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Impact of Trace Element Ions and Fe2+ on Anaerobic Food Waste Digestion

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Abstract

In Canada, about $31 billion worth of food is wasted annually. This amount of food waste (FW) ends up in landfills where it is naturally broken down by bacteria and releases methane to the atmosphere, a powerful greenhouse gas (GHG) which is 21 times more harmful to the environment than carbon dioxide (CO_2). This process can also be done in a controlled environment at wastewater treatment plants, in a process known as anaerobic digestion (AD). Diverting food waste from landfills to wastewater treatment facilities allows for the capture of the methane, which can be used as an energy source. Although AD is a relatively reasonable technology to treat food waste, digesters that take FW as the sole feedstock have been facing unstable performance and even process failure mainly because of the accumulation of volatile fatty acids (VFAs) which is linked to trace elements (TEs) deficiencies. TEs play a key role in stimulating the enzymatic activity of the methanogenic micro-organisms and enhancing metabolic pathways.

In this study, the importance of trace element (TE) background levels, especially Fe in the inoculum utilized for the mesophilic FW anaerobic digestion was assessed, while single and mixed ionic TEs were supplemented to batch reactors. Primarily, series of specific methanogenic activity (SMA) tests was carried out with acetate as substrate to observe the effects of TE ions supplementation on methanogenic activity. Subsequently in the biomethane potential tests (BMP), where FW was inoculated with the sludge and the effects of TE ions supplementation on the methane yield, maximum specific methane production rate ($SMPR_{max}$), and hydrolysis rate constant (K_h) were determined. The experiments were conducted first with an Fe-rich sludge and then repeated with a low-Fe sludge. Finally, the results of the two studies were compared in terms of methane yield and digestion kinetic parameters. SMA tests with the Fe-rich inoculum showed that Fe^{2+} (50-400 mg/L), Ni^{2+} (0.5-2 mg/L), Co^{2+} (0.1-0.5 mg/L), and Se^{4+} (0.005-0.05 mg/L) had drastic negative impacts on methane production rates. Mo^{6+} (2-20 mg/L) was the only TE ion that did not significantly affect SMA rates. On the other hand, with the low Fe sludge, the same ionic TE concentrations (Se^{4+} concentrations ranged from 0.1 mg/L to 0.8 mg/L) did not affect the methane production rate but 5 mg Mo^{6+}/L, 20 mg Mo^{6+}/L, and 400 mg Fe^{2+}/L addition increased the SMA rates by 28%, 22%, and 20%. Single and mixed ionic TE

supplementation to the batch reactors with the Fe-rich sludge led to the same values of $SMPR_{max}$ and K_h for TE dosed and control reactors, except for Fe^{2+} and Co^{2+} which reduced the K_h as well as Mo^{6+} which decreased the $SMPR_{max}$. Soluble ionic TE concentrations decreased significantly during the experiments. The estimated amount of free sulfides (S^{2-}) was well below the S^{2-} concentration required to precipitate all the TE ions i.e. Fe^{2+}, Ni^{2+}, Co^{2+}, Mo^{6+}, and Se^{4+}, therefore co-precipitation and adsorption onto iron sulfide complexes potentially played a significant role in reducing the soluble ionic TEs. In the second experiment with low Fe sludge, Ni^{2+}, Co^{2+}, Mo^{6+}, Se^{4+} and Fe^{2+} increased the biogas yield by 28%, 25%, 21%, 29%, 13%. In BMP tests however, Ni^{2+}, Co^{2+}, Mo^{6+}, Se^{4+}, and Fe^{2+} supplementation increased the methane yield (CH_4 per gram VS_{FW}) by 27%, 23%, 31%, 32%, 22%. This positive effect of methane production was never observed with AD of FW with Fe-rich sludge. Maximum specific methane production rates (based on the modified Gompertz model) as well as hydrolysis rate constants (K_h) resulted in the same values for all ionic TE dosed and control reactors. Exceptions were for Co^{2+} at 0.1 mg/L which reduced the K_h value by 33% as well as Fe^{2+} which at 100, 200, and 400 mg/L increased the K_h by 74%, 57%, and 42%, respectively. Moreover, all TEs decreased the digestion time (for 60% COD degradation) from 6.5 days (controls) to 2.5-4.5 days. Ni^{2+}+Co^{2+}+Se^{4+}, Ni^{2+}+Co^{2+}+Mo^{6+}, Mo^{6+}+Se^{4+}, and Ni^{2+}+Co^{2+} increased the methane yield by 6%, 9% 12%, and 16%, respectively. Maximum specific methane production rates based on modified Gompertz model and K_h values were the same for TE added and control reactors. A comparison between the performance of anaerobic digestion of FW with the two types of sludge indicates that methanogenic activity, methane yields (in all cases), and hydrolysis rate constant (in the Fe^{2+} case) were improved significantly when TEs were added to the batch reactors with low Fe inoculum, potentially due to the very different levels of Fe in the two inoculums which increase TE bioavailability. Thus, supplementing TEs in AD should be accompanied with a trace element background check in the sludge to make sure that Fe concentrations are not at the levels to promote co-precipitation and/or adsorption of ionic TEs onto the abundant Fe sulfide precipitates.

Keywords

Food waste; anaerobic digestion; trace elements; SMA; BMP; methane yield; hydrolysis rate constant; bioavailability; microbial community

To all strong, confident, and courageous girls who embrace challenges, persist during setbacks, and believe that being brave is more valuable than being perfect.

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Co-Authorship Statement

Two chapters of the thesis have been published or will be submitted for publication in referred journals.

Chapter 3: Impact of Trace Elements Supplementation on Mesophilic anaerobic Digestion of Food Waste Using Fe-rich Inoculum

Andisheh Yazdanpanah, Dara. S.M. Ghasimi, Min Gu Kim, George Nakhla, Hisham Hafez, Michele Keleman

Revisions under peer review at *Environmental Science and Pollution Research*

The primary authors of this chapter were Andisheh Yazdanpanah and Dara S.M. Ghasimi under the supervision of Dr. Nakhla. The experimental plan was developed by Yazdanpanah and Ghasimi with guidance from Dr. Nakhla while the execution of experiments, data collection and analysis were conducted by Yazdanpanah. Feedback on the manuscript was received from the co-authurs. The trace elements measurement with ICP-OES were conducted by Dr. Mingu Kim.

Chapter 4: Influence of Ionic Trace Element Supplementation on Mesophilic Food waste Anaerobic Digestion; The Impact of Fe in the Inoculum

Andisheh Yazdanpanah, George Nakhla, Mingu Kim, Michele Keleman

In preparation for submission to *Bioresource Technology*

The experimental plan was developed by Yazdanpanah with guidance from Dr. Nakhla while the execution of experiments, data collection and analysis, and drafting the manuscript was conducted by Yazdanpanah. Dr. Mingu Kim assisted with preparing and sending samples to Microbe Detectives lab for microbial analysis.

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List of Abbreviations and Symbols

ADS-HFe	Anaerobic Digested Sludge with a high Fe concentration (1.7 g Fe/L)
FWS-LFe	Food Waste digested sludge with a low Fe concentarion (0.26 g Fe/L)
$\Delta G°$	Gibbs free energy
AD	Anaerobic Digestion
AMPTS	Automated Methane Potential Tests System
ANOVA	Analysis of Variance
BMP	Biomethane Potential Test
CHP	Combined Heat and Power
CMP	Cumulative Methane Potential
COD	Chemical Oxygen Demand
F/M	Food to Microorganisms Ratio
FW	Food Waste
GHG	Greenhouse Gas
HRT	Hydraulix Retention Time
ICP–OES	Inductively Coupled Plasma- optical emission spectrometer
ISR	Inoculum to Substrate Ratio
K_h	Hydrolysis Rate Constant
K_{sp}	Solubility Product Constant
LCFA	Long Chain Fatty Acid
MSW	Municipal solid Waste
OLR	Organic Loading Rate
PC	Pearson Correlation
PCA	Principal Component Analysis

P_{max}	Ultimate Methane Production from Gompertz Model
SMA	Specific Methanogenic Activity
SMPR	Specific Methane Production Rate
SS	Suspended Solids
STP	Standard Temperature and Pressure
TE	Trace element
VFA	Volatile Fatty Acid
VS	Volatile Solid
VSS	Solatile Suspended Solid
WWTP	Wastewater Treatment Plant

Chapter 1

1. Introduction

1.1. Rationale

Anaerobic digestion (AD) is a process in which the organic matter is degraded by bacteria and biogas (mainly methane and carbon dioxide) is released and as the name suggests, it occurs in the absence of oxygen. AD is very effective in removing organics (COD). it also reduces the sludge volume, pathogens, and odor. A group of bacteria and archaea consume the organic carbon and convert it into biogas which is 70% methane (CH_4) and the balance is carbon dioxide (CO_2). This methane can be captured in an AD system, used as fuel for transportation, or sent to the combined heat and power plants (CHP) to produce heat and electricity. Moreover, the amount of produced excess sludge is very small and well stabilised which can be used as a fertiliser. Considering every day increasing energy demands and diminishing fossil fuel sources, AD is one of the most promising technologies for renewable energy.

FW is comprised of vegetables, meat, and dairy products and it is the largest fraction of municipal solid waste. In Canada, about $31 billion worth of food is wasted annually. This equates to about $868 worth of food wasted per person per year (The Ministry of Environment and Climate Change 2018). In 2015, about 2.3 million tonnes of food and organic waste was sent to disposal in Ontario. FW degrades into methane by naturally occurring anaerobic digestion in the environment if it is disposed to landfills and contribute to climate change. In 2015, greenhouse gas emissions from the waste sector accounted for 8.6 megatons of carbon dioxide, or approximately 5 per cent of Ontario's total greenhouse gas emissions from all sources (Environment and and Climate Change Canada). If FW were a country, it would be the third largest CO_2-emitting country in the world (Figure 1-1).

Besides the environmental issues associated with FW, it is worth mentioning that 250 km^3 of water and 28% of the world's agricultural area is used for the production of the 1.3 billion tons of FW (Parfitt et al. 2010). A research conducted recently in the United Kingdom reported that every dollar spent on food waste prevention and reduction returns an average of $14 in financial benefit for businesses (Hanson and Mitchell 2017). Thus, it is crucial to develop recycling systems to recover these natural resources. As a result, AD has become a very promising method for stabilizing FW and its transformation to renewable energy, as well as use of the digested FW as fertilizers production (Zhang et al. 2011a; Ariunbaatar et al. 2014).

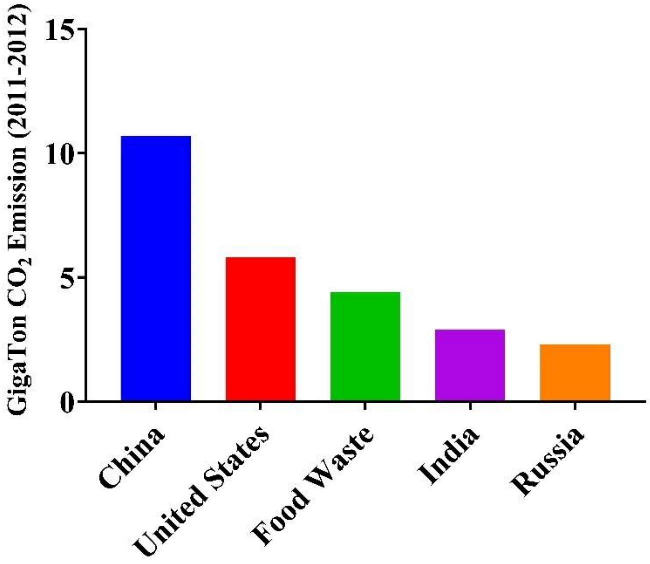

Figure 1-1 Global greenhouse gas emissions related to food waste (Clowes et al. 2017).

1.2. Research Objectives

The purpose of this study was to determine the importance of ionic TE concentrations in the mesophilic anaerobic digestion of FW. This study evaluated the hypothesis whether anaerobic FW digestion with Fe-rich inoculum (≈1.7 g Fe/L) would be improved using ionic TE supplementation individually and in mixtures. FW was also anaerobically digested with a low Fe content inoculum in batch tests and the results of the two studies of FW anaerobic digestion performance in terms of methane yield and digestion kinetic parameters were compared with each other. Since the primary focus of this study was to assess the impact of ionic TE additions on methanogens, specific methanogenic activity (SMA) tests were conducted with acetate as a readily biodegradable substrate and incorporating the same approach of TE ions supplementations were conducted. Subsequently FW was used as substrate in biomethane potential (BMP) tests to observe how TE ions supplementation affects the whole microbial community and overall process performance. It is worth mentioning that the two different inoculums are referred to as ADS-high Fe (ADS-HFe) and FWS-low Fe (FWS-LFe) for inoculums originating from digesters treating municipal biosolids and food waste, respectively.

1.3. Thesis organization

Chapter 1 presents an overall perspective of the thesis and the logic behind the emerging concept of energy recovery through anaerobic digestion of food waste. It includes a brief summary of the economical and environmental benefits that can be achieved by implementing this technology in wastewater treatment plants as well as the research objectives. In Chapter 2, the fundamentals and microbiology of anaerobic digestion are discussed along with an extensive literature review on the importance of trace elements as micronutrients in anaerobic digestion of food waste. Chapter 3 is a research paper entitled “Impact of trace elements supplementation on mesophilic anaerobic digestion of food waste using Fe-rich inoculum” published in the Environmental Research and Pollution Control Journal for review. Chapter 4 is a research article entitled “Influence of ionic trace element supplementation on mesophilic food waste anaerobic digestion; the impact of Fe in the inoculum” prepared for submission to *Bioresource Technology* Journal. Finally, Chapter 5 presents a summary of the main findings of this research.

1.4. References

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Chapter 2

2. Literature Review

2.1. Introduction to the microbiology of anaerobic digestion process

The anaerobic digestion of organic matter is comprised of four sequential steps of series and parallel processes: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis.

Several different species are involved in complex reactions of AD. The major groups that conduct the reactions depicted in Figure 2-1 (Lier et al. 2008) are: (1) fermentative bacteria, (2) hydrogen-producing acetogenic bacteria, (3) hydrogen-consuming acetogenic bacteria, (4) carbon dioxide-reducing methanogens, and (5) aceticlastic methanogens.

1) Hydrolysis: the fermentative bacteria excrete enzymes to complex, particulate material into soluble forms which can enter cell walls and membranes of the fermenters.
2) Acidogenesis: in this step, fermentative bacteria turn those dissolved material into volatile fatty acids (VFAs), alcohols, CO_2, H_2, NH_3, and H_2S, plus new cell material.
3) Acetogenesis: here the digestion products are converted into acetate, H_2, and CO_2 as well as new microorganisms.
4) Methanogenesis: acetate, CO_2, H_2, carbonate, formate, and methanol are turned into CH_4 and new cell material.

These processes are further explained in the following sections.

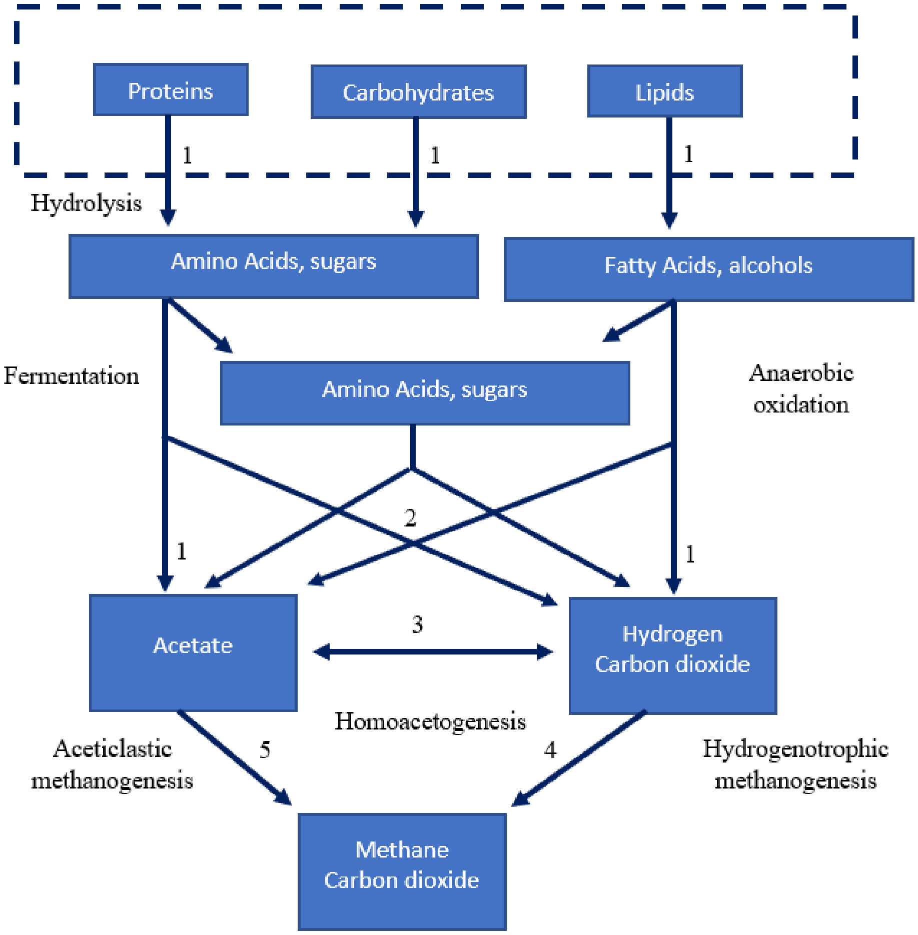

Figure 2-1 Successive reactions in anaerobic digestion. Numbers indicate the corresponding bacterial groups: 1. Hydrolytic and fermentative bacteria, 2. Acetogenic bacteria, 3. Homo-acetogenic bacteria, 4. Hydrogenotrophic methanogens, 5. Aceticlastic methanogens (Gujer and Zehnder 1983).

2.1.1. Hydrolysis

Bacteria cannot uptake particulate organic matter directly, therefore, hydrolysis is needed to break down those particles and make them available for microbial consumption. Most of the hydrolytic bacteria are in the two phyla *Firmicutes* and *Bacteroidetes* (Ling et al. 2017). *Clostridia*, a different family of *Firmicutes* have also been reported as contributors to hydrolysis (Sundberg et al. 2013; Vanwonterghem et al. 2014; Carballa et al. 2015). Hydrolysis is basically a surface phenomenon in which proteins are enzymatically hydrolysed to amino acids, polysaccharides to simple sugars and lipids to long chain fatty acids (LCFA). For particulate substrates, hydrolysis is the rate-limiting step in AD process.

However, this is not particularly due to the lack of hydrolytic enzymes but to the free available surface area of the particles and the structure of the substrate (Chandler et al. 1980; Mahmoud et al. 2003). It can be referred to as a process in which complex polymeric substrates are brought to soluble form for acidogenic bacteria consumption. Acetate and H_2 produced in this stage can be readily used by methanogens but all other VFAs should be catabolized through next steps to become ready to uptake by methanogens.

2.1.2. Acidogenesis

At this stage, amino acids, simple sugars, and LCFAs diffuse inside the bacterial cells and are subsequently fermented or anaerobically oxidized (Lier et al. 2008). Hydrolytic and non-hydrolytic microorganisms turn single monomers into VFAs, i.e. acetate, propionate, and butyrate as well as H_2, CO_2, lactic acid, ethanol, and ammonia (Figure 2-1).

Basically, the main products of sugars and protein disintegration are VFAs and carbonic acid, thus, this stage is called acidogenesis. Table 2-1 summarizes different sub-reactions in acidogenesis of sucrose. There is an indirect relationship between the prevailing H_2 concentration and the $\Delta G^{\circ\prime}$ of the reaction. Therefore, if hydrogenotrophic methanogens consume the H_2 rapidly, acetate will be the main end product. On the other hand, if the above-mentioned methanogens are not active and H_2 accumulates, these reactions shift towards production of more reduced compounds such as propionate, butyrate, lactate, and even alcohols.

Table 2-1 Acidogenesis of sucrose. The ΔG°′ are at 25°C (Lier et al. 2008).

Reactions	ΔG°′(kJ/mol)	Eq.
$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 4HCO_3^- + 8H^+ + 8H_2$	-457.5	(2.1)
$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 4HCO_3^- + 6H^+ + 4H_2$	-554.1	(2.2)
$C_{12}H_{22}O_{11} + 3H_2O \rightarrow 2CH_3COO^- + 2\ CH_3CH_2COO^- + 2HCO_3^- + 6H^+ + 2H_2$	-610.5	(2.3)

Among all AD stages, acidogenesis possesses the highest ΔG°′(Lier et al. 2008), making it the most rapid conversion step. Hence, sudden pH drops are possible due to overloading of anaerobic digesters. When the alkalinity is neutralized by the acids, pH starts to drop, thus increasing the VFAs concentrations and inhibiting methanogens' activity. This in turn, leads to even more VFA accumulation and pH drop. Acidogens and acetogens both comprise *Firmicutes*, *Clostridia*, and *Bacteroidetes*. These groups were also mentioned to be responsible for the hydrolysis process in the previous section, mainly because the ability to perform hydrolysis, acidogenesis, and acetogenesis are widespread among these bacteria (Ling et al. 2017).

2.1.3. Acetogenesis

The products of acidogenesis (short chain fatty acids other than acetate) are converted into acetate, H_2, and CO_2. Table 2-2 shows the reactions of propionate, butyrate, as well as lactate, ethanol, methanol, and even H_2 and CO_2 into acetate.

Long chain fatty acids (LCFAs) that have uneven C atoms typically produce propionate besides acetate. Butyrate and propionate are the most important intermediary products in AD processes. Acetogens are obligate hydrogen producers and high concentrations of H_2 inhibits their activity. Considering the positive ΔG°′ values for acetogenic conversion of propionate, butyrate, as well as ethanol and palmitate (an LCFA), these reactions are thermodynamically unfavorable. Thus, a balance between the H_2-producing acetogens and H_2-consuming methanogens is needed to maintain a low H_2 partial pressure and for the above-mentioned reactions to occur (Lier et al. 2008). This means that the disintegration of LCFAs strongly depends on electron accepting organism (methanogens). The bacterial groups responsible for acetogenesis are already discussed in the previous section.

Table 2-2 Acetogenic reactions with their corresponding free energy (ΔG°′) at 25°C, 1 atm pressure, and neutral pH (Lier et al. 2008).

Compound	Reaction	ΔG°′(kJ/mol)	Eq.
Lactate	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$	-4.2	(2.4)
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6	(2.5)
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.1	(2.6)
Propionate	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1	(2.7)
Methanol	$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O$	-2.9	(2.8)
Hydrogen-CO_2	$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$	-70.3	(2.9)
Palmitate	$CH_3 (CH_2)_{14}COO^- + 14H_2O \rightarrow 8CH_3COO^- + 7H^+ + 14H_2$	+345.6	(2.10)

2.1.4. Methanogenesis

This is the final step of AD process in which methanogens use H_2 as electron donor to reduce CO_2 and acetate to CH_4. It is the only stage in which the COD is removed in the gaseous form which is highly insoluble in water and leaves the reactor. Methanogens are obligate anaerobes and are often classified into two main groups: the aceticlastic (acetate reducing) and the hydrogenotrophic (hydrogen reducing) methanogens (Table 2-3). It is noteworthy to say that about 70% of the methane is produced from acetate and the rest originates from H_2 and CO_2.

Archaea are the only species that can accomplish methanogenesis; no known bacteria can produce methane (Ling et al. 2017). As discussed before, there are two types of methanogens: hydrogenotrophic and aceticlastic. *Methanosphaerula*, *Methanobacterium*, *Methanospirillum*, *Methanothermobacter*, *Methanomicrobiales*, *Methanoregula*, and *Methanoculleus* are considered to be hydrogenotrophs while *Methanosaeta*, *Methanosarcina*, *Methanobrevibacter*, and *Methanomicrobium* are aceticlastic methanogens (Ling et al. 2017).

Table 2-3 Methanogenic reactions with their corresponding free energy (ΔG°′) at 25°C (Lier et al. 2008).

Functional step	Reaction	ΔG°′(kJ/mol)	Eq.
Acetotrophic methanogenesis	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- + 2H_2$	-31	(1.11)
Hydrogenotrophic methanogenesis	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	-131	(1.12)

2.2. Strategies to improve the anaerobic digestion of food waste

As discussed thoroughly in Chapter 1, anaerobic digestion (AD) is an environmental-friendly alternative for the FW management; it has limited environmental footprints (Capson-Tojo et al. 2016) as well as high potential for renewable energy production (Zhang et al. 2014; Zamanzadeh et al. 2016; Capson-Tojo et al. 2016). For these reasons, investigating methods to enhance AD process of FW has become an exciting research field.

FW is a complex heterogeneous organic material that includes both highly recalcitrant and extremely biodegradable compounds, therefore, for choosing the best option to improve the AD performances focus should be placed on FW characteristics and composition. In other words, for lignocellulosic FW feedstock, hydrolysis is the rate limiting step, so pre-treatment would be a suitable strategy to improve AD performance while, in the case of soluble, readily biodegradable FW, inhibition is often caused by the accumulation of intermediary AD productions. In the later case, co-digestion with other substrates is suggested in order to stabilize the entire process as it could improve buffering capacity and reduce ammonia inhibition due to better carbon and nutrient balance (Wang et al. 2014). Both strategies are reviewed in the following sections.

2.2.1. Anaerobic co-digestion with other substrates to improve stability

Co-digestion of FW with other organic substrates is increasingly attracting research interest, for its numerous benefits such as promoting synergistic effects towards increasing biogas production and stabilizing digestate (El-Mashad and Zhang 2010; Liu et al. 2013a; Gou et al. 2014; Mata-Alvarez et al. 2014; Nghiem et al. 2017). The most common co-substrates used in the AD of FW in the literature are sewage sludge, animal manure, and agricultural wastes (Mata-Alvarez et al. 2014). The mixture ratios are selected based on the right nutrients and moisture balance, to avoid inhibition and to optimize methane production (Zhang et al. 2012; Braguglia et al. 2018). However, the wrong combination of co-substrates can lead to negative results. The most recent literature results regarding co-digestion of FW with different organic waste were studied and the results are discussed in this section.

Animal waste is one of the best co-substrates for anaerobic FW digestion because of its alkalinity, low C/N ratio, and most importantly, for its various macro- and micronutrients needed by microorganisms (Braguglia et al. 2018). Agyeman and Tao (2014) found that the co-digestion of FW with dairy manure in 50:50 VS-based ratio in a CSTR reactor at mesophilic conditions for 180 days resulted in 37% increase in methane yield at OLR of 2 kg VS m^{-3} day^{-1}. Another co-substrate for FW is sewage sludge which has low C/N ratio and low organic content that results in a C/N balance suitable for enhancing microbial activity and reducing intermediate accumulation such as ammonia (Dai et al. 2013; Liu et al. 2013b; Braguglia et al. 2018). In batch co-digestion tests, Naran et al. (2016) observed 53% increase in methane production by a 70:30 volume based ratio of FW with waste activated sludge (WAS). A 50:50 TS based ratio of FW and sewage sludge increased methane yields by nearly 3 times in batch mesophilic co-digestion study by Zhang et al. (2016).

Dai et al. (2013) operated a mesophilic semi-continuous digester at an SRT of 30 days with a 2.4:1 VS based ratio for FW with dewatered swage sludge and observed better stability and 10% higher methane yield compared to FW mono-digestion. As discussed thoroughly, co-digestion of FW with sewage sludge proves to increase FW digestion stability and enhance methane yields due to synergistic effects of using co-substrates (Mata-Alvarez et al. 2014).

Green waste are other possible co-substrates for FW anaerobic digestion. Chen et al. (2014) observed 18.7% increase in methane yield by a 4:6 VS based FW with green waste combination, compared to mono FW digestion. This is potentially due to the high content of recalcitrant lignin in green waste which reduces the biodegradation rate of FW (Drennan and DiStefano 2014) and hence, reducing VFAs accumulation (Chen et al. 2014; Haider et al. 2015). In a study by Jabeen et al. (2015), FW was co-digested with rice husk in a pilot scale plug-flow reactor and reactor stability as well as biogas production decreased as organic loading rate (OLR) increased. On the contrary, Owamah and Izinyon (2015) reported an increase in methane yields (up to 482 L CH_4 kg^{-1}VS) by increasing the OLR (up to 4.5 g VS $L^{-1}d^{-1}$) in co-digestion of FW with maize husk (FW to maize husk ratio of 3:1 on total mass basis). Yong et al. (2015) observed an increase in methane yields by increasing the FW content in co-digestion with straw in batch mesophilic reactors. However, several other studies showed inverse correlations between methane yield and substrate to inoculum ratios, in both mesophilic and thermophilic co-digestion of FW with green waste or rice husk (Liu et al. 2009; Haider et al. 2015).

There are several bottlenecks associated with co-digestion practicality such as inert impurities in FW, regulatory uncertainty, and impact on bio-solids agricultural use (Braguglia et al. 2018). Therefore, a multi-disciplinary approach is needed to overcome these challenges and to promote co-digestion full scale implementation (Nghiem et al. 2017).

2.2.2. Food waste pre-treatments and impact on AD performance

Since there are different pre-treatment methods which affect the enhancements in AD performance, it is difficult to choose one method as the ideal one mainly because of the lack of standardised protocols (Carlsson et al. 2012).

Although it is expected that increase in FW solubility would lead to higher methane yields, in practice these two parameters do not necessarily have a direct relationship with each other (Braguglia et al. 2018). Liu et al. (2012) in batch FW digesters with food to inoculum volumetric ratio of 0.2, observed around 8% reduction in methane yield while soluble sugars and soluble proteins increased by 114% and 204%, respectively, by thermal pre-treatment. Conversely, in batch mesophilic co-digestion of FW with sewage sludge (3:2, TS basis), Zhang et al. (2016) found a 347% increase in methane yield although soluble COD and soluble sugars decreased by 25% and 52%, respectively, by microwave pre-treatment of FW. Methane yield also decreased slightly (6%) despite a 16% increase in soluble COD in a study by Tampio et al. (2014), digesting autoclaved and untreated FW in semi-continuously fed mesophilic reactors at an OLR of 4 kg VS/m^3 d.

This suggests that the impact of different pre-treatment methods on substrate characterization should be better understood. Furthermore, most of the papers found in the literature in the area of FW pre-treatments were AD processes conducted in lab batch scale rather than continuous or semi-continuous. However, mechanical pre-treatments such as grinding or milling, with the aim of reducing particle size are already well stablished technologies which are successfully implemented in full-scale treatment plants (Braguglia et al. 2018). These methods are also less sensitive to substrate specific characteristics compared to thermal pre-treatments and they exclude the risk of inhibitory compounds formation, but their main drawback is high energy consumption (Braguglia et al. 2018). Another mechanical pre-treatment method for FW is high voltage pulse discharge (HVPD). In this method, a pulsed power supplier creates a rapidly pulsing, high-voltage electric field to break the cellular membranes and complex organic solids (Braguglia et al. 2018). Zou et al. (2016) first applied this pre-treatment method on FW samples and observed that pre-treated samples generated 134% higher methane than the control.

Biological pre-treatments are another option in which both enzymatic and aerobic methods are applied to intensify hydrolysis rate and improve methane production (Braguglia et al. 2018). Enzymes can be added either to the substrate prior to digestion as pre-treatment or directly in the digester. In the first case, there is a risk of fermenting bacteria consuming hydrolysed sugars so rapidly that leads to digester acidification and further inhibitions. Therefore, adding the enzymes directly in the digester is the most usual practice at full-scale (Carrere et al. 2016). The advantages of enzymatic over thermal and mechanical pre-treatments is less energy requirements but cost, process efficiency, and long required contact time (24 h at least) have been identified as major concerns; making it a less feasible option for full-scale plants (Uçkun Kiran et al. 2015; Yin et al. 2016). Another option is to aerate FW to increase production of hydrolytic enzymes which in turn increases the specific microbial growth and accelerates the hydrolysis rates (Ariunbaatar et al. 2014). In addition, this technology reduces VFAs accumulation and thus, improvs the start-up stability of AD (Braguglia et al. 2018). Rafieenia et al. (2017) investigated the efficiency of aeration pre-treatment with air flow rate of 5 L/h and the contact time of 24 hours, on different synthetic FW types in a two-stage AD system. In the second stage (methanogenesis), CH_4 production was higher for pre-aerated protein and carbohydrate-rich samples than in non-pre-aerated ones; on the other hand, pre-aeration of the lipid rich-substrate decreased the energy potential of FW compared to untreated sample.

Chemical pre-treatment is another method which includes the addition of strong acids, alkalis or oxidants to solubilise biopolymers of the organic compounds, making them more bioavailable for anaerobic microbial consumption. This pre-treatment strategy is very successful in achieving high levels of solubilisation but comes with the risk of chemical contamination and recalcitrant compounds formation (Braguglia et al. 2018). Thus, it might not be suitable for readily biodegradable substrates such as FW, as the high rate of carbohydrates degradation is directly proportional to VFA accumulation and subsequent methanogenesis inhibition (Ariunbaatar et al. 2014).

As mentioned earlier in this section, pre-treatment methods are very advantageous in increasing FW solubilisation and higher methane productions, yet there are disadvantages associated with them. Methanogenesis inhibition, recalcitrant products formation, long retention times, specificity of the enzyme, and energy costs are the main drawbacks, making pre-treatment strategy a less feasible approach to be applied to full-scale plants.

2.3. The importance of TEs as nutrients in FW anaerobic digestion

Lack of sufficient trace elements (TEs) during the long term digestion of FW has often been associated with poor stability and even process failure (Climenhaga and Banks, 2008; Zhang et al. 2011, 2015a, b; Banks et al. 2012; Tampio et al. 2014).

TEs play a significant role in the growth and activity of the methanogens involved in anaerobic digestion. According to the literature, TEs such as iron (Fe), cobalt (Co), nickel (Ni), selenium (Se), and molybdenum (Mo) have been found to be crucial for the activity of enzymes in methanogens (Zandvoort et al. 2006a; Feng et al. 2010; Pobeheim et al. 2011; Banks et al. 2012a; Qiang et al. 2012a; Karlsson et al. 2012; Facchin et al. 2013). Fe for instance, is utilized for catalysis and electron transport in the form of Fe-S (Fe_2S_2, Fe_3S_4, or Fe_4S_4 clusters). Additional Fe in the form of two Fe_4S_4 clusters is required by ferredoxins for electron transfer from H_2 to other methanogenesis enzymes (Daas et al. 1994). Another enzyme involved in methanogenesis is the Frh enzyme complex with an Fe-Ni active site and four Fe_4S_4 clusters which forms large aggregates, increasing metal requirements by approximately eight times (Orme-Johnson et al. 1987; Alex et al. 1990). Ni either binds to Fe–S clusters or to the centre of a porphyrin unique to methanogens, known as cofactor F_{430} (Tersteegen and Hedderich 1999). In fact, Ni-Fe enzymes are contained in all hydrogenases which oxidize H_2 and reduce ferredoxin, coenzyme F_{420}, among other electron carriers (Thauer et al. 2010). Aceticlastic methanogens use two metalloenzymes to convert the methyl group from acetate to (CH_3-H_4SPT). The most abundant metal-rich aceticlastic enzyme is CO dehydrogenase/acetyl-CoA synthase (Cdh), which separates the methyl group from acetyl-CoA and transfers it to CH_3-H_4SPT (Glass and Orphan 2012). The Cdh complex has one Fe_4S_4 cluster bound to an Ni–Ni site, four Fe_4S_4clusters and a $NiFe_4S_4$ cluster and reduces a 2×[Fe_4S_4] ferredoxin (Terlesky and Ferry 1988; Funk et al. 2004; Gong et al. 2008; Ferry 2010). Co can be found in cobamides involved in methyl group transfer. CH_3-H_4M(S)PT-coenzyme M methyltransferase (Mtr) which is used by all methanogens to transfer the methyl group from CH_3-H_4M(S)PT to HS-CoM, has two cobamide cofactors (with one Co each) and eight Fe atoms (Gartner et al. 1993; Zandvoort et al. 2006b; Glass and Orphan 2012). Methyl coenzyme M reductase (Mcr), another typical coenzyme in methanogenic pathways, is responsible for the reduction of CH_3-S-CoM to CH_4 and the production of CoM–CoB heterodisulfide with electrons sourced from HS-CoB (Glass and Orphan 2012). Mcr contains two coenzyme F_{430} Ni tetrapyrroles (Ermler et al. 1997). Mo is present in a pterin ($C_6H_5N_5O$) cofactor to catalyse two-electron redox reactions (Glass and Orphan 2012). Overall, it is highly suggested that FW should be either co-digested with other feedstocks rich in TEs or externally supplemented by TEs to establish stable AD performance (Qiang et al. 2012a; Zhang and Jahng 2012; Facchin et al. 2013; Tampio et al. 2014).

Thus far, various studies have highlighted the stimulatory effect of trace elements supplementation on enhancing the stability of anaerobic FW digestion with low Fe content inoculums (≤ 1000 mg Fe/L). A comprehensive literature survey yielded sixteen studies applying various ranges of TEs addition in batch and semi-continuous anaerobic digestion of FW (Table 2-4). In batch digesters at an inoculum-to-substrate ratio (ISR) of 0.44 by volume, Zhang et al. (2015b) achieved up to 36% increase in methane yield by supplementing reactors digesting FW using a 51.3 mgFe/L inoculum with a mixture of Fe (149 mg/L), Ni (6.6 mg/L), Co (2.5 mg/L), and Mo (6.1 mg/L). Similarly, Ariunbaatar et al. (2016) showed that in batch anaerobic FW digesters with relatively low Fe content inoculum (17.1 mg/L), up to 39% improvement in methane yields were obtained with addition of various concentrations of individual TEs (Fe, Ni, Co, Se) (Table 2-4).

Although batch tests do not reflect long-term digestion performance and stability, similar stimulatory effects have been reported in the few-continuously fed FW digestion studies. Zhang and Jahng (2012) showed the performance improvement of FW anaerobic digestion through the supplementation of Fe (100 mg/L), Co (2 mg/L), Mo (5 mg/L) and Ni (10 mg/L) in the reactor with the seed sludge at an Fe concentration of 99 mg/L during the stabilization of a single-stage reactor treating FW. Similarly, De Vrieze et al. (2013) achieved a stable methanation and higher methane production from co-digestion of FW with an iron-rich activated sludge (1350 mg/L). Addition of Co, Ni, and Mo also promoted stable digestion through enhanced degradation of volatile fatty acids (Feng et al. 2010). Karlsson et al. (2012) showed the positive impact of TEs (500 mg Fe/L, 0.5mg Co/L, and 0.25mg Ni/L) addition on the degradation efficiencies of VFAs, oleic acid (OA), and phenyl acetate (PA), as well as on microbial populations using lab-scale reactors that were fed semi-continuously at a hydraulic retention time (HRT) of 30 days. Banks et al. (2012) found that supplementation of Se (0.16 mg per kg fresh matter feed) and Co (0.22 mg per kg fresh matter feed) stabilized the performance of an AD treating FW at high organic loading rate (OLR) (5g VS/L/d), which suffered from propionic acid accumulation.

Studies also reported neutral and negative effects of TEs supplementation on digestion performance. For instance, Facchin et al. (2013) showed neutral or slightly negative impacts with inoculums originating from reactors with high background concentrations of trace elements (2 mg Ni/L, 0.3 mg Co/L, 0.7 mg Mo/L, and 0.4 mg Se/L) whereas with inoculum from reactors treating only FW (Ni 0.3 mg/L, Co 0.04 mg/L, Mo 0.17 mg/L, Se 0.04 mg/L), 45%-65% higher methane yields were achieved from FW with supplementation of mixed TEs (Co, Mo, Ni, Se, and W). Similar findings were also reported by Ariunbaatar et al. (2016)who did not observe any enhancement of the biomethane production from digestion of FW with elevated background concentrations of TEs (Fe of 120 mg/L, Co of 0.6 mg/L, Ni of 2.6 ng/L, Zn of 84.6 mg/L, Mn of 4.8 mg/L, Cu of 5.2 mg/L, Se of 0.3 mg/L, and Mo of 2.5 mg/L).

Optimum TE concentrations added to bioreactors were also different in the various studies. To better compare these studies, we converted the supplemented dosages to mg/L or mg/kg TS in the bioreactor. For example, in batch studies, 6.6 mg/L of Ni in the bottle enhanced the methane yield by 14% (Zhang et al. 2015c) while Facchin et al. (2013) showed a methane yield improvement of 15% at 0.6 mgNi/L, much lower than the 6.6 mg/L. Similarly, the optimum levels of Se were 0.05

mg/L (Facchin et al. 2013) and 0.006 mg/L (Ariunbaatar et al. 2016). Studies operating semi-continuous systems also showed divergent trace element concentrations for methane yield enhancement. For instance, Moestedt et al. (2016) showed higher enhancement of methane yield (8%) for the mixture of Fe (400 mg/L), Ni (0.5 mg/L), and Co (0.5 mg/L) than mixtures of Ni (0.5 mg/L) and Co (0.5 mg/L) in the reactor, five times higher than those reported by Nges and Björnsson (2012) who added Fe for digestion of a mixed substrate of beet roots, maize, and triticale. Based on the two studies providing trace element levels in digesters (Nges and Björnsson 2012; Moestedt et al. 2016), the observed levels of TE concentrations in the semi-continuous systems varied widely i.e. 66-400 mg/L for Fe, 0.5-2.8 mg/L for Co, 0.5-5.5 mg/L for Ni, indicating that optimum conditions could be system specific, potentially due to bioavailability.

Evidently, it is still a challenge to develop an appropriate TEs supplementation strategy due to considerable discrepancies in the literature with respect to the levels of optimum TEs and the lack of metal information in digestates

Table 2-4. Summary of TMs addition to FW digestion based on different studies reported in the literature

Reference	Reactor type	FW (mg/L)	Inoculum (mg/L)	Control and added metal amount	mg/kgTS in the bottles or bioreactors [a]	mg/L in the bottles or bioreactors [b]	Methane yield improvement (%) and findings [c]
(Facchin et al. 2013)	Batch (Inoculum to substrate ratio, VS/VS, ISR 2.5-3.3)	Ni (2.7) Co (<0.6) Mo (<0.6) Se (<0.3)	Ni (2.0) Co (0.3) Mo (0.7) Se (0.4)	Control	Ni (39.5), Co (6.3), Mo (13), Se (7.5)	Ni (2.0), Co (0.3), Mo (0.6), Se (0.4)	Not available (NA)
				Ni 5,10,20,100 mg/kgTS FW (the optimum 5)	40.5-59.5 (the optimum 40.5)	2.0-2.9 (the optimum 2.0)	3.9% at the optimum level
				Co 5,10,20,100 mg/kgTS FW (no optimum level)	7.3-26.3 (no optimum level)	0.4-1.3 (no optimum level)	Decline for all added metal concentrations
				Mo 3,6,12,60 mg/kgTS FW (no optimum level)	13.7-25.1 (no optimum level)	0.7-1.2 (no optimum level)	Decline for all added metal concentrations
				Se 0.5,1,2,10 mg/kgTS FW (no optimum level)	7.6-9.5 (no optimum level)	0.38-0.47 (no optimum level)	Decline for all added metal concentrations
		Ni (2.7) Co (<0.6) Mo (<0.6) Se (<0.3)	Ni (0.3) Co (0.04) Mo (0.17) Se (0.04)	Control	Ni (21.3), Co (2.7), Mo (3.6), Se (1.0)	Ni (0.3), Co (0.04), Mo (0.05), Se (0.02)	NA
				Ni 5,10,20,100 mgNi/kgTS FW (the optimum 100)	22.3-41.3 (the optimum 41.3)	0.34-0.63 (the optimum 0.63)	15% at the optimum level
				Co 5,10,20,100 mgCo/kgTS FW (the optimum 100)	3.7-22.7 (the optimum 22.7)	0.06-0.34 (the optimum 0.34)	11% at the optimum level
				Mo 3,6,12,60 mgMo/kgTS FW (the optimum 6)	4.2-15.6 (the optimum 4.8)	0..06-0.24 (the optimum 0.07)	43% at the optimum level
				Se 0.5,1,2,10 mgSe/kgTS FW (the optimum 10)	1.1-3.0 (the optimum 3.0)	0.02-0.0.5 (the optimum 0.05)	27% at the optimum level
	Batch	Fe (23)	Fe (51.3)	Control	Fe (1976), Co (60),	Fe (49), Co (1.5),	NA

(Zhang et al. 2015c)	(ISR 0.44)	Co (0.034) Mo (0.24) Ni (2.17)	Co (1.61) Mo (1.18) Ni (1.60)		Mo (45), Ni (65)	Mo (1.1), Ni (1.6)	
				Fe 20,50,100,200,500, 1000 mg/L (the optimum 100)	2776-41952 (the optimum 5974)	69-1049 (the optimum 149)	11% at the optimum level
				Co 0.5,1,2,5,10 mg/L (the optimum 1)	80-460 (the optimum 100)	2.0-11.5 (the optimum 2.5)	12% at the optimum level
				Mo 1,2,5,10,20 mg/L (the optimum 5)	85-844 (the optimum 245)	2.1-21.1 (the optimum 6.1)	12% at the optimum level
				Ni 2,5,10,20,50 mg/L (the optimum 5)	165-2064 (the optimum 265)	4.1-51.6 (the optimum 6.6)	14% at the optimum level
				Fe (100 mg/L)+Co (1 mg/L)	Fe (5974)+Co (100)	Fe (149)+ Co (2.5)	16%
				Fe (100 mg/L)+Mo (5 mg/L)	Fe (5974)+Mo (245)	Fe (149)+ Mo (6.1)	15%
				Fe (100 mg/L)+Ni (5 mg/L)	Fe (5974)+Ni (265)	Fe (149)+ Ni (6.6)	19%
				Co (1 mg/L)+Mo (5 mg/L)	Co (100)+Mo (245)	Co (2.5)+ Mo (6.1)	22%
				Co (1 mg/L)+Ni (5 mg/L)	Co (100)+Ni (265)	Co (2.5)+ Ni (6.6)	24%
				Mo (5 mg/L)+Ni (5 mg/L)	Mo (245)+Ni (265)	Mo (6.1)+ Ni (6.6)	23%
				Fe (100 mg/L)+Co (1 mg/L)+Mo (5 mg/L)	Fe (5974)+Co (100)+ Mo (245)	Fe (149)+ Co (2.5)+Mo (6.1)	23%
				Fe (100 mg/L)+Co (1 mg/L)+Ni (5 mg/L)	Fe (5974)+Co (100)+ Ni (265)	Fe (149)+ Co (2.5)+Ni (6.6)	26%
				Fe (100 mg/L)+Mo (5 mg/L)+Ni (5 mg/L)	Fe (5974)+Mo (245)+ Ni (265)	Fe (149)+ Mo (6.1)+Ni (6.6)	25%
				Co (1 mg/L)+Mo (5 mg/L)+Ni (5 mg/L)	Co (100)+Mo (245)+ Ni (265)	Co (2.5)+ Mo (6.1)+Ni (6.6)	28%

				Fe (100 mg/L)+Co (1 mgL)+Mo (5 mg/L)+Ni (5 mg/L)	Fe (5974)+Co (100)+ Mo (245)+Ni (265)	Fe (149)+ Co (2.5)+ Mo (6.1)+Ni (6.6)	36%
(Ariunbaatar et al. 2016)	Batch (ISR 2)	Fe (51.6) Ni (1.0) Co (0.2) Mo (0.5) Se (<DL)	Fe (17.1) Ni (0.1) Co (0.03) Mo (0.2) Se (0.1)	Control	Fe (566), Co (1.2), Ni (4.7), Se (<DL)	Fe (18.2), Co (0.04), Ni (0.15), Se (<DL)	NA
				Each supplemented concentration of Fe, Co, Ni, Se is 0.00625 mg/L.	Co (1.4)+Ni (4.9)+ Fe (567)	Co (0.04)+Ni (0.16)+ Fe (18.2)	11%
					Fe (567)	Fe (18.2)	39%
					Co (1.4)	Co (0.04)	24%
					Ni (4.9)	Ni (0.16)	26%
					Se (3.8)	Se (0.12)	34%
		Fe (123.1) Ni (2.7) Co (0.7) Mo (2.6) Se (0.3)	Fe (17.1) Ni (0.1) Co (0.03) Mo (0.2) Se (0.1)	Control	Fe (640), Co (1.7), Ni (6.5), Se (3.9)	Fe (20.6), Co (0.05), Ni (0.21), Se (0.13)	NA
				Se addition of 0.01 mg/L	Se (4.3)	Se (0.14)	30%
(Moestedt et al. 2016)	Semi-continuous (OLR 4.5kgVS/m^3/d, HRT 21d)	Ni (<0.2) Se (0.2 mg/kgTS) Fe (71-83)	NA	Fe (400 mg/L)	Fe (9640)	Fe (400)	Combination of Co and Ni showed 8% higher specific methane production than mixture of Co, Ni and Fe.
				Fe (400 mg/L)+Co (0.5 mg/L)	Fe (9640)+Co (12)	Fe (400)+Co (0.5)	
				Fe (400 mg/L)+Ni (0.5 mg/L)	Fe (9640)+Ni (12)	Fe (400)+Ni (0.5)	
				Fe (400 mg/L)+Co (0.5mg/L)+Ni (0.5 mg/L)	Fe (9640)+Co (12)+ Ni (12)	Fe (400)+Co(0.5)+ Ni (0.5)	
(Zhang and Jahng 2012)	Semi-continuous (OLR 2.2-4.5 kgCOD/m^3/d, HRT 20-30d)	Fe (3.17) Ni (0.19) Mo (0.025) Co (<0.03)	Fe (99) Ni (0.5) Mo (0.3) Co (0.14)	Control	Fe (354), Co (0.5), Mo (1.2), Ni (1.9)	Fe (94), Co (0.1), Mo (0.3), Ni (0.5)	209 L CH_4/kgVSfed (OLR 4.5 kgCOD/m^3/d, HRT 20d)
				Fe (100 mg/L)+Co (2 mg/L)+ Mo (5 mg/L)+Ni (10 mg/L)	Fe (730)+Co (8)+ Mo (20)+Ni (40)	Fe (194)+Co (2.1)+ Mo (5.3)+Ni (10.5)	446 L CH_4/kgVS$_{fed}$ (OLR 2.3 kgCOD/m^3/d, HRT30d)

				Fe (100 mg/L)+Co (2 mg/L)	Fe (730)+Co (8)	Fe (194)+Co (2.1)	390-450 L CH_4/kgVS$_{fed}$ (OLR 2.3-3.6 kgCOD/m^3/d, HRT 20-30d)
				Fe (100 mg/L)	Fe (730)	Fe (194)	359-409 L CH_4/kgVS$_{fed}$ (OLR 2.3-3.6 kgCOD/m^3/d, HRT 20-30d)
(Banks et al. 2012b)	Semi-continuous (OLR 2-5 kgVS/m^3/d, HRT 38-95d)	Co (<0.06) Fe (54) Mo (0.11) Ni (1.7) Se (<0.07)	Co (0.083) Se (0.05) Mo (0.29) Ni (2.9) Fe (173.7)	Control	Co (2) Se (1.5) Mo (7.7) Ni (79) Fe (4560)	Co (0.08) Se (0.05) Mo (0.27) Ni (2.7) Fe (157)	NA
				Co (1 mg/L), Se (0.2 mg/L), Mo (0.2 mg/L), Ni (1 mg/L), Fe (5 mg/L) A mixture of multiple metals with different combination	Co (3) Se (1.9) Mo (8.2) Ni (80) Fe (4605)	Co (0.1) Se (0.07) Mo (0.28) Ni (2.8) Fe (159)	At OLR 3 kgVS/m^3/day, when VFA increased to 10 g/L, the digester was added with Se, Co, Ni which decreased the VFA to <0.5 g/L
(Zhang et al. 2015b)	Semi-continuous (OLR 1-5.5 kgVS/m^3/d, HRT 40d)	Fe (50.2) Co (0.1) Ni (1.5) Se (0.1)	Fe (47.3) Co (0.1) Ni (1.5) Se (0.1)	Control	Fe (1761), Co (3) Ni (54), Se (4)	Fe (47.1), Co (0.07), Ni (1.46), Se (0.1)	NA
				A mixture of Fe (5 mg/L), Co (1 mg/L), Ni (1 mg/L), and Se (0.2 mg/L) was added in the food wastes	Fe (1764)+Co (3)+ Ni (55)+Se (4)	Fe (47.2)+Co (0.09)+ Ni (1.48)+Se (0.1)	The control without adding metals failed at OLR of 4 kgVS/m^3/day while the reactors supplemented a mixture of the selected metals showed stable performance at OLR of 4-5.5 kgVS/m^3/day with 480 L CH_4/kgVSfed at 5.5 kgVS/m^3/day)

(Zhang et al. 2015a)	Semi-continuous (OLR 4 kg VS/m^3/d, HRT 40d)	Fe (50 mg/L) Co (0.08 mg/L) Ni (1.46 mg/L)	Fe (47 mg/L) Co (0.07 mg/L) Ni (1.47 mg/L)	Control	Fe (1761), Co (3) Ni (54), Se (4)	Fe (47.1), Co (0.07), Ni (1.46), Se (0.1)	The control failed at OLR 4 kg VS/m^3/d but Fe+Co+Ni addition recovered the system. System failed at OLR 4d despite Co+Ni addition. Addition of Fe restored system from 130 to 440 L CH$_4$/kg VSadded
				Fe (5 mg/L)+ Co (1 mg/L)+ Ni (1 mg/L)	Fe (1764)+Co (3)+ Ni (55)	Fe (47.2)+Co (0.09)+ Ni (1.48)	
(Nges and Björnsson 2012)	Semi-continuous (OLR 1.5-5.5 kg/m^3/d, HRT 30-40d)	Different mixture of beet roots, maize, triticale Fe (24-37) Co (0.03-0.05) Mo (0.03-0.09) Ni (0.1-0.12)	Fe (852) Co (0.09) Mo (0.1) Ni (0.38)	Fe (30.5-46 mg/kg wet substrate) Ni (no addition-0.5 mg/ kg wet substrate) Co (1.8-2.0 mg/kg wet substrate) Mo (1.4-1.6 mg/kg wet substrate)	Digestates Fe (2200-2913) Co (93-104) Mo (517-696) Ni (150-191) at HRT 30d and OLR 3 kg/m^3/d	Digestates Fe (66-75.5) Co (2.4-2.8) Mo (15-17) Ni (4-5.55) at HRT 30d and OLR 3 kg/m^3/d	350-383 m^{3}CH$_4$/kgTS$_{added}$ at HRT 30d and OLR 3 kg/m^3/d
(De Vrieze et al. 2013)	Semi-continuous	Fe (9.5) Co (0.012) Ni (0.1) Ni (0.252)	NA	Ni (0.11mg/kgFW)+ Fe (0.07 mg/kgFW)+ Mo (0.02 mg/kgFW)+ Co (0.01 mg/kgFW)	NA	NA	Control (0.46 L CH$_4$/L/d) supplemented reactor (0.48 L CH$_4$/L/d)
(Feng et al. 2010)	Semi-continous (OLR 2.5 – 4 gVS/L/d, HRT 25d)	NA	NA	Se (0.8mg/L)+ W (1.8 mg/L)+ Co (0.06 mg/L)	NA	NA	860 L CH$_4$/kgVS$_{added}$ /day
				Se (0.008 mg/L)+ W (0.018 mg/L)+ Co (0.6 mg/L)	NA	NA	730 L CH$_4$/kgVS$_{added}$ /day

(Ortner et al. 2015)	Semi-continous (OLR 1-2.5 kg/m^3/d, HRT 80-20d)	NA	NA	Ni (14.2 mg/kgCOD)+ Co (6.1 mg/kgCOD)+ Mo (4.5 mg/kgCOD)+ Se (0.1 mg/kgCOD)	NA	NA	260-290 NLCH$_4$/kgCOD$_{added}$
(Karlsson et al. 2012)	Semi-continuous (OLR 2.1-2.5 gVS/L/d, HRT 30d)	NA	NA	Fe (500 mg/L)+ Co (0.5 mg/L)+ Ni (0.25 mg/L)	NA	NA	1140-1270 NLbiogas /kgVS$_{added}$ (metal added) 920-1250 NLbiogas /kgVS$_{added}$ (control)
(Climenhaga and Banks 2008)	Semi-continuous (OLR 1.45 kgVS/m^3/d, HRT 25, 50, 100d)	NA	NA	Fe (0.11 mg/L)+ Mo (0.001 mg/L)+ Co (0.1 mg/L)+ Ni (0.01 mg/L)+ Se (0.01 mg/L)	NA	NA	350-600 L CH$_4$/kgVS added (HRT 25d) for metal added while control failed. 400-600 L CH$_4$/kgVS $_{added}$) (HRT 50d) for metal added while control failed. 300-600 L CH$_4$/kgVS $_{added}$ (HRT 100d) for metal added while control failed.
(Qiang et al. 2012b)	Semi-continuous (OLR 1.9-6.3 kg/m^3/d, HRT 30-100d)	Fe (34.9 mg/L) Co (0.08 mg/L) Ni (<DL)	NA	R1: no addition R2: Co (1 mg/L)+ Ni (1 mg/L) R3:Fe (10mg/L)+ Co (1 mg/L)+ Ni (1 mg/L)	NA	NA	In R1, gas production decreased as HRT changed from 100d to 30d. In R2, gas production rate recovered and dropped at HRT of 30d. In R3, gas production rate was recovered and stabilized.

(Tampio et al. 2014)	Semi-continuous (OLR 2, 3, 4, 6 kg VS/m^3/d, HRT 117-58d)	Fe (0.03 mg/L)	NA	Fe (0.005 mg/kg digestate)+ Ni (0.001 mg/kg digestate)+ Mo (0.0002 mg/kg digestate)+ Se (0.0002 mg/kg digestate)	NA	NA	480 L CH_4/kgVS$_{added}$ at OLR 3 kg VS/m^3/d for untreated FW 420 L CH_4/kgVS$_{added}$ for autoclaved FW

a,b,c. Metal concentrations for the semi-continuous fed reactors conducted in Zhang and Jahng (2012), Banks et al. (2012), Wei et al. (2014) and Zhang et al. (2015c) were estimated using the TE levels of FW and, inoculum and the daily feeding rate derived from HRT and the reactor volume. The estimates may differ from the TE concentrations of the steady-state conditions which were not reported in the studies.

2.4. Synopsis of literature review

In summary, several strategies have been studied so far to enhance FW anaerobic digestion, including pre-treatment such as mechanical grinding, ultrasonication, microwave, thermal, chemical, and biological methods, as well as co-digestion with other substrates. However, as mentioned, these methods increase the risk of methane production inhibition and digester acidification, require long hydraulic retention times, include excessive costs for energy and material (enzymes), regulatory uncertainties, and impact on bio-solids agricultural use which make them less desirable technologies for practical purposes. TE supplementation on the other hand, is a reliable method which has proved to prevent and/or recover inhibition and enhance biomethane yields in the anaerobic digestion of FW. TEs act as micronutrients (co-factors) for various enzymatic reactions; promote microbial aggregation, enhance the activity of anaerobic microbes; and help to overcome sulfide toxicity through metal sulfide precipitation (Oleszkiewicz and Sharma 1990; De Vrieze et al. 2013; Ariunbaatar et al. 2016). However, at higher concentrations, TEs can become toxicants to the microbial biomass. These different effects of TEs depend on several factors such as background concentrations, bioavailability, and microbial uptake. Evidently, it is still a challenge to develop an appropriate TE supplementation strategy due to considerable discrepancies in the literature with respect to the lack of TE information in digestates and the levels of optimum TEs required to improve biomethane production from FW. Hence, the focus of this research was to assess FW anaerobic bio-methanation under TE supplementation with an emphasis on the importance of background TE levels in the inoculum and further determination of the optimum TE concentrations to improve a particular AD system.

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Chapter 3 [1]

3. Impact of trace elements supplementation on mesophilic anaerobic digestion of food waste using Fe-rich inoculum

3.1. Introduction

FW has enormous potential for renewable energy production through the AD process because of it's very high energy content (0.7-1.1 kWh L^{-1}), high biodegradability (up to 90%), and high water content (70-80%) (Banks et al. 2011a; Ma et al. 2011). However, AD operation on FW alone can suffer from instability due to VFA, ammonia, and sulfide inhibition (Demirel and Scherer 2011; Zhang et al. 2015a). Several ways such as pre-treatment, co-digestion with other substrates, and trace elements (TEs) supplementation are being investigated to enhance anaerobic FW digestion stability and stimulate the microbial activity (Demirel and Scherer 2011; Banks et al. 2012b; Zhang et al. 2015a). This research focuses on the effects of TEs addition on methane production and AD kinetics.

TEs play a key role in boosting enzymatic activity of the methanogenic micro-organisms and enhancing metabolic pathways (Zandvoort et al. 2006a). According to the literature, methanogens are depended on the presence and availability of Fe, Ni, Co, Se, and Mo. (Feng et al. 2010; Pobeheim et al. 2011; Banks et al. 2012a) Demirel and Scherer (2011) determined the cell TE content of 10 methanogens and observed that the TE content varies between the different species of different methanogens even when they are from the same genius and converting the same substrate. Thus, it is crucial to know the TE requirements and adequate dosages for optimized anaerobic digestion of a specific AD system.

The purpose of` this study was to determine the importance of TE background levels in the inoculum utilized in mesophilic FW anaerobic digestion. We assessed the anaerobic FW digestion with a low TE content inoculum in batch tests and compared the results of AD performance in terms of methane yield and digestion kinetic parameters with the ones of another study conducted by the same authors with the similar conditions but a high TE

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[1] A version of this chapter has been published in the Environmental Science and Pollution Research Journal.

content inoculum. Since our primary focus was to see the impact of TE additions on methanogens, we first conducted specific methanogenic activity (SMA) tests with acetate as a readily biodegradable substrate and incorporated the same approach of TE additions. Then we switched to using FW as substrate in biomethane potential (BMP) tests to see how this TE supplementation method affects the whole microbial community and overall process performance.

3.2. Materials and Methods

3.2.1. Food waste

Food waste samples were collected from the Grind2Energy systems (InsinkErator, WI) that process food waste from southeast WI supermarkets, and were stored in a cold room (4ºC) prior to conducting the cumulative methane production (CMP) tests. Total solids (TS) and volatile solids (VS) were measured on a weight basis (g/L) according to the standard methods for the examination of water and wastewater (APHA 2005). Different Hach methods were used to characterize total and soluble samples i.e. chemical oxygen demand (COD, 200-15,000 mg/L, method 8000), phosphorous (1-100 mg PO_4^{3-}/L, method 10121), sulphate (0-70 mg/L, method 8051), sulfide (0-0.7 mg/L, method 8131), volatile fatty acids (total VFA, 50-2,500 mg/L as acetic acid, molecular weight basis, TNT872 kits), and nitrogen (10-150 mg/L, method 10071). For analysis of all soluble parameters such as soluble COD, soluble nitrogen, and ammonia (0.4-50 mg/L, method 10031), sterile 0.45 µm membrane filter papers (VWR International, Canada) were used for the filtration of samples. FW Characteristics are presented in Table 3-1.

3.2.2. Inoculum

The Fe-rich inoculum was collected from a mesophilic digester treating primary and secondary sludge at the Stratford municipal wastewater treatment plant (WWTP) (Ontario, Canada). The characteristics of the inoculum and substrate are summarized in Table 3-1. The initial pH of the mesophilic inoculum sludge was 7.5 ± 0.1 prior to conducting the experiment. It should be mentioned that the inoculum used in this phase of the study is referred to as ADS-high Fe (ADS-HFe) throughout the entire thesis.

Table 3-1 Summary of the physical and chemical characteristics of inoculum and substrate (average ± standard deviation (number of analysis))

Parameter	Unit	Inoculum	Food Waste
TS	g/L	30 ± 2.7 (20)	142 ± 13.5 (20)
VS	g/L	17 ± 1.6 (20)	129 ± 16.8 (20)
TSS	g/L	31 ± 5.3 (15)	92 ± 6.6 (10)
VSS	g/L	15 ± 1.4 (15)	88 ± 8.0 (10)
TCOD	g/L	30 ± 2.9 (15)	259 ± 25.3 (15)
SCOD	g/L	1 ± 0.0 (15)	96 ± 1.9 (15)
TP	g/L	2.2 ± 0.2 (10)	5.8 ± 0.4 (10)
Sulfate	mg/L	30 ± 0.0 (10)	345 ± 7.1(10)
Sulfide	mg/L	40 ± 1.5 (10)	52 ± 0.5 (10)
TN	g/L	2.4 ± 0.1 (15)	6.6 ± 0.0 (15)
SN	g/L	0.0 ± 0.0 (15)	3.0 ± 0.1 (15)
N-Ammonia	g/L	1.0 ± 0.0 (10)	0.6 ± 0.0 (10)
Total VFA	g/L	0.2 ± 0.0 (10)	11.1 ± 0.1 (10)
Total Fe	mg/L	1681 ± 299 (3)	5 ± 1 (2)
Soluble Fe	mg/L	3 ± 2(3)	3 ± 1 (2)
Total Co	mg/L	0.05 ± 0.02(3)	<0.005 (2)
Soluble Co	mg/L	<0.005 (3)	<0.005 (2)
Total Mo	mg/L	0.3 ± 0.1(3)	0.04 ± 0.0 (2)
Soluble Mo	mg/L	<0.01(3)	<0.01 (2)
Total Ni	mg/L	1.5 ± 0.5 (3)	0.1 ± 0.0 (2)
Soluble Ni	mg/L	0.4 ± 0.6 (3)	<0.01 (2)
Total Se	mg/L	<0.05 (3)	<0.05 (2)
Soluble Se	mg/L	<0.05 (3)	<0.05 (2)

3.2.3. TE selection

The selection of TE and supplemented concentrations i.e. Fe^{2+} (50, 100, 200, 400 mg/L), Ni^{2+} (2, 5, 10, 20 mg/L), Co^{2+} (0.5, 1, 2, 5 mg/L), Se^{4+} (0.1,0.3,0.6,0.8 mg/L), and Mo^{6+} (2, 5, 10, 20 mg/L) were initially based on the survey of the literature (Ortner et al. 2015; Zhang et al. 2015c). The selected concentrations were used for SMA tests; however, the concentrations of Ni^{2+}, Co^{2+}, and Se^{4+} were further modified in the CMP tests to examine the impact of the TE levels over a wider range. The five TEs were prepared in solution using the following salts i.e. $FeCl_2{\cdot}4H_2O$, $NiCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $MoNa_2O_4{\cdot}6H_2O$, and Na_2O_3Se.

3.2.4. Gas chromatography (GC)

Methane content was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft 2mm, Restek). The temperatures of the column and the TCD detector were 90 and 105 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. No hydrogen was detected throughout the study.

3.2.5. TE analysis using ICP–OES (inductively coupled plasma–optical emission spectrometer)

Total and soluble TEs in the samples were analysed according to Standard Methods (3120) (APHA 2005). Total TEs include both dissolved and particulate forms. Acid digestion was used to extract TEs from the samples for the determination of total TEs. Briefly, an aliquot of the sample (2.5-3 g wet) added to 3 mL concentrated nitric acid (67%-70 %, Caledon Laboratories) was digested in a flask on a hot plate at 95-100 ºC for 3 hours. The digested sample was filtered through a syringe filter (0.45 μm) prior to testing. Similarly, to analyse soluble TEs in the sample, the undigested sample was initially diluted 10 times with distilled water. The diluted sample was then filtered through a 0.45 μm filter paper to collect soluble fraction. The pH of the filtrate was adjusted to below 2, using concentrated nitric acid (67%-70 %) prior to measurement. Then samples for both total and soluble TE measurement were poured individually into Autosampler vials. The detection limits of the analysts (mg/L) were 0.005 (Co, Fe), 0.01 (Mo, Ni), and 0.05 (Se).

TE analysis was conducted using inductively coupled plasma optical emission spectrometry ((ICP-OES Vista Pro Axial, Varian, Australia) with a flame temperature in the range from 6000 to 10000 K. ICP–OES was performed for both substrate (FW) and inoculum (anaerobically digested sludge, ADS-HFe) before the experiments. Total TE concentrations in the inoculum were 1681 mgFe/L, 0.05 mgCo/L, 0.3 mgMo/L, and 1.5 mgNi/L with Se levels below detection limit, indicating predominance of Fe. Similarly, FW contained 5 mgFe/L, 0.04 mgMo/L, 0.1 mgNi/L, with Co and Se concentrations below detection limits. The soluble fraction of Fe was 0.2% for the inoculum and 58% for the FW.

3.2.6. Specific Methanogenic Activity (SMA)

Two-third of the biologically produced methane in the methanogenic stage comes from the aceticlastic pathway (Rogers and Whitman 1991). Hence, the focus of the SMA tests conducted in this study was on the aceticlastic methanogens and this was evaluated by determining the maximum methane production rate using acetate as the substrate. Addition of acetate as the sole substrate circumvents other degradation steps prior to methanogenesis (hydrolysis, acidogenesis, acetogenesis) and thus, the substrate will be only consumed by aceticlastic methanogens, facilitating monitoring their activity during the AD process. SMA tests were performed at different TE (Fe, Ni, Co, Se and Mo) concentrations under mesophilic conditions (37°C) to assess the possible impacts TEs on methanogens. In this study, the SMA of the mesophilic sludge was determined using an Automated Methane Potential Test System (AMPTS_II, Bioprocess Control, Lund, Sweden).

The AMPTS system incubated 650 mL batch reactors containing the mesophilic inoculum and FW in a water bath at 37°C. CO_2 and H_2S were stripped from the biogas by leading the biogas through 100 mL bottles containing a 3M NaOH solution. Thereafter, the remaining gas which was methane, flowed into a gas flow cell with a calibrated volume. When the gas volume equaled the calibrated volume of the flow cell, the gas was released and recorded as one normalized volume at time t. The test continued until the daily methane production for three consecutive days was less than 1% of the total cumulative methane.

The SMA tests were conducted using sodium acetate (dehydrate) as the substrate with a COD concentration of 2 g/L. We performed the SMA tests in triplicates for the control bottles (without TEs) and duplicates for the bottles containing TEs using a working liquid volume of 0.4 L for SMA tests of Fe^{2+}, Ni^{2+} and Co^{2+} and 0.2 L for Se^{4+} and Mo^{6+}. It is noteworthy that a medium consisting of a mixture of macronutrients, trace elements and phosphate buffer solution was not added, enabling us to clearly evaluate the impacts of the used TEs on the methanogens without other additives' interferences. Prior to conducting the test, the headspaces of all flasks were purged with nitrogen gas for 3 minutes. The detailed summary of the SMA tests design is presented in Table 3-2.

Table 3-2 Summary of the SMA tests design for different TEs at various concentrations.

TEs	VSS [g/L]	Sludge/bottle [mL]	Sodium acetate/bottle [mL]	Working Volume [mL]	F/M
Fe^{2+} [50, 100, 200, 400 mg/L]	11.2	105.0	295.0	400.0	2.0
Ni^{2+} [2, 5, 10, 20 mg/L]	17.5	74.4	325.6	400.0	2.0
Co^{2+} [0.5, 1, 2, 5 mg/L]	17.5	74.4	325.6	400.0	2.0
Se^{4+} [0.1, 0.2, 0.4, 0.6 mg/L]	18.2	36.1	163.9	200.0	2.0
Mo^{6+} [2, 5, 10, 20 mg/L]	18.2	36.1	163.9	200.0	2.0

3.2.7. Cumulative biomethane production (CMP), assay experimental setup

The anaerobic biodegradability of the FW was performed according to the protocols described in Holliger et al. (2016) . Due to the large number of required batch bottles, the AMPTS system and three swirling-action shakers (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) were simultaneously used to monitor gas production.

The AMPTS system setup was described in section 2.1.6. The three shakers used for CMP testing were run at 150 rpm and 37°C to incubate batch samples in 300 mL (liquid volume of 200 mL) flasks. Biogas production from the batch bottles was measured twice a day at the beginning of the experiment and once a day at the middle of the experiment using a digital gas pressure meter (VWR® Traceable® Manometer Pressure, VWR International). The biogas samples collected from the headspace were stored in 9mL sample bottles (Vacuette® Serum Clot Activator Tubes, VWR) prior to methane analysis on the same day with a gas chromatograph (Model 310, SRI Instruments, Torrance, CA, refer to section 2.9). Biomethane production from the shakers was corrected for standard temperature and pressure (273K, 100 kPa). Methane production was calculated with a mass balance equation (Eq. 1), using biogas produced and its corresponding composition at each time interval:

$$V_{CH_4,i}=V_{CH_4,i-1}+C_{CH_4,i}\left(V_{G,i}-V_{G,i-1}\right)+V_{CH_4}(C_{CH_4,i}-C_{CH_4,i-1}) \quad \text{Equation 3-1}$$

where $V_{CH4,i}$ and $V_{CH4,i-1}$ are cumulative methane gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total biogas volumes in the current and previous time intervals, $C_{CH4,i}$ and $C_{CH4,i-1}$ are the fractions of methane gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, and V_{CH4} is the total volume of headspace in the reactor (López et al. 2007).

The CMP tests were performed using food (substrate) to microorganism (inoculum) ratio of 2 (F/M=2 gCOD/gVSS) corresponding to a volumetric ISR of 0.5, in triplicates for the blank and control bottles (no TE addition) and duplicates for bottles with TE supplementation. The selection of the F/M ratio was based on a previous batch anaerobic test conducted by the authors with an ISR of 3 (based on $gVS_{inoculum}/gVS_{substrate}$), the results of which are reported in Table 3-5. It was observed that Fe supplementation did not enhance the SMAs and had a neutral effect on CMPs. Thus, high substrate loading of FW was then employed to discount the high concentration of Fe in the inoculum to reduce the co-precipitation potential of the supplemented trace elements.

Every flask contained the same amount of inoculum. After adding the required amounts of inoculum and substrate, bottles were filled with distilled water to adjust the working volume. Prior to conducting the test, the headspaces of all flasks were purged with nitrogen gas for 3 minutes. It must be asserted that the observed trace element supplementation impacts were not influenced by accumulation of VFA or pH drops, as the final pHs were in the range of 7.00 to 7.15, expect for the Fe where the pHs varied from 7.35 to 7.43. No alkalinity was added to avoid interference with metal solubilities

The experimental design for the CMP tests at the F/M ratio of 2 (ISR of 0.5) is presented in Table 3-3. The working volumes of the digestion bottles were 0.2 L and the volume of inoculum in all bottles was fixed at 110 mL. Due to the slight variability in the characteristics of the inoculum and FW during the testing period (≈ 70 days), slightly different weights of FW were used to maintain the F/M ratio at 2 for all the experiments. It is noted that the COD:N:P ratio in the reactors ranged from 48:3:1 to 68:3:1 with an average of 61(±5.9):3:1.

Table 3-3 Design of CMP tests for individual and combined forms of TEs.

CMP tests using individual TEs	Inoculum/bottle [mL]	FW/bottle [g]	Inoculum [gVSS]	FW [gCOD]	Working Volume [mL]	F/M
Fe^{2+} [50, 100, 200, 400 mg/L] *	355	9.9	4.0	2.4	400	0.6
Fe^{2+} [50, 100, 200, 400 mg/L]	110.0	17.2	2.3	4.6	200	2.0
Ni^{2+} [0.5, 1, 1.5, 2 mg/L]	110.0	13.5	1.7	3.5	200	2.0
Co^{2+} [0.1, 0.2, 0.4, 0.5 mg/L]	110.0	16.0	2.1	4.2	200	2.0
Se^{4+} [0.005, 0.01, 0.02, 0.05 mg/L]	110.0	10.6	1.5	3.0	200	2.0
Mo^{6+} [2, 5, 10, 20 mg/L]	110.0	10.6	1.5	3.0	200	2.0
CMP tests using combined TEs						
Mo^{6+} [20 mg/L] +Se^{4+} [0.005-0.05 mg/L]	110.0	10.6	1.5	3.0	200	2.0
Ni^{2+} [0.5 mg/L] + Co^{2+} [0.5 mg/L] + Mo^{6+} [20 mg/L]	110.0	10.6	1.5	3.0	200	2.0
Ni^{2+} [0.5 mg/L] + Co^{2+} [0.5 mg/L] + Se^{4+} [0.005-0.05 mg/L]	110.0	10.6	1.5	3.0	200	2.0

* The first experiment with an iron supplementation at an ISR of 3 (equal to F/M of 0.6).

3.2.8. SMA rate calculation

SMA was calculated by obtaining the maximum slope of the cumulative methane production curve (mL/d) which typically occurred in the first 2 days, converting this slope (CH_4/day) to its COD equivalent mass, and dividing it by the initial mass volatile suspended solids (VSS) contributed by inoculum. The final values were expressed in g COD-CH_4/gVSS$_{inoculum}$/day. The detailed explanations for these calculations are presented in Appendix A.

3.2.9. Specific methane production rate (SMPR)

SMPR (expressed in ml CH_4/g VSS$_{inoculum}$/day) was obtained by subtracting the produced methane volumes (mL) between each two consecutive time intervals and dividing it by the time interval between those two readings (in days), and again dividing that by volatile suspended solids (VSS) of the inoculum in the bottle (in grams) (Raposo et al. 2006). An illustration for these calculations is presented in Appendix A.

3.2.10. Apparent hydrolysis rate constant (K_h)

Calculation of K_h was performed according to the protocol of Raposo et al. (2006). K_h describes the rate of degradation and typically follows a first-order kinetic model assuming normal growth (no inhibition, no lack of macro-nutrients or micro-nutrients) (Pfeffer 1974; Tong et al. 1990; Koch and Drewes 2014). When no intermediates accumulate, substrate hydrolysis can be regarded as the rate-limiting step. A first-order kinetic model can then be used for calculating the K_h as expressed in Eq. (2):

$$P = P_{max}[1 - e^{-K_h t}] \qquad \text{Equation 3-2}$$

Where,

P= net cumulative methane production from the CMP assay at time t (mL),

P_{max}= net ultimate methane yield from CMP assay at the end of the incubation time (mL),

K_h= first-order hydrolysis rate constant (1/d).

K_h can be derived from the slope of the linear regression line plotted for Ln ($1-P/P_{max}$) versus time (Figure 3-1D). The linearization was conducted by GraphPad Prism 7 software and the R^2 values of all the slopes were within 0.97 to 0.99.

3.2.11. Statistical Analysis

ANOVA (Analysis of Variances) tests were used in intergroup comparisons of $SMPR_{max}$, K_h, and total methane production in each batch for the control and TE supplemented reactors. In case a significant difference between the variables was observed, independent two samples t-test was used in comparisons between the control bottles values and TE added bottles values (separately for each TE). P values lower than 0.05 were considered statistically significant. The calculations were performed using Excel 2016, real statistics resource pack.

3.3. Results and Discussion

3.3.1. Impact of TEs on methanogens' activity (SMA tests)

As mentioned in section 3.2.2, the inoculum used in this study was Fe-rich sludge (ADS-HFe) collected from a mesophilic digester treating primary and secondary sludge. The TE concentrations in the inoculum were 1681 mg Fe/L, 0.05 mg Co/L, 0.3 mg Mo/L, 1.5 mg Ni/L, and <0.05 mg Se/L. Total COD concentration was 30 g/L with 3% being the soluble faction. Finally, total COD to VS and particulate COD to VSS ratios for the inoculum were 1.76 and 1.93, respectively.

SMA tests using acetate as a sole substrate were used to assess the maximum methane production rate of the methanogens in the seed sludge (Figure 3-2). These tests were conducted prior to exploring the impact of TEs individually and in mixtures on the FW digestion using CMP tests. The results of the SMA tests are summarized in Table 3-4. It should be noted that the difference in the SMA control rates in the Fe, Ni and Co, on one hand, with the ones from Se and Mo run, on the other hand, was due to the two different inoculums collected from the same WWTP over the testing period.

Fe^{2+} at all applied concentrations did not enhance the SMA rates (Figure 3-2). SMA rates of 0.29±0.007, 0.23±0.001, 0.23±0.003, 0.19±0.000 and 0.19±0.000 gCOD-CH_4/gVSS.d were found for the control, 50 mg Fe^{2+}/L, 100 mg Fe^{2+}/L, 200 mg Fe^{2+}/L, and 400 mg Fe^{2+}/L, respectively. As reported in Table 3-1, the inoculum used in this study contained a high concentration of Fe (≈ 1.7 g Fe/L). Thus, supplementing Fe^{2+} (50-400 mg Fe/L) in this study significantly reduced the SMA rate by 20% to 37% at the tested concentrations in comparison with the control (with no ionic TE addition). Similarly, Ni^{2+} severely reduced the SMA rates by 40% and 58% at 10 and 20 mg/L, respectively, compared to the control. SMA values for the control, 2 mg Ni^{2+}/L, 5 mg Ni^{2+}/L, 10 mg Ni^{2+}/L, and 20 mg Ni^{2+}/L were 0.38±0.014, 0.28±0.002, 0.26±0.000, 0.23±0.003 and 0.16 gCOD-CH_4/gVSS.d, respectively. Adding Co^{2+} at different concentrations to the SMA test bottles with mesophilic sludge resulted in 33%±0. 2% lower rate of methane production than the control which is the same case for all tested concentrations (0.25 gCOD-CH_4/gVSS.d). Se^{4+} added batch digester at 0.3 mg Se/L reduced the SMA rate by 26% An analysis of variance showed that addition of Mo^{6+} at different concentrations led to the same rate as the SMA control (0.28 gCOD-

CH_4/gVSS.d). According to the results of this study, none of the supplemented TE enhanced the SMA rates. Based on the SMA results, the applied concentrations of TEs, particularly for Ni^{2+}, Co^{2+}, and Se^{4+} in the CMP tests were modified.

Methane production yields (total methane productions) were also monitored in the SMA tests. Supplementing Fe^{2+} (50-400 mg/L) in this study significantly reduced the methane production by 50% at the tested concentrations in comparison with the control (with no TEs addition). Ni^{2+} drastically decreased the methane production by 45% (at 2 and 5 mg/L) and 75% at 20 mg/L compared to the control. Supplementing the batch bottles with Co^{2+}, significantly inhibited the methane production by 50%, in comparison with the control. An analysis of variances showed that the effects of Se^{4+} and Mo^{6+} addition on total methane production in SMA tests were not significant. Unlike the SMA rates, where only Mo supplementation in the range of 2-20 mg/L had a neutral impact, in terms of methane production, both Se^{4+} and Mo^{6+} had a neutral impact.

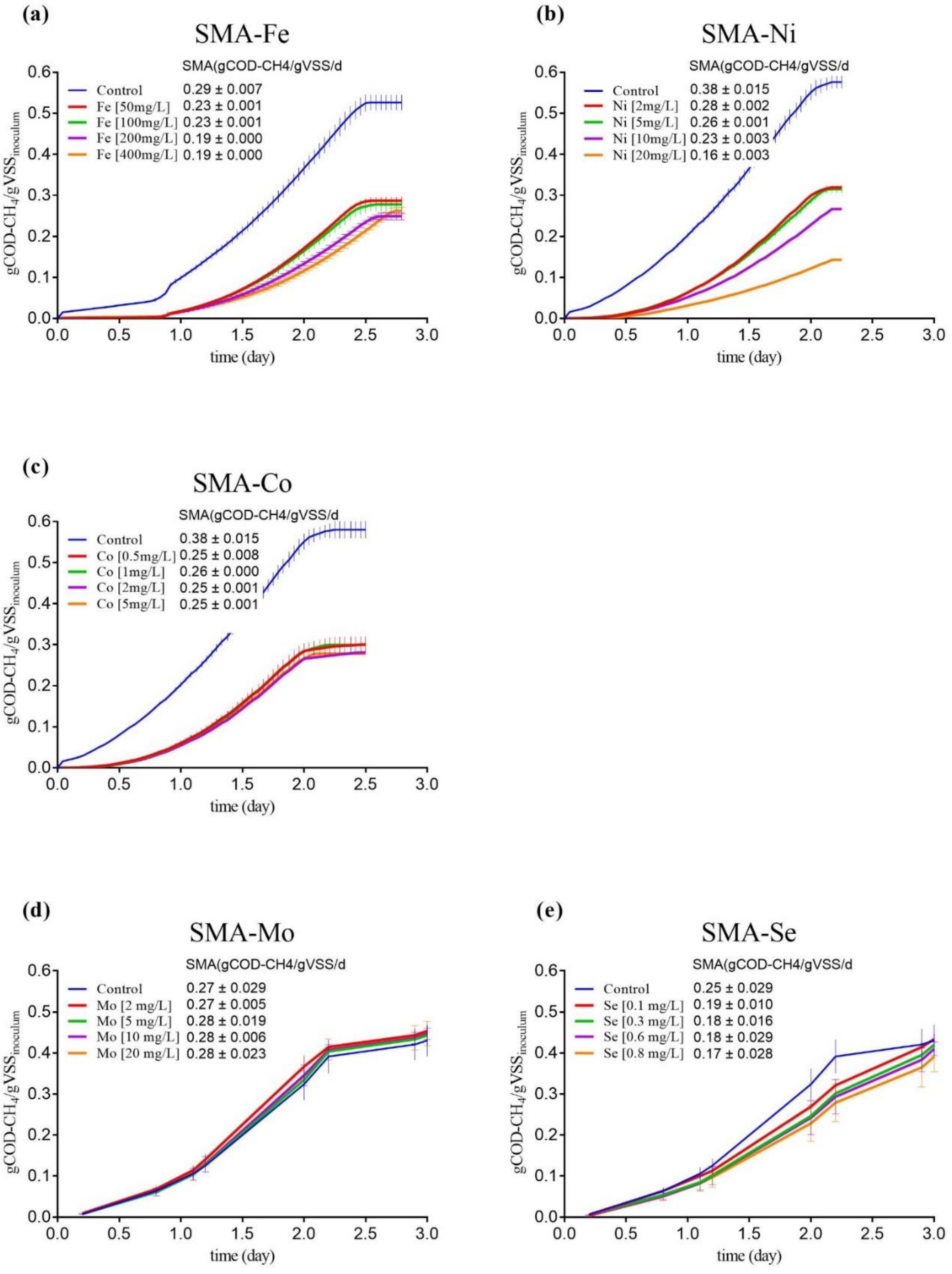

Figure 3-1 Effect of different TEs addition [Fe^{2+} (a), Ni^{2+} (b), Co^{2+} (c), Mo^{6+} (d), and Se^{4+} (e)] on mesophilic SMA tests, using acetate as a substrate at COD of 2 g/L.

3.3.2. Impact of individual TEs addition on FW digestion (CMP tests)

The substrate used in this study was FW collected from southeast WI supermarkets. The TE levels in the FW were 5 mg Fe/L, <0.005 mg Co/L, 0.04 mg Mo/L, 0.1 mg Ni/L, <0.05 mg Se/L. The total COD concentration in the FW samples were 259 g/L on average with 37% being the soluble fraction. The total COD to VS and particulate COD to VSS ratios for the FW were 2.00 and 1.85, respectively.

After assessing the impact of TEs on inoculum activity as reflected by SMA tests, the CMP tests with FW as the substrate were performed. It must be emphasized that the contribution of trace elements in FW were too low compared to the added TEs concentrations to adversely affect the methanogens. While the supplementation of trace elements in the Fe-rich inoculum did not improve methanogens' activity, the supplemented TE concentrations in the SMA tests i.e. Fe^{2+} (50-400 mg/L), Ni^{2+} (2-20 mg/L), Co^{2+} (0.5-5 mg/L), Se^{4+} (0.1-0.8 mg/L) are orders of magnitude higher than in FW. Thus, the impact of adding FW to digesters on methanogenic bacterial activity is indeed neutral since as shown in Table 3-1 total Fe, Ni, Co, and Se in FW averaged 5, 0.1, <0.005, <0.05 mg/L, respectively, substantially below externally added TEs' concentrations.

In this experiment, focus was placed on the impact of ionic TE addition individually on maximum specific methane production rate ($SMPR_{max}$) and the apparent hydrolysis rates constant (K_h) impacted by the addition of TEs individually during the first 6 days of batch digestion of FW. To study the impacts of designated TE ions on FW digestion, series of CMP tests were conducted at an F/M ratio of 2 corresponding to an ISR of 0.5 using Ni^{2+} (0.5, 1, 1.5, 2 mg/L), Co^{2+} (0.1, 0.2, 0.4, 0.5 mg/L), and Se^{4+} (0.005, 0.01, 0.02, 0.05 mg/L) as shown in Table 3-5. As previously mentioned, Fe^{2+} supplementation in the CMP tests was initially performed at an ISR of 3 which is equal to an F/M of 0.6. As shown in Table 3-5, Fe^{2+} addition at all concentrations (50 mg/L, 100 mg/L, 200 mg/L, and 400 mg/L) did not have any significant effects on $SMPR_{max}$ and K_h generated the same values for $SMPR_{max}$ and K_h as the control. Thus, considering the high Fe concentration in the inoculum, the F/M ratio was increased to reduce the Fe content of the batch reactors which is mostly originating from the inoculum. Therefore, after the first test, an F/M of 2 was selected for the rest of the experiments to minimize the impact of the high iron in the inoculum. The total

TEs concentrations in the batch bottles at an F/M of 2 (including inoculum and FW) after supplementation were 1117-1467 mg/L for Fe, 1.6-3.1 mg/L for Ni, 0.14-0.54 mg/L for Co, 2.2-20.2 mg/L for Mo and 0.005-0.05 mg/L for Se. Table 3-5 summarizes the values of P_{max}, $SMPR_{max}$, and K_h as well as the times used to establish the two aforementioned kinetic constants. It is apparent from Table 3-5 that for all tests at an F/M ratio of 2, the $SMPR_{max}$ occurred between day 6 and day 8. The same time interval was used to determine K_h, clearly indicating that, the anaerobic digestion of food wastes is limited by hydrolysis despite the soluble fraction of 37% (Table 3-1). Scrutiny of the $SMPR_{max}$ curve in Figure 3-1C indicates that a small peak was observed around day 3, potentially corresponding to the consumption of the soluble organics in the FW.

Fe^{2+} supplementation at all concentrations, with the exception of 400 mg Fe^{2+}/L, did not change the $SMPR_{max}$ significantly from the control value. On the other hand, K_h values were affected. Ni^{2+} addition resulted in relatively the same values for $SMPR_{max}$ and K_h at all concentrations i.e. 95 mLCH$_4$/gVSS.d and 0.17 1/d, respectively, on average basis, similar to the control. Co^{2+} at all concentrations resulted in the same $SMPR_{max}$ as the control but decreased the K_h by 12% at 0.2 mg/L and 40% at 0.4 mg/L and 0.5 mg/L. Adding Se at the relatively lower concentrations than the other TEs to the batch bottles resulted in similar values of $SMPR_{max}$ and K_h for the control and all applied concentrations of 44.2 mLCH$_4$/gVSS.d and 0.13 1/d, respectively, on average basis. Lastly, Mo^{6+} supplementation at 20 mg/L was the only case in which a statistically significant drop in K_h relative to the control was observed. $SMPR_{max}$ values were also decreased by 5%, 7%, and 10% in 5 mg/L, 10 mg/L, and 20 mg/L Mo^{6+} addition, respectively.

In general, it is concluded that individual supplementation of the TE ions to the batch flasks had marginally negative to neutral impacts on the kinetic parameters of FW digestion.

Table 3-4 K_h and maximum $SMPR_{max}$ values at different individual ionic TE supplementation and concentrations in batch digestion.

Fe^{2+*}

Parameter	Control	[50 mg/L]	[100 mg/L]	[200 mg/L]	[400 mg/L]
TE (mg/L in bottle)	1952	2002	2052	2152	2352
K_h (1/d)	0.58±0.042	0.60±0.017	0.53±0.038	0.51±0.02	0.56±0.012
Time interval for K_h calculation (days)	0.29 to 1.75	0.38 to 1.67	0.33 to 1.63	0.33 to 1.75	0.29 to 1.75
$SMPR_{max}$ (mLCH$_4$/gVSS.d)	84±5.5	87±4.0	81±4.1	81±3.9	83±1.1
Time of $SMPR_{max}$	1.38	1.38	1.42	1.38	1.54
P_{max} (mL)	718	730	635	664	686

* Results of the first batch experiment with Fe addition at an ISR of 3 corresponding to F/M of 0.6

Fe^{2+}

Parameter	Control	[50 mg/L]	[100 mg/L]	[200 mg/L]	[400 mg/L]
TE (mg/L in bottle)	1067	1117	1167	1267	1467
K_h (1/d)	0.14±0.005	0.14±0.001	0.14±0.006	0.12±0.004	0.11±0.002
Time interval for K_h calculation (days)	4 to 6.7	4.2 to 6.5	4.2 to 6.4	4.4 to 7.0	4.5 to 7.5
$SMPR_{max}$ (mLCH$_4$/gVSS.d)	101±3.7	100±2.6	96±1.5	89±3.3	84±4.1
Time of $SMPR_{max}$	6.00	5.83	6.00	5.92	5.92
P_{max} (mL)	1001	990	928	892	875

Ni^{2+}

Parameter	Control	[0.5 mg/L]	[1 mg/L]	[1.5 mg/L]	[2 mg/L]
TE (mg/L in bottle)	1.1	1.6	2.1	2.6	3.1
K_h (1/d)	0.18±0.016	0.17±0.006	0.15±0.021	0.16±0.024	0.16±0.014
Time interval for K_h calculation (days)	4.5 to 6.1	4.8 to 6.6	4.1 to 6.0	4.6 to 6.0	4.5 to 6.0
$SMPR_{max}$ (mLCH$_4$/gVSS.d)	102±3.5	102±6.6	99±7.9	95±9.2	92±2.9
Time of $SMPR_{max}$	6.33	5.17	6.33	5.33	5.25
P_{max} (mL)	824	835	764	774	744

Co^{2+}

Parameter	Control	[0.1 mg/L]	[0.2 mg/L]	[0.4 mg/L]	[0.5 mg/L]
TE (mg/L in bottle)	0.04	0.14	0.24	0.44	0.54
K$_h$ (1/d)	0.13±0.000	0.13±0.017	0.11±0.005	0.11±.004	0.08±0.008
Time interval for K$_h$ calculation (days)	5.7 to 7.4	5.3 to 7.2	4.8 to 6.6	4.8 to 6.8	4.9 to 6.7
SMPR$_{max}$ (mLCH$_4$/gVSS.d)	83±0.7	88±4.9	87±6.8	87±6.1	80±0.4
Time of SMPR$_{max}$	6.46	6.63	6.33	5.25	5.67
P$_{max}$ (mL)	930	930	740	744	738

Se^{4+}

Parameter	Control	[0.005 mg/L]	[0.01 mg/L]	[0.02 mg/L]	[0.05 mg/L]
TE (mg/L in bottle)	<DL (0.05)	0.005	0.01	0.02	0.05
K$_h$ (1/d)	0.12±0.008	0.13±0.015	0.13±0.029	0.13±0.001	0.13±0.003
Time interval for K$_h$ calculation (days)	4.6 to 7.7	4.6 to 6.9	4.6 to 5.6	3.8 to 7.7	4.6 to 7.7
SMPR$_{max}$ (mLCH$_4$/gVSS.d)	43±3.0	42±2.8	44±1.8	46±4.9	46±0.1
Time of SMPR$_{max}$	6.88	6.88	7.67	6.88	5.58
P$_{max}$ (mL)	484	452	446	467	468

* Since Se level in control was below the detection limit of 0.05, its levels in the supplemented bottles were presumed to be equal to the added concentration.

Mo^{6+}

Parameter	Control	[2 mg/L]	[5 mg/L]	[10 mg/L]	[20 mg/L]
TE (mg/L in bottle)	0.2	2.2	5.2	10.2	20.2
K$_h$ (1/d)	0.15±0.003	0.14±0.000	0.13±0.003	0.12±0.003	0.13±0.000
Time interval for K$_h$ calculation (days)	4.2 to 6.1	4 to 6.1	4.4 to 6.3	4.4 to 6.5	4.3 to 6.3
SMPR$_{max}$ (mLCH$_4$/gVSS.d)	89±0.3	91±1.3	84±0.6	83±1.0	81±0.6
Time of SMPR$_{max}$	5.00	4.96	5.00	5.12	5.25
P$_{max}$ (mL)	586	580	543	547	525

3.3.3. Impact of combined TEs addition on FW digestion (CMP tests)

To assess the impact of TEs mixtures on the digestion of FW, various combinations of TEs were tested. In this experiment, Ni^{2+}, Co^{2+}, and Mo^{6+} were used at concentrations of 0.5, 0.5 and 20 mg/L, respectively, and Se^{4+} at concentrations of 0.005, 0.01, 0.02, 0.05 and 0.1 mg/L. Figure 3-3 shows the net cumulative methane production during the first two weeks of batch digestion of FW (13.5 days).

An analysis of variance performed to assess the impact of combination of TE ions supplementation on methane yields showed that all combined forms produced the same amount of methane as the control during the first two weeks of digestion, i.e. there were no significant differences between the TE-supplemented and the control reactors in terms of total methane production.

K_h, $SMPR_{max}$ and TEs concentrations for each batch digestion are presented in Table 3-6. All FW digestion flasks supplemented by different mixtures of TEs showed similar values for K_h i.e. 0.08 1/d, on average basis, comparable to the controls. The analysis of variance for all combined ionic TEs at all concentrations showed no significant differences from the control values in terms of K_h. In addition, all combined ionic TEs showed the same $SMPR_{max}$ values as control reactors (an average of 45 $mLCH_4/gVSS.d$) with no significant differences.

These findings showed that individual supplementation of different ionic TEs had negligible impacts on FW digestion in terms of kinetics. Similarly, the use of TEs mixtures did not impact the $SMPR_{max}$ and K_h.

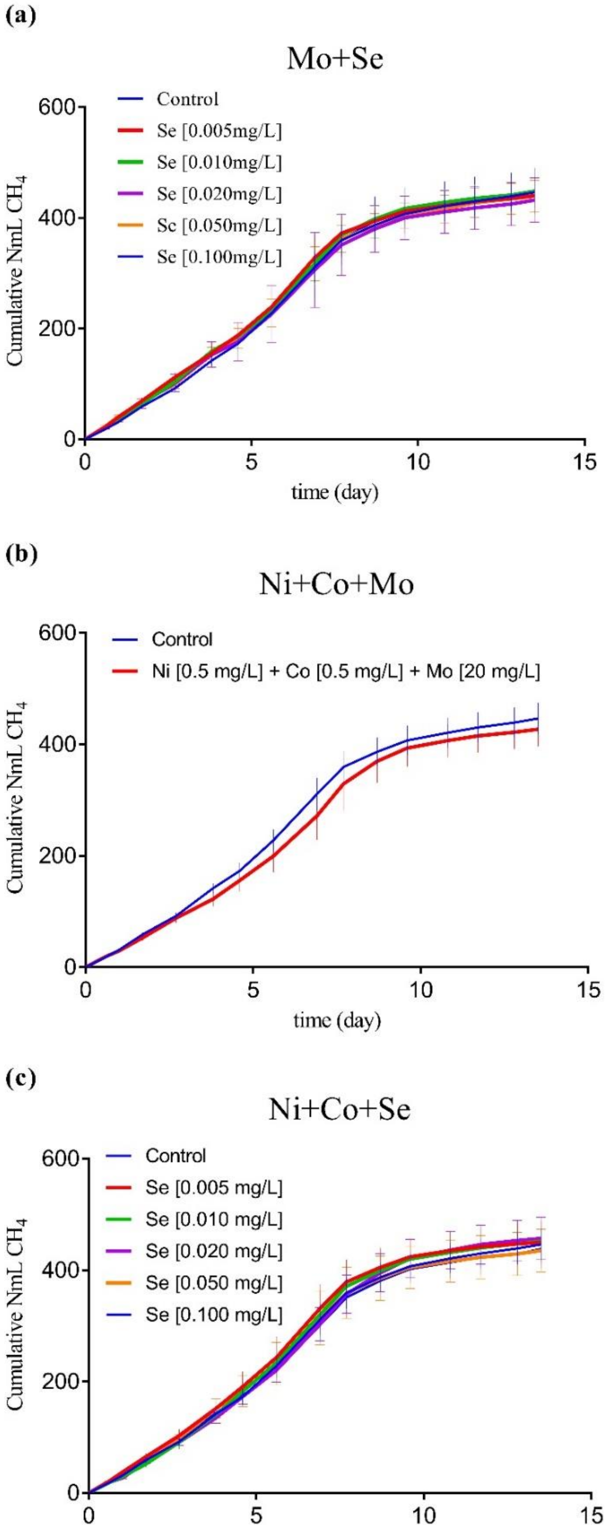

Figure 3-2 Impact of various ranges of combined TEs addition [(a) Mo^{6+}+ Se^{4+}, (b) Ni^{2+}+Co^{2+}+Mo^{6+}, and (c) Ni^{2+}+Co^{2+}+Se^{6+}) on the FW digestion at mesophilic conditions.

Table 3-5 K_h and $SMPR_{max}$ values at different ionic TE mixtures and concentrations in batch digestion

Mo^{6+} [20 mg/L] + Se^{4+} [at defined concentrations]

Parameter	Control	[0.005 mgSe^{4+}/L]	[0.01 mgSe^{4+}/L]	[0.02 mgSe^{4+}/L]	[0.05 mgSe^{4+}/L]	[0.1 mgSe^{4+}/L]
Mo^{6+} in bottle (mg/L)	0.2	20.2	20.2	20.2	20.2	20.2
Se^{4+} in bottle (mg/L) *	0.05	0.005	0.01	0.02	0.05	0.1
K_h (1/d)	0.08±0.008	0.08±0.006	0.08±0.014	0.07±0.013	0.08±0.002	0.08±0.010
Time interval for K_h calculation	2.7 to 7.7	5.6 to 7.7	5.6 to 7.7	4.6 to 7.7	5.6 to 7.7	4.6 to 7.7
$SMPR_{max}$ (mLCH_4/gVSS.d)	43±3.0	45±2.8	47±0.9	46±0.6	45±3.0	45±0.1
Time in which $SMPR_{max}$ was recorded	6.90	6.90	6.90	6.90	6.90	6.90
P_{max} (mL)	446	440	448	432	439	448

Ni [0.5 mg/L] + Co [0.5 mg/L] + Mo [20 mg/L]

Parameter	Control	Supplemented
Ni^{2+} in bottle (mg/L)	1.1	1.6
Co^{2+} in bottle (mg/L)	0.04	0.54
Mo^{6+} in bottle (mg/L)	0.2	20.2
K_h (1/d)	0.08±0.008	0.07±0.014
Time interval for K_h calculation	4.6 to 7.7	3.8 to 7.7
$SMPR_{max}$ (mLCH_4/gVSS.d)	43±3.0	48±3.9
Time in which $SMPR_{max}$ was recorded	6.90	7.70
P_{max} (mL)	446	427

Table 3-6 continued, K_h and $SMPR_{max}$ values at different ionic TE mixtures and concentrations in batch digestion

Ni^{2+} [0.5 mg/L] + Co^{2+} [0.5 mg/L] + Se^{4+} [at defined concentrations]

Parameter	Control	[0.005 mgSe^{4+}/L]	[0.01 mgSe^{4+}/L]	[0.02 mgSe^{4+}/L]	[0.05 mgSe^{4+}/L]	[0.1 mgSe^{4+}/L]
Ni^{2+} in bottle (mg/L)	1.1	1.6	1.6	1.6	1.6	1.6
Co^{2+} in bottle (mg/L)	0.04	0.54	0.54	0.54	0.54	0.54
Se^{4+} in bottle (mg/L) *	<DL (0.05)	0.005	0.01	0.02	0.05	0.1
K_h (1/d)	0.08±0.008	0.09±0.006	0.09±0.010	0.09±0.011	0.08±0.009	0.08±0.005
Time interval for K_h calculation	4.6 to 7.7	5.6 to 7.7	5.6 to 7.7	5.6 to 7.7	4.6 to 7.7	5.6 to 7.7
$SMPR_{max}$ (mLCH_4/gVSS.d)	43±3.0	45±2.6	45±3.2	46±3.8	42±4.3	43±3.1
Time in which $SMPR_{max}$ was recorded	6.90	6.90	6.90	7.70	6.90	7.70
P_{max} (mL)	446	450	452	457	435	438

* Since Se level in control was below the detection limit of 0.05, its levels in the supplemented bottles were presumed to be equal to the added concentration

It has been reported that TEs supplementation had either neutral or slightly negative effects with inocula originating from reactors with a high background level of TEs, such as those used for the co-digestion of biowaste and waste activated sludge (Facchin et al., 2013). However, with inocula from digesters treating only FW, it is highly plausible to observe increased methane yields and rates by supplementing TEs. Ariunbaatar et al. (2016) reported the increase of biomethane potential of a FW containing low concentrations of TEs whereas the same experiments did not result in an increased biomethane production when FW had an elevated background concentration of TEs. Therefore, assessing the inoculum and FW background TEs concentrations prior to adding any trace elements is indeed of high importance.

In this study, SMA rates were not enhanced by the TE supplementation to the reactors (Figure 3-2), and similarly in the CMP tests, methane production and kinetic values obtained from the TE supplemented reactors were almost the same as the controls (with no TE added). There are several studies in the literature showing significant positive effects of TE supplementation on hydrolytic bacteria. For instance, Zhu et al. (2007) observed a 400% increase in hydrogen production at 3.2 mg Fe/L addition to the batch reactors. Similarly, Yang and Shen (2006) could produce 164% more hydrogen from hydrolysing starch in batch mode at pH 8 when 30 mg/L Fe was added. Zhang and Shen (2006) also reported up to 185% (at 161 mg Fe/L) increase in hydrogen production form batch reactors. At 25 mg/L Ni addition, (Karadag and Puhakka (2010) was able to produce 71% more hydrogen. Thus, it seems reasonable to conclude that the negative effect of TEs on methanogenic archaea in the SMA tests was balanced out by its positive effect on hydrolytic bacteria in the CMP tests, hence TE addition to batch reactors in the CMP tests neutrally impacted the whole microbial community including both methanogenic and hydrolytic groups.

3.3.4. Fate of TEs

Soluble TE concentrations decreased significantly during the experiments except for Fe (Figure 3-4). The initial soluble ionic TE concentrations after supplementation were also low most probably due to rapid sorption and precipitation. The initial total Fe concentrations ranged from 1067 mg/L to 1467 mg/L. As evident from Figure 3-4A, Fe solubility was very low (initial soluble Fe concentrations were 0.03 mg/L to 3.42 mg/L). Furthermore, Figure 3-4A shows that final Fe solubility decreases with increasing initial soluble Fe concentration. Soluble Co concentrations of the final samples of CMP tests accounted for 29% of the initial soluble concentrations (Figure 3-4B). The initial soluble Co concentrations of 0.1-0.2 mg/L in the bottles supplemented with 0.1-0.5 mg Co/L decreased to <0.05 mg/L in the final samples. The soluble Co concentrations in the initial and final samples were 43±12% and 9±1% of the total levels, respectively, indicating a significant drop (≈79%) in soluble fraction. Similarly, Ni concentrations in the final soluble samples were 11% of the initial values (Figure 3-4C); soluble Ni levels were 2.1±1.3 mg/L (36±20% of the total Ni) for the initial samples and 0.21±0.16 mg/L (6±2% of the total Ni) for the final samples. Initial and final soluble concentrations of Mo were <0.01-18 mg/L (77±19% of the total Mo) and <0.01-5.6 mg/L (26±1% of the total Mo), indicating a substantial drop during the CMP tests (29%). Se concentrations were below the detection limits of 0.05 mg/L for both initial and final samples. The ranges of soluble TE fractions in this study were similar to the values reported by Gustavsson et al. (2013) who analysed soluble TE fractions in the samples from eight full-scale anaerobic digesters i.e. 4%-18% for Co, <2% for Fe, and <5% for Ni.

TE availability for growth and microbial uptake depends on speciation and metals need to be present as free forms or bound with biological ligands to be available to microorganisms (Oleszkiewicz and Sharma 1990; Adamo et al. 1996; Hassler et al. 2004; Kalis et al. 2007). Thus, bioavailability of metals is related to total metal concentration; precipitation, principally by sulfide, carbonate, and phosphate; complexation; and the kinetics of precipitation and complexation reactions (Callander and Barford 1983a). These anionic precipitates are mainly in the forms of carbonates, phosphates and sulfides (Callander and Barford 1983a; van der Veen et al. 2007; Carliell-Marquet et al. 2010). However, taking the solubility product constants (K_{sp}) into account, it is proven that TEs prefer to precipitate with the most anionic forms (CO_3^{2-}, S^{2-}, and PO_4^{3-}). The order of precipitation depends on the solubility products. According to previous studies, sulfide forms the most stable precipitates among all forms and is thermodynamically the most abundant product in the organic fraction in AD systems (Callander and Barford 1983a; Morse and Luther 1999; Fermoso et al. 2009). Sulfide may remove metal ions essential for growth, such as nickel, iron, and cobalt (Speece 1985)

Liu and Fang (1998) conducted an X-ray spectroscopy analysis on the cross section of UASB reactor granules and confirmed Ni and Fe sulfide precipitates formation under anaerobic conditions. In another study, Kaksonen et al. (2003) used sulphate reducing bacteria to produce sulfide and showed that 99.8% of the iron was precipitated as FeS and FeS_2. Similarly, van der Veen et al. (2007) found that sulfide could remove over 99% of heavy metal from acid mining sludge.

The level of sulfide in the inoculum and then in the batch reactors was relatively enough to make TE-sulfide precipitates (0.83 ± 0.1 mM sulfide). In a study on metal precipitation on digesters, Callander and Barford (1983) calculated the relative concentrations of gaseous H_2S, liquid H_2S, HS^-, and S^{2-} in a digester at pH of 7.3. In this study, the final pH during digestion was on average 7.23, as compared with 7.3 in the aforementioned study. Accordingly, HS^- and S^{2-} concentrations in this study, accounting for the difference in pH between the two studies were 63 % of the total sulphides i.e. 5.2×10^{-4} moles/litter.

This sulfide reacts with dissolved metals and form sulfide precipitates. The solubility constants for FeS, NiS, CoS, MoS at 35°C were $10^{-5.95}$, $10^{-19.4}$, $10^{-22.09}$, and 10^{-43} (Rex Goates et al. 1952; Emerson et al. 1983; Clark and Bonicamp 2000). Since the FeS solubility constant is significantly higher than other TE precipitates (Jacobs and Emerson 1982; Emerson et al. 1983), it is the dominant TE precipitating with S^{2-}. The estimated sulfide required to precipitate Fe based on the maximum soluble Fe concentration of 4.2 mg/L (0.075 mM/L) using the K_{sp} (FeS) of $10^{-5.95}$ in a working volume of 0.2 L, was 3.02×10^{-3} mole, which is substantially higher than the sulfide needed to precipitate other TEs. Using the maximum soluble Ni concentration of 1.03 mg/L with the K_{sp} (NiS) of $10^{-19.4}$ resulted in 4.39×10^{-16} mole sulfide. The same calculation was done for CoS (5.34×10^{-2} soluble mg Co/L and K_{sp} (CoS) of $10^{-22.09}$) and led to 1.80×10^{-17} mole sulfide.

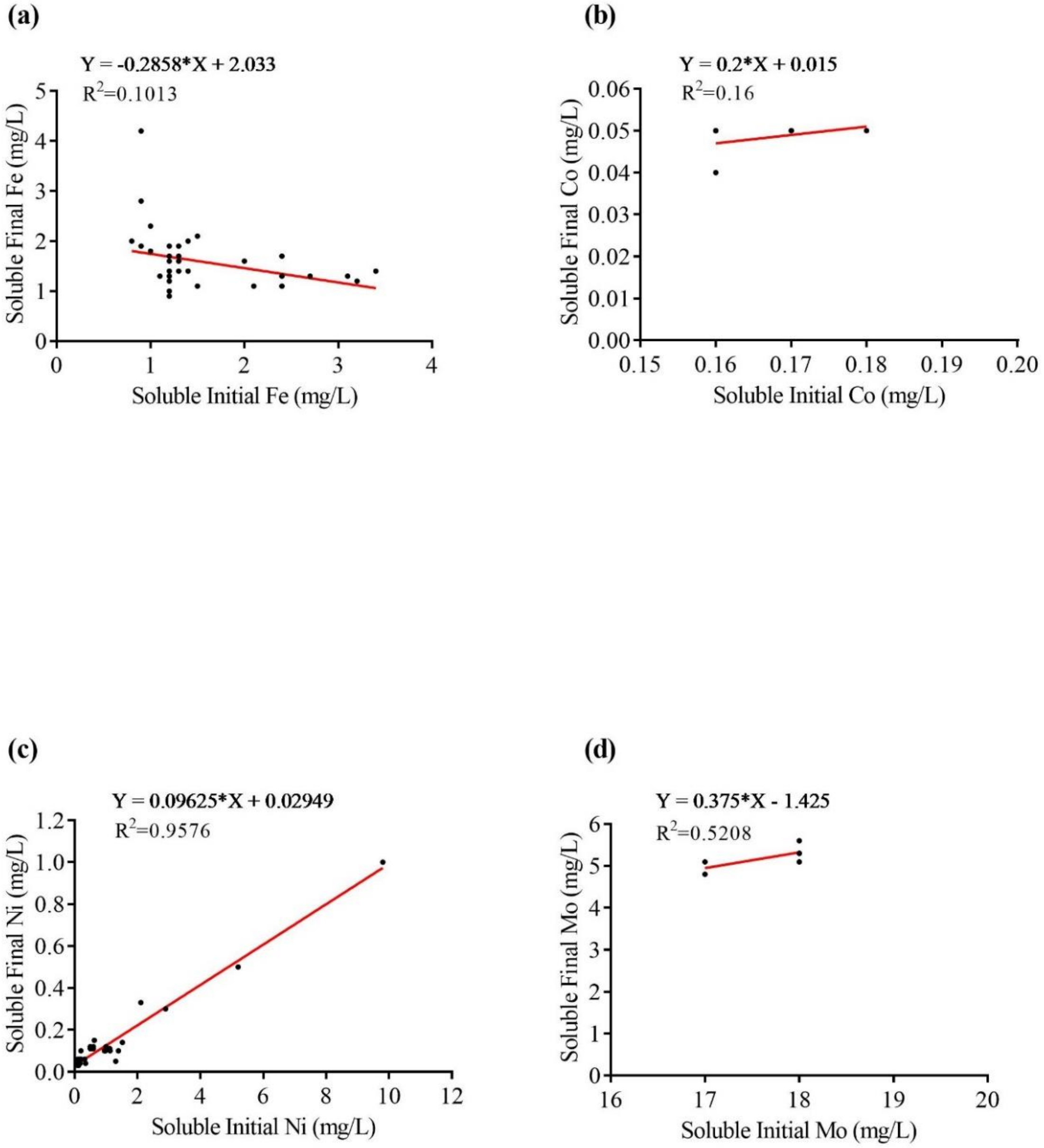

Figure 3-3 The correlation between initial and final soluble TE concentrations (a) Fe (b) Co (c) Ni (d) Mo.

Hence, since the amount of free sulfides (S^{2-}) based on the operational conditions of this study of 5.2×10^{-4} mole is well below the S^{2-} concentration of 3.02×10^{-3} mole required to precipitate all the TE ions i.e. Fe^{2+}, Ni^{2+}, Co^{2+}, Mo^{6+}, and Se^{4+}, it appears that co-precipitation and adsorption onto iron sulfide complexes played a significant role in reducing the soluble TEs. These processes have been studied in sediments with an excess of Fe over other metals (Huerta-Diaz et al. 1998; Cooper and Morse 1999; Morse and Luther 1999). Morse and Arakaki (1993) for instance concluded that those metals whose sulfide phases are less soluble than FeS exhibit an increase in partition coefficient. They observed considerable adsorption and coprecipitation of Co and Ni on FeS by calculating and comparing partition coefficients.

Thus, it is very likely that the high Fe concentration in the reactor lead to co-precipitation and/or adsorption of other metals on iron sulfide (Morse and Arakaki 1993). Carliell-Marquet et al. (2010) also reported a change in some TE speciation due to the daily iron dosing (from 300 $mg/L_{\text{dried digested sludge}}$ to 2100 $mg/L_{\text{dried digested sludge}}$). They observed that all metals measured, apart from iron, decreased in terms of their mass per mass of dried sludge over the 111-day iron dosing period and on average, metals concentrations decreased by 39% from their pre-dosing levels. TE incorporation is ferrous sulfide precipitates is governed by a kinetic competition in the exchange of the ligand bound with the TEs. Based on the orbital configuration and ligand field stabilization energy of the TE, Morse and Luther (1999) obtained an order of TE contents in FeS_2 and FeS precipitates metals to form co-precipitate. Hence, in reactors where Fe is supplied in relatively large quantities compared to other metals, adsorption/co-precipitation of other TEs on FeS_2 and/or FeS may become an important factor.

3.3.5. Environmental impact of TE supplementation

Anaerobically digested biosolids are typically used as fertilizers. The maximum concentrations of TE in biosolids expressed in mg/kg dry weight for land application in the province of Ontario (Canada) varies between TEs as e.g. 340 (Co), 420 (Ni), 34 (Se) (Canadian Council of Ministers of the Environment 2010). TE supplementation may be a concern for land disposal. The maximum TE concentrations of digestates in this study based on a 70% volatile solids destruction efficiency were estimated as 30 mgCo/kgTS, 140 mgNi/kgTS, and 5 mgSe/kgTS, indicating that the elevated

TE levels were still below the aforementioned levels for land application.

3.4. Conclusions

The impact of trace elements on methanogenic microorganisms and anaerobic microbial cultures was discerned from the SMA and CMP tests. For the Fe-rich inoculum used in this study, supplementing TEs had adversely affected the methanogenic activity, except Mo. In batch digestion of FW, supplementing Co, Ni, Se, Fe, and Mo individually did not enhance the maximum specific methane production rates and the apparent hydrolysis rate constants. Similar to the individual supplementation of TEs, the combination of different TEs addition had a neutral impact on methane yields, the apparent hydrolysis rates constant, and the maximum specific methane production rates. In addition, it must be asserted that in all TEs added reactors, the maximum daily methane was produced during the first six to eight days of digestion. However, since typical full-scale digesters have the solids retention time (SRT) of more than 15 days, this enhancement in digestion time is insignificant. Final soluble TE concentrations were 10%, 28%, and 29% of the initial soluble TE for Ni, Co, and Mo. It is apparent that the high concentration of Fe in the inoculum has led to a considerable decline in the bioavailability of TEs as evidenced by the significant drop in soluble TEs concentrations during digestion. This study revealed that the high Fe concentration in the inoculum, which is often the case in North America, reduces the concentration of free trace TEs via potential co-precipitations and thus does not result in any enhancement in digestion kinetic parameters ($SMPR_{max}$ and K_h) and methane yields. Overall, if batch methane potential test results are predictive of the full-scale digesters performance, the results of this study clearly show that contrary to many literature reports on the need to supplement TE in FW digestion to improve digestion efficiency, in Fe rich sludges, FW digestion might not be improved by TEs supplementation in the full-scale digesters. However, in full-scale digesters, over long periods of operation, pseudo-steady-state conditions which are mostly governed by the feedstock characteristics will prevail.

3.5. References

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Chapter 4

4. Trace element supplementation in mesophilic food waste anaerobic digestion: the impact of Fe in the inoculum

4.1. Introduction

Due to its high biodegradability and nutrient contents, food waste is an extremely desirable substrate for anaerobic digestion (AD), resulting in a high biochemical methane potential, about 0.44–0.48 m3 CH_4/kg of the FW volatile solid (VS_{FW}) (Banks et al. 2011b; Zhang et al. 2013). Although AD of FW is a great solution for GHG reduction and subsequent renewable energy production, digesters that take FW as the sole feedstock have been facing unstable performance and even process failure. This is basically due to the accumulation of volatile fatty acids (VFAs), ammonia and/or sulfide (Demirel and Scherer 2011; Zhang et al. 2015a) which is linked to trace element deficiencies (Banks et al. 2012c). This in turn limits the application of AD for treating FW at full scale (Climenhaga and Banks 2008; Banks et al. 2012d; Tampio et al. 2014).

One potential solution to enhance process stability is to add trace elements (TE) to digesters since food waste is deficient in some trace elements required for a stable AD process. TEs are crucially important in accelerating enzymatic reactions in the methanogenesis stage of AD process and improve metabolic pathways drastically (Zandvoort et al. 2006a). According to the literature, methanogens depend on the presence and availability of Fe, Ni, Co, Se, and Mo. (Feng et al. 2010; Pobeheim et al. 2011; Banks et al. 2012a).

Although enhancements and inhibitions of FW anaerobic digestion process with different TE additions have been very well studied (discussed in chapter 2), limited research have been done with a focus on the inoculum origin and its TE concentrations (especially Fe) affecting the whole anaerobic digestion process. The only study found in the literature was conducted by De Vrieze et al. (2013), in which kitchen waste (KW) was co-digested with an Fe-rich (1350 mg Fe/L) activated sludge (A-sludge) in thermophilic and mesophilic CSTR systems. It is noteworthy that the COD:N ratios of KW and A-sludge were 21.8 and 15.1, respectively. They observed that total net methane production increases by 320%, 430%, and 650% when 5:95, 10:90, and 15:85 volumetric ratios of KW with A-sludge are thermophilically digested, respectively. These volumetric ratios of 5:95, 10:90, and 15:85 volumetric ratios of KW and A-sludge correspond to Fe concentrations of 1283 mg/L, 1216 mg/L, and 1149 mg/L in the reactors, respectively. In mesophilic reactors however, 110%, 210%, and 326% increase in methane production occurred with 5:95, 10:90, and 15:85 volumetric ratios of KW with A-sludge. They concluded that the process failure that is a result of single KW anaerobic digestion can be overcome by co-digestion with A-sludge, which can be potentially due to the high Fe content in the A-sludge compared to the KW. This was the only paper found in the literature that have compared the FW anaerobic digestion performance of two different types of inoculum with different Fe concentrations.

TE bioavailability for microbial uptake highly depends on speciation. In other words, TEs must be In their free forms or bound with biological ligands to be readily available to microorganisms (Oleszkiewicz and Sharma 1990; Adamo et al. 1996; Hassler et al. 2004; Kalis et al. 2007), which is related to total metal concentration, precipitation, and complexation (Callander and Barford 1983a). TEs mainly form anionic precipitates that are often in the forms of carbonate, phosphate and sulphide (Callander and Barford; van der Veen et al. 2007; Fermoso et al. 2009; Carliell-Marquet et al. 2010). TEs prefer to precipitate with the most anionic forms (CO_3^{2-}, S^{2-}, and PO_4^{3-}). Among all precipitates, sulphide forms the most stable form and is thermodynamically the most abundant product in AD systems (Callander and Barford 1983a; Morse and Luther 1999; Fermoso et al. 2009).

Most metals can form sulfide minerals of low solubility. Thus, it is very likely that the high Fe concentration in the reactor lead to co-precipitation and/or adsorption of other metals on iron sulphide (Morse and Arakaki 1993). Carliell-Marquet et al. (2010) also reported a change in some TE speciation due to the presence of Fe-rich sludge. TE incorporation in ferrous sulphide precipitates is governed by a kinetic competition in the exchange of the ligand bound with the TEs. Based on the orbital configuration and ligand field stabilization energy of the TE, Morse and Luther (1999) obtained an order of TE contents in FeS_2 and FeS precipitates metals to form co-precipitate. Hence, in reactors where Fe is supplied in relatively large quantities compared to other metals, adsorption/co-precipitation of other TEs on FeS2 and/or FeS may become an important factor.

TE-sulfide precipitates have a very low solubility and as a result, they are one of the most important regulators of TE bioavailability (Callander and Barford 1983a, b). TE-sulfide solubility is however, dependent on organic and inorganic chelators and particle size (Jansen et al. 2005). Therefore, due to the complex pool of inorganic and organic matter in batch AD reactors, it is difficult to predict trace element requirements and bioavailability (Gustavsson et al. 2013).

Evidently, the lack of global knowledge of the TEs speciation is mainly due to the complexity of TE analysis in anaerobically digested sludge. Knowing TE requirements and selecting adequate dosages for optimized anaerobic digestion of FW requires taking background TE concentrations of FW and inoculum into consideration. However, there seems to be a lack of sufficient attention to initial TE concentrations in AD systems among different studies. Thus, the purpose of this study was to determine the importance of TE background levels, especially Fe, in the inoculum utilized in mesophilic FW anaerobic digestion. The anaerobic FW digestion was assessed with a low Fe content inoculum in batch tests and compared the results of AD performance in terms of methane yield and digestion kinetic parameters with the ones of another study (chapter 3) with the similar conditions but a high Fe content inoculum. Since the primary focus was to see the impact of TE additions on methanogens, specific methanogenic activity (SMA) tests were first conducted with acetate as a readily biodegradable substrate and various TE concentrations were supplemented to batch reactors. Subsequently, FW was used as the substrate in biomethane potential (BMP) tests to evaluate the effects of TE supplementation method on the whole microbial community and overall process performance.

4.2. Materials and Methods

4.2.1. Inoculum and food waste: sources and characterization

The sludge originating from a mesophilic anaerobic reactor in Dufferin, Toronto, Ontario treating food waste was used as inoculum. This inoculum is referred to as FWS-low Fe (FWS-LFe) throughout the entire thesis. Food waste samples were collected from southeast WI supermarkets and was provided by the Grind2Energy systems (InsinkErator, WI) that process food waste for energy production. Both inoculum and FW samples were stored in gas tight plastic containers in a cold room (4ºC) prior to conducting the SMA and BMP tests. Total solids (TS) and volatile solids

(VS) were measured on a weight basis (g/L) according to the standard methods for the examination of water and wastewater (APHA 2005). Total and soluble samples analysis were conducted based on Hach methods i.e. chemical oxygen demand (COD, 200-15,000 mg/L, method 8000), phosphorous (1-100 mg PO43-/L, method 10121), volatile fatty acids (total VFA, 50-2,500 mg/L as acetic acid, molecular weight basis, TNT872 kits), and nitrogen (10-150 mg/L, method 10071). Samples were filtered through sterile 0.45 µm membrane filter papers (VWR International, Canada) to obtain soluble fractions (for parameters such as soluble COD, soluble nitrogen, and ammonia (0.4-50 mg/L, method 10031)). Characteristics of the inoculum and FW can be found in Table 4.1.

Table 4-1 Summary of the physical and chemical characteristics of inoculum and substrate (average ± standard deviation (number of analysis))

Parameter	Unit	Inoculum	Food Waste
TS	g/L	40 ± 1.3 (10)	99 ± 2.2 (9)
VS	g/L	17 ± 0.3 (10)	89 ± 1.4 (9)
TSS	g/L	25 ± 1.0 (10)	73 ± 3.2 (6)
VSS	g/L	13 ± 0.6 (10)	70 ± 2.4 (6)
TCOD	g/L	28 ± 2.5 (10)	257 ± 10.6 (6)
SCOD	g/L	6.6 ± 0.2 (10)	95 ± 2.1 (6)
TP	g/L	1.4 ± 0.1 (10)	0.9 ± 0.3 (10)
TN	g/L	3.5 ± 0.2 (10)	3.6 ± 0.1 (10)
SN	g/L	3.0 ± 0.0 (10)	0.9 ± 0.0 (10)
N-Ammonia	g/L	2.7 ± 0.3 (10)	0.2 ± 0.0 (10)
Total VFA	g/L	1.1 ± 0.2 (10)	9.2 ± 0.1 (10)
Total Fe	mg/L	260 ± 47 (2)	1.98 ± 0.1 (2)
Soluble Fe	mg/L	4.4 (1)	1.11 ± 0.1 (2)
Total Co	mg/L	0.29 ± 0.00(2)	0.005 (1)
Soluble Co	mg/L	0.10 (1)	<0.005 (2)
Total Mo	mg/L	0.13 ± 0.01 (2)	0.01 ± 0.1 (2)
Soluble Mo	mg/L	0.02(1)	<0.01 (2)
Total Ni	mg/L	0.68 ± 0.08 (2)	0.02 ± 0.0 (2)
Soluble Ni	mg/L	0.20 (1)	<0.01 (2)
Total Se	mg/L	0.03 ± 0.01(2)	0.01 (1)
Soluble Se	mg/L	0.02 (1)	<0.05 (2)

4.2.2. TE selection

Fe^{2+} (50, 100, 200, 400 mg/L), Ni^{2+} (2, 5, 10, 20 mg/L), Co^{2+} (0.5, 1, 2, 5 mg/L), Se^{4+} (0.1,0.3,0.6,0.8 mg/L), and Mo^{6+} (2, 5, 10, 20 mg/L) were selected based on the survey of the literature (Ortner et al. 2015; Zhang et al. 2015c). The selected concentrations were used for SMA tests and then for the BMP tests in the next phase. The five TEs were prepared in solution using the following salts i.e. $FeCl_2 \cdot 4H_2O$, $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $MoNa_2O_4 \cdot 6H_2O$, and Na_2O_3Se.

4.2.3. TE analysis using ICP–OES (inductively coupled plasma–optical emission spectrometer)

Total and soluble TEs in the samples were analysed according to Standard Methods (3120) (APHA 2005). Total TEs include both dissolved and particulate forms. Acid digestion method was applied to extract TEs from the samples for the determination of total TEs. Briefly, 3 g of the sample added to 3 mL concentrated nitric acid (67-70%, Caledon Laboratories) was digested in a flask on a hot plate at 95-100 ^{o}C for 3 hours. The digested sample was filtered through a syringe filter (0.45 μm) for quantifying the concentration of total TEs using Inductively Coupled Plasma (ICP) (Vista-Pro, VARIAN). Similarly, to analyse soluble TEs in the sample, the original sample was initially diluted 10 times with distilled water, then filtered through a 0.45 μm filter paper to collect soluble fraction. The pH of the soluble fraction was adjusted to below 2, using concentrated nitric acid (67-70%) prior. Then samples for both total and soluble TE measurement were poured individually into Autosampler vials. The detection limits of the analysts (mg/L) were 0.005 (Co, Fe), 0.01 (Mo, Ni), and 0.05 (Se). TE concentrations of inoculum and FW are summarized in Table 4-1.

4.2.4. Specific Methanogenic Activity (SMA) tests using the AMPTS (Automated Methane Potential Test System)

Prior to adding FW as the substrate to the seed sludge, a set of specific methanogenic activity (SMA) tests were run with acetate as sole carbon and energy source to check the effect of TE ions addition on the activity of sludge. More than 60% of the biologically produced methane in the methanogenic stage comes from the aceticlastic pathway (Rogers and Whitman 1991). Therefore, acetate was used as the readily biodegradable substrate to focus on the aceticlastic methanogens and this was evaluated by determining the maximum methane production rate. When acetate is the sole substrate, other degradation steps prior to methanogenesis (hydrolysis, acidogenesis, acetogenesis) are skipped and thus, the substrate will be only consumed by aceticlastic methanogens, enabling us to monitor their activity during the AD process. Ionic trace elements (Fe^{2+}, Ni^{2+}, Co^{2+}, Se^{4+} and Mo^{6+}) were added to the reactors at mesophilic conditions (37°C) to observe their possible influence on methanogens' activity. SMA of the mesophilic sludge was determined using an Automated Methane Potential Test System (AMPTS_II, Bioprocess Control, Lund, Sweden).

The AMPTS system incubated 650 mL airtight batch reactors with 400 mL liquid working volume containing a mixture of mesophilic inoculum and acetate solution (2g COD/L) placed in a water bath at 37°C. CO_2 and H_2S were stripped from the biogas by leading the biogas through 100 mL bottles containing 80 mL 3M NaOH solution. Then the remaining gas which was methane, flowed into a gas flow cell with a calibrated volume. When the gas volume equaled the calibrated volume of the flow cell, the gas was released and recorded as one normalized volume at time t. The test was stopped after about 5 days since the SMA is to be read during the first few days of acetate digestion.

Sodium acetate (dehydrate) at 2 g COD/L was used as the substrate and the SMA tests were performed in triplicates for the control bottles (without TEs) and ionic TE supplemented bottles using a working liquid volume of 400 mL. The headspaces of all flasks were purged with nitrogen gas for 3 minutes prior to starting the test. The experimental design data is shown in Table. 4-2.

Table 4-2 Summary of the SMA tests design for different TE ions at various concentrations

TE ion	VS [g/L]	Sludge/bottle [mL]	Sodium acetate/bottle [mL]	Working Volume [mL]	I/S
Ni^{2+} [0.5, 1, 1.5, 2 mg/L]	16.6	104	296	400.0	3.0
Co^{2+} [0.5, 1, 2, 5 mg/L]	16.6	104	296	400.0	3.0
Mo^{6+} [2, 5, 10, 20 mg/L]	20.7	90	310	400.0	3.0
Se^{4+} [0.1, 0.3, 0.6, 0.8 mg/L]	20.7	90	310	400.0	3.0
Fe^{2+} [50, 100, 200, 400 mg/L]	20.4	91	309	400	3.0

It should be noted that the difference in volumes for inoculum and substrate used for the SMA tests was due to the two sets of inoculums collected from the same WWTP over the testing period.

Specific methane activity rate (SMA) was calculated by obtaining the slope of the accumulated methane production curve (mL/d), converting this number to its COD equivalence, and dividing it by the mass volatile suspended solids (VSS) in the reactor (inoculum). The final values were expressed in g COD-CH_4/gVSS/d. By looking at the graph, we estimated the maximum slope to lie in a certain time interval. Taking several smaller intervals between that period, we conducted a linear regression with GraphPad Prism7 and calculated the slopes to find the maximum slope the best with R^2. The same method was applied for all reactors and the data is presented in section 4.3.1.

4.2.5. Biomethane Potential (BMP) tests using the swirling shakers

The anaerobic biodegradability of the FW was performed according to the protocols (Holliger et al. 2016). Swirling shakers (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) were used for BMP tests to monitor biogas production. The shakers were run at 150 rpm and 37°C to incubate batch samples in 300 mL (liquid volume of 200 mL) flasks. The flasks were sealed with butyl rubber septa. Biogas production from the batch bottles was measured twice a day at the beginning of the experiment and once a day at the middle of the experiment using a digital gas pressure meter (VWR® Traceable® Manometer Pressure, VWR International). After each pressure measurement, the biogas in the head space was released and then the pressure in the headspace was measured again to make sure it is close to the ambient atmospheric pressure. Daily pressure differences were converted into biogas volumes using the following equation (El-Mashad and Zhang 2010):

$$V_{Biogas} = \frac{P.V_{headspace}.C}{R.T} \qquad \text{Equation 4-1}$$

Where V_{Biogas} = daily biogas volume (L), P = absolute pressure difference (mbar), $V_{headspace}$ = volume of the head space (L), C = molar volume (22.41 L mol−1), R = universal gas constant (83.14 L mbar K−1 mol−1), T = absolute temperature (K).

In addition, biogas samples were collected from the headspace of each reactor everyday and stored in disposable sample bottles (Vacuette® Serum Clot Activator Tubes 9 mL, VWR). The same day, their methane contents were determined with a gas chromatograph (Model 310, SRI Instruments, Torrance, CA, refer to section 2.9) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft 2mm, Restek). The temperatures of the column and the TCD detector were 90 and 105 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. The volume of injected biogas was 0.5 mL. No hydrogen was detected throughout the study. Biomethane production from the shakers was then corrected for standard temperature and pressure (273K, 100 kPa). The methane productions of blanks, containing all additions except the selected biowaste component, were determined to correct for the methane production of the inoculum due to the decay of methanogenic bacteria.

Methane production was calculated with a mass balance equation, using biogas produced and its corresponding composition at each time interval:

$$V_{CH_4,i} = V_{CH_4,i-1} + C_{CH_4,i}\left(V_{Biogas,i} - V_{Biogas,i-1}\right) + V_{CH_4}\left(C_{CH_4,i} - C_{CH_4,i-1}\right) \quad \text{Equation 4-2}$$

where $V_{CH_4,i}$ and $V_{CH_4,i-1}$ are cumulative methane gas volumes at the current (i) and previous (i-1) time intervals, $V_{Biogas,i}$ and $V_{Biogas,i-1}$ are the total biogas volumes in the current and previous time intervals, $C_{CH_4,i}$ and $C_{CH_4,i-1}$ are the fractions of methane gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, and V_{CH_4} is the total volume of headspace in the reactor (López et al. 2007). It is worth mentioning that all biogas volumes were recorded at 37°C and then converted to the equivalent volumes at standard temperature and pressure (STP) conditions, thus, all reported numbers regarding biogas and methane volumes are at 1 atm and 273.15 K conditions.

The BMP tests were performed using inoculum to substrate ratio (ISR; g $VS_{inoculum}$/g $COD_{substrate}$) of 3, in triplicates for the blanks, control bottles (no TE ions), and for bottles with TE ions (Table 4-3). All flasks contained 180 mL inoculum. After adding the required amounts of inoculum and substrate, bottles were filled with distilled water to adjust the working volume to 200 mL. Due to the slight variability in the characteristics of the inoculum and FW during the testing period (≈ 60 days), slightly different weights of FW were used to maintain the ISR at 3 for all the experiments. Prior to conducting the test, the headspaces of all flasks were flushed with nitrogen gas for 3 minutes.

Table 4-3 Summary of the BMP tests design for different TEs at various concentrations

TE ions	COD/bottle [g]	VS/bottle [g]	Sludge/bottle [mL]	FW/bottle [mL]	Water [mL]	Working Volume [mL]	I/S
Single TE ions	1.04	17.3	180	4.1	15.9	200	3.0
Mixed TE ions	1.85	30.1	180	9.5	10.5	200	3.0

It must be asserted that the observed trace element supplementation impacts were not influenced by accumulation of VFA or pH drops, as the final pHs were in the range of 7.5 to 8.5. Also, the COD:N:P ratio in the reactors ranged from 11:2:1 to 22:3:1 with an average of 18 (±2.7):3:1.

There were three parameters calculated after the BMP tests: methane yield, biodegradability, and apparent hydrolysis rate constant (K_h). Methane yield was calculated by dividing cumulative net methane production from each reactor (deducted from the average of three blanks) by the gram volatile solids of FW added to it. Biodegradability was measured by converting cumulative net methane productions to their corresponding COD values at STP conditions and calculating the fraction of COD degraded by dividing that COD equivalence to the COD of the FW added to the reactors. Calculation of K_h was performed according to the protocol of Raposo et al. (2006). K_h describes the rate of degradation and typically follows a first-order kinetic model assuming normal growth (no inhibition, no lack of macro-nutrients or micro-nutrients) (Pfeffer 1974; Tong et al. 1990; Koch and Drewes 2014). When no intermediates accumulate, substrate hydrolysis can be regarded as the rate-limiting step. A first-order kinetic model can then be used for calculating the K_h as follows:

$$CH_4 = CH_4^{ultimate}(1 - e^{-K_h . t}) \qquad \text{Equation 4-3}$$

Where, CH_4= net cumulative methane production from the CMP assay at time t (mL), $CH_4^{ultimate}$= net ultimate methane production at the end of the experiment calculated from the modified Gompertz model (Nielfa et al. 2015) using the BMP net cumulative methane production over the incubation time, K_h= first-order hydrolysis rate constant (1/d). K_h can be derived from the slope of the linear regression line plotted for $Ln(1 - CH_4/CH_4^{ultimate})$ against time. The linearization was done by GraphPad Prsim7 and the R^2 values ranged between 0.96 and 0.99.

4.2.6. Procedures for microbial analysis

Samples were taken from batch reactors with Fe, Ni, Co, Mo, and Se addition each at its optimum concentration as well as the control after the end of the experiments and then filtered through sterile 0.45 micrometer porous filter papers. Filtered samples were stored at -20 degrees C until shipment to Microbe Detectives LLC®. DNA was extracted and 16S rRNA genes were amplified and sequenced using V4 primers and Illumina MiSeq technology. Following standard sequence processing steps, sequences were binned into related groups and classified by taxonomy. Microbial community diversity was estimated by counting the number of species observed and calculating the Shannon's evenness index. Species observed indicates how many different types of microbes are present, while evenness indicates how evenly distributed their abundances are (Ling et al. 2017).

4.2.7. Statistical Analysis

Dunnett's multiple comparisons test were carried out as an ordinary single one-way ANOVA (Analysis of Variances) tests in intergroup comparisons of SMA, K_h, biogas production, and net cumulative methane production in each batch. In case a significant difference between the variables was observed, independent two samples t-test was used in comparisons between the control bottles values and ionic TE added bottles values (separately for each TE ion). P values lower than 0.05 were considered as statically significant. The calculations were performed using GraphPad Prism7 software.

4.3. Results and discussion

4.3.1. Impact of trace elements on sludge activity (SMA tests)

The inoculum used in this study was low-Fe sludge (FWS-LFe) collected from a mesophilic digester treating FW. The TE ion concentrations in the inoculum were 260 mg Fe/L, 0.29 mg Co/L, 0.13 mg Mo/L, 0.68 mg Ni/L, and 0.03 mg Se/L. Total COD concentration was 28 g/L with 23% being the soluble faction. Finally, total COD to VS and particulate COD to VSS ratios for the inoculum were 1.65 and 1.57, respectively.

Ni^{2+} at all applied concentrations did not affect SMA rates significantly compared to the SMA control, as no statistical differences were observed between Ni supplemented bottles and the control (Figure 4-1). SMA rates of 0.055±0.002, 0.047±0.001, 0.049±0.001, 0.049±0.000 and 0.049±0.002 gCOD-CH_4/gVSS/d were found for the control, 0.5 mg Ni^{2+}/L, 1 mg Ni^{2+}/L, 1.5 mg Ni^{2+}/L, and 2 mg Ni^{2+}/L, respectively. Similarly, Co^{2+} did not impact the SMA rates. SMA values for the control, 0.5 mg Co^{2+}/L, 1 mg Co^{2+}/L, 2 mg Co^{2+}/L, and 5 mg Co^{2+}/L were 0.039±0.003, 0.033±0.000, 0.037±0.002, 0.033±0.000 and 0.031±0.002 gCOD-CH_4/gVSS.d, respectively. Adding Mo^{6+} at different concentrations to the SMA test bottles with mesophilic sludge resulted in 28% and 22% higher rate of methane production than the control for 5 mg Mo^{6+}/L and 20 mg Mo^{6+}/L, respectively. Se^{4+} added batch digester at all concentrations resulted in insignificantly different SMA rates compared to the control (Figure 4-1). According to the results of this study, only Mo^{6+} supplementation at 5 mg Mo^{6+}/L and 20 mg Mo^{6+}/L resulted in 28% and 22% higher rate of methane production than the control, respectively. Fe^{2+} at 400 mg/L was another exception where it decreased the SMA rate by 20%.

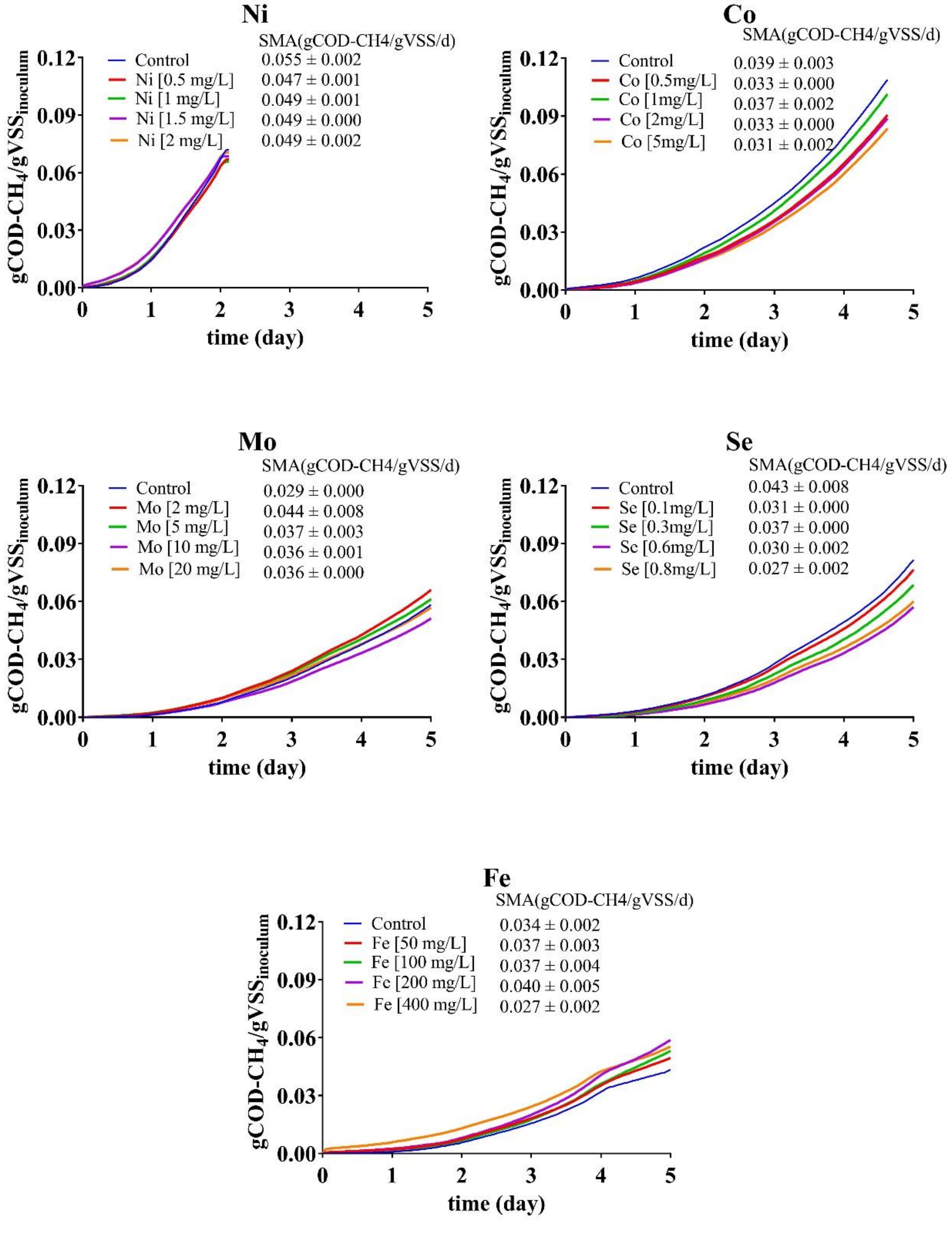

Figure 4-1 Effect of different TE ions addition at different concentrations on mesophilic digested sludge with low Fe content; SMA tests, using acetate as a substrate at COD of 2 g/L. SMA values obtained from each TE ion supplemented condition and controls are shown in front of the legends.

Figure 4-2 shows a comparison between the impact of TE ions supplementation to SMA tests with FW sludge-low Fe (FWS-LFe) and AD sludge- high Fe (ADS-HFe) . According to the first study with ADS-HFe (chapter 3), in contrast to the low Fe inoculum, ionic TE addition decreased the specific methane production rates in almost all cases. For the ADS-HFe SMAs ranged from 0.16-0.38 g COD-CH_4/ g $VSS_{inoculum}$/day while for the FWS-LFe, SMAs varied from 0.027 g COD-CH_4/ g $VSS_{inoculum}$/day to 0.057 g COD-CH_4/ g $VSS_{inoculum}$/day. It should be noted that the first SMA tests with ADS-HFe were conducted at an ISR of 0.5 where the second set of SMA tests with FWS-LFe were run at an ISR of 3. This explains the very different range of SMA numbers in the two studies. Also, the difference between the SMA rates in the two studies which might stem from the dissimilar sources of sludge, the ADS-HFe was from a digester treating primary and secondary sludge in a municipal wastewater treatment plant while the low Fe sludge originated from a digester treating FW. Nonetheless the focus here is observed SMA differences between each TE ion supplemented reactors and controls. With ADS-HFe, Ni^{2+} supplementation drastically reduced the SMA rates by 40% and 58% at 10 and 20 mg/L, respectively, compared to the control, while the differences between control and Ni^{2+} added reactors using low Fe sludge were not statistically significant. Adding Co^{2+} to the ADS-HFe at all concentrations reduced the rate of methanogenic activity by an average of 33% compared to the control. With FWS-LFe however, Co^{2+} supplementation did not show any significant improvement or inhibition. Mo^{6+} supplementation (2-20 mg/L) was the only case in the SMA test with ADS-HFe in which the methanogenic activity rates were neutrally impacted. Interestingly, this was also one of the only cases in the SMA tests with FWS-LFe where enhancements in SMA rates were observed. Using FWS-LFe, Mo^{6+} addition at 5 mg/L and 20 mg/L increased methane production rate by 28% and 22%, respectively. Se addition in the first study (ADS-HFe) at 0.3 mg/L reduced the SMA rate by 26% but it did not impact the rates in the tests with FWS-LFe. Finally, Fe^{2+} significantly reduced the SMA rate of the ADS-HFe by 20% (at 50 and 100 mg/L) to 37% (at 200 and 400 mg/L) in comparison with the control; and it decreased the methane production rates using the FWS-HFe by up to 20% at 400 mg/L addition.

In summary, ionic TE supplementation to reactors with FWS-LFe at the tested concentrations either improved methanogenic activity (Mo^{6+} addition at 5 and 20 mg/L and Fe^{2+} at 400 mg/L) or did not significantly affect the SMA (other TE ions except mentioned earlier), rather than inhibiting methane production (which was observed with ADS-HFe) probably due to the significantly lower Fe content in the inoculum of the second study. Comparing the different TE ion additions, it is observed that Mo^{6+} has a stronger effect on acetoclastic methanogens since it is the only case in which all concentrations ranging from 2-20 mg Mo/L, methane production was not inhibited, but either remained the same (with ADS-HFe) or were improved (with FWS-LFe) (Figure 4-2). Therefore, it can be concluded that Mo^{6+} is the only trace element among the tested TE ions which always has a positive impact on the methanogenic activity of the sludge.

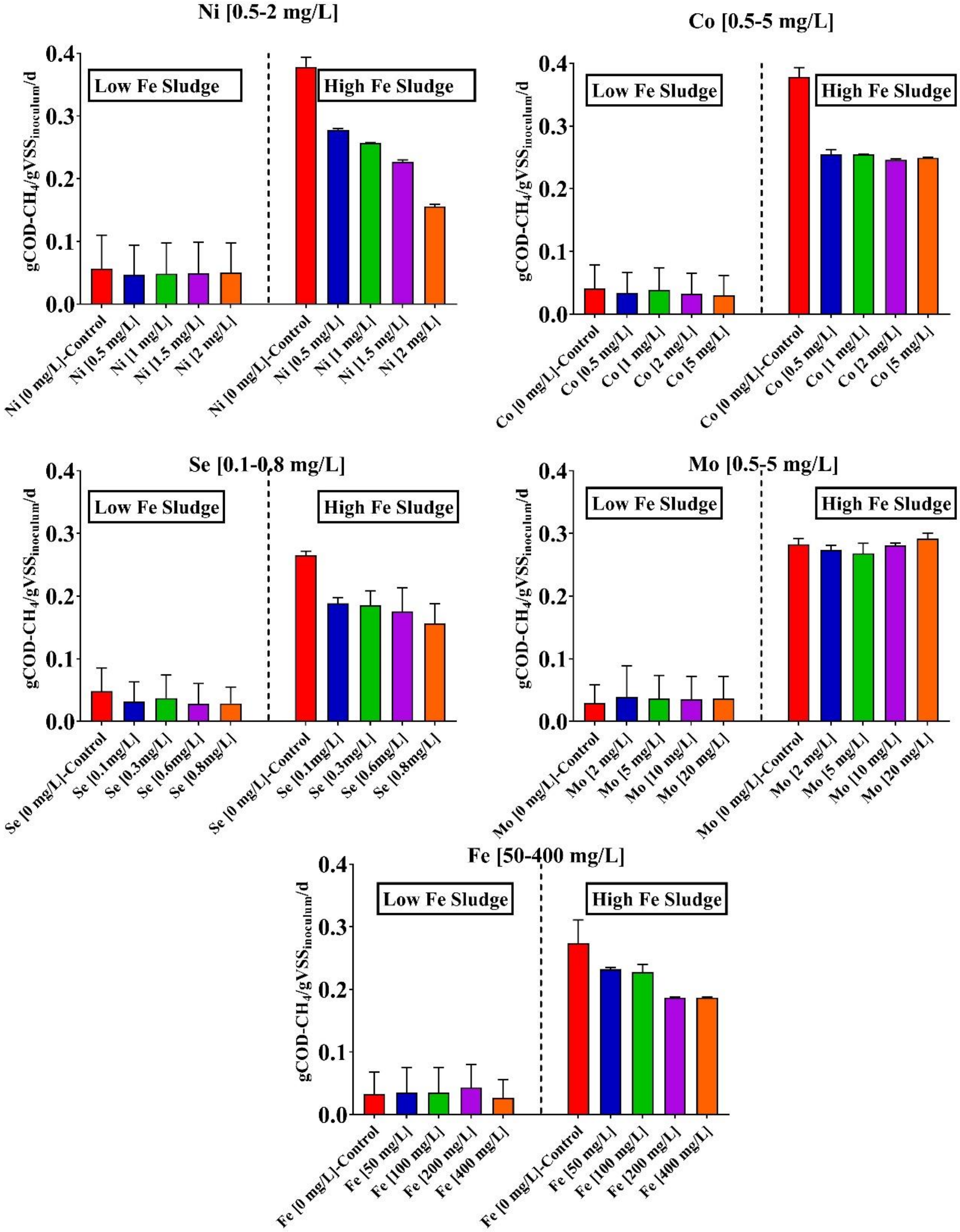

Figure 4-2 Comparison between the effect of different TE ions addition at different concentrations on two types of mesophilic digested sludge. One with low Fe content (FWS-LFe) and another with high Fe content (ADS-HFe); SMA tests, using acetate as a substrate at COD of 2 g/L

4.3.2. FW anaerobic digestion with single and mixed TE ions supplementation (BMP tests)

The substrate used in this study was originating from the same source as the previous study (FW collected from WI supermarkets), but its characteristics were slightly different. The ionic TE concentrations in the FW samples were 1.98 mg Fe/L, 0.005 mg Co/L, 0.01 mg Mo/L, 0.02 mg Ni/L, and 0.01 mg Se/L. Total COD concentration was 257 g/L with 36% being the soluble faction. Finally, total COD to VS and particulate COD to VSS ratios for the inoculum were 2.88 and 2.31, respectively.

After investigating the effects of TE ions addition has on methanogenic activity, BMP tests were conducted to assess the impact on FW anaerobic digestion, when the whole bacterial community is involved. The argument that the FW alone would add to the ionic TE content in the batch reactors is invalid, since TE ions concentrations in the FW sample where 1.98 mg/L, 0.005 mg/L, 0.01 mg/L, 0.02 mg/L, and 0.01 mg/L for Fe, Co, Mo, Ni, and Se, respectively which were very low compared to the seed sludge and externally supplemented concentrations, considering the fact that 4.1 mL FW for single ionic TE addition test and 9.5 mL FW for mixed ionic TE addition test was added to 180 mL sludge in the batch reactors (Table 4-1). In this section, the impact of single TE ions supplementation on FW degradability, hydrolysis rate constant, biogas yield, and specific methane production during anaerobic digestion is going to be discussed.

As mentioned in section 4.2.5, series of batch assays were set up at an ISR of 3 using Fe^{2+} (50-400 mg/L), Ni^{2+} (2-20 mg/L), Co^{2+} (0.5-5 mg/L), Se^{4+} (0.1-0.8 mg/L) (Table 4-3). Furthermore, to assess the impact of TE ions mixtures on the digestion of FW, various combinations of TE ions were prepared. Ni^{2+} [1mg/L] +Co^{2+} [0.1mg/L] +Se^{4+} [0.1, 0.2, 0.6, 0.8 mg/L], Ni[1mg/L]+Co^{2+} [0.1mg/L]+Mo^{6+} [2 mg/L], Mo^{6+} [2mg/L]+Se^{4+} [0.1, 0.2, 0.6, 0.8 mg/L], and Ni^{2+} [1mg/L]+Co^{2+} [0.1, 0.4, 0.5 mg/L] were prepared to investigate possible synergistic effects of adding multiple TE ions at the same time. Figures 4-3 and 4-4 show the net cumulative methane production during 19 days and 26 days of batch digestion for single and mixed TE ions supplemented reactors, respectively.

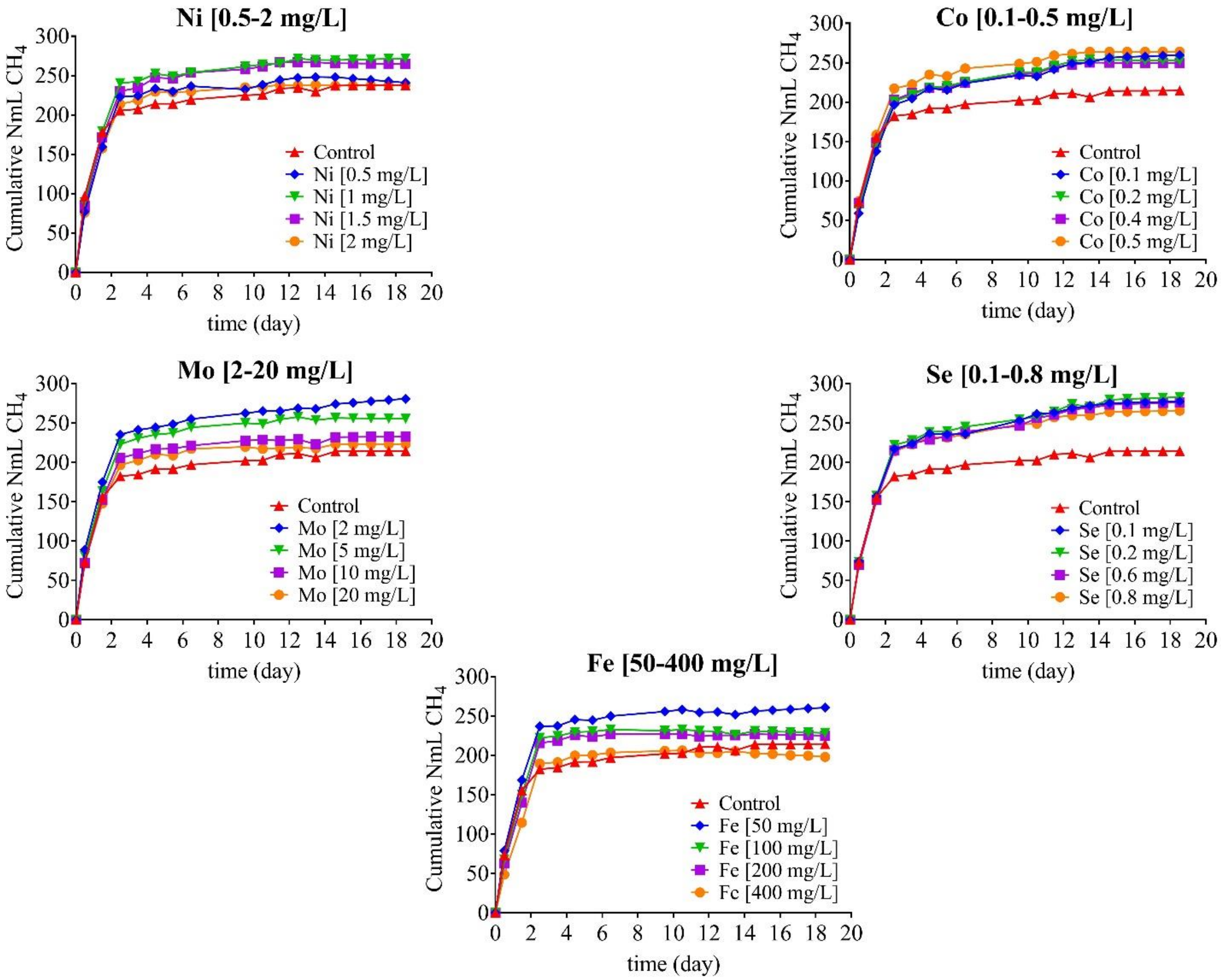

Figure 4-3 Cumulative net methane production profiles for reactors with single ionic TE supplementation and their corresponding controls (without TE ions addition).

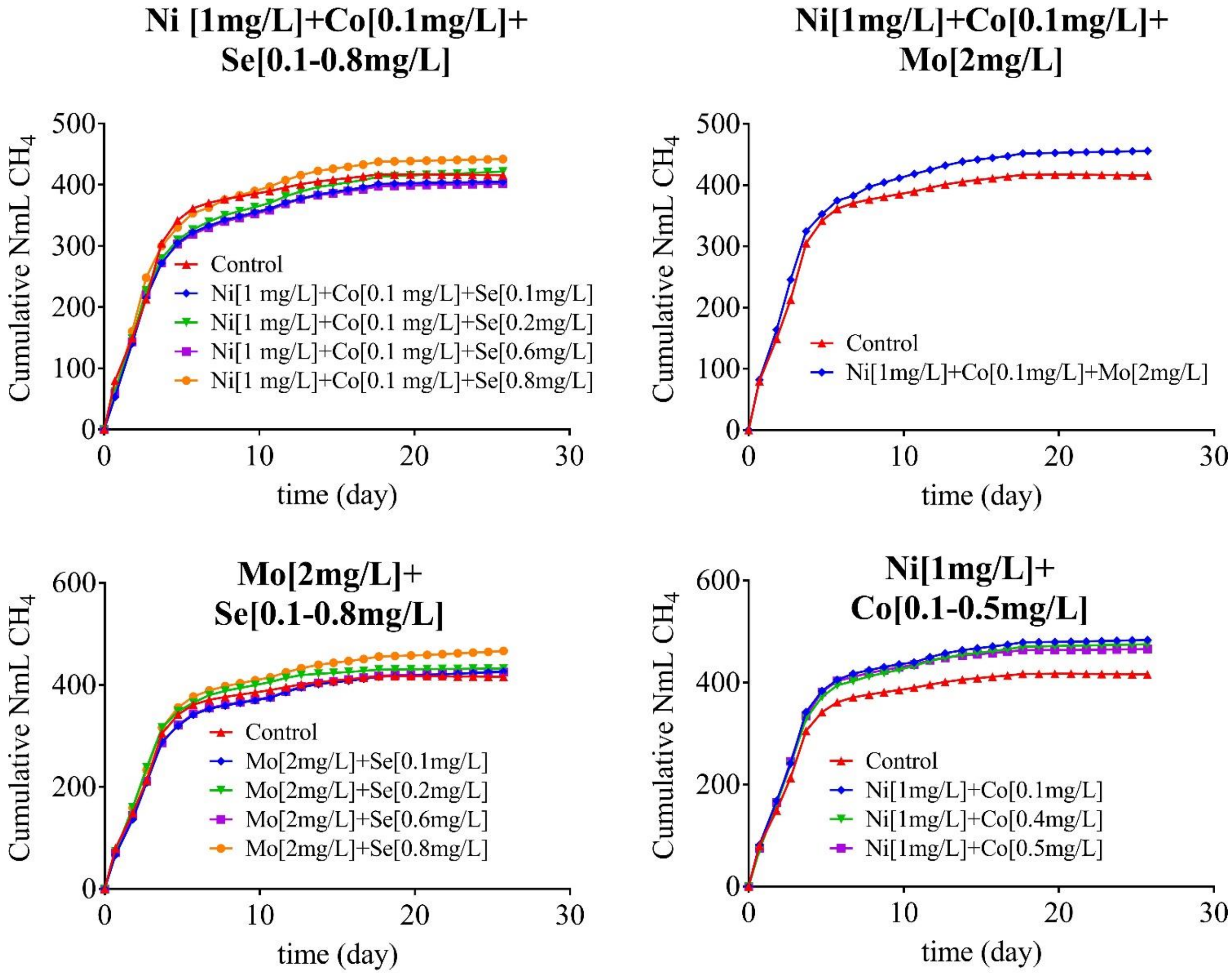

Figure 4-4 Cumulative net methane production profiles for reactors with mixed TE ions supplementation and their corresponding controls (without ionic TE addition).

Table 4-4 summarizes all the results taken from SMA and BMP tests while table 4-5 provides information about the BMP tests for the mixed ionic TE supplemented reactors and their controls. with FWS-LFe sludge.

Net biogas yield, which is biogas produced in the bottles minus biogas produced from the average of three blanks, divided by the gram VS added (FW) was measured in all reactors and compared to the controls. Statistical analysis was done to assess the significance of the differences and it was concluded that Ni^{2+} increased the biogas yield at all concentrations, ranging from 1240 mLbiogas/ gVS_{FW} to 1404 mLbiogas/ gVS_{FW} corresponding to 14%-28% enhancement. Similarly, Co^{2+} enhanced the biogas yield at all concentrations by up to 25% (130 mLbiogas/ gVS_{FW} to 1359 mLbiogas/ gVS_{FW}). Mo^{6+} improved biogas yield by 21% (1314 mLbiogas/ gVS_{FW}) and 18% (1287 mLbiogas/ gVS_{FW}) at 2mg/L and 5 mg/L, respectively. Among all the ionic TE added reactors, Se^{4+} had the best performance in terms of biogas yield, increasing it by an average of 29% (an average of 1399 mLbiogas/ gVS_{FW}). Finally, Fe^{2+} was able to increase the yield up to 13% (corresponding to 1230 mLbiogas/ gVS_{FW}) at both 100 mg/L and 200 mg/L. The analysis of variance test showed no significant difference between different ionic TE mixtures supplemented and control reactors in terms of biogas production. Biogas yield was the same for all mixed ionic TE supplemented reactors and the control with an average of 1338 mLbiogas/gVS_{FW}. The only exception was Ni^{2+} [1mg/L] +Co^{2+} [0.1mg/L] +Se^{4+} [0.1mg/L] in which biogas yield decreased by 12% (1210 mLbiogas/ gVS_{FW}).

Table 4-4 Summary of the SMA BMP test results for different ionic TEs at various concentrations

Ni^{2+}

Parameter	Control	Ni^{2+} [0.5 mg/L]	Ni^{2+} [1 mg/L]	Ni^{2+} [1.5 mg/L]	Ni^{2+} [2 mg/L]
SMA (mLCH$_4$/gVSS$_{inoculum}$.d)	0.055 ± 0.002	0.047 ± 0.001	0.049 ± 0.001	0.049 ± 0.000	0.049 ± 0.002
Biogas yield (mL/gVS$_{FW}$)	1090±44	**1240±89**	**1397±3**	**1404±8**	**1353±10**
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	594±113	667±49	**752±2**	**733±5**	659±5
K$_h$ (1/day)	0.74±0.1	0.77±0.1	0.73±0.1	0.73±0.1	0.85±0.2
Time to Reach 60% Biodegradability (day)	5.8	2.6	2.0	2.3	3.2

Co^{2+}

Parameter	Control	Co^{2+} [0.1 mg/L]	Co^{2+} [0.2 mg/L]	Co^{2+} [0.4 mg/L]	Co^{2+} [0.5 mg/L]
SMA (mLCH$_4$/gVSS$_{inoculum}$.d)	0.039 ± 0.003	0.033 ± 0.000	0.037 ± 0.002	0.033 ± 0.000	0.031 ± 0.002
Biogas yield (mL/gVS$_{FW}$)	1090±44	**1354±39**	**1349±41**	**1330±13**	**1359±13**
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	594±113	**719±16**	699±22	690±8	730±8
K$_h$ (1/day)	0.74±0.1	0.50±0.1	0.53±0.1	0.57±0.1	0.58±0.1
Time to Reach 60% Biodegradability (day)	5.8	3.8	3.7	3.7	2.8

Mo^{6+}

Parameter	Control	Mo^{6+} [2 mg/L]	Mo^{6+} [5 mg/L]	Mo^{6+} [10 mg/L]	Mo^{6+} [20 mg/L]
SMA (mLCH$_4$/gVSS$_{inoculum}$.d)	0.029 ± 0.000	0.044 ± 0.008	0.037 ± 0.003	0.036 ± 0.001	0.036 ± 0.000
Biogas yield (mL/gVS$_{FW}$)	1090±44	1314±5	**1287±52**	**1197±18**	1151±51
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	594±113	**778±3**	**707±3**	644±9	618±28
K$_h$ (1/day)	0.74±0.1	0.65±0.1	0.68±0.1	0.82±0.1	0.71±0.1
Time to Reach 60% Biodegradability (day)	5.8	2.2	2.6	4.7	5.2

Table 4-4 Continued

Se^{4+}

Parameter	Control	Se^{4+} [0.1 mg/L]	Se^{4+} [0.2 mg/L]	Se^{4+} [0.6 mg/L]	Se^{4+} [0.8 mg/L]
SMA ($mLCH_4/gVSS_{inoculum}.d$)	0.043 ± 0.008	0.031 ± 0.000	0.037 ± 0.000	0.030 ± 0.002	0.027 ± 0.002
Biogas yield (mL/gVS_{FW})	1090±44	**1388±62**	**1412±15**	**1424±22**	**1372±24**
Net Methane yield ($mLCH_4/gVS_{FW}$)	594±113	**768±35**	**783±9**	**765±12**	**735±13**
K_h (1/day)	0.74±0.1	0.53±0.0	0.54±0.0	0.52±0.1	0.55±0.0
Time to Reach 60% Biodegradability (day)	5.8	2.8	2.6	2.8	2.9

Fe^{2+}

Parameter	Control	Fe^{2+} [50 mg/L]	Fe^{2+} [100 mg/L]	Fe^{2+} [200 mg/L]	Fe^{2+} [400 mg/L]
SMA ($mLCH_4/gVSS_{inoculum}.d$)	0.034 ± 0.002	0.037 ± 0.003	0.037 ± 0.004	0.040 ± 0.005	0.027 ± 0.002
Biogas yield (mL/gVS_{FW})	1090±44	1274±32	**1229±22**	**1230±24**	1148±46
Net Methane yield ($mLCH_4/gVS_{FW}$)	594±113	**721±18**	632±13	623±13	548±20
K_h (1/day)	0.74±0.1	0.94±0.1	1.28±0.1	1.16±0.1	1.05±0.2
Time to Reach 60% Biodegradability (day)	5.8	2.0	2.7	3.8	NA

Table 4-5 Summary of the BMP test results for different ionic TE mixtures at various concentrations

Ni^{2+}[1mg/L] +Co^{2+} [0.1mg/L] +Se^{4+} [0.1, 0.2, 0.6, 0.8 mg/L]

Parameter	Control	Se^{4+} [0.1 mg/L]	Se^{4+} [0.2 mg/L]	Se^{4+} [0.4 mg/L]	Se^{4+} [0.8 mg/L]
Biogas yield (mL/gVS$_{FW}$)	1361±25	1210±41	1277±34	1259±19	1262±34
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	649±4	632±6	658±9	628±5	**691±6**
K$_h$ (1/day)	0.23±0.01	0.24±0.02	0.24±0.02	0.25±0.02	0.26±0.02
Time to Reach 60% Biodegradability (day)	3.5	3.3	3.3	3.2	3.1

Ni^{2+}[1mg/L]+Co^{2+} [0.1mg/L]+Mo^{6+} [2 mg/L]

Parameter	Control	Mo^{6+} [2 mg/L]
Biogas yield (mL/gVS$_{FW}$)	1361±25	1361±37
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	649±4	**712±11**
K$_h$ (1/day)	0.23±0.01	0.24±0.01
Time to Reach 60% Biodegradability (day)	3.5	3.3

Mo^{6+}[2mg/L]+Se^{4+}[0.1, 0.2, 0.6, 0.8 mg/L]

Parameter	Control	Se^{4+} [0.1 mg/L]	Se^{4+} [0.2 mg/L]	Se^{4+} [0.4 mg/L]	Se^{4+} [0.8 mg/L]
Biogas yield (mL/gVS$_{FW}$)	1361±25	1314±97	1335±38	1365±26	1413±38
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	649±4	666±12	675±13	664±9	**728±13**
K$_h$ (1/day)	0.23±0.01	0.20±0.01	0.25±0.01	0.22±0.01	0.22±0.01
Time to Reach 60% Biodegradability (day)	3.5	4.1	3.2	3.7	3.7

Table 4-5 Continued

Ni^{2+}[1mg/L]+Co^{2+}[0.1, 0.4, 0.5 mg/L]

Parameter	Control	Co^{2+} [0.1 mg/L]	Co^{2+} [0.4 mg/L]	Co^{2+} [0.5 mg/L]
Biogas yield (mL/gVS$_{FW}$)	1361±25	1452±103	1405±34	1388±6
Net Methane yield (mLCH$_4$/gVS$_{FW}$)	649±4	**755±5**	**742±12**	**727±1**
K_h (1/day)	0.23±0.01	0.21±0.01	0.23±0.01	0.25±0.01
Time to Reach 60% Biodegradability (day)	3.5	3.5	3.6	3.4

Figure 4-5 shows a comparison between specific net methane productions based on mass FW added in TE ions added versus the control reactors with statistically significant differences from the control bolded. Methane yields for FW anaerobic digestion with FWS-LFe ranged from 594-783 mLCH$_4$/gVS$_{FW}$. Ni^{2+} supplementation at 1 mg/L and 1.5 mg/L increased CH$_4$ per gram VS$_{FW}$ 27% and 23% corresponding to 752 mLCH$_4$/gVS$_{FW}$ and 733 mLCH$_4$/gVS$_{FW}$, respectively while it did not result in a statistically significant different methane yields at 0.5 mg/L and 2 mg/L. Co^{2+} added reactors at the lowest concentration (0.1 mg/L) increased the methane yield to 719 mLCH$_4$/gVS$_{FW}$, a 21% enhancement comparing to the control. 0.5 mg Co^{2+}/L supplemented reactors was another case in which Co^{2+} addition increased the methane yield to 730 mLCH$_4$/gVS$_{FW}$, improving it by 23%. Mo^{6+} supplementation only at the first two concentrations of 2 and 5 mg/L resulted in 778 mLCH$_4$/gVS$_{FW}$ and 707 mLCH$_4$/gVS$_{FW}$, equal to 31% and 19% higher methane yields, respectively. In comparison with the control, Se^{4+} at all concentrations enhanced methane yield, a statistically significant improvement of 24% to 32% (ranging from 735 mLCH$_4$/gVS$_{FW}$ to 783 mLCH$_4$/gVS$_{FW}$). Finally, Fe^{2+} addition did not affect methane production, except at the lowest concentration of 50 mg/L which resulted in 22% more methane per gram VS of the FW added to the reactors (721 mLCH$_4$/gVS$_{FW}$). Analysis of variances was performed to assess the impact of combination of TE ions supplementation on methane productions and it showed that Ni^{2+} [1mg/L]+Co^{2+} [0.1mg/L]+Se^{4+} [0.8] increased the methane production slightly (691 mLCH$_4$/gVS$_{FW}$ corresponding to 6% enhancement compared to the control). Mo^{6+} [2mg/L] +Se^{4+} [0.8] however, could enhance methane production up to 12% (728 mLCH$_4$/gVS$_{FW}$). Ni^{2+} [1 mg/L] +Co^{2+} combination was the best among the mixtures in terms of methane production enhancement since it increased the yield in all concentrations, resulting in 16% (755 mLCH$_4$/gVS$_{FW}$), 14% (742 mLCH$_4$/gVS$_{FW}$), and 12% (727 mLCH$_4$/gVS$_{FW}$) improvement at 0.1, 0.4, and 0.5 mg Co/L, respectively. Methane yield was slightly improved adding a mixture of TE ions, leading to an increase of only 10% at Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L]+Mo^{6+}[2 mg/L] (712 mLCH$_4$/gVS$_{FW}$).

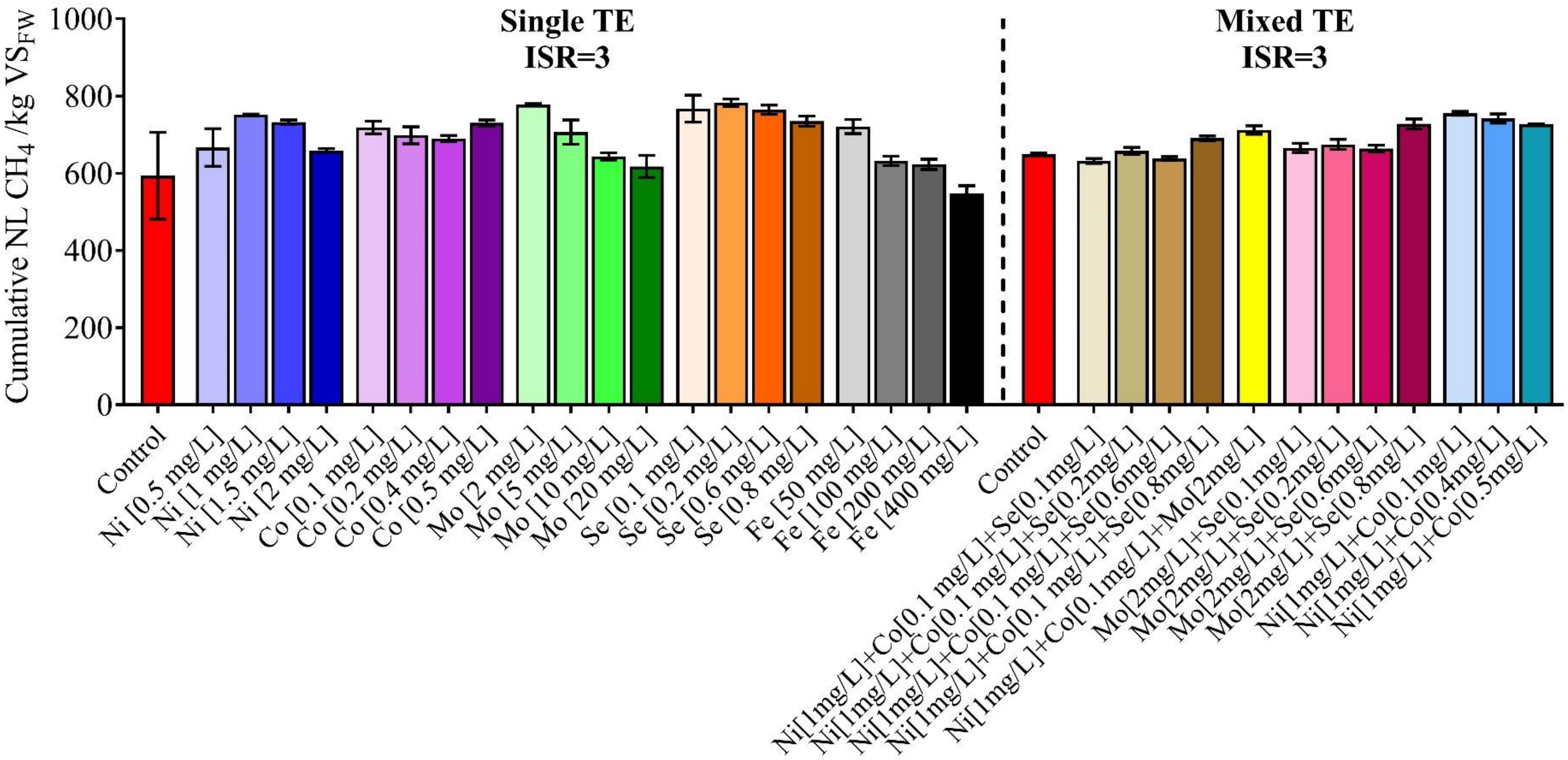

Figure 4-5 Net methane yield impacted by the trace elements addition using FWS-LFe to anaerobically digest FW.

A comparison between the BMP test results with FWS-LFe and ADS-HFe reveals that as opposed to the above-mentioned positive effects TE ions addition had on methane production, ionic TE supplementation did not improve ultimate methane production using ADS-HFe. In order to better compare the methane production yields, P_{max} values were calculated from Gompertz model (Shin et al. 2008) and then normalized based on gram COD added (FW) to each reactor.

The ultimate net methane yields in the BMP tests with FWS-LFe ranged from 194-254 mLCH_4/ g COD_{FW}, corresponding to 55%-73% biodegradability. When FWS-LFe was used in the BMP tests, Fe^{2+} addition at 50 mg/L increased net methane yield to 243 mLCH_4/ g COD_{FW} (12% increase compared to the control), while the rest of the Fe^{2+} concentrations did not change the ultimate methane yield significantly. Ni^{2+} supplementation on the other hand, increased ultimate methane yields to 230 mLCH_4/ g COD_{FW}, 252 mLCH_4/ g COD_{FW}, 249 mLCH_4/ g COD_{FW}, and 225 mLCH_4/ g COD_{FW}, which compared to the control, translates to improvements by 6%, 16%, 15%, and 4% at 0.5 mg/L, 1 mg/L, 1.5 mg/L, and 2 mg/L, respectively. Similar to Ni^{2+}, Co^{2+} addition successfully increased methane yields at all concentrations ranging from 229 mLCH_4/ g COD_{FW} to 243 mLCH_4/ g COD_{FW}, improving the yields by 7%, 7%, 6%, and 12% at 0.1 mg/L, 0.2 mg/L, 0.4 mg/L, and 0.5 mg/L, respectively. Mo^{6+} addition enhanced the ultimate methane yields drastically at all concentrations, with ultimate net methane yields varying from 208 mLCH_4/ g COD_{FW} to 249 mLCH_4/ g COD_{FW}; i.e. 16%, 17%, 15%, 12% enhancement at 2 mg/L, 5 mg/L, 10 mg/L, and 20 mg/L, respectively. Finally, Se^{4+} supplementation increase ultimate methane production by 17% and 10% at 0.1 mg/L and 0.2 mg/L, respectively. Combination of TE ions also successfully enhanced methane yields in a few cases. Ni^{2+}[1mg/L]+Co^{2+}[0.1 mg/L]+Se^{4+}[0.8mg/L] for instance, increase methane yield slightly (4%) compared to the control, leading to an ultimate net methane value of 230 mLCH_4/ g COD_{FW}. Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L]+Mo^{6+}[2mg/L] supplementation to the batch reactors resulted in 8% more ultimate methane yield (239 mLCH_4/ g COD_{FW}). Mo^{6+}[2mg/L]+Se^{4+}[0.1mg/L to 0.8mg/L] did not significantly increase methane yields, except for the Mo^{6+}[2mg/L]+Se^{4+}[0.8mg/L] at which a 9% increase in methane yield was observed (241 mLCH_4/ g COD_{FW}), significantly different from the control. However, among all mixed TE ions, Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L to 0.5mg/L] had the best performance in terms of ultimate methane yields; the mixture of Ni^2 and Co^2 improved yields by 15% (253 mLCH_4/ g COD_{FW}), 13% (249 mLCH_4/ g COD_{FW}) and 11% (246 mLCH_4/ g COD_{FW})

at Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L], Ni^{2+}[1mg/L]+Co^{2+}[0.4mg/L], and Ni^{2+}[1mg/L]+Co^{2+}[0.5mg/L], respectively.

In the BMP tests with Fe- rich sludge, ultimate net methane yields varied from 136 mLCH_4/ g COD_{FW} to 304 mLCH_4/ g COD_{FW}, corresponding to 38%-87% biodegradability. With Fe-rich sludge, in the first trial with Fe^{2+} supplementation at ISR of 3, ultimate methane yields decreased by 12% (264 mLCH_4/ g COD_{FW}), 8% (276 mLCH_4/ g COD_{FW}), and 4% (276 mLCH_4/ g COD_{FW}) at 100 mg/L, 200 mg/L, and 400 mg/L, respectively. The difference between ultimate methane yields in this Fe supplementation BMP test at ISR of 3 with the second study with FWS-LFe is probably due to the dissimilar sources of inoculum. However, the focus here is not on the absolute values bot on the differences between each TE ion supplemented reactor performance and the control.

The inoculum for the first study (ADS-HFe) was collected from a digester in a municipal wastewater treatment plant while the second one (FWS-LFe) was collected from a digester treating source segregated organic wastes (FW) as feed. The other BMP tests with Fe addition at an ISR of 0.5 with ADS-HFe again negatively affected methane yields; 7% (202 mLCH_4/ g COD_{FW}), 9% (194 mLCH_4/ g COD_{FW}), and 10% (190 mLCH_4/ g COD_{FW}) reduction at 100 mg/L, 200 mg/L, and 400 mg/L, respectively. Ni addition to the batch reactors with ADS-HFe, at 0.5 mg/L, 1 mg/L, 1.5 mg/L, 2 mg/L did not significantly affect methane yields; the ultimate yields was 143±5 mL CH_4/ g COD_{FW} on average for control and all Ni^{2+} added reactors. Co^2 addition to the FW batch reactors with ADS-HFe negatively affected methane yields and reducing them by 17% (176 mLCH_4/ g COD_{FW}) at 0.2 mg/L, 0.4 mg/L, and 0.5 mg/L. Similarly, Mo^{6+} addition at 5 mg/L, 10 mg/L, and 20 mg/L to the reactors with ADS-HFe reduced the methane yields by 7% (181 mLCH_4/ g COD_{FW}), 6% (182 mLCH_4/ g COD_{FW}), and 10% (175 mLCH_4/ g COD_{FW}), respectively. With Se^{4+} addition to the ADS-HFe batch reactors, no significant differences were observed regarding ultimate methane yields; resulting in an average of 145±4 mL CH_4/ g COD_{FW} for control and all Se^{4+} added reactors. Combination of metals in BMP tests with ADS-HFe did not significantly affect methane yields, led to an average of 148±3 mL CH_4/ g COD_{FW} for all mixed TE ions supplemented and control reactors.

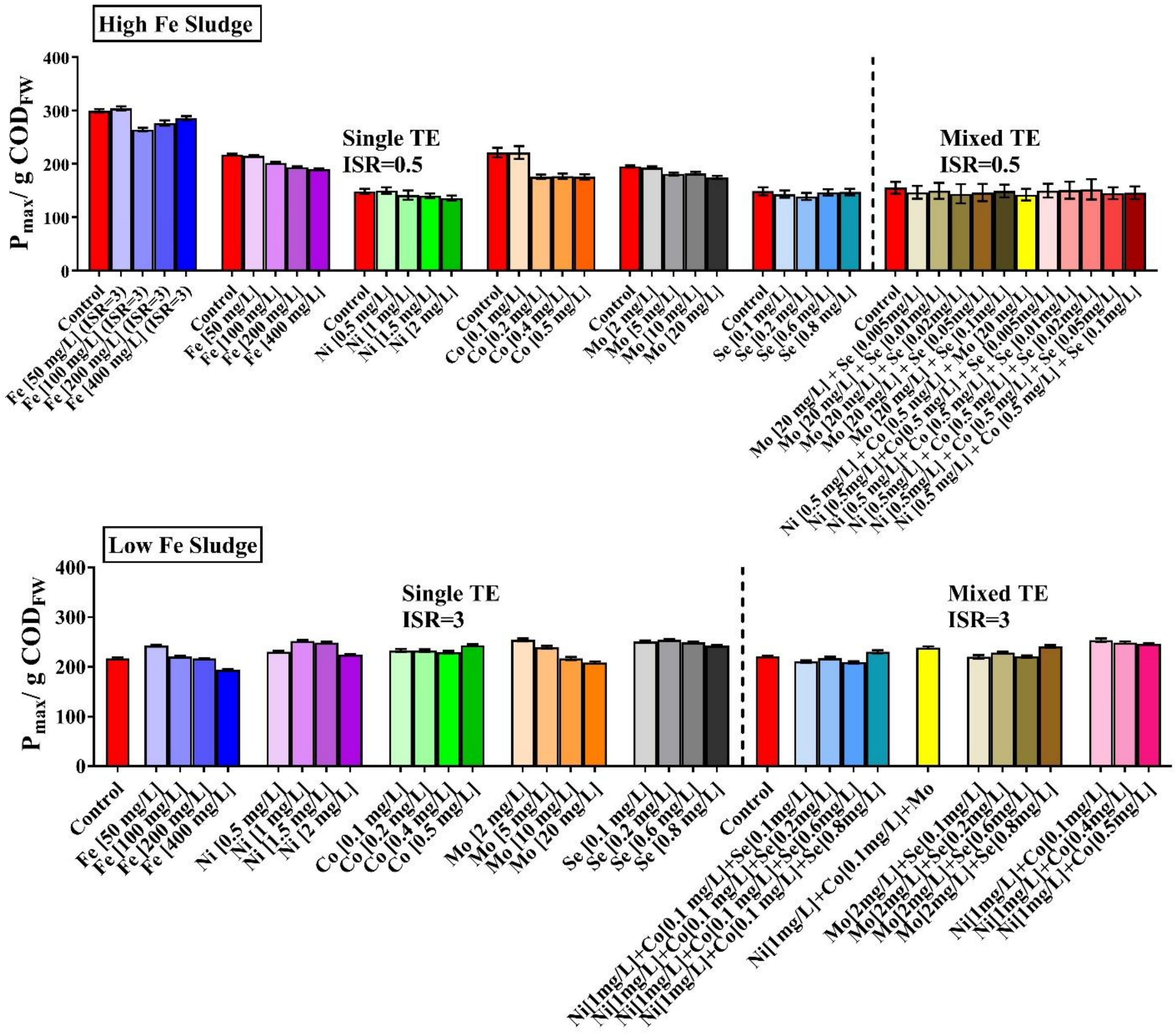

Figure 4-6 Comparison between maximum methane production normalized to mass of FW COD in the two studies.

Figure 4-7 shows the hydrolysis rate constant affected by ionic TE supplementation in the two studies of anaerobic digestion of FW with high and FWS-LFe.

Hydrolysis rate constant values for the BMP tests with ADS-HFe at an ISR of 3 ranged from 0.51 day^{-1} to 0.60 day^{-1} while it was 0.07 day^{-1}-0.18 day^{-1} at an ISR of 0.5. One-way ANOVA tests showed that in the BMP tests with ADS-HFe none of the single or mixed TE ions supplementations resulted in a significantly different K_h values compared to their corresponding controls.

Hydrolysis rate constant values for the BMP tests with FWS-LFe ranged from 0.26 day^{-1} to 1.29 day^{-1}. Performing a t-test analysis on the hydrolysis rate constant of control versus ionic TE supplemented reactors showed the following results for the BMP tests with the FWS-LFe. The single TE ions addition did not affect the hydrolysis rate constant significantly in any of the cases except Fe^{2+} at 100 mg/L in which K_h was increased by 60% compared to the control. Combined TE supplementation in the case of Ni^{2+}[1mg/L]+Co^{2+}[0.1 mg/L]+Se^{4+}[0.8mg/L] improved the hydrolysis rate constant by 21% but none of the other mixed ionic TE added reactors had significantly different K_h value compared to the control (0.23 1/day, on average basis). It should be noted that the K_h values were calculated from the linearization of the methane production between days 0 and 2.7, considering a first order degradation and the R^2 value of the linearization ranged from 0.98 to 0.99.

Comparing the K_h values affected by ionic TE supplementation in the two studies with low and high Fe, it is discerned that hydrolysis rate constant is not significantly affected in FW digestion with ADS-HFe, however it is improved with FWS-LFe in the cases of 100 mg Fe/L and Ni^{2+}[1mg/L]+Co^{2+}[0.1 mg/L]+Se^{4+}[0.8mg/L].

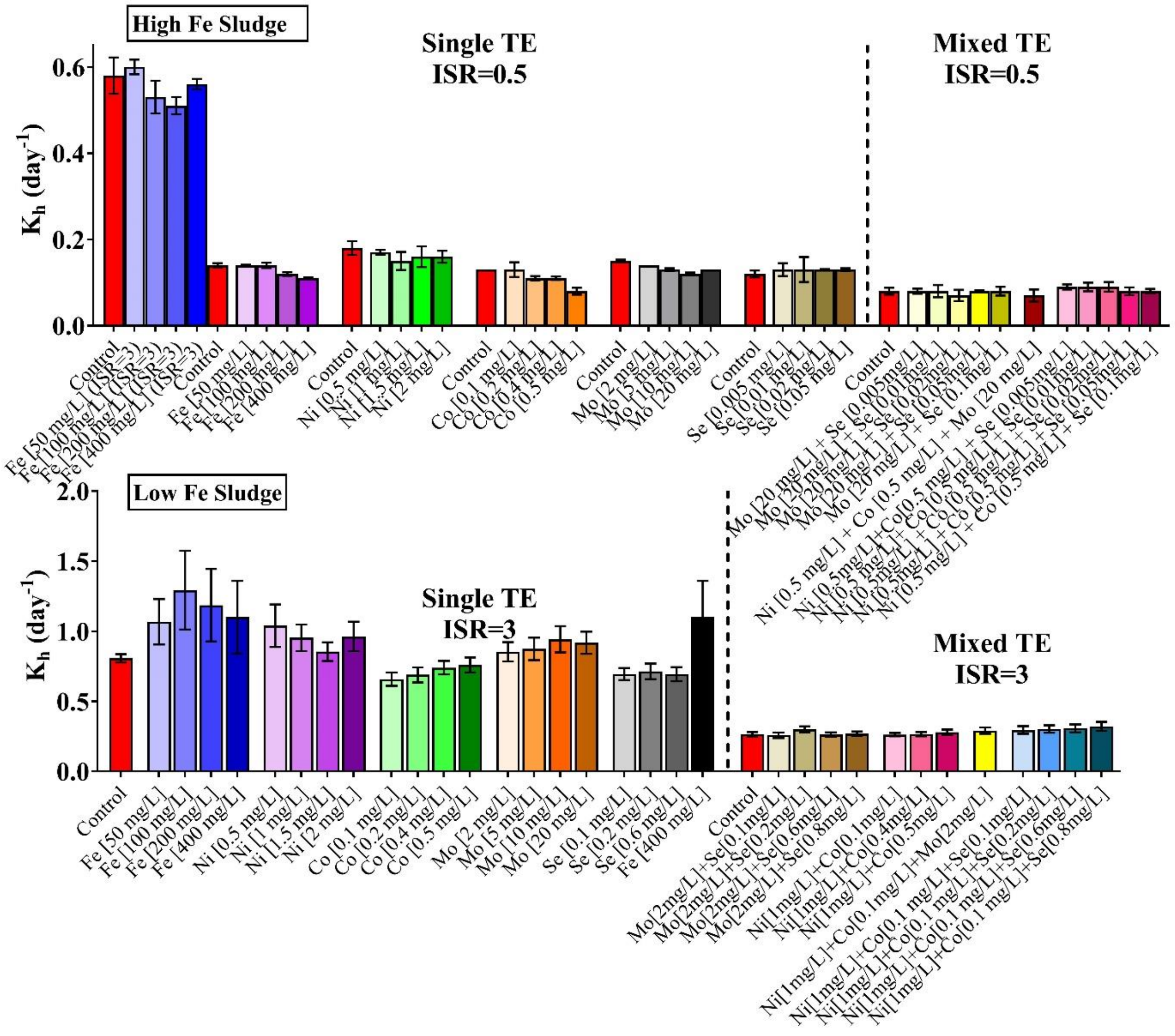

Figure 4-7 Hydrolysis rate constant affected by the trace elements addition using high and FWS-LFe to anaerobically digest food waste

Two other parameters were calculated for the study of FW anaerobic digestion with FWS-LFe: days to reach 60% COD_{added} biodegradability and maximum methane production rate (R_{max}). Comparing the K_h values affected by ionic TE supplementation in the two studies with low and high Fe, it is discerned that hydrolysis rate constant is not significantly affected in FW digestion with ADS-HFe, however it is improved in some cases with FWS-LFe.

The anaerobic degradability test gives a good indication of the rate and extent of degradation of particulate organic substrates (Koch and Drewes 2014). Therefore, another calculated parameter here is the anaerobic biodegradability of controls and different TE ions added reactors based on the net methane production. This parameter was calculated by dividing total methane production of each reactor by its maximum methane production potential, measured from COD content of FW added to each reactor and by assuming that every gram of COD produces 350 mL methane at STP conditions. The anaerobic biodegradability of the FW in the reactors were 53% to 78% in this study. Therefore, the focus here is on the determination of the time required to reach 60% biodegradability of the COD of the FW. This time was calculated by using the P_{max} value calculated by Gompertz model, theoretically expected methane production from 60% COD_{added} degradation considering the fact that each gram of COD is corresponded to 350 mL CH_4 at STP conditions, and hydrolysis rate constant, assuming that the degradation is fit to the first order model. As apparent from Table 4-5, in almost all cases, the TE ions addition shortened the degradation time. For the single TE ion addition test, it takes 5.8 days on average for the control reactors to degrade 60% of the FW while Ni^{2+}, Co^{2+}, Mo^{6+}, Se^{4+}, and Fe addition reduced it to an average of 2.5, 3.5, 3.1, 2.8, and 2.9 days, respectively. This means that ionic TE supplemented reactors can reduce the digestion time and hence, the solids retention time would potentially decrease 40% to 57% in continuous flow digesters. In the mixed TE ions supplementation test, 60% degradation occurred within 3.5 days for the control reactors, on average. The only combination that successfully reduced degradation time was Ni^{2+}[1mg/L]+Co^{2+}[0.1 mg/L]+Se^{4+}[0.1mg/L to 0.8 mg/L]; 3.2 days were required on average for these ionic TE supplemented reactors to reach 60% biodegradability. Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L]+Mo^{6+} also decreased this time to 3.3 days, on average, accelerating the degradation time by 6%. Time to degrade 60% COD was the same as control for all other mixtures of TE ions. It is noteworthy that these results cannot be compared solely with hydrolysis rate constant (K_h) results because the time required for 60% biodegradability is P_{max} devided by

K_h and both K_h and P_{max} values affect the degradability time.

R_{max} which is the maximum rate of methane production based on Gompertz model was also calculated for the batch reactors digesting FW with FWS-LFe and the results are presented in Figure 4-8. One-way ANOVA tests showed that R_{max} decreased by 26%, 22%, and 15% for Co^{2+} addition at 0.1 mg/L, 0.2 mg/L, and 0.4 mg/L, respectively while Ni^{2+} and Mo^{6+} addition did not have any significant impacts on methane production rates compared to the control. Furthermore, Se^{2+} at 0.6 mg/L and Fe^{2+} at 400 mg/L negatively affected the R_{max} 16% and 25%, respectively while other concentrations of Se^{2+} and Fe^{2+} did not affect it. Ni^{2+}[1mg/L]+Co^{2+}[0.1 mg/L]+Se^{4+}[0.1mg/L to 0.6 mg/L] decreased the R_{max} by 13% but Ni^{2+}[1mg/L]+Co^{2+}[0.1 mg/L]+Se^{4+}[0.8 mg/L] did not change the R_{max} significantly compared to the control. Mo^{6+}[2mg/L]+Se^{4+}[0.1mg/L] and Mo^{6+}[2mg/L]+Se^{4+}[0.6mg/L] reduced methane production rate slightly (11% and 9%, respectively). On the other hand, Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L] and Ni^{2+}[1mg/L]+Co^{2+}[0.5mg/L] increased the R_{max} by an average of 11%. It is worth mentioning that among all single and mixed ionic TE supplemented reactors, these two latter cases were the only ones which increased the maximum methane production rates. The R_{max} value calculated by the Gompertz model is equal to $P_{max} \times K$. This K is the rate constant of the degradation, similar to the K_h value described in this study, but with a difference. K_h is specific to the hydrolysis phase which is calculated by taking the slope of the first order degradation model during the first few days of the digestion while K is calculated based on the $P = P_{max}\exp(-\exp(K(t_0 - t)e + 1))$ and the whole digestion period from the beginning until the end of the BMP test is taken into consideration. Thus, when K values decrease although P_{max} values increase, R_{max} values would be lower than the control values.

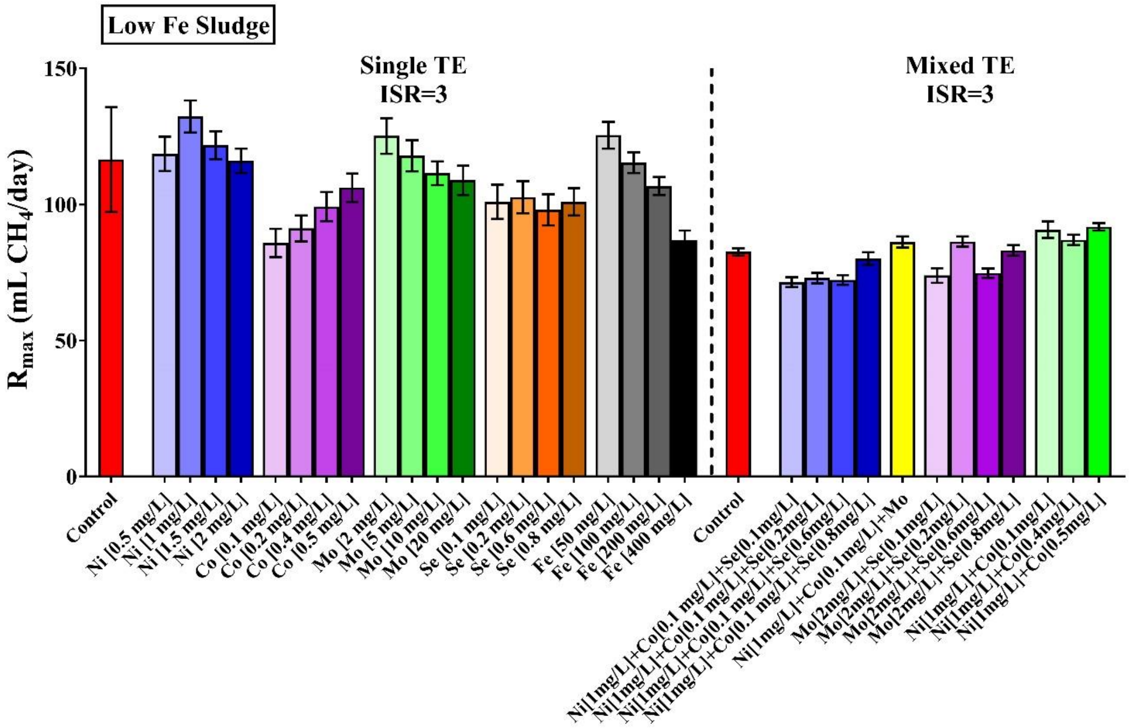

Figure 4-8 Maximum methane production rates calculated based on Gompertz model for the study of FW anaerobic digestion with FWS-LFe.

In general, it is concluded that Ni did not have a positive effect on methanogens based on the SMA tests (Figure 4-2), despite increasing the methane yield by 27% and 23% at at 1 mg/L and 1.5 mg/L, respectively. It also decreased the digestion time by 23%. Like Ni^{2+}, Co^{2+} did not improve SMA rates but enhanced the methane yield by 21% and 23% at 0.1 mg/L and 0.5 mg/L supplementation, respectively. Co^{2+} reduced the time needed for 60% biodegradation by 40%. Mo at 5 mg Mo^{6+}/L improved the methanogenic activity in SMA tests and methane yield in BMP tests by 28% 19%, respectively. At 20 mg/L, Mo^{6+} increased SMA rate by 22% and at the lowest concentration (2 mg/L), enhanced methane yield by 31%. Mo^{6+} impact on digestion time was also significant (46% enhancement). Se^{4+} did not improve SMA rates but was able to increase methane yield at all concentrations (up to 32%). Mo^{6+} also reduced digestion time by 52%. Fe^{2+} at 400 mg/L stimulated methanogenic activity by 20%, increase methane yield at 50 mg/L by 22%, and decreased digestion time from 5.8 days to less than 3 days.

Finally, a comparison between the single and mixed ionic TE added BMP results was done to better understand if the mixed ionic TE supplementation caused the synergistic effects in terms of COD biodegradability and methane production. In this comparison, the degradability of COD added as

FW is compared in each mixed ionic TE supplemented reactor with the degradability of other single ionic TE added reactors (the TEs that include in the mixture of the ionic TEs supplemented). Then a t-test was performed to assess the significant differences and the cases in which the mixed ionic TE added reactors performed better than their single ionic TE added associates are graphically illustrated in Figure 4-9. This comparison reflects that Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L]+Mo^{6+}[2 mg/L] increases the FW degradability by 15% compared to Mo^{6+} alone at 20 mg/L. Likewise, Ni^{2+}[1mg/L]+Co^{2+}[0.1mg/L] improves digestion up to 14% and 15% comparing with Ni added individually at 0.5 mg/L and 2 mg/L, respectively (Figure 4-7). FW degradability is also 13% higher with Ni^{2+}[1mg/L]+Co^{2+}[0.4mg/L] addition in comparison to Ni^{2} alone at 2 mg/L. Lastly, Mo^{2+}[2mg/L]+Se^{4+}[0.8 mg/L] was found to have 13% and 18% more methane production per COD added than Mo^{6+}[10mg/L] and Mo^{6+}[20mg/L], respectively.

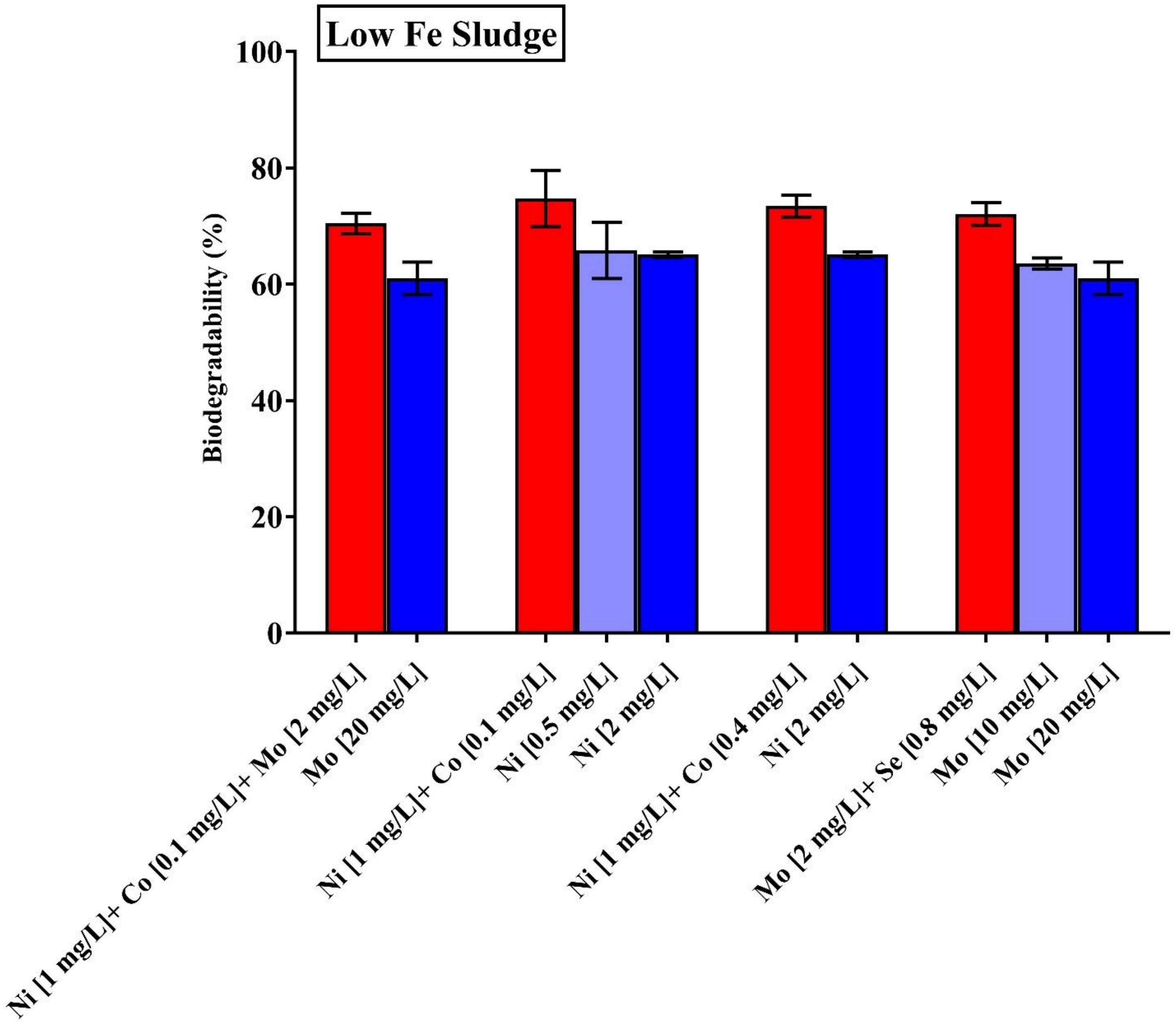

Figure 4-9 Comparison between FW biodegradability of single and mixed TE ions supplemented batch reactors. Biodegradability is defined as net cumulative methane per maximum expected methane calculated from COD (of FW) added.

4.3.3. Correlation of final soluble TE ion concentrations and BMP test results

The Pearson Correlation (PC) was determined with GraphPad Prism7 for analyzing the correlations between the final soluble TE ion concentrations after the BMP tests (single and mixed TE ion additions) and the test results (i.e. biogas yield, methane yield, and K_h value).

Figure 4-10 shows the BMP results versus final soluble Ni concentrations. PC showed a significant, moderately positive relationship between soluble final Ni and the biogas yield. Methane yield tend to increase at lower concentrations of Ni and then decrease at higher Ni concentrations. The relationship between K_h values and soluble Ni concentration seems to follow the similar trend. Considering all three graphs, in the range of soluble Ni concentrations of 0.14 mg/L to 20.09 mg/L, it seems that the optimum final soluble Ni concentration is around 0.25 mg/L.

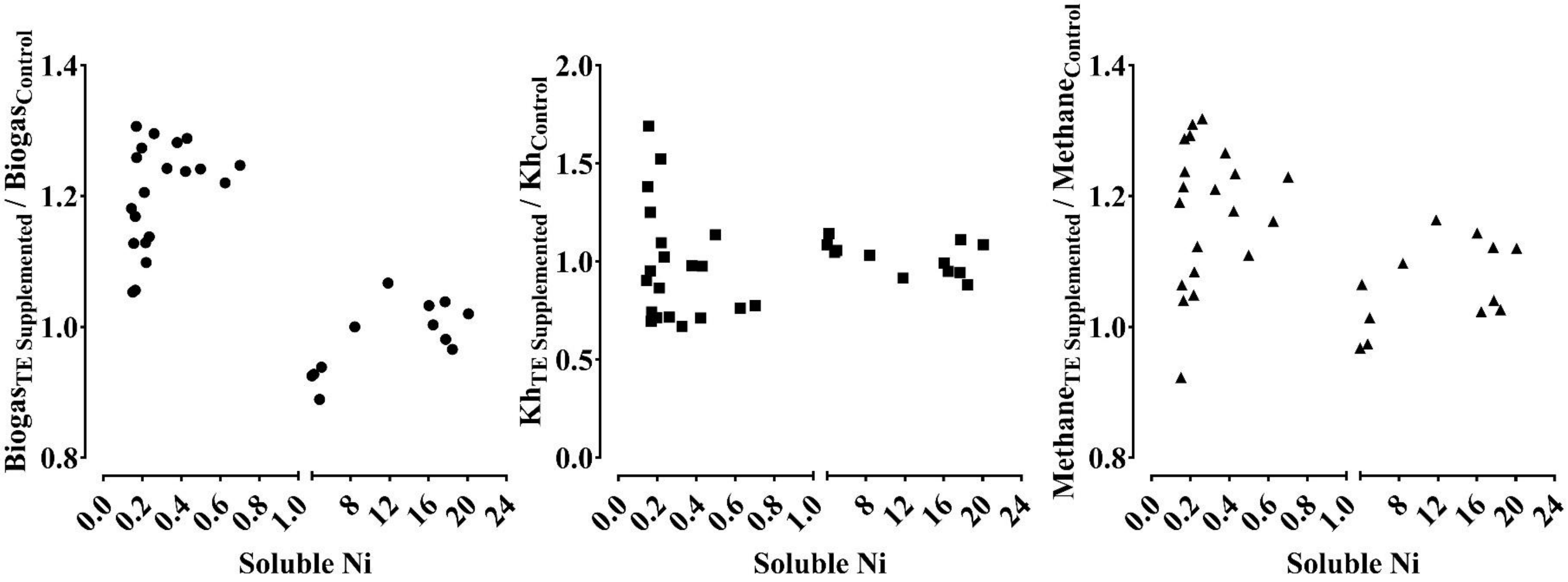

Figure 4-10 Relationship between final soluble Ni concentrations and BMP test results.

Figure 4-11 shows the BMP results versus final soluble Co concentrations. Pearson correlation showed that biogas yield does not have any significant correlation with soluble Co concentration although it moderately increases with increasing soluble Co. K_h values on the other hand, tend to decrease with increasing the soluble Co concentrations. Considering all three graphs, in the range of soluble Co concentrations of 0.17 mg/L to 0.53 mg/L, it is difficult to identify an optimum concentration for soluble Co concentration.

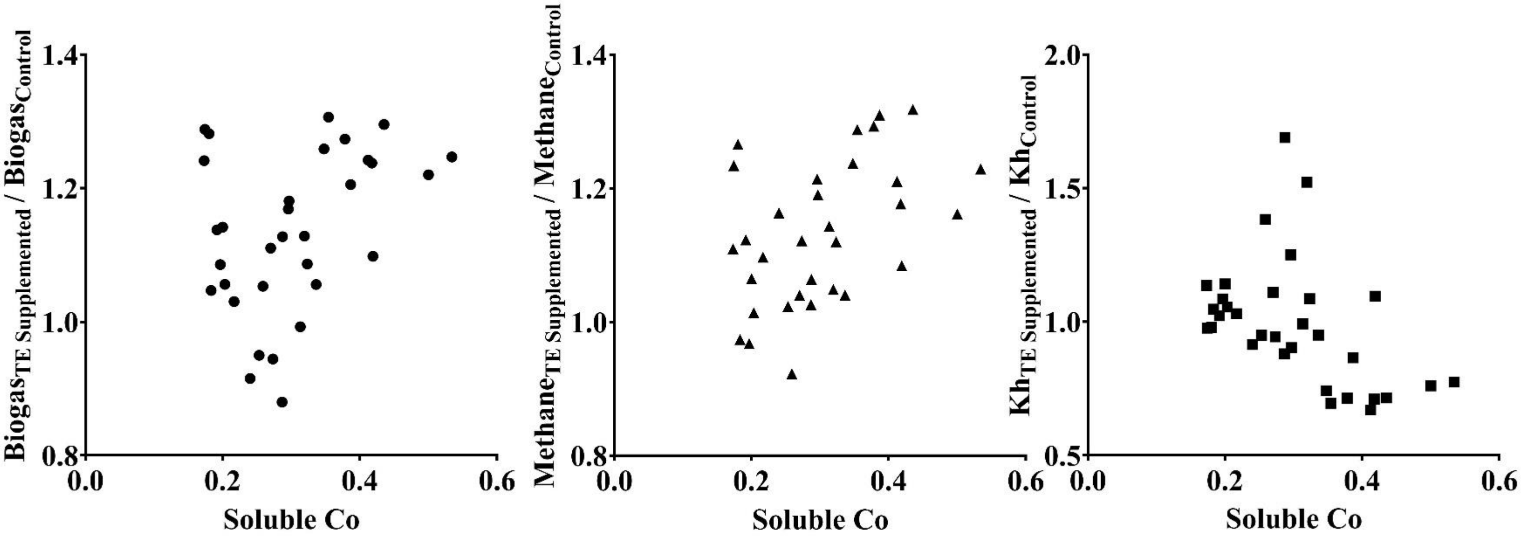

Figure 4-11 Relationship between final soluble Co concentrations and BMP test results.

Figure 4-12 shows the BMP results versus final soluble Mo concentrations. Biogas and methane yield have the same trend with soluble Mo concentrations. They first increase with increasing soluble Mo and then decrease. In the range of soluble Mo concentrations of 0.03 mg/L to 19.80 mg/L, both biogas and methane yield peak around 0.14 mg Mo/L. However, K_h values decrease with increasing soluble Mo as shown in Figure 4-12.

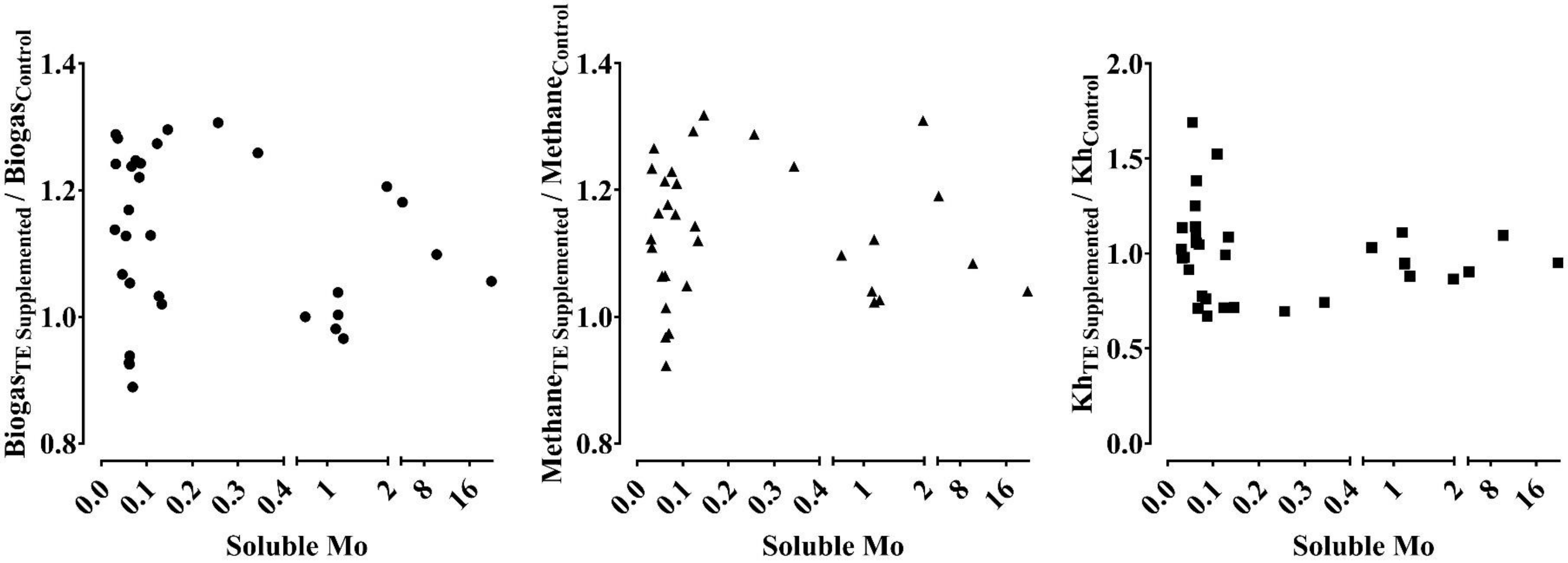

Figure 4-12 Relationship between final soluble Mo concentrations and BMP test results.

Figure 4-13 shows the BMP results versus final soluble Se concentrations. Pearson correlation did not lead to any significant correlations between the soluble Se concentrations and the BMP results. However, in the range of soluble Se concentrations of 0.001 mg/L to 0.286 mg/L, it seems that the optimum soluble Se concentration for all BMP parameters occurs at around 0.07 mg Se/L.

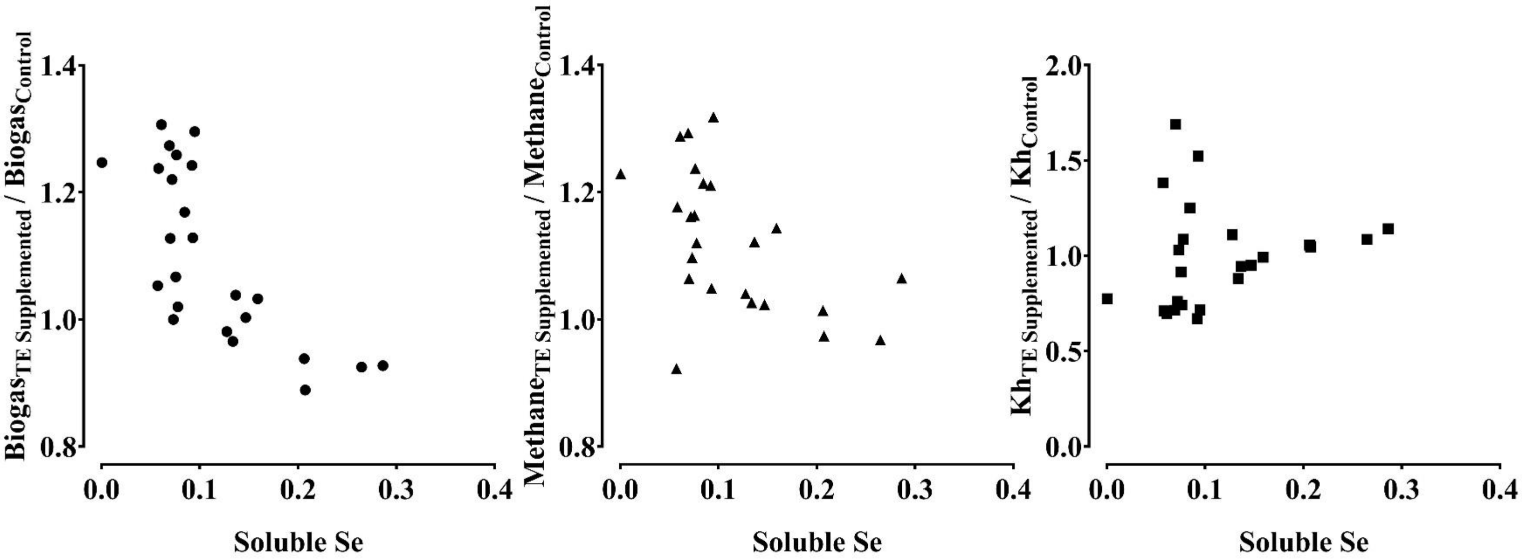

Figure 4-13 Relationship between final soluble Se concentrations and BMP test results.

Figure 4-14 shows the BMP results versus final soluble Fe concentrations. Similar to Se, soluble Fe does not seem to have a significantly positive or negative relationship with BMP test results. This was also proven by very low Pearson r values (≤0.2). Hence, as observed from Figure 4-14, in the range of soluble Fe concentrations of 4.01 mg/L to 37.93 mg/L, it is difficult to define an optimum soluble Fe concentration that gives the maximum biogas and methane yield as well as the best K_h value.

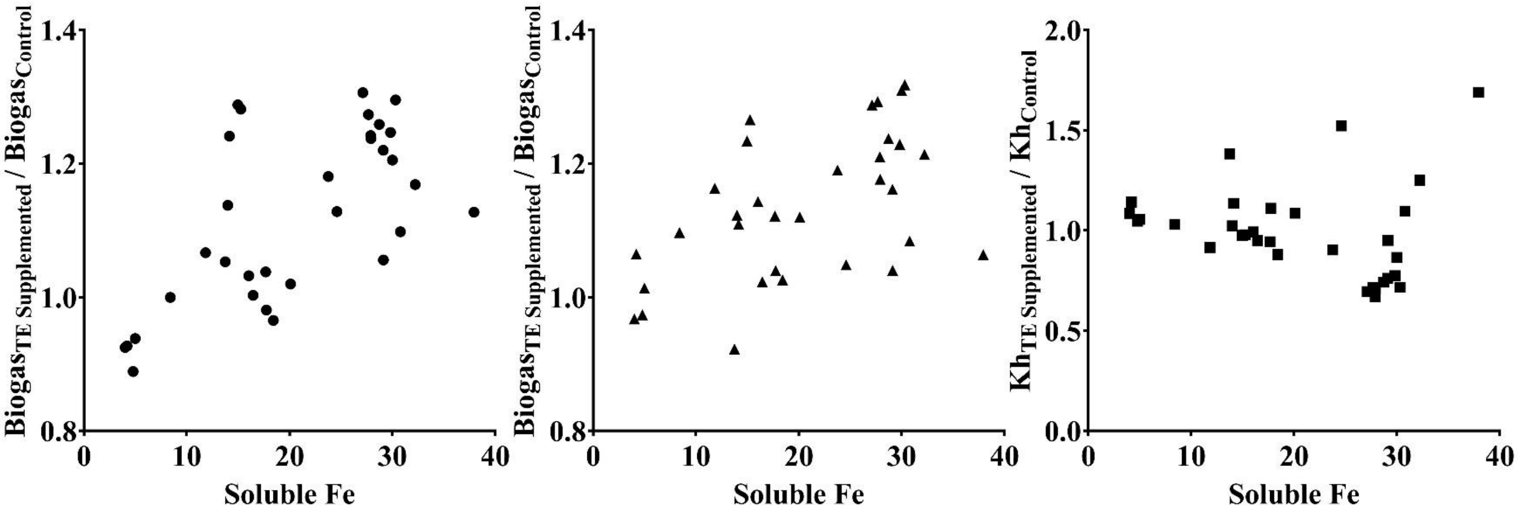

Figure 4-14 Relationship between final soluble Fe concentrations and BMP test results

Essential nutrients are needed crucial for microbial growth and survival in the biological treatment of wastewaters, including anaerobic treatment (Thanh et al. 2016). Therefore, it is important to have an AD system with adequate level of TEs to ensure an optimal performance (Choong et al. 2016). There are several types of wastewater that contain a sufficient amount of these nutrients, such as swine wastewater (Cestonaro do Amaral et al. 2014), while other types of wastewater such as Methanol wastewater (Fermoso et al. 2008), maize silage (Evranos and Demirel 2015), and wheat stillage (Schmidt et al. 2014) might need trace element supplementation to avoid nutrient deficiency.

In comparison with other types of biosolids, it seems that anaerobically digested sludge (ADS) possesses the highest TE content (Zorpas et al. 2001; Álvarez et al. 2002; Zaleckas et al. 2013; Jenkins et al. 2017). Table 4-7 shows the TE levels per COD mass of different municipal WWT biosolids as well as those values in the bioreactors and FW samples in this study. Considering the very low TE content of FW, ADS appears to be the most suitable option to provide the insufficient TE levels in FW anaerobic digestion.

Table 4-6 The range of trace element concentrations in different biosolids obtained from the literature (Zorpas et al. 2001; Álvarez et al. 2002; Zaleckas et al. 2013; Jenkins et al. 2017) versus the TE content of the bioreactors and FW samples in this study. All numbers in gTE/ gCOD.

	PS	TWAS	ADS	FW	Bioreactor
Co	0.07-0.18	0.04-0.08	0.22-0.67	2×10^{-5}	10^{-3}-10^{-1}
Fe	149-504	48-290	203-2597	8×10^{-3}-2×10^{-2}	2.64-7.29
Mo	0.12-0.34	0.16-0.21	0.25-0.94	4×10^{-5}- 2×10^{-4}	6×10^{-3}-3.81
Ni	0.12-8.28	0.26-10.05	1.31-21.94	8×10^{-5}-4×10^{-4}	3×10^{-2}-$\times10^{-1}$
Se				2×10^{-4}-4×10^{-4}	2×10^{-4}-2×10^{-2}

An extensive literature review was done in order to compare the optimum TE concentrations ibtained from this study with previous TE levels found in previous studies.

Scherer et al. (1983) analysed the TE composition of different methanogens and part of those results are presented in Table. The content of these elements in methanogens are in the order of Fe>>Zn>Ni>Cu≈Co≈Mo>Mn. This clearly shows that TEs play a significant role as the methanogens building elements (Choong et al. 2016).

Table 4-7 TE requirement of different methanogenic groups (Scherer et al. 1983)

Methanogen species	DSM-No	Fe (mg/L)	Ni (mg/L)	Co (mg/L)	Mo (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)
Methanobacterium bryantii	862	1350	150	20	25	250	5	160
Methanothermobacter marburgensis	2133	1500	110	10	25	50	<5	<10
Methanobrevibacter arboriphilicus	744	1300	65	15	40	440	5	20
Methanobrevibacter smithii	861	1100	95	90	25	630	5	25
Methanosarcina mazei	2053	720	95	35	<10	170	5	10
Methanosarcina vacuolate	1232	1600	70	30	30	260	15	40
Methanosarcina thermophila	1825	1600	150	50	<10	230	5	<10
Methanosarcina barkeri	800	1100	65	30	25	300	10	10
Methanosarcina barkeri	1538	1200	60	25	45	370	10	35
Methanosarcina barkeri	804	2150	135	60	60	130	5	10

Iannotti et al. (1981) isolated and characterized 130 strains of bacteria from a swine digester, and then divided them into 11 groups that included organisms identified as Peptostreptococcus, Peptococcus, Eubacterium, Lactobacillus, Bacteriodes, and unidentified genera plus miscellaneous facultative and strict anaerobes. The organisms required mixtures of known factors for growth plus unknown factors in crude extracts such as from digester fluid, swine manure extracts, and rumen fluid. They developed a medium that allowed the minimum growth for 80% of the isolates. The constituents of that medium in terms of trace elements are presented in Table 4-9. In that study, it was shown that deletion of trace elements from the medium in Table reduces the growth of most strains, indicating that digester fluid does not contain sufficient concentrations of these factors for maximum growth. When crude extract of swine manure was substituted for digester fluid, the growth of all groups of bacteria was drastically increased. The requirement for both known and unknown factors indicates that fermentative bacteria have complex requirements and this limits the ability to define optimum conditions (Speece 1985).

Table 4-8 Ionic TE requirement for 80% growth of anaerobic microorganisms (Iannotti et al. 1981).

Trace Element	Concentration
Fe^{2+}	20 µg/L
Mn^{2+}	8 µg/L
B^{3+}	52 µg/L
Co^{2+}	2 µg/L
Ni^{2+}	9 µg/L
Mo^{2+}	12 µg/L

The trace elements are relatively inexpensive to supplement. Most of the TEs are precipitated from the solution due to the presence of sulfide (Speece 1985). This in turn makes it difficult to determine their actual requirement. In a recommendation for optimal growth of methanogens, iron is added at approximately 10 mg/L; cobalt at 5 mg/L; and nickel, molybdenum, and selenium at 0.1 mg/L (Speece 1987). It should be noted that the inhibition threshold levels reported in this study of 0.25 mg Ni/L, 0.07 mg Se/L, and 0.14 mg Mo/L are in close agreement with the 0.1 mg/L reported by Speece (1987). Nutrient limitations will lead to a decreased rate of growth. Therefore, the search for possible stimulants highly depends on the nutrient media that the researcher is using and the situation is further complicated by the ecological interactions which must be considered (Speece 1985). Fore example, a compound which is stimulatory to a pure species of bacteria may have no effect on a mixed culture such as found in an anaerobic digester (Speece 1985). Mah et al. (1977) found that the rate of methane production differed in pure culture versus an enriched culture. They stated that the interactions demonstrated between methanogenic and nonmethanogenic species show that methane production in the mineral acetate enrichment is not the function solely of the organism catalyzing the split of acetate to methane and CO_2; it is a function of a community of organisms, each contributing nutrients to the common environment, and withdrawing others. Acetate decomposition to methane occurs far more rapidly in the community of mixed species than with the Methaonosarcina alone.

4.3.4. Characterization of microbial communities

Table 4-6 shows the various microbial communities (in gram VSS of the inoculum) for selected BMP tests. These BMP tests were chosen based on the maximum methane yields as well as the best COD closure. The BMP parameters chosen here for comparison are final VFA concentration (in gCH_3COOH/L), K_h (day-1), biogas yield (mL/ g VS), and methane yield (mL/ g VS).

Table 4-9 Summary of the BMP test results for selected TE supplemented reactors and their corresponding microbial analysis results (in g VSS of inoculum).

Parameter	**Control**	**Ni [1 mg/L]**	**Co [0.1 mg/L]**	**Mo [2 mg/L]**	**Se [0.2 mg/L]**	**Fe [50 mg/L]**
VFA (g/L)	1.43	0.78	0.74	1.48	1.51	0.83
Methane Production Rate (mL CH_4/day)	109	127	83	120	99	121
K_h (day^{-1})	0.74	0.94	0.73	0.50	0.54	0.65
Biogas yield (mL/g VS)	1090	1397	1354	1314	1412	1274
Methane Yield (mL/g VS)	594	752	719	778	783	721
Bacteroidetes	0.473	0.390	**0.613**	**0.591**	**0.569**	**0.670**
Firmicutes	1.060	**1.126**	**1.104**	0.935	**1.103**	1.002
Clostridium	0.012	**0.014**	0.011	0.011	**0.014**	0.008
Sum_Hydrolytic	1.534	1.516	1.717	1.526	**1.672**	**1.672**
Sum_Acido/Aceto	0.207	0.205	0.180	0.189	**0.237**	**0.220**
Methanosaeta	0.010	0.008	**0.017**	**0.010**	0.008	0.005
Methanosarcina	0.002	**0.003**	0.002	0.002	**0.003**	0.002
Methanobrevibacter	1E-04	3E-04	**0E+00**	1E-04	**0E+00**	5E-04
Methane Production – Aceticlastic	0.013	0.011	**0.019**	0.012	0.010	0.008
Methanobacterium	3E-04	1E-04	**0E+00**	**0E+00**	**0E+00**	**0E+00**
Methanospirillum	7E-04	5E-04	6E-04	6E-04	3E-04	3E-04
Methanoculleus	8E-04	**1E-03**	4E-04	6E-04	3E-04	5E-04
Methane Production – Hydrogenoclastic	2E-03	2E-03	1E-03	1E-03	6E-04	8E-04
Sum_Methanogenic	1E-02	1E-02	**2E-02**	1E-02	1E-02	9E-03

Table 4-10 Summary of the Pearson correlation r values between BMP test results for selected TE supplemented reactors and their corresponding microbial analysis results.

Parameter	VFA (g/L)	K_h (day^{-1})	Biogas yield (mL/g VS)	Methane Yield (mL/g VS)	Methane Production Rate (mL/day)
Bacteroidetes	0.39	-0.70	0.50	0.58	-0.09
Firmicutes	0.06	0.65	0.070	0.19	0.62
Clostridium	-0.16	0.27	0.11	-0.24	-0.17
Sum_Hydrolytic	0.50	-0.22	0.63	0.83	0.44
Sum_Acido/Aceto	0.91	-0.20	0.04	0.69	-0.11
Methanosaeta	-0.64	0.08	0.52	-0.24	0.21
Methanosarcina	0.24	0.35	-0.11	0.042	-0.01
Methanobrevibacter	0.26	0.25	-0.81	0.01	0.24
Methane Production - Aceticlastic	-0.66	0.17	0.44	-0.27	0.29
Methanospirillum	-0.99	0.24	-0.09	-0.86	-0.06
Methanoculleus	-0.54	0.77	-0.84	-0.76	0.19
Methane Production - Hydrogenoclastic	-0.76	0.72	-0.70	-0.89	0.15
Sum_Methanogenic	-0.72	0.16	0.42	-0.35	-0.69

All possible correlations between various microbial groups and BMP parameters i.e. K_h, biogas yield, methane yield, and final VFA concentrations were explored. Only the ones that are statistically significant with an absolute Pearson correlation r value grater than 0.65 are discussed here (Table 4-9).

The first group of bacteria involved in AD process are hydrolytic bacteria. They convert raw feedstocks into smaller organic molecules that can be used by other microbial groups (Ling et al. 2017). The majority of hydrolytic bacteria are in the two phyla *Firmicutes* and *Bacteroidetes* (Ling et al. 2017). Sum of *Bacteroidetes*, *Firmicutes*, and *Clostridium* was also calculated as the total hydrolytic bacteria which contributed to a significantly positive correlation with methane yield (Pearson r = 0.83).

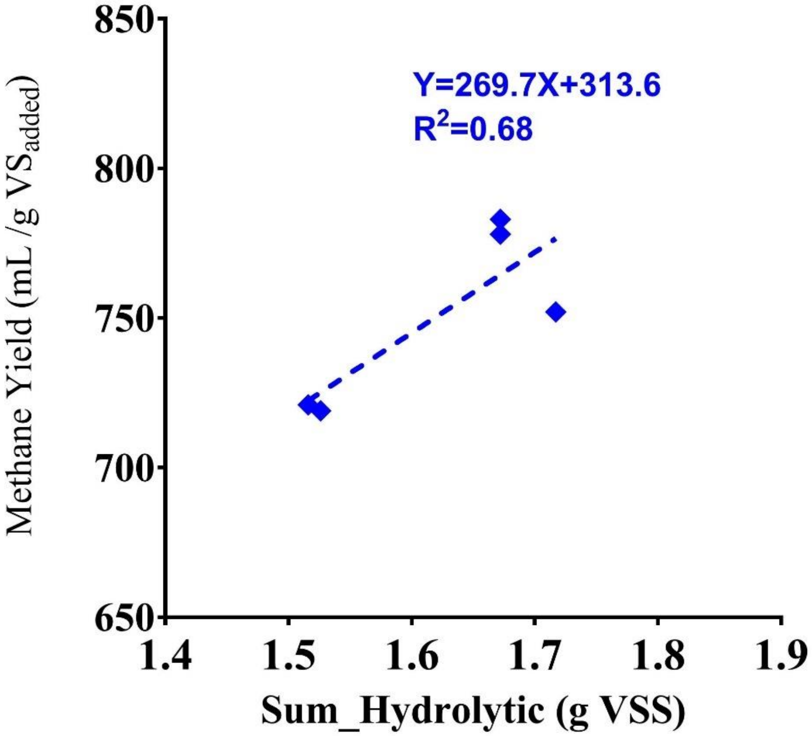

Figure 4-15 BMP results versus Hydrolytic population

Acidogenic and acetogenic bacteria form acetate and other organic acids from the products of hydrolysis. *Firmicutes*, including Clostridia, and *Bacteroidetes* along with *Proteobacteria* include acidogens and acetogens (Ling et al. 2017). Since the ability to perform hydrolysis, acidogenesis, and acetogenesis are widespread among bacteria and many groups can perform different functions depending on existing conditions, it is not feasible to identify which bacterial types are performing these preliminary metabolic steps (Ling et al. 2017).

- It is worth mentioning that *Proteobacteria* were not detected in the samples of this study.
- Final VFA concentrations were very well correlated with the sum of acidogenic and acetogenic bacteria (Pearson r = 0.91); the more the bacterial population, the higher the final VFA concentration which proves the fact that these populations are responsible for acid production in the AD process.
- According to Table 4-8, in cases where this population in the TE supplemented reactors was slightly more than the control reactor, 10%-19% higher methane yield was observed (Fe [50 mg/L] and Se [0.2 mg/L]).

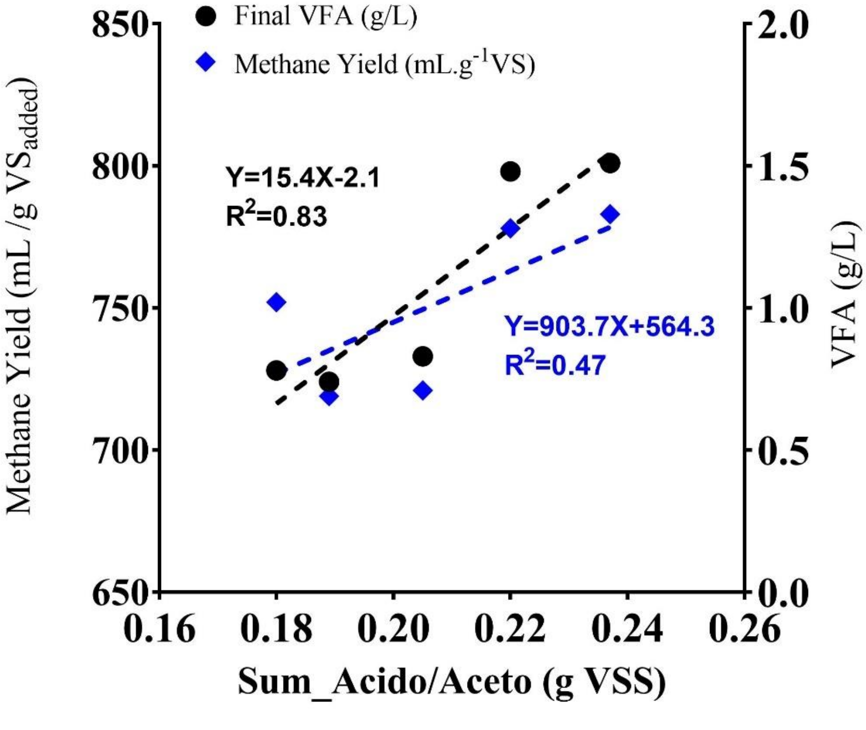

Figure 4-16 BMP results versus Acidogenic/Acetogenic population

Methanogens produce methane from acetate or hydrogen. Methanogenesis can only be accomplished by archaea; no known bacteria can produce methane (Ling et al. 2017).

- As could be expected, aceticlastic methanogens had a negative relationship with final VFA concentrations.

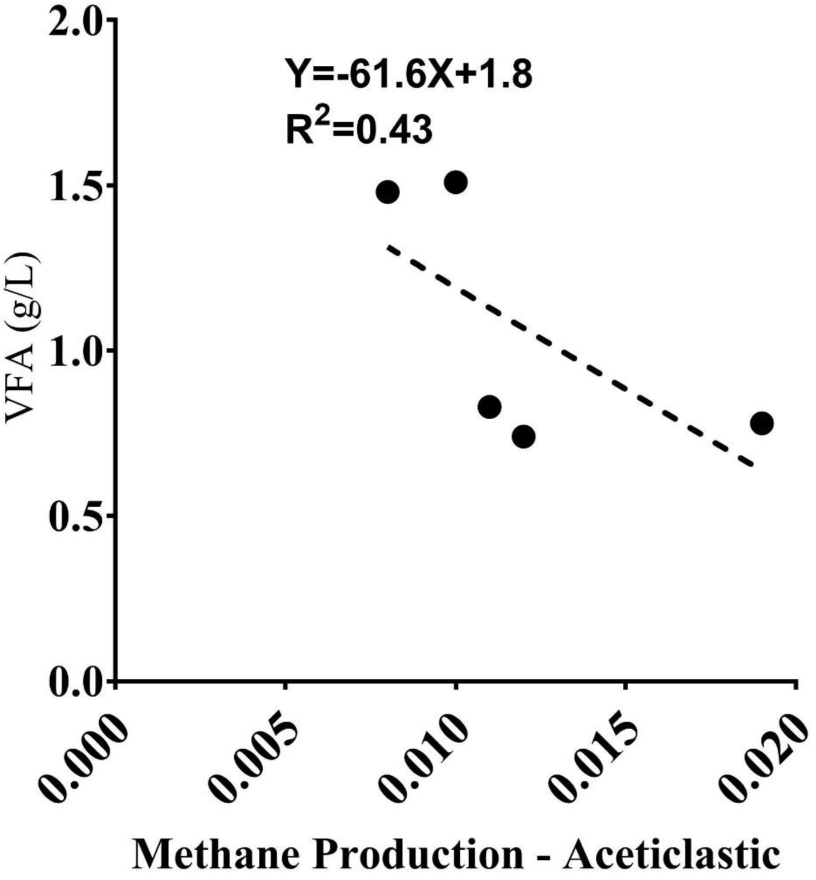

Figure 4-17 BMP results versus Aceticlastic Methanogenic population

- Negative relationships between the sum of all hydrogenoclastic methanogens and methane and biogas yields were observed. Surprisingly, there was a negative correlation between the final VFA concentrations and hydrogenoclastic methanogens.

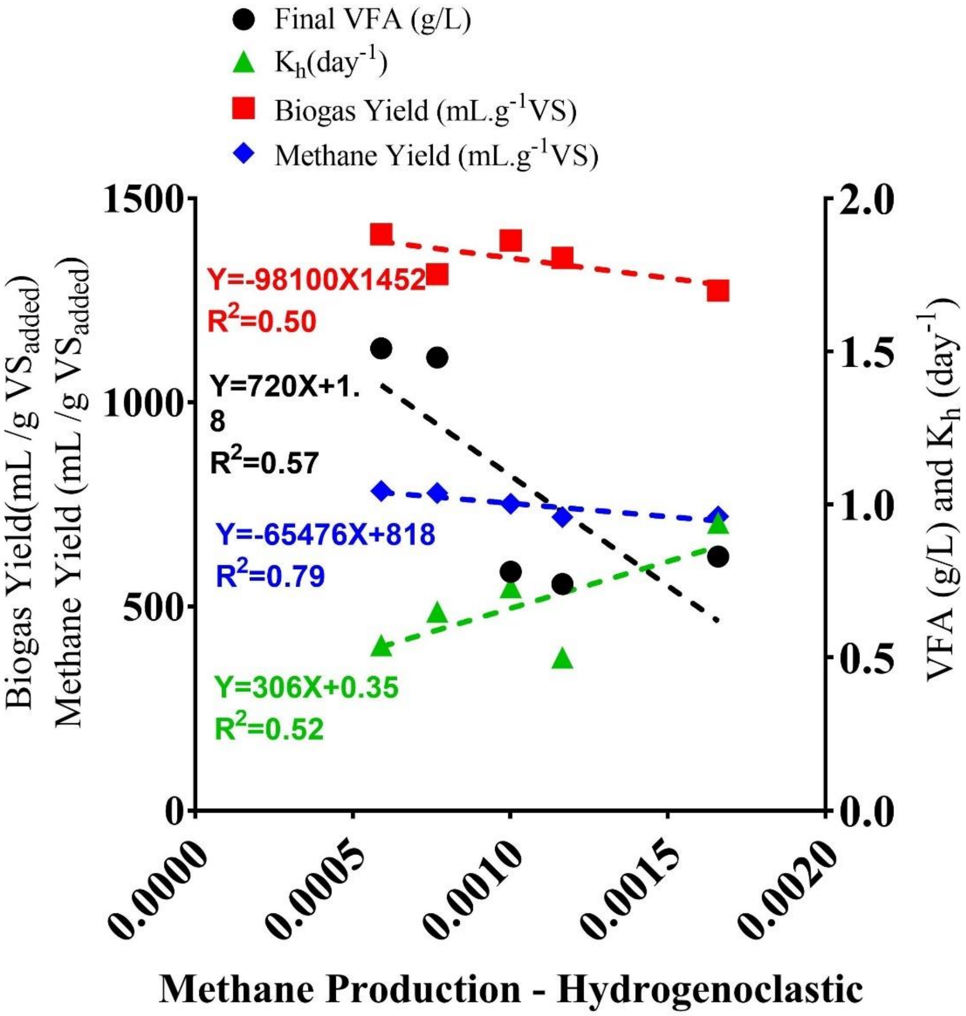

Figure 4-18 BMP results versus Hydrogenoclastic Methanogenic population

- Finally, acetoclastic and hydrogenoclastic methanogenic populations were combined to represent the whole methanogenic archaea and then were plotted versus the BMP parameters. A negative relationship was observed between the VFA concentration and methanogenic population while a moderately positive correlation was observed between biogas yield and methanogens population. Furthermore, there is an unexpected negative relationship between the population of methanogens and methane production rate.

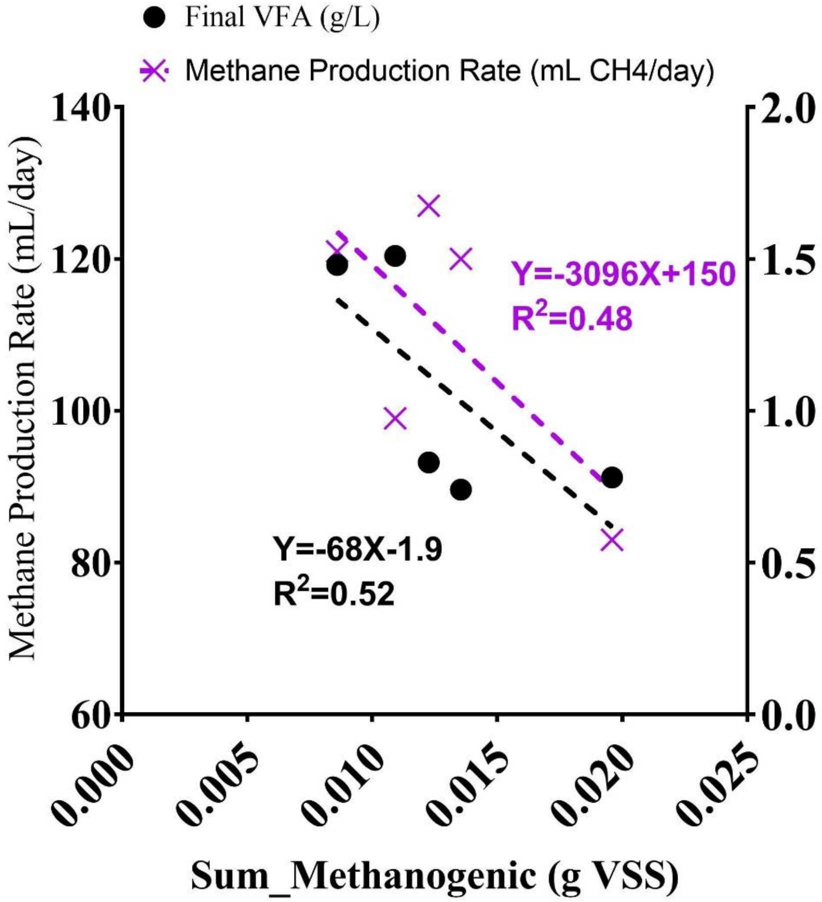

Figure 4-19 BMP results versus Methanogenic population

The correlations of microbial community analysis yielded the following expected outcomes:

- Firmicutes (a group of hydrolytic bacteria) positively correlated with K_h values.
- Methane yields increased with increasing hydrolytic bacteria as well as the sum of acitogenic and acetogenic populations.
- The sum of acitogenic and acetogenic populations had a positive correlation with final VFA concentrations.
- Aceticlastic methanogens had a negative relationship with final VFA concentrations.
- K_h and biogas yield increased with increasing Methanoculleus population which are hydrogenoclastic archaea.
- Kh had a positive relationship with total hydrogenoclastic methanogenic populations.
- Sum of all methanogenic archaea negatively correlated with the final VFA concentrations.

However, unexpectedly, the following correlations were observed:

- Bacteroidetes (a group of hydrolytic bacteria) negatively correlated with K_h values.
- Methanobrevibacter (a group of aceticlastic methanogens) had a negative relationship with biogas yield.
- Methanospirillum (a group of hydrogenoclastic methanogens) negatively correlated with both final VFA concentrations and methane yields.
- Methanoculleus negatively correlated with both biogas and methane yields.
- Sum of hydrogenoclastic methanogens had a negative relationship with final VFA concentrations.
- Sum of all methanogens had a negative correlation with methane production rate.

4.4. Conclusions

The influence of trace elements addition on methanogenic activity, biogas and methane yields, as well as the hydrolysis rate constant and anaerobic microbial cultures were discerned by conducting SMA, BMP, and microbial DNA-sequencing-based characterization. The results of this study using the FW sludge-low Fe inoculum (FWS-LFe) were compared to a previous study with the same experimental design but using a ADS-HFe. It was observed that supplementing TEs to the FWS-LFe did not significantly affect the methanogenic activity in terms of SMA rates except Mo case. Mo addition resulted in 28% and 22% higher rate of methane production than the control for 5 mg Mo/L and 20 mg Mo/L, respectively. Another exception was Fe at 400 mg/L which reduced the SMA rate by 20% comparing to the control. With the ADS-HFe, however, TE addition decreased the specific methane production rates in almost all cases, except for Mo which in the range of 2-20 mg/L had a neutral impact on methanogenic archaea. From this comparison, it can be concluded that the TE addition to the to the ADS-HFe inhibited the methanogenic activity where with the FWS-LFe, Mo and Fe additions were able to enhance the SMA rates, potentially due to the low Fe levels in the inoculum since the experimental designs were the same.

For the FWS-LFe, Ni positively affected methanogens, it increased the methane yield by 27% and 23% at 1 mg/L and 1.5 mg/L, respectively and also decreased the FW digestion time from 5.8 days to 2.5 days. Co on the other hand, enhanced the methane yield by 21% and 23% at 0.1 mg/L and 0.5 mg/L supplementation, respectively and reduced the digestion time by 40%. Mo at 2 mg/L and 5 mg Mo/L improved the methane yield in BMP tests by 28% and 19%, respectively. Mo impact on digestion time was also significant (from 5.8 days in control to 3.1 days). Se addition increased the methane yield at all concentrations (up to 32%) and decreased digestion time by up to 52% to 2.6 days. Fe increased methane yield at 50 mg/L by 22% and could decrease the digestion time by 50% to 3 days. Using the ADS-HFe, ultimate methane productions were not improved at any TE added case.

Comparing the K_h values affected by TE supplementation in the two studies with low and high Fe indicates that the hydrolysis rate constant was not significantly affected by TE addition in both cases, except Co and Fe addition with the FWS-LFe, which increased the hydrolysis rate constant by up to 33% and 74%, respectively.

From the trends between BMP results and soluble TE concentrations in the final samples, it was observed that the optimum final soluble TE concentrations for highest biogas yield, methane yield, and hydrolysis rate constant is around 0.25 mgNi/L, 0.14 mgMo/L, and 0.07 mgSe/L. It was difficult to determine an optimum concentration for Co and Fe due to data scatter.

Furthermore, microbiology test results showed that the sum of three main components of hydrolytic bacteria (Bacteroidetes, Firmicutes, and Clostridium) is positively correlated with methane yields. This indicates that the higher activity of hydrolytic bacteria which results in higher levels of soluble acids, readily available for methanogenic archaea, would lead to methanogenic activity stimulation and higher methane production. The sum of hydrogenoclastic and aceticlastic methanogens correlated positively with methane yields which suggests that TE supplementation has increased their population and hence, their propensity to digest more FW and produce methane.

High Fe concentration in the inoculum, reduced the concentration of free trace TEs via potential co-precipitations and thus did not result in any enhancement in digestion kinetic parameters ($SMPR_{max}$ and K_h) and methane yields and hence, in Fe rich sludges, FW digestion was not improved by TEs supplementation. However, in the anaerobic digestion of FW with FWS-LFe, it was showed that TE additions have several positive impacts on methane production and digestion kinetics.

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Chapter 5

5. Conclusions and recommendations

5.1. Conclusions

In the first phase of this study, using the Fe-rich sludge (≈ 1.7 g Fe/L) to investigate TE supplementation impacts on methanogens as well as anaerobic digestion of FW, the main findings were as follows:

I. In the SMA tests with acetate (2gCOD/L) as substrate, supplementing Fe (50-400 mg Fe/L) significantly reduced the SMA rate by 20% to 37% at the tested concentrations in comparison with the control (with no TEs addition). Similarly, Ni severely reduced the SMA rates by 40% and 58% at 10 and 20 mg/L, respectively, compared to the control. Adding Co resulted in 33%±0. 2% lower rate of methane production than the control which is the same case for all tested concentrations. Finally, Se added batch digester at 0.3 mg Se/L reduced the SMA rate by 26%. Only Mo addition at different concentrations led to the same rate as the SMA control. According to the results of this study, none of the supplemented TE enhanced the SMA rates.

II. Individual supplementation of the TEs had marginally negative to neutral impacts on the kinetic parameters of FW digestion. In CMP tests with FW as substrate and Fe-rich sludge as inoculum, Fe supplementation at all concentrations, except for 400 mg Fe/L, did not change the $SMPR_{max}$ and K_h significantly from the control value. Ni addition resulted in relatively the same values for $SMPR_{max}$ and K_h at all concentrations similar to the control. Co at all concentrations resulted in the same $SMPR_{max}$ as the control but decreased the K_h by 12% at 0.2 mg/L and 40% at 0.4 mg/L and 0.5 mg/L. Adding Se at the relatively lower concentrations than the other TEs to the batch bottles resulted in similar values of $SMPR_{max}$ and K_h for the control and all applied concentrations. Lastly, Mo supplementation at 20 mg/L was the only case in which a statistically significant drop in K_h relative to the control was observed. $SMPR_{max}$ values were also decreased by 5%, 7%, and 10% in 5 mg/L, 10 mg/L, and 20 mg/L Mo addition, respectively.

III. Similarly, the use of TEs mixtures in anaerobic digestion of FW with Fe-rich sludge did not impact the $SMPR_{max}$ and K_h.

IV. Soluble TE concentrations decreased significantly during the experiments except for Fe. Fe solubility decreased with increasing initial soluble Fe concentration. Soluble Co concentrations of the final samples of CMP tests had on average 79% reduction compared to their initial soluble concentrations. Similarly, Ni concentrations in the final soluble samples were 11% of the initial values. Soluble concentrations of Mo substantially dropped during the CMP tests to 29% of the initial value of 18 mg/L. Se concentrations were below the detection limits of 0.05 mg/L for both initial and final samples.

V. Since the FeS solubility constant is significantly higher than other TE precipitates, it is the dominant sulfide (other precipitated TEs were FeS, NiS, CoS, MoS). The estimated sulfide required to precipitate Fe was 3.02×10^{-3} mole, which was orders of magnitude higher than the sulfide needed to precipitate other TEs. Hence, since the amount of free sulfides (S^{2-}) based on the operational conditions of this study of 5.2×10^{-4} mole were well below the S^{2-} concentration of 3.02×10^{-3} mole required to precipitate all the metals i.e. Fe, Ni, Co, Mo, and Se, it appears that co-precipitation and adsorption onto iron sulfide complexes played a significant role in reducing the soluble TEs.

In the second study with the FWS-LFe (≈ 0.26 g Fe/L) and TE addition, the following results were observed regarding the impact of TEs on the methanogenic archaea and batch anaerobic FW digesters:

I. Ni, Co, and Se at all applied concentrations did not affect SMA rates significantly compared to the SMA control. However, adding Mo at different concentrations to the SMA test bottles with mesophilic sludge resulted in 28% and 22% higher rate of methane production than the control for 5 mg Mo/L and 20 mg Mo/L, respectively. Fe at 400 mg/L was another exception where the SMA rate increased by 20%.

II. Ni increased the biogas yield at all concentrations by 14% to 28%. Similarly, Co enhanced the biogas yield at all concentrations by up to 25%. Mo however, improved biogas yield by 21% and 18% at 2 and 5 mg/L, respectively. Among all the TE added reactors, Se had the best performance in terms of biogas yield, increasing it by an average of 29% by Se supplementation. Finally, Fe increased the yield up to 13% at both 100 mg/L and 200 mg/L.

III. Ni supplementation at 1 mg/L and 1.5 mg/L increased CH_4 per gram VS_{FW} by 27% and 23% to 752 and 733 $mLCH_4$/ g VS_{FW}, respectively. Co added reactors at the lowest concentration (0.1 mg/L) produced 21% more methane than the control per gram VS of added FW (719 $mLCH_4$/ g VS_{FW}). 0.5 mg Co/L supplemented reactors increased the methane yield by 23% to 730 $mLCH_4$/ g VS_{FW}. Mo supplementation at 2 and 5 mg/L led to 31% (778 $mLCH_4$/ g VS_{FW}) and 19% (707 $mLCH_4$/ g VS_{FW}) higher methane yield, respectively. Se at all concentrations enhanced methane yield by 24% up to 32% (735 to 783 $mLCH_4$/ g VS_{FW}). Finally, Fe addition did not affect methane production, except for 50 mg/L which resulted in 22% more methane yield (721 $mLCH_4$/ g VS_{FW}).

IV. Maximum methane production rate (R_{max}) decreased by 26%, 22%, and 15% for Co addition at 0.1 mg/L, 0.2 mg/L, and 0.4 mg/L, respectively. Ni and Mo addition however, did not have any significant impacts on methane production rates. Lastly, Se at 0.6 mg/L and Fe at 400 mg/L negatively affected the R_{max} by 16% and 25%, respectively. None of the single TE supplementations enhanced maximum methane production rates.

V. The hydrolysis rate constants of the control and TE supplemented reactors were not significantly different from each other. Exceptions include Co at 0.1 mg/L which reduced the K_h value by 33% to 0.5 day^{-1} and Fe which at 100, 200, and 400 mg/L increased the K_h by 74%, 57%, and 42% to 1.28 day^{-1}, 1.16 day^{-1}, 1.05 day^{-1}, respectively.

VI. Ni decreased the digestion time (for 60% COD degradation) from 5.8 days in the control to 2.5 days. Co, Mo, Se and Fe addition enhanced degradation time by 40%, 46%, 52%, and 51% to 3.5 days, 3.6 days, 2.7 days, and 2.8 days, respectively.

VII. Ni[1mg/L]+Co[0.1mg/L]+Se[0.8] and Ni[1mg/L] +Co[0.1mg/L] +Mo[2 mg/L] increased the methane yield slightly (6% and 9%, respectively to 691 and 712 $mLCH_4$/ g VS_{FW}). Mo[2mg/L] +Se [0.8] however, could enhance methane production by up to 12% to 728 $mLCH_4$/ g VS_{FW}. Ni [1 mg/L] +Co combination resulted in 16% (755 $mLCH_4$/ g VS_{FW}), 14% (742 $mLCH_4$/ g VS_{FW}), and 12% (725 $mLCH_4$/ g VS_{FW}) higher methane yields at 0.1, 0.4, and 0.5 mg Co/L.

VIII. R_{max} decreased by 13% with Ni[1mg/L]+Co[0.1 mg/L]+Se[0.1mg/L to 0.6 mg/L] supplementation. On the other hand, Ni[1mg/L]+Co[0.1 mg/L]+Se[0.8 mg/L] did not change the R_{max} significantly. Mo[2mg/L]+Se[0.1mg/L] and Mo[2mg/L]+Se[0.6mg/L] reduced methane production rate by 11% (74 $mLCH_4$/ day) and 9% (75 $mLCH_4$/ day), respectively. The only cases at which maximum methane production rates were improved were Ni[1mg/L]+Co[0.1mg/L] and Ni[1mg/L]+Co[0.5mg/L]; they both increased the R_{max} by an average of 11% (91 $mLCH_4$/ day).

IX. Time to reach 60% biodegradability slightly decreased with Ni[1mg/L]+Co[0.1mg/L]+Se[0.1 mg/L to 0.8 mg/L] addition by 8% to 3.2 days. Mo+Se mixture however, did not enhance the degradation time. Ni[1mg/L]+Co[0.1mg/L]+Mo[2 mg/L] also accelerated the degradation time by 6% to 3.3 days. Addition of Ni[1mg/L] +Co[0.1mg/L to 0.5 mg/L], on the other hand, did not lead to any significant enhancement on digestion time.

X. A comparison between the single and mixed TE added BMP results shows that Ni[1mg/L]+Co[0.1mg/L]+Mo[2 mg/L] increased the FW degradability by 15% compared to Mo alone at 20 mg/L. Likewise, Ni[1mg/L]+Co[0.1mg/L] improved digestion up to 14% and 15% comparing with Ni when added individually at 0.5 mg/L and 2 mg/L, respectively. FW degradability was also 13% higher with Ni[1mg/L]+Co[0.4mg/L] addition in comparison to Ni alone at 2 mg/L. Lastly, Mo[2mg/L]+Se[0.8 mg/L] was found to produce 13% and 18% more methane per COD added than Mo[10mg/L] and Mo[20mg/L], respectively i.e. 64% and 62% biodegradability.

XI. From the trends between BMP results and soluble TE concentrations in the final samples, it was observed that the optimum soluble Ni concentration with respect to enhancements in biogas yield, methane yield, and hydrolysis rate constant was 0.25 mg/L. This concentration was found to be 0.14 and 0.07 for Mo and Se while no optimum concentration was determined for Co and Fe due to data discrepancy.

XII. Microbiology test results showed that the hydrolytic bacteria population is positively correlated with methane yields. The sum of hydrogenoclastic and aceticlastic methanogens correlated positively with methane yields which suggests that TE supplementation has increased their population and hence, their activity to be able to digest more FW and produce more methane.

A comparison between the two studies shows that:

I. TE supplementation to reactors with FWS-LFe at the tested concentrations either improved methanogenic activity (Mo addition at all concentrations and Fe at 400 mg/L) or did not significantly affect the SMA (other TEs except mentioned earlier) although it inhibited methane production with ADS-HFe in all TE dosed reactors (except Mo, which had a neutral effect on SMA rates). This is probably due to the significantly lower Fe content in the inoculum of the second study. Mo has a stronger effect on acetoclastic methanogens since it is the only case in which at all concentrations ranging from 2-20 mg Mo/L, methane production is not inhibited, but stayed the same (with ADS-HFe) or improved (with FWS-LFe). Therefore, it can be concluded that Mo is the only trace element among the tested TEs which always has a positive impact on the methanogenic activity of the sludge.

II. Hydrolysis rate constant (K_h) is not significantly affected in both studies, except Co and Fe addition in the study with the FWS-LFe, which increased it by up to 33% and 74%, respectively.

5.2. Recommendations

The first study revealed TE supplementation to the batch anaerobic reactors reduces the concentration of free trace TEs via potential co-precipitations and thus, TE addition does not result in any enhancement in digestion kinetic parameters in BMP tests ($SMPR_{max}$ and K_h) as well as methane yields. Hence, in Fe rich sludges, FW digestion is not improved by TEs supplementation. However, in the second study (anaerobic digestion of FW with FWS-LFe) it was showed that TE additions have several positive impacts on methane production and digestion kinetics. Considering the fact that the experimental design for the two studies were similar, it can be concluded that the observed differences between the impact of TEs on FW anaerobic digestion results are due to the very different Fe concentrations in the two sludges (1.7 g/L versus 0.26 g/L). If BMP test results are considered as predictions to the actual full-scale AD performance, it is highly recommended that selecting TE dosages in the supplementation method be accompanied with a trace element background check in the sludge to make sure Fe concentration is not at the levels to promote co-precipitation and/or adsorption of added TEs onto the abundant Fe sulfide precipitates. However, in full-scale digesters, over long periods of operation, pseudo-steady-state conditions which are mostly governed by the feedstock characteristics will prevail.

Appendices

Appendix A. Supplementary material for Chapter 3

A sample calculation of the SMA rate for the control (average of 3 reactors) for the Co experimental setup is shown in Figure 3-1B shows the maximum slope between days 1 and 2. A linear regression with GraphPad Prism 7 was used to calculate the various rates between days 1 and 2; the maximum slope was found to be 0.29 mL CH_4/g $VSS_{inoculum}$/day from day 1.3 to day 2.1 with R^2 of 0.99. The same method was applied for all reactors and the data is presented in Table. 3-4. The R^2 of linearization ranged from 0.97 to 0.99 in this experiment.

Table A-1 SMA rate calculation for different TE supplemented reactors and their controls.

Fe

Parameter	Control	[50 mg/L]	[100 mg/L]	[200 mg/L]	[400 mg/L]
Time interval (days)	1.3 to 2.1	1.5 to 2.5	1.7 to 2.5	1.7 to 2.5	1.8 to 2.5
SMA (mL CH_4/g $VSS_{inoculum}$/day)	0.29 ± 0.01	0.23 ± 0.00	0.23 ± 0.00	0.19 ± 0.00	0.19 ± 0.00

Ni

Parameter	Control	[0.5 mg/L]	[1 mg/L]	[1.5 mg/L]	[2 mg/L]
Time interval (days)	1.5 to 2.0	1.7 to 2.0	1.5 to 2.0	1.5 to 2.2	1.1 to 2.0
SMA (mL CH_4/g $VSS_{inoculum}$/day)	0.38 ± 0.01	0.28 ± 0.00	0.26 ± 0.00	0.23 ± 0.00	0.16 ± 0.00

Co

Parameter	Control	[0.1 mg/L]	[0.2 mg/L]	[0.4 mg/L]	[0.5 mg/L]
Time interval (days)	1.4 to 1.8	1.3 to 1.8	1.3 to 1.9	1.5 to 1.9	1.5 to 1.9
SMA (mL CH_4/g $VSS_{inoculum}$/day)	0.38 ± 0.01	0.25 ± 0.01	0.26 ± 0.00	0.25 ± 0.00	0.25 ± 0.00

Se

Parameter	Control	[0.005 mg/L]	[0.01 mg/L]	[0.02 mg/L]	[0.05 mg/L]
Time interval (days)	1.2 to 2.2	1.2 to 2.2	1.2 to 2.2	1.2 to 2.2	1.2 to 2.2
SMA (mL CH_4/g $VSS_{inoculum}$/day)	0.25 ± 0.02	0.19 ± 0.01	0.18 ± 0.02	0.18 ± 0.03	0.17 ± 0.03

Mo

Parameter	Control	[2 mg/L]	[5 mg/L]	[10 mg/L]	[20 mg/L]
Time interval (days)	1.1 to 2.9	1.1 to 2.9	1.1 to 2.9	1.1 to 2.9	1.1 to 2.9
SMA (mL CH_4/g $VSS_{inoculum}$/day)	0.27 ± 0.03	0.27 ± 0.01	0.28 ± 0.02	0.28 ± 0.01	0.28 ± 0.02

A sample calculation of the $SMPR_{max}$ for the Co addition at 0.1 mg/L is presented in Figure A-

1C. Plotting methane production rates results in a curve which starts from almost a plateau, steeps to a peak, and then reaches a plateau again (Figure A-1C). The peak is reported as the maximum methane production rate ($SMPR_{max}$).

As mentioned in Chapter 3, a first-order kinetic model can then be used for calculating the K_h as expressed in Eq. (2):

$$P = P_{max}[1 - e^{-K_h t}] \qquad \text{Equation 0-1}$$

Where,

P= net cumulative methane production from the CMP assay at time t (mL),

P_{max}= net ultimate methane yield from CMP assay at the end of the incubation time (mL),

K_h= first-order hydrolysis rate constant (1/d).

K_h can be derived from the slope of the linear regression line plotted for Ln ($1-P/P_{max}$) versus time (Figure A-1D). The linearization was conducted by GraphPad Prism 7 software and the R^2 values of all the slopes were within 0.97 to 0.99.

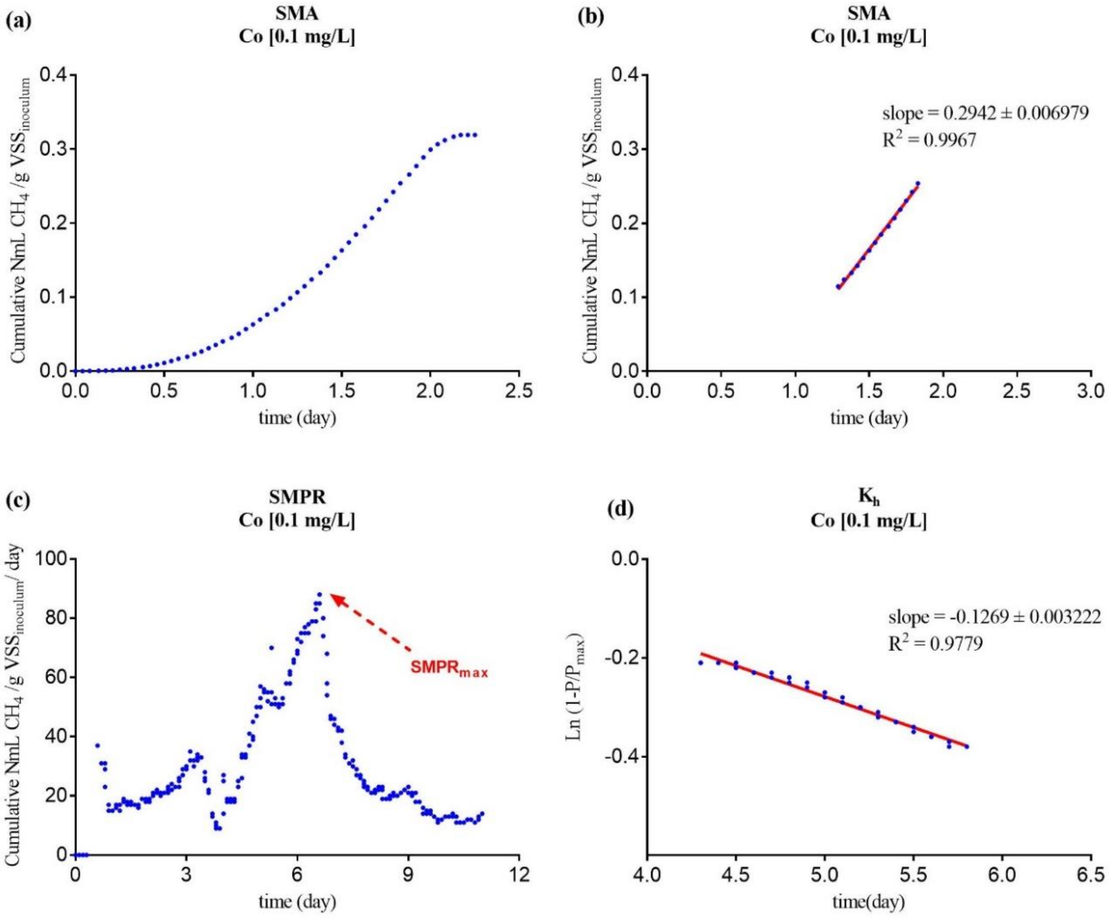


Figure A- 1 Sample kinetic rate and range of calculations for Co [0.1]. (a) SMA average, specific methane production of the average of triplicates; (b) Maximum SMA rate; (c) Temporal variations of specific methane production rate (d) first order apparent hydrolysis

Cumulative net methane production profiles were fit with Gompertz model based on the following equation

$$P = P_{max} \exp(- \exp\left(\frac{R_{max}}{P_{max}}(t_0 - t)e + 1\right))$$

where:

P is the net cumulative methane production

P_{max} is the ultimate net cumulative methane production

R_{max} is the maximum methane production rate

t_0 is the lag phase

t is the time at which methane production was recorded.

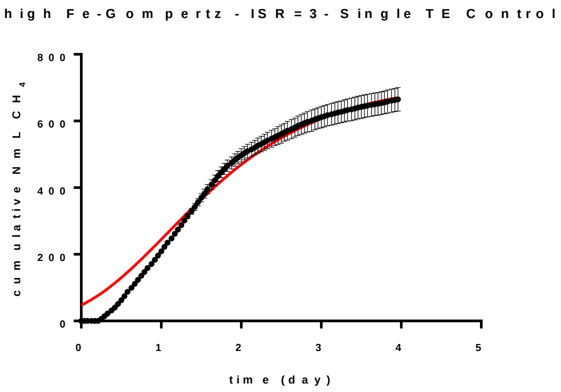


Figure A- 2 Gompertz modeling in the study with ADS-HFe- Fe addition control (ISR=3)

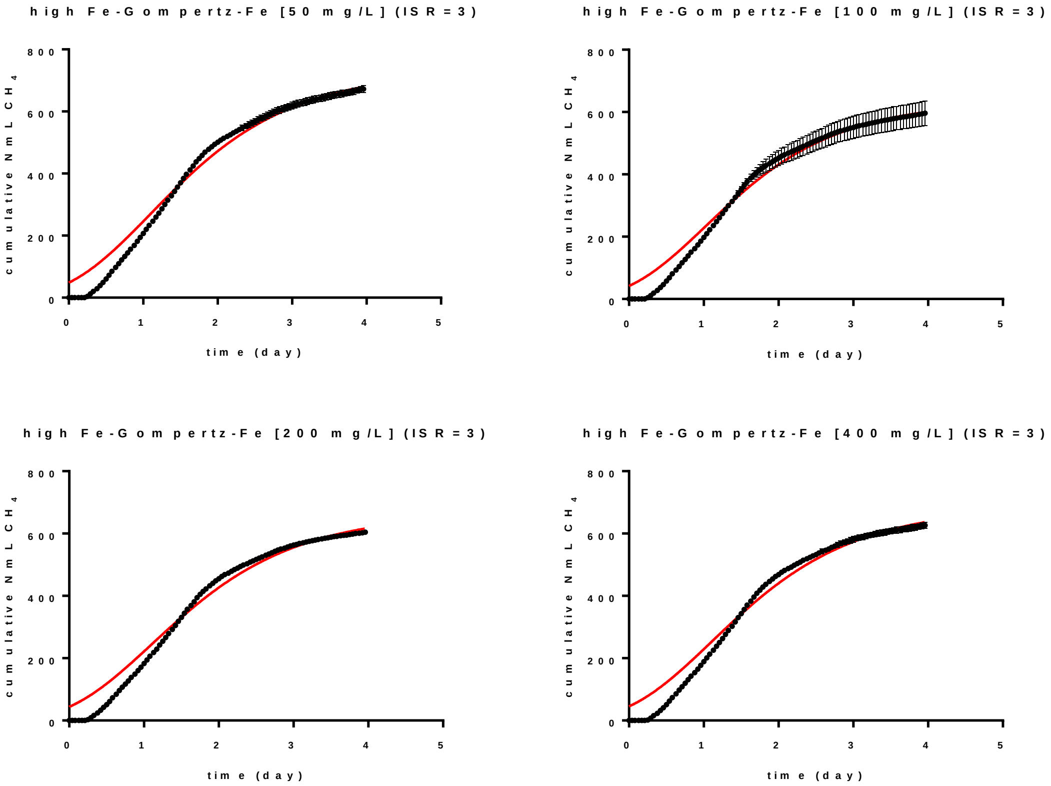


Figure A- 3 Gompertz modeling in the study with ADS-HFe- Fe[50-400 mg/L] addition (ISR=3)

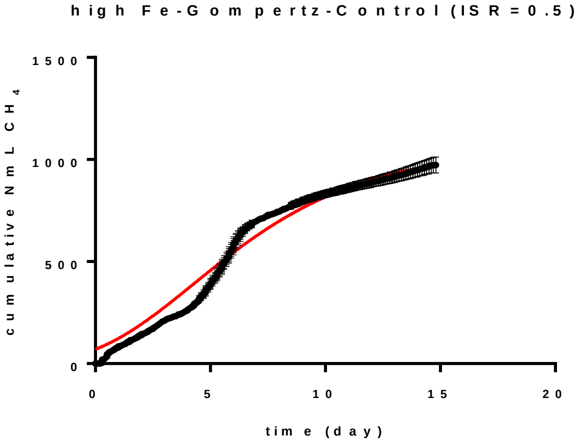


Figure A- 4 Gompertz modeling in the study with ADS-HFe- Fe addition control (ISR=0.5)

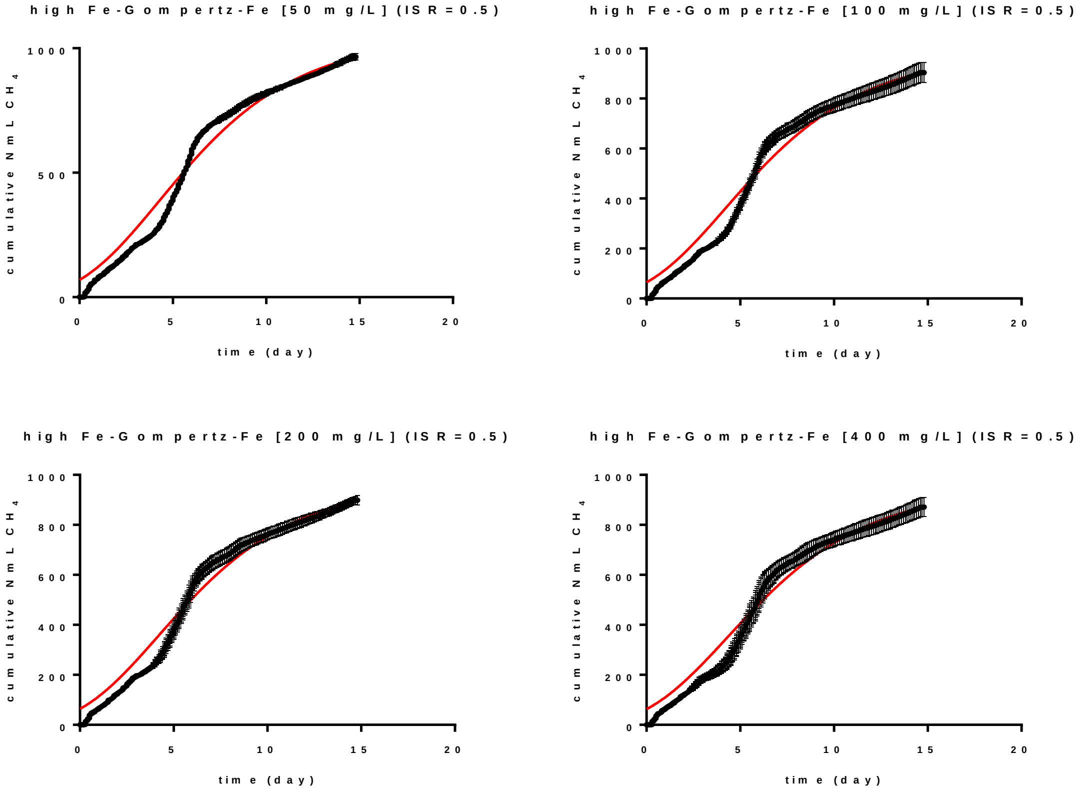


Figure A- 5 Gompertz modeling in the study with ADS-HFe- Fe [50-400 mg/L] addition (ISR=0.5)

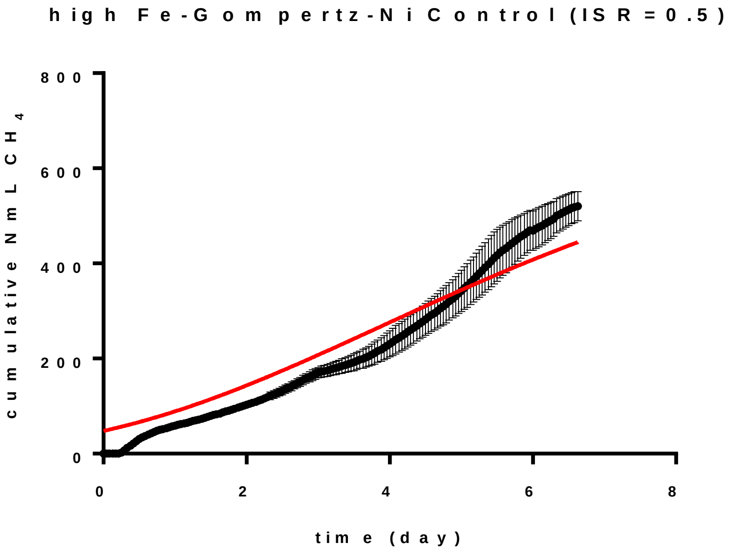


Figure A- 6 Gompertz modeling in the study with ADS-HFe- Ni addition control (ISR=0.5)

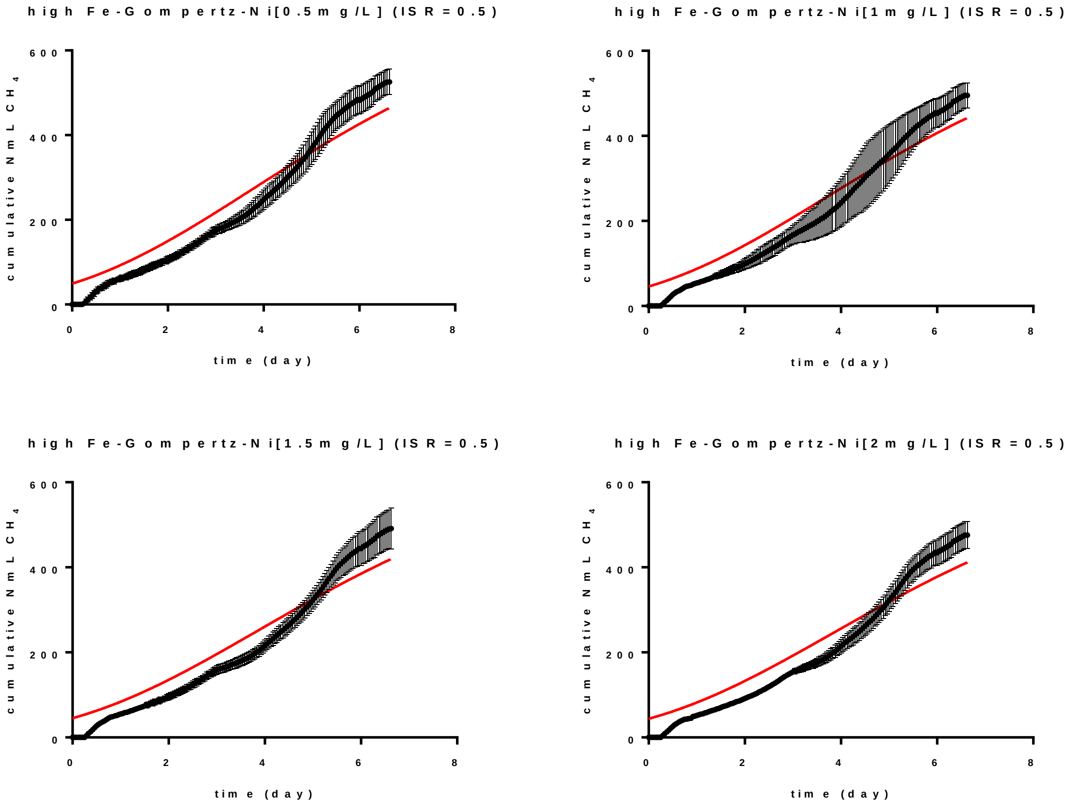


Figure A- 7 Gompertz modeling in the study with ADS-HFe- Ni [0.5-2 mg/L] addition (ISR=0.5)

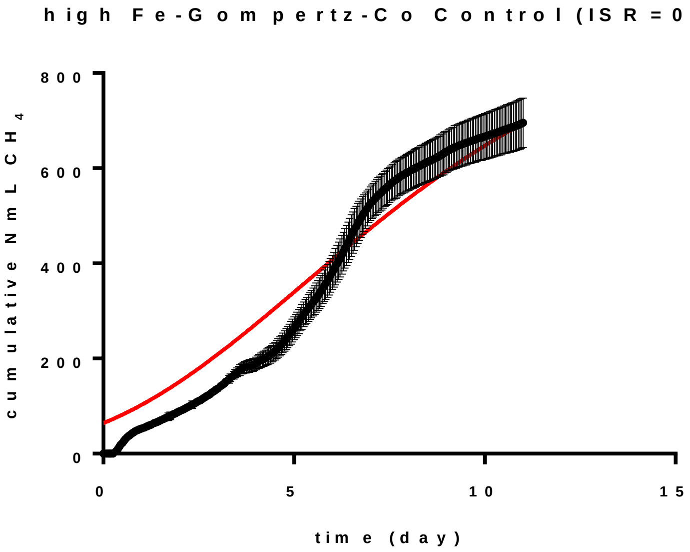


Figure A- 8 Gompertz modeling in the study with ADS-HFe- Co addition control (ISR=0.5)

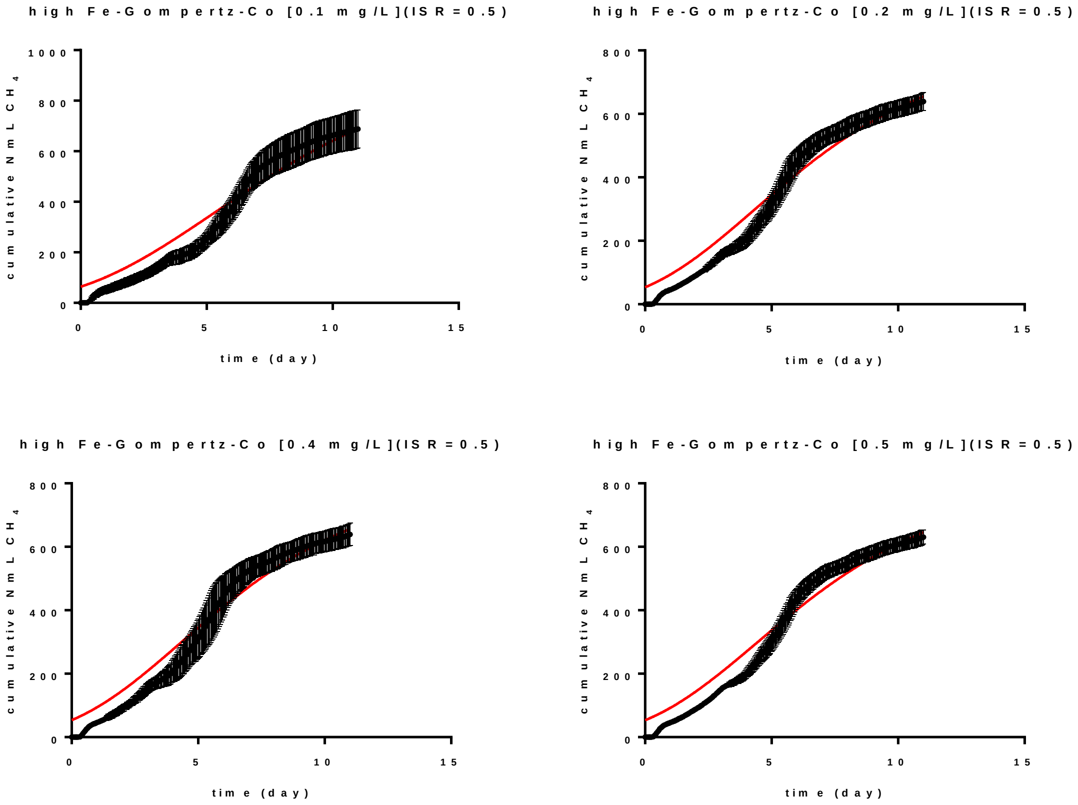


Figure A- 9 Gompertz modeling in the study with ADS-HFe- Co[0.1-0.5 mg/L] addition (ISR=0.5)

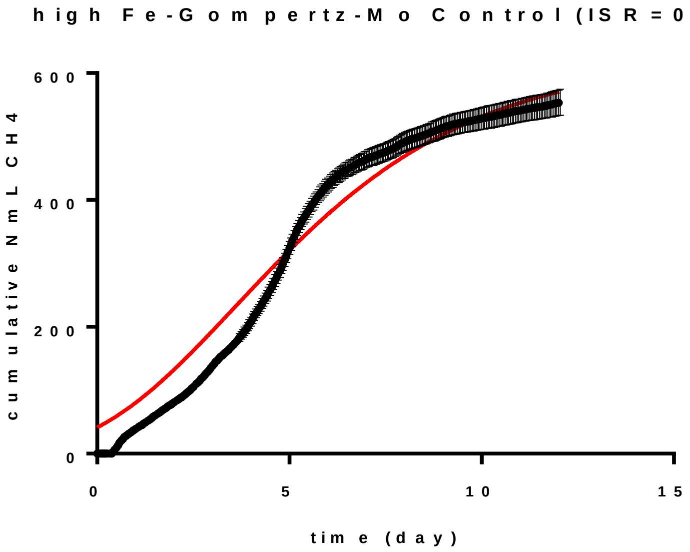


Figure A- 10 Gompertz modeling in the study with ADS-HFe- Mo control (ISR=0.5)

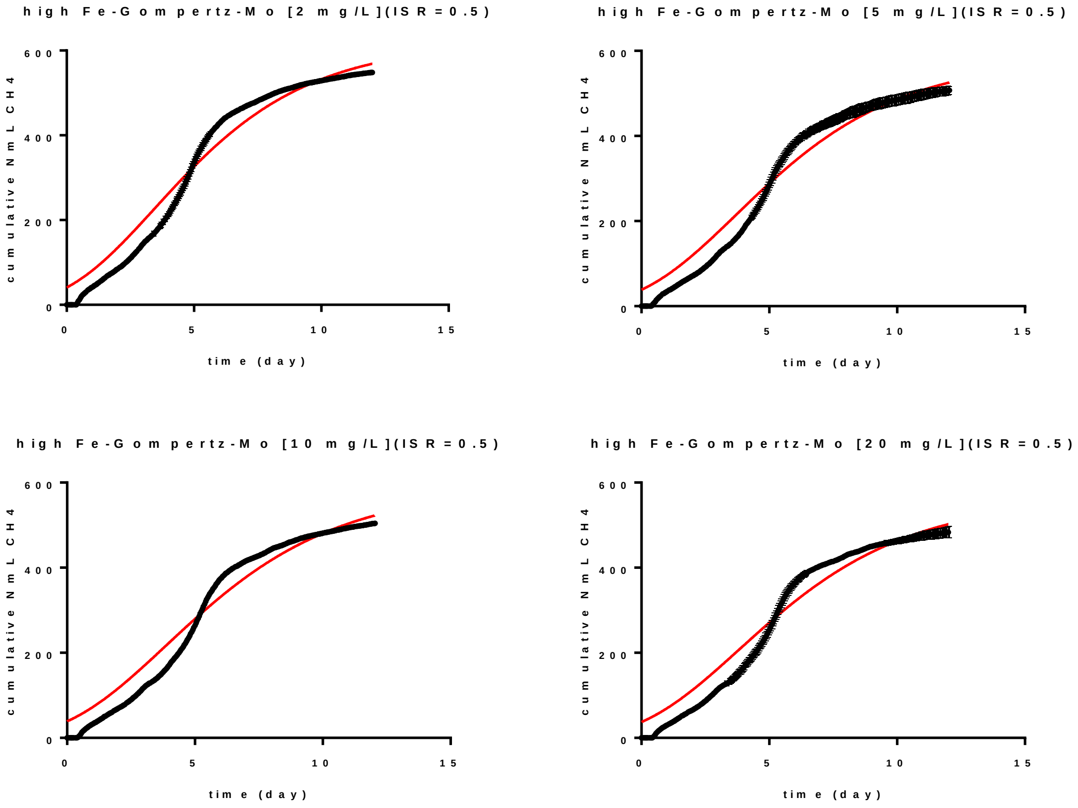


Figure A- 11 Gompertz modeling in the study with ADS-HFe- Mo[2-20 mg/L] addition (ISR=0.5)

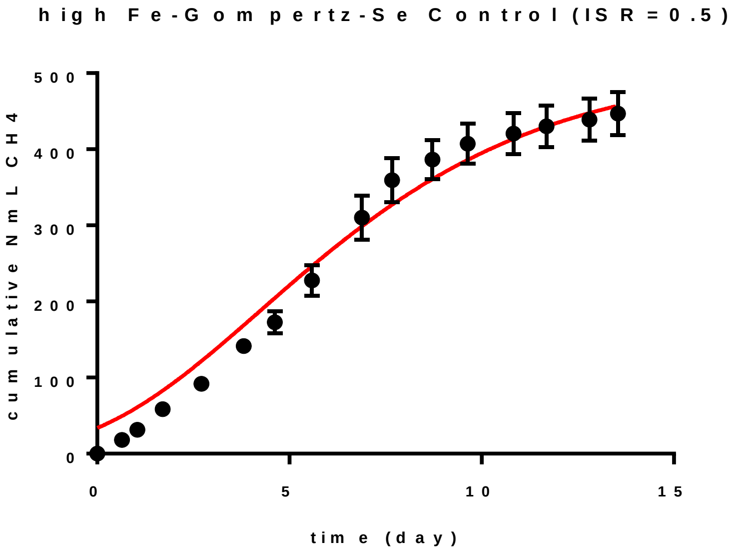


Figure A- 12 Gompertz modeling in the study with ADS-HFe- Se control (ISR=0.5)

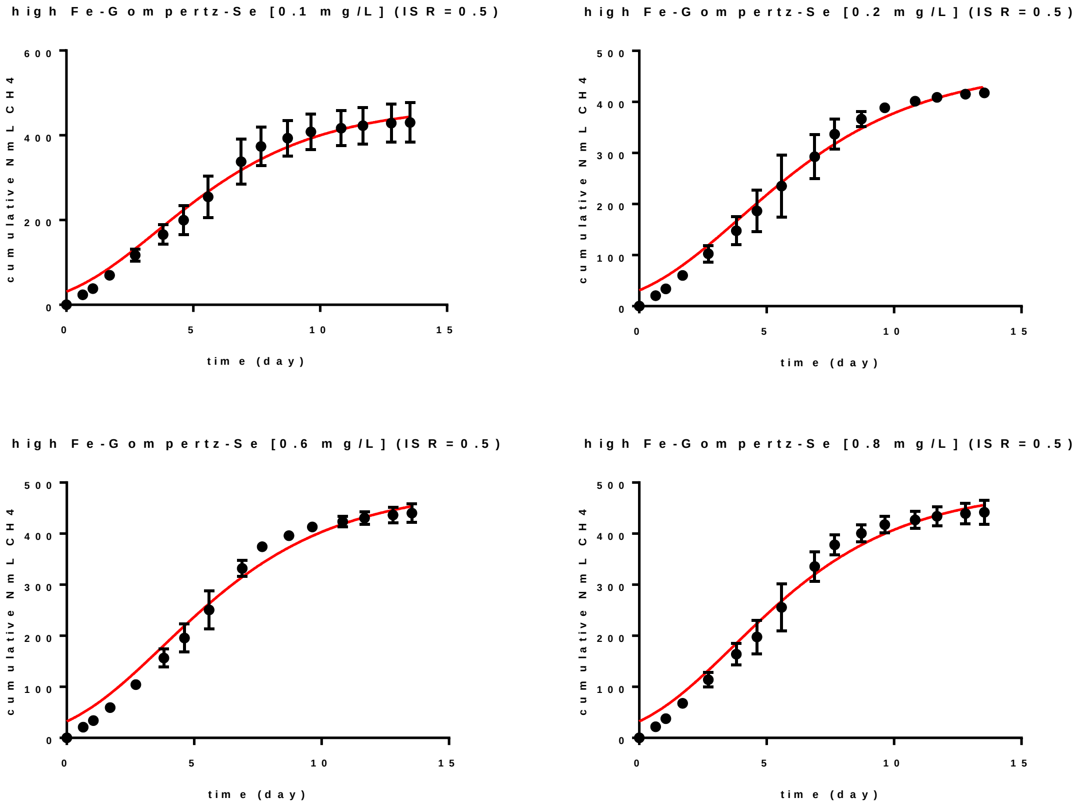


Figure A- 13 Gompertz modeling in the study with ADS-HFe- Se[0.1-0.8 mg/L] addition (ISR=0.5)

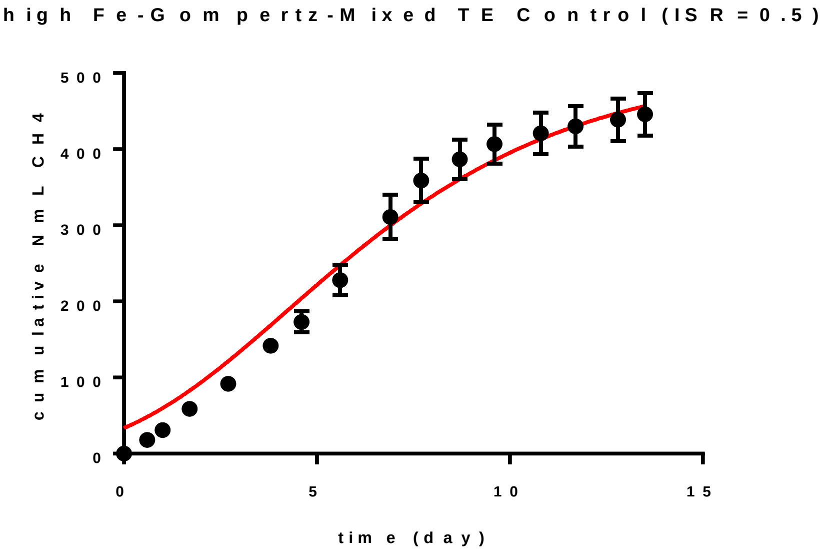


Figure A- 14 Gompertz modeling in the study with ADS-HFe- Mixed TE control (ISR=0.5)

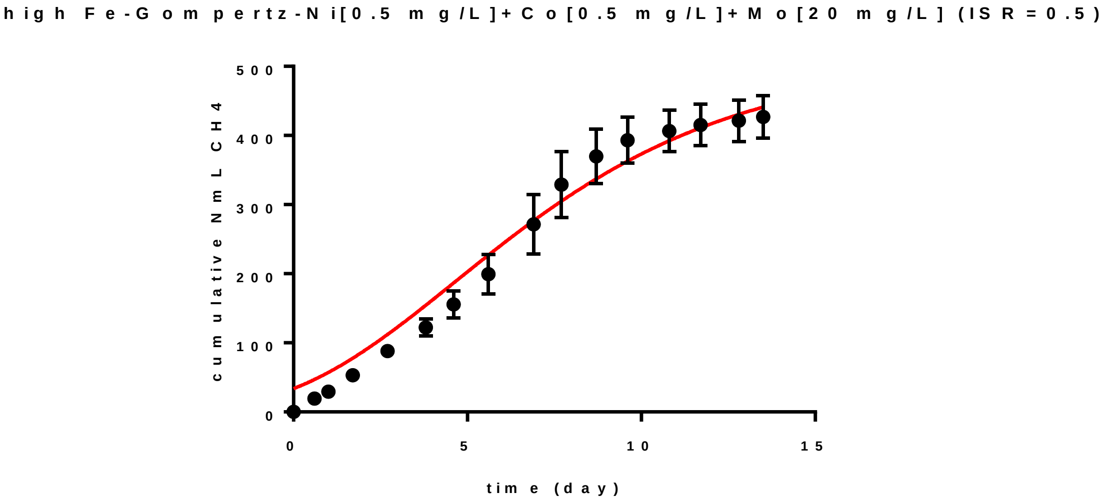
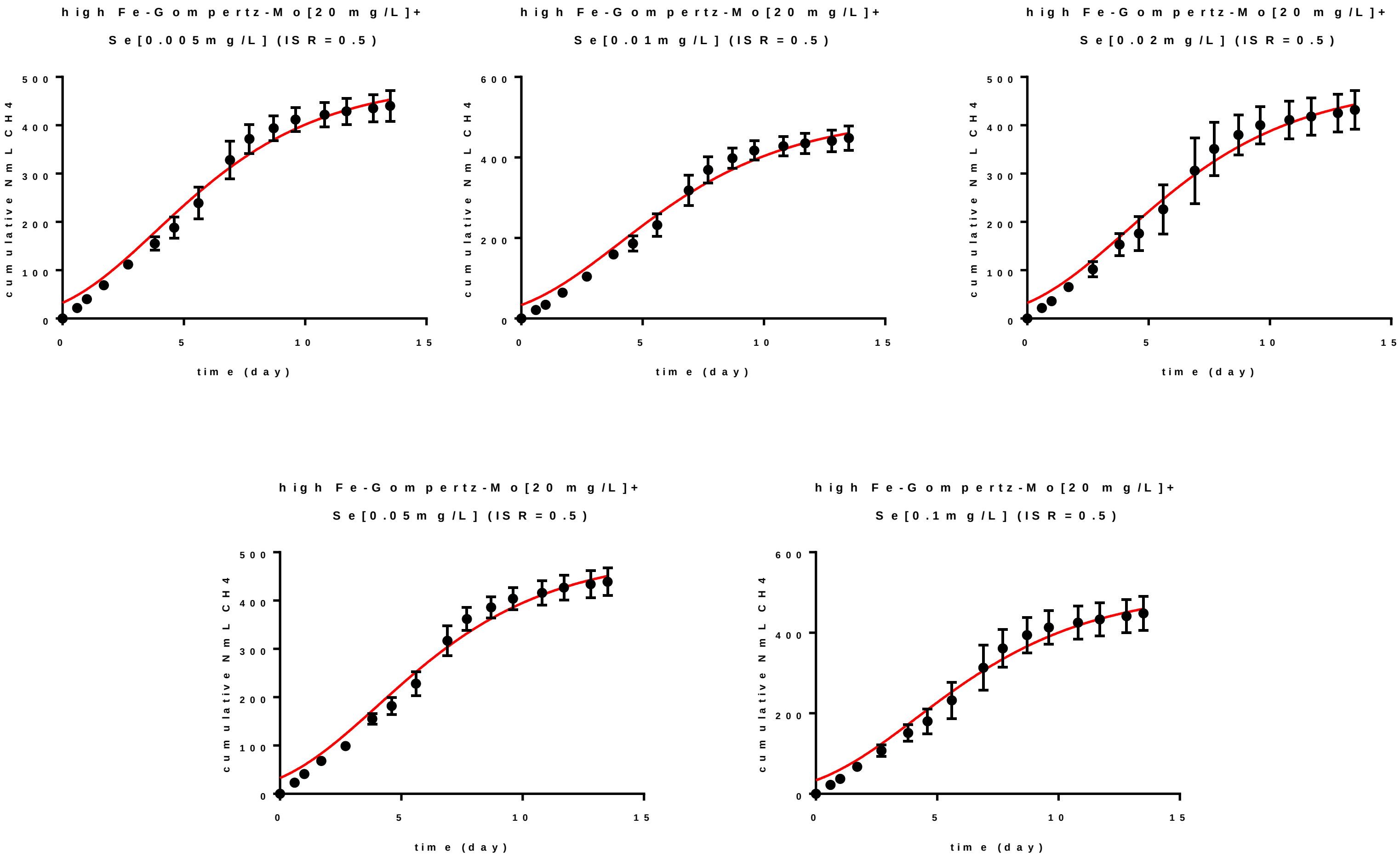


Figure A- 15 Gompertz modeling in the study with ADS-HFe- Ni[0.5mg/L]+Co[0.5mg/L]+Mo[2 mg/L] (ISR=0.5)


high Fe-Gompertz-Mo[20 mg/L]+ Se[0.005mg/L] (ISR=0.5)

high Fe-Gompertz-Mo[20 mg/L]+ Se[0.01mg/L] (ISR=0.5)

high Fe-Gompertz-Mo[20 mg/L]+ Se[0.02mg/L] (ISR=0.5)

high Fe-Gompertz-Mo[20 mg/L]+ Se[0.05mg/L] (ISR=0.5)

high Fe-Gompertz-Mo[20 mg/L]+ Se[0.1mg/L] (ISR=0.5)

Figure A- 16 Gompertz modeling in the study with ADS-HFe- Mo[20mg/L]+Se[0.005-0.1 mg/L] (ISR=0.5)

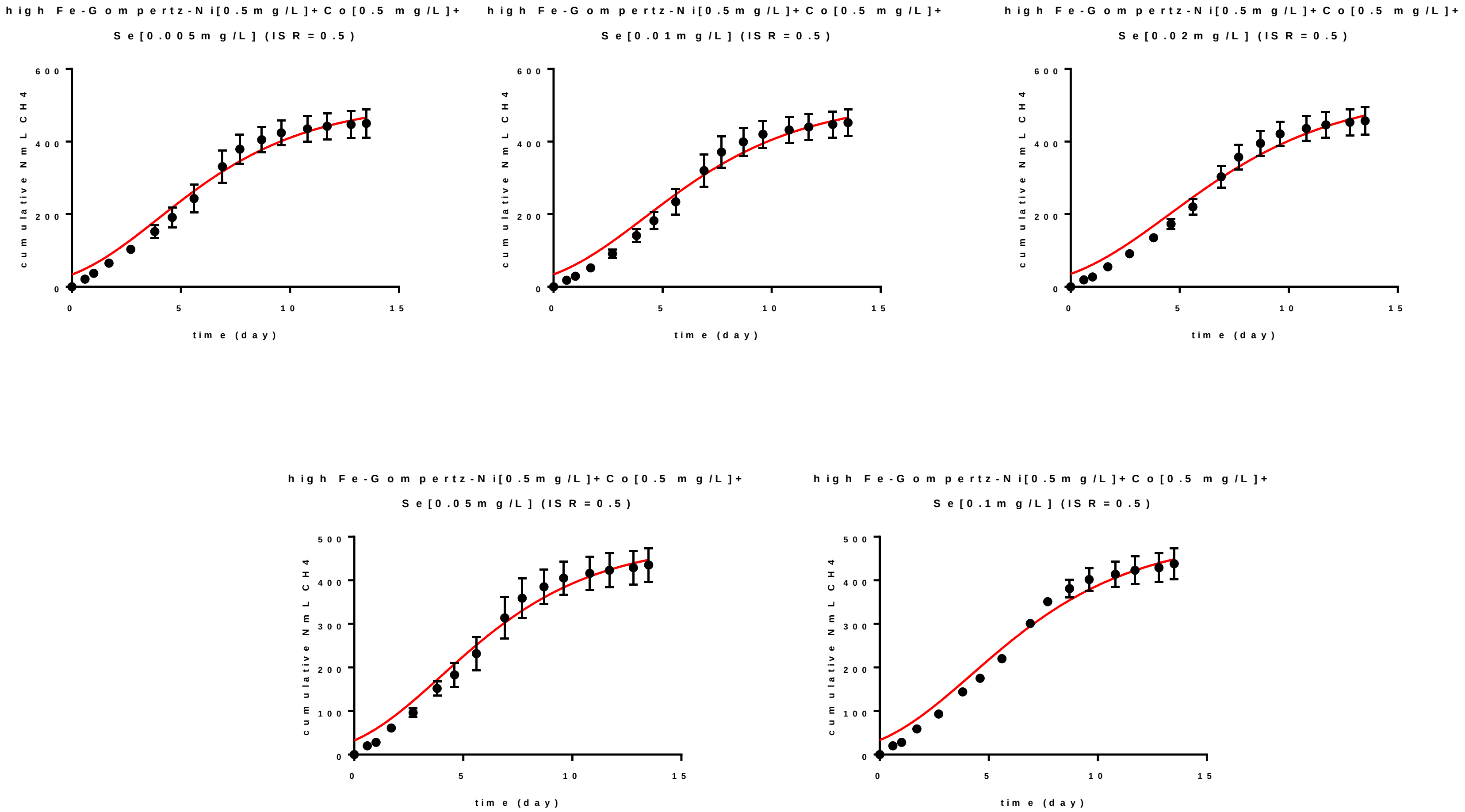


Figure A- 17 Gompertz modeling in the study with ADS-HFe- Ni[0.5mg/L]+Co[0.5mg/L]+Se[0.005-0.1mg/L] (ISR=0.5)

Appendix B. Supplementary material for Chapter 4

A sample curve fitting of cumulative methane production versus time with Gompertz model. The modified used model is $P = P_{max}\exp(-\exp\left(\frac{R_{max}}{P_{max}}(t_0 - t)e + 1\right))$ where:

P is the net cumulative methane production

P_{max} is the ultimate net cumulative methane production

R_{max} is the maximum methane production rate

t_0 is the lag phase

t is the time at which methane production was recorded.

Table B-1. shows the results of the methane production curve fitting with Gompertz model. Also, all figures which show the curve fitting with Gompertz model are presented in this section.

Table 0-1 methane production curve fitting for TE supplementation to batch reactors with FW and FWS-LFe.

TE Supplemented Reactor	Experimental Cumulative Methane (NmL)	P_{max} (NmL)	R_{max} (NmL/day)	Curve Fitting R^2
Single TE Supplementation Control	217.7	226.8	150.9	0.95
Ni [0.5 mg/L]	244.1	240.5	118.6	0.97
Ni [1 mg/L]	274.8	263.7	132.3	0.98
Ni [1.5 mg/L]	267.9	260.1	121.8	0.98
Ni [2 mg/L]	241.3	234.8	116.1	0.98
Co [0.1 mg/L]	262.8	243.5	85.9	0.95
Co [0.2 mg/L]	255.6	243.2	91.2	0.97
Co [0.4 mg/L]	252.5	239.9	99.2	0.97
Co [0.5 mg/L]	267.1	254.4	106.2	0.97
Mo [2 mg/L]	284.1	265.7	125.2	0.97
Mo [5 mg/L]	258.5	250.3	117.9	0.97

Mo [10 mg/L]	235.8	226.7	111.5	0.98
Mo [20 mg/L]	226.4	218.1	108.9	0.97
Se [0.1 mg/L]	280.5	262.23	101	0.95
Se [0.2 mg/L]	286.1	265.7	102.7	0.96
Se [0.6 mg/L]	279.4	260.2	98.1	0.97
Se [0.8 mg/L]	268.8	253.8	101	0.97
Fe [50 mg/L]	263.8	254.1	125.5	0.98
Fe [100 mg/L]	231.6	230.7	115.4	0.99
Fe [200 mg/L]	228.3	226.4	106.8	0.99
Fe [400 mg/L]	201.2	202.8	86.9	0.98
Mixed TE Supplementation Control	410.9	408.3	82.6	0.98
Ni[1mg/L]+Co[0.1 mg/L]+Se[0.1mg/L]	404.7	389.6	71.5	0.97
Ni[1mg/L]+Co[0.1 mg/L]+Se[0.2mg/L]	421.6	402	73.02	0.97
Ni[1mg/L]+Co[0.1 mg/L]+Se[0.6mg/L]	402.0	386.4	72.3	0.97
Ni[1mg/L]+Co[0.1 mg/L]+Se[0.8mg/L]	444.4	425.6	80.2	0.96
Ni[1mg/L]+Co[0.1mg/L]+Mo	455.7	441.1	86.2	0.97
Mo[2mg/L]+Se[0.1mg/L]	444.9	407.2	73.9	0.95
Mo[2mg/L]+Se[0.2mg/L]	432.1	422.4	86.4	0.98
Mo[2mg/L]+Se[0.6mg/L]	425.0	408.1	74.8	0.97
Mo[2mg/L]+Se[0.8mg/L]	466.3	445.9	83.2	0.97
Ni[1mg/L]+Co[0.1mg/L]	465.5	468.3	90.8	0.95
Ni[1mg/L]+Co[0.4mg/L]	474.8	459.6	86.9	0.98
Ni[1mg/L]+Co[0.5mg/L]	465.4	455.1	91.8	0.99

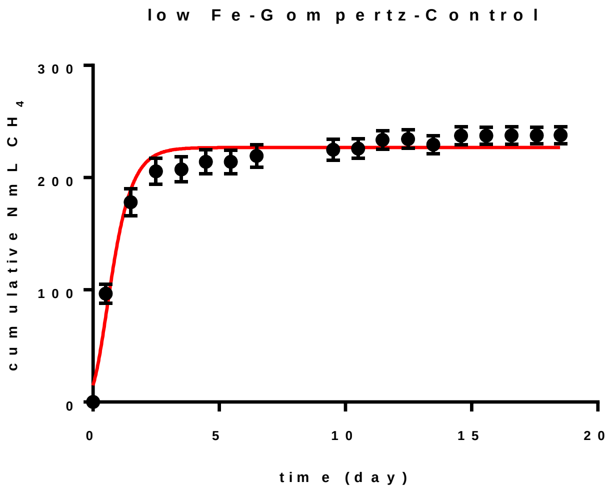


Figure B- 1 Gompertz modeling in the study with FWS-LFe - Single TE Control reactor

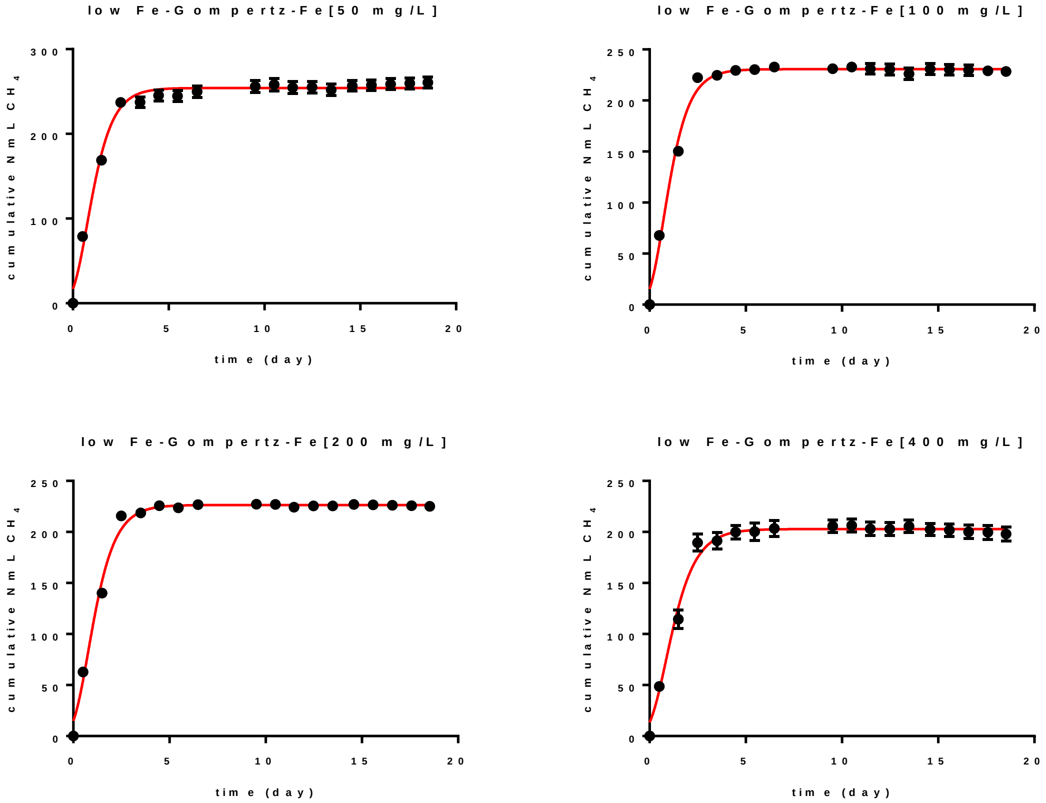


Figure B- 2 Gompertz modeling in the study with FWS-LFe - Fe [50-400 mg/L] addition

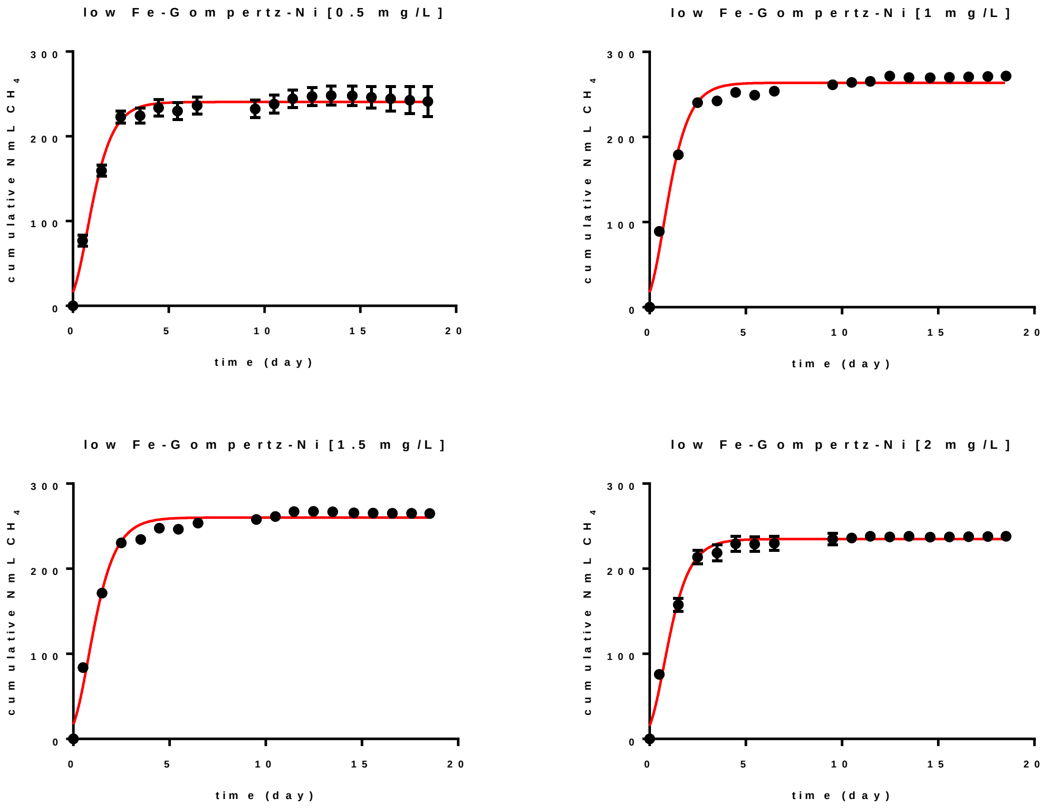


Figure B- 3 Gompertz modeling in the study with FWS-LFe - Ni [0.5-2 mg/L] addition

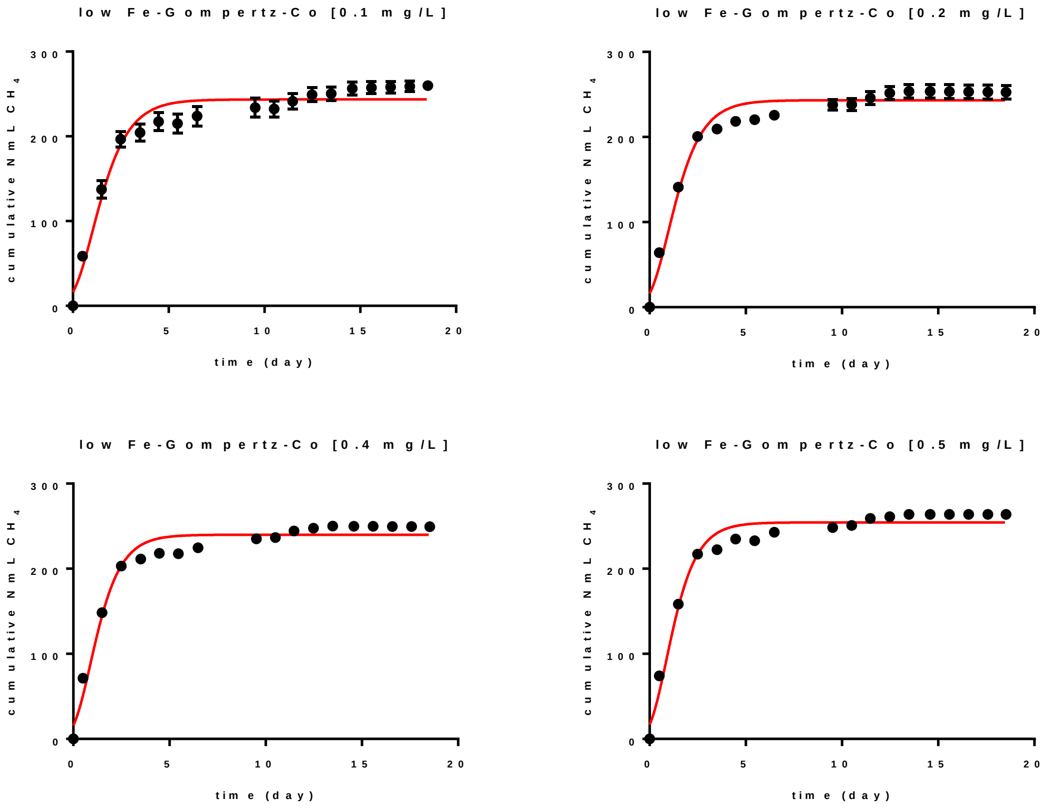


Figure B- 4 Gompertz modeling in the study with FWS-LFe - Co [0.1-0.5 mg/L] addition

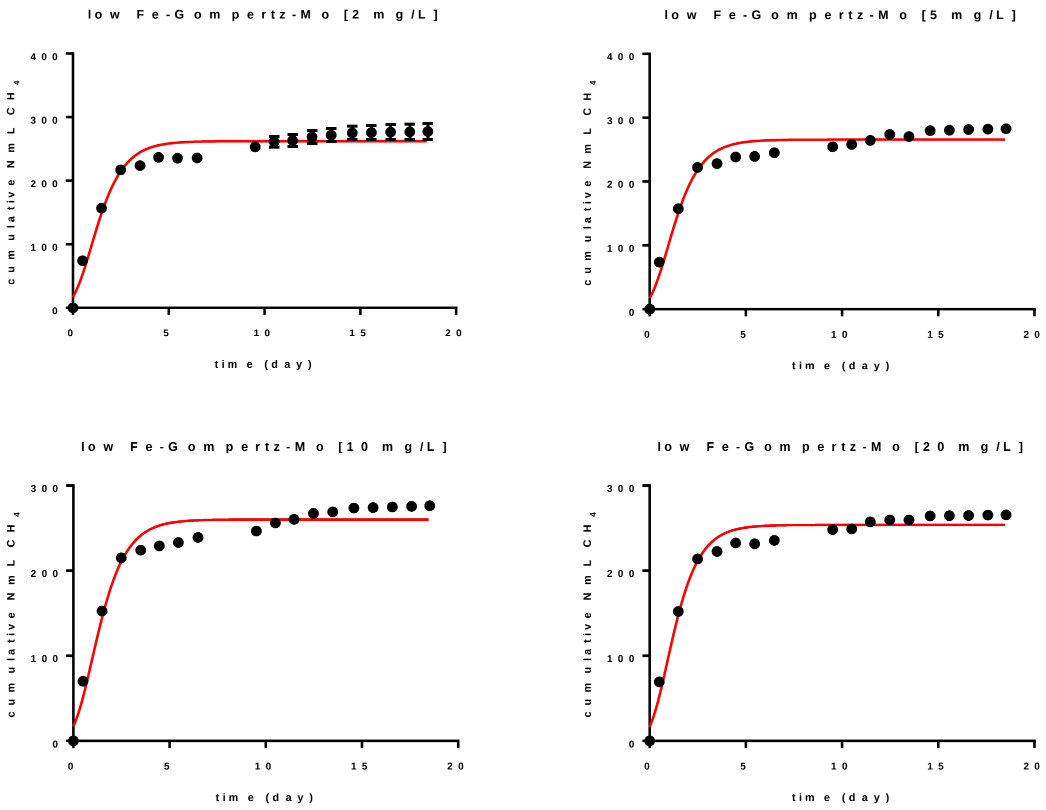


Figure B- 5 Gompertz modeling in the study with FWS-LFe - Mo [2-20mg/L] addition

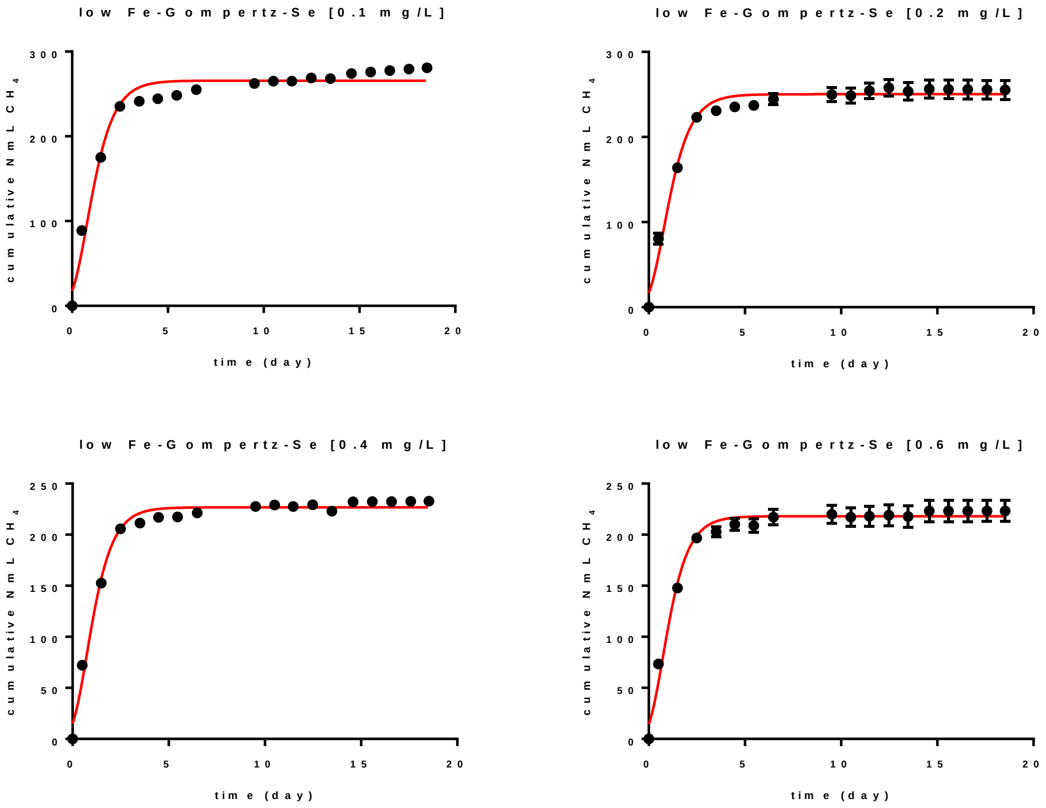


Figure B- 6 Gompertz modeling in the study with FWS-LFe - Se [0.1-0.8 mg/L] addition

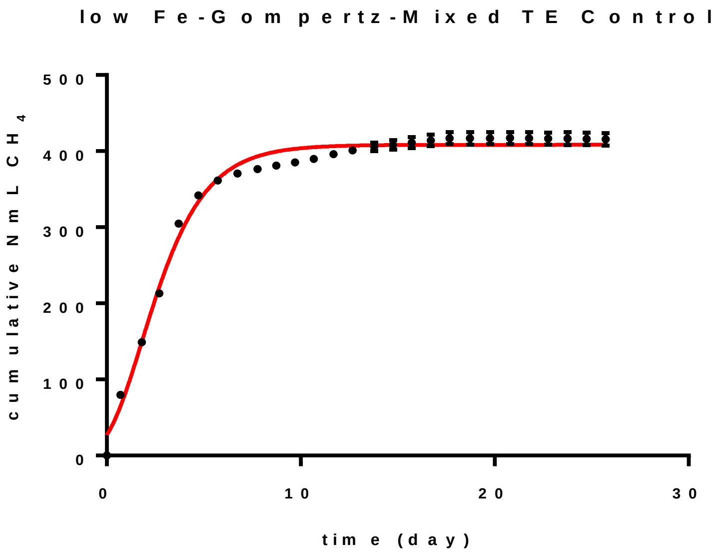
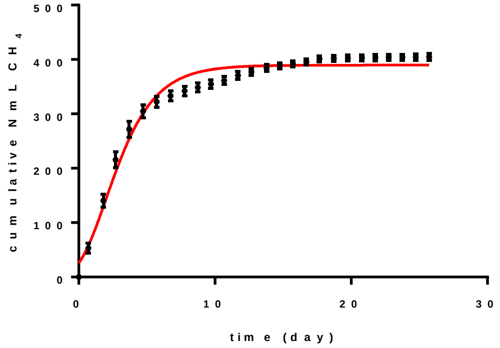
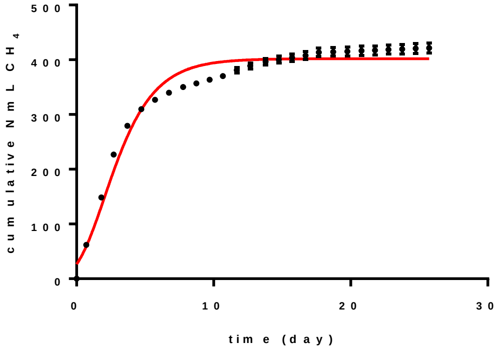


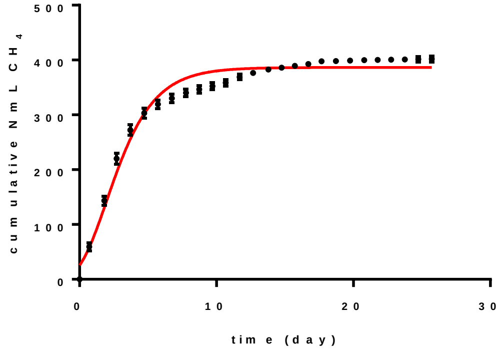
Figure B- 7 Gompertz modeling in the study with FWS-LFe - Mixed TE Control reactor low Fe-Gompertz-Ni[1mg/L]+Co[0.1 mg/L]+Se[0.1mg/L]



low Fe-Gompertz-Ni[1mg/L]+Co[0.1 mg/L]+Se[0.2mg/L]



low Fe-Gompertz-Ni[1mg/L]+Co[0.1 mg/L]+Se[0.6mg/L]



low Fe-Gompertz-Ni[1mg/L]+Co[0.1 mg/L]+Se[0.8mg/L]

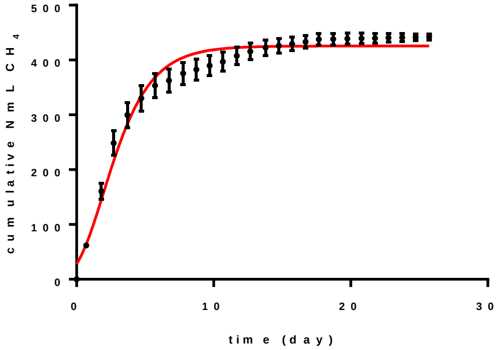


Figure B- 8 Gompertz modeling in the study with FWS-LFe - Ni[1 mg/L]+Co[0.1 mg/L]+Se [0.1-0.8 mg/L] addition

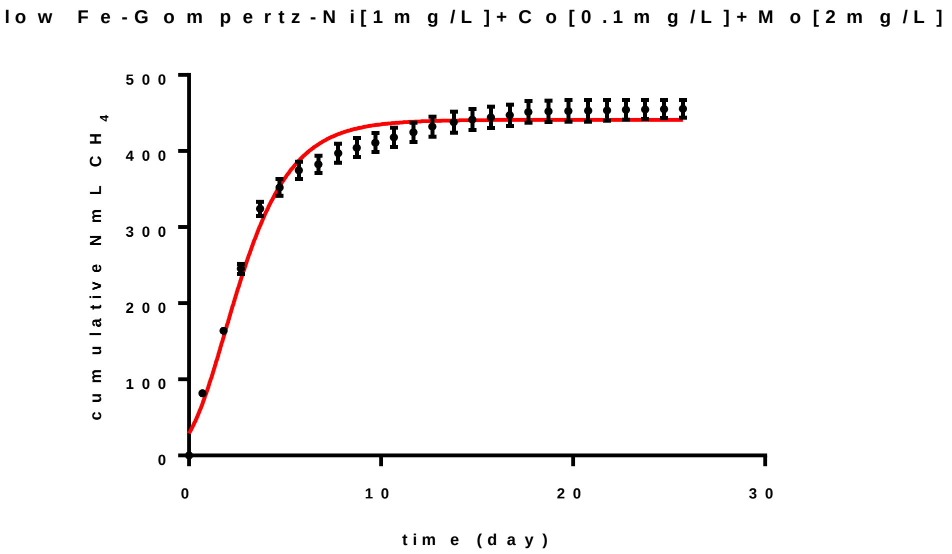


Figure B- 9 Gompertz modeling in the study with FWS-LFe - Ni[1 mg/L]+Co[0.1 mg/L]+Mo [2 mg/L] addition

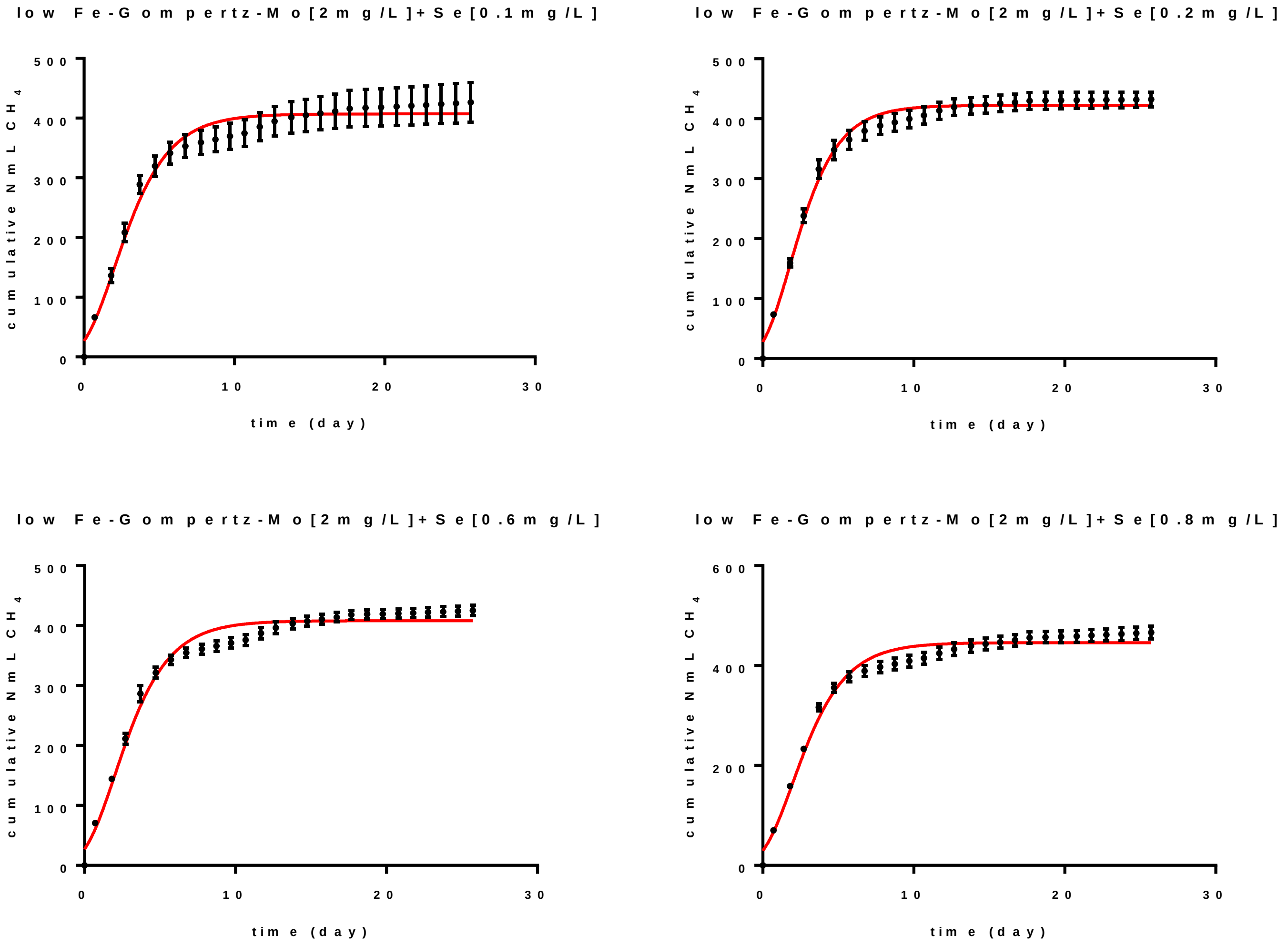


Figure B- 10 Gompertz modeling in the study with FWS-LFe - Mo[2 mg/L]+Se [0.1-0.8 mg/L] addition

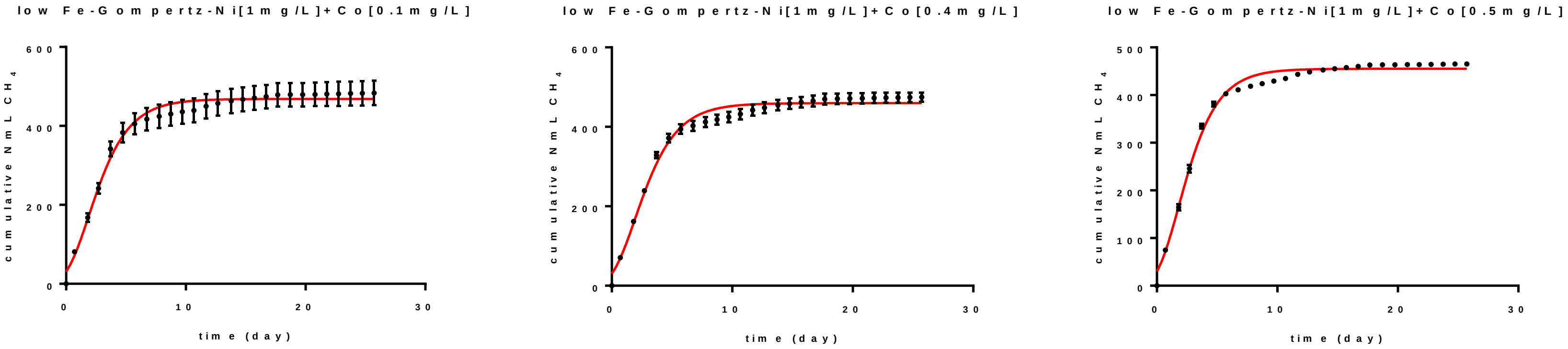


Figure B- 11 Gompertz modeling in the study with FWS-LFe - Ni[1 mg/L]+Co [0.1-0.5 mg/L] addition

Microbial community changes versus BMP results for different groups of bacteria and archaea

- *Firmicutes* did not correlate significantly with the BMP parameters.

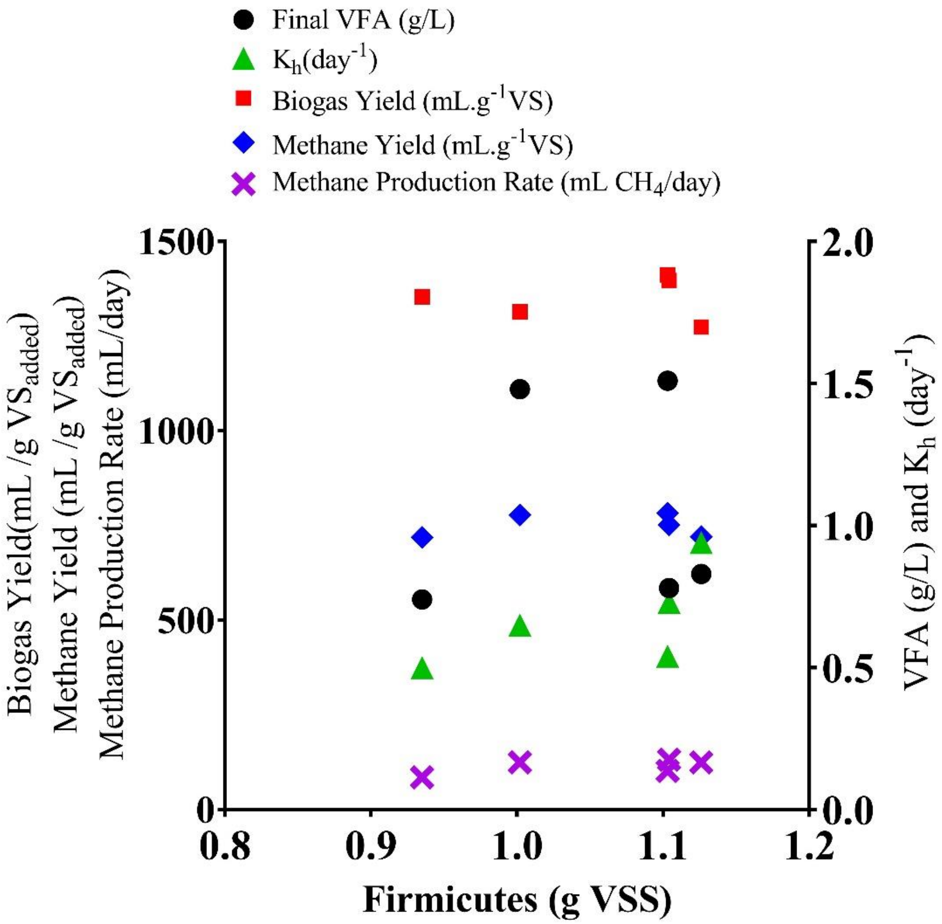


Figure B- 12 BMP results versus Firmicutes population

- Pearson correlation showed that biogas and methane yield have a moderately positive relationship with *Bacteroidetes*. This may indicate that the higher population of *Bacteroidetes* in all selected TE supplementations compared to the control has contributed to the higher soluble acids production for methanogens' consumption and hence, resulted in higher biogas and methane yields. Other studies (Sundberg et al. 2013; Vanwonterghem et al. 2014; Carballa et al. 2015) found Clostridia, a different family of *Firmicutes*, were abundant contributors to hydrolysis.

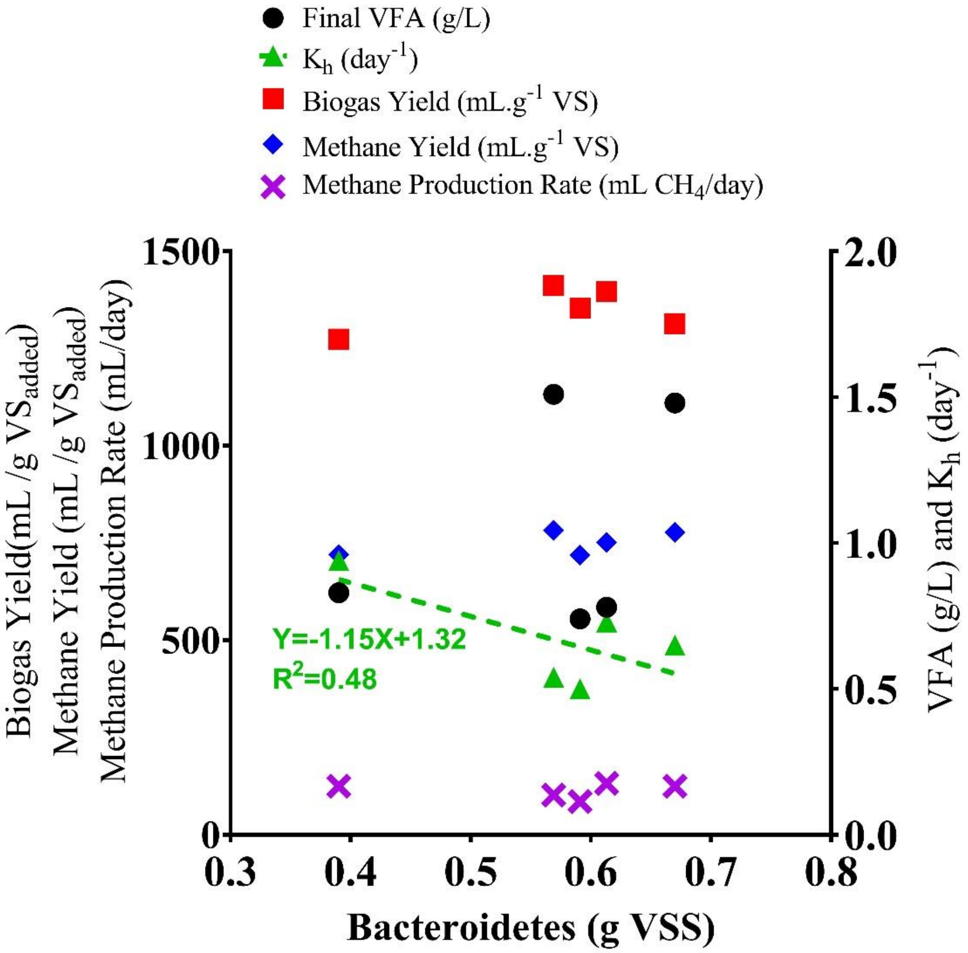


Figure B- 13 BMP results versus Bacteroidetes population

- Similar to *Firmicutes*, Clostridia did not correlate significantly with the BMP parameters.

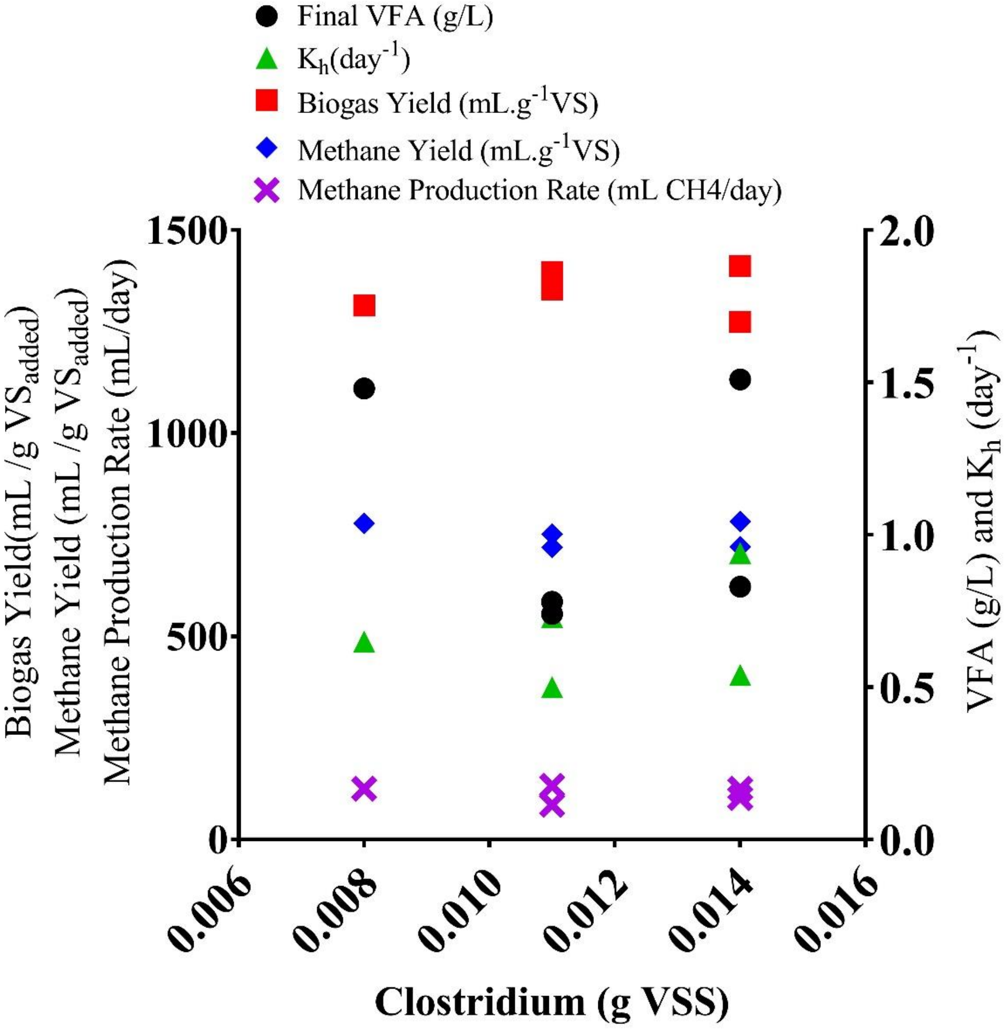


Figure B- 14 BMP results versus Clostridium population

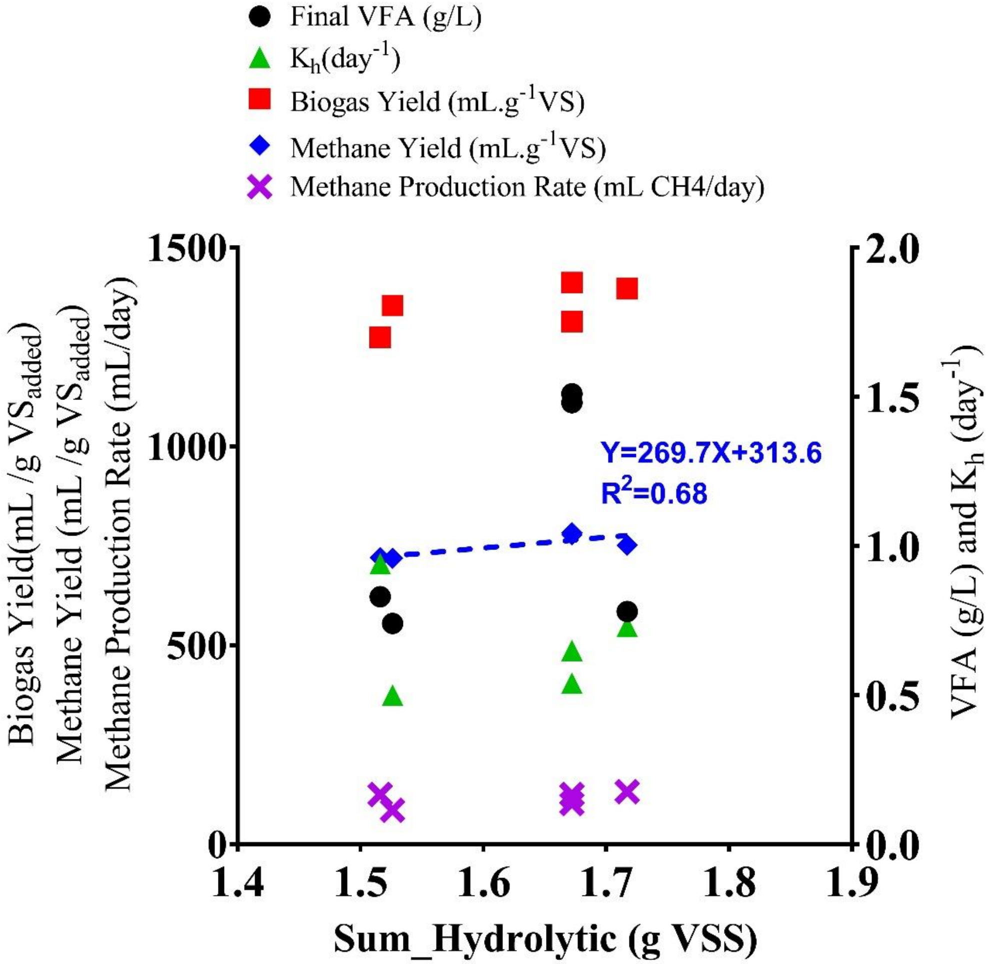


Figure B- 15 BMP results versus Hydrolytic population

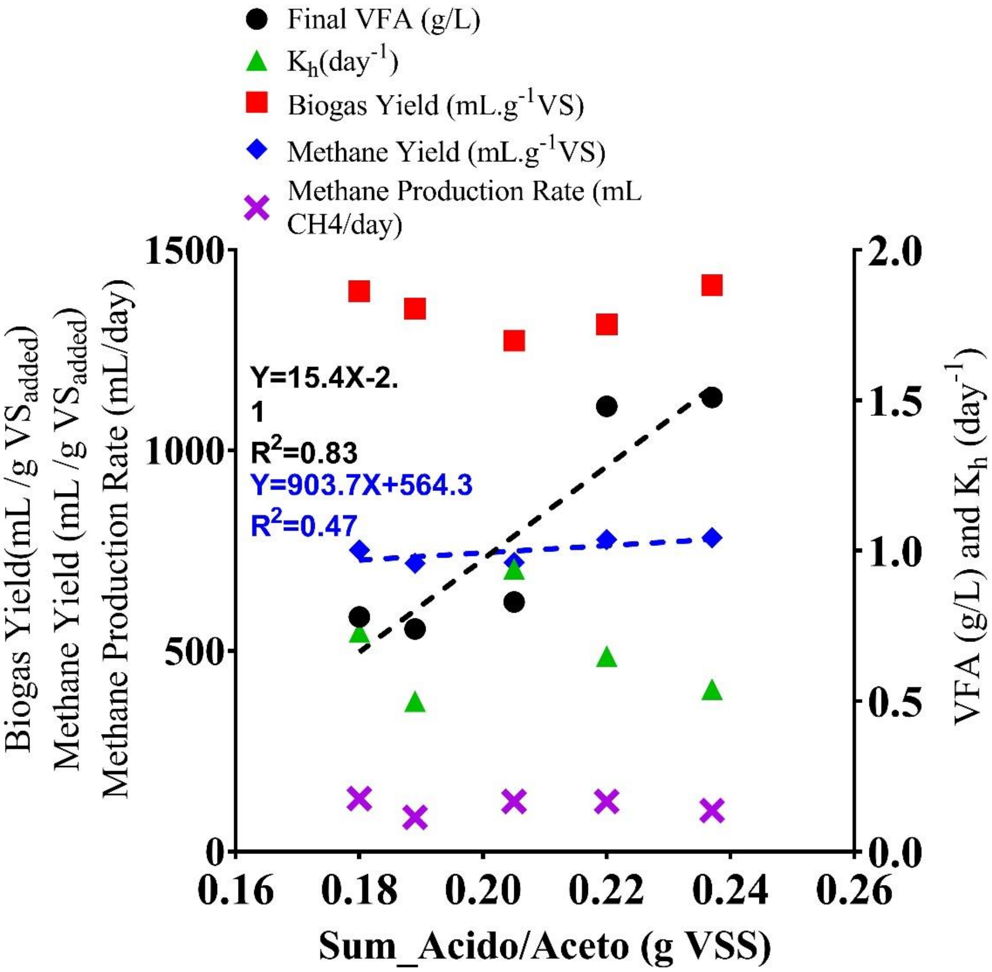


Figure B- 16 BMP results versus Acidogenic and Acetogenic population

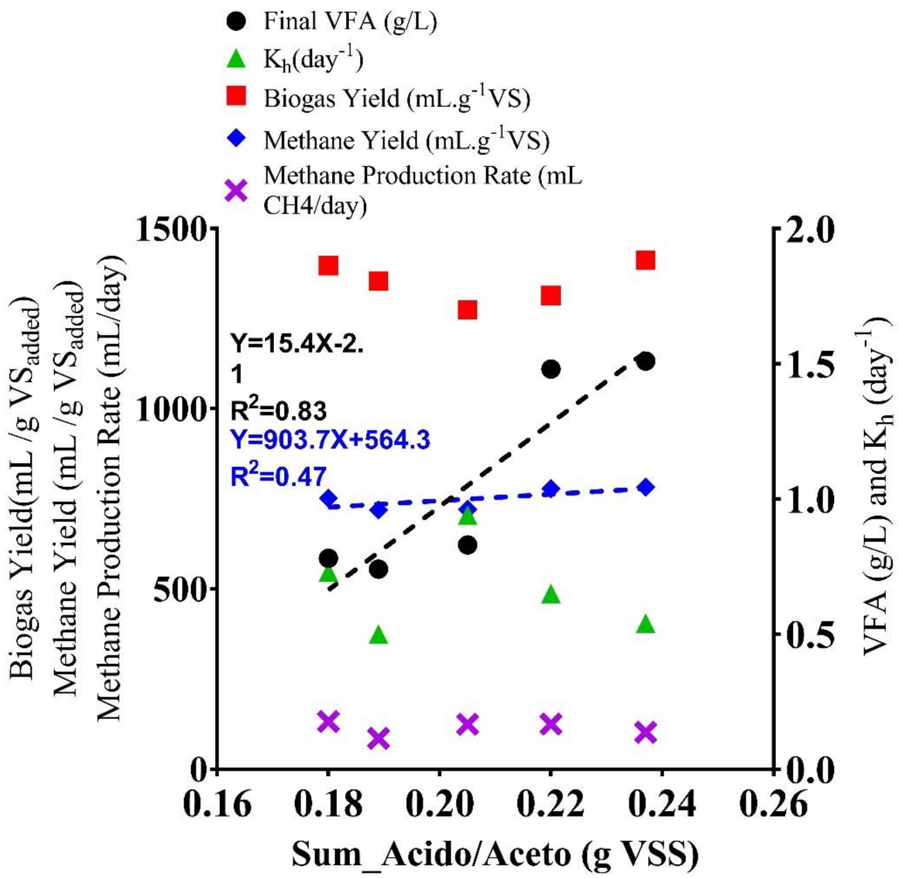


Figure B- 17 BMP results versus Methanosaeta populations

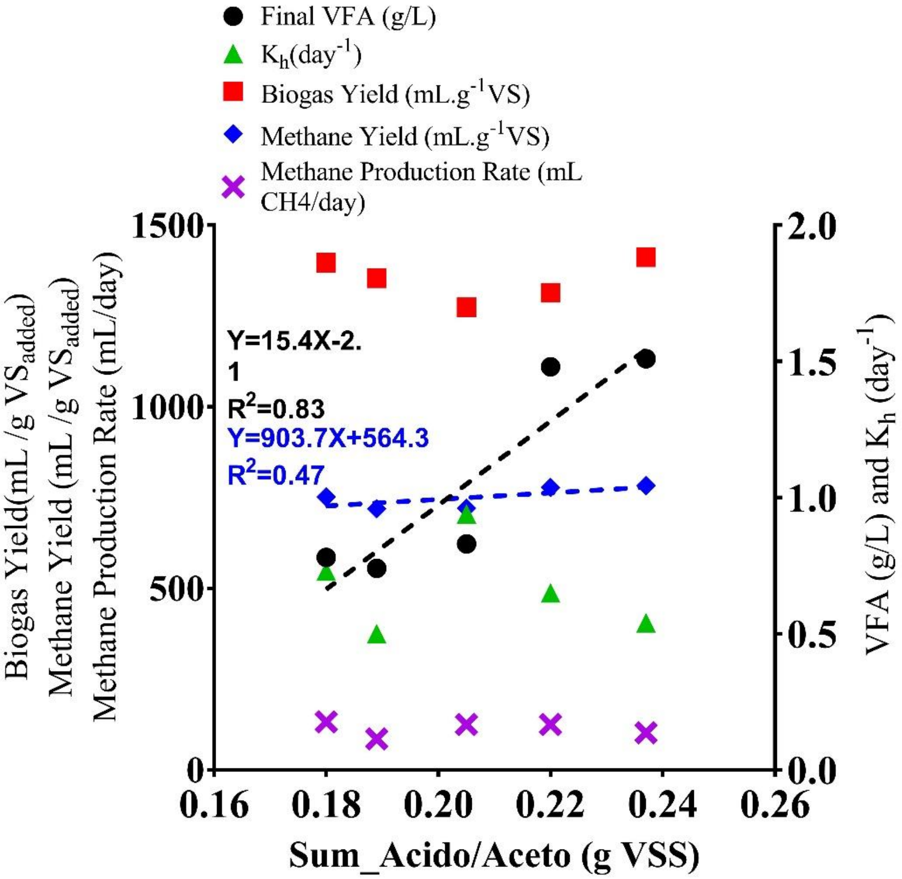


Figure B- 18 BMP results versus Methanobrevibacter populations

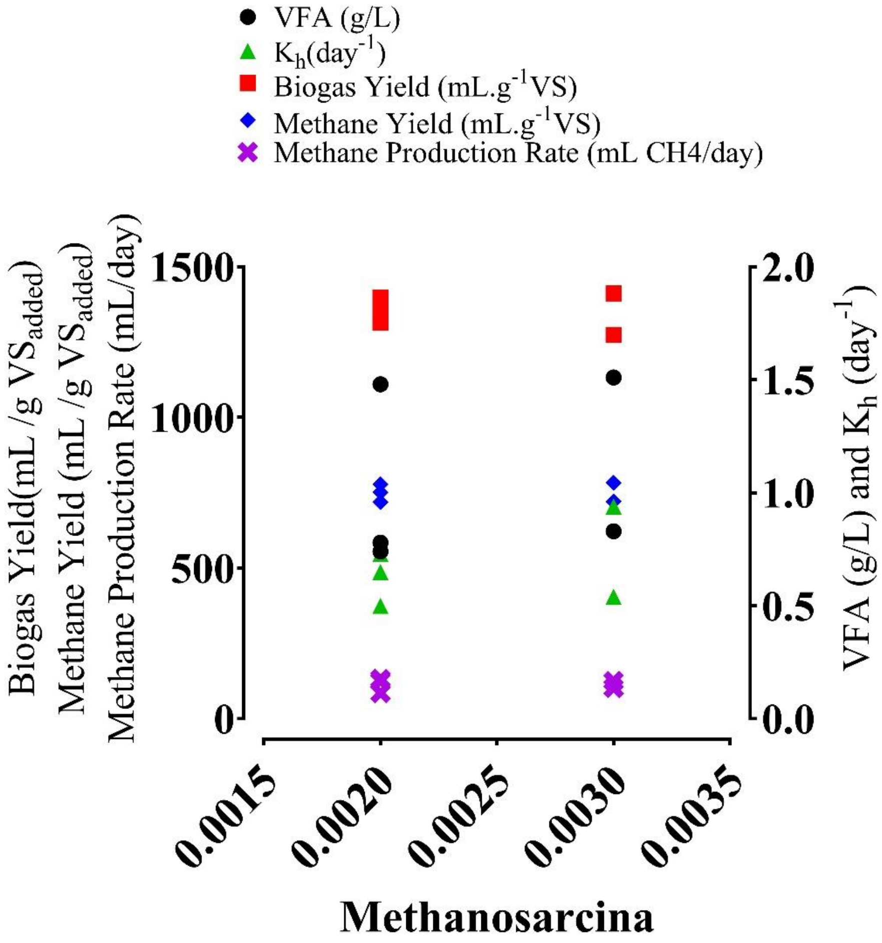


Figure B- 19 BMP results versus Methanosarcina populations

- *Methanobrevibacter*, *Methanosaeta*, and *Methanosarcina* make the acetoclastic methane producing population. *Methanosaeta* were observed to have a negative relationship with VFA concentration as expected. Surprisingly, *Methanobrevibacter* had a negative relationship with biogas yield. Furthermore, Methanosarcina had a slightly positive correlation with K_h values.

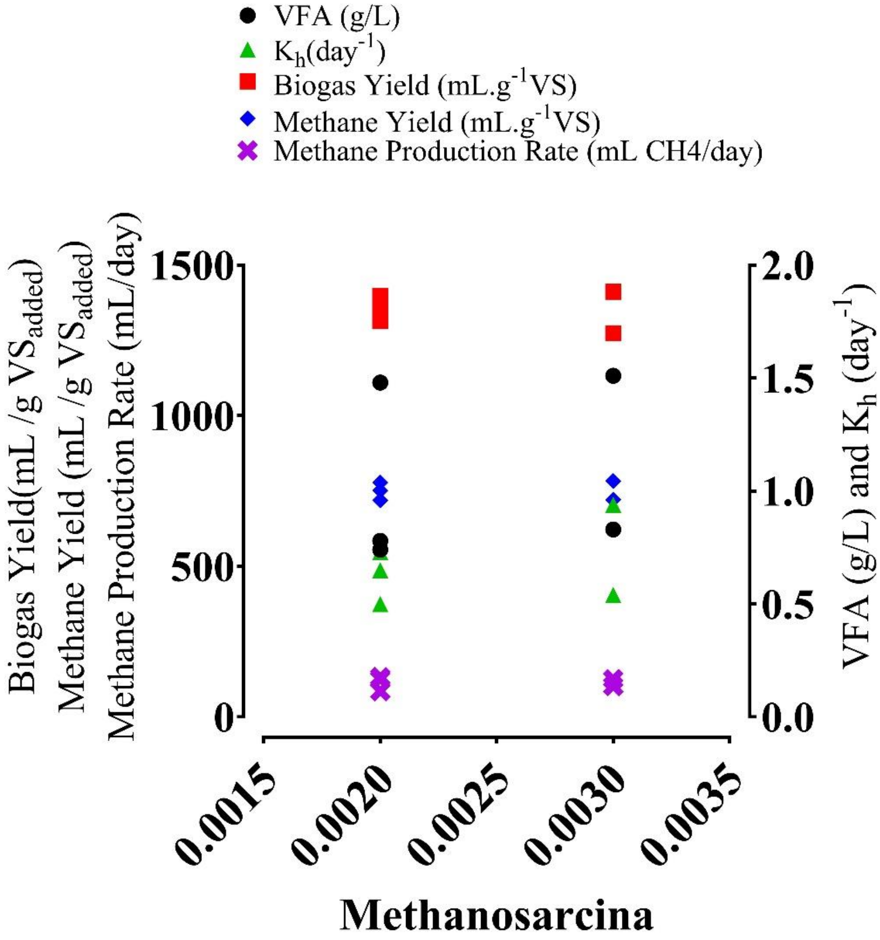


Figure B- 20 BMP results versus Methanosarcina populations

Methanospirillum and *Methanoculleus* are hydrogenotrophic, meaning that they get their energy from hydrogen produced by other microbes in the digester (Ling et al. 2017).

- Unexpectedly, *Methanospirillum* had a perfectly negative relationship with VFA concentration, and had a positive relationship with methane yield.

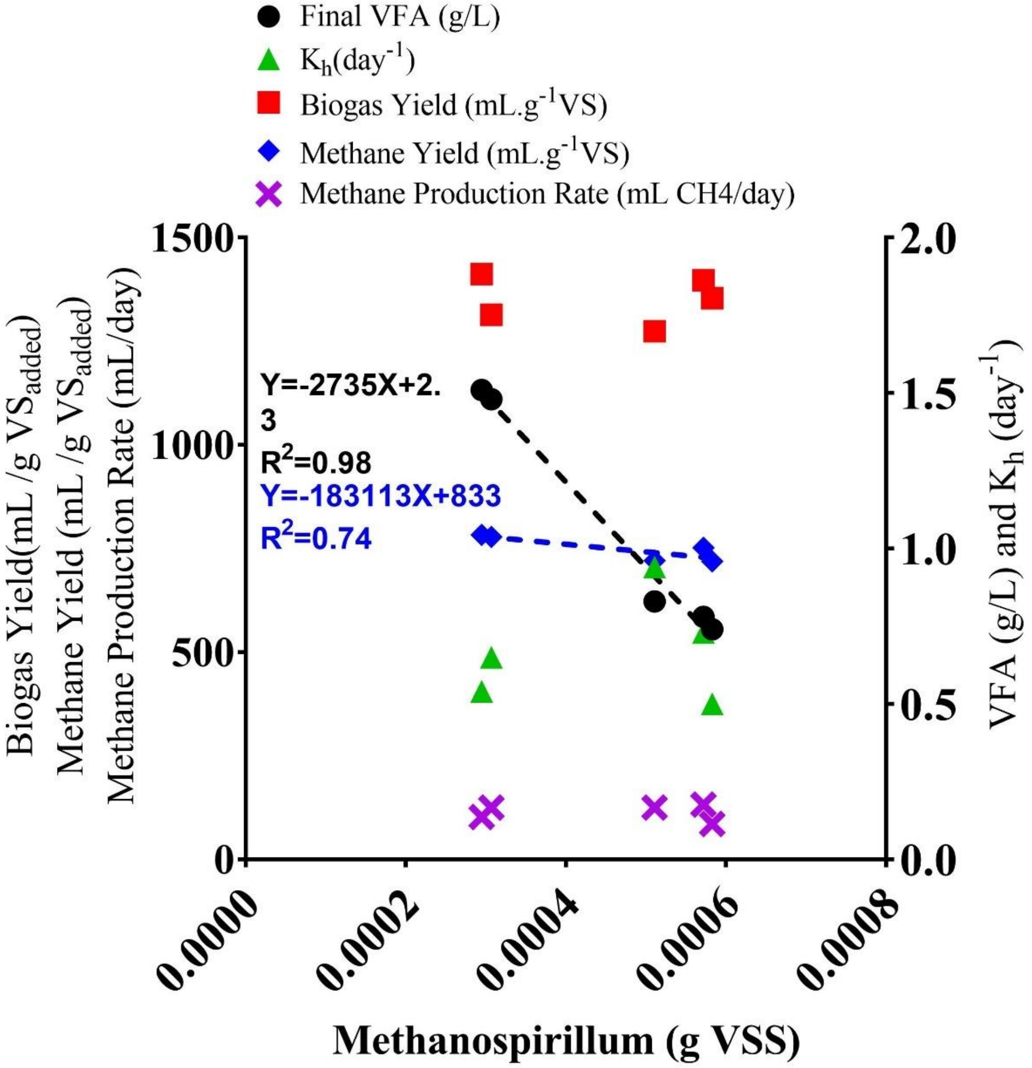


Figure B- 21 BMP results versus Methanospirillum population

- Moreover, methane yield, biogas yield, and VFA concentration decreased with increasing *Methanoculleus* population.

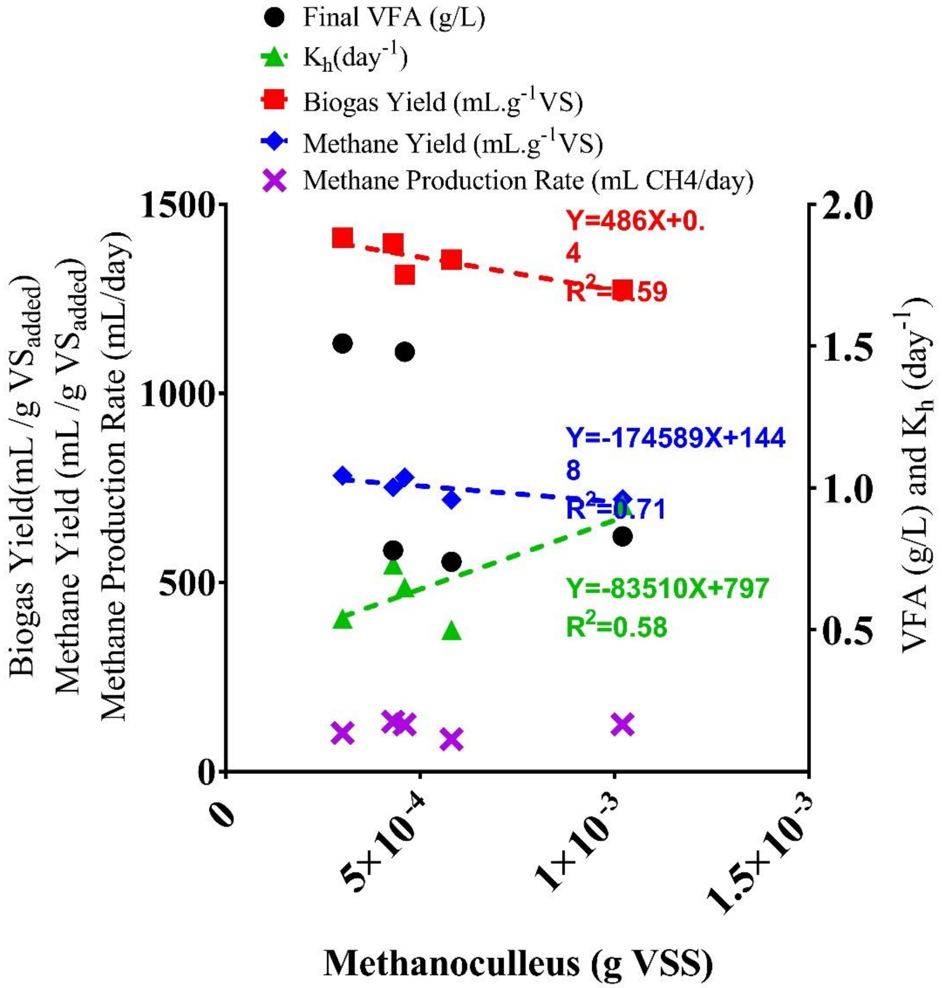


Figure B- 22 BMP results versus Methanoculleus population

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7th International Congress on Biofuels and Bioenergy, October 2-4, 2017 Toronto, Canada (oral presentation) Andisheh Yazdanpanah, Dara S.M. Ghasimi, Mingu Kim, George Nakhla, M. Keleman. Effect of Trace Metals Addition on Food Waste Anaerobic Digestion

Eurasia Waste Management Symposium, May 2-4, 2018 Istanbul, Turkey (oral presentation) Andisheh Yazdanpanah, Dara S.M. Ghasimi, Mingu Kim, George Nakhla, M. Keleman. Impact of Trace Metals Supplementation on Food Waste Anaerobic Digestion