

Effect of laboratory iron dosing on metal and phosphorus behaviour in anaerobic digesters

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ABSTRACT

Iron salts are used at wastewater treatment works to remove phosphorus for the final effluent as the excess of phosphorus in this effluent can result in eutrophication. The sludge rich in iron and phosphorus generated after the addition of iron must be stabilized before disposal usually by anaerobic digestion. This research investigated the effect of different iron salts at different ratios iron:phosphorus on anaerobic digestion of iron and phosphorus rich sludge by measuring biogas and methane production and the destruction of organics as well as the effect on phosphorus removal. Iron and phosphorus inorganic profiles were also studied of samples generated before and after digestion in order to establish any relationship between the content of iron and phosphorus in the bioavailable fraction and biogas and methane production. The approach used in this research was direct comparison of iron-dosed activated sludge and non iron-dosed activated sludge, using the iron-dosing laboratory method developed for Smith and Carliell-Marquet (2009).

Results from this research showed that iron has not always a detrimental effect on anaerobic digestion as biogas and methane production. From the same amount of volatile solid fed and similar destruction between iron-dosed activated sludge and non iron-dosed activated sludge, iron dosed as ferric sulphate at molar ratio iron:phosphorus 0.6:1 generated around 9% more biogas and 6% more methane than non iron-dosed activated sludge and iron dosed as ferrous sulphate at molar ratio iron:phosphorus 1.2:1 generated around 9% more biogas and 7% more methane than non iron-dosed activated sludge. However when iron was dosed as ferric sulphate at molar ratio iron:phosphorus 1.2:1 produce approximately the same biogas and 11% less methane than non iron-dosed activated sludge. The phosphorus removal efficiency was greater in all the experiments within the range 91.5-99.69%.

No relationship between bioavailable iron and phosphorus and biogas or methane production was found. Although concentrations closer of 75 mg/l of bioavailable phosphorus should be further studied as a threshold of unstable digestion or biogas reduction as the greater production of biogas and methane was generated for samples closer to this limit.

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“The whole point of getting things done is knowing what to leave undone.”

Lady Reading

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LIST OF SYMBOLS AND ABBREVIATIONS

~	Approximately
>	Greater than
≤	Minor or equal than
%	Percentage
AA	Atomic absorption
AAS	Atomic absorption spectrometry
Abs	Absorbance
AD	Anaerobic Digestion
Al	Aluminium
Al ₂ (SO ₄) ₃	Alum
AlPO ₄	Aluminium phosphate
am	Before noon
APHA	American public health association
AS	Activated Sludge
atm	Atmosphere(s)
BOD	Biochemical oxygen demand
BOD:N:P	Biochemical oxygen demand to nitrogen and phosphorus ratio
BPR	Biological phosphorus removal
BTU	<i>British Thermal Unit</i>
Ca	Calcium
CaCO ₃	Calcium Carbonate

cc/conc	Concentrate
CH ₄	Methane
CHP	Combined heat and power
CO ₂	Carbon dioxide
COD	Chemical oxygen demand
C:P	Carbon to phosphorus ratio
CPR	Chemical phosphorus removal
°C	Degree(s) Celsius
Defra	Department for environmental food and rural affairs
Dig	Digested sludge
DO	Dissolved oxygen
DW	Distilled water
EC	European community
EDTA	Ethylenediaminetetraacetic
e.g.	For example
<i>et al.</i>	And others
Fe	Iron
Fe(II)	Ferrous iron
Fe(III)	Ferric iron
Fe:P	Iron to phosphorus ratio
FeCl ₃	Ferric chloride
Fe:OH	Iron to hydroxyde ratio
FePO ₄	Iron phosphate
FeSO ₄	Ferrous sulphate
Fe ₂ (SO ₄) ₃	Ferric sulphate

ft ³	Cubic feet
g	Gram(s)
GC	Gas chromatograph
g/l	Grams per litre
h	hour
H ₂	Hydrogen
HCl	Hydrochloric acid
HCO ₃	Bicarbonate
HNO ₃	Nitric acid
H ₂ O	Water
H ₂ SO ₄	Sulphuric acid
IA	Intermediate alkalinity
KF	Potassium fluoride
Kg	Kilogram(s)
KH ₂ PO ₄	Potassium dihydrogen phosphate
KNO ₃	Potassium nitrate
l	Litre
LCFA	Long chain fatty acids
M	Molar concentration
m ³	Cubic meter(s)
min	Minute(s)
Mg	Magnesium
mg	Milligram(s)
mg/l	Milligrams per litre
µg/l	Micrograms per litre

ml	Millilitre(s)
µm	Micrometre(s)
Mn	Molecular mass
MO	Micro-organism(s)
N	Nitrogen
N	Normal concentration (as a concentration unit)
Na ₂ EDTA/ Na-EDTA	Di-sodium ethylenediaminetetraacetic dyhydrate
NaOH	Sodium hydroxide
NH ₃	Ammonia
NH ₄ ⁺	Ammonium ions
(NH ₄)Mo ₇ O ₂₄ ·4H ₂ O	Ammonium molybdate
NH ₄ VO ₃	Ammonium metavanadate
nm	Nanometre(s)
Na ₃ PO ₄ ·12H ₂ O	Tri-sodium phosphate
Na ₄ P ₂ O ₇	Tetra-sodium pyrophosphate
OH	Hydroxide
Ortho-P	Orthophosphate
P	Phosphorus
PA	Partial alkalinity
p.e.	Population equivalent
pH	Hydrogen ion concentration
Pm	After noon
ppm	Parts per million
RAS	Return activated sludge
ROW	Reverse osmosis water

rpm	Revolutions per minute
RR	Ripley's ratio
S	Sulphur
SE	Sequential extraction
SD	Standard deviation
SEM	Scanning electron microscopy
SetS	Settle sewage
TA	Total alkalinity
TS	Total solids
TOC	Total organic carbon
UK	United kingdom
UPW	Ultra pure water
UWWTD	Urban wastewater treatment directive
VFA	Volatile fatty acids
VS	Volatile solids
WW	Wastewater
WWT	Wastewater treatment
WWTW	Wastewater treatment work

CHAPTER 1

INTRODUCTION

Phosphorus (P) is an essential element for the growth of plants and as such is known as a nutrient. Excess quantities of P, together with an excess of main plant nutrient, nitrogen, can result in eutrophication. Eutrophication is defined by Pierzynsky *et al.* (2000) as “an increase in the fertility status of natural waters that causes accelerated growth of algae or water plants”. Iron (Fe) salts can be used to remove phosphorus from wastewater, generating a sludge rich in P and Fe. P removal processes have increased in Europe due to the establishment of the Urban Wastewater Treatment Directive (UWWTD).

Anaerobic digestion (AD) process is widely used in UK to stabilise sludge before disposal. During this process biogas is generated. This biogas is made up of around 70% methane and 30% carbon dioxide, which can be used to obtain energy. Sludge generated when Fe is used in wastewater treatment (WWT) plants to remove P seems to generate less biogas than normal sludge. These above issues are presented within the following sections of Chapter 1.

1.1 Phosphorus removal in wastewater treatment

P in sewage comes from human, domestic and industrial waste. Average of P concentration in the wastewater (WW) in the UK, range from 5-20 mg/l as total P, of which 1-5 mg/l is the organic fraction and the rest inorganic (Gray, 2004).

Conventional biological wastewater treatment (WWT) does not remove enough P to meet the level stipulated in the UWWTD. Therefore, the application of advanced WWT techniques is required to reduce P discharges. The technique used to remove P from the WW will depend on some factors relevant to the operation of the WWT plant. This technique can be biological or chemical.

Biological P removal (BPR) processes require the establishment of a population of micro-organisms (MOs) which have the ability to store poly-phosphate in excess of their normal metabolic requirements (Forster, 2003). Thus, accumulation of P in the biomass is achieved, which can then be removed with the waste sludge stream.

Chemical P removal (CPR) is usually preferable to BPR because is easy to control the process and does not require a specific amount of carbon in the treated WW (Bolzonella *et al.*, 2004), which is not common in municipal WW in the UK. In addition to this, CPR is easy to retrofit and high efficiencies, around 80-90% can be achieved. The CPR is typically undertaken with the addition of Fe or aluminium (Al) salts or lime that forms a precipitate of sparingly soluble phosphates. This precipitate is removed as sludge. As a consequence of CPR, the volume of sludge increases between 37-97% and its chemical composition and physical sludge characteristics changes (Yeoman *et al.*, 1988). Fe salts are more widely used than Al salts.

P removal is further discussed in section 2.2 of the literature review.

1.2 The Urban Wastewater Treatment Directive

The UWWTD sets the limits on the total P concentration in effluents which are discharged into “sensitive areas”¹. Typical limits are 2 mg/l P and 15 mg/l N for 10,000-100,000 population equivalent² (p.e.) works and 1 mg/l P and 10 mg/l N for > 100,000 p.e. works. The UWWTD defines “eutrophic-sensitive” waters as waters which are eutrophic or may become eutrophic unless protective action is taken. Guideline are set, which the Environment Agency must follow to categorise water as sensitive, in the UK.

As a result, there were 234 areas designated as eutrophic in the UK in 2008 (Defra, 2008), therefore additional treatment to reduce the level of P in discharges is necessary, such as nutrient removal. The use of ferric salts help to achieve the discharge levels, but also increase the energy usage³ and the raw material consumption, and the release of Fe in the effluent has an environmental impact on the receiving water body (Defra, 2009).

¹ Sensitive areas: “an area designated under the Directive according to three criteria: (a) waters that are, or have the potential to become, eutrophic if no protective action is taken. (b) drinking water sources that contain or could contain more than 50mg/l of nitrate if no protective action is taken. (c) waters in need of protective action to meet the requirements of other Directives. Waste water discharges over 10,000 p.e. that pollute Sensitive Areas need treatment that relates to the designation criterion or criteria” (Defra, 2002) .

² Population equivalent: “The unit of measure used to describe the size of a waste water discharge. 1 population equivalent is the biodegradable load (matter) in waste water having a 5-day biochemical oxygen demand (BOD) of 60g of oxygen per day. Population equivalent doesn’t necessarily reflect the actual population of a community” (Defra, 2002)..

³ This increase is due to the incorporation of raw material to the process; an increase in the solids content in the basin produces an increase in the velocity of the agitation system hence higher energy consume.

1.3 Effect of Iron rich sludge in Anaerobic Digestion

Over the years a number of different substances have been used as precipitants, the most common chemical are alum, ferric chloride, ferric sulphate, ferrous sulphate (copperas) or lime. These chemical have been used to improve precipitation of P or suspended solid removal (Gossett *et al.*, 1978). When Fe is added to the process, there is more than one reaction that takes place, and therefore the relationship between the metal salt required and P in solution is not stoichiometric⁴ (Fytianos *et al.*, 1998; Jenkins *et al.*, 1971). Fe can be added as Fe(II) or Fe(III). Thus if Fe is added as Fe(II) it is necessary to aerated the wastewater so that Fe(II) will be oxidised to Fe(III). Not all Fe will be converted to Fe(III), although conversion of 77% after 30 min at high pH level (7.5-8.0) where reported for Thistleton *et al.* 2001. The formation of Fe(III)-phosphates is preferable to the formation of Fe(II)-phosphates because the Fe(II)-phosphates forms flocs that settle poorly (Nielson, 1996) and the limits cannot be achieved if solids and high P content are discharge in the final effluent.

The effect of CPR on AD has been widely discussed over the years. There are some authors who report no effect of CPR on AD (Ghyoot and Verstraete, 1997; Grigoropoulos *et al.*, 1971); whilst other claim CPR adversely affects AD (Dentel and Gosset, 1982; Gosset *et al.*, 1978; Johnson *et al.*, 2003; Smith and Carliell-Marquet, 2008; Yeoman *et al.*, 1990). A reduction in the biogas production as a result of the AD of Fe-dosed sludge was the most common adverse effect reported for the researchers cited above. This decrease results in energy lost, as biogas is a renewable energy source. This decrease was not related to a toxicity produced for Fe (Dentel and Gosset, 1982; Gosset *et al.*, 1978), but it was related to the organic material in the WW, particularly these with high protein or lipid content (Dentel and Gosset, 1982; Gosset *et al.*, 1978). A critical review of previous work within this area is presented in section 2.3 of the literature review.

Fe is considered one of the essential micronutrients for AD. Particularly, methanogens has a high Fe requirement. Its role is diverse on AD; it can perform as an enzyme, a terminal electron acceptor or oxidation/reduction agent. Not all Fe can be used for MOs, only Fe considered bioavailable. The use of the inorganic fractionations methods allow to know the bioavailable fraction of Fe and P before and after digestion. In addition, when Fe is reduced to Fe(II) under anaerobic conditions, it reacts with the ammonia formed during the anaerobic fermentation (Ivanov *et al.*, 2002). Therefore the addition of Fe

⁴ The relationship between reactants and products in a balanced chemical reaction. In the case of the reaction between Fe and P this ratio is 1:1.

into the process has two beneficial effects, control the ammonia toxicity and as nutrient for MOs, as well as P precipitation.

To summarise there have been many researches about how Fe affects AD, but it is difficult to compare these results to reach any definite conclusions about whether CPR is really detrimental to biogas output at a WWT work (WWTW). This is because all the studies use different sludges from different locations and do not consistently report all sludge parameters and experimental conditions (e.g. VS feed or ratio Fe:P dosed). Another experimental factor that introduces more variability is the lack of consistency in dosing systems. In some papers the method of laboratory dosing is simply not reported (Dentel and Gossett, 1982; Yeoman *et al.*, 1990); or commonly the Fe is slug dosed into the sludge (Grigoropoulos *et al.*, 1971) and the implications for the resulting inorganic speciation have not been considered. The studies that use full-scale Fe-dosed sludges do not suffer from the dosing method problems, but are not able to directly compare a non Fe-dosed sludge with a Fe-dosed sludge as these sludges are usually obtained from different sites, introducing a host of additional site-specific variables (Ghyoot and Verstraete, 1997). There is a novel method to determine biogas potential of Fe-dosed activated sludge (Smith and Carliell-Marquet, 2009). This method was designed in order to simulate a full-scale activated sludge (AS) simultaneous precipitation dosing-system considering various factors (see below) which were not considered by others authors, while eliminating the disadvantages of doing the experiments with full-scale Fe-dosed sludge:

- Fe addition: instead of slug dosing Fe into the aeration tank, Fe was dosed little by little continuously. It permits to control pH and dissolved oxygen concentration in the aeration tank. In addition, this method considers various factors as Fe oxidation/reduction, P availability, time of reaction and food availability. This approach simulates simultaneous precipitation of P through Fe-dosing into an aeration tank of a full-scale AS plant.
- P and Fe accumulation: in a full-scale WWTW there is a return stream of AS, which would be rich in P and Fe if the plant is using CPR. This stream returns Fe and P to the aeration basin, and the accumulation of Fe and P depends on the molar ratio Fe:P used in the plant and the solids retention time of the AS. The typical concentration of P for full-scale Fe-dosed AS is 40 mg/gTS (Oikonomidis, 2007) based on AS plants with solids retention time of approximately 10 days. This is the calculation base.
- Microorganisms (MOs) feed: in a full-scale WWTW settled sewage would be continuously feeding the aeration tank, hence supplying food for the MOs. Any Fe dosed into the tank would react with an actively respiring

microbial population, which might change the inorganic speciation. For this reason, the dosing system in this research also has an organic input in the form of settled sewage.

This method is applied to two samples of the same AS, the difference between both samples at the end of the process is that one of them have been simulated with the addition of Fe and P, and the other one was dosed with ultrapure water (UPW) in order to conserve the same working volume. Both samples undergo the same process, which should, theoretically, allow direct comparison between the samples which has been Fe-dosed and the one has not been Fe-dosed.

The effect of Fe rich sludge on AD is further discussed in section 2.3 of the literature review.

1.4 Knowledge gap

Since Fe salts are commonly used to remove P from WW, there have been several previous investigations on CPR and the influence of these salts on AD. When these previous investigations are reviewed, it is difficult to compared the results, as different parameters are selected to report the effect of Fe on AD, and the conditions imposed for the experiments are also different (some authors compare results obtained using different VS feed or sludge from different WWTW where they are introducing other factors, as sludge age, composition, etc.). Moreover, most of the results previously reported for Fe salts are related to the use of FeCl_3 added to the pre-precipitation position, without specified Fe:P dosing ratios (e.g. Gosset *et al.*, 1978; Johnson *et al.*, 2003; Smith and Carliell-Marquet, 2008; Yeoman *et al.*, 1990). Nowadays, dosing FeSO_4 to the co-precipitation position is the most common practice in the full-scale WWTW which use CPR (Forster, 2003; Smith, 2006).

Smith (2006) developed and used a novel method to simulated the AS process. The inorganic profiles (Fe and P) obtained for the AS dosed using this method was similar to real Fe-dosed sludge collected for four WWT plants (Smith and Carliell-Marquet, 2009). This method allows obtaining the direct effect on AD produced for the addition of Fe salts.

This research investigates the effect of Fe on AD using different Fe salts which are used in full-scale plants and adding these salts to the AS in a ratio Fe:P lower than the common used for this plants due to the recent environmental impact found for the release of Fe in the effluent. (Defra, 2010).

In addition of the comparison of biogas volume and composition, inorganic profiles of sludge will be investigated. This fractionation of the inorganic material

of the sludge gives information about the distribution of Fe and P in Fe-dosed and non Fe-dosed sludge before and after digestion. The bioavailable fraction is the most important fraction in both sequential fractionation procedures as it gives information about how available Fe and P are for MOs. Will it be beneficial for AD as well as productive to remove P from the WW using a low ratio Fe:P?.

It is hypothesised that the use of Fe salts at Fe:P ratios under the stoichiometric (0.6:1) or briefly higher (1.2:1) will remove P in order to accomplish the levels stipulates from the UWWTD and may enhance AD (biogas production).

These ratios were chosen after reviewing literature about P removal and effect of Fe on AD. Thistleton found 83% P removal using FeCl_2 at ratio Fe:P 0.6:1 Jenkis *et al.*, also found great percentage of P removal using 0.7:1 Fe:P ratio for FeSO_4 addition where P “was removed by a mechanism other than precipitation” (Jenkis *et al.*, 1970). Other reason for choosing this ratios was that in most of the researchers where the impair effect was reported, the Fe:P ratio used was 2:1 or higher.

1.5 Aim and Objectives

The main aim and objectives relate to this aim are presented within this section.

1.5.1 Aim

The principal aim of this research is to establish whether Fe-dosed AS produces less biogas than non Fe-dosed AS when subjected to batch AD tests. The Fe-dosed AS sludge is generated in a laboratory Fe-dosing system using three different Fe salts dosed at two different ratios of Fe:P.

1.5.2 Objectives

The objectives of this research which relate to the Aim are:

Objective 1 - to use a laboratory Fe-dosing unit to produce Fe-dosed AS that is comparable to non Fe-dosed AS in all aspects other than changes produced by Fe-dosing.

Objective 2 – To use different Fe salts (FeCl_3 , $\text{Fe}_2(\text{SO}_4)_3$, FeSO_4) to produce Fe-dosed AS in a laboratory dosing unit and to use these

sludges to compare the methane produced from batch AD tests, with that obtained from AD of non Fe-dosed sludge.

Objective 3 – To use different dosing ratios of Fe:P to produce Fe-dosed AS in a laboratory dosing unit and to use these sludges to compare the methane produced from batch AD tests, with that obtained from AD of non Fe-dosed sludge.

Objective 4 – To use sequential extraction methods to compare the inorganic fractionation profiles of laboratory-generated Fe-dosed and non Fe-dosed AS, prior to, and after, batch digestion tests.

1.6 Thesis layout

Following the Introduction in Chapter 1 is a Literature Review (Chapter 2), where principles of anaerobic digestion, chemical phosphorus removal, the effect of coagulant on anaerobic digestion and the inorganic fractionation techniques are critically reviewed. Chapter 3 provides a summary of materials, experimental methods and procedures used in this research. The results are presented, interpreted and discussed in Chapter 4, and finally Conclusions and Recommendations are outline in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

Within this chapter is introduced a brief resume about anaerobic digestion (section 2.1). In section 2.2 some fundamental aspects of phosphorus removal and some parameters which affect this removal are introduced. The impact of coagulant on anaerobic digestion is reviewed (section 2.3). Finally, the methods used for metal and phosphorus fractionation are introduced and reviewed (section 2.4).

2.1 Principles of Anaerobic Digestion

Sludge is generated during primary and secondary wastewater treatment (WWT). This sludge had to be treated before can be disposed. The wide stabilization treatment used is anaerobic digestion (Arnaiz *et al.*, 2006). This stabilization has some advantages over aerobic digestion, such as do not need to be aerated hence the energy requirements are lower and produce a reduction in the volume of sludge. In addition to that AD produce a gas called biogas made approximately 70% methane (CH_4) and 30% carbon dioxide (CO_2) (Foster, 2003).

The biochemical reactions that occur during AD are divided into four processes: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The last three are biological processes, whereas the hydrolysis stage is enzymatic. Each process results in intermediary products which are further broken down in the following stages. Figure 2.1 shows the stages of the AD process. In the final stages of the degradation process, there is a co-dependence and competition among three kinds of bacteria, acetate forming, sulphate reducing and methanogens. Acetate forming grows in a symbiotic relationship with methanogens and sulphate reducing bacteria (SRB) because it can only survive under very low concentrations of hydrogen. SRB and methanogens compete for the same substrate, acetate and hydrogen available. It is crucial that the concentration of hydrogen are low enough to avoid inhibition or intoxication of the acetate forming bacteria, and high enough to feed the methanogens sufficiently to allow energy conservation and growth (Schink and Friedrich, 1994).

positive effect of Fe on methanogenic fermentation is that in the presence of Fe(II) ions, SRB lose their competitiveness due to the activity of FeRB (Ivanov *et al.*, 2002).

2.1.2 Importance of bioavailability of Phosphorus and Iron on Anaerobic Digestion

The concentration of soluble metals or phosphate is usually considered to be indicative of the bioavailable fraction. Bioavailability is a complex and evolving concept (Bacon *et al.*, 2008), but has recently been defined as the “degree to which chemicals present in the soil may be absorbed or metabolized by a human or ecological receptor or are available for interaction with biological systems” (Bacon and Davidson, 2008).

Carliell-Marquet (2001) stated that the speciation between the soluble and solid phases is a dynamic phenomenon dependant on the waste treated, the concentration of individual metals and competition between them for adsorption and complexation sites. In addition, Callander and Barford (2006) concluded that the bioavailability of non alkali metals in digesters is “influence by precipitation by sulphides, carbonates and sometimes phosphates, complexing as organic chelates and ion pairs, and possibly by rates of formation of precipitates and soluble complexes”.

Fe is the main essential micronutrient meanwhile P is the most important macronutrient on AD (Metcalf and Eddy, 2003). Nutrient limitation, especially trace metals, would result in a decreased rate of methane formation from acetate. An analysis of the elemental composition of methanogenic bacteria shows that the methanogens have a requirement for: Mg=Ca>Fe>Zn>Ni>Co=Mo>Cu>Mn (Carliell-Marquet, 2001). All methanogens appear to require nickel (Ni), cobalt (Co), and Fe for growth (Zandvoort, 2006; Zhang *et al.*, 2003). Due to the highest concentration of Fe in the cell biomass of methanogens, Fe is considered one of the most important essential metals for AD; this importance is related to its redox property and its requirement for some enzymes such as Fe-sulphur proteins (Carliell-Marquet, 2001).

Peffer and White (1964) found that Fe supplementation at low concentrations (200 and 400 mg/l) stimulated the digestion through the increase in Volatile Fatty Acids (VFA) degradation and methane production. Horban and van den Berg (1979) also reported an increase in the methane production with the addition of Fe between 290 and 580 mg/l. However, Peffer and White (1964) found that higher concentrations of Fe (600 and 800 mg/l) depressed the soluble phosphate limiting MOs activity. On the other hand, high concentration

of soluble phosphate (75 mg/l) results in significant increase of VFA and consequently an unstable system (Peffer and White, 1964). Moreover, low concentration of soluble phosphate is necessary for an efficient AD, thereby a fine balance exists between the optimum and inhibitive concentration of Fe and P. Horban and van den Berg (1979) found the optimum soluble Fe concentration between 11 and 111 mg/l.

2.2 Phosphorus removal

2.2.1 Introduction

Development for P removal started in the 1950s in response to the issue of eutrophication and the need to reduce the levels of P entering surface waters (Morse *et al.*, 1997).

The needs for P removal processes have increased in Europe due to the establishment of the Urban Wastewater Directive. This was proposed by the European Community (EC) in 1991. The Directive set discharge limits for some of the established sanitary determinants, e.g. biological oxygen demand (BOD) and suspended solids, as well as the nutrients, for rivers and designated “sensitive areas” (House of Lords, 1991; EC, 1992). Typical limits are 2 mg/l P and 15 mg/l N for 10,000-100,000 p.e. works and 1 mg/l P and 10 mg/l N for >100,000 p.e. works.

Phosphate can be removed from WW chemically or biologically. Traditionally the removal of P has been achieved by the addition of coagulants to the WW at same particular point during the treatment process. However recent advances in our understanding of how P is taken up by micro-organisms (MOs) has led to the development of biological removal processes, although such systems are not as yet widely used (Metcalf and Eddy, 2003).

2.2.2 Chemical Phosphorus removal

P can be precipitated out of solution by the addition of coagulant. To remove P the coagulants need to be added at the correct dosage rate, at some stage during the treatment cycle, and subsequently removed by sedimentation. Chemical precipitation primarily removes the orthophosphates, the other forms of P being more difficult to remove.

Three coagulants are used, lime, aluminium salts or iron salts, with the orthophosphate combining with the metal cations. The level of precipitation in a

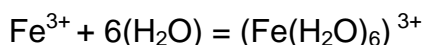
WWTW depends on the pH of the system, the type of metal salt used and on the degree of mixing of the metal salt into the sewage (Thistleton *et al.*, 2001). However the mechanisms of CPR are poorly understood and data reported in the literature have often been contradictory (Thistleton *et al.*, 2001).

2.2.2.1 Iron salts

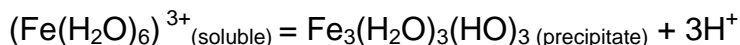
The major advantages of using Fe salts for precipitation are the low cost and the sludge produced has excellent dewatering properties (Yeoman *et al.*, 1990). Ferric chloride (FeCl_3) or sulphate ($\text{Fe}_2(\text{SO}_4)_3$) and ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), also known as copperas, are all widely used for P removal, although the actual reactions are not fully understood as these treatments involve the formation of hydroxides as well as phosphates.

These reactions are explained by Forster (2003) in the following way:

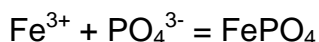
Initially, ions are formed:



The positive charges of the trivalent ions cause the bonds within the water molecules to polarize, leading to the liberation of protons and the formation of an insoluble hydrated metal hydroxide



The reaction is pH dependent, with progressively more protons being released under alkaline conditions. This precipitate is gelatinous and enmeshes any particle found in the sewage, including the precipitated Fe-phosphate



2.2.2.2 Strategies for Phosphorus removal

There are three variations on the addition of coagulants, each requiring different modifications to the AS plant. These are known as:

1. Pre-precipitation: the chemical is dosed before primary sedimentation and phosphate is removed in the primary sludge. Around 90% of the total P concentration can be removed. This also achieves significant BOD and suspended solids removal (Lees *et al.*, 2000).
2. Simultaneous precipitation: the chemical is dosed directly into the aeration tank and phosphate removed in secondary sludge. Dosing into

the aeration tank means that it is possible to use cheaper ferrous salts which would then be oxidized to the ferric state in the aeration tank. The oxidation of the ferrous salts will use oxygen, in the theory $0.15 \text{ g O}_2/\text{gFe}^{2+}$. This will need to be considered in design calculations, but de Haas *et al.* (2001) have shown that the oxygen demand due to Fe will be only a small part of the overall oxygen demand. The dose would depend on the concentration of phosphate in the settled sewage with an Fe:P ratio of 2:1 being typical (Smith, 2006). Dosages generally fall in the range of 1 to 3 metal ion:P molar ratio (Metcalf and Eddy, 2003).

3. Post-precipitation: the chemical is dosed after the final tank. Although a final effluent of excellent quality is produced and smaller chemical is required, this approach is not generally favoured because this treatment requires an additional reactor and sedimentation tank as well as tertiary treatment for solids removal.

2.2.2.3 Parameters which influence chemical precipitation

There are three parameters reported in the literature which have a notable influence in the precipitation of Fe salts: pH, DO and redox potential.

In a Fe-orthophosphate system, phosphate removal is independent of pH below an Fe:P molar ratio of 1.5:1 (Yeoman *et al.*, 1988). At ratios above this value, pH has an increasing influence (De Haas *et al.*, 2000). The optimum pH for phosphate precipitation with ferric ion is the range between pH 4.0 and 5.0 (De Haas *et al.*, 2000; Fytianos *et al.*, 1997; Thistleton *et al.*, 2002), although at higher pH significant phosphate removal can be achieved (Thistleton *et al.*, 2002), while for ferrous ion is close to pH 8.0 (Thistleton *et al.*, 2001). According to De Haas *et al.* (2000) CPR is strongly pH dependent for FeSO_4 and FeCl_3 , if pH falls below 7.0, the P removal efficiency decreased. At a process $\text{pH} \geq 7.2$, the P removal efficiency increased to a maximum of 80% for FeSO_4 and 100% for FeCl_3 (De Haas *et al.*, 2000). Thistleton *et al.* (2002) found that a total P removal of 80% was achieved at dose of 1.48:1 molar ratio Fe:P using FeCl_3 . Lees *et al.* (2000) also used FeCl_3 at molar ratio Fe:P 0.5:1 obtained P removal over 90%, DO level in the range of 3-5 mg/l and pH range was no specified. If Fe is dosed as Fe(II) the DO and the redox potential used to achieve the conversion to Fe(III) play an important role. Not all Fe(II) is converted to Fe(III). According to Thistleton *et al.* (2001), to get a good conversion, DO concentration have to be within the range 1-3.5 mg/l and redox potential has to be positive. The optimal range of pH for WWT process is 6-8 (De Haas *et al.*, 2000), pH close to neutral are required for the discharge of the final effluent. At pH higher than 8, Fe-hydroxy complex formation are favoured (De Haas *et al.*,

2000), accentuate this effect with high doses of coagulant and poor mixing (Thistleton *et al.*, 2001).

Having as a reference the work completed for the researchers cited above, pH and DO concentration were set up between 7-8 for pH and 1-3.5 mg/l for DO concentration. The range of work for these parameters was maintained in all the experiments for all Fe salts used. The use of the same conditions in all the experiments mimic the difference between the different Control sludges generated in the experiments.

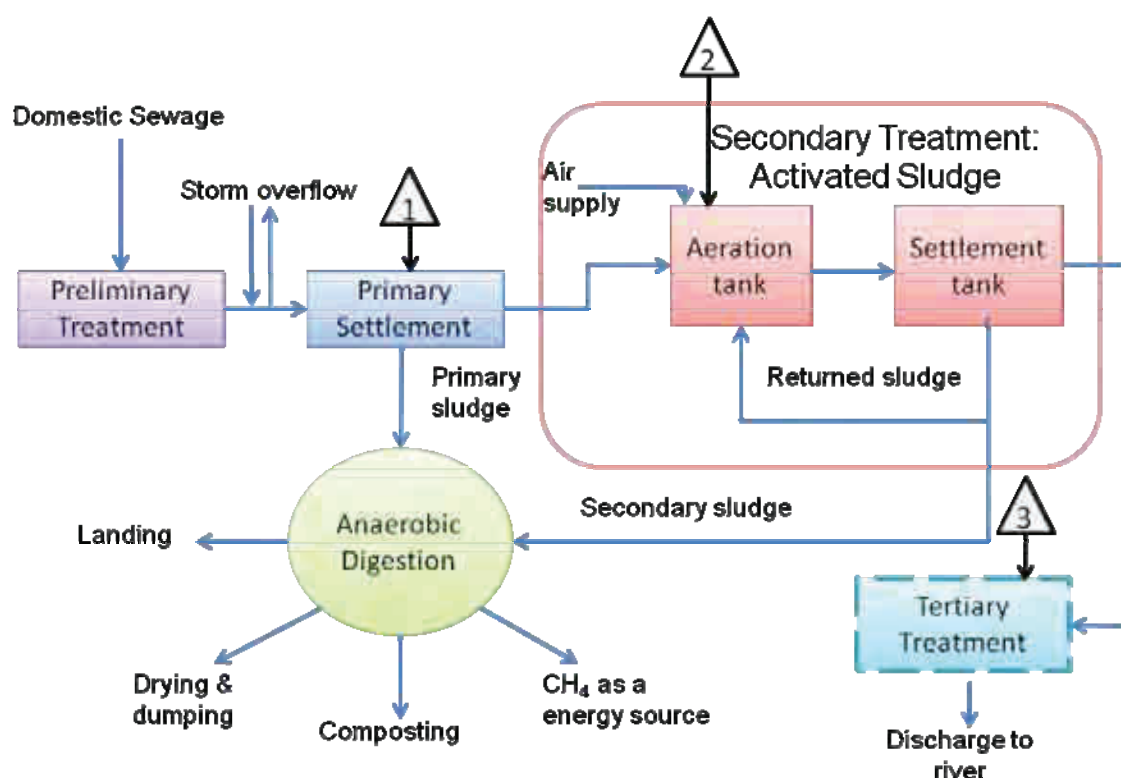


Figure 2.2 – Stages of wastewater treatment where the three possible locations of coagulant addition are represented with a black triangle.

2.3 Review of the Impact of chemical Phosphorus removal on Anaerobic Digestion

There are a lot of results reported in the literature about how Fe salts affect CPR, based on physical and chemical properties of the sludge (De Hass *et al.*, 2000; Fytianos *et al.*, 1997; Jenkins *et al.*, 1970; Lees *et al.*, 2000; Thistleton *et*

al., 2001 and 2002; Yeoman *et al.*, 1990) but there are just a few reports about how these salts affect AD (Ghyoot and Verstraete, 1997; Grigoropoulos *et al.*, 1971; Gosset *et al.*, 1978; Johnson *et al.*, 2003; Smith and Carliell-Marquet, 2008; Yeoman *et al.*, 1990). There are two groups of results obtained for previous research:

1. Chemical coagulants impaired AD of the dosed sludge.
2. Chemical coagulants did not have a detrimental effect on AD digestion of the dosed sludge.

Table 2.1 – Anaerobic digestion performance parameters reported in the literature

	Biogas production	Methane production	VS	COD	Alkalinity	Bioavailability of Fe and P
Dentel and Gosset, 1982	✓					
Yeoman <i>et al.</i>, 1990	✓	✓			✓	
Gosset <i>et al.</i>, 1978	✓	✓	✓	✓		
Johnson <i>et al.</i>, 2003	✓	✓				
Smith and Carliell-Marquet, 2008	✓					✓
Ghyoot and Verstraete, 1997	✓	✓	✓	✓		✓
Grigoropoulos <i>et al.</i>, 1971	✓		✓	✓		✓

2.3.1 Chemical coagulants impaired Anaerobic Digestion

According to Dentel and Gosset (1982) chemical coagulation of organic materials with alum or FeCl_3 causes a decrease in anaerobic digestibility (gas production) of the resulting sludge but this effect was not attributable to toxicity or nutrient limitation. Gosset *et al.* (1978) studied the effect of alum and FeCl_3 on domestic WW also and determined that the use of coagulant reduced

performance of AD (reduced total gas production, methane production, COD reduction and VS destruction) and as well as Dentel and Gosset (1982) this effect was not attribute to Fe toxicity. Both of them attributed this effect to the decrease of the biodegradability of substrates. Moreover, Dentel and Gosset (1982) assigned it to the association of the substrates with metal hydroxide flocs while Gosset *et al.* (1978) assigned it to the nitrogen contained in the organic materials. Alum and FeCl_3 were used as a coagulant for Yeoman *et al.*, (1990) and concluded that chemical coagulants have an adverse effect on AD in terms of methane production and alkalinity, but no reason for this adverse effect was reported. The origin of the sewage used for Yeoman *et al.* (1990) was a mixture of industrial and domestic WW.

Johnson *et al.* (2003) and Smith and Carliell-Marquet (2008) investigated the effect of Fe-dosing on domestic WW and found that Fe-dosed sludge produce less biogas than non Fe-dosed sludge but higher methane concentration. No clear reason was given for Johnson *et al.*, (2003) however Smith and Carliell-Marquet (2008) found a correlation between impaired AD and the concentration of bioavailable Fe and P. Lowers levels of Fe and P were found in Fe-dosed samples where biogas production was reduced.

2.3.2 Chemical coagulants did not have a detrimental effect on Anaerobic Digestion

Jenkins *et al.* (1970) reviewed CPR in domestic WW and concluded that any detrimental effect on AD could be assigned to the presence of Fe(III) and Al(III) in sludge resulting from phosphate precipitation. Ghyoot and Verstraete (1997) and Grigoropoulos *et al.* (1970) agreed with this conclusion. Grigoropoulos *et al.* (1970) through use of Al salts within a molar ratio Al:P 1.3-1.9:1 and Ghyoot and Verstraete (1997) through use FeCl_3 in a molar ratio Fe:P 0.8:1. Both of them found that the P precipitated was not released to the supernatant during AD.

In addition to the authors cited above Pfeffer and White (1964) and Horban and van den Berg (1979) as it was mentioned earlier studied the effect of Fe on AD. Both of them found that Fe supplementation at low concentration (between 200 and 580 mg/l) enhanced methane production and increased the VFA degradation. The use of higher concentrations (600-800 mg/l) produced the opposite effect, although Horban and van den Berg (1979) found that the inhibitory effect produced for the addition of Fe (1160 mg/l) was not permanent, the first two to four days after the addition.

2.2.3 Summary

Reviewing literature about effect of CPR on AD, there are some aspects which hamper to can compare the results obtained for different researchers. These aspects are: AD performance parameters, experimental conditions, dosing system, kind of sludge and length of trials.

There is not a specific way to report AD performance, so the results obtained and their interpretation depend on the researcher criteria. For example, Dentel and Gosset (1982) highlight that digesters fed with CPR sludge are 69% less efficient in terms of methane volume per mass of VS fed from Grigoropoulos *et al.* (1970) results.

All researchers reported biogas production in addition to other parameters as a parameter which represents AD performance, but this parameter (biogas production) is not representative without data about VS or COD fed or destroyed. It is expected a higher biogas production for higher VS loading, although there is a limit which the digester is overload. The length of the trial also influence the quantity of biogas produced. Gosset *et al.* (1978), Smith and Carliell-Marquet (2008) and Smith and Carliell-Marquet (2009) used small digesters to do their experiments for 15, 12 and 13 days as a detention time. However, others authors did bench-scale experiments, where the duration of the experiment is longer because of the set up time, acclimatization of MOs and stabilization of the system (Dentel and Gosset, 1982; Johnson *et al.*, 2003; Yeoman *et al.*, 1990). Gosset *et al.* (1978) and posterior Dentel and Gosset (1982) found that the longer the trial, the less adverse effect produce the coagulant on the digestion, ie, the differences between non dosed sludge and dosed sludge on AD decrease over time.

With exception of Smith and Carliell-Marquet (2009) and Ghyoot and Verstraete (1997), in any of the investigations cited above, the method used to dose the metal salts was reported. Some authors decided to dose the samples in the laboratory artificially (Gosset *et al.*, 1978; Grigoropoulos *et al.*, 1970; Smith and Carliell-Marquet, 2009; Yeoman *et al.*, 1990), others authors nevertheless, decided to use samples collected from a full-scale plants (Dentel and Gosset, 1982; Johnson *et al.*, 2003; Smith and Carliell-Marquet, 2008). Authors as Grigoropoulos *et al.* (1970) and Yeoman *et al.* (1990) used a mix of primary and secondary dosed sludge, which dilute or modify the effect of Fe on AD; therefore it adds more variables to the experiments.

The ratio Fe:P was not specified in most of the investigations (Dentel and Gosset, 1982; Gosset *et al.*, 1978; Johnson *et al.*, 2003; Smith and Carliell-Marquet, 2008; Yeoman *et al.*, 1990) which makes really difficult to get an idea of the effect of Fe on AD, because according to Dentel and Gosset (1982)

depending on Fe added to the process, the effect expected for this Fe on AD will be different; increasing this effect with the dose increases (Gosset *et al.*, 1978).

All these issue mentioned above makes difficult compare results from different authors or use the results obtained as guide. In addition, not all authors give an explanation or thought about what can impair AD. Johnson *et al.* (2003) Yeoman *et al.* (1990) only compared the results obtained for the digestion of Fe-dosed sludge and non Fe-dosed sludge. However other authors suggest and/or explain the results obtained. The three most common reasons reported in the literature for the adverse effect produced by metal salts are: organic composition of sludge and P and Fe bioavailability.

P is a macronutrient vital for the growth of MOs (Gerardi, 2003), therefore P had to be present in a form that MOs can use. The P threshold or the optimum P concentration is still unknown, so to ensure a successful AD, sewage sludge should contain suitable concentrations of P (Horban and van den Berg (1979); Peffer and White, 1964; Smith, 2006). Grigoropoulos *et al.* (1970), Ghyoot and Verstraete (1997) found that P do not release during AD, but they did not find adverse effect due to P limitation. Dentel and Gosset (1982) neither attributed the adverse effect of Fe on AD to P limitation, although Peffer and White (1964) found a decrease in the methane production when higher concentration of Fe where added. Only Jenkis *et al.* (1970) found P release on AD through the decrease in pH levels and neither any adverse effect on AD was reported. Nevertheless, Gosset *et al.* (1978) and Dentel and Gosset (1982) did not attributed Fe impairment to toxicity but to the organic composition of sludge; WW with high protein or lipid content. The association of this kind of substrate with metal affects the biodegradability of these substrates, “producing a barrier to enzymatic hydrolysis” (Gosset *et al.*, 1978). The higher concentration of bioavailable P in RAS, the higher is the biogas production obtained; this correlation was found for Smith and Carliell-Marquet (2008). They also suggested that the biogas production expected decrease with increasing the mass of Fe in the feed sludge.

There seems to be a consensus that CPR does not cause toxicity on AD (Dentel and Gosset, 1982; Gosset *et al.*, 1978; Grigoropoulos *et al.*, 1970), but nevertheless, digestion is sometimes impaired by dosing of sludge for P removal.

2.4 Metal and Phosphorus Sequential Extraction Methods

Over the past three decades, interest has increased markedly in the use of indirect approaches such as sequential chemical extraction (Bacon and Davison, 2008). In sequential extraction (SE), a series of reagents is applied to the same sample to sub-divide the total metal content. The vigour of the treatment generally increases through the steps of the procedure, from initial mild conditions (e.g. shaking with water) to the use of much harsher reagents (e.g. hot mineral acid). The elements extracted early in the process thus generally those most weakly bound to the solid phase. Hence, they have greater potential mobility, and environmental impact, than those released later.

The reagents used were selected on the basis of their ability to remove analytes from specific, major, sediment phases; either by exchange processes or by dissolution of the target phase (Bacon and Davison, 2008). Extractions steps also correspond with, or at least represented extremes of, important changes in environmental conditions that could affect metal binding in sediments: acidification, reduction and oxidation.

In this research the use of SE to differentiate Fe and P bioavailable fraction is the most important function of this procedure. This importance lies on the relationship found for Smith and Carliell-Marquet (2008) between bioavailable P and biogas production.

2.4.1 Metal Fractionation Method

2.4.1.1 Stover method adapted by Smith

This method refers to a method developed by Stover *et al.* (1976), which consists of five fractionations: (a) exchangeable metals, (b) adsorbed metals, (c) organically bound metals, (d) carbonate precipitates and (e) sulphide precipitates.

This method was tested on RAS and digested sludge samples and modified by Smith in 2006. The stages of the fractionate process are shown in Table 2.2.

Table 2.2 – The Successive stages of the “Stover” sequential extraction method used to fractionate metals modify by Smith in 2006

	Reagent	Concentration	Extraction time	Liquid:solid ratio ml:g TS
Smith (2006)	KNO ₃	1 M	12 h	50:1
	KF	0.5 M (pH 6.5)	12 h	70:1
	Na ₄ P ₂ O ₇	0.1 M (pH 6.5)	12.5 h	70:1
	Na ₂ EDTA Dihydrate	0.1 M	16 h	70:1
	HNO ₃	1 M	16 h	50:1
	Aqua Regia	4 %	~1 h	Sludge pellet + 1.3 ml conc. HNO ₃ + 2.7 ml conc. HCl

2.4.1.2 Sposito Method

Sposito *et al.* (1982) reported this fractionation procedure to be experimentally precise for the determination of: (a) exchangeable, (b) sorbed, (c) organic, (d) carbonate, (e) sulphide. This SE was modelled based on Stover *et al.* (1976) method. This method was modified and verified by Pichtel *et al.* in 2007 for sewage sludge compost.

The reagent strengths, extractions times and liquid:solid ratios for both SE methods are shown in Table 2.3 and Table 2.4.

Table 2.3 – The “Sposito” sequential chemical extraction method

	Reagent	Concentration	Extraction time	Liquid:solid ratio ml:g TS
Sposito <i>et al.</i> (1982)	KNO ₃	0.5 M	16 h	12.5:1
	De-ionised H ₂ O		6 h	12.5:1
	NaOH	0.5 M	16 h	12.5:1
	Na ₂ EDTA Dihydrate	0.05 M	6 h	12.5:1
	HNO ₃	4 M	16 h (80°C)	12.5:1

Table 2.4 – The “Pichtel” sequential chemical extraction method

	Reagent	Concentration	Extraction time	Liquid:solid ratio ml:g TS
Pichtel <i>et al.</i> (2007)	De-ionised H ₂ O		6 h	12.5:1
	KNO ₃	0.5 M	16 h	12.5:1
	NaOH	0.05 M	16 h	12.5:1
	Na ₂ EDTA Dihydrate	0.05 M	6 h	12.5:1
	HNO ₃	4 M	16 h (80°C)	12.5:1

2.4.1.3 Method used in this research

The method used in this research is based in the method used for Smith (2006) and method used for Pichtel *et al.* (2007). The main reasons for choosing the Pichtel method were:

- The suppression of the KF fraction, because KF is a toxic reagent and the adsorbed metal can be extracted in the UPW fraction used in this research.
- The use of NaOH instead of Na₄P₂O₇ in order to can measure P levels in the EDTA fractions.

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The method sequence of reagent addition, quantities and length of reaction time is shown in Table 2.5.

The main changes introduced in the method used for Pichtel (2007) were in the extraction time and in the liquid:solid ratio ml:gTS. These changes were considered appropriated after testing them; the comparison between the method used in this research and the method used for Pichtel *et al.* (2007) is presented in Appendix A.

Table 2.5 – Metals fractionation scheme

	Reagent	Concentration	Extraction time	Liquid:solid ratio ml:g TS
This Research	UPW		6 h	50:1
	KNO ₃	1 M	12 h	50:1
	NaOH	0.05 M	12 h	50:1
	Na ₂ EDTA Dihydrate	0.1 M	12 h	70:1
	HNO ₃	1 M	12h	50:1
	Aqua Regia	3:1	~1 h	Sludge pellet + 2 ml conc. HNO ₃ + 6 ml conc. HCl

This method will be used to fractionate RAS and digested sludge samples. The interpretation of fractions for both kinds of samples can be observed in the Table 2.6 and 2.7.

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Table 2.6 – Interpretation of fraction profiles and fraction storage in RAS (Adapted from Table 2.10 Smith, 2006)

Reagent	Fractions in RAS	Storage
Supernatant	Soluble Metal complexes	Frozen
UPW	Soluble Metal complexes	Frozen
KNO₃	Metal bound to sludge by electrostatic attraction (Stover <i>et al.</i> , 1976)	Frozen
NaOH	Organically bound	Frozen
Na₂EDTA Dihydrate	Metal from Metal-phosphates Metal from Metal-carbonates Fe from Fe-hydroxides and Fe-hydroxy-phosphates. Mg from Mg-hydroxides	Refrigerated
HNO₃	Metal from Metal-sulphides	Refrigerated
Aqua Regia	Metal from Metal-sulphides	Refrigerated

Table 2.7 – Interpretation of fraction profiles and fraction storage in digested sludge
(Adapted from Table 2.11 Smith, 2006)

Reagent	Fractions in digested Sludge	Storage
Supernatant	Soluble Metal complexes	Frozen
UPW	Soluble Metal complexes	Frozen
KNO ₃	Metal bound to sludge by electrostatic attraction (Stover <i>et al.</i> , 1976)	Frozen
NaOH	Organically bound	Refrigerated
Na ₂ EDTA Dihydrate	Metal from Metal-phosphates Metal from Metal-carbonates	Refrigerated
HNO ₃	Metal from Metal-sulphides	Refrigerated
Aqua Regia	Metal from Metal-sulphides	Refrigerated

The word Metal in Tables 2.6 and 2.7 refers to Fe, it can be in form of Fe(III) or Fe(II). In RAS samples, most of the compounds formed by Fe, it will be in form of Fe(III), while in digested samples the compounds form by Fe will be in the form of Fe(II) (Smith, 2006).

2.4.2 Phosphorus Fractionation Method

The method used by Uhlmann *et al.* (1990) was chosen for P fractionation. This method has four target phases: (a) water soluble P, (b) redundant-soluble P, (c) organically bound and associated with Fe and Al, and (d) Ca bound with P. The sequence of reagents used, length and liquid:solid ratio for this method is shown in Table 2.8.

Table 2.8 – Successive stages of the “Uhlmann” sequential extraction method used to fractionate phosphorus

	Reagent	Concentration	Extraction time	Liquid:solid ratio (ml:g TS)
Uhlmann et al., 1990	De-ionised H ₂ O (deoxygenated)	pH 6.2	12 min	100:1
	Bicarbonate	0.11 M	30 min	50:1
	Dithionite			
	De-ionised H ₂ O	pH 6.2	5 min	50:1
	NaOH	1 M (pH 13.76)	18 h	50:1
	De-ionised H ₂ O	pH 6.2	5 min	50:1
	HCl	0,5 M (pH 0.6)	18 h	50:1
	De-ionised H ₂ O	pH 6.2	5 min	50:1

This method was modified by Carliell-Marquet (2001) and Smith (2006). Carliell-Marquet (2001) replaced the Bicarbonate Dithionite reagent with a two-stage reaction using an acetate buffer. The modification enabled to be extracted from struvite and the solubilisation of calcium carbonate (prior to NaOH extraction) but Bicarbonate Dithionite caused analytical interference (Carliell-Marquet, 2001). The method was further modified by Smith (2006) with the elimination of all water rinses, except for the one between the NaOH and HCl extractions (Smith, 2006). The new procedure resulted in a quicker total extraction time and as such, “enables work to be carried out between the hours of 7 am and 11 pm and so meet health and safety requirements” (Smith, 2006).

Table 2.9 – “Uhlmann” sequential extraction method modified by Smith (2006)

Smith (2006)	Reagent	Concentration	Extraction time	Liquid:solid ratio (ml:g TS)
	De-ionised H ₂ O	UPW	20 min	60:1
	Acetate buffer	0.1 M (pH 5.2)	45 min	60:1
	Acetate buffer	0.1 M (pH 5.2)	30 min	60:1
	NaOH	1 M	18 h	60:1
	De-ionised H ₂ O	UPW	5 min	60:1
	HCl	0,5 M	18 h	60:1
	Aqua Regia	3:1	~1 h	Sludge pellet + 2 ml conc. HNO ₃ + 6 ml conc. HCl

Finding from the fractionation of P using the Smith (2006) modified Uhlmann method is presented in Table 2.9.

It is possible to correlate results from the Metal and the P extraction methods. For instance, the compounds formed for Fe and P can be extracted in the EDTA fraction in the Metal method and in the NaOH fraction in the P method (Smith, 2006).

As the method used for Metal fractionation, this method will be used to fractionate RAS and digested sludge samples. The interpretation of these fractions can be observed in the Table 2.10.

Table 2.10 – Interpretation of fraction profiles with regard phosphorus recovery in both activated and digested sludge samples and storage (Adapted from Table 2.9 Smith, 2006)

Reagent	Recovery of P in RAS and digested sludge		Storage
Supernatant	Soluble P complexes		Frozen
UPW	P weakly bound to sludge particles that can be “washed off”		Frozen
Acetate buffer	P for struvite	P adsorbed to CaCO_3	Frozen
Acetate buffer	Some P from very soluble (amorphous) Ca-P precipitates.		Frozen
NaOH	Soluble reactive P from Fe, Al or Mg-phosphates; P from organic phosphates Fe-hydroxides (Choi <i>et al.</i> , 2009)		Refrigerated
HCl	P from Ca-phosphates		Refrigerated
Aqua Regia	Residual, nothing specific		Refrigerated

2.4.3 Limitations of Sequential Extractions

In the literature there are several procedures for the SE of metal and P. In this section of the literature review, a brief review about the weakness of these procedures is introduced.

The main function of SE is divided the element content of a sample in different portions which can be extracted for a particular reagent under particular conditions. The election of the reagent plays an important role within the extraction procedure. The reagent is selected to target a specific phase, but it cannot be guaranteed. In addition, SE is formed for several fractions, hence it is not only important to choose the appropriate reagent to extract a specific phase but also the sequence of which is applied.

In order to improve the interpretation of the results of SE in terms of binding of trace of metal to specific mineral, Bacon and Davidson (2008) suggest to apply additional techniques as X-ray-based or analytical techniques to the residues of

each stage in the extraction. In addition to this, Bacon and Davidson (2008) suggest the use of at least two independent SE procedures, as the amount of metal is strongly dependent on the extractant and the procedure used.

There are several reasons why SE does not determine quantitatively the trace metals associated with specific mineral phases in environmental solids. Bacon and Davidson (2008) highlight the following weakness of SE procedures:

- Re-distribution of analytes among phases during extractions.
- Non-selectivity of reagents for target phases.
- Incomplete extraction.
- Precipitation of “new” mineral phases during extractions.

In addition in this study:

- The difficulty of getting a representative sample as sludge is not homogeneous.
- Analysis of anaerobic samples under aerobic conditions (digested samples).

Presentation and interpretation of the results are also an important issue related to SE. The presentation of the results as percentage without giving concentration data, generate confusion beside misinterpretation of the results. Bacon and Davidson (2008) consider that the data interpretation depend on the context and aim of the study.

Although there are issues with SE, it is thus now widely accepted and adopted. The approach has led to improved understanding of behaviour of elements, and generated large amounts of useful data (Bacon and Davidson, 2008).

CHAPTER 3

MATERIALS AND METHODS

Within this chapter, the main methods and materials used throughout this research are presented. This chapter is split into three main sections:

1. Methods and Experimental set-up – this section details the methods and the experimental set-up used in this research:
 - a. Laboratory Dosing Activated Sludge with Iron
 - b. Batch Test Digesters
2. Metal and Phosphorus Analysis – this section details the analytical methods used to extract and analyse Metal and Phosphorus in sludge samples.
3. General Sludge Analysis – this section details the analytical methods use in this research.

3.1 Methods and Experimental set-up

The two methods reported in this section are relating to the four principal objectives. Objective 1 was to set up and use the novel laboratory method developed for Smith and Carliell-Marquet (2009); Objective 2 was investigated the effect of different Fe salt in the digestion process; Objective 3 was investigated the answer of the digester to different quantities of Fe dosed in the aeration tank; and Objective 4 was to use the information obtained from the inorganic fraction profiles to complement Objective 2 and 3.

3.1.1 Laboratory Dosing Activated Sludge with Iron

The procedure followed in this research to dose return activated sludge (RAS) with Fe was based on the procedure tested and validated by Smith and Carliell-Marquet, (2009). Typically a mix of primary and secondary sludge is fed to the digester and thus the direct impact of chemical dosing is not measurable (Smith and Carliell-Marquet, 2008). According to Smith and Carliell-Marquet (2008), the use of RAS allows more direct correlations to be made between chemical dosing and AD.

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The sludge samples used in this research were collected from Kidderminster WWTW, which is a non Fe-dosed plant. These samples were returned activated sludge (RAS), settled sewage (SetS) and digested sludge. In this experiment just RAS and SetS were used.

The RAS was thickened to a total solid (TS) concentration of approximately 7 g/l. The TS concentration of SetS was measured.

The Fe is added to simulate simultaneous precipitation, so Fe is added directly into the AS chamber where the biomass is active. The proportion of RAS and SetS used was in a ratio between 60:40 and 70:30 to produce an active biomass and to have a TS typical concentration of 3.5 g/l in the AS chamber.

The molar ratios of Fe:P used by WWTW are between 1:1 – 3:1 (Metcalf and Eddy, 2003). The most common molar ratio is 2:1. Previous research using this dosing system (Smith and Carliell-Marquet, 2009) showed that sludge generated with a dosing ratio of 2:1 Fe:P produced less methane in batch digestion tests. Lower molar ratios (0.6:1 and 1.2:1) were chosen for this research to investigate whether these Fe:P ratios would have a similar effect on methane production (objective 3). The chemical form which Fe is added also influences the P removal. So Fe is added in form of Fe(II) and Fe(III) (objective 2). The source of P used was $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$.

Table 3.1 – Fe salts used in this research

Form	Compound	Molecular weight (g/mol)
Fe(III)	FeCl_3	162.21
Fe(III)	$\text{Fe}_2(\text{SO}_4)_3$	399.89
Fe(II)	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	278.01

One problem encountered related to the amount of Fe to add. In a full-scale AS plant, Fe is dosed directly into the aeration chamber and the dosing rates are known, but these rates cannot be used in the laboratory test because they would not account for Fe accumulation due to the return stream. So the concentration of Fe needed in the laboratory test will be based on the molar ratios and the accumulated P concentration at full-scale after several retention times (Smith, 2006). This concentration was measured in five different Fe-dosed AS, it was found to be approximately 40 mgP/gTS (Oikonomidis, 2003; Smith, 2006).

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The procedure followed to calculate the quantities of Fe and P needed is described in the next list:

- Thicken RAS until 7 g/l of TS and measure Total P. Measure TS and Total P of SetS.
- Calculate volume necessary to obtain 3.5 g/l TS in 3 litres mixing RAS and SetS.
- Obtain quantity of P in each portion:

$$P_{RAS} = \text{Total-P}_{RAS} * V_{RAS}$$

$$P_{SetS} = \text{Total-P}_{SetS} * V_{SetS}$$

- Measure TS and Total P of the mix.
- Concentration of P desired in the mixed RAS:SetS: 40 mg/gTS
P desired in 3.5 g/l TS: 140 mg.

- The quantity of P accumulate is:

$$P_{accum} = 140 \text{ mg/l} - P_{SetS} - P_{RAS}$$

- Mass of $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ necessary = $P_{accum} * (\text{Mn Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O} / \text{Mn P})$
- Quantity of Fe is necessary to add: $\text{Fe}_{total} = \text{Fe}_{dose} + \text{Fe}_{accum}$

$$\text{Fe}_{Dose} = P_{SetS} * \text{ratio}$$

$$\text{Fe}_{accum} = P_{accum} * \text{ratio}$$

- Calculations are done in mass, so the ratio values used are in mass:

Molar ratio Fe:P	Mass ratio Fe:P
1.2:1	2.16:1
0.6:1	1.08:1

- Mass of Fe to add = $\text{Fe}_{total} * (\text{Mn reagent} / \text{Mn Fe})$
The reagents used are shown in Table 3.1.
- The quantities of chemical were calculated to dissolve in 30 ml of UPW, so these values were extrapolated to 100 ml, because some liquid is necessary to clean the pump ducts used to dose the Fe and P reagents.

Once all the Fe and P had been added, the sludge was left to mature. It was stirred and aerated for 10.5 hours approximately. After that, biogas production, P removal and metal profiles were investigated.

The whole dosing process are summarize in the Figure 3.1.

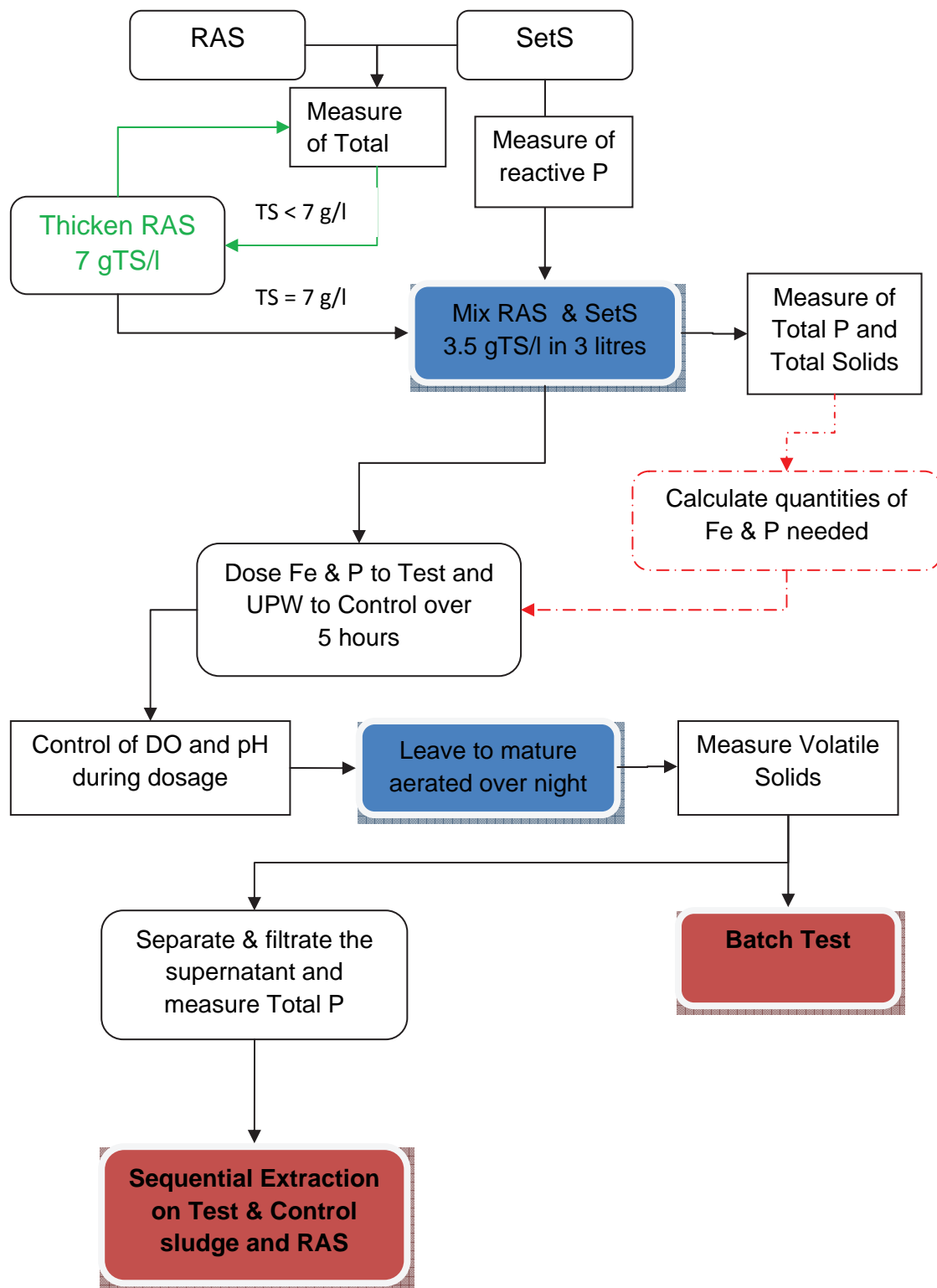


Figure 3.1 – Flow diagram of Fe-dosing procedure (Adapted from Figure 4.4 Smith, 2006)

3.1.1.1 Set up

Two vessels of 10 litres were used as AS chamber. In one of them Fe and P was dosed, called Test, while the other one, Control UPW was dosed instead of Fe and P. Both vessels were aerated and stirred. Three peristaltic pumps were used to dose the Fe and P reagents into the Test vessel and UPW into the Control vessel.

The apparatus used for Fe-dosing system in the laboratory were:

- pH meter
- DO meters
- Stirrer controllers
- Air flow controllers

These apparatus are shown in Figure 3.2.

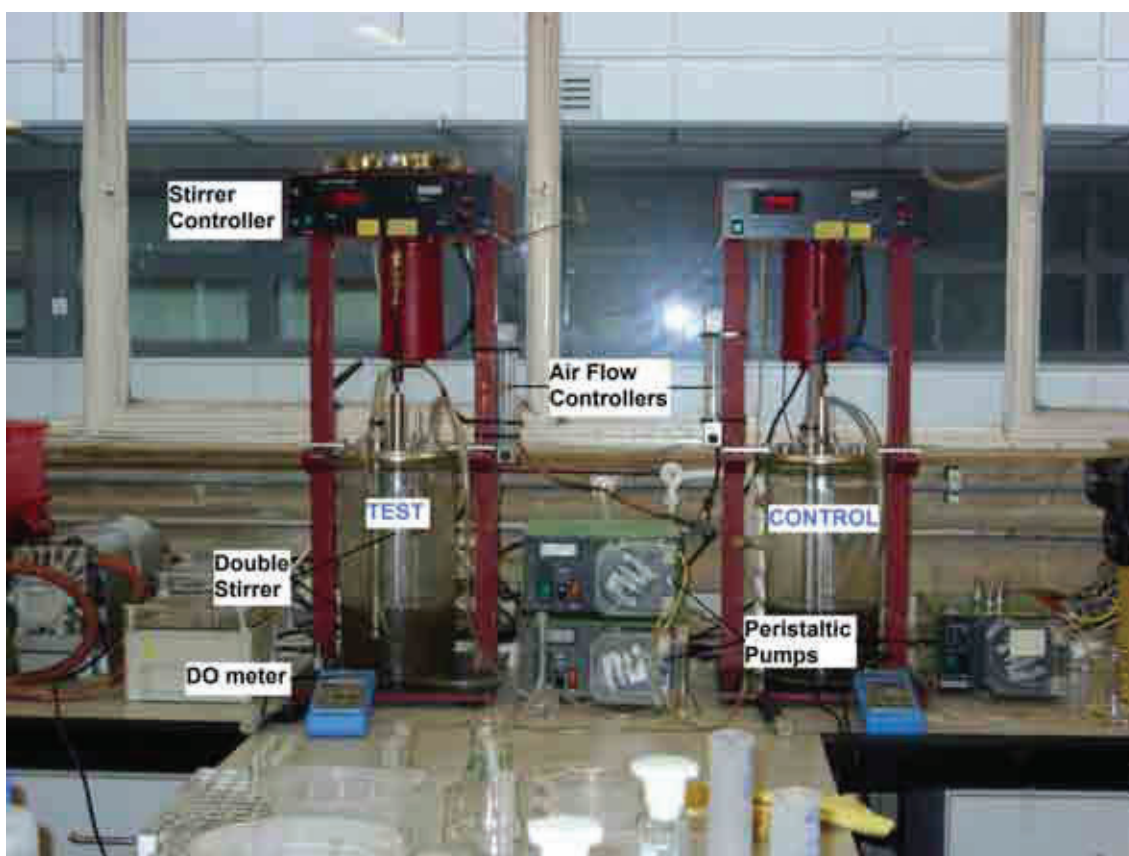


Figure 3.2 – Photograph of the Fe-dosing apparatus

To perform the Fe-dosing system adequately there are several factor to account, but not all of them can be controlled. The parameters of control used in this procedure are detailed in the Table 3.2.

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Table 3.2 – Parameters of control for Fe-dosing system

Parameters	Range	Comments
Working volume	3 litres	Volume necessary to realize: Batch test and sequential extraction (SE)
pH	Between 7-8 is required for Fe(II) to oxidise to Fe(III) and react with the available P.	To increase the pH, drops of NaOH 4M was used.
Oxygen availability	DO levels: Between 1 and 3.5 mg/l	Fe has to be in form of Fe(III) to react with the P.
Stir	0.40 rpm	Use always the same
Dose	Add 3 ml of Fe and P compounds every 30 min for 5 hours to the Test vessel, and 6 ml of UPW to the Control vessel.	
Maturation time	Around 10.5 hours	Leave the mixture to mature overnight.

The main changes introduced in this research in the dosing-system used for Smith (2006) were in the working volume and in the dispensed quantity and time of dosage.

3.1.2 Batch Test Digesters

Digestion is the following step in the process after the dosage (aeration tank). The samples used in these experiments were the Test and Control samples generated after dosage and the digested sludge collected for Kidderminster.

This test was done the day after the collection, so any sample had to be stored. The Test and Control samples were used after the maturation period, while the digested sample was kept in the 35 °C room.

Digested sludge from Kidderminster was used as a seed in all the batch tests realised. This sludge was sieved to remove large lumpy biomass before used. Total solids (TS) and volatile solids (VS) were measured in all samples, because the quantity needed is function of the VS concentration. In order to minimize factors that may influence and be able to compare experiments, the same concentration of VS was used (to maintain equals levels of organic material available for degradation). This value was 0.5 gVS for digested sludge and 0.4 gVS for Test and Control.

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Serum bottles of 120 ml were used as batch digesters. The working volume was 100 ml, so the biogas generated could accumulate in the remaining 20 ml. Test and Control samples had to be thickened to fill inside the 100 ml work volume.

Once the quantity necessary of digested sludge and Test or Control sludge were added to the serum bottle, it was sealed with a butyl rubber septum and aluminium crimp and kept at a constant temperature in the 35 °C room. Three samples were digested:

- Test sludge seed with digested sludge.
- Control sludge seed with digested sludge.
- Digested sludge (it is needed to add UPW to fill up to the 100 ml).

Each sample was tested in triplicate. The test ran until biogas production subsided, usually after 11-12 days.

Biogas accumulated in the 20 ml headspace of the bottles was measured and analysed everyday to establish the percentage of methane (CH₄) and carbon dioxide (CO₂) and the total volume produced.



Figure 3.3 – Photograph of serum bottles

3.1.2.1 Analysis of Biogas Composition

Biogas was sampled before the gas volume was recorded. Around 1.2 ml of biogas was used to inject in the gas chromatograph (GC) and this volume was included in the total volume of biogas collected. A 1 ml plastic syringe attached to a needle was used for sampling as follows:

1. The septum is pierced by the needle and 1.2 ml of biogas is collected in the syringe.
2. The syringe is removed from the septum and the needle is pricked in a small plastic circle that serves as a stopper.
3. Take the stopper out and expel any additional biogas until the 1 ml mark is reached.
4. Inject the sample into the GC for analysis.

The needles used were small and short to ensure that the pierced holes in the septum were small and the needle tip did not touch the sludge. Needles were replaced when they became blunt.

The GC used for analysis the biogas was a Cygnus Chromatograph Ai Cambridge GC94. The system uses a column packed Spherocarb (mesh sized 80-100). The column temperature was set to 150 °C. The carrier gas was helium, and the flow rate was 30 ml/min. The size of the standards and samples was 1ml.

The GC was calibrated using three standards: 1) 100% CH₄, 0% CO₂; 2) 50% CH₄, 50% CO₂; 3) 0% CH₄, 100% CO₂. The GC identified three gases, air, CH₄ and CO₂. The air was a result of the sampling method and did not represent an actual portion of the biogas generated. For this reason, the percentages and volumes reported are all based on the assumption that the biogas is purely CH₄ and CO₂.

3.1.2.2 Measurement of Volume of Biogas generated

To measure the volume of biogas generated a manometer was used. The final biogas volumes reported were based on standard temperature (25 °C) and standard pressure (1 atm) using the next equation:

$$\text{Volume of gas (ml)} = \frac{P_{lab} \times V_{read} \times T_{st}}{T_{lab} \times P_{st}}$$

Where:

P_{lab} = atmospheric pressure of the laboratory (mmHg \pm 5)

V_{read} = gas volume read from the manometer (ml ± 0.1)

$T_{st} = 25\text{ }^{\circ}\text{C}$

$T_{lab} = 35\text{ }^{\circ}\text{C}$

$P_{st} = 760\text{ mmHg}$

One of the extreme of the manometer is connected to a tube in which extreme there is a needle. This needle pierces the septum and the biogas goes into the manometer moving the liquid inside the manometer. The difference between volumes gives the biogas volume generated. Biogas volume was measure twice a day during the first days of the trial and once a day when the production of biogas slow down.

3.2 Metal and Phosphorus Analysis

Within this section Metal and P analysis are described together with the acid washing glassware and samples preparation procedures. The extraction used to know the total content of metal and P and the orthophosphate method are also described.

3.2.1 Acid Washing

All glassware and plastic ware associated with the analysis of metal or P concentrations were acid washed to remove inorganic contamination using the following procedure:

1. Wash the glassware in hot water with P-free soap.
2. Rinse the glassware thoroughly with tap water (5 times) and reverse osmosis water (ROW) (3 times).
3. Soak the glassware overnight in 10% HCl.
4. Rinse glassware in ROW (3 times).
5. Soak the glassware overnight in ROW.
6. Dry the glassware in a hot cabinet.

3.2.2 Sequential Extraction Procedures

The sequential extraction (SE) methods used in this research are detailed in section 2.4 of the literature review. SE methods for both Metal¹ and P were carried out in triplicate on Test and Control, Test and Control digested (Test Dig and Control Dig) and RAS samples.

Both the Metal and P SE procedure are similar, except for reagent volume, type of reagent used, time of incubation and kind of samples storage. Both procedures are detailed in the followings sections.

Table 3.3 shows the planning followed in this research, where SE procedure and the general sludge analysis are summarized.

Table 3.3 – Programme of sequential extractions and general sludge analysis (adapted from Table 4.4 Smith, 2006)

Time	Monday	Tuesday	Wednesday	Thursday	Friday
7:00	Preparation of Solutions	TS/VS Dosed Sludge Alkalinity, pH COD, Ortho-P	(F) KNO ₃ (8:30) NaOH		
8:00	6 Tins in oven	Acid Digestion Total M & P		(F8:30) HCl	
9:00	RAS, SetS & Dig Reception			(F) EDTA (9:30) HNO ₃	Acid Digestion of Metal SE pellets
10:00	TS/VS, Alkalinity, pH COD, TOC	Sludge pellet Freeze Supernatant		Ortho P analysis EDTA fraction	
11:00					
12:00	RAS & SetS	(S12) DW	(F) NaOH UPW	Acid Digestion of P SE pellets	
13:00	3 Tins in oven		(S14:30) HCl		
14:00			Dilute NaOH fraction		
15:00	Starting DOSE FeCl₃ 2.16:1	BATCH TEST FeCl₃	Ortho P analysis NaOH fraction		
16:00	Digested Sludge				
17:00	TS/VS, Alkalinity, pH	(S17) UPW			
18:00	Digested Sludge	Acetate			
19:00	3 Tins in oven	(F) DW (19:30) KNO ₃			
20:00	End DOSE	(S20) NaOH			
21:00	LEAVE TO MATURE		(F) NaOH (21) EDTA	(F) HNO ₃	
22:00					

	Metals SE
	P SE
	Acid Digestion

¹The term Metal refers to iron (Fe).

3.2.2.1 Sample preparation for Sequential Extraction

The sample preparation procedure was the same for all kind of samples; the only difference was the quantities of sample used, as the volume of digested sample was limited to 100 ml in the batch test. The steps involved in the samples preparation are summarised in the following list:

1. Measure TS of the digested sludge (the TS concentration of RAS was done before).
2. Take the volume necessary to **contain 0.5 g of TS** for RAS samples and **0.25 g of TS** for Test, Control, Test digested and Control digested samples and pour it in the centrifuge tubes.
3. Form the pellets by centrifuging: 6000 rpm for 15 min.
4. Decant supernatant into 100/50 ml flask; pass through a 540 Whatman filter. Rise it with UPW up to 100/50 ml. Suck the supernatant through a 45 µm-pore Whatman filter to ensure the sample contained no insoluble material.
5. Transfer the sample into an acid-washed 120/60 ml bottle and frozen.

3.2.2.2 Metal Procedure

The different stages of the metal SE procedure together with quantities of reagent, reagents and samples storage are detailed in the following paragraphs.

1st extraction

1. Add 25/12.5 ml **UPW** to the sludge pellet and shake vigorously to re-suspend (25 ml in the case of RAS samples and 12.5 ml in the case of Test, Control, Test digested and Control digested samples).
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 30 min.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 100/50 ml flask; pass through a 540 Whatman filter.
5. Repeat step 1-4 three times.
6. Rinse it with UPW up to 100/50 ml. Bottle the sample and frozen.

2nd extraction

1. Add 25/12.5 ml of **KNO₃ 1M** to the sludge pellet and shake vigorously to re-suspend.
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 12 hours.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 50/25 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 50/25 ml. Bottle the sample and frozen.

3rd extraction

1. Add 25/12.5 ml of **NaOH 0.05M** to the sludge pellet and shake vigorously to re-suspend.
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 12 hours.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 50/25 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 50/25 ml. Bottle the sample and frozen.

4th extraction

1. Add 35/17.5 ml of **Na₂-EDTA 0.1M** to the sludge pellet and shake vigorously to re-suspend.
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 12 hours.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 100/50 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 100/50 ml.
5. Add 25 ml of **De-ionised H₂O** to the sludge pellet and shake vigorously for 5 minutes. Decant again into the 100/25 ml flask, pass through a 540 Whatman filter. Make up to 100/50 using UPW.
6. Bottle the sample and refrigerate.

5th extraction

1. Add 25/12.5 ml of **HNO₃ 1M** to the sludge pellet and shake vigorously to re-suspend.

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2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 12 hours.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 50/25 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 50/25 ml. Bottle the sample and refrigerate.

6th extraction

1. Transfer the sludge pellet into a 250 ml glass beaker, using **30 ml UPW**.
2. Add **6/3 ml Analar-grade HCl and 2/1 ml Analar-grade HNO₃**.
3. Place the beaker on the hotplate and boil the solution until approximately 10 ml remains.
4. Remove the beaker from the hotplate, allow the solution to cool and add further **6/3 ml Analar-grade HCl, 30 ml UPW and 2/1 ml Analar-grade HNO₃**.
5. Return the beaker on a hotplate and boil the solution until approximately 10 ml remains.
6. Leave the solution to cool before filtering through a 540 Whatman filter into a 100/50 ml flask.
7. Rinse the filter paper and beaker with UPW before diluting the sample to volume with UPW.
8. Bottle the sample and keep refrigerate.

3.2.2.3 Phosphorus Procedure

The filters used in this procedure were cleaned following the procedure described in Standard Methods (424 A, APHA AWWA WPCF, 1985):

- Soak 50 filters in 2 litres DW for 24 hour or soak 50 filters 2 litres DW for 1 hour, change DW, and soak filters an additional 3 hours.
- Determination of a blank value to ensure consistency in washing and to evaluate different lots of filters.

As in the previous point the different stages of the P SE procedure, volume of reagents, reagents and samples storage are detailed below.

1st extraction

1. Add 30/15 ml of **UPW** to the sludge pellet and shake vigorously to re-suspend (30 ml in the case of RAS samples and 15 in the case of Test, Control, Test digested and Control digested samples).

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2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 20 minutes.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 50/25 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 50/25 ml. Bottle the sample and frozen.

2nd and 3rd extraction

1. Add 30/15 ml of **Acetate buffer 0.1M** to the sludge pellet and shake vigorously to re-suspend.
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 45 minutes.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 50/25 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 50/25 ml. Bottle the sample and frozen.
5. Do this extraction twice.

4th extraction

1. Add 30/15 ml of **NaOH 1M** to the sludge pellet and shake vigorously to re-suspend.
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 18 hours.
3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 100/50 ml flask; pass through a 540 Whatman filter.
5. Add 30 ml of **UPW** to the sludge pellet and shake vigorously for 5 minutes. Decant again into the 100/50 ml flask; pass through a 540 Whatman filter (the volume total of liquid is 60 ml). Make up to 100 using UPW.
6. Pipette 25 ml of the 100/50 ml into a 50/25 ml flask, to get a sample 0.15M NaOH..
7. Bottle the sample and refrigerate.

5th extraction

1. Add 30/15 ml of **HCl 0.5M** to the sludge pellet and shake vigorously to re-suspend.
2. Place in an end-over-end shaker in a constant temperature room 25°C and leave for 18 hours.

3. Remove the tubes from the shaker and centrifuge at 6000 rpm for 15 minutes.
4. Decant supernatant into a 50/25 ml flask; pass through a 540 Whatman filter. Rinse it with UPW up to 50/25 ml. Bottle the sample and refrigerate.

6th extraction

1. Transfer the sludge pellet into a 250 ml glass beaker, using **30 ml UPW**.
2. Add **6/3 ml Analar-grade HCl and 2/1 ml Analar-grade HNO₃**.
3. Placing the beaker on a hotplate and boil the solution until approximately 10 ml remains.
4. Remove the beaker from the hotplate, allow the solution to cool and add further **6/3 ml Analar-grade HCl, 30 ml UPW and 2/1 ml Analar-grade HNO₃**.
5. Return the beaker on a hotplate and boil the solution until approximately 10 ml remains.
6. Leave the solution to cool before filtering through a 540 Whatman filter into a 100/50 ml flask.
7. Rinse the filter paper and beaker with UPW before diluting the sample to volume with UPW.
8. Bottle the sample and keep refrigerate.

3.2.3 Acid Digestion (Total Metal)

The method was used to know the total metal and P contained. The method followed in this research was based in the one used by Smith (2006) and the one described in Standard Methods (302 C, APHA AWWA WPCF, 1985).

It is the same procedure described in the 6th extraction of Metal and P SE procedures, but in this case the quantity and state of the samples are different. The first step is different while the rest of the procedure is the same.

1. Pipette 20 ml sludge for RAS, Test, Control samples and 10 ml of digested samples of Test digested, Control digested and Digested into a 250 ml glass beaker.
2. Follow from the second to the seventh point detailed in the 6th extraction of P Procedure.

3.2.4 Measurement of Metal and Phosphorus Concentration

The Atomic Absorption Spectrometry (AAS) instrument (Perkin Elmer AAnalyst 800) was used to measure the concentration of Fe in the extracted samples generated in the SE procedures.

Fe contained in the supernatant and the UPW fraction was measured using the graphite furnace technique meanwhile remaining fractions of the Fe procedure were measure using the flame system (air-acetylene).

Four standards plus the blank were used to calibrate the AAS when the graphite furnace and flame technique were used. In order to avoid false results one standard was measure every 9 samples, also at the beginning to ensure a good calibration. If the results of the standards were more than 10% out, the AAS was stopped and a cleaning-up was done. If the error persisted a new calibration was done.

Any sample was measure in triplicate, so the results were accepted if the standard deviation of the three replicates was $\leq 10\%$ for low concentrations and $\leq 5\%$ for high concentrations.

Table 3.4 – Concentration of standards, range and wavelength used for Fe and P analysis

Technique	Standards	Range	Wavelength (nm)
Furnace	50, 100, 200, 400 $\mu\text{g/l}$	20-200 $\mu\text{g/l}$	248.3
Flame	2, 10, 20, 40, 50 mg/l	2-40 mg/l	248.3
Plasma	1, 20, 40, 60, 80, 100 mg/l	1-100 mg/l	177.5

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It relies therefore heavily on Beer-Lambert law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbital for a short amount of time by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy (the power) put into the flame or the furnace is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer-Lambert law, to calculate

how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured. For example for Fe, the wavelength is 248.3 nm, so the light measure at this wavelength is proportional to the amount of Fe in the sample.

P concentrations were measured using a sequential plasma emission spectrophotometer (ICP) Thermo Jarrell Ash Atomscan16. Five standards plus the blank were used to calibrate the ICP. Results obtained from the ICP follow the same procedure than when the AAS was used.

3.2.5 Total phosphate method

Total phosphate (total P) is determined by some form of sample digestion to convert all phosphate forms to orthophosphate, followed by determination of the orthophosphate through the vanadate-molybdate method described in next section. This section describes the digestion method adapted for the method Standard Methods (424 A, APHA AWWA WPCF, 1985)

Samples were pipetted (20 ml) into 50 ml beaker with 1 ml of sulphuric acid concentrate and 0.5 g potassium persulphate. The beaker were placed on the hotplate and boiled until approximately 10 ml remained. The solution was allowed to cool and pH were adjusted to 7.5-8 using NaOH 0.1M or 0.1M H₂SO₄. Then samples were made up to 50 ml and the orthophosphate contained was determined using the vanadate-molybdate method.

3.2.6 Orthophosphate Determination

The term orthophosphate is used to denominate the soluble reactive phosphate. This method was applied in the laboratory dosing experiment. This method is also used after the acid digestion to know the total P in the RAS and SetS (section 3.1.1).

The Vanadomolybdophosphoric Acid Colorimetric Method (based in the Standard Method 424 D, APHA AWWA WPCF, 1985) is described below.

Reagent:

- Hydrochloric acid, HCl, 1+1. The acid concentration in the determination is not critical but a final sample concentration of 0.5 N is recommended. Pipette 25 ml HCl concentrate (cc) into 500 ml flask made up to 500 ml with ROW.

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- Vanadate-molybdate reagent: mix 20 ml of Solution A plus 20 ml of Solution B, add 19.4 ml HNO₃ cc and make up to 100 ml.
 - 1) Solution A: ammonium molybdate 10 %. Dissolve 25 g ammonium molybdate, (NH₄)Mo₇O₂₄·4H₂O in 200 ml ROW (+2.5 ml ammonium hydroxide), make up to 250 ml.
 - 2) Solution B: ammonium metavanadate 0.25 %. Dissolve 0.625 g ammonium metavanadate, NH₄VO₃, by heating to boiling in 200 ml ROW. Cool add 3 ml HNO₃ cc. Cool Solution B to room temperature and make up to 250 ml.
- Standard phosphate solution: dissolve in ROW 0.3582 g anhydrous KH₂PO₄ and dilute to 250 ml. (1000 ppm of PO₄).
- Standard 50 ppm: take 12.5 ml of 1000 ppm standard into a 250 ml flask and made up to 250 ml using ROW.

Calibration curve:

Concentration PO4 (ppm)	0	10	20	30	40	50
Volume Standard 50 ppm (ml)	0	1	2	3	4	5
Volume ROW (ml)	5	4	3	2	1	0
Concentration P (ppm)	0	3.26	6.52	9.78	13.04	16.31

Volume final = 5 ml

Procedure:

- Adjust pH between 7.5-8 using hydrochloric acid, HCl, 1+1.
- Place 5 ml of sample a boiling tube.
- Add 2 ml vanadate-molybdate reagent.
- Prepare a calibration curve using the standards. Plot absorbance versus phosphate concentration.
- Allow the reaction for 30 min.
- Measure absorbance of sample versus a blank at a wavelength of 470 nm (wavelength range 420-490 nm). The Spectrophotometer Cecil CE292 Series 2 was used to measure the absorbance.

3.3 General Sludge Analysis

Within this section the different analytical methods used to assess the physicochemical properties of sludge are described. In the Table 3.5 is shown the parameters which have been analysed in the different types of sludge samples. The parameters in red were not possible to analyse.

All the following parameters were measured in triplicate for all the sludge samples.

3.3.1 Total and Volatile Solid

The main procedure is described in Standard Method (209 F, APHA AWWA WPCF, 1985).

Clean tins were introduced in a muffle furnace at 550 °C for 1 hour. These tins were stored in desiccators until needed. Once the tins were cool, the tins were weighed (W1). Samples were pipette (25 ml of RAS, Test and Control or 10 ml of digested sludges) into the pre-weigh tins. After that the tins were placed into an oven at 105°C for 2 hours approximately (until all the liquid were evaporated). Then the tins were let cool in the desiccators, weighted (W2) again and transferred to the 500 °C muffle for at least 1 hour. Then the tins were cooled and weighted (W3). Concentration of TS and VS can be calculated using the following equations:

$$TS = (W2 - W1)/Volume \quad TS \text{ g/l}$$

$$VS = (W2 - W3)/Volume \quad VS \text{ g/l}$$

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Table 3.5 – Parameters analysed in any type of sludge

PARAMETRES	RAS	Test & Control Sludge	Digested Sludge (All samples)
Total solids	✓	✓	✓
Volatile solids	✓	✓	✓
pH	✓	✓	✓
Chemical Oxygen Demand	✓	✓	
Volatile Fatty Acids	✓	✓	✓
Alkalinity	✓	✓	✓
Ripley's ratio	✓	✓	✓
Particle size	✓	✓	✓
Density	✓	✓	✓
Viscosity	✓	✓	✓
N as NH_4^+	✓	✓	✓
S as S^{2-}	✓	✓	✓
P	✓	✓	✓
Fe	✓	✓	✓
Mg	✓	✓	✓
Ca	✓	✓	✓
Orthophosphate	✓	✓	✓
Biogas Volume			✓
CH ₄ %			✓
CO ₂ %			✓

3.3.2 Chemical Oxygen Demand (COD)

The COD is used as a measured of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.

The procedure selected to measure COD is the closed reflux colorimetric method described in Standards Methods (508 C, APHA AWWA WPCF, 1985).

This method works in the principle that most of the organic matter is oxidized by a boiling mixture of chromic and sulphuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate. After digestion, the remaining unreduced potassium dichromate Oxygen consumed in that reaction is measure against standards at 600 nm using a spectrophotometer, as the intensity of green colour generated is proportional to the concentration of oxygen in the sample.

Samples were diluted in order to be in the range of measure: 0-500 mg O₂/l.

2 ml of samples were pipetted into boiling tube, after that 0.1 ml of silver nitrate was added to avoid that chloride ions interfered in the process. FICODOX was the reagent used instead of potassium dichromate and sulphuric acid. So 3.8 ml of this reagent were added into the boiling tube and the caps were screwed. Ones everything inside the boiling tube was mixed carefully, the tubes were placed in a block digester preheated at 105 °C for 2 hours. Five standards were prepared to build the calibration curve: 100, 200, 300, 400 and 500 mg/l. The standard and the sample were prepared together, and the procedure was applied to both. A blank with DW were done as well. When the 2 hours were gone, the tubes were let cool before to measure the absorbance at 600 nm in the Cecil CE292 Series 2 spectrophotometer. A calibration curve was constructed and the concentration in the samples was calculated using the equation of the line. The results are expressed in O₂ mg/l.

3.3.3 Alkalinity and Ripley's Ratio

The method used is based in the titration method described in Standards Methods (403, APHA AWWA WPCF, 1985). The volume of sample took was 30 ml for RAS, Test and Control and 10 ml for all digested samples. The sample was pipetted into a beaker with a small magnet stirrer to keep the sample well mixed. This titration was realized with 0.1 N sulphuric acid normalized. The pH-meter was used as indicator of the end points of pH: 5.75, 4.5 and 4.3. The end point of pH 4.5 is used for total alkalinity calculation, while the orders end points are used to calculate the Ripley's Ratio just in digested samples.

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Alkalinity is a measure of the ability of a solution to neutralize acids to the equivalence point of carbonate or bicarbonate. Total Alkalinity (TA) is calculated as mg CaCO₃ using the following equation:

$$TA = \frac{Vac \times Nac \times 50000}{Vs}$$

Where:

TA = Total alkalinity (mg CaCO₃/l)

Vac = Volume of standard acid used to reach end point of pH 4.5 (ml)

Nac = Normality of standard acid used (0.1N)

Vs = Volume of sample used (ml)

Parameters as alkalinity or volatile fatty acids (VFAs) give an idea about the digester stability. Jenkins *et al.* (1983) defined Intermediate Alkalinity (IA) and Partial Alkalinity (PA). The alkalinity between pH 8.3 and 5.75 it considered as a PA, where around 80% of that is due to bicarbonate alkalinity and rest is due to VFAs and the alkalinity between pH 5.75 to 4.3 is called IA, where it is at the contrary, around the 80% is due to VFAs and the 20% account for bicarbonate alkalinity.

Ripley's Ratio (RR) is similar to the IA:PA ratio. Ripley *et al.* (1986) state that IA:PA values below 0.3 are good and values as high as 0.8 are indicative of a stressed digester. The equation to calculate RR is:

$$RR = \frac{V2 - V1}{V1}$$

Where:

RR = Ripley's Ratio

V1 = Volume of standard acid used to reach end point of pH 5.75 (ml)

V2 = Volume of standard acid used to reach end point of pH 4.3 (ml)

CHAPTER 4

RESULTS AND DISCUSSION

This chapter is split into three main sections, the first part is related to the second and third primary objectives, the second part is related to the fourth objective outline in Chapter 1, and finally in the third part, the results presented in the section earlier are discussed.

- In Section 4.1, different chemical forms of Fe, and different dosing ratios of Fe:P are investigated for their effect on metal fractionation and anaerobic digestion characteristics of activated sludge produced from a laboratory dosing unit.
- Comparison of Inorganic Fractions is presented in section 4.2.
- The results of this research are discussed in section 4.3.

4.1 Effect of different kind of iron-compounds at two molar ratios in the digestion (Objective 2 and 3)

This section contains a comparison of the biogas production from batch anaerobic digestion (AD) of Fe-dosed activated sludge (AS), when dosed with three different Fe salts. This was carried out to establish whether different salts of Fe at different concentrations have the same effect in the digestion. The dosage molar ratios Fe:P compared within this section are 1.2:1 and 0.6:1 and the three Fe salts used were FeCl_3 , $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 . The methods used are outlined in Chapter 3 Section 3.1.1.

The six experiments were done on different dates, as is shown in Table 4.1.

Table 4.1 – Date of experiments

Reagent	Ratio Fe:P	Sample Collection	Laboratory Fe dosing test	Anaerobic batch test
FeCl_3	1.2:1	19/01/2010	19/01/2010	20/01 – 02/02/2010
$\text{Fe}_2(\text{SO}_4)_3$	0.6:1	25/01/2010	25/01/2010	26/01 – 08/02/2010
FeSO_4	1.2:1	02/02/2010	02/02/2010	03 – 17/02/2010
$\text{Fe}_2(\text{SO}_4)_3$	1.2:1	25/05/2010	25/05/2010	26/05 – 07/06/2010
FeSO_4	0.6:1	01/06/2010	01/06/2010	02 – 14/07/2010
FeCl_3	0.6:1	02/06/2010	02/06/2010	03 – 15/07/2010

4.1.1 Biogas and methane production from batch digestion of laboratory iron-dosed activated sludge

Fe was dosed into the laboratory-scale aeration chamber, generating two samples of AS: the Test sample (Fe-dosed) and the Control sample (non Fe-dosed). Both Test and Control samples of AS were subjected to exactly the same procedure, with the exception of Fe-dosing. Hence, any differences between the two samples should result from Fe-dosing of that sludge. Test and Control AS samples were then anaerobically digested using serum bottle batch tests as described in section 3.1.2. The biogas volumes and methane fractions were measured as described in Chapter 3, section 3.1.2.1 and 3.1.2.2.

These experiments were done using sludge from Kidderminster WWT plant (RAS, SetS and digested sludge). Batch digestion test were carried out in triplicate. Serum bottles of 120 ml were used to carry out the batch tests. Biogas from the serum bottle digesters was measured typically over 11 or 12 days. Figures 4.1 to 4.6 show the average biogas production from triplicate digestion experiments, for FeCl_3 , $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 at the Fe:P ratios given in Table 4.1. 'Test' indicates results from batch digesters treating Fe-dosed AS, 'Control' indicates biogas production from batch digesters treating non-dosed AS and 'Dig' indicates the biogas produced from the digested sludge inoculum without any additional AS.

- Low ratio Fe:P 0.6:1

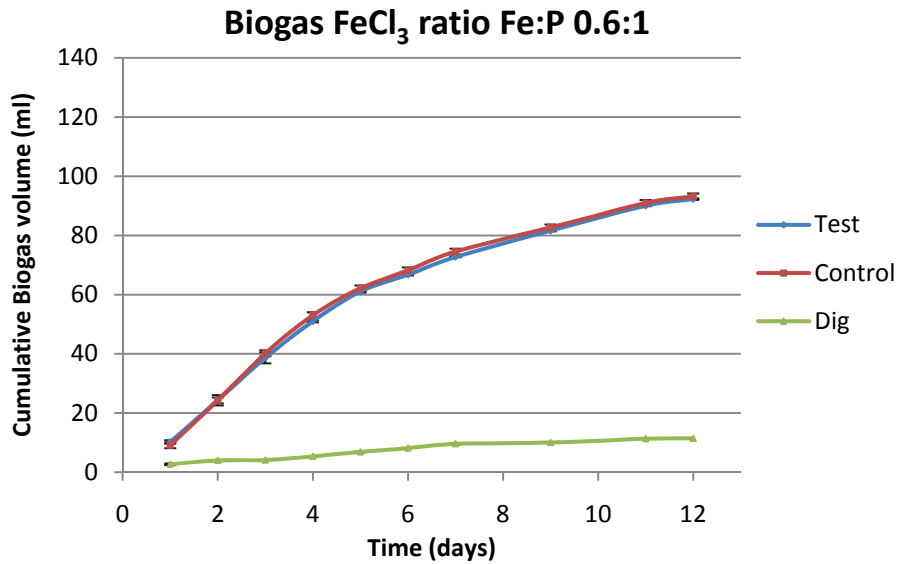


Figure 4.1 – Cumulative biogas volumes produced from the dosage of RAS with FeCl_3 using Fe:P molar ratio of 0.6:1

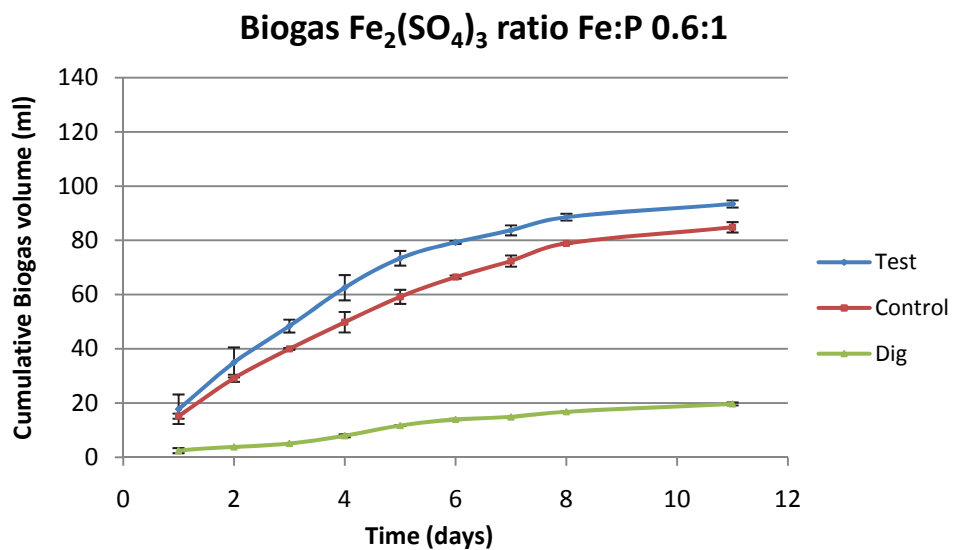


Figure 4.2 – Cumulative biogas volumes produced from the dosage of RAS with $\text{Fe}_2(\text{SO}_4)_3$ using Fe:P molar ratio of 0.6:1

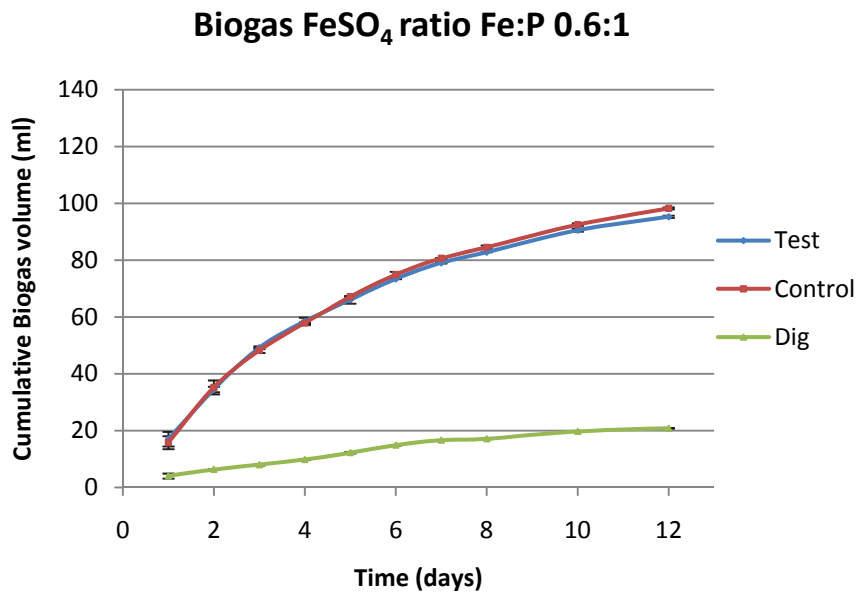


Figure 4.3 – Cumulative biogas volumes produced from the dosage of RAS with FeSO_4 using Fe:P molar ratio of 0.6:1

- High ratio Fe:P 1.2:1

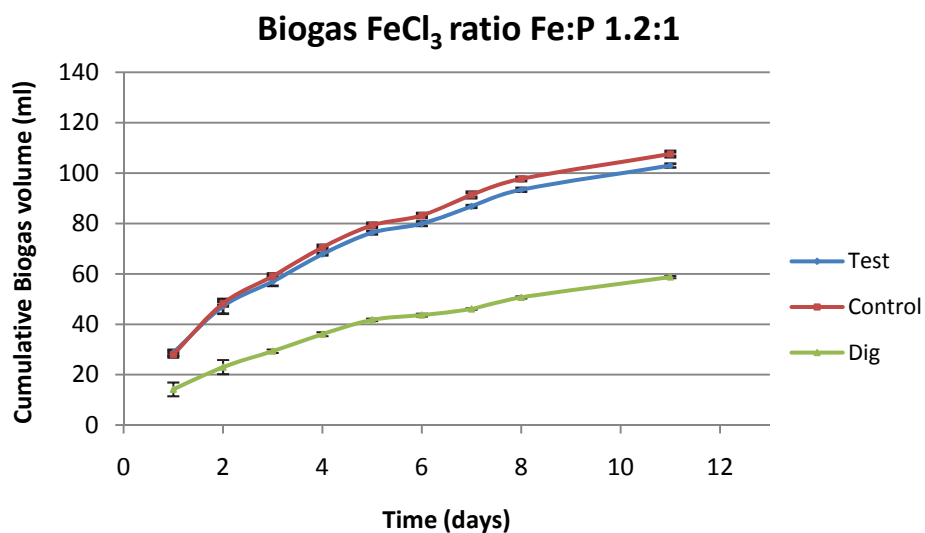


Figure 4.4 – Cumulative biogas volumes produced from the dosage of RAS with FeCl_3 using Fe:P molar ratio of 1.2:1

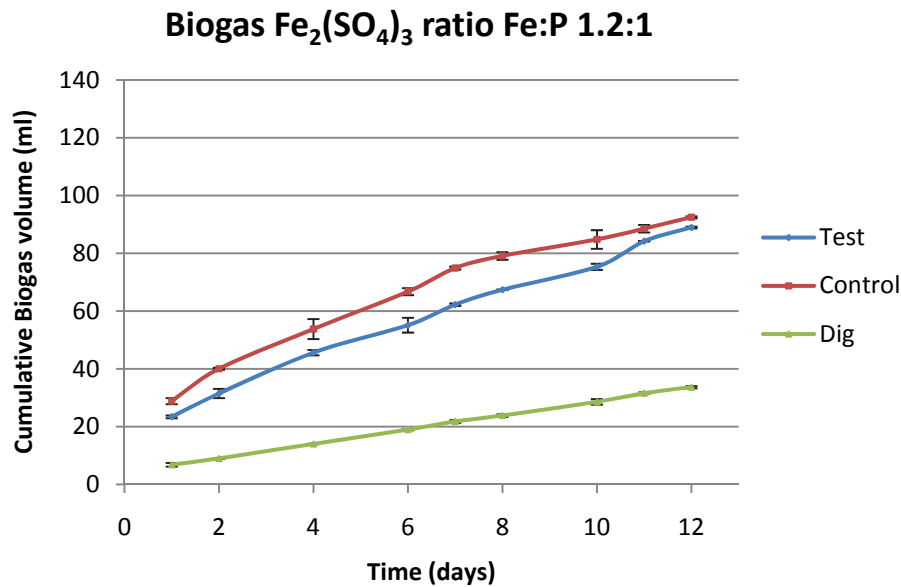


Figure 4.5 – Cumulative biogas volumes produced from the dosage of RAS with $\text{Fe}_2(\text{SO}_4)_3$ using Fe:P molar ratio of 1.2:1

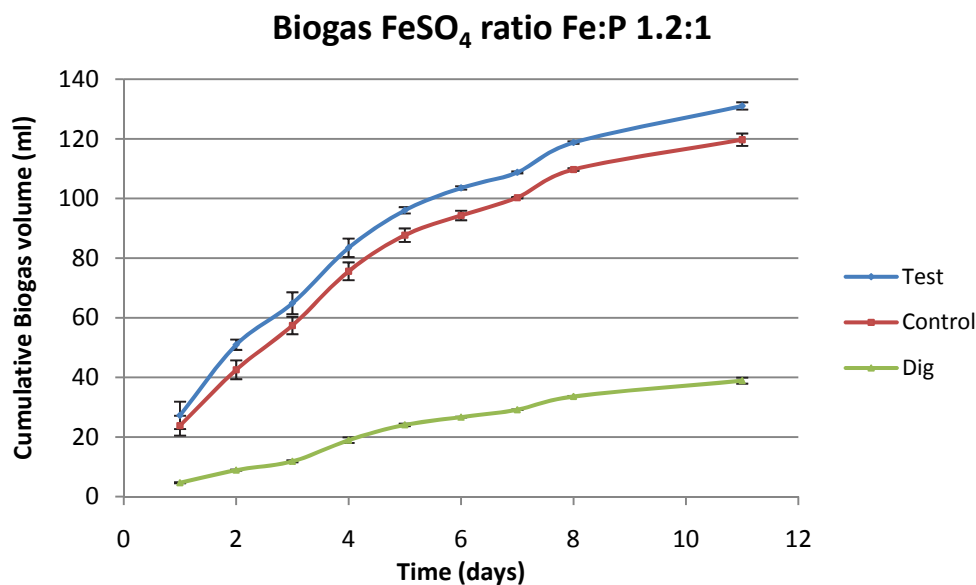


Figure 4.6 – Cumulative biogas volumes produced from the dosage of RAS with FeSO_4 using Fe:P molar ratio of 1.2:1

Figures 4.1 and 4.3 show that the biogas production for Test and Control AS samples was similar, specifically this difference was 1% and 3% in the

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experiments where FeCl_3 and FeSO_4 were used as a dosage at low ratio. This difference was also low, 5% and 4%, when FeCl_3 and $\text{Fe}_2(\text{SO}_4)_3$ were dosed at high ratio. Nevertheless, when $\text{Fe}_2(\text{SO}_4)_3$ was dosed at low ratio and FeSO_4 was added at high ratio this difference was 9%.

A two sample t Test was applied to all the samples to compare if the difference of biogas volume produced for Test and Control were significant. The result of this test with a significance level of 0.1 (90% confidence) resulted in no difference between Test and Control in the experiment where FeCl_3 was dosed at low ratio and $\text{Fe}_2(\text{SO}_4)_3$ was dosed at high ratio. However, this difference between both samples (Test and Control) was significant for $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 low ratio and FeCl_3 and FeSO_4 high ratio. Therefore, there were two experiments where Fe did not produce any significant effect (FeCl_3 low ratio and FeSO_4 high ratio), two experiments which showed that Fe decreased biogas production (FeSO_4 low ratio and FeCl_3 high ratio) and another two experiments where Fe produced an increase in biogas production. If 0.05 significance level is applied (95% confidence), the results obtained are different. At this level of significance there were just two experiments where the difference between the biogas generated for Test and Control was significant. These experiments are the experiments where Fe-dosed AS produced more biogas than non Fe-dosed AS ($\text{Fe}_2(\text{SO}_4)_3$ low ratio and FeSO_4 high ratio).

The interpretation and posterior discussion of the results of biogas production, net biogas production and methane production are based on the 90% confidence level.

Table 4.2 supplements Figures 4.1 to 4.6 and details the volumes of biogas produced for Test and Control samples.

From Table 4.2 it is apparent that the higher difference in the biogas production between Test and Control was 9%. In both experiments Control AS did not produce as much biogas as Test AS. In the other experiments, however, the opposite effect was observed, Control AS produced more biogas than Test AS, although this difference in the biogas generated was lower (3%-5%).

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Table 4.2 – Details of volume of biogas produced

	Iron salt	Biogas volume Test	Biogas volume Control	Trial duration	Increment ¹	Reduction ²	90% confidence level
Low ratio	FeCl ₃	92.30 ±1.86	93.20 ±1.64	12		1%	None difference
	Fe ₂ (SO ₄) ₃	93.44 ±2.72	84.84 ±2.60	11	9%		Significant different
	FeSO ₄	95.30 ±2.57	98.25 ±2.46	12		3%	Significant different
High ratio	FeCl ₃	107.89 ±2.91	113.84 ±1.81	11		5%	Significant different
	Fe ₂ (SO ₄) ₃	88.93 ±2.56	92.47 ±3.46	12		4%	None difference
	FeSO ₄	137.17 ±3.66	125.51 ±3.33	11	9%		Significant different
¹ Test AS produces more biogas than Control AS							
² Control AS produces more biogas than Test AS							

The rate of biogas production is other parameter which can be analysed. It is based on the biogas volume produced during the first 24 to 30 hours of the batch test and “gives an indication of either how hydrolysable the feed sludge was, or how acclimatised the seed sludge was to the feed” (Smith, 2006).

Table 4.3 – Rate (ml/day) of biogas production

	Iron salt	Test	Control	Dig	Test net ¹	Control net ¹
Low ratio	FeCl ₃	24.23 ±1.75	24.27 ±1.64	4.00 ±0.12	20.23	20.27
	Fe ₂ (SO ₄) ₃	35.00 ±1.84	29.16 ±1.32	3.80 ±0.08	31.20	25.36
	FeSO ₄	34.50 ±0.95	35.23 ±1.01	6.33 ±0.15	28.17	28.90
High ratio	FeCl ₃	41.15 ±1.16	41.26 ±0.90	19.10 ±0.70	22.05	22.16
	Fe ₂ (SO ₄) ₃	31.50 ±1.60	40.15 ±0.35	9.00 ±0.06	22.50	31.15
	FeSO ₄	44.00 ±1.74	38.56 ±2.33	7.40 ±0.73	36.60	31.16
¹ Net biogas production						

The greatest rate of biogas production was observed in the experiment where FeCl₃ and FeSO₄ were dosed at high ratio. This rate was pretty similar for Test (41.15 ml/day) and Control (41.26 ml/day) when FeCl₃ was added and different

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for FeSO_4 experiment (44.00 and 38.56 ml/day for Test and Control respectively).

It should also be noted that the biogas production for Dig and Control samples was different for each experiment as fresh digested sludge inoculum and RAS were collected for each experiment. Figures 4.7 and 4.8 represent the results obtained for these samples in the different experiments. Both figures show different volume of biogas generated depending on the experiment. In Figure 4.7 is appreciated that the digested sludge (“Dig”) used in the experiment where FeCl_3 was dosed at molar ratio Fe:P 1.2:1 produced 58.72 ml of biogas the greatest production, meanwhile the Dig used in the experiment where FeCl_3 was dosed at molar ratio Fe:P 0.6:1 produced 11.43 ml of biogas, the least biogas production. The use of different digested sludge inoculum introduces yet another variable into the process, so in order to avoid that, net biogas production was calculated, i.e. biogas produced from digestion of AS only, which is calculated by subtracting the Dig biogas production from the total biogas production. Net biogas volume is presented in Figure 4.9 and 4.10.

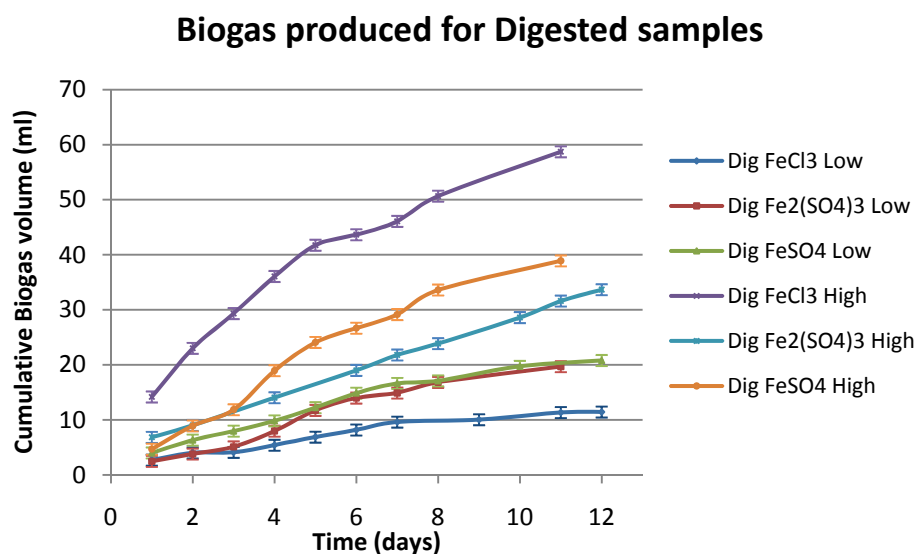


Figure 4.7 – Cumulative Biogas volume produced for Dig samples in the six experiments realized at low and high ratio

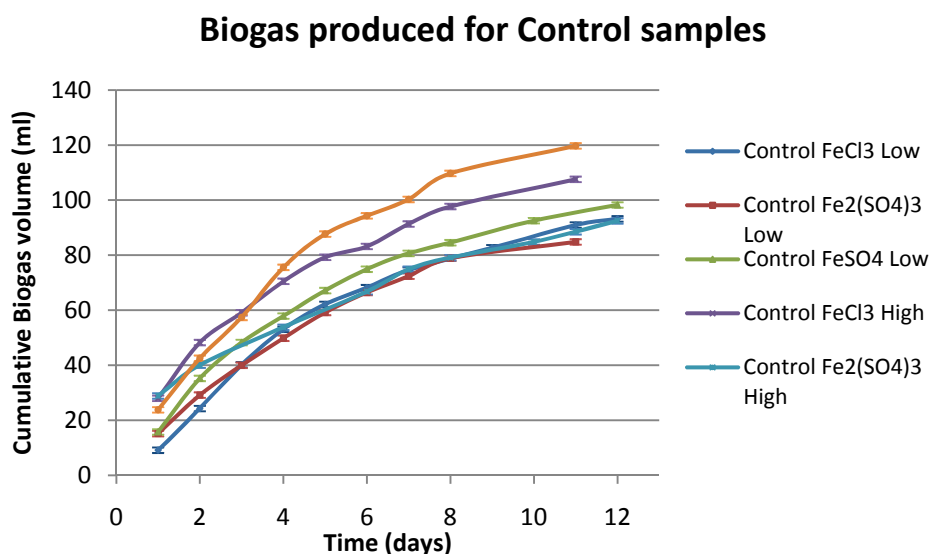


Figure 4.8 – Cumulative Biogas volume produced for Control samples in the six experiments realized at low and high ratio

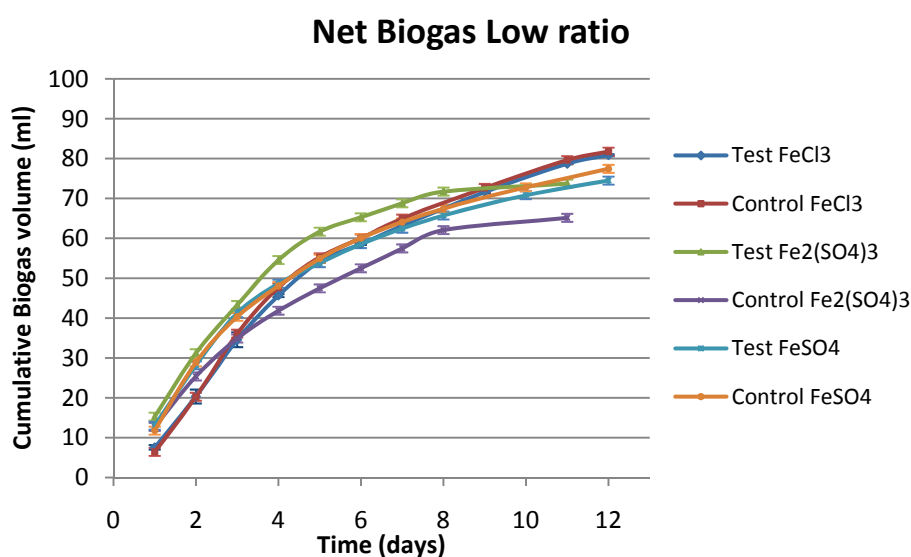


Figure 4.9 – Net Biogas Volume produced from simulated Fe-dosed sludge (Test) with FeCl₃, Fe₂(SO₄)₃ and FeSO₄ and non Fe-dosed sludge (Control) at molar ratio Fe:P 0.6:1

The net biogas production obtained using the three different Fe-salts at low Fe:P ratios were pretty similar in volume of biogas generated and tendency. Table 4.4 supplements Figure 4.9. In this table is appreciated that by the end of the trial the AS used in the experiment where FeCl₃ was dosed produced 81.77 ml and the iron-dosed AS 80.87 ml; the difference between both biogas productions was less than 1%, and this difference was not significant with a

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90% confidence level, reason why in this case Fe had no detrimental effect. However, using the same confidence level, when Fe was dosed as FeSO_4 , it reduced the production of biogas around 3.8%, (74.50 ml for Test versus 77.45 ml for Control), although when $\text{Fe}_2(\text{SO}_4)_3$ was added the opposite effect was observed, Fe produced an increase in the biogas production of 11.6% (73.75 ml and 65.16 ml for Test and Control respectively), higher than when FeSO_4 was dosed.

Table 4.4 – Net biogas volume and percentage of CH_4 at low ratio Fe:P 0.6:1

	FeCl_3		$\text{Fe}_2(\text{SO}_4)_3$		FeSO_4	
	Test	Control	Test	Control	Test	Control
Net Volume biogas (ml)	80.87	81.77	73.75	65.16	74.50	77.45
Net Volume biogas a day(ml/day)	6.74	6.81	6.71	5.92	6.21	6.45
Average % CH_4	59.81 ± 3.81	60.25 ± 1.31	62.55 ± 4.00	63.87 ± 3.41	67.25 ± 1.40	63.00 ± 3.20

When net biogas production is plotted for higher Fe:P ratios (Figure 4.10), the graph obtained is completely different. On the top of the graph highlight are the results obtained for the experiment where FeSO_4 was used as dosage, the net biogas production was 92.15 ml and 80.83 ml for Test and Control respectively. The net biogas generated using FeCl_3 and $\text{Fe}_2(\text{SO}_4)_3$ was much lower, 44.26 ml for Test and 48.36 ml for Control where the net volume generated in the experiment where FeCl_3 was dosed and 55.27 ml and 58.80 ml for Test and Control when $\text{Fe}_2(\text{SO}_4)_3$ was dosed. also In addition, the trend followed for Test and Control samples in the case of FeCl_3 and $\text{Fe}_2(\text{SO}_4)_3$ was unusual. Usually, the biogas production increases sharply at the beginning of the batch test and levels off at the end. In these cases (FeCl_3 and $\text{Fe}_2(\text{SO}_4)_3$) the biogas production was approximately the same during the whole experiment. It may be due to the RAS used in these experiments, because either Test or Control samples have the same performance. This reduction in the biogas production can be due to the sludge age. Roseff (2010) observed a reduction of 9% for sludge age from 1 to 4 days older and the curve obtained for the 4 days older sludge had the same trend that the one obtained in this research for FeCl_3 and $\text{Fe}_2(\text{SO}_4)_3$ AS. This theory is also supported for Bolzonella *et al.* (2005), who investigated biogas production of AS from AD with different sludge age and concluded that the longer sludge age, the lower volume of biogas.

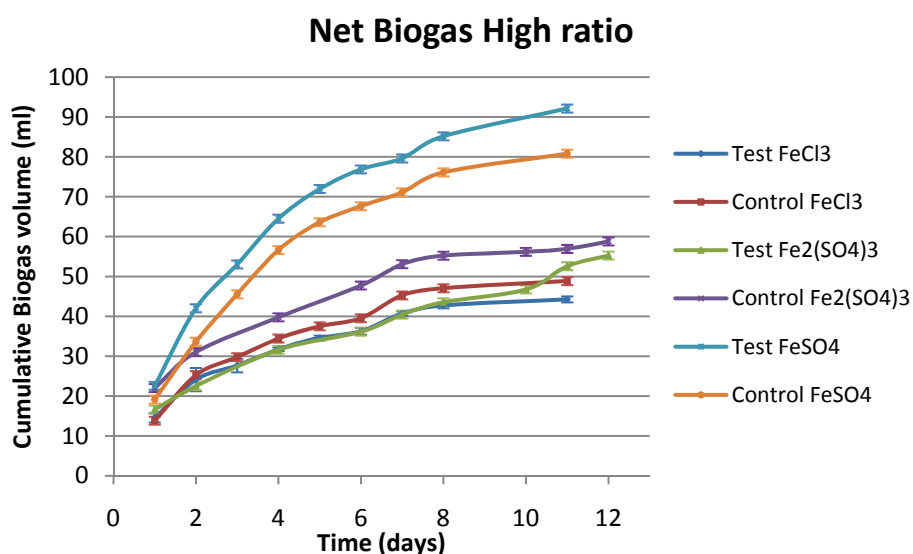


Figure 4.10 – Net Biogas Volume produced from simulated Fe-dosed sludge (Test) with FeCl₃, Fe₂(SO₄)₃ and FeSO₄ and non Fe-dosed sludge (Control) at molar ratio Fe:P 1.2:1

Table 4.5 – Net biogas volume and percentage of CH₄ at high ratio Fe:P 1.2:1

	FeCl ₃		Fe ₂ (SO ₄) ₃		FeSO ₄	
	Test	Control	Test	Control	Test	Control
Net Volume biogas (ml)	44.26	48.36	55.27	58.80	92.15	80.83
Net Volume biogas a day(ml/day)	4.02	4.44	4.61	4.90	8.38	7.29
Average % CH₄	73.68*	74.71*	66.50	70.67	68.22	69.45
	±1.81	±3.84	±3.05	±2.82	±5.79	±1.80
*These percentages are not representative because in this experiment the percentage of methane was measured just the last 4 days of the experiment.						

Figure 4.11 and 4.12 show methane production instate of biogas production for both ratios studied.

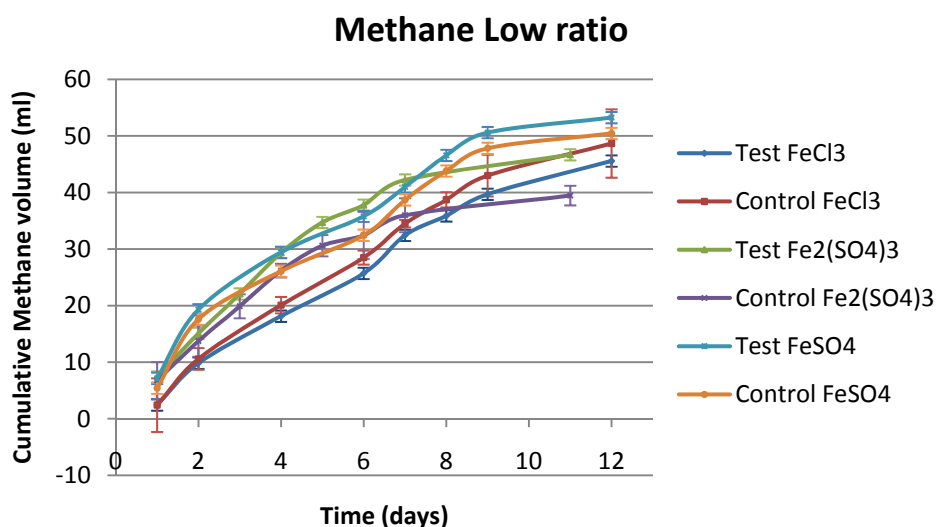


Figure 4.11 – Methane Volume produced from simulated Fe-dosed sludge (Test) with FeCl_3 , $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 and non Fe-dosed sludge (Control) at molar ratio Fe:P 0.6:1

Total methane production for experiments realised at molar ratio Fe:P 0.6:1 were within the range 46-54 ml. In the experiments where $\text{Fe}_2(\text{SO}_4)_3$ was dosed Test (50.56 ml) produced higher methane volume (90% confidence level) than Control (46.68 ml). The addition of Fe did not produce any effect on methane production (0.1 significant levels) in the experiment where FeCl_3 and FeSO_4 were added. The greatest methane production at this ratio was generated for the pair Test-Control when FeSO_4 was dosed, 53.25 ml and 50.45 ml for Test and Control respectively.

The methane production of Test and Control when $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 were dosed at high ratio were also different (90% confidence level). At this ratio methane production of Control (60.71 ml) was higher than Test (54.11 ml) when $\text{Fe}_2(\text{SO}_4)_3$ was dosed and when FeSO_4 was added, on the contrary, Test (81.85 ml) generated higher volume of methane than Control (77.41 ml). The last experiment mentioned produced the higher methane volume at the end of the experiment for both samples (Test and Control) matching with the greatest biogas production, 131.05 ml (Test) and 119.73 ml (Control), but not with the greatest percentage of average of methane in the biogas, 70.67% for Control in the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at high ratio and 68.22% for Test in the experiment where FeSO_4 were added at high ratio.

What it cannot be appreciated neither from Figure 4.11 and 4.12 are that Test and Control samples produced the same percentage of methane in biogas (90% confidence level), except in the case of FeSO_4 low ratio and $\text{Fe}_2(\text{SO}_4)_3$ high ratio where this difference was significant. In the experiment FeSO_4 low

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ratio Test produced higher percentage of methane and on the contrary the highest percentage of methane was produced for Control in the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at high ratio. This information is detailed in the Tables 4.4 and 4.5.

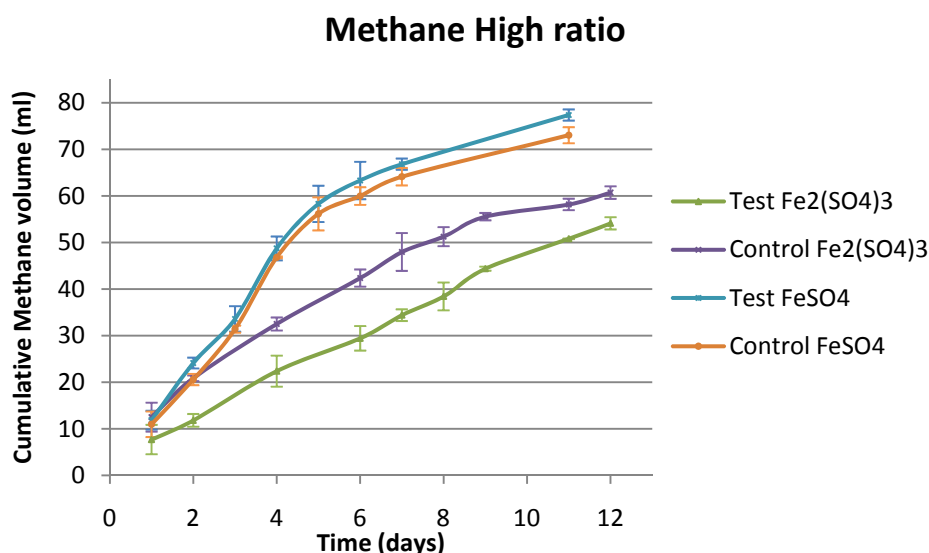


Figure 4.12¹ – Methane Volume produced from simulated -dosed sludge (Test) with $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 and non Fe-dosed sludge (Control) at molar ratio Fe:P 1.2:1

In order to compare the methane volume generated in the experiment where FeCl_3 was dosed at high ratio, the methane volume of last four days of the experiment has been calculated. This volume is illustrated in Table 4.6.

Table 4.6 – Methane production during the last four days of the trials

Fe salt	Low ratio		High ratio	
	Test	Control	Test	Control
FeCl_3	19.68 ml	19.52 ml	19.63 ml	21.55 ml
$\text{Fe}_2(\text{SO}_4)_3$	20.01 ml	17.33 ml	21.37 ml	15.81 ml
FeSO_4	17.63 ml	17.03 ml	19.05 ml	16.89 ml

¹ FeCl_3 results are not show in this graph due to the methane and carbon dioxide fractions were not possible measure during the first days of the trial.

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From Table 4.6 is appreciated that the methane production during the last four days of the trials in the experiments where FeCl_3 and FeSO_4 was dosed at low and high ratio was similar for Test and Control (no significant difference for 90% confidence level). However, this difference between both samples was significant in the experiments where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at both ratios, beside of Test (20.21 ml low ratio and 21.37 ml high ratio) produced more methane than Control (17.33 ml low ratio and 15.81 ml high ratio). Should be noted that the total methane production in the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was added at high ratio was higher for Control 60.71 versus 54.11 ml for Test which indicated that at the effect of Fe in the trial was not the same during the whole trial. This observation is discussed in section 4.3.

In addition to volume of biogas generated and methane fraction, there are other parameters which influence digestion. These parameters are presented in Table 4.7 and 4.8. In these tables is apparent that pH values after dosage (pH feed) were within the working range 7-8. This pH increased after digestion in all the experiments, it is most likely to be due to the accumulation of NH_4^+ , although this was not measured during these experiments. The values of alkalinity for Test and Control samples after dosing were in the range of 200-300 mgCaCO_3/l and 2000-2500 mgCaCO_3/l after digestion. These values are within the optimum working range. Higher values of alkalinity were found in Control samples before and after digestion in all the experiments. It indicates that the addition of iron salts into the aeration tank produces a decrease on alkalinity. Ripley's Ratio was also measured in order to have an idea about the digester stability; the value of this parameter was lower than 0.3 in all the experiment realised, which according to Ripley *et al.* (1986) indicated stable digestion.

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Table 4.7 – Parameters of the laboratory Fe-dosed batch test at low ratio Fe:P 0.6:1

Sample	FeCl ₃			Fe ₂ (SO ₄) ₃			FeSO ₄		
	Test	Control	Dig	Test	Control	Dig	Test	Control	Dig
pH feed	7.54 ±0.02	7.77 ±0.01	7.83 ±0.06	7.36 ±0.06	7.82 ±0.01	7.93 ±0.10	7.41 ±0.02	7.50 ±0.02	7.83 ±0.06
Alkalinity after dosing	239 ±19.3	289 ±19.3	3339 ±9.0	186 ±5.0	222 ±16.5	3392 ±39.7	189 ±19.3	194 ±9.6	3389 ±9.0
Biogas Volume (ml)	92.30 ±0.60	93.20 ±2.50	11.43 ±0.46	93.44 ±2.72	84.84 ±7.30	19.68 ±0.96	95.30 ±3.09	98.25 ±5.73	20.80 ±0.63
Net Biogas Vol (ml)	80.87	81.77		73.75	65.16		74.50	77.45	
Average of % CH ₄ in biogas	59.81 ±3.81	60.25 ±1.31	17.08 ±1.51	62.55 ±4.00	63.87 ±3.14	27.77 ±33.00	67.25 ±1.40	63.00 ±3.20	38.95 ±1.57
Methane Volume (ml)	46.87 ±1.31	48.67 ±1.51	1.30 ±1.30	50.56 ±3.92	46.68 ±3.44	4.67 ±2.06	53.25 ±3.40	50.45 ±5.19	5.19 ±1.57
Net Volume of CH ₄ (ml)	45.57	47.37		45.89	42.01		48.06	45.25	
% VS destroyed	52.56	54.33	43.00	51.00	52.89	38.20	35.00	36.11	23.00
Ripley's Ratio	0.18 ±0.06	0.16 ±0.02	0.27 ±0.01	0.18 ±0.08	0.20 ±0.09	0.14 ±0.02	0.23 ±0.02	0.28 ±0.00	0.29 ±0.02
pH post digestion	8.46 ±0.01	8.49 ±0.01	7.91 ±0.01	8.45 ±0.08	8.49 ±0.02	8.38 ±0.02	8.46 ±0.01	8.47 ±0.02	7.78 ±0.01
Alkalinity after digestion	2050 ±35.4	2283 ±56.4	1983 ±44.1	1383 ±57.8	1550 ±66.8	1950 ±51.7	2033 ±35.4	2217 ±60.7	2072 ±74.7
% COD removal	90.77	89.75		93.23	92.21		94.31	92.70	

The experiments done at the lower Fe:P ratio, the percentage of VS destroyed was high (35-50%) and quite similar for Test and Control samples. Although the percentage of VS destroyed for Control samples was slightly higher, around 3.5%. Nevertheless, the percentage of VS destroyed for samples dosed at ratio Fe:P 1.2:1 was lower than the samples dosed allow ratio, this reduction was in the range of 16-38%. Moreover at this ratio is appreciated the same difference between Test and Control samples, where percentage of VS destroyed was higher for Control samples, although this difference was 4% where FeCl₃ was dosed and 13% and 12% for the experiments where Fe₂(SO₄)₃ and FeSO₄ were

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dosed respectively. The percentage of VS destroyed for Dig samples was pretty lower.

Table 4.8 – Parameters of the laboratory Fe-dosed batch test at high ratio Fe:P 1.2:1

Sample	FeCl ₃			Fe ₂ (SO ₄) ₃			FeSO ₄		
	Test	Control	Dig	Test	Control	Dig	Test	Control	Dig
pH feed	7.69 ±0.05	7.55 ±0.02	7.84 ±0.03	7.48 ±0.07	7.75 ±0.05	8.11 ±0.10	7.42 ±0.06	7.51 ±0.05	7.53 ±0.02
Alkalinity after dosing	433 ±0.0	450 ±16.7	3467 ±68.1	200 ±0.0	261 ±9.6	3378 ±77.0	278 ±28.8	289 ±47.1	3789 ±41.0
Biogas Volume (ml)	102.99 ±7.14	107.58 ±1.81	58.72 ±4.23	91.87 ±2.39	95.60 ±3.21	35.73 ±1.88	131.05 ±20.52	119.73 ±7.37	38.90 ±1.75
Net Biogas Vol (ml)	44.26	48.36		55.27	58.80		92.15	80.83	
Average of % CH ₄ in biogas	73.68 ¹ ±5.07	74.71 ¹ ±3.84	69.29 ¹ ±4.37	66.50 ±3.05	70.67 ±2.82	44.68 ±0.74	68.22 ±5.79	69.45 ±1.80	52.75 ±7.71
Methane Volume (ml)				54.22 ±3.05	60.71 ±2.82	13.81 ±0.74	81.85 ±5.79	77.41 ±1.80	19.58 ±2.77
Net Volume of CH ₄ (ml)	40.37	44.40		40.30	46.90		62.27	57.83	
% VS destroyed	43.56	45.55	2.67	21.67	24.89	8.71	31.00	35.22	3.60
Ripley's Ratio	0.26 ±0.05	0.27 ±0.04	0.20 ±0.03	0.29 ±0.00	0.21 ±0.00	0.21 ±0.02	0.22 ±0.03	0.22 ±0.03	0.22 ±0.06
pH post digestion	8.41 ±0.04	8.40 ±0.05	8.26 ±0.13	8.44 ±0.01	8.48 ±0.04	8.77 ±0.04	8.38 ±0.05	8.28 ±0.02	8.06 ±0.06
Alkalinity after digestion	2048 ±67.8	2233 ±60.7	2992 ±77.0	2417 ±82.8	2467 ±28.8	2400 ±86.6	2017 ±25.8	2100 ±35.4	2017 ±22.5
% COD removal	85.08	87.89		94.58	93.86		89.05	87.86	

¹ Methane and carbon dioxide fraction in the biogas was not possible to measure during the first days of the trial; therefore these values cannot be compared with the results of the other experiments.

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Summary:

The addition of Fe to AS had not generated a pattern, it means each experiment had a different result. Fe had a different effect in the biogas production (90% confidence level) as is shown below:

No effect: FeCl_3 at low Fe:P ratio and $\text{Fe}_2(\text{SO}_4)_3$ high Fe:P ratio.

Detrimental effect: FeSO_4 low Fe:P ratio and FeCl_3 high Fe:P ratio.

Improvement effect: $\text{Fe}_2(\text{SO}_4)_3$ low Fe:P ratio and FeSO_4 high Fe:P ratio.

Marking that, the difference in the biogas production when the improvement effect was shown was 9% (net biogas volume) in both experiments and however in the experiments where Fe produced detrimental effect, this difference was between 3-5%.

The AS used when FeSO_4 was dosed at molar ratio Fe:P 1.2:1 produced the greatest net volume of biogas. In this experiment non Fe-dosed sludge (80.83 ml over 11 days) did not produce as much biogas as Fe-dosed sludge (92.15 ml over 11 days). Fe produced the same effect (increased biogas production) in the experiment where $\text{Fe}_2(\text{SO}_4)_2$ was dosed at molar ratio Fe:P 0.6:1. The addition of Fe into the process produced an increase in the biogas production of 8.59 ml meanwhile this increase was 11.32 ml when FeSO_4 was dosed at molar ratio Fe:P 1.2:1.

Having a look net volume of biogas generated in the different experiments, the AS used in the experiment where FeSO_4 was dosed at molar ratio Fe:P 1.2:1 can be compared (net volume of biogas) with the AS used when FeCl_3 was dosed at molar ratio Fe:P 0.6:1, as the non Fe-dosed AS produced 81.77 ml (over 11 days) meanwhile the AS used in the FeSO_4 experiment produced 80.83 ml. Nonetheless, Fe had no effect on AD in the FeCl_3 experiment (90% confidence level).

However if methane production results are analysed (Table 4.6, 4.7 and 4.8) the effect of Fe on AD (90% confidence level) is pointed below:

No effect: FeCl_3 and FeSO_4 low Fe:P ratio.

Detrimental effect: $\text{Fe}_2(\text{SO}_4)_3$ high ratio.

Improvement effect: $\text{Fe}_2(\text{SO}_4)_3$ low ratio and FeSO_4 high ratio.

As when biogas production was compared, there are two experiments where the addition of Fe enhanced methane production. The highest methane production was observed in the experiment where FeSO_4 was dosed at high ratio for both samples, Test (81.85 ml) and Control (77.41 ml).

Variables not associated with Fe were introduced in the experiments. These variables may impact AD, hence the comparison of the experiments is difficult. Factors as sludge age, bioavailable P or soluble Fe can influence the digestion and therefore the answer of the system due to the addition of Fe can be different. This issue cited above is discussed in section 4.3.

4.2 Comparison of Inorganic Fractions (Objective 3)

RAS, Test, Control, Test digested and Control digested samples, were sequentially extracted to separate Fe and P into different fractions. These samples can be divided into two groups, depending on digestion. Profiles of RAS, Test and Control represent samples before digestion, while Test Dig and Control Dig profiles were done after digestion. The Fe and P fractionation profiles of these samples were compared alongside fractionation obtained for the different iron salts.

4.2.1 Iron fractions

These fractions give an idea about the relationship between Fe and other compounds and how this Fe interacts with MOs in the digester. The addition of Supernatant, UPW and KNO_3 fraction are considered to be bioavailable. This means that MOs can access to this proportion of Fe in the sludge. Soluble Fe will be extracted in the Supernatant and UPW fraction, meanwhile KNO_3 will extract Fe bound to sludge. Fe organically bound will be extracted on NaOH fraction, while Na-EDTA reagent will extract Fe associated with carbonates and phosphates (Fe-phosphates, Fe-hydroxides and Fe-hydroxy-phosphates) and Fe associated with sulphides will be extracted in the HNO_3 and Residual fractions.

Figures from 4.13 to 4.18 illustrate the concentration and distribution of Fe. The numbers on the top of the bars are the total concentration of Fe (as the addition of the fractions) in each sample and the numbers inside of the bars indicated the concentration for each fraction. In these figures concentration is reported as mg Fe per gram of TS.

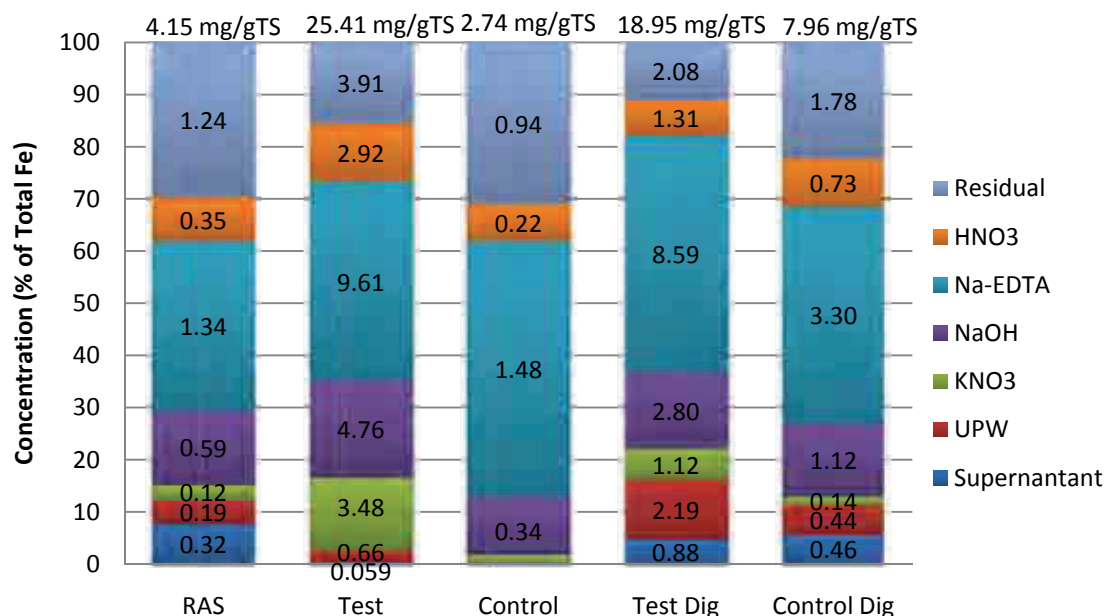


Figure 4.13 – Iron fractionation profiles showing the relative differences between samples before and after digestion for FeCl_3 at Fe:P dosing ratio of 0.6:1

In the Figure 4.13 is apparent that the bioavailable fraction in Control before digestion was really low (2%) and the Residual fraction quite high (31%). The percentage of Fe in the bioavailable fraction for Test increased from 16.5% to 22.1%, nevertheless the concentration of Fe in the fraction was the same before and after digestion, 4.2 mgFe/gTS. The content of Fe in the KNO_3 fraction for Test was higher than the content in RAS and Control, 18% and 3% respectively, however, the content of Fe in the Residual fraction of Test sample was lower (15%) than the content of Fe in RAS (30%) and Control (31%). The proportion of Fe in the Na-EDTA fraction increased after digestion and also bioavailable fraction. There was more Fe binding phosphates and hydroxides (Na-EDTA) and more Fe available for MOs after digestion. The digested sludge used in this experiment as inoculum was the one which generated the lowest volume of biogas, 11.43 ml.

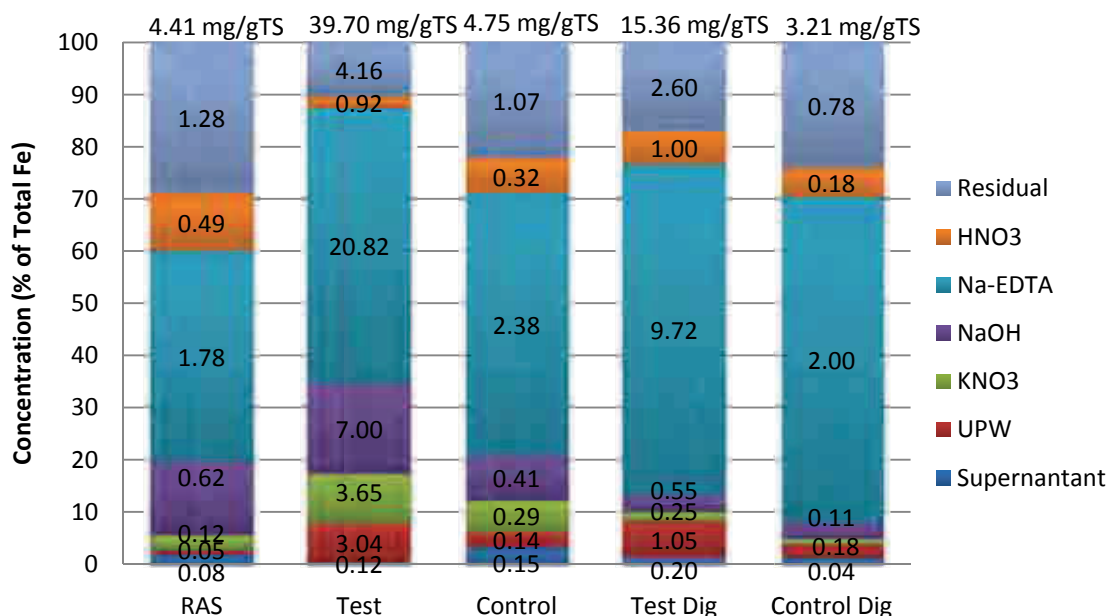


Figure 4.14 – Iron fractionation profiles showing the relative differences between samples before and after digestion for $\text{Fe}_2(\text{SO}_4)_3$ at Fe:P dosing ratio of 0.6:1

When $\text{Fe}_2(\text{SO}_4)_3$ was used as dosage (Figure 4.14) and Test and Control profiles are compared it is apparent that there was an increase in the Fe organically bound (NaOH fraction) and the soluble Fe (UPW fraction) and also the Fe bound to sulphides (HNO_3 fraction and in the Residual fraction). After digestion HNO_3 fraction, Na-EDTA fraction and Residual increased, however NaOH and bioavailable fraction declined. The Fe contained in the bioavailable fraction seems to be used by MOs and the Fe in the NaOH fraction redistributed to the Na-EDTA and Residual fractions.

In Figure 4.15 highlight the proportion of bioavailable fraction in all the fractionation profiles represented. Bioavailable fraction of Control before digestion was unusually high 1.09 mgFe/gTS taking 26.2% of the total Fe extracted, it likely to be due to the high concentration of Fe in the SetS used in this experiment. The high percentage of bioavailable fraction (20.9% for Test and 26.2% for Control) and NaOH fraction (17.9% and 16.4% Test and Control respectively) after digestion were also unusual. The Residual fraction of Control was higher than Test before and after digestion, 26.4% and 25.8% for Control and 14.6% and 19.2% for Test.

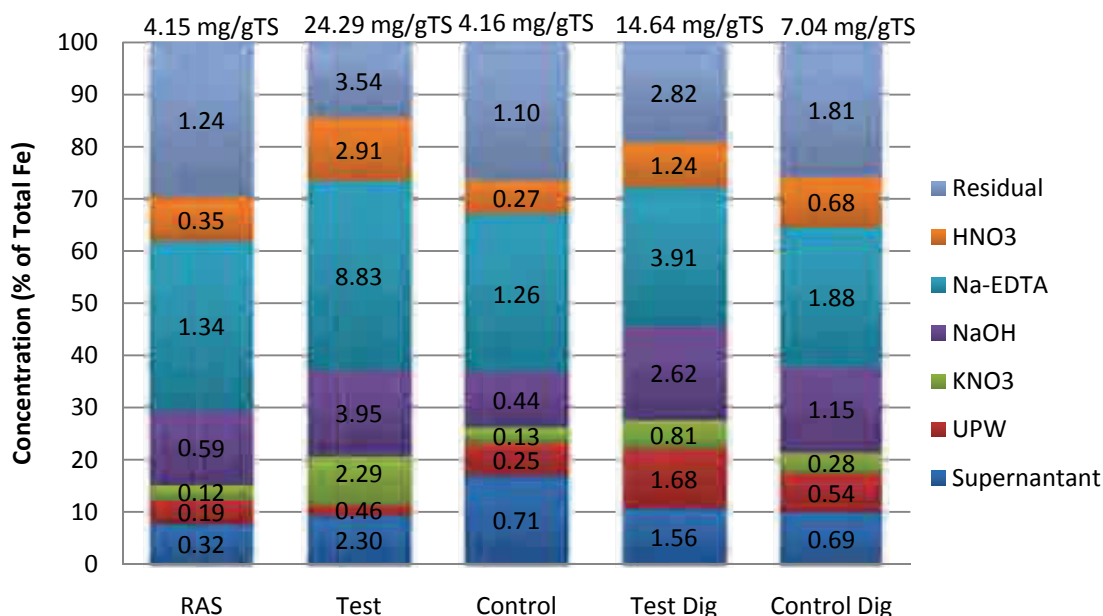


Figure 4.15 – Iron fractionation profiles showing the relative differences between samples before and after digestion for FeSO_4 at at Fe:P dosing ratio of 0.6:1

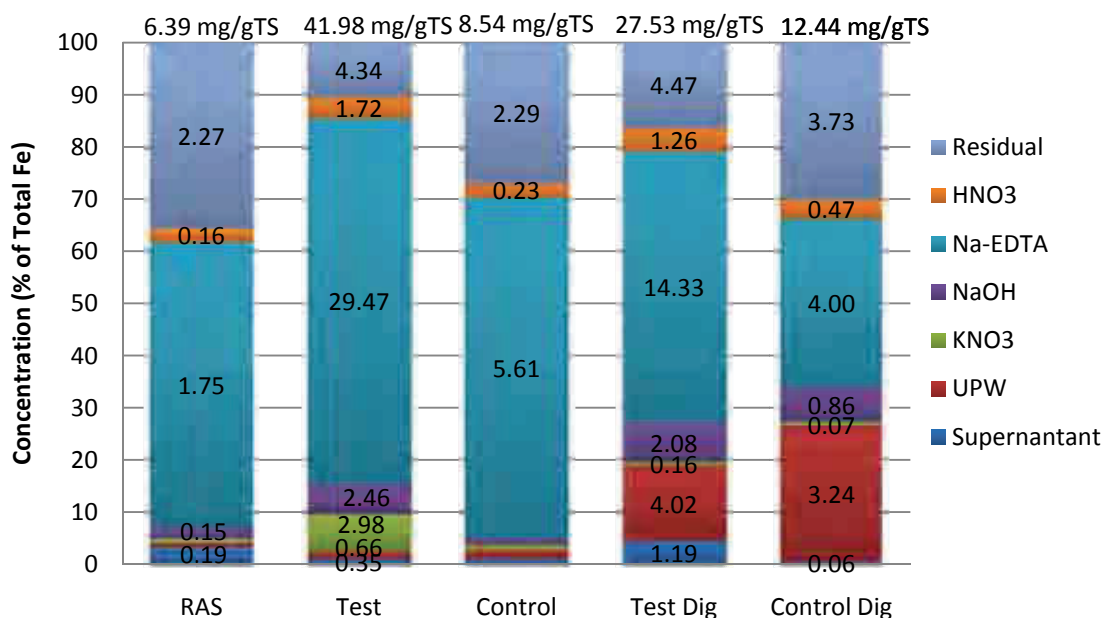


Figure 4.16 – Iron fractionation profiles showing the relative differences between samples before and after digestion for FeCl_3 at Fe:P dosing ratio of 1.2:1

In Figure 4.16 is observed that the small proportion of HNO_3 was conserved before and after digestion. However the Na-EDTA fraction was huge before digestion, taking 68.1% and 63.2% for Test and Control respectively. This

proportion of Fe in the Na-EDTA fraction dropped after digestion due to mainly the increase of Fe in the UPW fraction. The digested sludge used in this experiment produced the greater volume of biogas, which can be related to the high amount of Fe found in the UPW fraction after digestion. It means that during digestion soluble Fe was released or the content of soluble Fe in the digested sludge used in this experiment was elevated. Both RAS and Control presented low concentration of Fe in the bioavailable fraction (4.8% and 3.7%), meanwhile this concentration was a bit higher for Test (9.5%) due to the Fe extracted in the KNO_3 fraction.

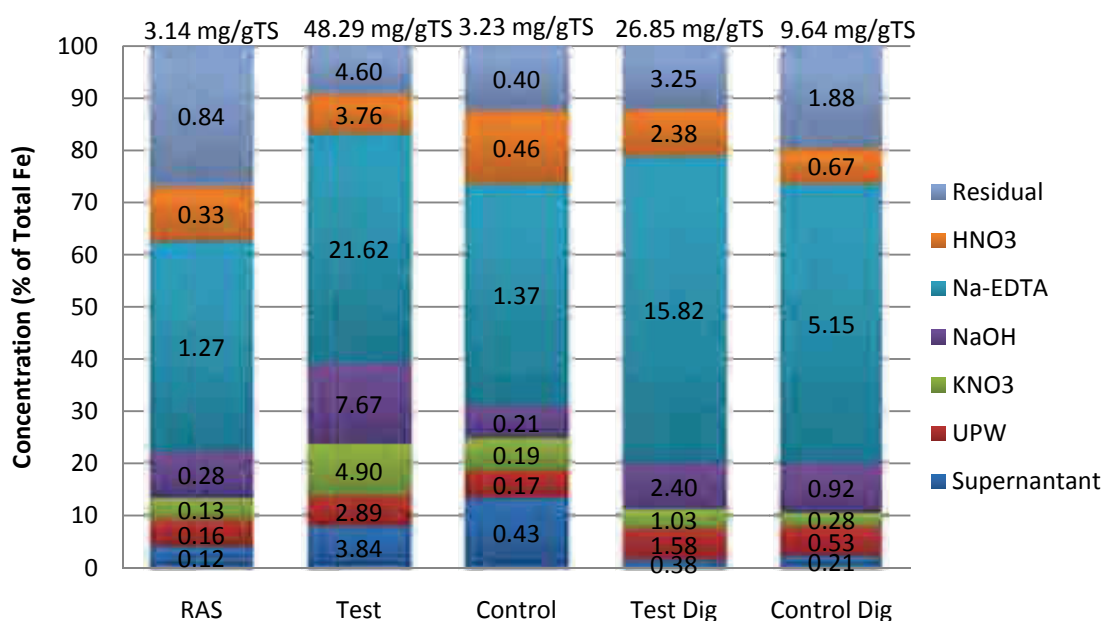


Figure 4.17 – Iron fractionation profiles showing the relative differences between samples before and after digestion for $\text{Fe}_2(\text{SO}_4)_3$ at Fe:P dosing ratio of 1.2:1

From Figure 4.17 is appreciated that bioavailable fraction of Test and Control before digestion was 23.6% and 24.6% respectively. This percentage of Fe in this fraction decreased after digestion to 11.1% and 10.6% for Test and Control. However, the content of Fe in the Na-EDTA fraction increased after digestion from 43.9% (21.6 mgFe/gTS) to 58.9% (15.8 mgFe/gTS) for Test samples and from 42.3% (1.4 mgFe/gTS) to 53.4% (5.2 mgFe/gTS); therefore the concentration of Fe of Test samples fell while the concentration of Fe of Control increased.

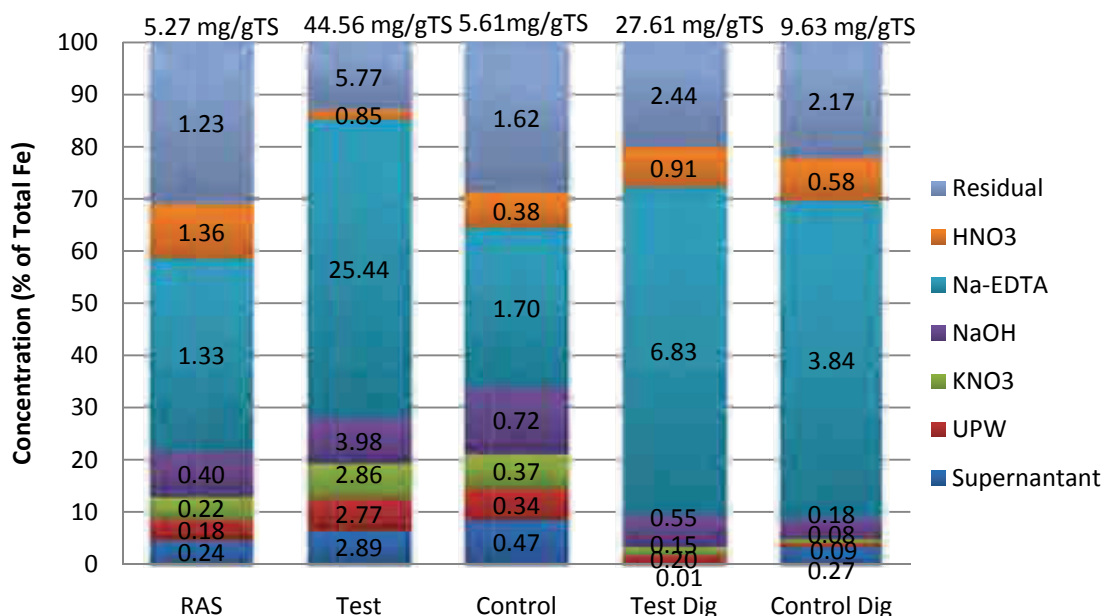


Figure 4.18 – Iron fractionation profiles showing the relative differences between samples before and after digestion for FeSO_4 at Fe:P dosing ratio of 1.2:1

In Figure 4.18 is observed the same trend for bioavailable and Residual fractions that was observed in the experiment above. Bioavailable fraction was 10% higher for Control sample before digestion. This fraction was 21.2% for Control and 11.1% for Test. In addition, the percentage of Fe in this fraction decreased after digestion for both digested samples, taking only 3.3% and 6.1% for Test and Control respectively. However, the percentage of Fe in the Na-EDTA fraction increased after digestion in 14% for Test and 23% for Control.

Should be remarked the low concentration of Fe after digestion in this experiment, 0.36 mgFe/gTS and 0.44 mgFe/gTS for Test and Control respectively. In addition to Test and Control AS used in this experiment generated the greatest volume of biogas. But, Control AS (119 ml) did not generate as much biogas as Test AS (131 ml).

The figure 4.19 shows the fractionation profiles of the different RAS used in this research. Residual fractions were pretty high in the range between 24.8% and 49%, which is most likely be due to a considerable portion of Fe has not able to be extracted in the fractions before. Na-EDTA fractions were also high what indicated that most of Fe was removed as Fe-hydroxides, Fe-phosphates, Fe-hydroxy phosphates or Fe-carbonates. The HNO_3 fraction in the case of FeSO_4 H is pretty high, 27.4% (1.36 mgFe/gTS) in comparison with the rest of experiments. The higher production of biogas matches with the use of this RAS.

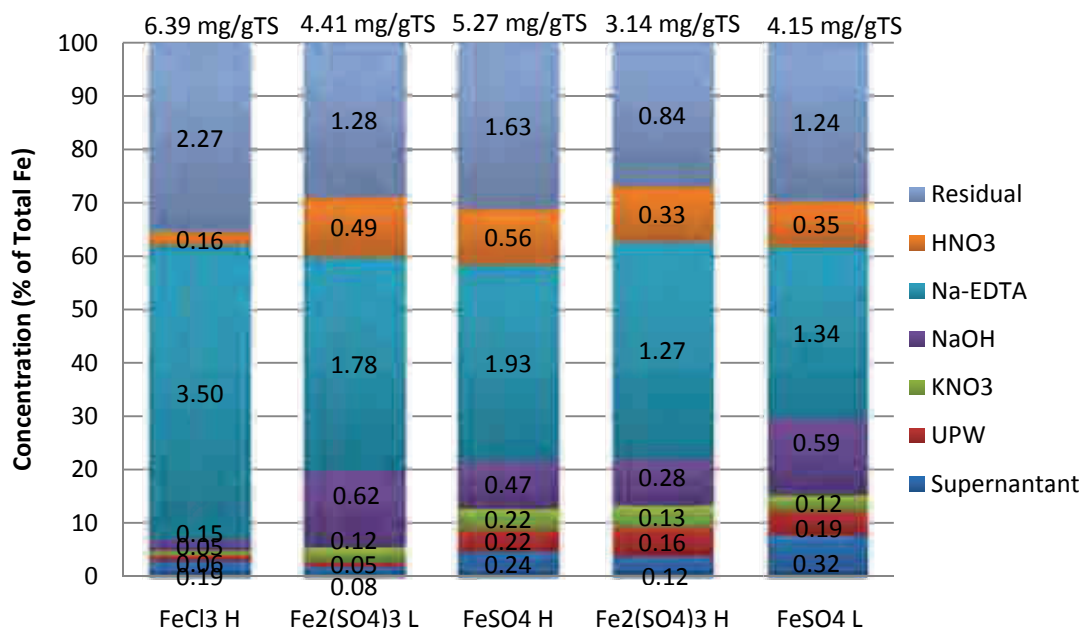


Figure 4.19 – Iron fractionation profiles showing the relative differences among the different RAS² used in the laboratory Fe-dosing experiments

Total Fe concentration of Test samples generated in this research was lower than the typical Fe-dosing RAS due to the typical molar ratio Fe:P used for WWT plant is 2:1 higher than the ratios used in this research, 0.6:1 and 1.2:1. Total Fe concentration measured were similar than total Fe concentration generated for the mass balance calculation. These calculation are described below for the experiment where FeCl₃ was dosed at molar ratio Fe:P 0.6:1.

- Concentration of Fe added was 80.21 mgFe/l, which was equivalent to 21.33 mgFe/gTS as the TS concentration was 3.76 g/l.
- The total Fe concentration measured for Test and Control was 24.18 mgFe/TS and 2.86 mgFe/gTS, hence the calculated total Fe concentration in the Test AS should be: $2.86 + 21.32 = 24.19$ mgFe/gTS

Total Fe concentration calculated 24.19 mgFe/gTS was the same as total Fe concentration measured 24.18 mgFe/gTS. In Table 4.9, the mass balance calculation results are detailed for all experiments. The highest difference between total Fe concentration measured and calculated was 0.05 mgFe/gTS.

² The same RAS was used for the experiments where FeCl₃ and FeSO₄ were used as dosage at low ratio.

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Table 4.9 – Mass balance calculation of the total iron concentration

		Measured		Calculated	
	Fe salt	Fe in Test	Fe in Control	Fe added	Fe in Test
Low ratio	FeCl ₃	24.18	2.86	21.33	24.19
	Fe ₂ (SO ₄) ₃	40.64	4.92	35.76	40.68
	FeSO ₄	25.22	3.29	21.90	25.19
High ratio	FeCl ₃	41.25	6.59	34.64	41.23
	Fe ₂ (SO ₄) ₃	49.29	3.23	46.01	49.23
	FeSO ₄	47.60	5.69	41.90	47.59

* All units are in mgFe/gTS

Summary:

In all the figures shown above the Residual fraction of Control samples before and after digestion was always higher than Test samples. It can be likely due there was a high portion of Fe associated to sulphide or the extractants were not able to extract the Fe from the fractions before, probably due to flocs properties rather than the saturation of the reagent, because the total content of Fe in Test samples was higher in all the experiments although the percentage of the Residual fraction was lower in these samples. The bioavailable fraction decreased after digestion for Test and Control samples in the experiments where AS was dosed with Fe₂(SO₄)₃ at low and high ratio and FeSO₄ high ratio. Meanwhile, this fraction increased after digestion in the other three experiments, FeCl₃ low and high ratio and FeSO₄ low ratio.

The majority of the additional Fe in the Fe-dosed AS was mainly removed in the NaOH and Na-EDTA fractions, it means that most of the Fe was organically bound or associated with phosphate and hydroxide. More Fe was extracted in the Na-EDTA fraction in Fe-dosed AS (53%) than in non Fe-dosed AS (41%). If the NaOH extractant was saturated with Fe, it is probable that some Fe organically bound could be extracted in the Na-EDTA fraction.

4.2.2 Phosphorus fractions

Within this section the results obtained for P fractionation are discussed. The bioavailable fraction is formed for the addition of Supernatant, UPW fraction and acetates fractions. Soluble P and P weakly bound to sludge particles will be extracted in the supernatant and UPW fraction respectively, meanwhile P associated with struvite and CaCO₃ and P associated with Ca will be extracted in the acetate fractions. NaOH will extract Fe and organic phosphates and HCl will extract P from Ca-phosphates.

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Figures from 4.20 to 4.25 illustrate the concentration and distribution of P. As in the figures before the numbers on the top of the bars represent the total concentration of P (as the addition of the fractions) for this sample and the numbers inside the bars indicated the concentration of each fraction.

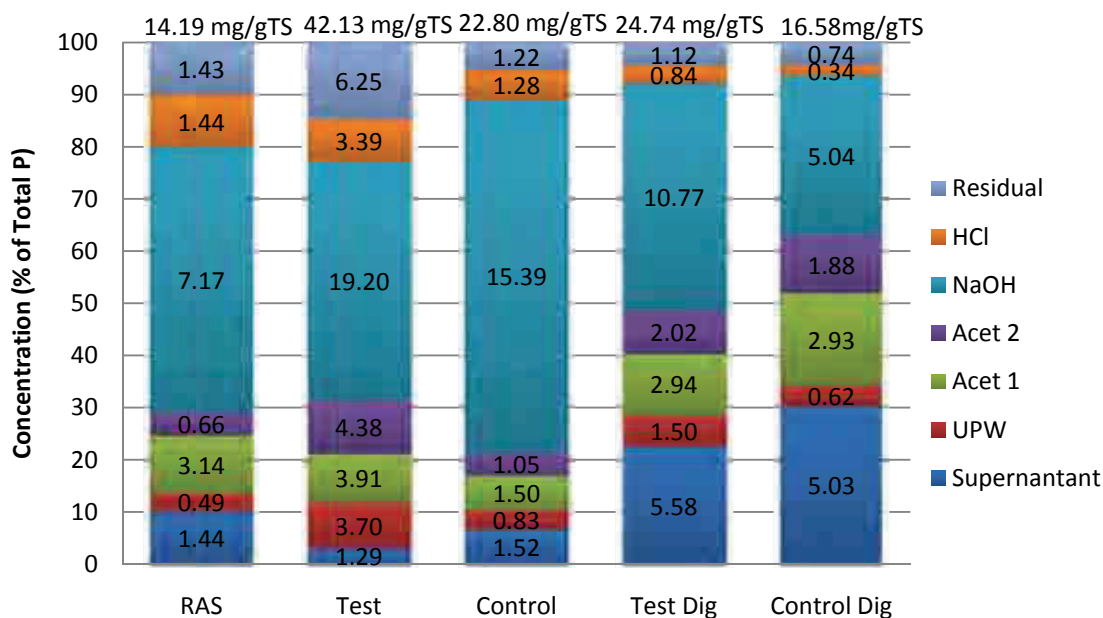


Figure 4.20 – Phosphorus fractionation profiles showing the relative differences between samples before and after digestion for FeCl_3 at Fe:P dosing ratio of 0.6:1

It is apparent in Figure 4.20 that Control presented a high content of P in the NaOH fraction, 15.39 mgP/gTS taking account for 67.5% of the total P extracted, which indicates that the most P in this sample was in form of Fe, Al or Mg-phosphate or part of organic phosphate. The concentration of P in the bioavailable fraction increased after digestion. In the case of Test, the percentage of P increased from 31.5% to 48.6%. The increase of this fraction was higher for Control, from 21.5% to 63.1%. Both Test and Control samples presented a huge content of P in the Supernatant (soluble P complexes) after digestion, particularly 5.58 mgP/gTS (22.5%) and 5.03 mgP/gTS (30.3%) for Test and Control respectively. In addition, after digestion Residual and HNO_3 fractions were low, around 8%-6.5%, it means that the concentration of P associated with sulphide was low, 1.8 mgP/gTS for Test and 1.1 mgP/gTS for Control.

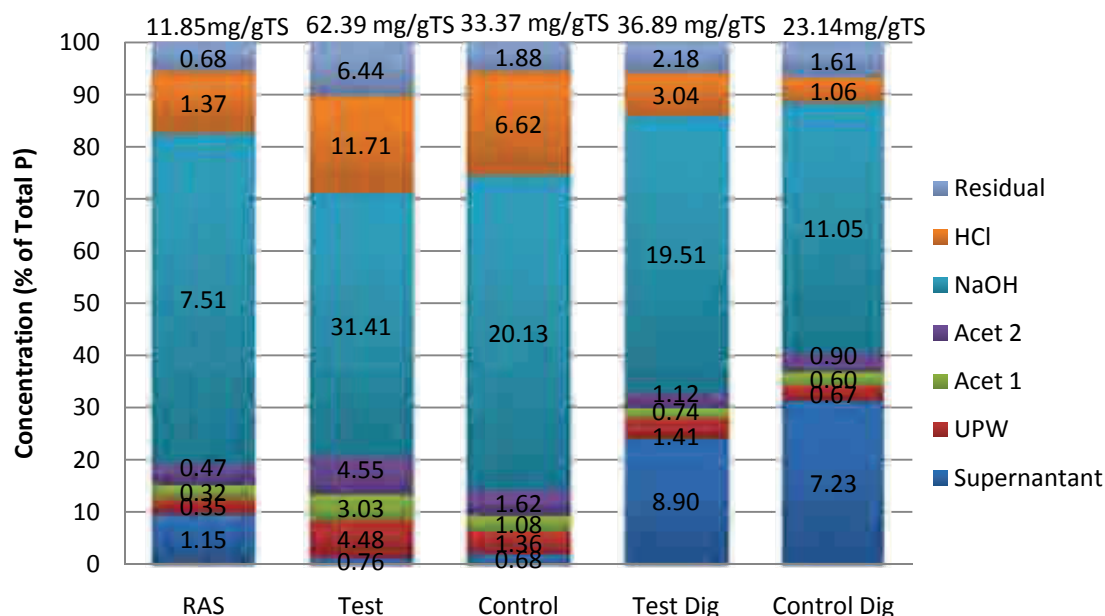


Figure 4.21 – Phosphorus fractionation profiles showing the relative differences between samples before and after digestion for $\text{Fe}_2(\text{SO}_4)_3$ at Fe:P dosing ratio of 0.6:1

In Figure 4.21, the NaOH fraction was the highest fraction of the profile before digestion, 63.4% for RAS, 50.4% for Test and 60.3% for Control. Bioavailable fraction at the contrary took the lowest portion of the fractionation profile, 19.3% for RAS, 20.6% for Test and 14.2% for Control. As in the above fractionation profile, bioavailable fraction climbed after digestion focusing this increase in the supernatant. The concentration of P in this fraction was 8.9 mgP/gTS for Test Dig and 7.2 mgP/gTS for Control Dig, accounting for 24.1% and 31.3% respectively.

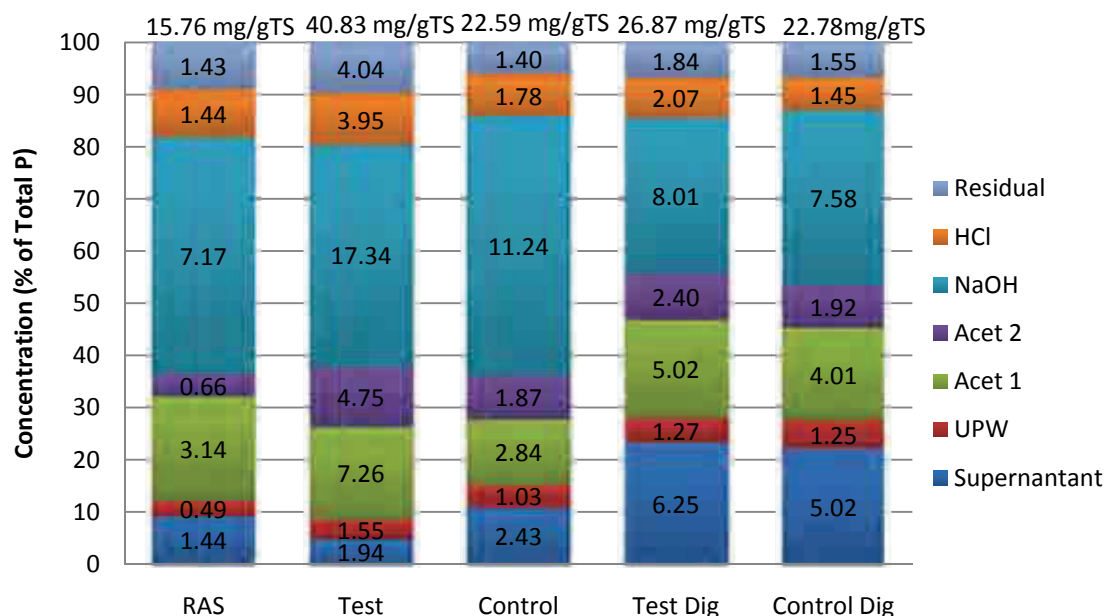


Figure 4.22 – Phosphorus fractionation profiles showing the relative differences between samples before and after digestion for FeSO_4 at Fe:P dosing ratio of 0.6:1

It is apparent in Figure 4.22 and should be remarked the high P concentration extracted in the Acetate 1 fraction, which indicates that there was a great portion of P forming struvite or adsorbed to CaCO_3 . In addition, the bioavailable fraction accounted for approximately 38% for Test and 36% for Control of the total P before digestion, increasing to 55% and 52% after digestion respectively, what means that the bioavailable fraction followed the same trend that the two profiles showed above, as beside of the high content of P in the Acetate 1 fraction, the content of P was also high in the Supernatant after digestion. Test and Control profiles after digestion were pretty similar, although the total P content was different, 26.9 mgP/gTS for Test Dig and 22.8 mgP/gTS for Control Dig.

In Figure 2.23 is observed that Residual and HCl fractions were quite high before digestion, 13.9% and 25.6% for Test and 16.8% and 29.9% for Control. Bioavailable fraction however, was low, especially in Test 7.4% (1.7 mgP/gTS). This fraction rose after digestion, but in this case not only Supernatant fraction rose but also UPW fraction increased from 0.9% (0.4 mgP/gTS) to 12.0% (3.6 mgP/gTS) for Test sample and from 2.9% (0.8 mgP/gTS) to 13.3% (3.4 mgP/gTS) for Control sample. On the contrary, in the profiles after digestion the Residual and HCl fractions accounted for a bit of the total P (no more than 16%).

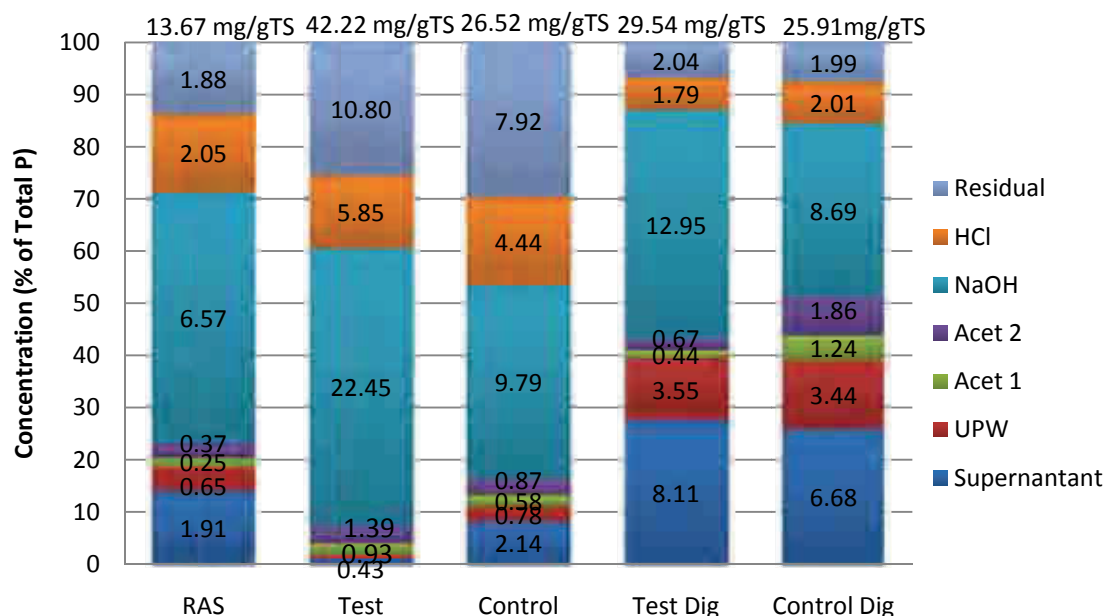


Figure 4.23 – Phosphorus fractionation profiles showing the relative differences between samples before and after digestion for FeCl_3 at Fe:P dosing ratio of 1.2:1

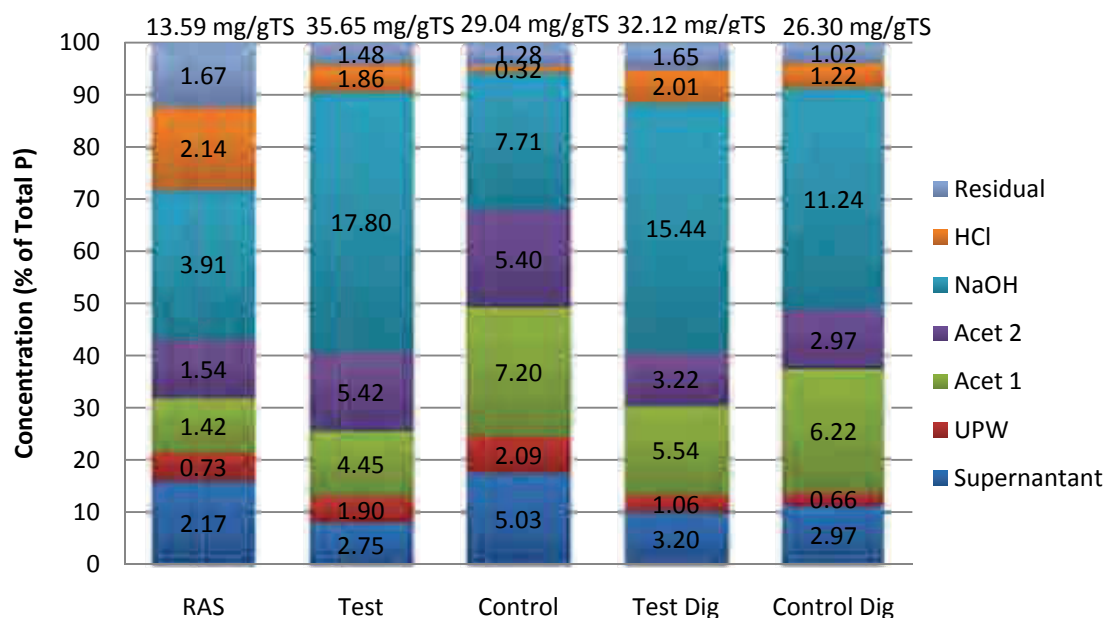


Figure 4.24 – Phosphorus fractionation profiles showing the relative differences between samples before and after digestion for $\text{Fe}_2(\text{SO}_4)_3$ at Fe:P dosing ratio of 1.2:1

It is apparent from Figure 4.24 that the bioavailable fraction was huge in all the profiles represented. Specifically, Acetate 1 and Acetate 2 fractions, what means that there was a high content of P absorbed to CaCO_3 , forming struvite

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or associated with Ca precipitated. Bioavailable fraction for Test before and after digestion took the same percentage, nonetheless, for Control the percentage of this fraction dropped after digestion around 20%, redistributing this P mainly to the NaOH fraction, increasing 16.2%, thus there was more P associated with Fe, Al or Mg-phosphate or organic phosphates after digestion.

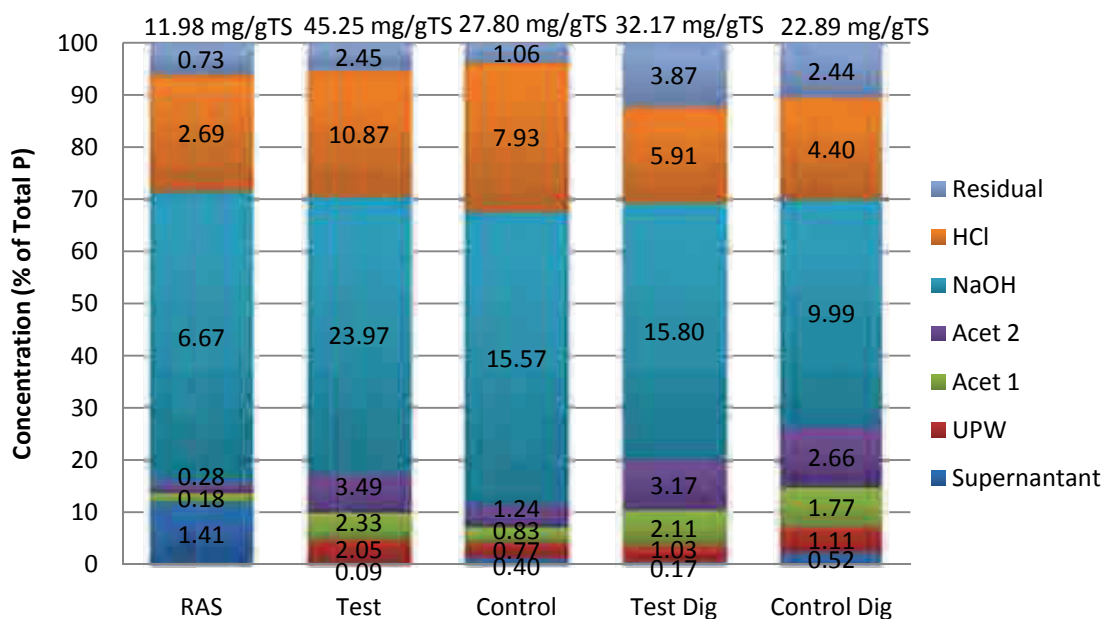


Figure 4.25 – Phosphorus fractionation profiles showing the relative differences between samples before and after digestion for FeSO_4 at Fe:P dosing ratio of 1.2:1

In all profiles shown in Figure 4.25 is apparent that the dominant fraction was the NaOH fraction and beside the bioavailable fraction was pretty low, although this fraction increased after digestion. This increase was 2.6% and 14.8% for Test and Control samples respectively. The HCl fraction was also high in all the profiles. These fractionation profiles had the higher proportion of P in the HCl fraction. Before digestion this proportion was 24.0% for Test and 28.5% for Control and after digestion this fraction account for 18.4% for Test Dig and 19.2% for Control Dig.

In Figure 4.26 is shown the different profiles generated from the RAS used in the experiments. The profile generated for the RAS collected between January and February presented low bioavailable fraction and high NaOH fraction in comparison with the RAS collected between May and June. The total P concentrations of all RAS used in the experiments were within the range 11.85-15.76 mgP/gTS, much lower than the typical concentration of Fe-dosed AS 40 mgP/gTS.

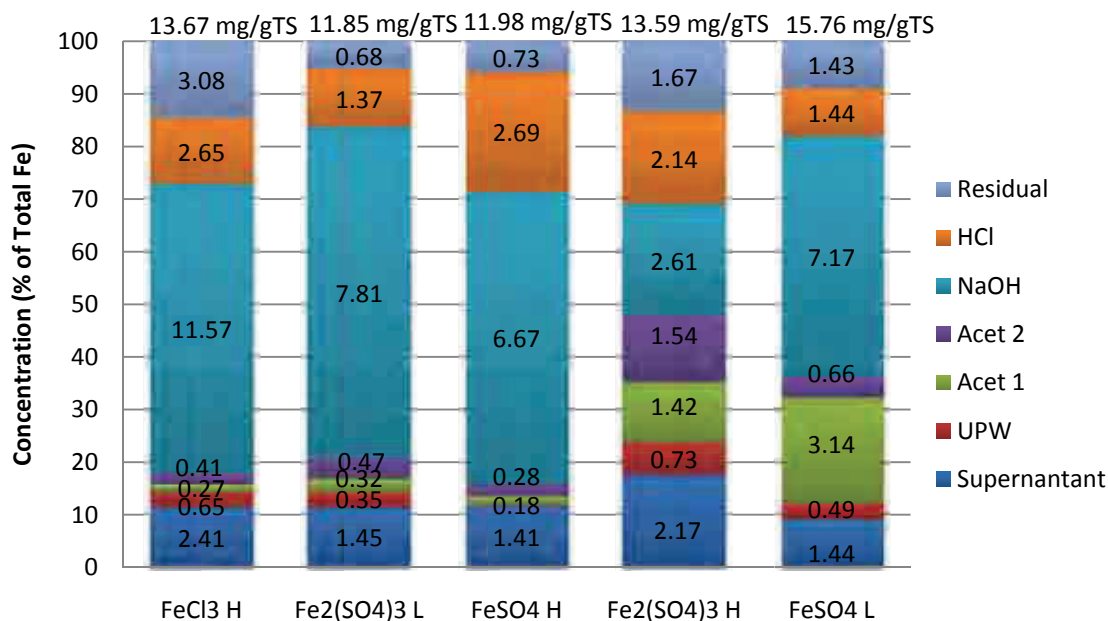


Figure 4.26 – Phosphorus fractionation profiles showing the relative differences among the different RAS³ used in the laboratory Fe-dosing experiments

Summary:

In most of the experiment NaOH fraction was the highest fraction in all P fractionation profiles, except in the experiments where the Acetate 1 fraction took a great portion of the profile (Figure 4.22 and 4.24). The content of P on the UPW fraction increased after digestion which indicates that soluble P was released from the digestion. In addition, quantity of P bioavailable was greater for digested samples in all the experiments less in the experiment where Fe₂(SO₄)₃ was dosed at ratio Fe:P 1.2:1 (Figure 4.24). Residual and HCl fraction decreased after digestion in all experiments less when FeSO₄ was dosed at ratio Fe:P 1.2:1, where Residual fraction increased, decreasing HCl fraction after digestion.

The total P concentration for Test sample was close to 40 mgP/gTS (total P of typical Fe-dose sludge) except in the experiment where Fe₂(SO₄)₃ was dosed at ratio Fe:P 0.6:1 (Figure 4.21). In this experiment the total P concentration of Test was 62.4 mgP/gTS higher than normal concentration (40 mgP/gTS), due to miscalculation mistake, the concentration of TS in this experiment was 2.32 g/l lower than the stipulated, 3.5 g/l.

³ The same RAS was used for the experiments where FeCl₃ and FeSO₄ were used as dosage at low ratio.

4.2.3 Phosphorus Removal Efficiency

In order to satisfy the Urban Wastewater Treatment Directive (UWWTD), the phosphorus removal efficient was measured, as the reason of dosed sludge with Fe is to remove P. The UWWTD stipulates that WWTWs with a population equivalent >100,000 need to achieve a P removal of either 80% or produce an effluent with a final P concentration of less than 1 mg/l.

The amount of P removed during the laboratory dosing was calculated as a percentage by: 1) measuring the P available in SetS, 2) calculating the amount of P added as $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, depending on the mass ratio choose, 3) measuring the total P in the final effluent. This procedure is detailed in section 3.1.1.

The percentage of P removed was measured using the next equation:

$$\text{Percentage P removal} = \frac{(P \text{ added} + P \text{ SetS}) - P \text{ in effluent}}{(P \text{ added} + P \text{ SetS})} \times 100$$

The percentage of P removed after 5 hours of Fe addiction and 10.5 hours of maturation and the final P concentration in the effluent are detailed in Table 4.10.

Table 4.10 – Percentage of P removed and concentration of P in the final effluent.

Reagent	% P removed	P concentration in the effluent (mg/l)
FeCl₃ L	95.71	3.21±0.20
Fe₂(SO₄)₃ L	97.55	1.91 ±0.24
FeSO₄ L	96.80	2.43 ±0.24
FeCl₃ H	98.67	1.07 ±0.20
Fe₂(SO₄)₃ H	91.45	6.86 ±0.73
FeSO₄ H	99.69	0.21 ±0.02

In all cases, the P removal was achieved as all percentages were higher than 90%, however the final effluent concentration measured in the Fe-dosed AS supernatant was higher than 1 mg/l in all experiments less in the last one, even though the limit detailed in the UWWTD was met.

4.3 Discussion of Results

The principal aim of this research was to establish whether Fe-dosed AS produced less biogas than non Fe-dosed AS, when subjected to batch AD tests. Within the following section the main results are discussed in light of some existing theories.

Effect of Fe-dosing in biogas and methane production

The main issues discussed within this section are related to the methods used for previous researchers, organic content of sludge used in the experiments and Fe and P soluble, bioavailable and total concentrations.

It is difficult to compare the results obtained in this research with the results reported in the literature, because the methods of Fe-dosing were often not reported in accounts of previous research (Dentel and Gosset, 1982; Yeoman *et al.*, 1990) or the quantities of Fe dosed were not specified (Gosset *et al.*, 1978; Johnson *et al.*, 2003; Smith and Carliell-Marquet, 2008; Yeoman *et al.*, 1990). It is probably because the main objective of these authors was to report the effect of Fe on AD, therefore they paid less attention to the AS process. Smith and Carliell-Marquet (2009) reported a laboratory Fe-dosing system which had taken into account accumulation of Fe and P in the system, addition of Fe in multiple doses (avoiding the formation of Fe-hydroxide products produced in a single dose), pH, DO concentration and food availability. Hence, the use of this method allows control of the AS process, dosing Fe in multiple doses and leaving the sludge to mature. Parameters such as pH and DO were also used to control the process. The feed conditions used in this research also specify 0.5 gVS for digested sludge and 0.4 gVS for Test and Control AS. On the other hand, the use of the same quantity of VS do not necessarily ensure the same feed, as the use of the same concentration of VS cannot include living MOs (Arnaiz *et al.*, 2005), ie, part of this feed can be inert mass, and this portion will not be always the same. This inert mass could also influence TS measured, therefore although all experiments were realised under same conditions some inert mass can be taking account from the beginning of the experiment.

These experiments allow for an interpretation of cause-effect, considering biogas production and methane production as the effect and Fe-dosing the cause. This interpretation can be done when the same RAS, SetS and digested sludge is used, ie, when results from Test and Control are compared. The comparison among different experiments is possible but it is important to highlight the introduction of external variables, as the samples (RAS, SetS and

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digested sludge) were sampled from the same WWT plant on different dates, hence the matrix of these samples and the concentration of COD, P or Fe change. Composition of sludge may influence in the digestion, specially sludge with high content in protein and lipid (Dentel and Gosset, 1982; Gosset et al., 1978), because Fe precipitates become “enmeshed” within and around the floc, therefore the hydrolysis of this substrates take longer, thus the biogas volume generated will decrease. This theory is not supported from the result obtained in this research as the VS destruction for both samples, Fe-dosed and non Fe-dosed AS, was similar; besides the biogas generated for Fe-dosed AS was higher although the VS destruction was lower ($\text{Fe}_2(\text{SO}_4)_3$ low ratio and FeSO_4 high ratio). The composition of the sludge used in this research was not analysed. All samples were collected from the same WWT plant, Kidderminster; hence, the composition of the sludge should be similar in all experiments. In this study although the influent COD concentration was different for each experiment, the COD removal efficiency was similar (90% confidence level) for Test and Control and in all experiments within the range between 85.08 and 94.31%. Oikonomidis *et al.* (2010) also found that the chemical addition of Fe(II) and Fe(III) salts did not affect COD removal efficiency.

Peffer and White (1964) related the formation of Fe-phosphate precipitated (when high concentration of Fe was dosed) with a reduction of soluble P “limiting” P for the MOs and therefore suppressing the biogas production. The results obtained in this research do not support this theory as the concentration of soluble P (Supernatant) and bioavailable fraction increased after digestion likely because soluble P could release during digestion and not re-precipitated as a ferrous phosphate due to the low Fe concentration in the system as the ratio used in this research to dose Fe into the system where lower (molar ratio Fe:P 0.6:1 and 1.2:1) than usual (molar ratio Fe:P 2:1). Only Jenkins *et al.* (1970) using a molar ratio Fe:P 0.7:1 found P release on AD. On the contrary, Dentel and Gosset (1982), Grigoropoulos et al. (1970) and Gyhoot and Verstraete (1997) in addition to the author cited above did not relate the impairment of the digestion to a P limitation.

Peffer and White (1964) also tried to find the threshold concentration of soluble P necessary for the growth of MOs, without success. To identify the threshold concentration of P below which MOs become impaired is difficult (almost impossible) due to the properties of sludge (as organic composition, concentration of metals or P or COD concentration), which change as equilibriums shift during the digestion processes.

The dose of Fe at low molar ratios Fe:P within this research can influence AD in a different way that the authors who used higher molar ratios as Fe:P 2:1 or higher. Smith (2006) found a negative correlation between the mass of Fe in the

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feed to the AD and the biogas production using samples from 13 WWT plants, this correlation was not found in this research, it most likely be due to the mass of Fe in this research (24-49 mgFe/gTS) was much lower than the mass of Fe reported for Smith (35-119 mgFe/gTS). Smith (2006) also found a positive correlation between bioavailable P and rate of biogas. In this research the greatest rate of biogas and methane production was generated for the Fe-dosed AS which the lowest concentration of P in the bioavailable fraction (6.46 mgP/gTS) which coincides with the average concentration of bioavailable P in the non Fe-dosed AS (6.46 mgP/gTS) reported for Smith (2006) and she also observed that the biogas and methane production was higher for these non Fe-dosed AS, with an average of biogas volume and methane volume of 169 ml and 123 ml, while Fe-dosed AS produced an average of 149 ml of biogas volume and 114 ml of methane volume. However, the average concentration of bioavailable P in the Fe-dosed AS reported for Smith (2006) was 0.93 mgP/gTS much lower than the concentration obtained in this research (average of 11.90 mgP/gTS) likely due to as it was mentioned earlier, the low ratios used in this research, there was not enough Fe in the system to re-precipitated all the release P as a ferrous phosphate.

If instead of biogas production, methane production is analysed it is found that there were two experiments where the methane production was higher for Fe-dosed AS. These experiments were $\text{Fe}_2(\text{SO}_4)_3$ low ratio and FeSO_4 high ratio (Table 4.7 and 4.8). The bioavailable Fe concentration after digestion of the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at low ratio was low, 0.67 mgFe/gTS although not as low as 0.36 mgFe/gTS, the concentration in the FeSO_4 high ratio experiment. Nonetheless, the bioavailable P concentration after digestion was higher in this experiment 12.17 mgP/gTS. It should be noted that the bioavailable Fe concentration before digestion was low for the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at low ratio, 6.80 mgFe/gTS in comparison with 8.5 mgFe/gTS in the FeSO_4 high ratio experiment.

The biodegradability of the AS used in the experiment where FeSO_4 was dosed at high ratio was also the greatest, as the rate of biogas for this AS was 36.60 ml and 31.16 ml for Fe-dosed and non Fe-dosed AS respectively. The values of bioavailable Fe and P were 0.36 mgFe/gTS and 6.49 mgP/gTS for Fe-dosed AS and 0.44 mgFe/gTS and 6.06 mgP/gTS for non Fe-dosed AS. Furthermore, in this experiment was also achieved the greatest P removal (99.69%) and the lowest concentration of P in the final effluent (0.21 mg/l). The SetS used in this experiment also had the greatest concentration of COD, 561 mg/l. This COD concentration was similar to concentration of the SetS used in the other experiment where Fe-dosed AS produced more biogas than non Fe-dosed AS ($\text{Fe}_2(\text{SO}_4)_3$ low ratio), 541.67 mg/l. The COD concentration in the rest of the experiments was lower, between 237 and 461 mg/l. It seems that COD

concentration around 550 mg/l enhance the positive effect of Fe on AD. In summary, in this research the experiment which generated the greatest volume of biogas contained the lowest concentration of bioavailable Fe and P in the digested sludge; these concentrations are similar to the concentrations reported for Smith (2006). It is possible that the high bioavailable P concentrations obtained in the others experiments (average of 12.99 and 11.62 mgP/gTS for Fe-dosed and non Fe-dosed AS respectively) inhibit digestion to be above the threshold, as the greatest volume of biogas and methane was achieved for the samples (Test and Control) generated in the experiment where FeSO_4 was dosed.

Some authors found that the adverse effect of Fe on AD is reduced over time. Dentel and Gosset (1982) reported that periods up to 82 days of digestion reduced the adverse effect of Fe on AD due to chemical coagulation, but did not eliminate the differences between Test and Control. Gosset *et al.* (1978) also reported the same effect after 125 days. However, Horban and van der Berg (1979) observed this effect during the first two to four days after the addition, when high level of Fe (1160 mg/l) was added to the digester. Similar effect to that outline above was observed in this research when methane production during the last four days of the trials was analysed (Table 4.6). The clear example of this behaviour was the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at high ratio, where if total volume of methane is analysed Test produced lower volume than Control, but if the volume of methane generated during the last four days of the trial is analysed instead of total volume of methane, Test produced higher volume of methane, therefore the adverse effect produced for Fe in this experiment was at the beginning of the trial reducing over time. When the same Fe salt was used at low ratio the volume of methane generated for Test was also higher than the volume generated for Control, although in this case, this behaviour was also observed when total methane volume was compared. Meanwhile, in the rest of the experiments the volume of methane generated during the last four days of the trial was similar (90% level of confidence) for Test and Control.

Fe and P fractions

Within this section will be discussed Fe and P concentrations with limits stipulated for and stable and favourable digestion of Fe-dosed AS and change in the mechanism of control of precipitation.

Smith (2006) studied Fe and P profiles from 13 different WWT plants (6 Fe-dosed and 7 non Fe-dosed) and found than the percentage of Fe organically bound in the non Fe-dosed AS was higher (56%) than the Fe-dosed AS (31%).

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In this research the result obtained were opposite to the one obtained for Smith (2006), although the fractionation method used in both researches was different (section 2.4.1). The percentage of Fe organically bound (NaOH fraction) in the non Fe-dosed AS was 8.5% and 13.8% for the Fe-dosed AS. The percentage of bioavailable Fe fractions after digestion of the two experiment where Fe-dosed AS produced more biogas than non Fe-dosed AS ($\text{Fe}_2(\text{SO}_4)_3$ low ratio and FeSO_4 high ratio) were really low 9.8% for $\text{Fe}_2(\text{SO}_4)_3$ (0.67 mgFe/gTS) and 3.2% (0.36 mgFe/gTS) for FeSO_4 in comparison with the rest of the experiments, average of 20% (2.25 mgFe/gTS).

The supplementation of Fe to anaerobic digesters was studied for Peffer and White (1964) and found that the supplementation of Fe to anaerobic digester with low content of bioavailable Fe produced an increase in the biogas production. This theory can explain the result obtained for the experiment where Fe-dosed AS produced more biogas than non Fe-dosed AS, as in these experiments the content of bioavailable Fe was really low for Fe-dosed ($\text{Fe}_2(\text{SO}_4)_3$ low ratio: 1.50 mgFe/gTS; FeSO_4 high ratio: 0.89 mgFe/gTS) and non Fe-dosed AS ($\text{Fe}_2(\text{SO}_4)_3$ low ratio: 0.15 mgFe/gTS; FeSO_4 high ratio: 0.47 mgFe/gTS) even though the content of bioavailable Fe of AS was high before digestion, 6.80 and 0.58 mgFe/gTS for Test and Control of $\text{Fe}_2(\text{SO}_4)_3$ experiment and 8.52 and 1.19 mgFe/gTS for Test and Control of FeSO_4 experiment. The concentration of bioavailable P in the experiment where FeSO_4 was dosed at high ratio as it was mentioned earlier was also the lowest concentration of P, 6.46 mgP/gTS, nevertheless this P concentration was higher and similar to the concentration of the other experiments for both samples in the experiment where $\text{Fe}_2(\text{SO}_4)_3$ was dosed at low ratio (12.17 mgFe/gTS for Test and 9.41 mgFe/gTS for Control). The concentration of Fe and P in mg/gTS on bioavailable fractions after digestion were converted to concentration in mg/l to check if Fe concentration in this fraction where in the optimum range stipulated for Horban and van den Berg (1979) and if P concentration was lower than 75 mg/l as Peffer and White (1964) stipulated for an stable digestion. Concentration of soluble Fe was higher than 12 mg/l in the two experiments where non Fe-dosed AS produced higher biogas than Fe-dosed AS. But if methane production is analysed in the experiment where FeSO_4 was dosed at low ratio, this production was higher for Fe-dosed AS (48.06 ml) than non Fe-dosed AS (45.25 ml), as well as soluble Fe was 19.94 mg/l and soluble P was 79.25 mg/l a bit higher than the level of 75 mg/l stipulated for Peffer and White (1964). However the concentration of soluble Fe was lower than 11 mg/l in the other two experiments where methane production and biogas production were higher for Fe-dosed AS. Concentration of soluble P was really different for these two experiments, 107.07 mg/l for $\text{Fe}_2(\text{SO}_4)_3$ low ratio and 1.91 mg/l for FeSO_4 high ratio. If bioavailable concentration are analysed, concentration of bioavailable Fe was lower than 111 mg/l, but all of them was higher than 11

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mg/l except in the experiment where FeSO_4 was dosed at high ratio, where bioavailable Fe was out of this range (lower than 11 mg/l) as well as in this experiment the concentration of bioavailable P was lower than 75 mg/l. The opposite situation was observed in the experiment where $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 were dosed at low ratio, where the concentration of bioavailable P was higher than 75 mg/l (146 mg/l and 189.57 mg/l respectively) and however the methane production was higher for Fe-dosed AS. In summary the results obtained in this research do not agree with the limits stipulates for Horban and van den Berg (1979) and Peffer and White (1964). However if the threshold of the unstable digestion produced for high concentration of bioavailable P (instead of soluble P) were 75 mg/l, the data obtained in this research would support that theory, as the only experiment where the bioavailable P concentration was lower than 75 mg/l was when FeSO_4 was dosed at high ratio.

Table 4.11 – Concentration of soluble and bioavailable Fe and P in samples after digestion

		Fe concentration (mg/l)				P concentration (mg/l)			
		Soluble		Bioavailable		Soluble		Bioavailable	
	Fe salt	Test	Control	Test	Control	Test	Control	Test	Control
Low ratio	FeCl_3	11.70	5.61	55.59	22.57	74.21	61.37	159.87	127.61
	$\text{Fe}_2(\text{SO}_4)_3$	2.41	0.83	18.05	11.61	107.07	77.79	146.41	101.25
	FeSO_4	19.94	8.78	51.76	19.22	79.25	63.90	189.57	155.31
High ratio	FeCl_3	14.77	0.75	66.77	41.86	100.65	82.97	158.48	164.19
	$\text{Fe}_2(\text{SO}_4)_3$	4.12	2.40	32.55	11.64	34.72	33.89	141.27	146.28
	FeSO_4	0.34	3.31	9.99	5.18	1.91	5.74	72.88	66.84

Carliell-Marquet *et al.* (2010) found that the content of Fe in the Residual and HNO_3 fraction of digested sludge decreased with the addition of Fe to the process, and this introduced a change in the mechanism of the control of the Fe solubility in the digestion. The solubility of Fe in the digestion of non Fe-dosed AS was controlled by ferrous sulphide precipitation meanwhile ferrous carbonate was increasing in importance when Fe is added to the system (Carliell-Marquet *et al.*, 2010). In this research the effect of Fe-dosing in the mechanism of control of Fe solubility found for Carliell-Marquet *et al.* (2010) was observed in the fractioned profile of samples before and after digestion (section 4.2.1).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Within this chapter principal conclusion related to the main aim and objective are presented in section 5.1 followed by the recommendations and further work in this area, section 5.2.

5.1 Conclusions

The principal aim of this research was to establish whether iron-dosed activated sludge produces less biogas than non-dosed activated sludge when subjected to batch anaerobic digestion tests and also investigated the change in the inorganic fractions produced for Fe-dosing. The principle conclusions and conclusions related to the objectives address to the main aim are listed below.

Principle conclusions

- No relationship was found among Fe-salt, molar ratio Fe:P dosed, biogas production and methane production.
- Fe has not always a detrimental effect on AD (in terms of biogas production and methane production).
- When Fe was dosed as FeCl_3 , at molar ratio Fe:P 0.6:1 had no effect on anaerobic digestion as Fe-dosed AS and non Fe-dosed AS produced the same volume of biogas and methane. However when this Fe salt (FeCl_3) was dosed at molar ratio Fe:P 1.2:1 Fe impaired the digestion of Fe-dosed AS through the production of 4% less biogas than non Fe-dosed AS.
- When $\text{Fe}_2(\text{SO}_4)_3$ was used as source of Fe, at molar ratio Fe:P 0.6:1 Fe improved the digestion; as Fe-dosed AS generated 9% more volume biogas and 8% more volume of methane than non Fe-dosed AS, although the content of methane in both AS samples was the same (around 63%). At molar ratio Fe:P 1.2:1 both AS samples generated the same volume of biogas approximately 93 ml, however non Fe-dosed AS generated a biogas richer in methane (6%) than Fe-dosed AS, in terms

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of methane volume it was an 11% less methane generated, therefore at this ratio Fe inhibits anaerobic digestion.

- When the precipitant Fe salt used was FeSO_4 , at molar ratio Fe:P 0.6:1 Fe had no effect on digestion as Fe-dosed AS and non Fe-dosed AS produced the same volume of biogas and methane, however, at molar ratio Fe:P 1.2:1, digestion was enhanced as Fe-dosed AS generated approximately 9% more biogas and 7% more volume of methane than non Fe-dosed AS.
- No correlation was found between bioavailable Fe or mass of Fe in the Fe-dosed AS and production of biogas.

Objective 1 – To use a laboratory Fe-dosing unit to produce Fe-dosed AS that is comparable to non Fe-dosed AS in all aspects other than changes produced by Fe-dosing.

The Fe-dosing method developed for Smith and Carliell-Marquet (2009) allows the comparison between the Fe-dosed AS generated with the non Fe-dosed due to Fe addition.

- The Fe-dosed AS (Test) generated in all experiments was representative of typical Fe-dosed sludge in terms of total P concentration (40mgP/gTS or 140 mg/l) and Fe and P distribution. The majority of the additional Fe dosed to the system was mainly removed in the Na-EDTA (53%) fraction of the Metal fractionation method. This fraction (Na-EDTA) represents Fe bounded or associated with phosphate and hydroxide. The main fraction of P extracted using the P fractionation method was NaOH fraction (48%) where soluble reactive P from Fe, Al or Mg-phosphate is extracted.
- 91.5-99.69% of P was removed from effluent meeting the legislative discharge consent concentration.

Objective 2 and 3 – To use different Fe salts (FeCl_3 , $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4) at two molar ratios Fe:P (0.6:1 and 1.2:1) to produce Fe-dosed AS in a laboratory Fe-dosing system and compare the biogas and methane produced from batch test anaerobic digestion.

- No pattern was produced for the addition of Fe salts at different ratios studied.
- The direct comparison between Fe-dosed and non Fe-dosed AS was possible due to the use of the same SetS, RAS and digested sludge in each individual experiment. However, the comparison among the different Fe-dosed and non Fe-dosed AS generated in the different

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experiments was influence for the use of different SetS, RAS and digested sludge, therefore in order to be able to compare the samples generated in the different experiments the same feed (0.4g VS of AS) and mass of digested sludge (0.5g VS) was used in the batch test.

- The use of lower molar ratios Fe:P in this research showed that the addition of Fe did not always impair the digestion (methane production) but enhance the digestion. The experiment where $\text{Fe}_2(\text{SO}_4)_3$ and FeSO_4 were dosed at low and high ratio respectively were examples of the improvement produced on AD due to Fe-dosing.

Objective 4 – To use sequential extraction methods to measure Fe and P fractions in laboratory Fe-dosed AS and non Fe-dosed AS, before and after batch digestion test.

- A modification of the method used for Pichtel *et al.* (2007) was used to fractionate sludge for Fe characterisation. The modification realised for Smith (2006) on the method used for Uhlmann *et al.* (1990) was used to fractionate sludge for P.
- The comparison of the concentrations of bioavailable P after digestion showed that concentrations lower than 75 mg/l enhanced the biogas and methane production of Fe-dosed and non Fe-dosed sludge digestion as it was suggested for Pepper and White (1964). The greatest biogas production, 131 ml for Test and 120 ml for Control and methane production, 82 ml for Test and 77 ml for Control was generated in the experiment where the concentration of bioavailable P was 73 mg/l and 67 mg/l for Test and Control respectively, in the experiment where FeSO_4 was dosed at molar ratio Fe:P 1.2:1. In this experiment the content of Fe in the bioavailable fraction was also low after digestion, 0.89 mgFe/gTS for Test and 0.47 mgFe/gTS for Control.
- The addition of Fe to the process produced a change in the mechanism of Fe solubility in the digestion. The increase of 10% of Fe extracted in Na-EDTA fraction and the decrease of 9% Fe in the HNO_3 plus Residual fraction showed the increase of importance of ferrous carbonate in the control of Fe solubility in the digester decreasing ferrous sulphide in importance.

5.2 Recommendations and Further work

The laboratory dosing method used in this research allows the on-site comparison of Fe-dosed AS and non Fe-dosed AS when one Fe salt was dosed. The direct comparison of the effect of more than one Fe salt at different dosage ratios on AD will be more interesting than the single comparison realised in this research. It could be possible if jar test equipment was used. If this system was used the better ratio and Fe salt could be easier identify. It would allow the use of different dosing ratios depending on the organic and chemical composition of the SetS, effluent, AS and digested sludge over the different season during the year. The use of lower quantities of Fe would save companies 40% of the money invested in the coagulant and if beside the addition of Fe has no detrimental effect on AD or enhance the digestion (as production of biogas and methane) the advantage for water companies will be double.

There are further recommendations for further work:

- The effect of FeSO_4 at molar ratio Fe:P 1.2:1 should be further studied using different sludges from different WWT plants to prove the positive effect of Fe on AD when is dosed as FeSO_4 using the same dosing system and conditions used in this research.
- This research took into account chemical concentration, properties and characteristics of sludge. It would be interesting to measure organic composition in addition to know the diversity of MOs in the sludges used (RAS and digested sludge). It would be also interesting to fractionate the organic composition.
- There will interesting to investigated the impact and behaviour of FeRB in anaerobic digester feeding with Fe-dosed AS depending on the total concentration of Fe and the distribution of Fe in the sludge, as there is no reporting about this topic in the literature.
- The impact of the bioavailable concentration of P lower than 75 mg/l should be further investigated in order to establish this limit as a detrimental for AD as well as the effect of different concentrations of bioavailable Fe on AD, as there was no relationship found in this research.

REFERENCES

- Arnaiz C., Gutierrez J. & Lebrato J.(2006) Biomass stabilization in anaerobic digestion of wastewater sludges. *Bioresource technology*, 97, 1179-1184.
- APHA-AWWA-WEF (1985) Standard methods for the examination of water and wastewater (16th edition). Washington, D.C.
- Bacon, J. R., & Davidson, C. M. (2008) Is there a future for sequential chemical extraction? *Analyst*, 133(1): 25-46.
- Bell, P. F., James, B. R., & Chaney, R. L. (1991) Heavy-metal extractability in long-term sewage-sludge and metal salt-amended soils. *Journal of Environmental Quality*, 20(2): 481-486.
- Bolzonella, D., Pavan, P., Battistoni, P., & Cecchi, F. (2005) Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. *Process Biochemistry*, 40(3-4): 1453-1460.
- Bond, D. R., & Lovley, D. R. (2002) Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. *Environmental Microbiology*, 4(2): 115-124.
- Bouda, M., Hammy, F., Mercier, G., & Blais, J. F. (2009) Chemical Leaching of Metals from Wastewater Sludge: Comparative Study by Use of Three Oxidizing Agents H₂O₂, FeCl₃, and Fe-2(SO₄)(3). *Water Environment Research*, 81(5): 523-531.
- Callander I.J., & Barford J.P. (1983) Precipitation, Chelation, and Availability of Metals as Nutrients in Anaerobic Digestion. I Methodology. *Biotechnology and Bioengineering*, 25, 1947-1957.
- Callander I.J., & Barford J.P. (1983) Precipitation, Chelation, and Availability of Metals as Nutrients in Anaerobic Digestion. II Applications. *Biotechnology and Bioengineering*, 25, 1959-1972.
- Carliell-Marquet, C., (2001), The effect of phosphorus enrichment on metal and phosphorus fractionation in anaerobically digested sludge, PhD thesis, Civil Engineering, Loughborough University.
- Carliell-Marquet, C., J. Smith, I. Oikonomidis & A. Wheatley (2010) Inorganic profiles of chemical phosphorus removal sludge. *Proceedings of the Institution of Civil Engineers-Water Management*, 163, 65-77.
-

-
- Chao, W., Li, X. C., Wang, P. F., Zou, L. M., & Ma, H. T. (2006) Extractable fractions of metals in sewage sludges from five typical urban wastewater treatment plants of China. *Pedosphere*, 16(6): 756-761.
- Chen, Y., J. J. Cheng & K. S. Creamer (2008) Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, 99, 4044-4064.
- Choi, H. J., Choi, C. H., & Lee, S. M. (2009) Analyses of phosphorus in sewage by fraction method. *Journal of Hazardous Materials*, 167(1-3): 345-350.
- Clark, T., Burgess, J. E., Stephenson, T., & Arnold-Smith, A. K. (2000) The influence of iron-based co-precipitants on activated sludge biomass. *Process Safety and Environmental Protection*, 78(B5): 405-410.
- Clark, T., & Stephenson, T. (1999) Development of a jar testing protocol for chemical phosphorus removal in activated sludge using statistical experimental design. *Water Research*, 33(7): 1730-1734.
- Convery, John J. (1970) The use of physical-chemical treatment techniques for the removal of phosphorus. New York Water Pollution Control Association, New York.
- de Haas, D. W., & Greben, H. A. (1990) Phosphorus fractionation of activated sludges from modified bardenpho processes with and without chemical precipitant supplementation. Paper presented at the 15th Biennial Conf of the International Assoc on Water Pollution Research and Control, Kyoto, Japan.
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. 2(000a) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal Part 1: Literature review. *Water Sa*, 26(4): 439-452.
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. (2000b) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal Part 2: Method development for fractionation of phosphate compounds in activated sludge. *Water Sa*, 26(4): 453-466.
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. (2000c) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal Part 3: Experimental periods using alum. *Water Sa*, 26(4): 467-483.
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. (2000d) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal Part 4: Experimental periods using ferric chloride. *Water Sa*, 26(4): 485-504.
-

-
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. (2001a) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal - Part 6: Modelling of simultaneous chemical-biological P removal - Review of existing models. *Water Sa*, 27(2): 135-150.
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. (2001b) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal - Part 5: Experimental periods using a ferrous-ferric chloride blend. *Water Sa*, 27(2): 117-134.
- de Haas, D. W., Wentzel, M. C., & Ekama, G. A. (2001c) The use of simultaneous chemical precipitation in modified activated sludge systems exhibiting biological excess phosphate removal - Part 7: Application of the IAWQ model. *Water Sa*, 27(2): 151-168.
- Defra (2002) EC Urban Waste Water Treatment Directive. <http://ww2.defra.gov.uk>.
- Defra (2009) Designated nutrient sensitive areas. <http://ww2.defra.gov.uk>.
- Defra (2010) Urban Waste Water Treatment Directive: Progress and problems. <http://www.utilityweek.co.uk/features/europe/urban-wastewater-treatment-dir.php>.
- Degroot, C. J., & Golterman, H. L. (1990) Sequential fractionation of sediment phosphate. *Hydrobiologia*, 192(2-3): 143-148.
- Delvasto, P., Ballester, A., Munoz, J. A., Gonzalez, F., Blazquez, M. L., Igual, J. M., Valverde, A., & Garcia-Balboa, C. (2009) Mobilization of phosphorus from iron ore by the bacterium *Burkholderia caribensis* FeGL03. *Minerals Engineering*, 22(1): 1-9.
- Dentel SK. and Gosset JM. (1982) Effect of chemical coagulation on anaerobic digestibility of organic materials. *Water Research*, 16, 707-718.
- Eckenfelder, W. Wesley (1995) *Activated sludge treatment of industrial wastewater*. Technomic, Lancaster, Penn.
- Fitamo, D., Itana, F., & Olsson, M. (2007) Total contents and sequential extraction of heavy metals in soils irrigated with wastewater, Akaki, Ethiopia. *Environmental Management*, 39(2): 178-193.
- Flores, L., Blas, G., Hernandez, G., & Alcala, R. (1997) Distribution and sequential extraction of some heavy metals from soils irrigated with wastewater from Mexico city. *Water Air and Soil Pollution*, 98(1-2): 105-117.
-

-
- Forster, C. F., Christopher F. (2003) Wastewater treatment and technology. Thomas Telford, London.
- Frossard, E., Bauer, J. P., & Lothe, F. (1997) Evidence of vivianite in FeSO₄-flocculated sludges. *Water Research*, 31(10): 2449-2454.
- Fuentes, A., Llorens, M., Saez, J., Soler, A., Aguilar, M. I., Ortuno, J. F., & Meseguer, V. F. (2004) Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere*, 54(8): 1039-1047.
- Fytianos K., Voudrias E. and Rainkos N. (1998) Modelling of phosphorus removal from aqueous and wastewater samples using ferric iron. *Environmental Pollution*, 101, 123 – 130.
- Gerardi M.H. (2003) The microbiology of anaerobic digesters. John Wiley and Son, Inc. Hoboken, New Jersey.
- Ghyoot W. and Verstraete W. (1997) Anaerobic digestion of primary sludge from chemical pre-precipitation. *Water Science and Technology*, 36 (6-7): 357-365.
- Gosset J., McCary P., Wilson J. and Evans D. (1978) Anaerobic digestion of sludge from chemical treatment. *Journal of the Water Pollution Control Federation*, 50, 533-542.
- Grigoropoulos S., Vederr R. and Max D. (1971) Fate of aluminium-precipitated phosphorus in activated sludge and anaerobic digestion. *Journal of the Water Pollution Control Federation* Vol. 43 (12) pp 2366-2382.
- Gray, N. F. (1990) Activated sludge: theory and practice. Oxford University Press, Oxford.
- Horban D. & van der Berg L. (1979) Effect of iron on conversión of acetic acid to methane during methanogenic fermentation. *Journal of Applied Bacteriology*, 47, 153-159.
- Ivanov, V., S. L. Kuang, V. Stabnikov & C. H. Guo (2009) The removal of phosphorus from reject water in a municipal wastewater treatment plant using iron ore. *Journal of Chemical Technology and Biotechnology*, 84, 78-82.
- Jenkins D., Ferguson J. and Menar A. (1971) Chemical Processes for phosphate removal. *Water Research*, 5, 369-387.
- Johnson, D. K., Carliell-Marquet, C. M., & Forster, C. F. (2003) An examination of the treatment of iron-dosed waste activated sludge by anaerobic digestion. *Environmental Technology*, 24(8): 937-945.
-

-
- Larner, B. L., Seen, A. J., & Townsend, A. T. (2006) Comparative study of optimised BCR sequential extraction scheme and acid leaching of elements in the certified reference material NIST 2711. *Analytica Chimica Acta*, 556(2): 444-449.
- Lees, E. J., Noble, B., Hewitt, R., & Parsons, S. A. (2001a). The impact of residual coagulant on downstream treatment processes. *Environmental Technology*, 22(1): 113-122.
- Lees, E. J., Noble, B., Hewitt, R., & Parsons, S. A. (2001b). The impact of residual coagulant on the respiration rate and sludge characteristics of an activated microbial biomass. *Process Safety and Environmental Protection*, 79(B5): 283-290.
- Lovley, D. R. & E. J. P. Phillips (1986) Organic-matter mineralization with reduction of ferric iron in anaerobic sediments. *Applied and Environmental Microbiology*, 51, 683-689.
- Lovley, D. R. & E. J. P. Phillips (1987) Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction. *Applied and Environmental Microbiology*, 53, 2636-2641.
- Lovley, D. R. & E. J. P. Phillips (1988) Novel mode of microbial energy-metabolism organic-carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, 54, 1472-1480.
- Madoni, P., Davoli, D., & Guglielmi, L. (1999) Response of sOUR and AUR to heavy metal contamination in activated sludge. *Water Research*, 33(10): 2459-2464.
- Metcalf & Eddy, In. (1991) *Wastewater engineering: treatment, disposal, and reuse* (3rd edition). McGraw-Hill, London.
- Mogens Henze (2002) *Wastewater treatment: biological and chemical processes* (3rd edition). Springer, London.
- Morse, G. K., Brett, S. W., Guy, J. A., & Lester, J. N. (1998) Review: Phosphorus removal and recovery technologies. *Science of the Total Environment*, 212(1): 69-81.
- Nielsen, J. L., & Nielsen, P. H. (1998) Microbial nitrate-dependent oxidation of ferrous iron in activated sludge. *Environmental Science & Technology*, 32(22): 3556-3561.
- Nielsen, P. H. (1996) The significance of microbial Fe(III) reduction in the activated sludge process. Paper presented at the 18th Biennial Conference of the International Association on Water Quality, Singapore.
-

-
- Oikonomidis, I. (2007) Iron accumulation in activated sludge as a result of chemical phosphorus removal : effect on Floc properties and biological activity, PhD thesis, Civil Engineering, Birmingham University.
- Oikonomidis, I., L. J. Burrows & C. M. Carliell-Marquet (2010) Mode of action of ferric and ferrous iron salts in activated sludge. *Journal of Chemical Technology and Biotechnology*, 85, 1067-1076.
- Peffer J. & White J. (1964) The role of iron in anaerobic digestion in *Proceedings of 19th Industrial Waste Conference*, Purdue University, USA 887-901.
- Paul, E., Laval, M. L., & Sperandio, M. (2001) Excess sludge production and costs due to phosphorus removal. *Environmental Technology*, 22(11): 1363-1371.
- Pichtel, J., & Anderson, M. (1997) Trace metal bioavailability in municipal solid waste and sewage sludge composts. *Bioresource Technology*, 60(3): 223-229.
- Pichtel, J., & Bradway, D. J. (2008) Conventional crops and organic amendments for Pb, Cd and Zn treatment at a severely contaminated site. *Bioresource Technology*, 99(5): 1242-1251.
- Pierzynski, G. M., Sims, J. T., and Vance, G. F. (2000). Soil phosphorus and Environmental quality. *In "Soils and Environmental Quality"*, 155-207. CRC Press, Boca Raton.
- Rasmussen, H., & Nielsen, P. H. (1996) Iron reduction in activated sludge measured with different extraction techniques. *Water Research*, 30(3): 551-558.
- Roseff, G. (2010) How the Age of Waste Biomass Affects Renewable Energy Production, MEng Project, Civil Engineering, Birmingham University.
- Singer P. (1972) Anaerobic control of phosphate by ferrous iron. *Journal of the Water Pollution Control Federation*, 44 (4): 663-669.
- Smith J. A. (2006) An Investigation into the Anaerobic Digestibility of Iron-dosed Activated Sludge, PhD thesis, Civil Engineering, Birmingham University.
- Smith, J. A., & Carliell-Marquet, C. M. (2008) The digestibility of iron-dosed activated sludge. *Bioresource Technology*, 99(18): 8585-8592.
- Smith, J. A., & Carliell-Marquet, C. M. (2009) A novel laboratory method to determine the biogas potential of iron-dosed activated sludge. *Bioresource Technology*, 100(5): 1767-1774.
-

-
- Solis, G. J., Alonso, E., & Riesco, P. (2002) Distribution of metal extractable fractions during anaerobic sludge treatment in southern Spain WWTPs. *Water Air and Soil Pollution*, 140(1-4): 139-156.
- Sposito, G., Lund, L. J., & Chang, A. C. (1982) Trace-metal chemistry in arid-zone field soils amended with sewage-sludge. 1 Fractionation of Ni, Cu, Zn, Cd, and Pb in solid-phases. *Soil Science Society of America Journal*, 46(2): 260-264.
- Sposito, G., Holtzclaw, K. M., Levesque, C. S., & Johnston, C. T. (1982) Trace-metal chemistry in arid-zone field soils amended with sewage-sludge. 2 Comparative-studio of the fulvic acid fraction. *Soil Science Society of America Journal*, 46(2): 265-270.
- Sposito, G., Levesque, C. S., Leclaire, J. P., & Chang, A. C. (1983) Trace-metal chemistry in arid-zone field soils amended with sewage-sludge.3 Effect of time on the extraction of trace-metals. *Soil Science Society of America Journal*, 47(5): 898-902.
- Stabnikov V.P., Tay S.T.-L., Tay D.-Kh, & Ivanov V.N. (2004) Effect of Iron Hydroxide on Phosphate Reoval during Anaerobic Digestion of Activate Sludge. *Applied Biochemistry and Microbiology*, 40, 376-380.
- Stover, R. C., Sommers, L. E., & Silviera, D. J. (1976) Evaluation of metals in wastewater-sludge. *Journal Water Pollution Control Federation*, 48(9): 2165-2175.
- Straub, K. L., Benz, M., & Schink, B. (2001) Iron metabolism in anoxic environments at near neutral pH. *Fems Microbiology Ecology*, 34(3): 181-186.
- Tessier, A., Campbell, P. G. C., & Bisson, M. (1979) Sequential extraction procedure for the speciation of particulate trace-metals. *Analytical Chemistry*, 51(7): 844-851.
- Thistleton, J., Clark, T., Pearce, P., & Parsons, S. A. (2001) Mechanisms of chemical phosphorus removal - 1-iron (II) salts. *Process Safety and Environmental Protection*, 79(B6): 339-344.
- Thistleton, J., Berry, T. A., Pearce, P., & Parsons, S. A. (2002) Mechanisms of chemical phosphorus removal II - Iron(III) salts. *Process Safety and Environmental Protection*, 80(B5): 265-269.
- Tiyapongpattana, W., Pongsakul, P., Shiowatana, J., & Nacapricha, D. (2004) Sequential extraction of phosphorus in soil and sediment using a continuous-flow system. *Talanta*, 62(4): 765-771.
-

-
- Uhlmann, D., Roske, I., Hupfer, M., & Ohms, G. (1990) A simple method to distinguish between polyphosphate and other phosphate fractions of activated-sludge. *Water Research*, 24(11): 1355-1360.
- Van Hullebusch, E. D., Utomo, S., Zandvoort, M. H., & Lens, P. N. L. (2005) Comparison of three sequential extraction procedures to describe metal fractionation in anaerobic granular sludges. *Talanta*, 65(2): 549-558.
- Wild, D., Kisliakova, A., & Siegrist, H. (1997) Prediction of recycle phosphorus loads from anaerobic digestion. *Water Research*, 31(9): 2300-2308.
- Xie, J. Z., Chang, H. L., & Kilbane, J. J. (1996) Removal and recovery of metal ions from wastewater using biosorbents and chemically modified biosorbents. *Bioresource Technology*, 57(2): 127-136.
- Xie, W. M., Wang, Q. H., Ma, H. Z., Ohsumi, Y., & Ogawa, H. I. (2005) Study on phosphorus removal using a coagulation system. *Process Biochemistry*, 40(8): 2623-2627.
- Yeoman, S., Lester, J. N., & Perry, R. (1990) The effects of chemical phosphorus precipitation on anaerobic-digestion. *Environmental Technology*, 11(8): 709-720.
- Yeoman, S., Lester, J. N., & Perry, R. (1992) Phosphorus removal and its influence on particle-size and heavy-metal distribution during wastewater treatment. *Environmental Technology*, 13(10): 901-924.
- Yeoman, S., Lester, J. N., & Perry, R. (1993) Phosphorus removal and its influence on metal speciation during waste-water treatment. *Water Research*, 27(3): 389-395.
- Yeoman, S., Stephenson, T., Lester, J. N., & Perry, R. (1988) The removal of phosphorus during wastewater treatment: a review. *Environmental Pollution*, 49(2): 183-233.
- Zandvoort, M. H., van Hullebusch, E. D., Feroso, F. G., & Lens, P. N. L. (2006) Trace metals in anaerobic granular sludge reactors: Bioavailability and dosing strategies. Paper presented at the International Symposium on Environmental Biotechnology, Leipzig, Germany.
- Zhang, P., Chen, Y. G., & Zhou, Q. (2009) Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: Effect of pH. *Water Research*, 43(15): 3735-3742.
- Zhang Y., Zhang Z., Suzuki K., & Maekawa T. (2003) Uptake and mass balance of trace metals for methane producing bacteria. *Biomass & Bioenergy*, 25, 427-433.
-

Zheng, X., Tong, J., Li, H. J., & Chen, Y. G. (2009) The investigation of effect of organic carbon sources addition in anaerobic-aerobic (low dissolved oxygen) sequencing batch reactor for nutrients removal from wastewaters. *Bioresource Technology*, 100(9): 2515-2520.

Appendix A

Comparison of two methods for Metal sequential extraction

The comparison between the Fe concentration extracted in the different fractions of the SE method used in this research and the SE method used for Pichtel *et al.* (2007) are shown in table below.

Reagent	Method used in this research (mgFe/gTS)	Pichtel <i>et al.</i> 2007 (mgFe/gTS)	Deviation between both methods
Supernatant	0.17 ±0.01	0.18 ±0.08	0.01
UPW	2.90 ±0.18	3.46 ±0.22	0.36
KNO ₃	4.20 ±0.31	3.95 ±0.27	0.29
NaOH	7.92 ±0.82	8.08 ±0.89	0.75
Na ₂ EDTA Dihydrate	13.61 ±0.72	13.83 ±0.85	0.67
HNO ₃	2.43 ±0.12	1.78 ±0.23	0.39
Aqua Regia	1.42 ±0.18	1.13 ±0.15	0.23
Total	32.65	32.42	

A two sample t Test was applied to all the fractions to compare whether the difference between the concentrations of Fe extracted for both methods was significant. The result of this test with a 99% confidence level concluded that there is no significant difference in the concentrations obtained for both methods.

Appendix B

Mass balance of the iron-dosing system

Fe and P concentration of the different samples used in this research to calculate the Fe and P necessary to simulate Fe-dosing system are presented in the table below:

Fe Salt	P SetS (mg/l)	P RAS (mg/l)	P added (mg/l)	P added (mg/gTS)	Fe added (mg/l)	Fe added (mg/gTS)
FeCl₃ L	7.76	74.87	66.98	21.33	80.21	17.81
Fe₂(SO₄)₃ L	6.38	69.68	71.03	30.62	82.97	35.76
FeSO₄ L	7.76	72.78	68.10	18.11	82.35	21.90
FeCl₃ H	9.87	89.63	52.97	13.87	132.32	34.64
Fe₂(SO₄)₃ H	11.48	71.25	70.79	18.20	176.98	45.50
FeSO₄ H	6.55	77.92	65.67	18.29	150.43	41.90

It should be remained that the calculation base was 40mgP/gTS or 140 mg/l of P for TS concentration of 3.5 g/l in the Fe-dosed AS after dosing.