direct comparison between the controls and test reactors once zinc and nitrate dosing started; providing confidence that any observed change in performance resulted from the salt addition. The control reactors 2 and 3 and zinc test reactors 1 and 3 showed a percentage of methane between 40% and 60%, while the control reactor 1 and zinc reactor 2 showed lower values between 20% and 30% (Figure 3.2.b and Figure 3.2.c). Those percentages were maintained between the first testing stages when there was no salt addition as well as for 15 mg and 50 mg Zn/L in the reactor (Figure 3.2.b and Figure 3.2.c). Methane concentration was reduced to zero in the test reactors 1 and 3 and to 10% for test reactor 2 when the concentration of 700 mg Zn/L was aimed for in the reactors from day 100 to 126 (Figure 3.2.c). A lower inhibition was observed in a batch experiment from Lin *et al.*, where a 50% inhibition of methane was caused by 690 mg Zn/L addition using an inoculum from a UASB reactor with 1 d HRT. In the same experiment, a lower concentration, 270 mg Zn/L, produced 50% of methane inhibition using an inoculum from the same reactor with 2 d HRT [27]. In a continuous study by Leighton and Foster, a reduction of 53% of methane production was observed at 3 mM of Zn (196.14 mg Zn/L) addition using a UASB. COD removal efficiency in that study was recovered once heavy metal addition was stopped into their reactors. In that study, they found that zinc could attach to exchangeable sites and organic compounds in the sewage sludge, suggesting an accumulation of zinc inside the reactor [24]. Our previous study with the same source of sewage sludge obtained an improvement of double production of biogas and methane at 760 mg Zn/L when compared to the control and a toxic effect at 1676 mg Zn/L in a BMP test (Chapter 2). The different effect with the previous experiments with the same condition and sewage sludge (Chapter 2) could result from a synergistic/antagonistic effect with other compounds such as sulphate, the counter ion of the salt used [13].



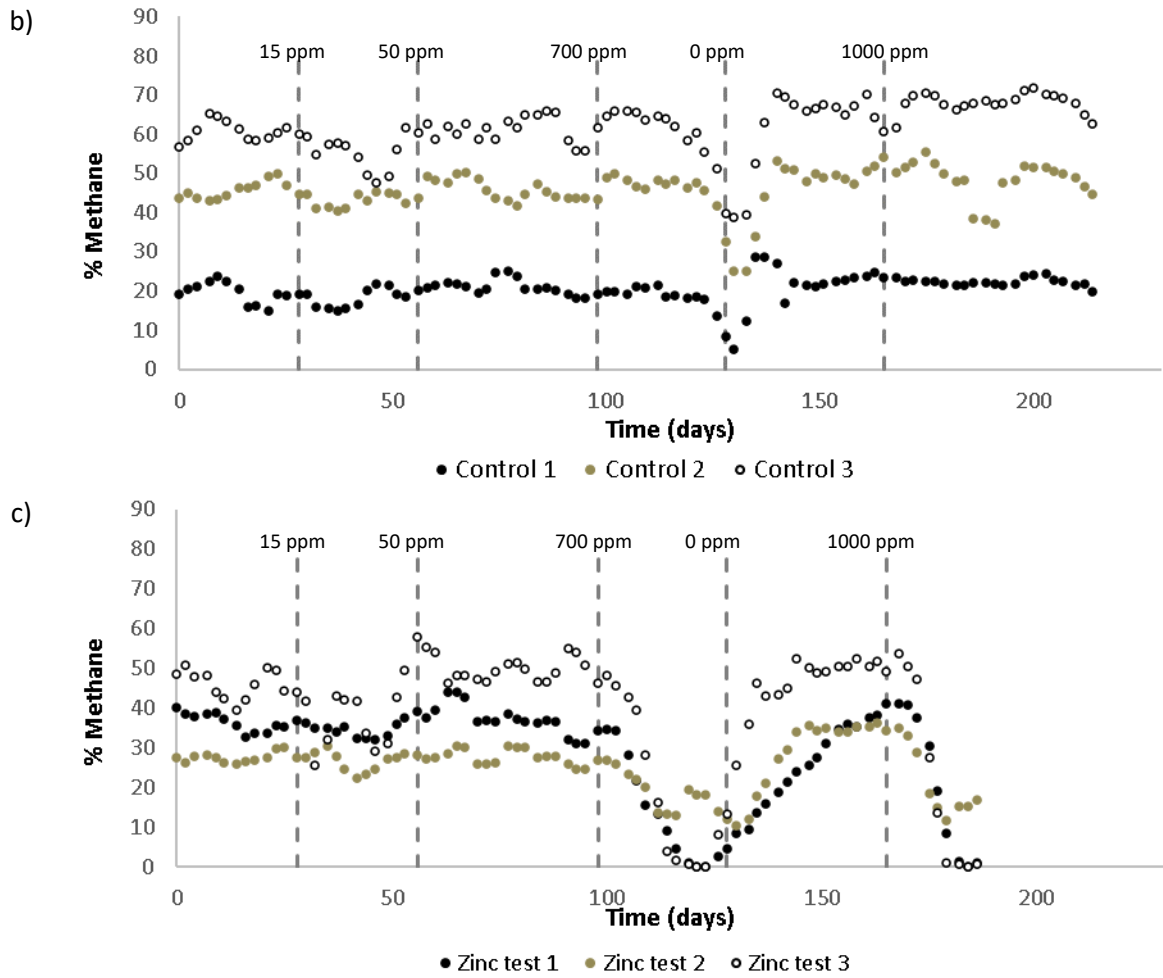


Figure 3.2. Performance data for semi-continuous control and test reactors dosed with zinc, where a) daily biogas production represented as 7 days moving average and as an average of the replicate units and b & c) methane concentration for control and test reactors, respectively, represented as 7 days moving average. Vertical lines represent the points where the dose of zinc in the test digesters was changed.

Note – the drop in CH_4 content observed for control digesters between days 126 and 142 was related to leaks in the experimental rig.

Between the initial stage with no addition of salt and the addition of zinc to reach 50 mg Zn/L, the TVFA levels remained below 2000 mg Acetate_{eq}/L and most of them below 1000 mg Acetate_{eq}/L (Figure 3.3.b). There was an increasing trend in TVFA concentration at the same time as the methanogens were inhibited in the test units when the concentration of 700 mg Zn/L was targeted in the reactors, with an accumulation rate of 255 mg Acetate_{eq}/L/d, reaching values between 3891 and 2394 mg Acetate_{eq}/L on day 119 (Figure 3.3.b and Figure 3.2). Stopping the zinc addition reduced TVFA accumulation with an utilisation rate of -150 mg Acetate_{eq}/L/d, reducing concentration inside of the test reactors to 161 mg Acetate_{eq}/L as measured in the digestate, which was close to the 75 mg Acetate_{eq}/L recorded for control units. Resuming zinc addition to reach 1000 mg Zn/L increased TVFA accumulation again up to values between 3202 and 2549 mg Acetate_{eq}/L on day 182 with an accumulation rate of 170 mg Acetate_{eq}/L/d on the same period as the second drop in biogas and methane production in the zinc test reactors was recorded (Figure 3.3.b, Table 3.2 and Figure 3.2). The simultaneous accumulation of TVFA and decrease of biogas and methane production

could indicate the inhibition of zinc was affecting more methanogens than acetogens. When looking at individual VFAs, acetate levels increased to a range between 600 mg Acetate/L and 800 mg Acetate/L when 700 mg Zn/L was aimed in the test reactors, and a further increase to a value of 1029 mg Acetate/L on day 182 which corresponds with the phase when 1000 mg Zn/L was targeted (Figure 3.4.c). Zinc addition did not affect the concentration of propionate which mostly remained below 400 mg Propionate/L with few points at 600 mg Propionate/L along different periods. The most extreme point in propionate was a value of 2731 mg Propionate/L on day 154 in test reactor 1, but propionate was below detection for the rest of that period (Figure 3.4.d). A previous study by Lin *et al.*, reported similar inhibition in VFA degradation when zinc concentrations between 56 and 200 mg Zn/L were tested in a batch study using granular sludge [27]. On the contrary, Yu and Fang observed an inhibition of TVFA and acetate production at a zinc concentration of 300 mg/L [39]. Yenigün *et al.* observed a decrease of acetate, propionate and butyrate when using glucose as a carbon source when zinc concentration was increased, but, the effect was reduced if copper concentration was between 1 and 5 mg Cu/L [40]. Both behaviours were found in a continuous study using a UASB as one of the reactors in two-phase digester system by Leighton and Foster where zinc addition accumulated TVFA and further addition of zinc showed an inhibition of acidogenesis as TVFA levels were reduced [24].

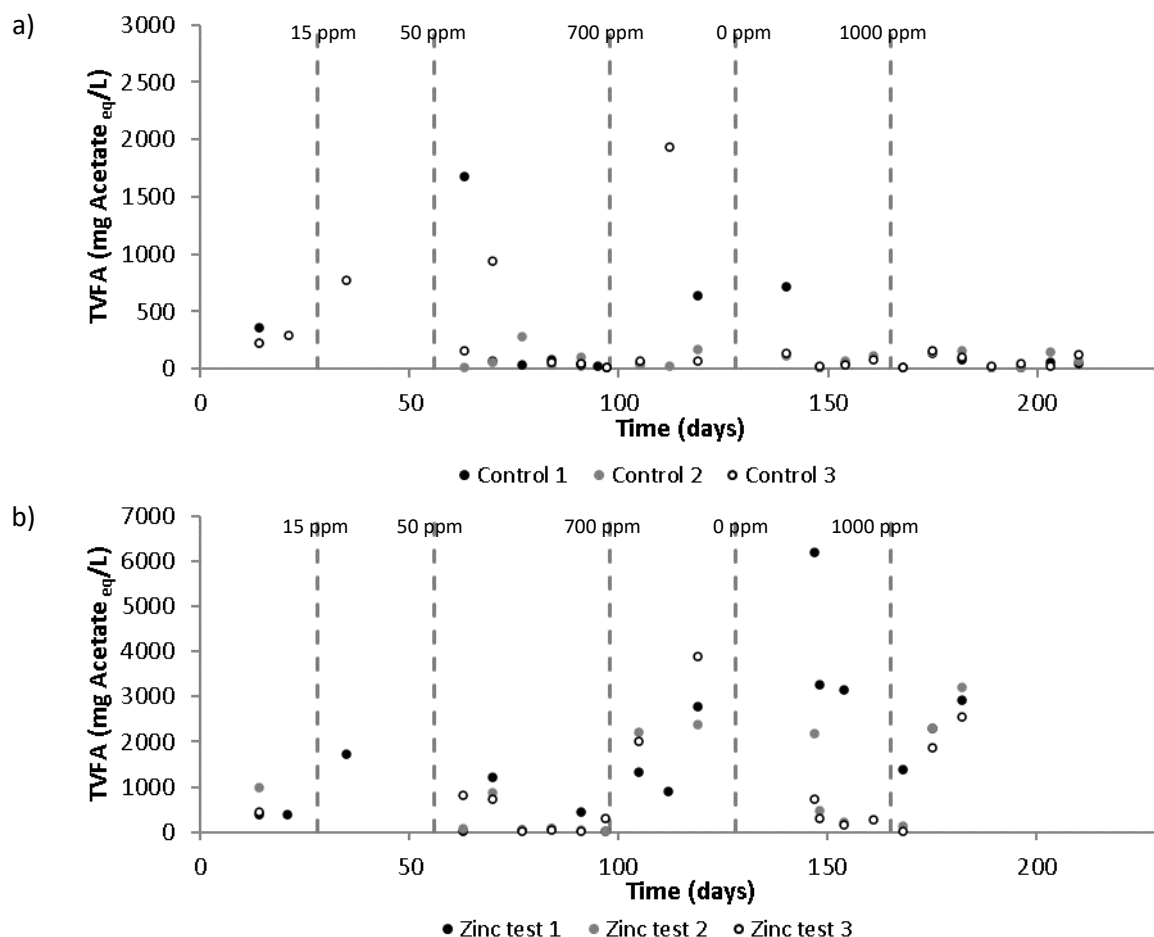


Figure 3.3. TVFA a) Control and b) Zinc test data with the different additions of zinc to the test reactors.

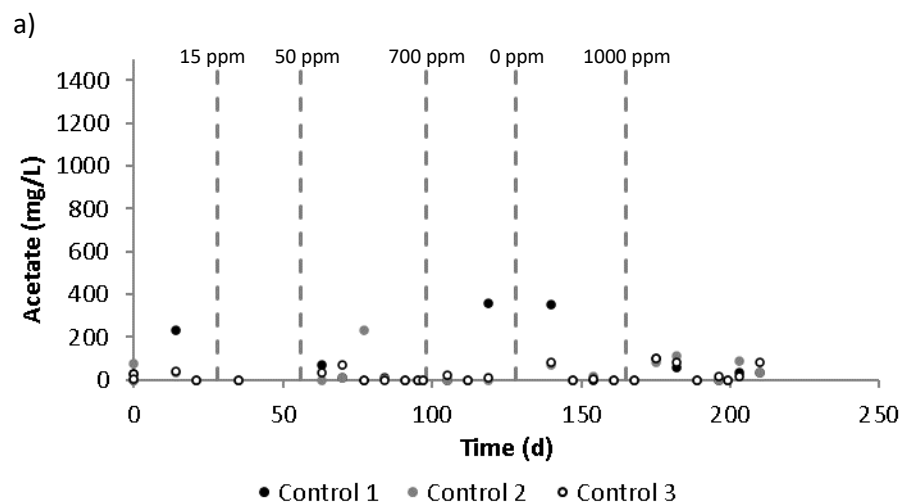
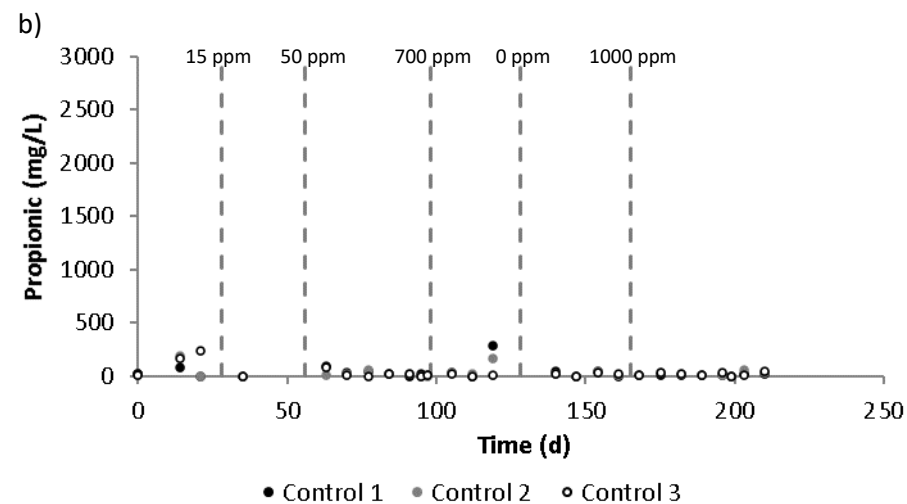
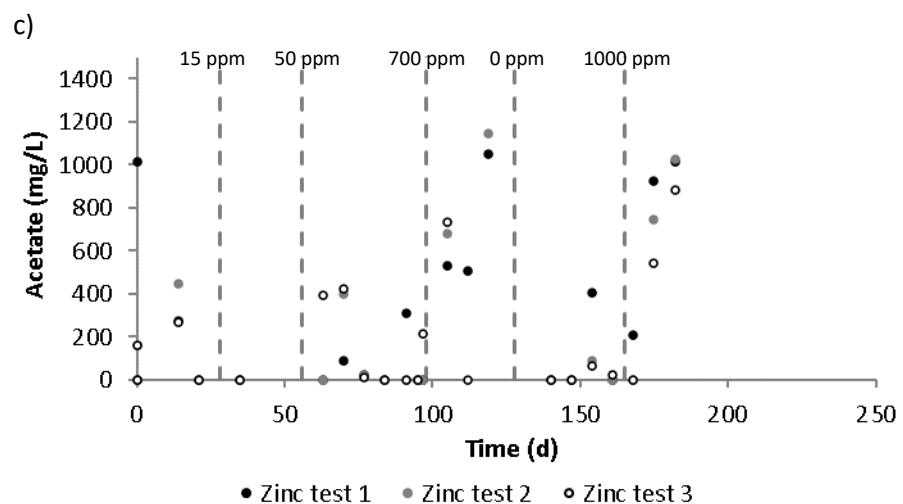
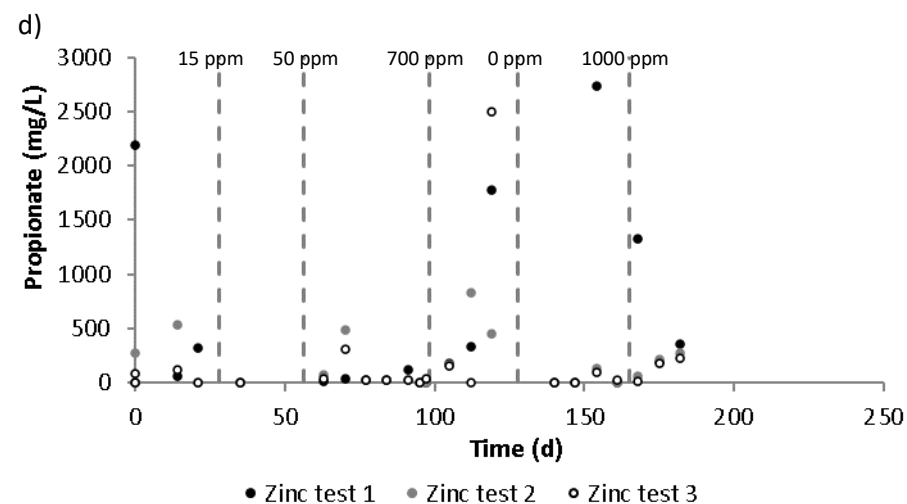


Figure 3.4. Control and Zinc test data for a & c) acetate (mg/L) and b & d) propionate concentration (mg/L), with different additions of zinc to the test reactors.

The pH of the control reactors in the first period (0 to 26 days) was between 7.4 and 8.6 and then stabilized at between 6.5 and 7 from day 35 (Figure 3.5.a). This pH is within the optimal for AD that has been reported to be 6.8-7.2 [41]. Zinc test reactors showed a stable pH between 6.5 and 7 until day 105. The pH then varied between 5 and 7.5 during the period from 105 d to 154 d and an even wider range was observed between days 177 and 186 with pH values from 4 to 7 (Figure 3.5.b). This decrease in the pH was attributed to zinc inhibition and resulted in an imbalance of the reactors [41,42]. The pH drop, VFA accumulation and lack of methane formation (Figure 3.5.b, Figure 3.3.b, and Figure 3.2.c) evidenced a suppression of methanogenic activity, linked to permeation of undissociated acids through the microorganisms' membrane [41].

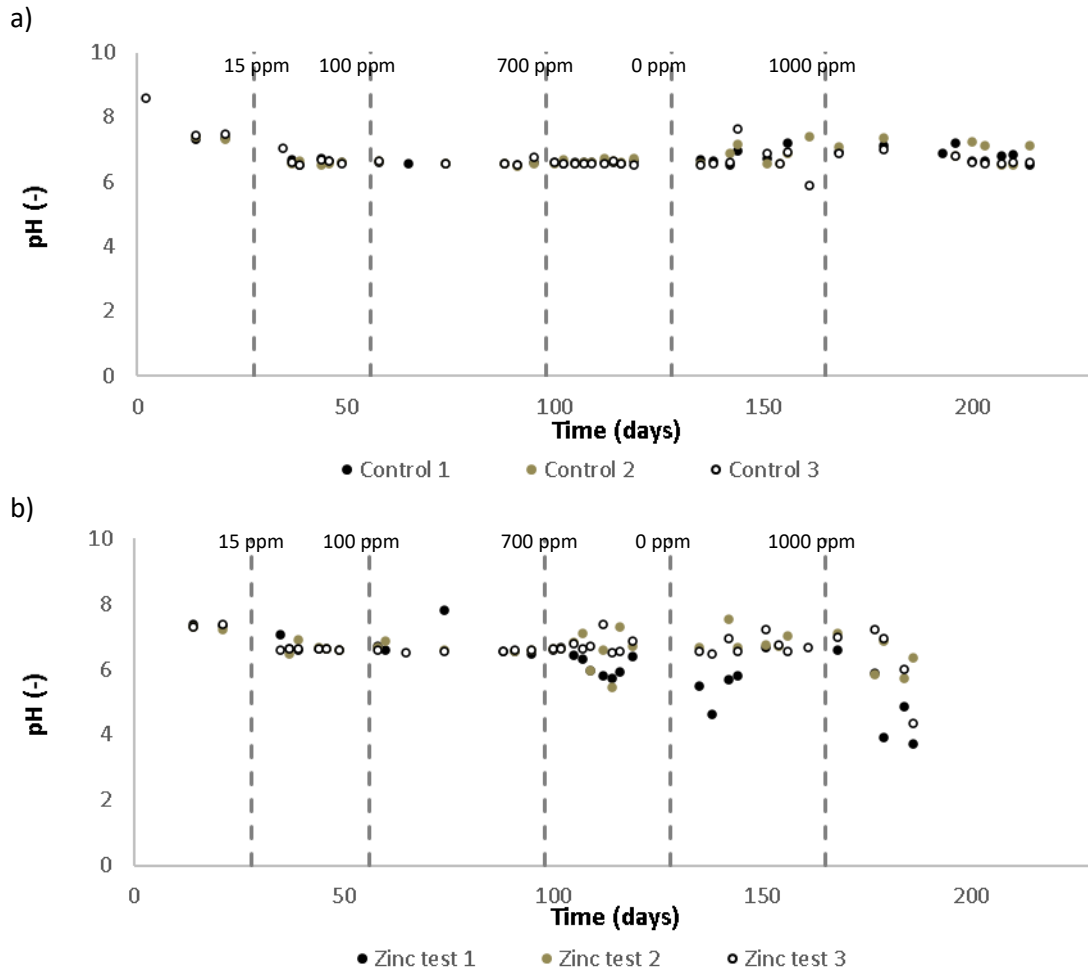


Figure 3.5. Control (a) and Zinc test (b) pH data, with the different additions of zinc to the test reactor.

The concentration of zinc and sulphate in the outlet digestate was a maximum of 0.7 mg Zn/L and 30 mg SO₄/L for the control reactors and of 0.8 mg Zn/L and 45 mg SO₄/L for the zinc test reactors during the early stages of operation (Table 3.1). Similar concentrations were also maintained for the lower concentrations of zinc tested, with values between 1-0.4 mg Zn/L and 1-0.8 mg Zn/L in the digestates of the control and test reactors, respectively, when the test units contained 50 mg Zn/L.

Both zinc and sulphate concentrations in the test units outlet increased after a level of 700 mg Zn/L was aimed in the reactors, with an increase of 36 mg Zn/L/d and 620 mg SO₄/L/d, reaching concentrations of 1030 mg Zn/L and 30201 mg SO₄/L in the period between days 98 and 126 (Table

3.2 and Table B2 in appendix). The higher release of soluble zinc with the digestate than the target concentration of zinc (700 mg/L) on day 98 was caused by an accumulation of zinc in the reactor as a consumption of zinc is not expected, but it could be in the solid fraction of sewage sludge in the form of a precipitate or adsorbed into the organic matter. After a cumulative addition of 4700 mg Zn/L (700 ppm calculated inside the reactor), the dosing of Zinc sulphate was stopped and the biogas and methane productions were recovered (Figure 3.2). During that period, the zinc and sulphate levels in the test digesters outlet decreased to a range of 26-0.3 mg Zn/L and 7422-11 mg SO₄/L, respectively. At a higher concentration of zinc in the semi-continuous system of 1000 mg Zn/L, the concentration of zinc and sulphate in the test reactors affected the AD performance but the outlet did not show as high accumulation of zinc and sulphate as in the previous doses of zinc, 700 mg Zn/L, with maximum values of 1 mg Zn/L and 23 mg SO₄/L, respectively (Table B2 in appendix). Therefore, the decrease of performance of the zinc reactors might be because of a sudden release zinc and sulphate in the reactor from the accumulation as a precipitate or other solid form.

The values previously described as targeted concentrations inside the reactor (Table 3.1) do not consider the accumulation of zinc in the reactor solids resulting from continuous addition of zinc. Figure 3.6 accounts for the accumulation of zinc in the reactor and the addition of new zinc mass with the feed. Assuming that the mixing was enough to have a CSTR reactor, the concentration in the reactor should be equal to the concentration in the outlet. Therefore, the observable difference between the calculated accumulated zinc concentration in the reactor and measured soluble concentration of zinc in the outlet is the amount of zinc that was attached to the solid fraction of sewage sludge or precipitated in the reactor as non-soluble zinc. The difference between calculated and measured can be appreciated in various periods. First, there is an increase in the calculated zinc concentration to around 350 mg Zn/L between days 65 to 93, but the measured dissolved concentration in the digestate remained around 1 mg Zn/L. Second, the increase of zinc concentration between 93 and 119 days also shows a difference between measured and calculated levels. For example on day 112, the measured concentration was 2750 mg Zn/L, while the calculated concentration was 3466 mg Zn/L (Figure 3.6), indicating that a significant part of the zinc partitioned to the solid phase of the digestate and was hence not measured when analysing the solids free fraction. In that period, the biogas and methane productions were reduced to almost 0 mL/% VS red/d (Figure 3.2). Therefore, the reductions in biogas and methane could be caused by the zinc accumulation within the solid phase of the sludge. Comparing this result to the previous research on batch systems using sewage sludge and inoculum from the same treatment facility and at the same temperature, the concentration identified as inhibitory in this study is almost double than the one found to be toxic on the batch tests, which was 1676 mg Zn/L concentration inside the reactor (Chapter 2). The aimed concentration of that period for the test semi-continuous reactors was 700 mg Zn/L, which is a similar zinc concentration to the one added in the batch experiment that inhibited 41% of the biogas production, 746 mg Zn/L (Chapter 2). In the same period of the semi-continuous experiment, sulphate was present in concentrations 30 times higher than the concentration studied in Chapter 2, 1466 mg SO₄/L in the reactor that showed 8% and 16% decrease of biogas and methane respectively (Table B2 in appendix). Therefore, the high sulphate concentrations may have also contributed to the reduced performance in the semi-continuous ADs. The test reactors received clean feed without zinc sulphate from day 119, resulting in a drop in zinc (Figure 3.6). A further increase of zinc addition increased the calculated zinc concentration in the

outlet to 5105 mg Zn/L. The experimental data at that period was measured only at the beginning of that period with an 1100 mg Zn/L difference with a concentration from 1.35 to 0.65 mg Zn/L on day 168 (Figure 3.6). As previously commented, the differences between the calculated (total) and measured (soluble) concentrations of zinc in the digestate could be caused by an increase of zinc adsorbed onto the sewage sludge [22,24,27]. Previous research by Braga *et al.* obtained that up to 73% of the non-soluble zinc in sewage sludge samples could be found in the organic matter or as a sulphide precipitate, with a further 1 to 25% of the total non-soluble zinc present as carbonate or exchangeable zinc [43].

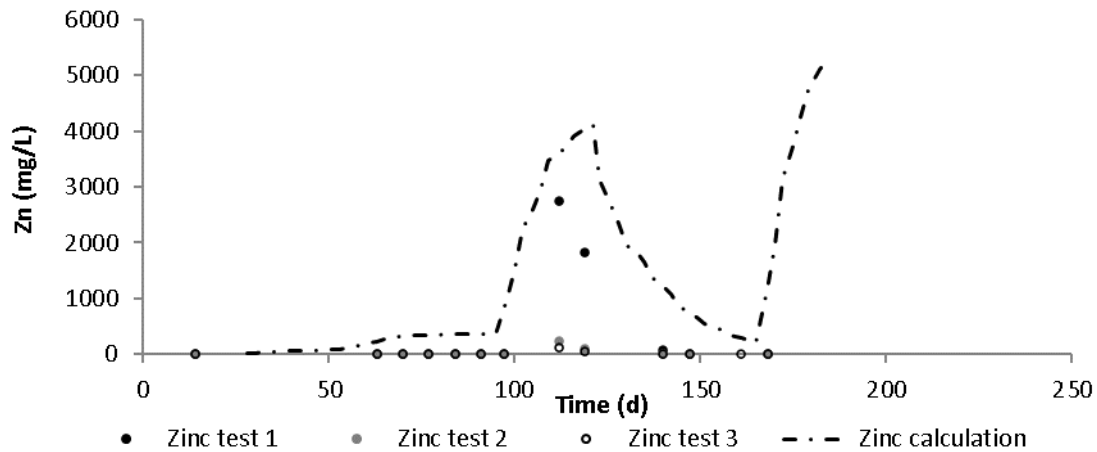


Figure 3.6. Concentration of Zinc in the digestate of semicontinuous anaerobic digesters spiked with zinc sulphate. Values are reported as concentrations measured in the digestate's solids free fraction (soluble zinc) for individual replicates (Zinc test 1, 2 and 3) and concentrations calculated as 'accumulated' in the reactors when considering the continuous spiking with zinc sulphate ($(\text{mass Zn in reactor} + \text{mass Zn added} - \text{mass Zn removed}) / \text{reactor volume} = \text{Zinc calculated}$).

Table 3.2. Rate of change (slope) of biogas and digestate concentrations for control (C) and zinc test (Zn) digesters for each of the experimental periods.

<i>Period</i>	No addition		15 mg Zn/L		50 mg Zn/L		700 mg Zn/L		Recovery		1000 mg Zn/L	
<i>Days</i>	0-25		28-53		56-95		98-126		128-163		165-186	
<i>Slopes</i>	C	Zn	C	Zn	C	Zn	C	Zn	C	Zn	C	Zn
<i>Biogas (mL/% VS removed/d/d)</i>	1.52	0.81	1.52	2.20	0.14	-0.42	-3.96	-4.20	0.07	7.23	0.96	-5.4
<i>% Methane/d</i>	-0.009	-0.07	0.2	0.3	-0.09	-0.1	-0.4	-1	0.5	0.7	-0.4	-2
<i>TVFA (mg Acetate_{eq} /L/d)</i>	2	-31	-	-	-17	-16	24	255	-10	-150	7	170
<i>% Acetate/d</i>	-5	-8	-	-	0	0	1	1	-2	-1	5	2
<i>% Propionate/d</i>	-3	-2	-	-	1	0	0	2	0	-2	-3	-3
<i>% Removal VS/d</i>	0.2	0.6	0.1	-0.2	-0.1	-0.1	0.01	0.1	-1	-0.8	0.5	0.8
<i>TAN (mg-N/L/d)</i>	10	56	71	11	-0.4	-142	-68	-92	9	21	-52	-115
<i>FAN (mg-N/L/d)</i>	-6	5	-3	0.2	16	-15	-6	-6	5	3	-6	-8
<i>CODs (mg/L/d)</i>	8	16	-	-	-52	-80	-8	124	-22	-	34	-3
<i>Na (mg/L/d)</i>	-	-	-	-	0.9	1.3	-0.5	27	-0.3	-7.6	-	-
<i>Zn (mg/L/d)</i>	-	-	-	-	0	0	0	36.1	0	-0.7	-	-
<i>NO₃ (mg/L/d)</i>	3	3	-	-	1	0	-1	-1	0	0	1	1
<i>SO₄ (mg/L/d)</i>	-3	-6	-	-	3	5	4	424	1	-194	2	-2
<i>pH (-)</i>	-0.01	-0.01	-0.03	0.004	-0.003	-0.001	0.001	-0.01	0.01	0.01	0.04	-0.04

3.3.2. Impact of nitrate concentration in semi-continuous ADs

Nitrate test reactors showed similar performance to the control units except for the targeted nitrate concentration in the reactor of 100 and 2000 mg NO₃/L (Figure 3.7.a) (Table B1 in appendix). There was a decreasing trend in daily biogas formation in nitrate dosed digesters when spiked to 15 mg NO₃/L, with a later recovery to yields similar to the control units after 1.2 HRT of being exposed to that nitrate dose (Figure 3.7.a), however, this difference was not statistically significant as RMSE_n of the test reactors was close to those without addition (Table B1 in appendix). Contrarily, addition of 100 mg NO₃/L resulted in significant differences between test and control units, with a RMSE_n value of 0.43. The differences resulted from a great variability in biogas production in nitrate-spiked reactors, whose performance ranged from similar values to the control ADs to higher biogas formation of up to 151 and 243 mL/% VS red/d at days 70 and 88, suggesting an improved biogas production due to nitrate addition. Both the control reactors and the nitrate test reactors with 300 mg NO₃/L showed a decreasing trend in biogas formation, with slopes from -0.14 mL/%VS red/d/d and -0.27 mL/% VS red/d/d between 98 d and 147 d, which might be due to feedstock variation over that period (Table 3.3). Addition of nitrate to a level of 700 mg NO₃/L started to impact the units' performance, although differences remained non-statistically significant as RMSE_n was 0.27 which is close to 0.22 from non-addition period (Figure 3.7.a) (Table B1 Table B in appendix). A further increase in nitrate concentration to 2000 mg NO₃/L in the test reactors resulted in a rapid reduction of daily biogas production, with a drop to 20-38 mL/% VS red/d as opposed to 80-100 mL/% VS red/d achieved in the control reactors (Figure 3.7.a).

The biogas methane content was constant between 16% to 25%, 43% to 55% and 47% to 72% for the control reactors 1, 2 and 3, and 35% to 46%, 11% to 24% and 45% to 67% for the nitrate test reactors 1, 2 and 3 for most of the experiment (Figure 3.7.b and Figure 3.7.c), except for days 119 to 140 when there was a drop in the percentage of methane, as previously described, due to leakages in the experimental rig. Another exception to this almost constant trend was for the test digesters from day 184, when the concentration in the test reactors was aimed at 2000 mg NO₃/L and resulted in a decrease in methane concentration in one of the triplicates (nitrate test 3) reaching a value of 32% at day 203, which equated to a drop of 37% with regards to the period with 700 mg NO₃/L (Figure 3.7.c). There has been previous research at 2500 mg NO₃-N/L nitrate that showed inhibition of methane production [25]. On the contrary, in another study by Barber and Stuckey, nitrate addition between 176 and 37 mg NO₃/L was beneficial as methane production increased by 67% [32], while no clear positive impact on biogas formation was recorded in the current trials (Figure 3.7.a).

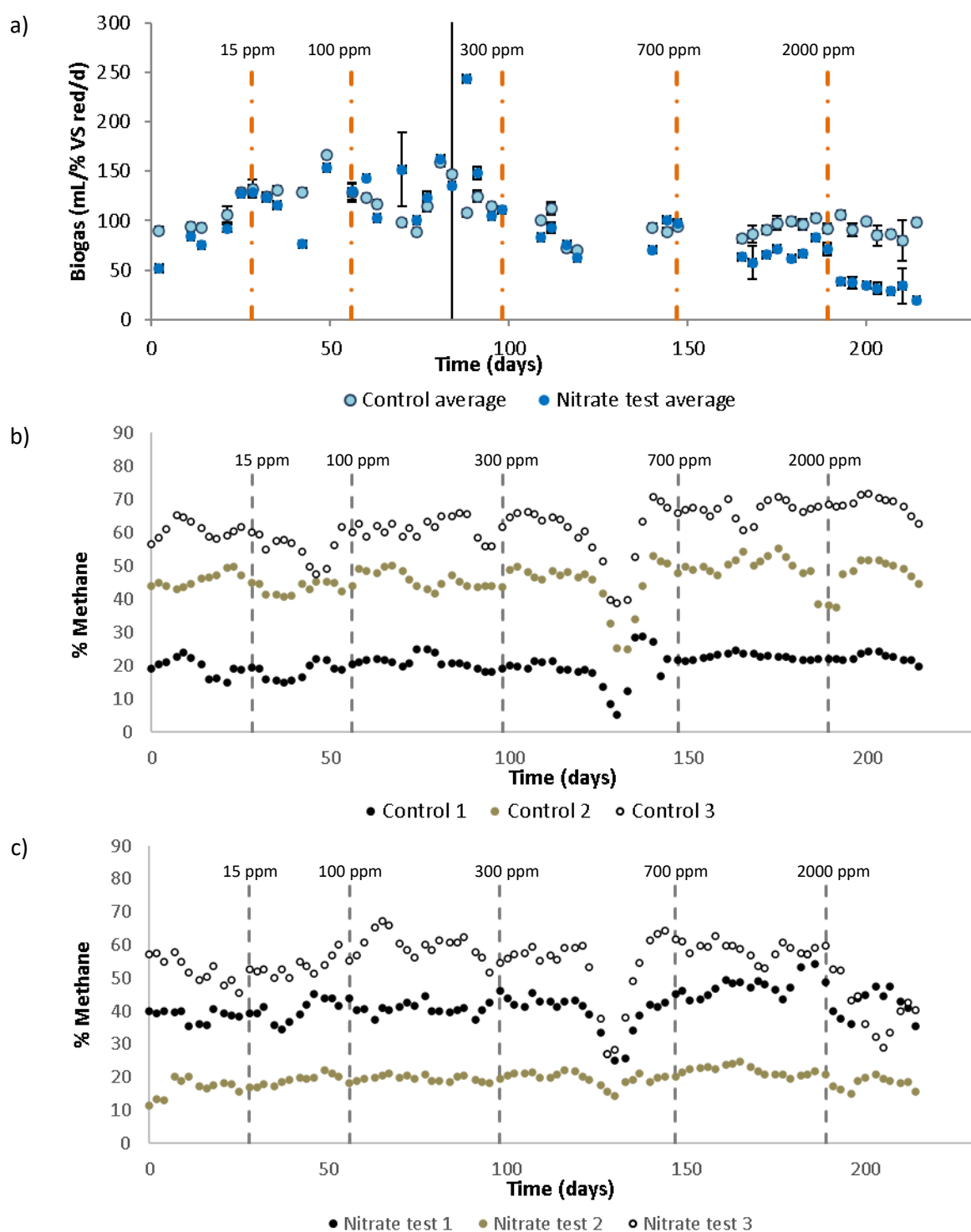


Figure 3.7. Performance data for semi-continuous control and test reactors dosed with nitrate, where a) daily biogas production is represented as 7 days moving average and as an average of the replicate units and b & c) are the methane concentrations for control and test reactors, respectively, represented as 7 days moving average for each separate replicate. Vertical lines represent the point where the dose of nitrate in the test digesters was changed.

Note – the drop in CH₄ content observed between days 123 and 137 was related to leaks in the experimental rig.

During the periods of no nitrate addition, 15 mg NO₃/L and 100 mg NO₃/L, TVFA levels mostly remained below 1000 mg Acetate_{eq}/L for both control and test units. Few days with higher concentrations were recorded like the TVFA values of 1675 mg Acetate_{eq}/L and 2641 mg Acetate_{eq}/L

for control and test reactors, respectively, at day 63, but they were short punctual changes not representative of sustained trends (Figure 3.8.a & Figure 3.8.b). Nitrate additions to reach levels of 700 and 2000 mg NO₃/L in the reactors, resulted in an increasing trend in TVFA levels, with an accumulation rate of 55 mg Acetate_{eq}/L/d and 258 mg Acetate_{eq}/L/d of TVFA up to 3022 mg Acetate_{eq}/L and 4078 mg Acetate_{eq}/L at days 182 and 210, respectively (Figure 3.8.b) (Table 3.3). Regarding the individual VFAs, there was an increase in the nitrate reactors of acetate and propionate, which were both below 400 mg/L in the control units (Figure 3.9). On day 182 d up to 1200 mg Acetate/L and 900 mg Propionate/L were recorded during the period that nitrate test reactors were aimed to have 700 mg NO₃/L, which might indicate the start of inhibition of methanogenesis at that period, that was not fully observable on the biogas and methane trends (Figure 3.9). Further increase of nitrate up to 2000 mg NO₃/L showed first a decrease and later an increase of acetate and propionate reaching values of 3224 mg Acetate/L and 481 mg Propionate/L respectively while TVFA levels remained at values of 4078 mg Acetate_{eq}/L on day 210 (Figure 3.9). An increase of acetate was observed as well by Yi *et al.* when nitrate was added using a COD/NO₃ ratio of 8 which was suggested to be because of growth of denitrifiers, which consume preferably propionate and butyrate, over methanogens [26].

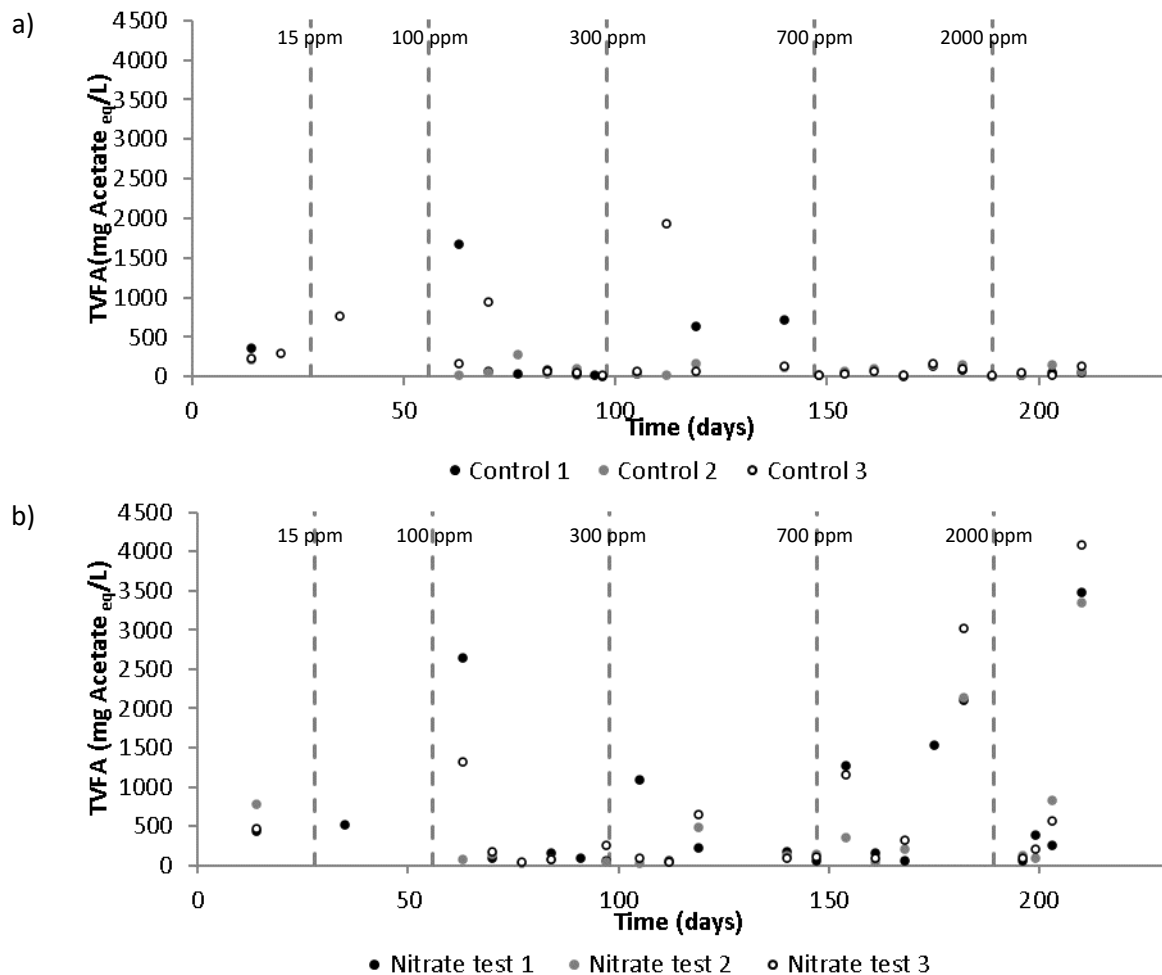


Figure 3.8. Profile of TVFA concentrations for a) Control and b) Nitrate test digesters with the different additions of nitrate to the test reactors.

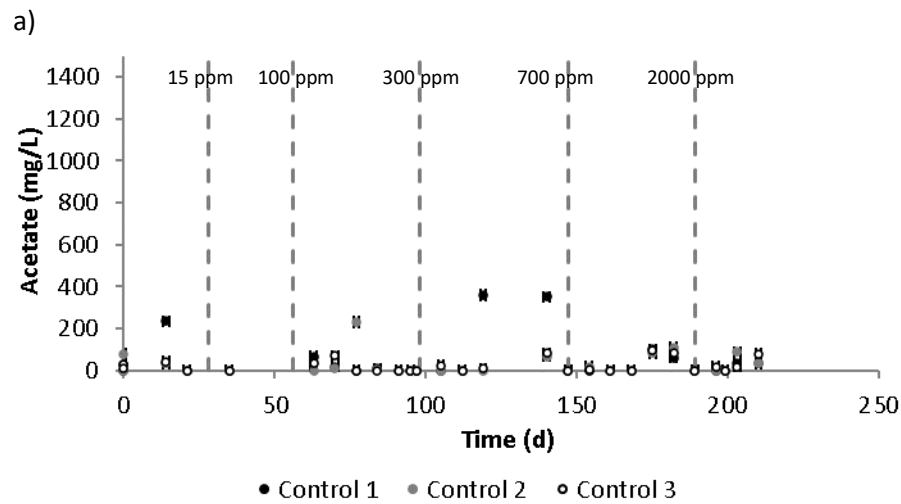
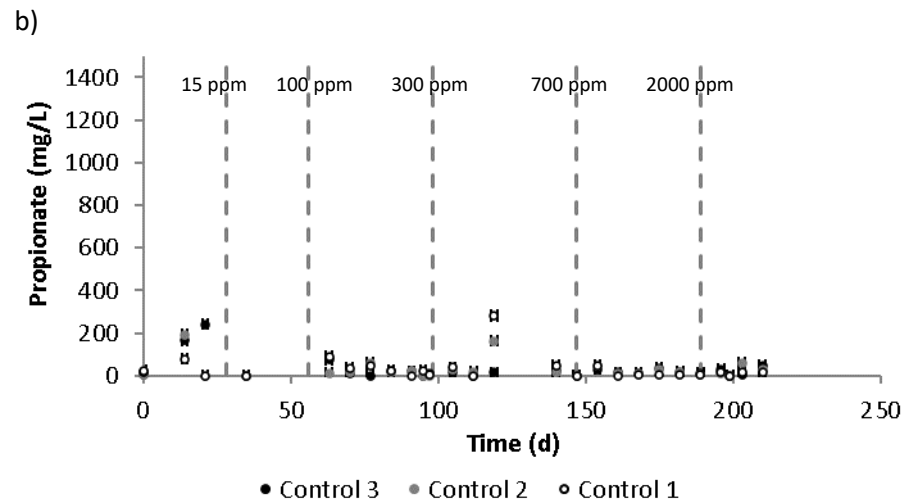
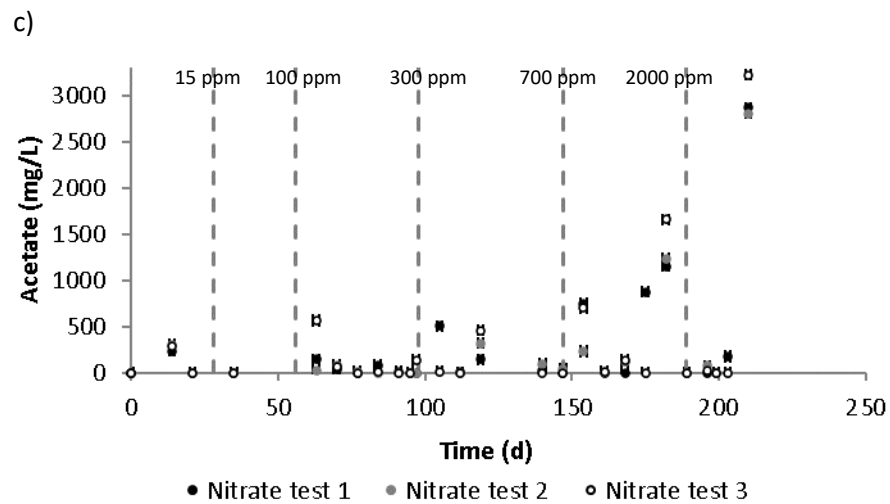
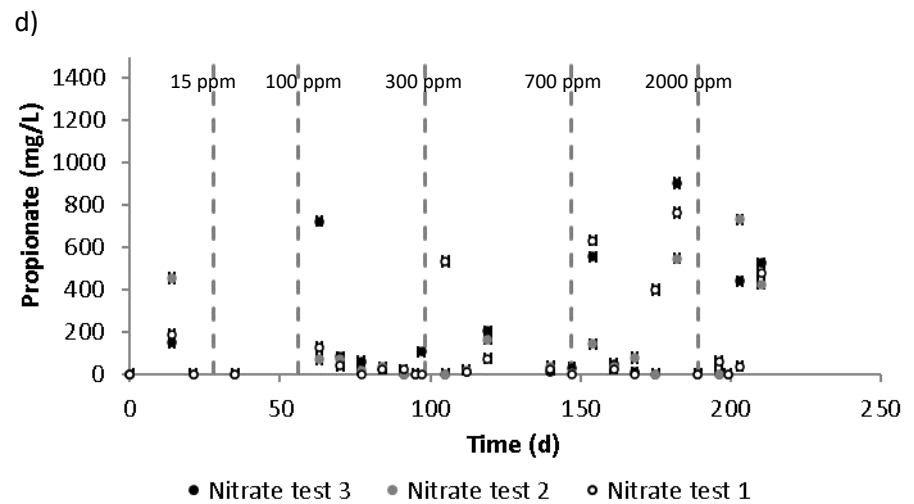


Figure 3.9. Control and Nitrate test data for a & c) acetate (mg/L) and b & d) propionate concentration (mg/L), with different additions of nitrate to the test reactors.

The pH showed stable values between 7.5 and 6.5 for all the reactors throughout the experiments (Figure 3.10.a & Figure 3.10.b), which were within the optimum pH values reported for stable AD [41]. The pH did not decrease even at the highest concentration tested in the current study, despite the reactor reduced methane performance and TVFA accumulation. The pH decrease because of the unbalance in the reactors could have been masked by the pH increase due to denitrification. In a study by Yi *et al.*, pH increased to a value up to 7.8 decreasing later on down to 7.6 when they were studying simultaneous denitrification and methanogenesis with addition of nitrate in a ratio 8 C/NO₃-N using a mesophilic batch test [26]. An increase of pH was observed as well in a study by Ghyselbrech *et al.* linked to the consumption of protons during the conversion of nitrate to nitrogen gas by denitrification [44].

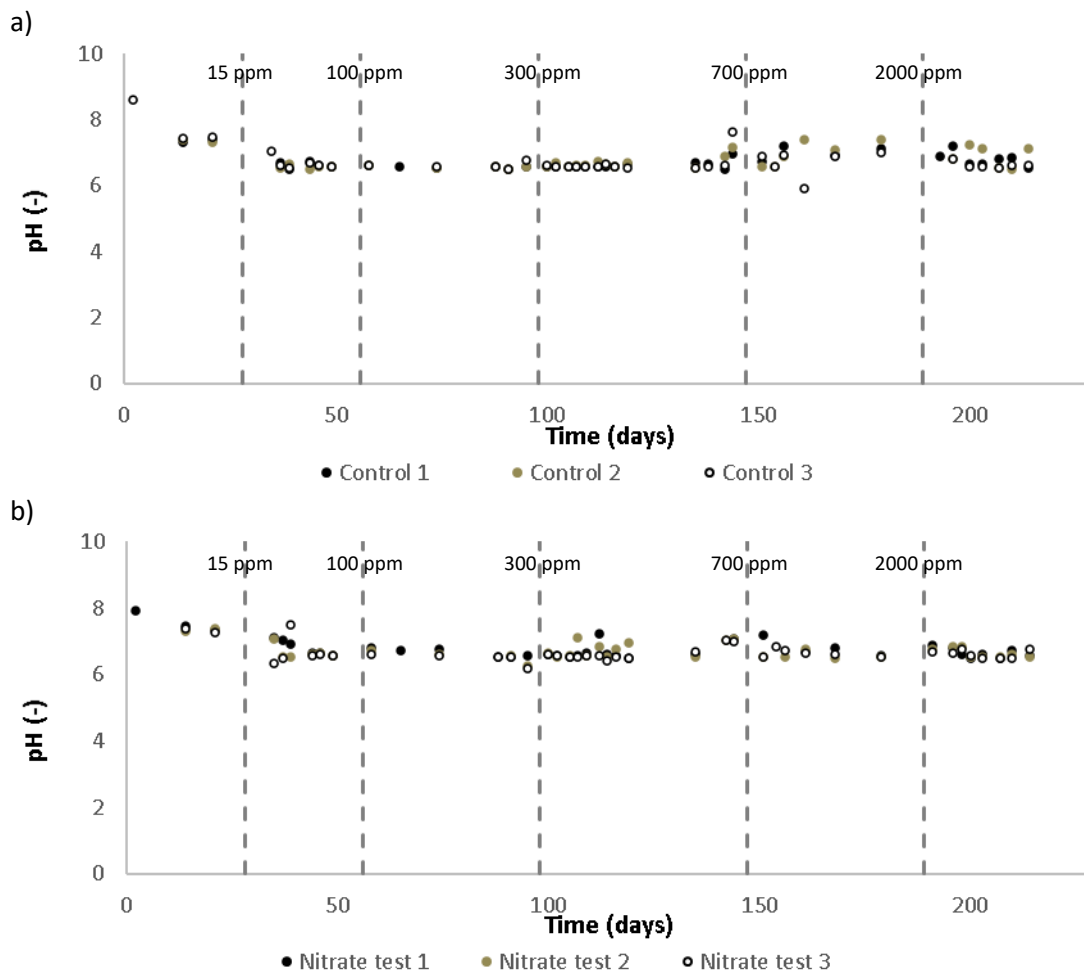


Figure 3.10. Control reactors (a) and Nitrate test reactors (b) pH data, with the different additions of nitrate to the test reactors marked in vertical lines.

The concentration of nitrogen compounds is important when nitrates are added to the system, as it can be transformed by denitrification into other compounds with the potential to negatively impact methane production [25]. TAN and nitrate concentrations reduced in the reactors. Nitrate dosed and control reactors had similar TAN trends suggesting that any decrease resulted from variations in the feedstock for the periods from non-addition to 700 mg NO₃/L in the reactor. An addition of nitrate to reach 2000 mg NO₃/L decreased TAN with a slope of -17 mg TAN/L/d leading to a concentration range between 3461 and 3700 mg TAN/L, while there was a TAN increase in the control reactors

with concentrations from 4251 to 5217 mg TAN/L (Table 3.3 and Table B3 in appendix). The reduction in ammonia levels in the test reactors during the period of 2000 mg NO_3/L could indicate that denitrifiers could degrade the nitrite converted from the nitrate added and the TAN to nitrogen competing for the carbon source with the methanogens [44]. Moreover, nitrate levels in the digestate did not increase despite its addition to the test reactors (Table 3.3), with its concentration remaining on ca. 20 mg NO_3/L . The nitrite concentration, which has been reported to accumulate in AD reactors when nitrate is added [25], was below the detection limit throughout the trials. The relatively low concentrations of nitrate measured in the digestates from the test ADs suggest that the inhibition observed in biogas production may have resulted from other nitrogen compounds formed from nitrate rather than from the nitrate itself, from the sodium contained in the salt used for spiking (sodium nitrate); or from the competition between denitrifiers and methanogens. Sodium concentration was observed to increase at a rate of 116 mg Na/L/d when a concentration of 2000 mg NO_3/L was targeted in the reactor (Table 3.3). Nonetheless, sodium concentration in the digestate remained between 2033 mg Na/L and 4453 mg Na/L, which was below the value of 6165 mg Na/L found to be inhibitory when using sodium chloride in batch digesters (Table B3 in appendix) (Chapter 2). By-products from denitrification, such as nitric oxide, nitrous oxide and hydroxylamide, have been observed to inhibit methanogenesis [21,44,45]. These compounds were not measured in the current study, although prevalence of some can be inferred from the operating conditions inside the ADs as previous studies have seen that the ratio of nitrous oxide and nitrogen depends on pH, oxygen content and copper concentration [45]. In this study, the environment was anaerobic, the pH was kept between 7.5 and 6.5 and an average copper concentration of 0.9 mg Cu/L (14 μM) was measured, which indicate a predominance of nitrogen over nitrous oxide [45]. Therefore, nitrogen gas will be the predominant nitrogen species in the gas phase. Another explanation for the low biogas yields might be the competition between denitrifiers and methanogens, which would consume nitrate and produce nitrogen gas reducing the percentage of methane in the biogas. In the study by Yi *et al.*, there was a decrease of methane percentage from 68% in the control to 44% in the reactor with nitrate addition because of an increase of nitrogen and carbon dioxide gases due to activity of denitrifiers and facultative anaerobes [26]. The biogas and methane decrease at the highest concentration of nitrate tested might have resulted from the competition with other microorganisms such as denitrifiers for the organics, which could have limited organic material availability for the methanogens.

The nitrate concentration in the outlet (digestate) calculated from salt addition with the spiked feedstock was of 200 mg NO_3/L between days 67 and 93 (Figure 3.11). This calculated outlet concentration of nitrate reached a value of 319 mg NO_3/L on day 100 (Figure 3.11), which is a similar concentration to that resulting in a 15% methane reduction in the batch trials, 332 mg NO_3/L (Chapter 2). The calculated concentration further increased to 1587 and 4240 mg NO_3/L in the outlet on days 165 and 210, respectively (Figure 3.11). Nonetheless, the measured concentration in digestate samples was around 20 mg NO_3/L most of the time and reached 40 mg NO_3/L on days 175 and 182 for nitrate reactors 1 and 2 (Figure 3.11). A higher concentration, 71 mg NO_3/L was observed on nitrate test reactor 3 on day 20 (Figure 3.11). The vast difference between calculated and measured nitrate levels might indicate a chemical or biological reaction of the nitrate in the reactor. As previously commented, the reduced level of nitrate in the outlet could be caused by denitrifiers that consumed nitrate to produce nitrogen gas, which could cause a competition

between denitrifiers and methanogenesis. However, there was not a reduction of biogas or methane between days 28 and 189 despite the differences between nitrate measured and calculated excluding the competition for organic matter between denitrifiers and methanogenesis during that period (Figure 3.7). These differences in nitrate concentrations observed between the feed and outlet suggest a removal, possibly through denitrification, in which the nitrate would be converted to nitrogen gas. The nitrogen gas potentially produced was evaluated by calculating the difference between the nitrate added to the feedstock and the concentration in the digestate, which will be referred to as nitrate consumed, and used to calculate the equivalent moles of nitrogen gas. The moles of nitrogen obtained were then converted to volume with “The Ideal Gas Law” and then reported as fraction of the total biogas volume (in %). The percentage of methane in the biogas did not change despite the increase of nitrogen. This is because the nitrogen produced was significantly lower than the methane percentage and even below the resolution of the methane measurement with a maximum of 0.5% (Figure B1 in appendix). Therefore, it is possible that the nitrate consumed formed nitrogen gas without decreasing the methane fraction in the biogas.

Further addition of nitrate up to 2000 mg NO_3/L decreased the biogas and methane production with a consumption of nitrate as well. Therefore, from day 189 to day 210, there could have been a competition between denitrifiers and methanogens, or an inhibition because of the formation of by-products from an incomplete denitrification. Ghyselbrecht et al. also observed a decrease of biogas and nitrate without nitrite accumulation when nitrate was added between concentrations of 1.25 and 5 mg $\text{NO}_3\text{-N}/\text{L}$, which suggested that nitrogen gas production by denitrifiers reduced the concentration of nitrate and a by-product from denitrification called hydroxylamide caused the inhibition of methanogenesis reducing the biogas production [44]. Therefore, directly or indirectly the denitrifiers seems to be involved in the decrease of biogas and methane performance.

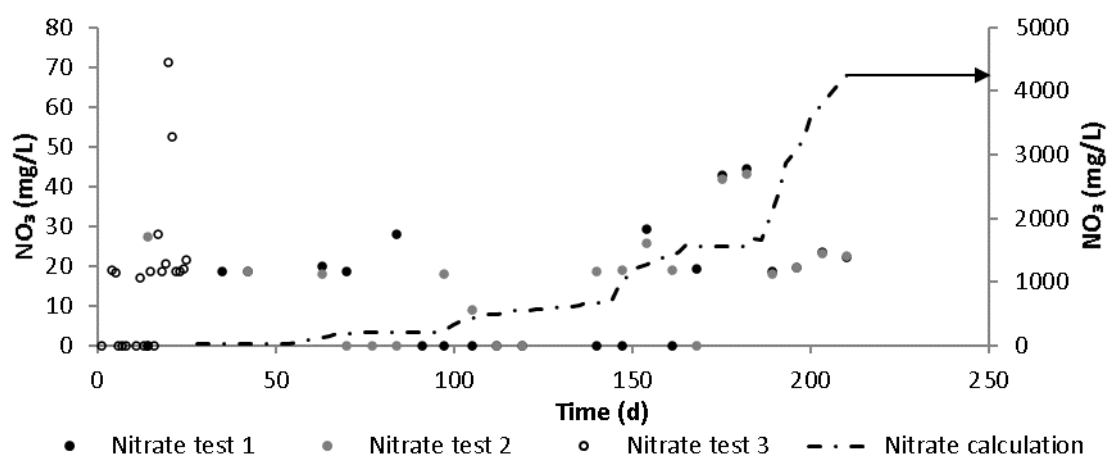


Figure 3.11. Concentration of nitrate in the digestate of semicontinuous anaerobic digesters spiked with sodium nitrate. Values are reported as concentrations measured in the digestate’s solids free fraction (soluble nitrate) for individual replicates (Nitrate test 1, 2 and 3) and concentrations calculated as ‘accumulated’ in the reactors when considering the continuous spiking with sodium nitrate sulphate ((mass NO_3 in reactor + mass NO_3 added - mass NO_3 removed)/reactor volume = Nitrate calculated).

Table 3.3. Rate of change (slope) of biogas and digestate concentrations for control (C) and nitrate test (NO_3^-) digesters for each fo the experimental periods.

<i>Period</i>	No addition		15 mg NO_3/L		100 mg NO_3/L		300 mg NO_3/L		700 mg NO_3/L		2000 mg NO_3/L	
<i>Days</i>	0-25		28-53		56-95		98-126		128-163		165-186	
<i>Experiment</i>	C	NO_3	C	NO_3	C	NO_3	C	NO_3	C	NO_3	C	NO_3
<i>Biogas (mL/% VS feed/d/d)</i>	1.52	2.84	1.52	0.35	0.14	0.84	-0.14	-0.27	0.30	-0.48	-0.37	-1.40
<i>% Methane/d</i>	-0.01	-0.1	0.2	0.2	-0.09	-0.06	-0.07	-0.08	-0.07	0.02	-0.2	-0.2
<i>TVFA (mg Acetate_{eq} /L/d)</i>	2	-	-	-	-17	-36	6	-1	3	55	4	258
<i>% Acetate/d</i>	-5	0	-	-	0	0	1	1	-2	-1	5	2
<i>% Propionate/d</i>	-3	-2	-	-	1	-1	-1	0	-1	0	-1	0
<i>% Removal VS/d</i>	0.2	0.8	0.1	-0.1	-0.1	-0.05	0.4	0.2	0.2	0.2	0.7	-0.1
<i>TAN (mg-N/L/d)</i>	10	37	71	62	-0.4	-161	-30	-18	26	19	69	-17
<i>FAN (mg-N/L/d)</i>	-6	8	-3	1	16	-6	-1	-0.3	-1	1	8	-4
<i>CODs (mg/L/d)</i>	8	9	-	-	-52	-79	14	-52	0	38	-	-
<i>Na (mg/L/d)</i>	-	-	-	-	0.9	2	0	15	-1.3	28	1.3	116
<i>Zn (mg/L/d)</i>	-	-	-	-	0	0	0	0	0	0	0	0
<i>NO_3 (mg/L/d)</i>	3	-1	-	-	0	0	0	0	0	1	1	0
<i>SO_4 (mg/L/d)</i>	-3	-6	-	-	1	0	5	-3	-1	-1	0	0
<i>pH (-)</i>	-0.01	-0.002	-0.03	-0.04	-0.003	-0.002	0.001	0.01	0.00	-0.01	-0.02	-0.01

3.4. Conclusions

The semi-continuous experiments showed no negative impact on digester performance when zinc and nitrate were added to reach concentrations between 15-50 mg Zn/L and 15-700 mg NO₃/L in the tests reactors. A further increase of zinc to 700 and 1000 mg Zn/L resulted toxic for biogas and methane formation, with null biogas rate (0 mL/% VS red/d) and no methane in the gas phase. Between the period of 700 mg Zn/L and the period of 1000 mg Zn/L, there was a period without zinc spiking, which resulted in recovery of biogas and methane production. The zinc trials resulted in an accumulation of zinc, and sulphate in the reactors, which could suggest that continuously adding the metal and sulphates to the AD will accumulate them inside the digester in a later stage. Nitrate addition up to 2000 mg NO₃/L reduced biogas production from 98 mL/% VS red/d in control ADs to 21 mL/% VS red/d in test units, with a simultaneous drop in methane content of 5-20%. Nitrate levels in the digestate of test reactors suggested that denitrifiers were present when levels of 15 to 700 mg NO₃/L were maintained in the ADs, without hindering methane production or competing with methanogens acetoclastic pathway. An increase of nitrate addition to reach 2000 mg NO₃ in the reactor showed a negative impact on biogas and methane production, which seemed to be caused by competition by denitrifiers or by-products formation from denitrification process. Therefore, addition of TWs to ADs with high levels of zinc or nitrate need to be carefully controlled, as zinc and nitrate inhibition could be prevented by alternating with less concentrated TWs or by blending the wastes before feeding to sustain concentrations in the ADs below 700 mg Zn/L and 2000 mg NO₃/L. According to typical TWs composition (Chapter 2), most TWs could be added to the digester unit and maintain digestate concentrations below those levels as long as TW addition remains within a maximum of 10% of the feedstock volume. Feeding TWs in proportions >10% of the incoming feed would require careful management of the TWs loads to minimise the risk of inhibition. For example, TWs that can potentially increase the zinc concentration in the reactor above 700 mg/L have to be mixed with other TWs with very low levels of zinc to provide dilution and maintain the overall concentration below inhibitory or toxic levels. Similarly, TWs addition should be balanced to maintain the overall nitrate concentration below 2000 mg/L in the reactor, which was shown to impact biogas and methane production.

3.5. References

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CHAPTER 4: Membrane Concentration of Low Strength Trade Wastes for Anaerobic Co-digestion with Sewage Sludge

Olivia Berzal de Frutos ^a, Marta García ^a, Yadira Bajón Fernández ^a, Marc Pidou ^a

^a Cranfield Water Science Institute (CWSI), School of Water, Energy and Environment (SWEE), Cranfield University, Bedford MK43 0AL, United Kingdom

Abstract

Trade wastes (TWs) could be treated by water utilities dealing with municipal wastewater. They are generally treated with the municipal wastewater in a biological nutrients removal (BNR) system when the TWs have a chemical oxygen demand (COD) lower than 50 g/L or with the sewage sludge in anaerobic digestion (AD) when their COD content is above 50 g/L. The anaerobic co-digestion (Aco-D) of TWs with sewage sludge can improve digestion by balancing nutrients, increase soluble organics and reduce toxicity. Low strength TWs could contain nutrients and organic in high enough concentrations to increase the energy demand of the BNR plant through increased aeration required to breakdown the increased load. In view of reducing the impact and maximising the benefits of these lower strength TWs, they could be treated in the AD after increasing their organic content with a pre-concentration step.

The aim of this study was to understand the concentration of TWs with microfiltration (MF) and ultrafiltration (UF) membranes and the impact of the resulting concentrates in Aco-D with sewage sludge. The results showed that MF performed better in leather, resin, food and paper TWs, while UF performed better in personal care products, food, paper and leather TWs. Most of the concentrates in biological methane potential tests showed beneficial or non-effect, especially the chemical TW concentrate from the MF showed a beneficial value of 17%. Resin TW concentrate was the only one that showed an inhibitory effect for methane production suggesting that this TW will not be suitable for their use in a pre-concentration step before AD. The best economical benefit was found in food and chemical TWs concentrate from UF.

4.1. Introduction

When treating trade wastes (TWs) in municipal wastewater treatment facilities, there are two main routes available, through the main treatment train by mixing with the municipal wastewater generally in biological nutrients removal (BNR) systems, or through the sewage sludge treatment route by feeding to anaerobic digestion (AD). The route through which the TWs are treated is often dependant on their organic content. Indeed, some utilities regulate treatment based on chemical oxygen demand (COD), with TWs with COD concentrations below 50 g/L treated in the BNR, between 50 g/L and 250 g/L going directly to the AD for co-digestion (Aco-D) and for wastes with a COD concentration above 250 g/L they are directed to storage in high strength tanks (HST) and controlled addition to the AD to avoid shocks to the system. Aco-D of TWs with sewage sludge can use the spare capacity of AD reactors frequently available in wastewater treatment plants (WWTP), help improving the digestion of the respective substrates by balancing the organics and nutrients levels, increase the load of degradable organics and dilute possible inhibitors present in TWs that prevent their biological treatment as sole substrates, all leading to an improvement of the digestion

process and an increase in biogas production [1–6]. Alternatively, the lower strength TWs (<50 g/L in COD), although typically added at significantly lower rates than the flow of municipal wastewater in the WWTP, can still contain considerable levels of organics, nutrients and other pollutants as compared to municipal wastewater and consequently are likely to increase the overall load on the BNR plant. The increased load in organics may provide a benefit as an additional carbon source for the BNR process to balance the C/N ratio. To illustrate, TWs such as cassava stillage and glycerol wastewaters have been found to improve nutrients removal in municipal WWTPs because of their high organic content [7]. However, the increased load in nutrients, organics and possible toxic compounds present in TWs such as landfill leachate, might lead to an increase of the energy demand for the aeration and low removal of organics and nutrients [8]. As an alternative, to avoid such negative impact of TWs addition in the main treatment train of the WWTP, some of the lower strength TWs could be sent to the AD plant after a pre-treatment stage focussed on concentrating the wastes to sustain required organic loads for the digestion process.

Several methods are available to concentrate wastewater, for example evaporation, centrifugation and membrane filtration. Membrane treatment has gain importance in wastewater treatment nowadays. There are four main types of membranes depending on the pore size/selectivity, i.e. microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) [9]. The last two types are high pressure membranes which have typically been used for water reclamation and are generally the last step of the treatment train after significant pre-treatment. The high pressures required makes their operation energy intensive and prohibitive for the application considered here [10]. Therefore, the focus of this study was on low pressure MF and UF membranes for the concentration of the low strength TWs, with the concentrate being sent for AD and the filtrate likely to need further treatment through the BNR plant before discharge.

There have been previous studies about the concentration of TWs for different goals. Capar *et al.* studied the concentration of silk industry wastewater with UF and NF membranes to recover sericin. To avoid overload of those filtration methods, a pre-treatment step was applied using centrifugation and/or MF membrane. MF as pre-treatment suffered fouling because of the high concentration of proteins in that wastewater with a reduction of 26% COD, 79% colour and 99% turbidity in the permeate. UF after the MF and centrifugation decreased the COD between 58-65%, colour by 85-99%, turbidity by 96-99%, TS by 46-60%, proteins by 61-70% and flux declined by 58-88%. There was a better removal with NF with a reduction of COD of 93-99%, colour of 100%, turbidity of 100%, TS of 91-99%, proteins of 85-100% and flux declined by 70-75% [11], clearly highlighting the potential to concentrate organics and other compounds from TWs but also the impact to be expected on the operation of the membranes with such wastes. The other TW that has shown to gain value by concentration is pulp and paper wastewater. In a publication by Liu *et al.*, MF and UF membranes were used to concentrate a type of pulp and paper wastewater and the possibility to recover or reuse the concentrate was investigated. In that study, they achieved a COD removal of 79% for UF and 37% for MF, removals of TS and VS of 25% and 50% for both MF and UF, and removal of lignin of 90% and 80% for MF and UF, respectively. It was suggested that lignin could be extracted for its use in adhesives [12]. In those previous studies, the concentration of organics in TWs following membrane treatment was 26-37% in MF, and 79% in UF without pre-treatment [11,12]. Therefore, the possibility of obtaining valuable resources, including organics, which should be biodegradable to

be used in AD reactors, with membrane technologies for TWs concentration has already been demonstrated; although there is limited understanding of the impact on other compounds which in higher concentrations may inhibit the AD process. In Chapter 2, 8 groups of TWs were defined based on the source type including landfill leachate, glycol/beverages/alcohol, chemical, food, soapy waste, oily containing waste, sludge and other. From those types, a range of TWs with low organic content, below 50 g COD/L, could be concentrated, with 55% of the landfill leachates, 4% of glycol/beverage/alcohol, 14% of food, 13% of chemical, 2% of oil containing waste, 2% of soapy waste, 6% of sludge and 5% of others (Chapter 2). However, chemical industry wastewaters generally contain a wide range of toxic, hazardous and harmful compounds which are mostly hardly biodegradable [13]. Paper industry wastewater is formed by hardly biodegradable and inhibitory compounds, such as lignocellulose, resin acids, sulphur and organ chlorine compounds [14]. Food industry wastewater is known to be high in organic content especially proteins and fats that could be degraded to volatile fatty acids (VFAs), free ammonia nitrogen (FAN) and long-chain fatty acids (LCFAs) resulting in inhibition to the AD [15–18]. Leather industry wastewater could contain refractive organic and inorganic compounds that could affect the Aco-D, such as polyphenolic tannins, nitrogen compounds, chromium, sulphide and chloride [19,20]. And personal care products industry wastewater might contain contaminants of emerging concern that can bio-accumulate and affect living organisms, such as fragrances, triclosan and benzophenone-3 [21]. Nonetheless, these TWs also present benefits; for example, LCFAs have a higher potential to produce methane than carbohydrates [18], and those benefits can be exploited if Aco-D of TWs with sewage sludge is closely managed to dilute possible toxicity [15]. Food TWs has a potential to show a higher benefit than chemical, paper, leather and personal care TWs because of their higher biodegradability (13-21).

In order to maximise the value proposition for the treatment of TWs on municipal wastewater treatment sites, it then becomes essential to study the use of TWs concentrates as substrate for Aco-D with sewage sludge. The aim of this study was then to understand the impact of real concentrated TWs on Aco-D with sewage sludge. The specific objectives towards that aim were: i) to investigate the concentration of real TWs with MF and UF membranes, ii) to evaluate the levels of organics and other compounds, including AD inhibitors, present in the concentrates depending on membrane selectivity; and iii) to determine the potential of concentrated TWs to enhance the AD of sewage sludge. The current work will address the gap in knowledge in understanding the fate of specific pollutants contained in real TWs when concentrated with MF and UF membranes; their subsequent impact, positive or negative, on the Aco-D with sewage sludge and further contribute to establishing guidance for TW selection on sites.

4.2. Material and methods

4.2.1. Feedstock, inoculum and trade wastes

The sludge used as a substrate for the AD trials consisted of a mixture of primary sludge (PS) and secondary activated sludge (SAS) from a WWTP in the West Midlands, England. The inoculum was the digestate from the same site. TWs which are currently treated on municipal WWTPs were collected from several sites in the West Midlands, England. The substrate, TWs and inoculum were stored at 4 °C on the day of sampling. The samples were allowed to reach room temperature for 24 hours before the start of the experiments. The TWs selected were a landfill leachate and wastewaters from the chemical, paper, resin, food and personal care products industries. They were selected because of their range of characteristics while also having a 'low' organic concentration, below 50 g/L in COD, as compared with typical TWs that are used for Aco-D with sewage sludge (Table 4.1).

4.2.2. Membrane filtration

Membranes used for the concentration of TWs were a tight UF membrane with a 10 kDa molecular weight cut-off (MWCO) and a MF membrane with a 300 kDa MWCO, both from Pall laboratories with a filtration area of 41.8 cm². They were inserted in a dead-end filtration stirred cell model 8400 from Millipore. The applied pressures were 3.2 bar for the 10 kDa membrane and 3.2 bar and 1.8 bar for 300 kDa. The clean water permeability was measured with distilled water before each filtration test to obtain a baseline for the membranes used before the filtration of the TWs. The flux (J in L/m²/h or LMH) and permeability (K in L/m²/h/bar) were calculated for each filtration trial according to Equation 4.1 and 4.2, respectively.

$$J = \frac{\Delta V_p}{A_f \cdot \Delta t} \quad (4.1)$$

$$K = \frac{J}{P} \quad (4.2)$$

Where ΔV_p in L is the volume of permeate produced over the time Δt in h, A_f is the filtration surface area in m² and P is the applied pressure in bar.

The concentration factor by volume (CF_V) was calculated as the volume of original TWs filtered (V_{TW}) divided by the volume of the concentrate left at the end of the filtration (V_{con}) (Equation 4.3).

$$CF_V = \frac{V_{TW}}{V_{con}} \quad (4.3)$$

Concentration of TWs was continued until the concentrate reached a concentration of 50 g/L COD in the concentrate, the lower limit for wastes to be typically taken to AD treatment. The concentration factor (CF) was calculated after the filtration test dividing the concentrate COD content (C_m) by the feed concentration of the TWs (C_F) (Equation 4.4).

$$CF = \frac{C_m}{C_f} \quad (4.4)$$

New membranes were used for each TW tested. If needed for a specific TW, the membrane used was cleaned between sets of filtrations. The membrane was first gently washed with a soft sponge and rinsed with DI water, followed by a chemical cleaning achieved by soaking the membrane in 0.01 M sodium hydroxide (NaOH) overnight. Finally, the membrane was rinsed and left in DI water until its next use. The effectiveness of the membrane cleaning step was measured as the percentage of the flux recovery $\%J_{rec}$ (Equation 4.5) calculated as the ratio of the clean water permeability after cleaning (K_A) over clean water permeability before the filtration trial (K_B).

$$\%J_{rec} = \frac{K_A}{K_B} \times 100 \quad (4.5)$$

4.2.3. Batch AD tests

Biological methane potential (BMP) tests were used to understand the impact of concentrated TWs addition in sewage sludge AD. The BMP tests were carried out in 125 mL serum glass bottles in triplicates consisting in a blank with only inoculum, a control with inoculum and substrate, and test reactors containing inoculum, sewage sludge as 90% mass of feed, and concentrated TWs as 10% mass of feed. All the digesters were operated with an inoculum to feedstock volatile solids (VS) ratio of 1:2 considering the VS of the sewage sludge and TWs. The temperature of the bottles was kept at 38 °C using an incubator with continuous shaking. The tests were ended when the daily biogas production variation was less than 1% [22,23].

Biogas (Y_{Bg} , mL/g) and methane (Y_M , mL/g) yields were calculated from their accumulated production in mL ($AccV_s$) subtracted by the accumulated biogas production from the blank in mL ($AccV_B$) and normalising over the mass of VS fed to the reactor of sewage sludge and TWs (g VS_f) (Equations 4.6 and 4.7).

$$Y_{Bg} = \frac{AccV_s - AccV_B}{g\ VS_f} \quad (4.6)$$

$$Y_M = \frac{AccV_s - AccV_E}{g\ VS_f} \quad (4.7)$$

The average TWs maximum yield for biogas or methane (Y_{TW}) was then divided by the average control one (Y_C) and multiplied by 100 to obtain the percentage of performance ($\%Y$) over each control as there were three different controls (Equation 4.8).

$$\%Y = \frac{Y_{TW}}{Y_C} \cdot 100 \quad (4.8)$$

Biogas production kinetics were estimated using First-order kinetics and Gompertz modified models [22,24] Equations 4.9 and 4.10, respectively.

$$1^{\text{st}} \text{ order kinetics} \quad P(t) = P_{\max} \cdot (1 - e^{(-k_h \cdot t)}) \quad (4.9)$$

$$\text{Gompertz modified model} \quad P(t) = P_{\max} \cdot e^{\left(-e^{\left(\frac{k \cdot (t - \lambda)}{P_{\max}} + 1 \right)} \right)} \quad (4.10)$$

Where $P(t)$ is the production of biogas at time t (d); $P_{\max}$ is the maximum biogas production or biogas yield (mL biogas/g VS); k_h is the hydrolysis constant (d^{-1}); k is the specific rate constant (mL biogas/g VS/d), and λ is the lag phase time constant (d).

The differences between both models and the real data were calculated using normalised root mean square error (RMSE_n) and r^2 (equations 4.11 and 4.12) [25,26].

$$\text{RMSE}_n = \left(\frac{1}{n} \sum_{i=1}^n \left(\frac{X_i - Y_i}{Y_i} \right)^2 \right)^{1/2} \quad (4.11)$$

$$r^2 = \left(\frac{m(\sum_{i=1}^n X_i Y_i) - (\sum_{i=1}^n X_i)(\sum_{i=1}^n Y_i)}{\left(\left[m(\sum_{i=1}^n X_i^2) - (\sum_{i=1}^n X_i)^2 \right] \left[m(\sum_{i=1}^n Y_i^2) - (\sum_{i=1}^n Y_i)^2 \right] \right)^{1/2}} \right)^2 \quad (4.12)$$

Where X_i is the predicted value of biogas (mL $\text{CH}_4/\text{g VS}$), and Y_i is the measured value of biogas (mL $\text{CH}_4/\text{g VS}$).

Once the yields of biogas and methane and the kinetics were calculated, the observed response for each concentrated TWs was associated with beneficial, non-effect, inhibitory, inhibitory for kinetics and toxic. Beneficial is defined as the samples that yielded biogas or methane above the value of the control. Non-effect samples had values close to the control. There are two types of inhibition depending on what is affected, if the yield is affected then it is called inhibition, if there is a lag phase, then it is an inhibition of kinetics. Toxic is referred to the samples that had less biogas production than the blank and hence a negative yield.

4.2.4. Analytical methods

Sludge and TWs samples were analysed before and after the BMP tests. Partial alkalinity (PA), intermediate alkalinity (IA), total solids (TS) and VS were measured using standard methods [27,28]. The measurement of pH was carried out with a Mettler Toledo MA235 pH/ion analyser. Total and soluble COD, total ammonia nitrogen (TAN), and total nitrogen (TN) were analysed using cell test kits from Millipore (VWR, UK) and a spectrometer Spectroquant NOVA 60 from the same company. Solid free samples for analyses of soluble COD, TAN, VFA, ions and metals were obtained after centrifuging at 5000 rpm for 8 hours and filtering the supernatant through 0.45 μm Millipore syringe filters. FAN was calculated from the TAN concentration and pH value using Equation 4.13 [29].

$$[\text{FAN}] = \frac{[\text{TAN}]}{1 + \frac{10^{\text{pH}}}{K_a}} \quad (4.13)$$

Where [FAN] is the FAN concentration (mg/L), [TAN] is TAN concentration (mg/L), and K_a is the dissociation constant.

Shimadzu prominence high-performance liquid chromatography (HPLC) was used to analyse VFA levels from 1 mL of the acidified sample that was stored in a freezer at -20 °C. The mobile phase for the HPLC consisted of a 0.5 mM sulphuric acid solution with an injection flow rate of 1 mL/h, and a Rerex 150 column [30]. The acidification consisted of addition of 10 μ L of 96% sulphuric acid to 9 mL of sample. A Dionex ICS-1600 ion chromatography (IC) was used for the determination of Nitrate, Nitrite, Sulphate, Phosphate, Fluoride, Chloride, and Bromide with a mobile phase of 9 mM Sodium carbonate and an AS 9HC column. Finally, a NexION 350D inductively coupled plasma (ICP) mass spectrometer was used to measure cations (Calcium, Magnesium, Potassium, Sodium, Manganese, Zinc, Cobalt, Molybdenum, Nickel, Copper, Iron, Selenium, Chromium, and Tungsten, Lithium, Aluminium, Silica, Titanium, Arsenic, Cadmium, Lead, Mercury, and Antimony). ICP samples were acidified with 100 μ L of >68% nitric acid in 10 mL of diluted soluble sample without solids.

Gas samples were characterised for biogas volume and methane content. Biogas was measured using a 1 M hydrochloric acid trap with an inverted measuring cylinder to measure liquid displacement. The biogas was collected in a 10 mL syringe from the measuring cylinder after pushing it through a 0.45 μ m Millipore syringe filter to avoid humidity intrusion for methane analysis with a Servomex 1440 gas analyser (Surrey, England, UK).

4.2.5. Statistics

ANOVA analysis was applied to determine if the differences between the biogas and methane yields from the control and tests reactors were statistically significant. ANOVA was performed by a Microsoft excel package "Data analysis". ANOVA uses the F-test to analyse the similarity between groups by comparing their variances. The similarity between control and sample units occurred when the F-value (F) was below the F-critical value (F_{crit}). On the contrary, when F was above F_{crit} , the control and sample had statistically different yields (Table 1 in appendix).

4.2.6. Economics

In order to evaluate the economic benefits associated with the reduction in load to the BNR and the increase in methane production in the AD by concentrating TWs and diverting them from the BNR to the AD plant, an example WWTP was used. Information such as the average municipal wastewater flow (177600 m³/d), the municipal wastewater characteristics ([COD] = 568 mg/L, [suspended solids] = 242 mg/L and [NH₃-N] = 32 mg/L), the typical flow of TWs going to the inlet of the works (289 m³/d), and the feed flow to the AD reactor (1248 m³/d), were all obtained from a real site. Economic values were then obtained for the methane production from AD and the aeration consumption reduction when TWs going to the BNR process are diverted to the AD.

The income from methane (I_M) (£/y) was obtained by multiplying the methane yield from the tests Y_M (mL methane/gVS), by the VS mass concentration in the feed (m_{VS}) (gVS/L feed), methane density (ρ) (0.628481 g/L at 38°C), a conversion factor for methane to energy (C) (50.1 kJ/g methane) [31]

and also assuming a 35% efficiency, conversion from kJ to kWh, a feed-in tariff (t_f) (0.046 £/kWh) [32], and the yearly flow of feed (Q_f) (L/y) (Equation 4.14).

$$I_M = Y_M \cdot m_{VS} \cdot p \cdot C \cdot 0.35 \cdot 0.000278 \cdot t_f \cdot Q_f \quad (4.14)$$

Then the benefit from adding concentrated TWs was calculated for each concentrate tested by subtracting the estimated income from the control (sewage sludge only) to the estimated income from the mixture of sewage sludge and each of the concentrated TWs as 10% of the feed.

The saving in aeration cost associated with the diversion of some of the TWs (10% of the feed flow to the AD) was calculated by subtracting the cost of the power consumption for the aeration in the BNR during the treatment of the mixture of municipal wastewater and TWs with the reduction in TWs to initial cost for the mixture with the full flow of TWs. The cost for aeration (C_E) (£/y) was calculated by multiplying the power required for the blowers in the treatment plant (P_b) (KW), determined by calculating the oxygen demand for treatment depending on the characteristics of each of the mixtures and based on standard design of the treatment plant, assuming continuous daily operation (24 hours), by the energy price E_e (0.03384 £/kWh) [33], and adjusted for a year (365 days) (Equation 4.15). The oxygen requirement was calculated as the sum of the oxygen needed for COD removal (0.65 Kg O₂/Kg COD), nitrification (4.34 Kg O₂/Kg NH₄-N) and endogenous respiration (0.024 Kg O₂/Kg MLSS) minus oxygen produced during denitrification (2.85 Kg O₂/Kg NO₃-N).

$$C_E = P_b \cdot 24h \cdot E_e \cdot 365d \quad (4.15)$$

4.3. Results and discussion

4.3.1. Trade wastes, feed and inoculum characteristics

This section describes the characteristics of the feed as sewage sludge, the inoculum, the TWs and their concentrates after UF and MF membranes filtration. The characteristics reported in this section will provide an insight into the possible effects of adding the TW concentrates to the AD and the efficiency of membrane concentration.

An important factor to filter TWs is the CF, as it will affect the operation of the membrane system and the volume of TW concentrates produced. The CF required for the TWs with both MF and UF was low for the food, chemical, resin and paper wastes with values of 1.61 and 1.52, 2.56 and 2.44, 3.04 and 2.96, and 3.81 and 3.85, respectively (Table 4.1 and Table 4.2). Due to their lower initial COD concentrations, higher CFs of 7.58 and 7.94 and 9.12 and 9.12 were required for the leather and personal care product wastes with the MF and UF membranes, respectively (Table 4.1 and Table 4.2). Higher CFs mean that the concentration stage will have to be operated for longer periods of time at a fixed rate to achieve the greater recoveries needed, but they will produce a smaller volume of concentrate assuming a fixed volume of TWs being delivered to the sites. Therefore, the initial COD of the TWs selected will impact the suitability of the method.

The sewage sludge as well as the resin, chemical and personal care products TWs showed a pH below 6 that might negatively affect the AD process. Additionally, these TWs had low alkalinity with values as low as 0 mg CaCO₃/L for IA, which might be problematic for AD performance (Table 4.1 and Table 4.2). Nevertheless, mixing those TWs with high alkalinity sewage sludge could compensate for the potential negative effect on biogas and methane production. Moreover, the inoculum pH is generally higher with an average of 7.8 (Chapter 1), again helping mitigate any slightly acidic pH of TWs. As expected, solids increased in the concentrates and full rejection was achieved when concentrating chemical TWs with UF using a CF_v (concentration factor by volume) of 2.64 and personal care concentrated by MF and UF with a CF_v of 2.22 and 1.46 respectively (Table 4.1 and Table 4.2).

The TAN in the MF concentrate for the paper TW was 1400 mg TAN/L which based on previous work (Chapter 1) could be inhibitory resulting in 10% reduction of the biogas and methane yield if feed directly to the reactor without co-digestion with sewage sludge. The pH was not measured for the concentrates, but the high pH of the TWs fed to the membrane, 8.1, might increase the concentration of FAN in the concentrate of MF of paper industry above the TWs start value of 64 mg FAN/L up to 201 mg FAN/L if pH is kept constant (Table 4.2). Nevertheless, this value of FAN will be lower than the inhibitory value observed in Chapter 1 which identified as 255 mg FAN/L in reactors spiked with ammonium chloride.

The concentration of ions in the concentrates was expected to decrease as neither UF nor MF are able to reject dissolved ions or inorganic salts [34]. On the contrary to what was expected, the concentration of chloride doubled for the food industry TW from an initial value of 4634 mg Cl/L up to 9228 and 9418 mg Cl/L in the concentrate of MF and UF, respectively. Chloride in food industry might be contained in cleaning agents that could be big enough particles to be rejected by the UF and MF membrane [35–37]. Chloride measurement required a filtration of 0.45 µm to measure soluble, which is bigger than the pore size of the membranes used with 10 kDa and 300 kDa for UF and MF respectively. This difference in pore size allows for measuring colloids and proteins besides the soluble fraction in the analysis of chloride in the concentrate [38]. Therefore, the chloride concentrated in food industry should be from a colloidal compound that contains chloride as it was able to pass through 0.45 µm but not through the MF or UF membranes. Paper and personal care products TWs had a similar initial concentration of chloride, 4364 mg Cl/L, but the concentration decreased in the concentrates of the MF and UF to around 100 and 600 mg Cl/L, respectively, demonstrating in this case a low rejection of what is expected to be mostly soluble chloride. In the personal care products and food wastes, the concentration of sodium was also high with 1020 mg Na/L and 20900 mg Na/L, respectively, in the un-treated TWs, which further doubled or tripled in the MF and UF concentrates of the personal care products TW. The paper industry and resin industry wastes had a lower concentration of sodium at levels of 427 mg Na/L and 183 mg Na/L, but it was doubled in the concentration of TWs up to 773 mg Na/L and 436 mg Na/L, respectively, in the concentrate of UF. Similarly to chloride, the difference in pore sizes between the filter used for obtaining the solid free fraction of samples before analysis and the membranes used in the filtration of the TWs suggested that the sodium should be in a colloidal form, which is small enough to pass through a 0.45 µm filtrate, but was concentrated by the MF and UF membranes used [38]. The highest concentration of sodium was found in the food TW with a decrease from 20900 mg/L to

15700 and 14700 mg Na/L in the concentrates from the MF and UF suggesting that sodium in food TWs was in a smaller form than a colloid (Table 4.1 and Table 4.2). Comparing all these values with the values found in BMP tests (Chapter 2), concentrates from food, paper and personal care products TWs could be inhibitory if digested as sole substrates or their loads are not carefully managed in Aco-D because of their chloride levels in the MF and UF concentrates, while sodium levels are a concern in food TWs in the MF and UF concentrates. In the literature, a higher value of chloride in the feedstock, 5500mg/L, was observed to decrease performance in AD treating tannery wastewater [39]. Alternatively, paper and personal care products TWs contained chloride within the range previously studied, up to 4634 mg Cl/L in the reactor (Chapter 2), and are not expected to have a negative effect. For the personal care products TW, the concentration of sodium increased in the concentrates of the UF membrane up to 3000 mg Na/L, which is still lower than the inhibitory concentration found in a previous research of 6165 mg Na/L when spiking digesters with sodium chloride (Table 4.1 and Table 4.2) (Chapter 2).

The paper and personal care products TWs had also a high concentration of copper and zinc. The concentrate of the paper industry reached 860 and 315 mg Cu/L using MF and UF with a CF_V of 5.66 and 3.91 (Table 4.1 and Table 4.2). Despite the pores on UF being smaller there was a lower concentration retained than with the MF, which could result from more significant fouling and pore blocking in the MF further increasing its selectivity and reducing the passage of those elements through the membrane [40]. The copper levels recorded were within the range resulting in inhibition in previous experiments, where addition of copper chloride to reach 22 mg Cu/L decreased the methane yield by 61% (Chapter 2). The zinc concentration increased when paper and personal care products TWs were concentrated from 30 and 2 mg Zn/L up to 174 and 116 mg Zn/L using MF and up to 1882 and 82 mg Zn/L using UF, respectively (Table 4.1 and Table 4.2). A complete rejection of zinc was achieved during the concentration of the resin TW in MF as the CF_V was 3.23 that matched the concentration of zinc in the un-treated resin TWs characteristics.

Table 4.1. TWs, concentrates, inoculum and feed characteristics.

	Feed (PS and SAS mixture)	Inoculum	Resin industry			Leather industry			Chemical industry		
			TW	Conc. MF	Conc. UF	TW	Conc. MF	Conc. UF	TW	Conc. MF	Conc. UF
pH	5.3±0.2	-	5.2±0.02	-	-	6.3±0.1	-	-	5.2±0.01	-	-
COD total (mg/L)	46029±50	-	17817±379	54100±1096	52733±5126	6262±180	47450±13251	49700±926	20137±437	51550±2718	49200±7
COD soluble (mg/L)	5000±0	-	4707±15	-	-	2588±36	-	-	12233±247	-	-
Concentration factor COD	-	-	-	3.04	2.96	-	7.58	7.94	-	2.56	2.44
Concentration factor volume	-	-	-	3.23	8.49	-	8.20	8.23	-	4.01	2.53
TS (%)	4.47±0.61	2.19±0.23	-	3.66±0.09	3.12±0.02	0.48±0.006	2.79±0.02	3.29±0.49	0.81±0.005	1.88±0.13	2.08±0.01
VS (% over TS)	77±1	65±0.95	-	95±2.8	94±0.18	74±0.7	91±1.8	80±11	66±1	74±3.8	78±0.05
TAN (mg-N/L)	1921±5	-	31±0.4	59±1	72±5	21±1	72±6	425±44	75±12	76±4	76±1
FAN(mg-N/L)	0.49±0.47	-	0.006±0.0003	-	-	0.05±0.01	-	-	0.01±0.002	-	-
PA (mg CaCO ₃ /L)	2630±30	-	75±0.9	-	-	682±38	-	-	682±38	-	-
IA (mg CaCO ₃ /L)	328±10	-	0±0	-	-	278±8	-	-	0±0	-	-
Cl (mg/L)	126±3	124±16	29±1	7±0.4	ND	193±23	190±0	38±1	1238±47	700±20	696±9
NO ₃ (mg/L)	ND	20±0.03	ND	19±0	ND	13±12	35±13	ND	37±10	ND	19±0
SO ₄ (mg/L)	15±2	14±3	253±6	ND	ND	175±26	126±0	ND	45±23	ND	5±0
Na (mg/L)	196±6.95	142±2	183±12	163±10	436±5	1020±59	786±22	699±74	2110±153	1330±90	1820±102
Al (mg/L)	0.7±0.1	0.4±0.2	6±0.8	2±0.2	23±1	3±0.1	2±0.2	2±0.2	5±0.5	3±0.4	6±0.5
Cu (mg/L)	0±0.01	0.005±0.2	0.03±0.09	0.1±0.01	0.02±0.1	2±0.1	1±0.03	0.5±0.005	0.08±0.02	0.5±0.01	0.2±0.03
Zn (mg/L)	0.8±0.03	2±0.3	2±0.2	5±0.2	12±0.9	2±0.1	2±0.06	0.7±0.1	2±0.2	2±0.2	7±0.5
As (mg/L)	0.07±0.007	0.02±0.07	0±0.003	0.003±0.004	0.002±0.007	0±0.002	0.003±0.001	0.003±0.002	0.0007±0.003	0.007±0.005	0.01±0.008
Hg (mg/L)	0.005±0.0006	0.007±0.02	0.1±0.01	0.1±0.01	0.02±0.03	0.001±0.0005	0.01±0.001	0.02±0.001	0.003±0.006	0.03±0.007	0.04±0.001

ND: Not detected; Conc.: Concentrate

Table 4.2. TWs, concentrates, inoculum and feed characteristics.

	Food industry			Paper industry			Personal care products industry		
	TW	Conc. MF	Conc. UF	TW	Conc. MF	Conc. UF	TW	Conc. MF	Conc. UF
pH	6.3±0.2	-	-	8.1±0.04	-	-	5.8±0	-	-
COD total (mg COD/L)	32837±6314	52817±225	49750±1386	13033±671	49633±2743	50175±318	34800±2805	52750±996	52750±1361
COD soluble (mg COD/L)	11650±350	-	-	4320±370	-	-	3000±54	-	-
Concentration factor	-	1.61	1.52	-	3.81	3.85	-	1.52	1.52
Concentration factor volume	-	3.92	1.60	-	5.68	3.91	-	2.22	1.46
TS (%)	4.05±0.1	4.54±0.09	4.65±0.03	0.81±0.01	3±0.03	2.92±0.007	0.31±0.02	2.08±0.09	2.20±0.02
VS (% over TS)	42±2	48±0.17	48±0.44	76±1.8	75±0.39	78±0.44	55±3.3	72±1.8	73±1.9
TAN (mg-N/L)	545±7	591±92	1292±3	462±12	1440±38	222±0	14±1	24±3	43±1
FAN(mg-N/L)	1.4±0.6	-	-	64±6	-	-	0.01±0.0008	-	-
PA (mg CaCO ₃ /L)	2833±424	-	-	1929±250	-	-	0±0	-	-
IA (mg CaCO ₃ /L)	7076±1357	-	-	981±136	-	-	122±15	-	-
Cl (mg/L)	4634±963	9228±672	9418±347	4634±963	125±4	101±14	4634±963	651±59	642±15
NO ₃ (mg/L)	ND	ND	ND	ND	ND	19±0	ND	59±4	ND
SO ₄ (mg/L)	126±23	ND	ND	126±23	ND	ND	126±23	ND	5±0
Na (mg/L)	20900±1310	15700±565	14700±922	427±10	697±56	773±45	1020±50	1970±28	3000±174
Al (mg/L)	2±0.1	7±0.4	0.7±0.1	2±0.09	3±0.3	4±0.4	1±0.08	4±0.1	4±0.5
Cu (mg/L)	0.06±0.01	0.6±0.02	0.03±0.02	59±1	860±64	315±18	0±0.02	0.1±0.01	0±0.03
Zn (mg/L)	2±0.06	18±1	0.4±0.1	30±0.3	174±11	188±9	2±0.2	116±2	82±6
As (mg/L)	0±0.006	0.03±0.005	0.001±0.005	0.03±0.001	0.04±0.002	0.05±0.002	0.002±0.008	0.006±0.004	0.007±0.004
Hg (mg/L)	0.004±0.001	0.03±0.001	0.02±0.002	0.0008±0.0009	0.01±0.002	0.005±0.0006	0.004±0.0005	0.01±0.0008	0.008±0.0007

ND: Not detected; Conc.: Concentrate

4.3.2. Filtration

As expected, because of the smaller pore size of the UF membrane over the MF, the clean permeability and the TWs permeability were smaller for the UF with a range of 121 and 536 LMH/bar than for the MF with a range of 539 and 1349 LMH/bar (Table 4.3, Figure 4.1 and Figure 4.2). The pore size also affects the type of fouling. Choi *et al.* observed that bigger particles were blocking the pores more in MF than in UF, but in UF there was a more important adsorption of small elements on the membrane pores [40]. This behaviour would explain the difficulty in cleaning some of the UF membranes and their lower recovery compared with the MF. All MF membranes showed at first a rapid decrease in the permeability with a later stabilization. On the contrary, the UF membranes showed a flatter tendency with lower permeability than MF. This agrees with Choi *et al.*'s results, as they found a longer decrease of flux with MF than UF before the stabilization of the flux [40].

Table 4.3. Permeability with clean water before the start of the experiments.

TWs	Start Permeability (LMH/bar)	
	MF	UF
Chemical industry	1089	209
Food	849	121
Paper	862	265
Personal care	1349	536
Leather	1162	231
Resin	539	392

Most of the TWs concentrated with MF had a starting permeability of around 15-20 LMH/bar except for the personal care product and chemical TWs that started below 5 LMH/bar, highlighting an increased fouling propensity for the latter two. Nonetheless, all permeability values were smaller by one or two orders of magnitude than the clean water permeability for MF membranes, which was between 1349 and 862 LMH/bar except for the membrane used on TW resin that had a permeability of 539 LMH/bar (Table 4.3).

The use of resin TW with the MF membrane decreased the permeability to 7.65 LMH/bar in less than 10 minutes with a slope of -144 LMH/bar/h, and then, remained between 7.65 and 5.87 LMH/bar for another 3 h. Afterwards, the membrane was cleaned and used again to reach the aimed organic concentration in the concentrate and the recovery of membrane permeability was only about 11%, demonstrating the presence of irreversible fouling and highlighting the challenge to be faced in terms of operation of such system at full scale. The clean water permeability after cleaning reached a maximum of 160 LMH/bar, which was 70% lower than the clean permeability of that membrane before the experiment, 539 LMH/bar. Further treatment of resin TW reduced the permeability to 6 LMH/bar with a slope of -550.7 LMH/bar/h (Figure 4.1.a and Table 4.4). There was a high concentration of non-soluble COD being 74% of the total COD. Therefore, the step decrease at the beginning of the experiment might be because of solid fraction of the COD (Table 4.1).

The membrane used for the leather TW showed a sharp decrease of the permeability with a slope of -189.6 LMH/bar/h and a stable trend after 6 min of operation with a slope of -0.8 LMH/bar/h (Table 4.4). The first stage of the curve started at 21 LMH/bar decreasing to 12 LMH/bar in 6 minutes. The second stage decreased from 12 LMH/bar at 6 min from the start of the experiment to 6.6 LMH/bar at 5.2 h. The membrane recovery after the cleaning was slightly higher than for resin TWs with 29% of the initial clean water permeability. After the cleaning, the starting permeability with the leather TW was recovered up to 21.7 LHM/bar with a faster decrease than before, as the slope was -241.4 LMH/bar/h, to reach 6.7 LMH/bar in 2 min from the cleaning and 5.41 h from the start of the experiment (Figure 4.1.b and Table 4.4). The TS in the experiment concentrated from 0.48% TS in the raw leather TWs to 2.79% TS in the concentrate from leather TW at the end of the experiment (Table 4.1).

For the personal care products TW concentration two membranes were used as the fouling was significant compared with the other TWs. The clean water permeability was 1349 LMH/bar which decreased to 3 LMH/bar for the first membrane and 2 LMH/bar for the second membrane when TWs was added. These low permeability values were constant during 4 and 9 hours, respectively (Figure 4.1.c). Despite being easily fouled, the concentration of TS in the concentrate was smaller than for the previous TWs with 2%, and the CF_v was 2.22, which suggest that there might be other components besides solids blocking the flow through the membrane as with that CF_v should have a shorter filtration than other TWs (Table 4.2).

The paper TW MF membrane started with a clean water permeability of 862 LMH/bar that decreased to a starting permeability of 13 LMH/bar when TWs was added and further decreased to 5 LMH/bar in 5 minutes with a slope of -29.7 LMH/bar/h where it remained until 4 hours when the membrane was cleaned (Table 4.3, Figure 4.1.d and Table 4.4). The recovery of the clean water permeability of the membrane after its cleaning was 44.1%, which is the highest recovery observed for MF membranes. After the cleaning, the starting permeability with paper TW was 19 LMH/bar followed by a decrease with a slope of -186.7 LMH/bar/h on permeability of TW reaching 7 LMH/bar at 7 h from the start of the experiment (Figure 4.1.d and Table 4.4). The main difference in concentration from the paper TW and the other TWs is pH, as it is the most basic one with a value of 8.1 (Table 4.1). There might be an interaction between the pH and the fouling that allowed for a higher recovery of the membrane used.

The MF membrane used for the food TW filtration had a distillate water permeability of 849 LMH/bar (Table 4.3). The food TWs filtration started with 19 LMH/bar decreasing to 6 LMH/bar in half an hour with a slope of -30 LMH/bar/h, similar to the one of paper TW first filtration, and to 3 LMH/bar in 2.5 h. The recovery of the clean water permeability after cleaning for the MF membrane used for the food TWs was 38%, which is also close to the one of paper filtration in MF. After the cleaning, the start permeability reached 27 LMH/bar and decreased to 4 LMH/bar with a slope of -23.7 LMH/bar/h after 1.5 h from the cleaning (Figure 4.1.e and Table 4.4). Despite the high TS content of food TWs, 4.05%, the MF fouling was less severe than for other TWs tested as the recovery was the second highest one. This better recovery could be due to the fact that this TW has high levels of salts with sodium and chloride

concentrations of 15700 mg Na/L and 9228 mg Cl/L, which impacted the double layer in the organic matter and then prevented its attachment to the membrane [41,42] (Table 4.2).

Finally, the clean water permeability of the membrane used for MF of chemical TWs was 1089 LMH/bar. Chemical TWs permeability varied from 4 to 1 LMH/bar during 7h with the smallest slope in MF at the beginning of the experiment, -0.2 LMH/bar/h. Despite being the second lowest recovery of distillate permeability after the cleaning, 20%, the start point and the decreasing slope were higher than at the filtration before the cleaning (Table 4.3). After the cleaning, the clean water permeability was 216 LMH/bar at 7.36 h from the start of the experiment decreasing when TWs was added to the membrane reactor with a slope of -189.4 LMH/bar/h and after 2.4 min there was a variation with a slope of -2.4 LMH/bar/h to reach 8 LMH/bar at the end of the experiment at 9.3 h (Figure 4..f and Table 4.4).

Table 4.4. Rates of decrease in permeability over time for the different TWs in MF and UF.

<i>TWs</i> ↓	<i>Stage</i> →	Slope MF (LMH/bar/h)				Slope UF (LMH/bar/h)					
		1.1	1.2	2.1	2.2	1.1	1.2	2.1	2.2	3.1	3.2
Resin		-143.8	-0.3	-550.7		-1.9					
Leather		-189.6	-0.8	-241.4		-3.7	-0.8	-20.9	-0.2		
PC		0.07		-0.02		-7.7	1.1				
Paper		-29.7	-0.3	-186.7	-1	-33.8	-0.03				
Food		-30	-0.07	-23.7	0.4	-73		-49.2	-0.6	-78.5	-0.5
Chemical		-0.2		-189.4	-2.4	-3.6	-0.7	-8.6	-0.02	0.08	-0.8

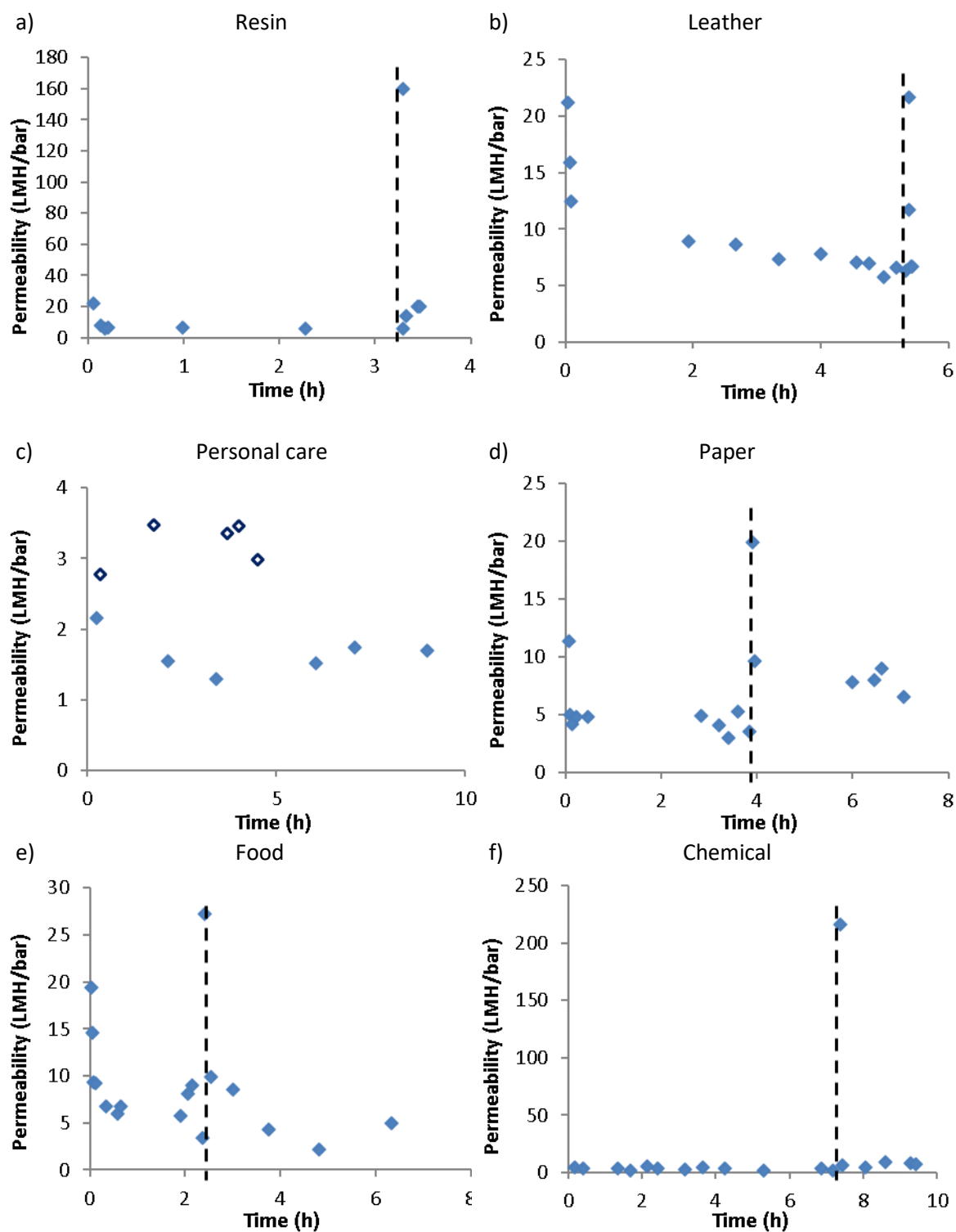


Figure 4.1. Permeability of TWs MF a) resin, b) leather, c) personal care, dark blue empty first membrane and light blue filled second membrane, d) paper e) food, f) chemical with a dark blue line representing the cleaning of the membrane.

The clean water permeability for the UF membrane used for the resin TW was 392 LMH/bar (Table 4.3). Resin TWs permeability was constant at 3 LMH/bar during 8.5 h with a slope of -1.9

LMH/bar/h (Figure 4.2.a and Table 4.4). The high content of TS 3.12% in the concentrate of this experiment explains the reduced starting permeability with TWs (Table 4.1).

The permeability with clean water for the membrane used for leather TWs was lower than the one used for resin with 231 LMH/bar (Table 4.3). For the leather TW, 2 membranes were used; the first membrane had a start permeability of 9 LMH/bar which decreased to 5 LMH/bar in 15 min with a slope of -3.7 LMH/bar/h, staying around that value for 1.5 h; the second membrane had a start permeability of 7 LMH/bar decreasing to 3.5 LMH/bar at 10 min with a steeper slope of -20.9 LMH/bar/h, being constant at that value until the end of the experiment (Figure 4.2.b and Table 4.4). The level of TS was 3.29% which was close to the one for the concentrate of UF of resin TW (Table 4.1 and Table 4.2).

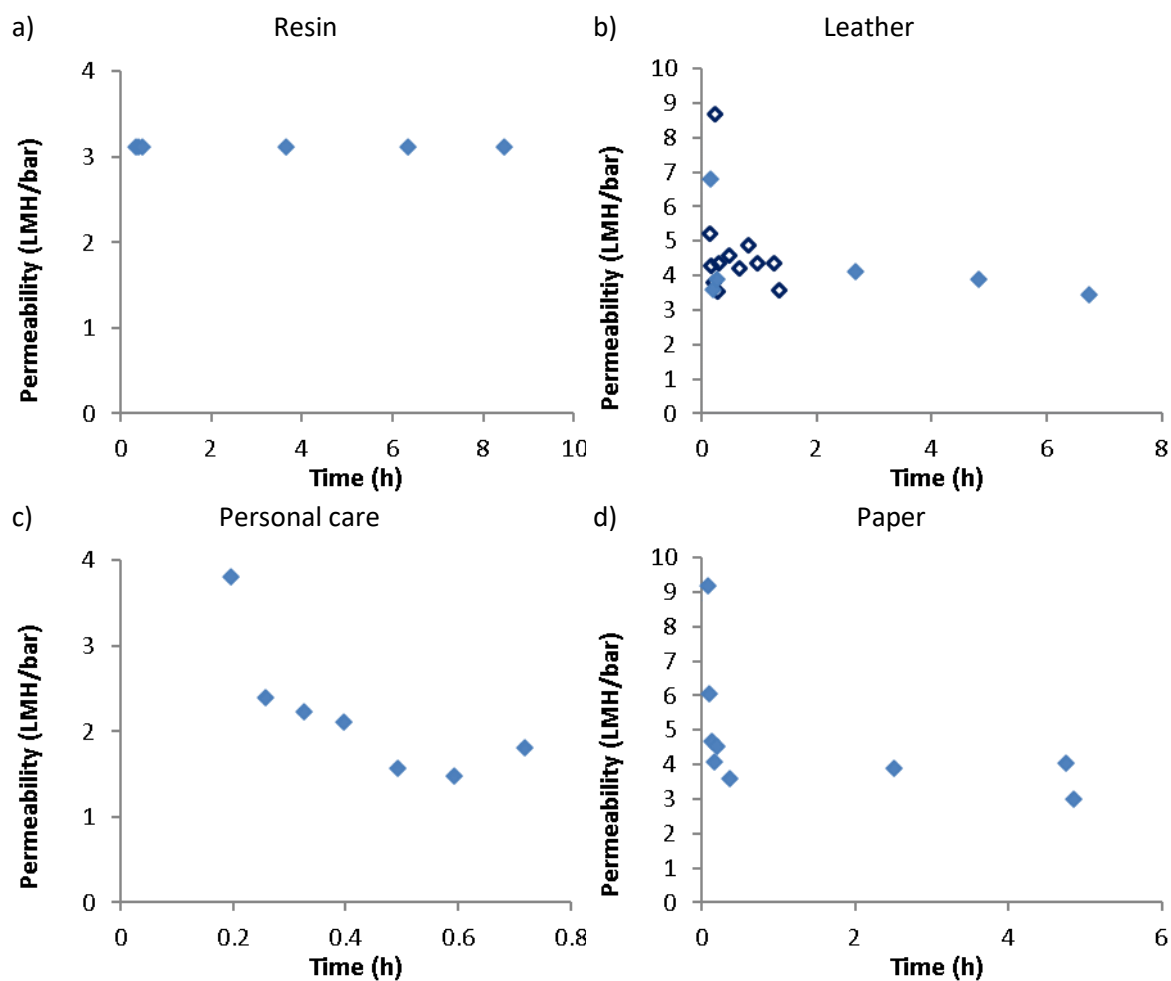
The clean water permeability of the UF membrane for the personal care product TW of 536 LMH/bar was the lowest initial permeability obtained for the UF membranes (Table 4.3). The personal care product TW addition decreased from a start membrane permeability of 4 LMH/bar to 2 LMH/bar in less than 1h with a slope of -7.7 LMH/bar/h (Figure 4.2.c and Table 4.4). Despite the low permeability, it should be noted that the filtration was quite efficient and short, less than one hour, probably because of the low CF_V required, 1.46 (Table 4.2).

The UF membrane used with the paper TW showed a clean water permeability similar to the membrane used in leather TW, 265 LMH/bar (Table 4.3). The starting permeability with paper TW was 9 LMH/bar and it decreased to 3.5 LMH/bar in 20 min then reaching 3 LMH/bar in 5 h with slopes of -33.8 and -0.03 LMH/bar/h, respectively (Figure 4.2.d and Table 4.4). The TS content in the concentrate was high at 2.92% compared with un-treated TWs, but similar to the TS from MF concentrate of the same TW (Table 4.2).

The clean water permeability of the UF membrane used for the food TW was the lowest with 121 LMH/bar (Table 4.3). The starting permeability with the food TW was the highest at 22 LMH/bar that then lowered to 7 LMH/bar in 10 min with a slope of -73 LMH/bar/h and recovered after the cleaning up to 17 LMH/bar with a later decrease to 8 LMH/bar in 20 min with a slope of -49.2 LMH/bar/h (Table 4.4). The recovery of the clean water permeability was the highest one with a value of 162.6%, which suggests an improvement of performance contrary to the reduction of performance of the membrane during the cleaning that it is generally accepted. The increase of performance after the cleaning could be caused by the presence of a chemical in the membrane to preserve it from the production site to its final use that could increase the resistance of the membrane. A second cleaning at 2h of the experiment recovered the clean water permeability by 53% and the trade wastes permeability up to 20 LMH/bar, lowering again to 5 LMH/bar in 2 min with a slope of -78.5 LMH/bar/h and reaching 1 LMH/bar after 4 h from the start of the experiment with a slope of -0.5 LMH/bar/h (Figure 4.2.e and Table 4.4). Filtration of food TWs improved in performance after the cleaning, which was surprising as it was the TW with higher TS in the concentrate with a value of 4.65%. Similarly to the MF, there was a high concentration of sodium and chloride in the concentrate

of the UF, 14700 mg Na/L and 9418 mg Cl/L that could have reduced fouling of the membrane (Table 4.2).

The clean water permeability for the UF membrane used for the chemical TW was 209 LMH/bar (Table 4.3). Chemical TWs permeability varied between 5 LMH/bar to 4 LMH/bar during 1 h with a slope of -3.6 and -0.7 LMH/bar/h (Figure 4.2.f and Table 4.4). The first cleaning recovery of distillate permeability was 43.5%. After the first cleaning, the permeability of TW decreased from 4 LMH/bar to 3 LMH/bar with a slope of -8.6 and -0.02 LMH/bar/h (Figure 4.2.f and Table 4.4). The second cleaning recovery of clean water permeability was much higher than the first one, 118.7%. The TWs permeability after the second cleaning at 6 h showed a permeability of 4 LMH/bar reduced to 3.5 LMH/bar after 1.5 h from the second cleaning with a slope of 0.08 LMH/bar/h. At 12 h from the start of the experiment, the permeability was reduced to 1 LMH/bar with a slope of -0.8 LMH/bar/h (Figure 4.2.f and Table 4.4). The filtration of the chemical TW with UF performed worse than food TW in the UF, even though the raw chemical TW had a TS content of 0.81% compared with raw food TWs with 4.05% (Table 4.1 and Table 4.2). This might again result from the higher concentration of ions that might reduce the fouling on the membrane.



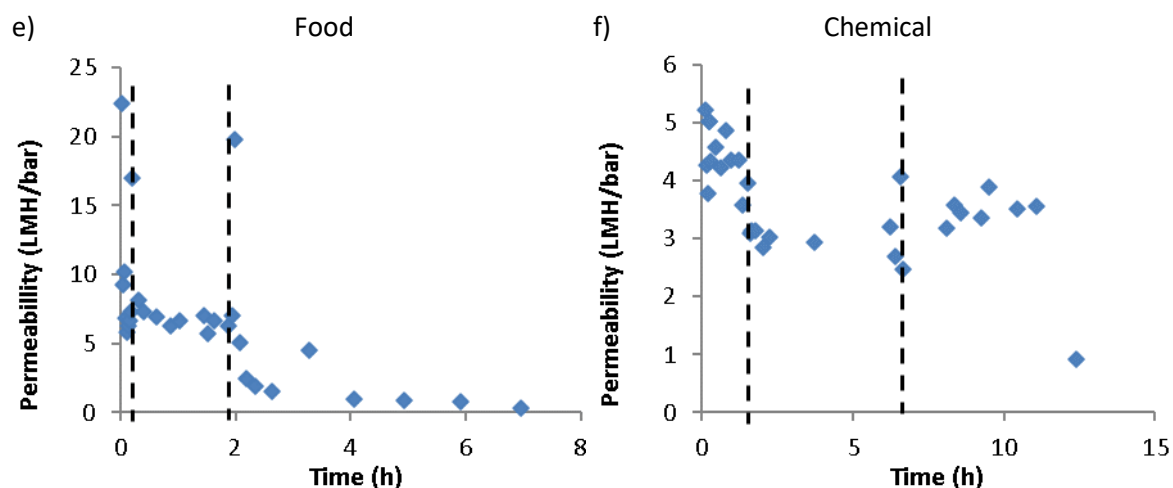


Figure 4.2. Permeability of TWs UF a) Resin, b) leather, dark blue empty first membrane and light blue filled second membrane, c) personal care, d) paper ink, e) food, f) chemical with a dark blue line representing the cleaning of the membrane

In the MF, the food TW reached a lower permeability than the resin TW after 3 h of the experiment with values of 3 LMH/bar and 6 LMH/bar respectively. Alternatively, the resin TW performed worse than the leather TW as the time required for the permeability to drop to 6 LMH/bar was shorter with 3 h for the resin TW against 5 h for the leather TW. However, after cleaning, the leather TW displayed a shorter time, 2 min, than the resin TW to reach a permeability value between 7 and 6 LMH/bar, suggesting more irreversible fouling. The paper TW performed worse than all the other TWs mentioned as a permeability of only 5 LMH/bar was reached within 5 min. The personal care products TW displayed the lower starting permeability, 3 and 2 LMH/bar, but it remained constant throughout the experiments of 4 and 9 hours for the two membranes used, demonstrating rapid but stable fouling. Finally, the chemical TW showed a starting permeability of 4 LMH/bar reaching 1 LMH/bar over 7 h. After cleaning, the permeability was higher, but the time of the experiment was shorter with 2 h again suggesting presence of irreversible fouling. Therefore, the use of the personal care products and chemical TWs might be problematic in MF from an operational point of view.

In the UF, the personal care products TW showed the shorter time to achieve the desired concentration <1 h with a permeability variation from 2 LMH/bar to 4 LMH/bar. Despite the low permeability, the CFV required was small enough to have a fast filtration time. the food TW showed the best starting permeability with 22 LMH/bar, with an increase up to 4 h of the time needed to achieve the desired concentration. The paper and leather TWs showed similar starting permeabilities at 9 LMH/bar. Nonetheless, with the leather TW two membranes were needed while paper used only one membrane to achieve the desired concentration. The longest to filtrate were the resin and chemical TWs, 8.5 h and 12 h, due to their low starting permeabilities of 5 LMH/bar and 3 LMH/bar respectively. MF seemed to be performing better with leather, resin, food and paper TWs while the UF showed better performance with the personal care, food, paper and leather TWs.

Even though these laboratory scale experiments are not modelling full scale conditions, they provide some understanding on the fouling and other limitations that could be encountered at full scale. The filtration performance will depend on the CF_V and the permeability. Moreover, the cleaning performance will affect the reuse of the membranes, hence affecting the cost of buying the membrane or chemicals needed. A maximum CF_V of 10 is suggested as a higher value will be unsuitable for this treatment route because of the duration of the filtration step and the energy required as the TWs that had a CF_V above 8 the experiments lasted between 6.7 and 8 h in resin MF, and leather MF and UF (Figure 4.1, Figure 4.2, Table 4.1 and Table 4.2). If membrane-based pre-concentration is applied on treatment plants, it should be noted that CF_V will vary depending on TWs COD content and will require a change of method when different TWs are concentrated. The MF performed better with the resin, leather and chemical TWs while personal care products and paper TWs performed better with the UF. The food TW was the only one that performed similarly in UF and MF filtration. Comparing the shape of the filtration graphs with previous research, MF membranes showed a quick decrease between -30 to -187 LMH/bar/h, which is indicative of pore blocking, followed by a slowly decrease to almost a constant permeability which could be related to a cake layer formation. Personal care TWs and chemical TWs before cleaning showed an almost constant permeability from the beginning of the experiments suggesting a quicker formation of the cake layer [43] (Table 4.4). TWs concentrated using UF showed low permeability slopes between 0 and -8 LMH/bar/h suggesting as well a quick formation of cake layer with the exception of the paper and food TWs that had first a steep slope between -34 and -73 LMH/bar/h with a later period of slope decrease to almost 0 LMH/bar/h [43] (Table 4.4). Similar behaviour was observed by Lin *et al.* that found that a small pore size, 10 KDa, membrane tend to be fouled by a gel layer while the higher pore membrane, 100 KDa, showed an inner pore blocking together with a gel layer formation [44].

4.3.3. Impact of concentrated TWs addition to sewage sludge ADs

The concentrates produced from each waste with both types of membranes were then co-digested with sewage sludge in BMP tests (Figure 4.3 as an example, Figure C1 in appendix for all).

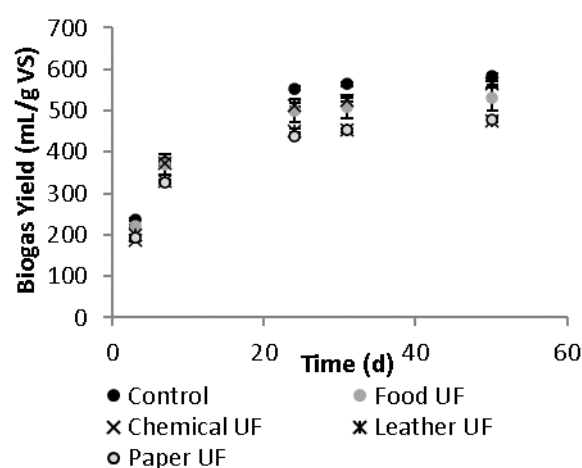


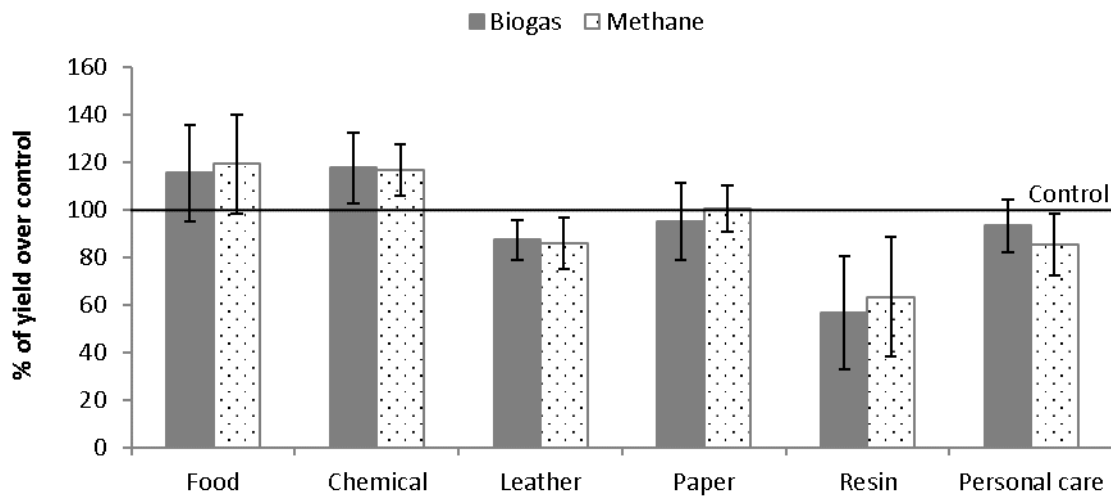
Figure 4.3. Example of cumulative biogas production for BMP with concentrated TWs as 10% of feed and sewage sludge as 90% of feed.

The chemical and food TWs concentrates from the MF resulted in biogas uplifts when compared with control units treating only sewage sludge of 18% and 15% for biogas and 17% and 19% for methane (Figure 4.4). Biogas quality in terms of methane content remained unaltered, except for the UF concentrate of the chemical TW, which resulted in significantly higher methane levels, 399 mL/g VS, respect the control, 339 mL/g VS ($F > F_{crit}$, Table C1 in appendix). Alternatively, the food TW concentrate from the MF did not show a statistical difference due to the high variance of the experiment, 3623 mL²/g VS² for biogas and 550 mL²/g VS² for methane ($F < F_{crit}$, Table C1 in appendix). The MF concentrates from the paper, personal care products and leather TWs resulted in similar or slightly lower yields than the control reactors for biogas and methane, although those differences were found not to be statistically significant ($F < F_{crit}$, Table C1 in appendix). Finally, the resin TW concentrate from the MF showed an average reduction of 43% and 37% of biogas and methane respectively from the control values (Figure 4.4), but there was not a statistically significant difference between them due to the high variance of the experiment, 15671 mL²/g VS² for biogas and 6185 mL²/g VS² for methane ($F < F_{crit}$, Table C1 in appendix). Therefore, the MF concentrate of chemical TW is beneficial for sewage sludge AD methane production and the rest of the TWs showed statistically non-effect for the digestion.

The average methane production of food, chemical and personal care TWs from UF increased by 15%, 21% and 12%, respectively, from the control (Figure 4.4), but they were not significantly beneficial because of their high variances, 16367, 907 and 174 mL²/g VS² for biogas and 9637, 366 and 532 mL²/g VS² for methane respectively. There was a significant inhibition of biogas in the leather and paper TWs UF concentrates and inhibition of methane for the resin UF concentrate with a reduction of 19%, 18% and 35%, respectively (Figure 4.4). The paper TW UF concentrate contained a concentration of copper that might negatively impact sewage sludge digestion and the resin TW concentrate in UF was characterised by a low pH of 5.2, which increased to 6 when mixed with sewage sludge and inoculum for the BMP tests but remained below the optimum of 6.5-7.5 for AD. The concentrate of TWs from paper

industry had a concentration of 315 mg/L of copper which is above the inhibitory values found in the literature where a 21% methane inhibition was found with the addition of 50 mg/L of copper in an ESGB [45]. Resin TW UF concentrate contain a value of zinc of 12 mg/L which is above the value found in a sucrose batch test that showed 50% inhibition of the methanogens at 7.5 mg/L of zinc [46]. Chemical TW had similar pH value than resin TW, 5.2, but the higher partial alkalinity of 682 mg CaCO₃/L could have limited a negative effect of the chemical TW concentrates (Table 4.1 and Table 4.2). The rest of TWs concentrates did not show a significant impact.

a)



b)

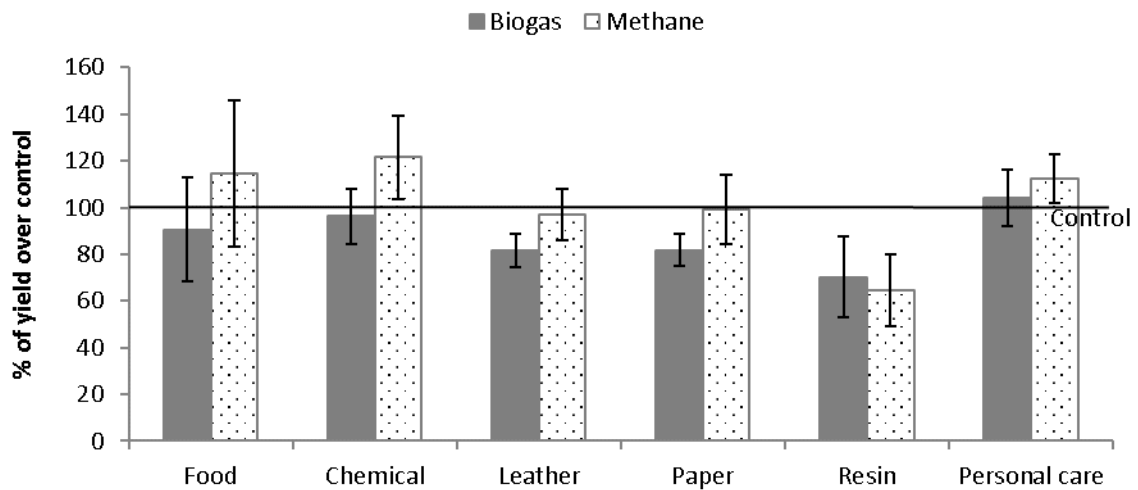


Figure 4.4. Percentage of yield over the control for biogas and methane when co-digesting sewage sludge with concentrates from (a) MF and (b) UF.

Therefore, most of the concentrated TWs with MF and UF showed a beneficial effect or non-effect for methane production except for the resin UF concentrate that inhibited methane production. Previous research on co-digestion of TWs and sewage sludge has reported beneficial impacts, although a pre-concentration step has not been previously investigated. Xie

et al. obtained a beneficial effect in the co-digestion of sewage sludge and paper TWs compared with their respective mono-digestion, 159 and 157 mL/g VS respectively, compared with 368 mL/g VS in Aco-D [5]. Benito-Mora *et al.* reported an improved methane production when co-digesting olive mill TWs and sewage sludge with values ranging from 353 and 374 mL/g VS compared with mono-digestion of sewage sludge at 333 mL/g VS [47].

First-order kinetics and Gompertz model were applied to investigate biogas kinetics in the BMP experiments. Both models were used to provide an estimate of the hydrolysis constant using First-order kinetics and to account for the lag phase using Gompertz model [13]. Gompertz model gives a better estimate for biogas yield when there is a lag phase whilst First-order kinetic is preferred for biogas yield estimate when there is no lag phase. In the current research, there was a better fitting with the Gompertz model for most of the tests except for the control for the experiment, and experiments using the MF concentrates of the leather, resin and personal care products TWs which showed a better fit in First-order kinetics as r^2 was higher and RMSE_n lower than in the Gompertz model.

The kinetics of biogas formation showed that the digestion in the control reactors was faster than when the concentrates of the chemical, leather and paper TWs from UF were mixed with the feedstock. The Gompertz model showed that R_m decreased from 70 mL/g VS/d to 60 mL/g VS/d when chemical TW UF concentrate was used with a good fitting in that model as r^2 was 0.952 and 0.983 and RMSE_n 0.08 and 0.04 respectively (Table 4.5). The leather and paper TWs UF concentrates showed slower digestion as R_m was also 60 mL/g VS/d in Gompertz model with a r^2 0.959 and 0.957 respectively and RMSE_n 0.06 in both showing a good fitting, and they had a smaller biogas yield, 80% of the control yield that had a R_m of 70 mL/g VS/d (Table 4.5). Therefore, the substrate degradation rate was slightly negatively impacted when those concentrates were added. The personal care products and resin TWs concentrate from UF had also slower digestion than the control units with k_h from the First-order model decreasing from 0.36 d⁻¹ to 0.32 and 0.34 d⁻¹ when they were added (Table 4.5). Nevertheless, R_m in the Gompertz model increased from 86 mL/g VS/d in the control to 117 mL/g VS/d and 121 mL/g VS/d when the resin and personal care products TWs UF concentrates were used while the lag phase also increased up to 0.94 d and 0.5 d, respectively (Table 4.5). Gompertz model showed a better fitting than First-order kinetics in those two TWs with r^2 values of 0.956 and 0.986 and RMSE_n of 0.06 and 0.03 (Table 4.5). Therefore, the addition of those two concentrates seems to produce a delay because of the increase on lag phase and a later increase on substrate degradation because of the increase on R_m .

Food TWs concentrate from MF showed an improved degradation rate as R_m increased from 86 to 100 mL/g VS/d in Gompertz model with a good fitting of r^2 and RMSE_n with values of 0.984 and 0.03 respectively (Table 4.5). Contrary, in MF concentrates for the chemical and paper TWs, the digestion using the First-order model showed a smaller k_h from 0.36 d⁻¹ in the control to 0.34 d⁻¹ and 0.31 d⁻¹ with a fitting to that model of 0.923 and 0.908 r^2 and 0.07 and 0.08 RMSE_n respectively. The lag phase from the Gompertz model was slightly higher than the control for those two concentrates with a value of 0.10 d for the chemical TW concentrate and

0.15 d for the paper TW concentrate, as opposed to the 0.01 d of the control units (Table 4.5). However, the chemical TW concentrate from the MF showed an increase on the R_m using the Gompertz model suggesting an improved performance on the substrate degradation (Table 4.5). For both of those concentrates Gompertz model showed a better fitting than First-order kinetics with values of r^2 and $RMSE_n$ of 0.980 and 0.04 for both the chemical and paper TWs concentrate in MF. These results suggest that food and chemical concentrates from MF were beneficial on degrading the substrate while the paper concentrate from MF delayed substrate degradation. The leather TW concentrated with the MF showed a better fitting using First-order kinetics with r^2 and $RMSE_n$ values of 0.883 and 0.03, respectively, showing an increase on k_h up to 0.41 d^{-1} (Table 4.5). On the contrary, the resin and personal care products TWs concentrates from the MF had slow digestion as k_h and R_m in First-order and Gompertz model decreased from 0.28 d^{-1} and 50 mL/g VS/d to 0.23 d^{-1} and 25 mL/g VS/d in resin, and 0.24 d^{-1} and 42 mL/g VS/d in personal care. In these two last TWs, there is a better fitting for First-order kinetics with a r^2 of 0.957 and 0.94904 for resin and personal care MF concentrates, respectively, and a $RMSE_n$ of 0.03 and 0.04 for resin and personal care MF concentrates, respectively, suggesting a slow degradation of the substrate when those concentrates were added (Table 4.5).

Table 4.5. Kinetic parameters using 1st order and Gompertz models for biogas production.

	1st order				Gompertz model				
	$k_h\text{ (d}^{-1}\text{)}$	$P_{\max}\text{ (mL/g VS)}$	$RMSE_n$	r^2	$R_m\text{ (mL/g VS/d)}$	$\lambda\text{ (d)}$	$P_{\max}\text{ (mL/g VS)}$	$RMSE_n$	r^2
Control *	0.20	607	0.13	0.829	70	0.00	555	0.08	0.952
Control **	0.36	323	0.07	0.918	86	0.01	319	0.04	0.973
Control ***	0.28	275	0.03	0.951	50	0.00	268	0.04	0.935
Food UF *	0.21	546	0.13	0.816	68	0.00	503	0.06	0.963
Chemical UF *	0.16	592	0.19	0.756	60	0.00	531	0.04	0.983
Leather UF *	0.21	491	0.13	0.832	61	0.00	451	0.06	0.959
Paper UF *	0.21	487	0.14	0.811	60	0.00	448	0.06	0.957
Resin UF **	0.32	224	0.09	0.875	117	0.94	221	0.06	0.956
Personal care UF **	0.34	345	0.07	0.932	121	0.50	339	0.03	0.986
Food MF **	0.36	381	0.06	0.938	100	0.00	375	0.03	0.984
Chemical MF **	0.34	386	0.07	0.923	103	0.10	380	0.04	0.980
Paper MF **	0.31	313	0.08	0.908	79	0.15	307	0.04	0.980
Leather MF ***	0.41	238	0.03	0.883	57	0.00	237	0.04	0.861
Resin MF ***	0.23	160	0.04	0.957	25	0.00	152	0.06	0.903
Personal care MF ***	0.24	258	0.04	0.949	42	0.00	248	0.05	0.921

*First experiment BMP, ** Second experiment BMP, and *** Third experiment BMP

4.3.4. Pre-concentration Economical Benefit

Once the methane production was calculated the economic value of that benefit or cost was evaluated. Moreover, assuming that AD flow of 10% was composed by the TWs studied in this paper a reduction from the BNR aeration was calculated based on the organics and nutrients from the TWs studied. The highest benefit from using concentrated TWs in AD instead of sending that TWs to the BNR were from the chemical and food TWs concentrates from both MF and UF with a benefit of £106,549, £177,012, £161,149 and £159,698 per year, respectively (Figure 4.5). The next TWs concentrates to show a benefit are the concentrates of the leather TW from the UF, the paper TW from both the MF and UF and the personal care products TW from the UF with a value of £40,383, £5,274, £52,596 and £39,468 per year (Figure 4.5). Paper concentrated with the MF showed a benefit from the avoided cost of the aeration as it showed a value of £10,366 per year (Figure 4.5). The resin TW concentrates from both MF and UF, and leather and personal care products concentrates from the UF showed a loss of £123,573, £139,685, £29,932 and £45,700 per year as the production of methane was reduced and the avoided cost for aeration was not enough to compensate these losses (Figure 4.5). The UF concentrates showed to have most benefit when added to the AD except for the resin concentrates showing that TWs concentrates could provide an economic benefit.

The resin, leather and chemical TWs were classified as chemical TWs in Chapter 2 accounting for 13% of TWs below 50 g COD/L, food TW represented 14% of all TWs below 50 g COD/L, and the personal care products might belong to soap containing TWs group, which was 2% of all the TWs below 50 g COD/L. Therefore, the two TWs that generates the highest benefit, food and chemical, represents 27% of the total TWs.

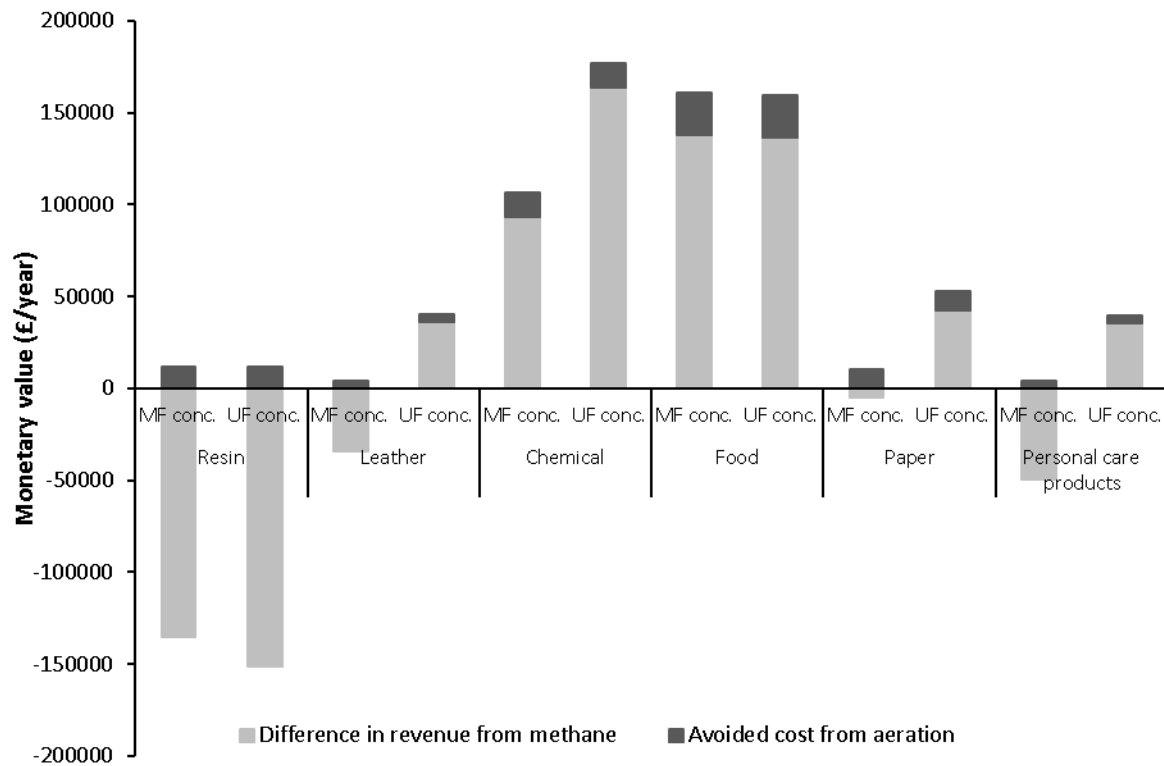


Figure 4.5. Income of biogas production from AD and reduction in energy consumption from BNR because of using the pre-concentration step in part of the TWs going to BNR to use them in Aco-D with sewage sludge

4.4. Conclusion

The use of MF and UF as pre-concentration step for TWs before Aco-D of TWs with sewage sludge was studied. The filtration experiments provided an understanding of the fouling of the membrane and performance. Most of the MF membranes showed a first step decrease in permeability with an almost constant permeability afterwards suggesting a first inner blocking occurred and then a cake layer was formed. UF did not show that initial rapid decrease and most of the TWs showed an almost constant permeability suggesting that a cake layer was formed more quickly than for MF. The resin, leather and chemical TWs showed a better performance in the MF, while the personal care products and paper TWs performed better in the UF. The food industry was the only one that performed similarly in UF and MF membranes. The performance order with MF was leather > resin > food > paper > personal care > chemical and in UF was personal care > food > paper = leather > resin = chemical. Therefore, chemical TWs will be a difficult TWs to operate with both membranes.

Most of TWs kept the biogas and methane productions obtained in sewage sludge ADs, with biogas uplifts of 17% recorded for the MF concentrate of the chemical TW. Nonetheless, the use of concentrates from UF for the leather and paper TWs reduced biogas production by 18-19%, but not methane production that was kept to similar values as the control. The only concentrate that reduced significantly the methane was the resin TW when UF was used. Therefore, the use of MF as a pre-concentration step will not decrease AD performance. Most of the TWs showed an economic beneficial value with the UF concentrates ranging from £5,274 to £177,012 year, except for the resin TWs that did not show any beneficial economic value in any of the concentrates, suggesting a beneficial value from the pre-concentration of TWs before AD. Therefore, the use in Aco-D with sewage sludge of concentrated TWs from chemical and food industries using UF membrane has a potential to improve biogas production.

4.5. References

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CHAPTER 5: Implications of the Work: Perspectives on the Utilisation of Trade Wastes in Anaerobic Co-digestion of Sewage Sludge

The aim of this thesis was to understand the effects of trade wastes (TWs) addition in anaerobic co-digestion (Aco-D) with sewage sludge to support TWs selection in view of maximising the benefits and reducing the risks of process inhibition. This aim was addressed in the previous Chapters (Chapter 2-4) which demonstrated the importance of pollutant levels from the TWs, their possible accumulation or acclimation in the anaerobic digestion (AD) system, all highlighting the need for controlled addition and more specifically for balancing the loads. This understanding of Aco-D of sewage sludge and TWs would be helpful for water utilities to select TWs that can be dosed into sewage sludge treating ADs without hindering biogas and methane production. This section will discuss the implications of this work to the industry, the selection of TWs for Aco-D with sewage sludge, and the mechanisms behind that Aco-D of sewage sludge and TWs. The key findings of this thesis are:

- Inhibition was observed for ammonia, sulphate, zinc, chloride, copper, aluminium and arsenate in the range of concentration studied in this work.
- 10% and 1% addition of TWs volume to AD of sewage sludge will be problematic for 14% and 1% of the TWs studied respectively.
- Nitrate addition in semi-continuous sewage sludge reactors caused non-inhibition up to 700 mg/L and a negative effect at 2000 mg/L because of the denitrifiers competition or generation of toxic by-products such as NO and N₂O.
- Zinc addition in a semi-continuous system treating sewage sludge showed inhibition at 700 mg/L and an accumulation of zinc in the solid fraction because of precipitation or adsorption to organic matter.
- Pre-concentration of TWs prior AD seemed to have a potential to increase the energy produced and reduce the energy demand from BNR in certain TWs, such as chemical and food TWs when UF was used.

5.1. Is there a benefit for the water industry to treat trade wastes in anaerobic co-digestion with sewage sludge?

The benefits of co-digesting sewage sludge with TWs are i) revenue from the gate fee, ii) an increase in energy production that will depend on TWs characteristics and load to the reactor and iii) a reduction of energy consumption in the main wastewater treatment line.

The water utilities accepting TWs will benefit from the additional revenue resulting from gate fees to be paid by the waste producers [1]. Considering the data from Chapter 2 gathered from

real TWs characteristics obtained from a water company and using the Mogden formula, which is the formula used by water utilities in England to calculate the cost of TWs based on their organics and solids content and volume to be treated [2], the prices can then be calculated. TWs currently regarded as suitable to be fed to the anaerobic digesters had a potential income range for water utilities of 49-639 £/m³ and the ones stored for controlled dosing to AD (chemical oxygen demand (COD) > 250 g/L) had a range of 578-830 £/m³. TWs that are mixed with the sewage at the head of the works for treatment in the biological nutrients removal (BNR) process have a potential income range of 0.65-87 £/m³. This additional revenue can constitute a benefit for water utilities even when accepting TWs on site that do not directly yield biogas and methane increases in the anaerobic digesters. Currently, TWs accepted on UK sites are treated in the BNR units, due to the perception of this process to be more toxic resistant than AD [3] and to the current legislation limitations for co-digesting different feedstocks in sewage sludge digestion centres [4]. Economic benefits to water utilities could be further increased if TWs currently treated within the main sewage treatment line could be diverted to the ADs, which would result in less aeration demand to treat the additional organics and nutrients in the BNR units. Considering the example wastewater treatment plant (WWTP) from Chapter 4, this represents a revenue for the lower strength TWs of about £68,000 to £9 million per year and an additional £2 to £30 million per year for the mid-strength wastes that could be sent to the AD (assuming 10% of feed load).

The results from Chapter 2 and 4 demonstrated that it is possible to identify ranges of pollutants concentrations and/or specific TWs with which an increase in biogas and more importantly methane production over sewage sludge alone is achieved. The increase in energy produced from the bio-methane will provide additional revenue (or savings if used on site) for the water utilities. To illustrate, TWs that result in an increase of total ammonia nitrogen (TAN) concentration in the reactor to levels of 635 to 1460 mg TAN/L have been seen as beneficial to sewage sludge digestion, with increases of methane production of 9% in biological methane potential (BMP) tests (Chapter 2), corresponding to an increase of £141,425 per year in income for the example WWTP from Chapter 4. Greater benefits would be expected when treating real TWs, as the addition of soluble organics that would further benefit the digestion process was not accounted for in those experiments. Hence, those concentrations that were regarded as resulting in “non-effect” in Chapter 2 could have a potential for improving the performance of the AD of sewage sludge due to organic matter conversion, as long as none of the TW constituents reach inhibitory or toxic concentrations. Concentrated TWs such as chemical TWs with microfiltration showed an improved methane production of 17% above the control, equating to an estimated economic benefit of £93375 per year for the example WWTP (Chapter 4). Therefore, TWs with individual constituents in concentration ranges resulting in “beneficial” are encouraged to be co-digested with sewage sludge as they will have the potential to increase biogas and methane production when organic fraction is considered. The ranges of pollutants determined in the current work will then be of critical importance for the industry to determine the appropriate wastes and loads to be added to the AD plants to ensure optimum operation and benefits. Finally, the diversion of some of the TWs currently

sent to WWTPs to the AD plants was shown, by reducing the load of organics and nutrients onto the WWTP, to reduce the energy use of the biological system and consequently would allow the utilities to achieve savings. To illustrate, for the wastes tested in Chapter 4, savings of between £3,786 and £23,433 per year were calculated for the example WWTP. Overall, a reduction in energy use on WWTPs combined to an increase in renewable energy production through AD plants would also enable the water utilities to achieve the renewable energy targets set for the industry [5].

Finally, it is also important to consider that the stabilisation of sewage sludge in AD has the additional advantage of reducing malodours, pathogen content and volume, which, in the United Kingdom, ultimately allows for digestate to be recycled to agricultural land as an organic fertilizer rich in nutrients [6,7]. However, the addition of TWs to anaerobic digesters could increase the content of heavy metals and other pollutants in the final bio-solids, which might hinder this recycling route [8]. Metals fractionation between the solid and liquid phases of sludge are critical in determining metal levels in final dewatered sludge cakes and hence need to be further investigated. The possible adsorption of zinc into the sludge solid phase was observed in Chapter 3 as there was a difference between the calculated outlet zinc concentration for the test reactors and the measured soluble one, which was hypothesised to result from the adsorption of zinc on the solids of sewage sludge or precipitate formation. Therefore, it is critical to further investigate metal fate on digestates, particularly but not limited to those resulting from Aco-D of TWs with sewage sludge, and its implication in land application of sewage sludge.

5.2. How can water utilities select TWs based on their characteristics to improve the decision-making process?

As discussed in Chapter 2, TWs should be grouped by their characteristics rather than by their origin. BMP and semi-continuous experiments spiked with different concentrations of typical TWs' constituents allowed for TWs to be divided into beneficial, non-effect, inhibitory and toxic depending on their load to the digesters. This division of TWs by characteristics could be shaped in a more complex decision-support tool (e.g. excel or application based) which could suggest safe TWs loads based on concentrations of its constituents. To further improve the accuracy of this tool, the synergistic and antagonistic effects of multiple TWs' constituents in AD could be investigated. A further improvement would be to consider opportunities for blending different TWs in order to balance the toxicity of some by diluting with less concentrated TWs.

Chapter 2 identified a TWs/sewage sludge volumetric ratio of 10/90 as the most suitable to minimise toxicity of TWs (Chapter 2), with only 13.7% of TWs constituting an inhibitory risk at this load out of the 160 TWs investigated. Nonetheless, if only 1% of TWs is added to sewage sludge reactors only 0.6% of TWs will be inhibitory. There were also some TWs with low enough concentrations of the compounds of interest to be considered in higher loads than 10% or for them to be of use for dilution of TWs with high concentration of the compounds of

interest. Some of the more diluted TWs currently treated in the main wastewater treatment line could also be used to balance benefit and inhibition of TWs going to the ADs. Therefore, some TWs initially considered as inhibitory or toxic could still be treated in Aco-D if they are stored and slowly mixed with less concentrated TWs to avoid inhibitory concentration on the AD reactors.

Furthermore, managing variable TWs that would need mixing or other pre-treatments before AD requires storage tanks whose cost could be around £1290 for 9.255 m³ capacity [9] for storage and mixing of TWs to control their characteristics when arriving at the site. To get better control of TWs added and their characteristics, the use of quick tests for TWs characterisation should be paramount, such as the use of cell test kits or titration. As TWs analyses are not currently carried out for every batch, there is a need for a more detailed characterisation strategy on site. Methods to do analysis such as COD, solids, pH, ammonia and sulphate might be available on site. However, tests for heavy metals or chloride might not be so common in water utilities and samples are sent to external laboratories for their analysis. Therefore, the use of cell tests kits or titrators to analyse the key components such as the ones studied in Chapter 2 will allow keeping a track of the TWs characteristics and ensure precise addition to the AD in suitable ranges. Other methods that could be used in water utilities are BMP tests to observe the biodegradability of the substrate, but this method has limitations to understand the acclimation and accumulation of the compounds and additional information from the literature and characterisation data from TWs should be considered.

Understanding acclimation of microbial communities to inhibitory compounds may, together with mixing different TWs, enable treatment of wastes initially categorised as inhibitory. A division of the studied TWs according to their impact on AD was carried out in Chapter 2, but trials with zinc and nitrate in Chapter 3 resulted in different inhibitory concentrations in the continuous experiments compared with batch systems. This was because of accumulation and transformation of the compounds when continuously added to sewage sludge digesters. The semi-continuous systems could cope with a nitrate concentration of up to 700 mg NO₃/L using sodium nitrate in the reactor as nitrate was being consumed showing non-effect on biogas and methane production, compared with the inhibitory concentration of nitrate of 174 mg NO₃/L using sodium nitrate observed in batch units. Zinc was not consumed but adsorbed to the solids in sewage sludge or precipitated in the semi-continuous reactor. However, this process was overcome by the zinc concentration added to the reactor reducing methane production to zero when 700 mg Zn/L was aimed increasing the soluble zinc concentration to 2750 mg Zn/L. Contrary, the batch units showed a production below the blank when the concentration of zinc was 1676 mg Zn/L using zinc sulphate. Nonetheless, the semi-continuous system was able to recover from zinc inhibition. Therefore, once the reactor suffered from a high dose of heavy metals it could be recovered by feeding sewage sludge with low concentrations of that metal, with results of Chapter 3 suggesting that recovery could take place within 1 to 2.4 hydraulic retention time (HRT), which equated to 16 to 33 days in the trials conducted. Accumulation of heavy metals such as zinc could be avoided in full-scale assets by alternating feeding of TWs

rich on heavy metals with more diluted wastes, or by suitable blending of TWs prior to dosing to the ADs.

Therefore, the limits found in this study (Chapter 2) for concentrations of typical TWs constituents that allow stable digestion together with a more detailed characterisation of TWs on-site should suffice to inform which TWs arriving to site can be co-digested in existing sewage sludge treatment assets. Selection could be further improved by the continuous experiments (Chapter 3), which highlighted the possible accumulation of heavy metals and the importance of blending TWs of different characteristics or alternating periods where TWs with high and low concentrations of heavy metals are dosed to AD. Future research should focus on quick tests for TWs characterisation and TWs division based on characterisation, which could be enhanced by the use of machine learning algorithms.

In Chapter 2, two types of inhibition were described: yield and kinetic inhibitions. Yield inhibition showed the effect on biogas and methane production which could be used to know the manageable concentration of that pollutant in the system and give a limit of TWs addition for water utilities. Lag phase was in most cases lower than the HRT in the AD reactor treating sewage sludge on actual sites, which is minimum 14 days. Nonetheless, lag phases longer than the unit HRT were observed in the experiments with 760 mg Zn/L of zinc sulphate, 1309 mg Al/L of aluminium sulphate with a pH of 5.3 and those lowering the pH to 5.2 and 4.5, where lag phases of 21 d, 50 d, 16 d and 25 d respectively were recorded. The TWs with those characteristics could result in a decrease of biogas production when dosed in continuous ADs, as the resulting lag phases were greater than the reactor's HRT (14 days).

There was an accumulation of volatile fatty acids (VFA) in copper, zinc and nitrate experiments that suggested inhibition of methanogenic communities but not of the acidogenesis phase (Chapter 2 and Chapter 3). Copper addition in batch experiments up to 811 mg Cu/L inhibited methanogenic activity increasing VFAs up to 613 mg Acetate/L (Chapter 2). Zinc was inhibiting the methane production at 1676mg Zn/L in batch test and 700 and 1000 mg Zn/L in semi-continuous increasing VFAs up to 2109 mg Propionate/L in batch and 1029 mg Acetate/L in semi-continuous (Chapter 2 and Chapter3). Nitrate on semi-continuous experiments showed a decrease of methane at 2000 mg NO_3/L increasing VFAs up to 4078 mg Acetate equivalent ($\text{Acetate}_{\text{eq}}$)/L (Chapter 3). The inhibition of methanogens could be avoided by treating TWs with those characteristics in two-stage digestion reactors with sewage sludge, as it will allow the acetogens to grow in the first stage and potentially the methanogens will be in a less stressful environment in the second reactor.

The decision-support tool to be created from the previous mentioned points should consider first the concentration of COD. If the TW is higher than 50 g COD/L, its biodegradability will determine the potential use of that TW as co-digestate with sewage sludge (Figure 5.1). If the TW is biodegradable, the concentration of inhibitors will be considered (Figure 5.1). TWs with low concentration of inhibitors or high concentration of inhibitors but mixed with other TWs to reduce enough their inhibition will be treated in Aco-D with sewage sludge (Figure 5.1).

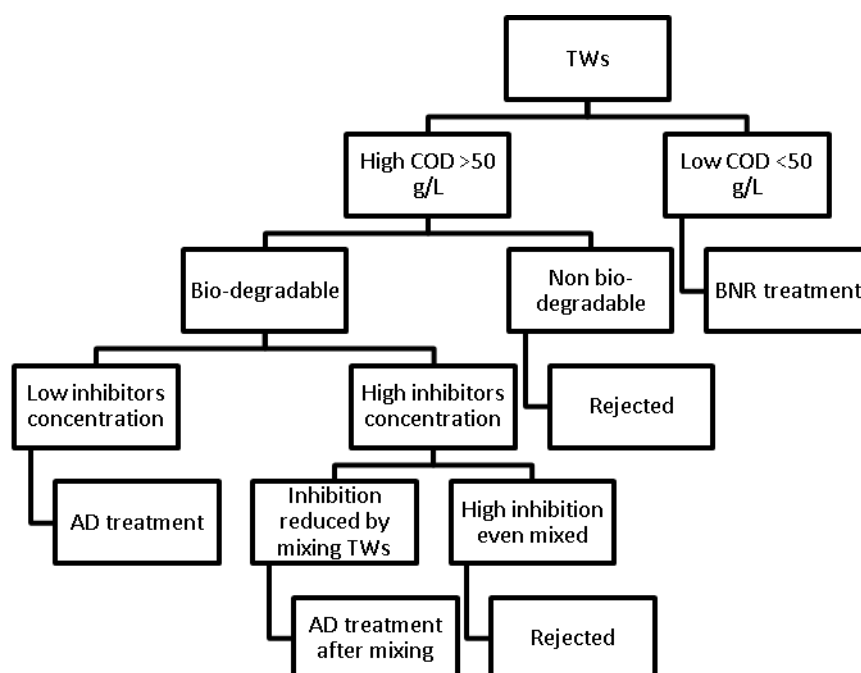


Figure 5.1. Diagram decision-support tool description

Comparing the real TWs experiments and the individual compounds from TWs experiments, it is observable that there might be synergistic and antagonistic effects with other compounds in TWs. The MF concentrate for the paper TW mixed with sewage sludge was high in copper, 860 mg Cu/L (Table 4.2), and it should have shown inhibition which was not observed in Chapter 4. This effect might differ if this sample was used in a continuous reactor as copper might attach to the organic matter [10] and accumulate like it was observed for zinc in Chapter 3. Moreover, other concentrated TWs that could have a potential negative effect were resin, chemical and personal care products TWs that showed a pH below the optimum range for anaerobic digestion, 6.5-7.5. The resin TW showed a statistically lower value of methane yield than the control probably due to its low pH, 5.2, and low partial alkalinity of 75 mg CaCO₃/L (Table 4.1). The chemical and personal care products concentrates showed a statistically similar value to the control (Chapter 4). The chemical TW showed a low pH, 5.2, but a high partial alkalinity, 682 mg CaCO₃/L (Table 4.1). The personal care products TW showed a higher pH than resin and chemical TWs, 5.8 (Table 4.2). Therefore, there are differences in the results obtained for each compound and the real TWs experiments, and further study is needed in the antagonistic and synergistic effects in AD.

5.3. Reference

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CHAPTER 6: Conclusions and Future Work

6.1. Conclusions

This project studied the potential for co-digesting trade wastes (TWs) in sewage sludge digesters, to maximise the benefits and reduce the risk of inhibition. This was achieved by studying the impact that key individual components of TWs exert when digested with sewage sludge in batch and continuous experiments, in order to inform TW loads and dosing strategies safely implementable in full-scale assets.

The characteristics of 160 real TWs were investigated and key constituents identified as: ammonia, chloride, nitrate, sulphate, aluminium, copper, zinc, arsenic and mercury. Their effect was examined in biological methane potential tests (BMP) which allowed finding different ranges of non-effect, beneficial, inhibitory and toxic impact. Yield inhibition was observed for ammonia, sulphate, zinc, copper and aluminium, while lag phase inhibition was recorded on the experiments adding zinc, aluminium, sulphate, copper, ammonium and arsenate (Objective 1). The different ranges of non-effect, beneficial, inhibitory and toxic were used to identify the optimum TW load into ADs, which was defined as 10% of TWs and 90% of sewage sludge in the feedstock, which showed inhibition on 13.7% of TWs. Most of the TWs that showed a potential inhibition with 10% of TWs addition belong to the chemical, 15 TWs, and to the glycerol/beverage/alcohol group, 9 TWs. Reducing the TWs addition to 1% of the total feed should reduce the TWs negatively affected to a 0.6% from the 160 TWs studied (Objective 2).

Opportunities and impact for digesting TWs in sewage sludge anaerobic digestion (AD) could be further increased when considering the ability of microbial communities to adapt to inhibitory or toxic compounds, as well as the possible transformations of those inhibitors. Nitrate resulted in an inhibitory effect at a target concentration of 2000 mg NO_3/L in semi-continuous trials, which greatly differs from inhibitory levels on batch units where there was a 10% decrease of biogas and methane yield at 92 mg NO_3/L . The effect of nitrate in the semi-continuous experiment was attributed to denitrifying microorganisms, which compete with methanogens for organic substrate and produce toxic by-products to the methanogens. Contrarily to the positive effect to nitrate levels on semi-continuous experiments, other TWs constituents increased their negative impact when fed to ADs over long periods. Zinc resulted in process inhibition when targeting concentrations of 700 and 1000 mg Zn/L leading to a complete suppression of the methane production with, however, a recovery after 700 mg Zn/L addition, which were lower than the level of 1676 mg Zn/L resulting in no production of biogas or methane in BMP units. Reactor performance was recovered after 16-33 days when zinc spiking stopped, reinforcing the importance of alternating feeding of high and low strength TWs and, even better, blending TWs before anaerobic treatment (Objective 3).

Pre-concentration of lower organic content TWs for use in AD with microfiltration (MF) and ultrafiltration (UF) membranes was demonstrated. MF performed better when leather, resin, food and paper TWs were concentrated due to their high permeability, while the UF performed better when personal care, food, paper and leather TWs were treated because of the low concentration factor by volume of the personal care TWs and the high permeability of the other TWs. The trials highlighted the complex nature and high fouling propensity of the TWs and their concentrates. Pre-concentrating these low organic content TWs before anaerobic co-digestion (Aco-D) with sewage sludge resulted in biogas and methane yields generally equal to the control units or higher for the chemical TWs concentrated with MF. Nonetheless, the resin TW concentrates did lead to a decrease in performance being unsuitable for this application. Overall, this study demonstrated that the use of concentrates from MF or UF membranes will generally benefit Aco-D with sewage sludge as most of the TWs showed non- or beneficial effects with an increase of up to 17% in methane production. The work highlighted that by concentrating and diverting TWs typically send to the main wastewater treatment plant for the treatment to the AD, the avoided impact on the operation of the wastewater treatment plant and the increased methane production in the AD led to benefits ranging from £5,274 to £177,012 per year for the UF concentrates, clearly demonstrating the potential of this application especially for food and chemical TWs (Objective 4).

6.2. Future perspectives

In this research, some key knowledge has been observed to be missing in the current literature that will be described in the following lines:

- The current study generally investigated the individual impact of compounds (Chapter 2 and 3) but in some instances the presence of other compounds impacted the behaviour in the system (e.g. different effects for the different salts used for a single compound of interest). This highlights the need for further research in antagonistic and synergistic effects to better understand how the limits of inhibition for certain compounds could vary. The current literature in synergistic or antagonistic effect is limited and further research on elements that are commonly found together in a waste is necessary to find limits in the inhibition towards anaerobic microorganism. In Aco-D, the elements of both wastes should be considered as the elements commonly found in one of the wastes used for Aco-D may not be present in the other and may lead to reaction, which ultimately will lead to antagonistic and synergistic effects. From the perspective of Aco-D of sewage sludge with TWs, a better understanding of these effects will help further with the selection and mixtures of TWs. In order to fill this gap in knowledge, the most common elements in TWs should be added first in pairs to observe their effect in a sewage sludge AD pilot. Once this experiment is done, some TWs with those elements could be added in a sewage sludge AD pilot to see the similarities. A model could be created with both experiments to simulate the effects of

adding TWs in AD of sewage sludge. In order to confirm that model, a long-term pilot with mixed TWs could be run evaluating its performance.

- This study only studied zinc and nitrate in semi-continuous experiments, but there were other key elements for Aco-D of sewage sludge with TWs that were studied in batch test only. Other studies investigated the impact of different compounds in continuous experiments, but there is a lack of knowledge on continuous experiments using aluminium, mercury and arsenic, all of interests for TWs applications. Further research on continuous systems will broaden the understanding of acclimation of the AD system and again could also improve the decision-making process for the selection of TWs.
- The pre-concentration step discussed in this thesis is only a proof of concept and although, some of the benefits of the application were demonstrated, further research is needed before its full-scale implementation. Future work should first focus on investigating the effect of TWs on filtration performance in more realistic conditions (e.g. cross-flow filtration), which would enable the scaling up of the concept. If the lab scale experiment is successful, a pilot study would be necessary before applying on-site. Furthermore, this study only considered one TW at a time in the membrane and AD reactors while for the real applications, TWs will be mixed so the impact of mixtures should also be investigated on both steps.
- The economic benefit analysis of the pre-concentration step before AD did not consider the membrane capital and operational costs, such as building the membrane unit, membrane cleaning, membrane replacement and the operators required to manage that additional treatment. Based on the additional research mentioned above, a more detailed business case could then be established to ascertain the economic viability of the proposed concept.
- Heavy metals in general should be further studied in Aco-D of sewage sludge and TWs, especially the speciation in the digester and their bio-availability and effect in sewage sludge application to land. In this research, zinc was shown to accumulate in the solid fraction of the sewage sludge, which has a potential to have a negative effect if applied to land. Therefore, further research on the application of bio-solids from Aco-D of TWs and sewage sludge is important to avoid any damage in the ecosystem. An experiment with a continuous AD reactor with sewage sludge and metal addition could be carried out. The analysis of the outlet concentration of heavy metals on the organic phase partition, inorganic salts precipitate and soluble fraction would provide that information. Moreover, the existing total concentration of those heavy metals in the sewage sludge is necessary to understand the fraction of heavy metal being accumulated in the solids. Those experiments could be done at different concentration to observe the effect on their fraction distribution.
- The current information on this thesis together with the future work previously described could be used to build an exhaustive decision-support tool to select the mixing and loading of TWs in the AD of sewage sludge. The decision-support tool could be improved by the use of machine learning algorithms to be applied to the

characterisation data and compare it with the biogas and methane yield. The division of TWs with machine learning algorithms could also improve the selection of TWs for previously suggested experiments with real TWs. Once the division of TWs, the synergistic/antagonistic effects on continuous experiments, and the proof of concept for the pre-concentration are done, a decision-support tool could be built. Afterwards, there is potential of transforming that tool into a mobile application for water utilities.

Appendix A

Table A1. Statistics of the biogas and methane yield using ANOVA compared with the control test (if $F > F_{crit}$ they are different).

<i>CuSO₄</i>	<i>mg Cu/L</i>	20	32	73	123	167
Biogas	F	0.77	0.01	0.26	0.18	0.15
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	0.00	0.61	0.03	0.19	1.64
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>Al₂(SO₄)₃</i>	<i>mg Al/L</i>	85	210	771	1308	1534
Biogas	F	0.14	0.00	24.59	39.19	309.83
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	0.13	10.82	29.84	155.46	1728.87
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>NaCl</i>	<i>mg Cl/L</i>	1066	1941	2821	3666	4535
Biogas	F	22.36	71.65	13.08	5.07	19.78
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	4.29	2.99	1.67	5.07	20.12
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>NaSO₄</i>	<i>mg SO₄/L</i>	162	308	600	1032	1466
Biogas	F	5.18	59.24	66.89	247.08	47.18
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	65.10	115.08	193.43	715.63	204.86
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>CuCl₂</i>	<i>mg Cu/L</i>	22	91	198	501	804
Biogas	F	802.48	823.00	567.04	292.74	2496.73
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	446.98	1105.54	239.50	241.92	4729.63
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>AlCl₃</i>	<i>mg Al/L</i>	82	203	760	1312	1531
Biogas	F	5.69	19.16	284.00	2483.70	3709.46
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	8.59	11.85	549.89	4231.14	4233.74
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>ZnCl₂</i>	<i>mg Zn/L</i>	10	61	87	592	1307
Biogas	F	0.22	0.23	6.07	1348.11	2973.37
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	0.00	4.87	3.76	1421.18	2923.37
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>NH₄SO₄</i>	<i>mg NH₄/L</i>	1460	1602	1764	2201	3405
Biogas	F	3.61	0.03	10.75	47.23	28.78
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	36.82	4.68	49.21	19.93	18.83
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>NaNO₃</i>	<i>mg NO₃/L</i>	92	174	254	332	427
Biogas	F	100.75	11.85	176.30	43.46	1.36
Biogas	F_{crit}	7.71	7.71	7.71	7.71	7.71
Methane	F	684.23	80.07	270.19	653.99	65.37
Methane	F_{crit}	7.71	7.71	7.71	7.71	7.71
<i>HgSO₄</i>	<i>mg Hg/L</i>	25	20	920	239	495

Biogas	F	1.63	7.65	15.86	110.65	8.19
Biogas	Fcrit	7.71	7.71	7.71	7.71	7.71
Methane	F	13.91	42.45	1.96	11.47	23.17
Methane	Fcrit	7.71	7.71	7.71	7.71	7.71
NH₄Cl	mg NH ₄ /L	976	1958	2946	3923	5162
Biogas	F	0.09	0.16	0.87	5.62	28.21
Biogas	Fcrit	7.71	7.71	7.71	7.71	7.71
Methane	F	0.57	0.00	0.06	1.73	23.79
Methane	Fcrit	7.71	7.71	7.71	7.71	7.71
NaHAsO₄	mg As/L	0.1	0.4	2	4	17
Biogas	F	0.43	5.15	0.09	0.77	0.39
Biogas	Fcrit	7.71	7.71	7.71	7.71	7.71
Methane	F	0.34	1.15	0.04	5.17	0.59
Methane	Fcrit	7.71	7.71	7.71	7.71	7.71
Al+OH/pH	pH 5	pH 4.5	pH 6.55	Al 608 mg/L	Al 262 mg/L	Al 464 mg/L
Biogas	F	37.14	54.77	2.26	13.43	8.29
Biogas	Fcrit	18.51	18.51	18.51	18.51	18.51
Methane	F	600.01	1761.45	0.97	17.10	18.25
Methane	Fcrit	18.51	18.51	18.51	18.51	18.51

Appendix B

Table B1. Comparison of biogas production (mL/% VS red/d) in zinc and nitrate reactors against the control reactors in their different feeding periods using RMSE_n.

Zn	RMSE_n	NO₃	RMSE_n
Non-effect	0.15	Non-effect	0.22
15 ppm	0.15	15 ppm	0.19
100 ppm	0.20	50 ppm	0.43
700 ppm	0.81	300 ppm	0.16
0 ppm	0.50	700 ppm	0.27
1000ppm	0.74	2000 ppm	0.62

Table B2. Ranges of biogas and methane formation as well as digestate properties at different periods of the zinc experiment for control (C) and zinc (Zn) reactors.

<i>Period</i>	<i>No addition</i>		<i>15.0</i>		<i>100.0</i>		<i>700.0</i>		<i>Recovery</i>		<i>1000.0</i>	
<i>Days</i>	0-25		28-53		56-95		98-126		128-163		165-186	
<i>Max-Min</i>	C	Zn	C	Zn	C	Zn	C	Zn	C	Zn	C	Zn
<i>Biogas (mL/% VS red/d)</i>	129-90	122-67	166-125	176-95	158-89	162-91	117-70	90-3	94-89	125-66	103-82	88-2
<i>% Methane</i>	45-36	40-32	45-31	40-23	47-36	47-33	47-28	37-5	57-19	43-11	52-35	47-4
<i>TVFA (mg Acetate eq/L)</i>	288-271	604-390	770-770	0.1-0.1	618-26	941-38	976-13	11457-114	325-17	3040-264	149-13	2892-516
<i>% Acetate</i>	32-0.1	59-0.1	0.1-0.1	0.1-0.1	64-0.1	47-0.1	23-0.1	64-4	55-0.1	52-9	73-0.1	34-0.5
<i>% Propionate</i>	68-0.1	66-0.1	0.1-0.1	0.1-0.1	77-23	49-27	72-45	79-3	69-9	57-8	57-9	76-7
<i>% Removal VS</i>	54-42	51-18	60-47	64-43	56-39	52-35	47-32	44-10	61-31	49-28	49-30	47-23
<i>TAN (mg-N/L)</i>	1193-1043	3829-1014	1962-836	2270-792	2634-1824	4728-763	4563-2124	3960-1927	4708-2487	4731-2407	5103-4370	5000-2924
<i>FAN (mg-N/L)</i>	112-47	314-43	47-35	66-20	502-86	439-23	175-39	155-11	286-40	136-23	155-72	144-5
<i>CODs (mg/L)</i>	1325-934	1425-678	-	-	1943-868	2249-1135	800-672	2756-885	1329-879	-	983-376	3038-2835
<i>Na (mg/L)</i>	77-77	74-4	-	-	223-74	152-89	73-61	798-120	98-72	346-128	72-72	128-128
<i>Zn (mg/L)</i>	0.7-0.7	0.8-0.8	-	-	1.2-0.4	1.4-0.8	0.8-0.6	1030-0.4	1-0.5	26-0.3	0.5-0.5	1-1
<i>NO₃ (mg/L)</i>	18-0.1	18-0.1	19-19	19-19	25-0.1	13-0.1	18-0.1	15-0.1	12-0.1	7.4-0.1	39-0.1	16-0.1
<i>SO₄ (mg/L)</i>	30-7	45-6	7-7	7-7	233-10	204-14	252-5	30201-0.1	252-13	7422-11	41-10	23-0.1
<i>pH (-)</i>	8-7.4	7.8-7.3	7.2-6.7	6.8-6.7	6.7-6.5	7-6.7	6.7-6.7	6-6.9	7.3-6.7	7 -5.7	7.3-6.6	7-5.6

Table B3. Ranges of biogas and methane formation as well as digestate properties at different periods of the nitrate experiment for control (C) and nitrate (NO₃) reactors.

<i>Period</i>	No addition		15		50		300		700		2000	
<i>Days</i>	0-25		28-53		56-95		98-126		128-163		165-186	
<i>Min-Max</i>	C	NO₃	C	NO₃	C	NO₃	C	NO₃	C	NO₃	C	NO₃
<i>Biogas (mL/% VS red/d)</i>	129-90	127-52	166-125	154-76	158-89	243-100	117-70	111-63	103-82	97-57	106-80	71-20
<i>% Methane</i>	45-36	43-30	45-31	45-32	47-36	44-34	57-19	44-19	52-35	49-37	51-38	43-26
<i>TVFA (mg Acetate eq/L)</i>	288-271	559-559	770-770	-	618-26	1349-39	976-13	455-50	149-13	2420-98	83-13	3636-93
<i>% Acetate</i>	32-0.1	52-52	0.1-0.1	-	64-0.1	42-16	55-0.1	67-3	73-0.1	62-8	61-0.1	84-8
<i>% Propionate</i>	68-0.1	36-0.1	0.1-0.1	-	77-23	73-17	72-9	26-11	69-9	37-2	63-28	84-1
<i>% Removal VS</i>	54-42	50-18	60-47	53-39	56-39	57-28	61-32	55-38	51-30	54-30	62-40	51-28
<i>TAN (mg-N/L)</i>	1193-1043	2876-851	1962-836	2729-844	2634-1824	6087-676	4563-2124	3736-2293	5103-2487	4889-2471	5217-4251	3700-3461
<i>FAN (mg-N/L)</i>	112-47	470-56	47-35	38-21	502-86	233-24	175-39	145-48	286-40	155-110	187-75	143-85
<i>CODs (mg/L)</i>	1325-934	1420-984	-	-	1943-868	2376-1267	1329-672	1256-485	983-376	2398-1335	-	-
<i>Na (mg/L)</i>	77-77	79-79	-	-	223-74	302-238	73-61	934-250	98-72	2033-892	105-73	4453-2033
<i>Zn (mg/L)</i>	0.7-0.7	0.7-0.7	-	-	1-0.4	1-0.6	1-0.6	1-0.2	0.7-0.5	0.7-0.2	0.9-0.4	1-0.2
<i>NO₃ (mg/L)</i>	18-0.1	9-0.1	19-19	19-19	25-0.1	9-0.1	18-0.1	12-0.1	39-0.1	52-6	18-0.1	22-18
<i>SO₄ (mg/L)</i>	30-7	40-0.1	7-7	7-7	233-5	51-6	252-5	1061-2	252-10	286-3	17-11	6-0.1
<i>pH (-)</i>	8-7.4	8.6-7	7.2-6.7	7.6-6.7	6.7-6.5	6.8-6.7	6.8-6.7	7.2-6.4	7.3-6.6	7.2-6.4	7-6.8	6.9-6.4

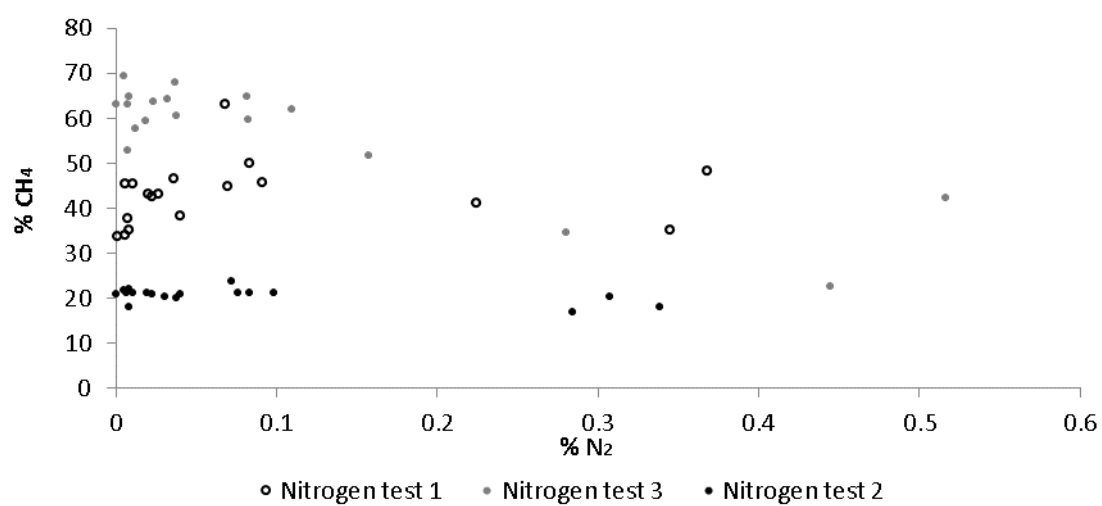
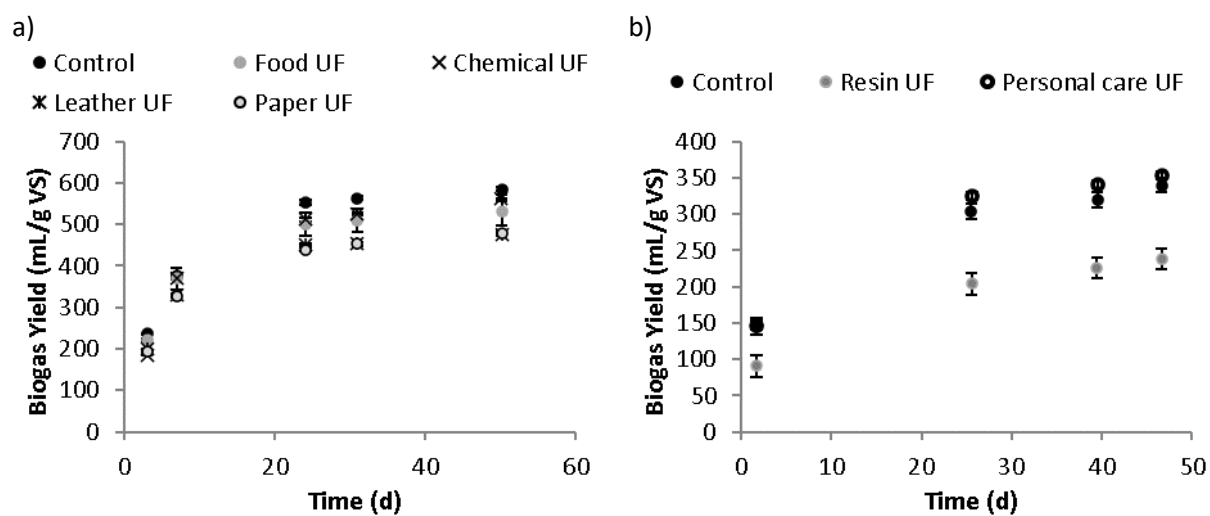


Figure B1. Nitrogen and methane production during the semi-continuous experiments with nitrate addition.

Appendix C

Table C1. Statistics of biogas and methane to measure the similarity between the BMPs with the concentrates and the control.

	Food MF	Chemical MF	Leather MF	Paper MF	Resin MF	Personal care MF
Biogas F	1.23	2.62	2.39	0.20	3.30	1.19
Biogas Fcrit	7.71	7.71	7.71	7.71	7.71	7.71
Methane F	6.89	13.85	2.23	1.23	2.18	2.20
Methane Fcrit	7.71	7.71	7.71	7.71	7.71	7.71
	Food UF	Chemical UF	Leather UF	Paper UF	Resin UF	Personal care UF
Biogas F	0.52	0.73	33.21	34.29	3.84	0.20
Biogas Fcrit	7.71	7.71	7.71	7.71	7.71	7.71
Methane F	0.31	1.46	0.03	0.002	32.79	0.11
Methane Fcrit	7.71	7.71	7.71	7.71	7.71	7.71



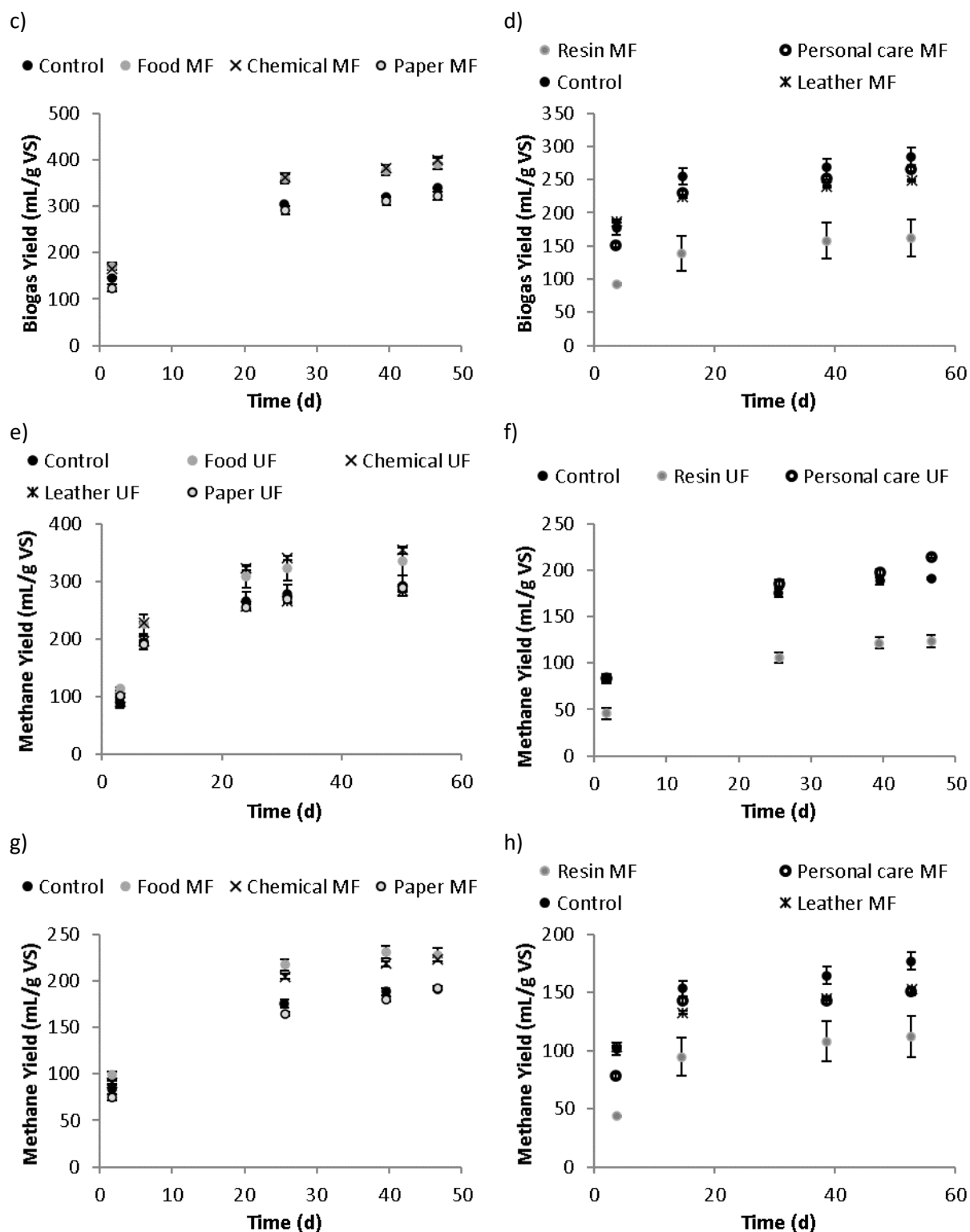


Figure C1. Biogas and methane curve for BMP with concentrated TWs and sewage sludge.