Alexandra Prodănescu

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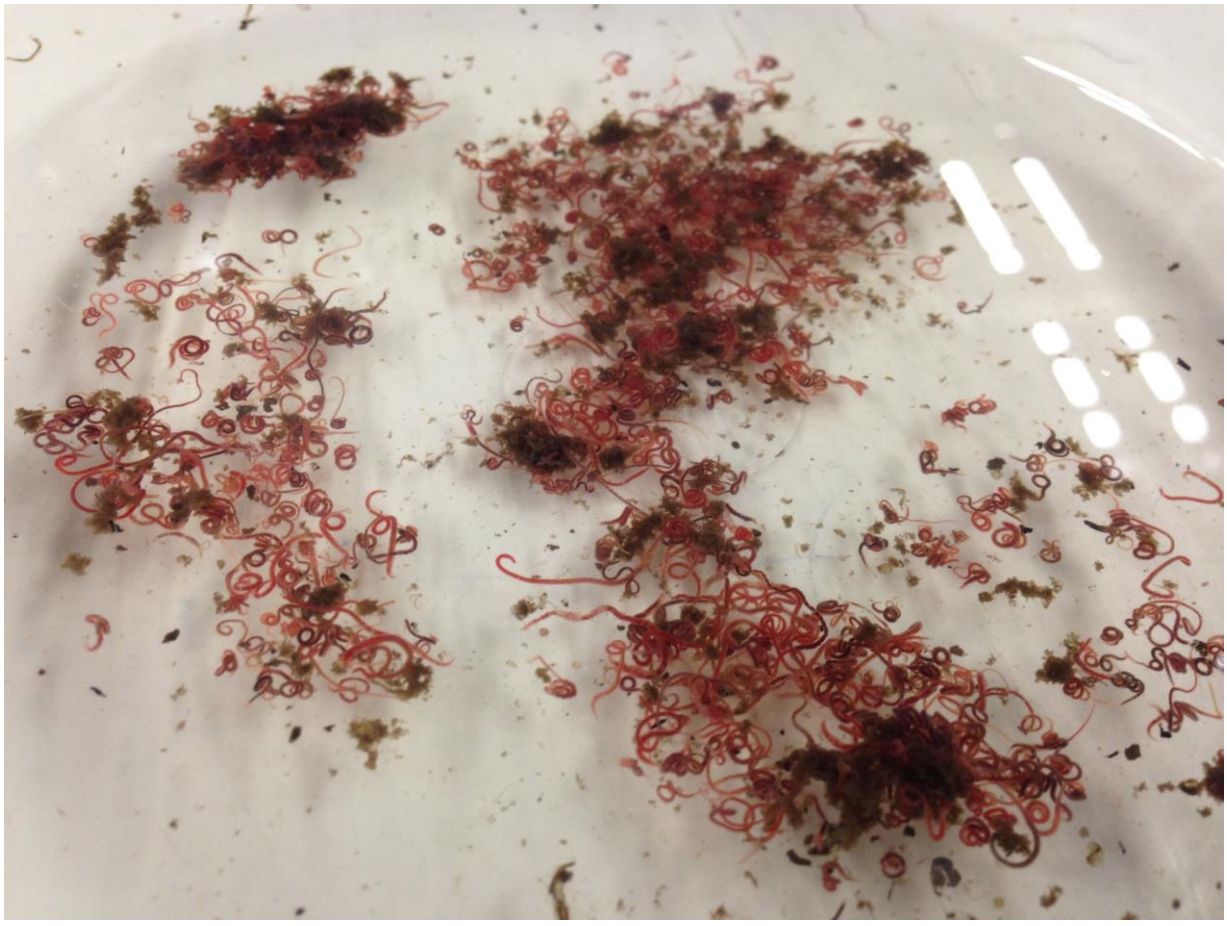
Additional thesis report



TU Delft

Worm predation on excess activated sludge: Solids reduction capabilities and biochemical changes

Additional thesis report

Alexandra Prodănescu

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Concept

Aerobic, anaerobic and worm predated sequential batch digestion experiments were performed on waste activated sludge in order to compare the mechanisms and performances of these three digestion processes. To this aim, the *Tubifex Tubifex* specie was chosen to simulate the worm predation solids reduction process.

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1. Introduction

1.1. Introduction

Domestic and industrial wastewaters treated through a biological aerobic process result in an excess of waste activated sludge mostly consisting of organic and inorganic compounds and microorganisms [Ratsak & Verkuijlen, 2006; Hendrickx et al., 2009a], of which some components such as heavy metals, organic micropollutants and pathogens have a hazardous nature [Serrano et al., 2016]. Due to its potential threat to human health and environmental protection [Wei et al., 2009], it is necessary for waste activated sludge to be stabilized; a process aiming at removing or decreasing volatile solids and pathogens content, as well as odor [Park et al., 2006].

However, the costs of treatment and disposal are substantial and stringent regulations limit discharge methods alternatives [Ratsak & Verkuijlen, 2006; Hendrickx et al., 2009b; Wei et al, 2009; Serrano et al., 2016]. In consequence, innovative strategies and processes for excess activated sludge reduction are assiduously sought for [Huang et al., 2007; Liang et al., 2006b].

One approach is microfauna predation; a method using organisms, belonging to the metazoan or protozoa group and naturally predating on bacteria, for excess sludge reduction [Wei et al., 2003; An & Chen, 2008]. Feeding at higher trophic level means a more inefficient energy conversion rate and, thus, a more substantial amount of energy being used for the maintenance, growth and reproduction processes of the organisms; the result in a more limited population growth for a given amount of substrate consumed [Ratsak & Verkuijlen, 2006; Wei et al., 2003].

Natural development of aquatic worms' population in the aeration tanks of the WWTP is common [Hendrickx et al., 2009a] and, due to their higher position on the food chain, they are considered a fitting candidate for reduction and mineralization of bacterial biomass and organic compounds [Wei et al., 2003]. Moreover, the worm predation digestion promises advantages such as reduced cost due to low energy input and no additional pollution [Liang et al., 2006a; Liang et al., 2006b; Wei et al, 2009]. However, there are disadvantages to be dealt with: sensitivity of worms to various compounds [Huang et al., 2007; Liang et al., 2003; Chapman et al., 1982] and physical conditions [Huang et al., 2007; Chapman et al., 1982; Zhang et al., 2013; Hendrickx et al., 2009b; Buys et al., 2008], issues with retaining the population in the reactor and with maintaining a constant growth [Wei & Liu, 2005; Wei et al., 2009].

Numerous studies have already proven the sludge reduction capabilities of aquatic worms (Table 1). For this reason, this study aims to better understand the mechanisms behind sludge degradation through microfauna predation and not solely observe its effects: rate and extent of digestion. Moreover, between the studies of Park et al., [2006], where a differentiation was made between aerobic and anaerobic digestion regarding the targeted compounds during degradation, and of de Valk et al., [2016], where it was suggested that worm predation targets similar compounds as the aerobic digestion, it was considered equally important to have an extended comparison between aerobic, anaerobic and worm

predated digestions regarding their degradation processes. Furthermore, this was an opportunity to evaluate the possibility and benefits of implementing a two steps digestion with consecutive worm predation and anaerobic digestion.

1.2. Characteristics of Tubifex tubifex

Tubifex tubifex is a worm from the Tubificidaeis family of the Oligochaeta order present in oxic areas of sediments or in aeration steps of wastewater treatment plants. It is formed of red colored segments and measures 100 mm. [Liang et al., 2006a]. The feeding is done through the frontal part of the worm, whilst the posterior part serves for the defecation and the oxygen uptake functions [Hendrickx et al., 2009a]. Suitable conditions for development are important in order to maintain a stable or growing population of T.Tubifex. For worms belonging to the Oligochaeta order a temperature of 15-35^{o}C [Huang et al., 2007; Chapman et al., 1982; Zhang et al, 2013], accompanied by a mild to high aeration of 2-8 mg O_2/L [Hendrickx et al., 2009b; Buys et al., 2008], and a pH in between 3.6 and 10.5 are considered suitable survival conditions [Chapman et al., 1982]. Salinity manifests its toxicity of 50% lethal concentration at 5100 mg/L according to Huang et al., [2007] and at 9000 mg/L according to Chapman et al., [1982].

Ammonia is a known toxicant for worms, with a lethal dose LD50 of 880 mg/L [Huang et al., 2007] for T.Tubifex and of 264 mg/L [Liang et al., 2003] for A.hemprichi. The maximum acceptable dose for long term contact was determined to be 20-50 mg/L [Liang et al., 2003] for A.hemprichi.

Other reported harmful compounds for T.tubifex are heavy metals with some of the most toxic being: copper with a 24h-LC50 of 2.5 mg/L reported by Huang et al., [2007], and mercury and cadmium with 96h-LC50 of 0.14 mg/L and 0.32 mg/L, respectively, tested by Chapman et al., [1982]. Rathore & Khangarot, [2002] determined the T.tubifex 96h-LC50 at 20^{o}C for a series of heavy metals with the following results: mercury 0.048 mg/L; copper 0.0923 mg/L; chromium 2.720 mg/L; cadmium 5.91 mg/L; zinc 14.74 mg/L; nickel 18.97 mg/L; iron 125.42 mg/L; cobalt 179.71 mg/L; manganese 275.70 mg/L and lead 514.19 mg/L.

Worms belonging to the Oligochaeta order have a reported composition of 63% proteins, 25% fat, 7% sugars, 6% ash, 11-13% nitrogen and 0.9-2.2% phosphorus of the dry worm weight [Elissen et al.,2010] [Hendrickx et al., 2010]. A typical dry worm weight (“DW”) and wet worm weight (“WW”) ratio is 0.12-0.14 [Buys et al., 2008] or 0.15 [Hendrickx et al., 2010].

This microfauna occurs naturally in the aeration tanks of the wastewater treatment plants [Hendrickx et al., 2009a], and reduces and mineralizes organic components in sludge and forms: CO_2, H_2O, new worm biomass and faeces [Buys et al., 2008]. Worms appear to digest and use for biological functions 21- 30% of the amount of sludge ingested; the remaining 70- 79% is excreted as worm faeces which naturally present a lower biodegradable organic fraction than the consumed sludge [Hendrickx et al., 2009a].

[Buys et al., 2008] determines the optimum worm to sludge ratio around 0.41-0.61 g TSS worm/g TSS sludge, whilst [Huang et al., 2007] considers it to be around 1600- 2500 mg worm/L, or 0.64- 1 g worm/g VSS sludge, and [Zhang et al, 2013] reports it at 11-12 g worm/L, or 3.67- 4 g wet worm / g TSS sludge. It The optimum worm concentration is important, as excessively dense worm populations result in a

decrease of predation efficiency due to the restricted contact surface and inability of the microfauna to access substrate and oxygen [Huang et al., 2007].

1.3. Sludge reduction capabilities of Tubifex tubifex

Table 1 presents the results of different studies that examined the solids reduction capabilities of worm predation, as a method for excess activated sludge handling. From the viewpoint of solids removal capacity, most researchers are in accord about the potential of this method. However, the is no consensus over exact values, which appear to span over a broad range. This may stem from the considerations that studies utilize different worm species and reactor types. Other limitations of the studies are: the ratio of worms compared to the available substrate is not quantified; the effect of endogenous respiration is not considered ; or the units of the measurements are not adequately presented.

For the case of T. Tubifex predation, both for batch and continuous systems, similar VS reduction rates were found by researchers. However, variable VS reduction extents were measured for worm predation excluding endogenous respiration, and there seems to be little accord over the degree of participation of endogenous respiration in the worm predation process. Common denominators found between researches are the solubilization of organic carbon and the release of NH_4^+, NO_3^- and PO_4^{2-} from floc. The study of de Valk et al., [2016] examined the process in detail and observed a strong decrease in floc proteins, accompanied by a light decrease in floc polysaccharides with constant amounts of floc humics; whilst all soluble major EPS components were found stable. The study analyzed cation occurrence and noticed a decrease in soluble Ca^{2+} and $Fe^{3+/2+}$, and an increase in soluble Mg^{2+} and Al^{3+}.

1.4. Sludge reduction capabilities of aerobic, anaerobic and combined sub-sequential digestion

Table 2 reviews common effects of aerobic and anaerobic digestion, as well as of the combination of the two, excluding the participation of predatory grazing.

According to research papers, aerobic digestion results in a large increase in soluble polysaccharides, Ca^{2+} and Mg^{2+}, and a moderate increase in soluble proteins, NH_4^+, K^+ and NO_3^-. On the contrary, in the opinion of researchers, anaerobic digestion results in a large increase in soluble proteins, NH_4^+, K^+ and $Fe^{3+/2+}$, and either a stable or a small increase in soluble polysaccharides, Ca^{2+} and Mg^{2+}.

Individually aerobic and anaerobic digestion differ in VS reduction capabilities by a relatively moderate margin. However, by making the two processes consecutive , regardless of order, the same VS destruction is achieved. Moreover, each stage behaves similarly to the individual process.

Table 1 Review of worm predation process

Reference	Worm type	Reactor type	Control	Solids reduction ("SR")	Growth rate	Other compunds	Observations
[Hendrickx et al., 2009a]	L.variegatus	Continous	Yes	22-30% VSS SR of "WP incl. ER"; of which 41 vs 59% and 71 vs 29% as "WP excl. ER" vs "ER"	0.013/d or 0.13 g dry worms/g VSS	-	-
[Liang et al., 2006a]	T. Tubifex	Batch	No	0.52- 0.64 mg VS/mg worms,d	-	-	-
[Huang et al., 2007]	T. Tubifex	Continous	No	0.45 mg VS/ mg worms,d	-	Release of 0.09 mg sCOD/ mg worms,d ; 0.03 mg NH3/ mg worms,d ; in 0.0006 mg P/ mg worms,d.	-
[Buys et al., 2008]	L.Variegatus	Batch	Yes	37.8-40.5% VSS SR of "WP incl. ER"; 17.7-20.4% vs 20.1% SR of "WP excl. ER" vs "ER"	-	-	-
[Wei & Liu, 2005]	T. Tubifex	Continous	Yes	63% vs 43% TSS SR of "WP incl. ER" vs "ER" 49% vs 33% VSS SR of "WP incl. ER" vs "ER"	-	Release of 7% TOC, 9% P and NH4+ and NO3-.	-
[Hendrickx et al., 2011]	L.Variegatus	Continous	No	11% TSS SR 16% VSS SR 0.138 mg TSS/mg wet worms,d	0.015/d or 0.18 g dry worms/g TSS	Release of 55 mg (NH4+ +NO2- + NO3-) / g TSS; 17 mg P / g TSS.	-
[Hendrickx et al., 2010]	L.Variegatus	Continous	No	21% TSS SR 26% VSS SR	0.8 g dry worms/g TSS	Reduction of 42% Total COD and 39% Total N Mineralization and release of 58.0 g NH4-N /kg TSS ; 25.8g PO4-P/kg TSS.	"AN"→ "WP" 42% TSS SR; "WP"→ "AN" 50% TSS SR and 40% lower CH4 production.
[Liang et a.l, 2006b]	A.Hemprichi	Continous	No	0.53-6.32 mg VS/mg dry worms ,function of F/M and SRT	-	-	-
[Hendrickx et al., 2009b]	L.Variegatus	Continous	No	6.2-11.8 mg TSS digested/g wet worms,d 36-77% TSS SR	-	-	-
[de Valk et al., 2016]	T. Tubifex	Batch	Yes	47% vs 9% VS SR for "WP incl. ER" vs "ER"	-	Removal of 7.2 g COD/d for "WP incl. ER" vs 1.7 g COD/d for "ER".	↘↘ proteins, ↘polysaccharides and ≈humics in floc. No increase in soluble fractions. ↘Ca2+ and Fe3+/2+ soluble.↗ Mg3+ and Al3+ soluble.
[Tamis et al.,2011]	A. Furcatus	Continous	Yes	0.5-1 g TSS /g dry worms,d 30-40% vs 14% TSS SR "WP incl. ER" vs "ER" 50% VSS SR	-	Release of sCOD; 70 g (NH4 + NO2 +NO3)/ kg TSS; 8 g PO4-P/kg TSS.	"WP"→ "AN" ↗ digestability; 65% VSS SR.

Table 2 Review of aerobic and anaerobic digestion processes

Reference	Aerobic digestion ("AIR")	Anaerobic digestion ("AN")	"AN"→"AIR"	"AIR"→"AN"
[Park et al., 2006]	↗↗ soluble polysaccharides, Ca2+ and Mg2+ ↗ soluble proteins and NH4+	↗↗soluble protein ,NH4+, Fe3+/2+, K+ ↗soluble Ca2+ and Mg2+, ≈ polysaccharides	45.6% VS SR 1st stage→ 17.5% VS SR 2nd stage 1st stage -↗ soluble protein, NH4+ and K+ ; ↘Ca2+ 2nd stage-↗ soluble divalent cations; fluctuating polysaccharides;↘ proteins and NH4+	49.5% VS SR 1st stage→13.1% VS SR 2nd stage 1st stage -↗soluble polysaccharides, Ca2+ and Mg2+ ; 2nd stage- ≈ soluble protein and ↗ NH4+;↘ polysaccharides, Ca2+ and Mg2+
[Novak et al., 2003]	↗↗ soluble polysaccharides, Ca2+ and Mg2+ ↗ soluble proteins, K+ and NH4+ 2x more polysaccharides released compared to proteins Glucosidase ↘, stops day 10 Peptidase ↘ to zero, but recovers day 10	↗↗soluble protein,↗ K+ and NH4+ ↗ soluble polysaccharides , ≈ Ca2+ and Mg2+ 3-5x more proteins released compared to polysaccharides Glucosidase ≈ Peptidase ↘	-	-
[Foladori et al. 2015]	Cell lysis Degradation of released compounds	Cell death but no lysis Solubilization and hydrolysys of nonbacterial material ↗ soluble COD and NH4+	-	-
[Park & Novak, 2007]	31% VS SR ↗↗solution polysaccharides and divalent cations ↗ soluble NH4+ and NO3-	39% VS SR ↗↗solution proteins and NH4+	-	-

2. Materials and methods- Experimental approach

2.1. Experimental tracks

The research consisted of six combinations of two sub-sequential digestion stages: one aerobic stage and one anaerobic stage. The aerobic digestion stage was either a 7 days worm predated digestion ("WP"), a 7 days endogenous respiration ("ER") or a 40 days extended endogenous respiration ("AIR"). The anaerobic stage was a 40 day anaerobic digestion ("AN"). To this aim, digestion batch experiments were undertaken. All experimental tracks were performed in biological triplicate. Complete measurements were performed in the beginning, stage switch and end of each experimental track. Additionally, two partial intermediary measurements were executed for the AIR and AN stage.

Due to scheduling issues, the exact number of days was later modified. The experimental tracks and their actual timelines are presented in Table 3:

Sequencial digestion steps		Abreviation	Start	Sampling 1	Sampling 2	Switch	Sampling 3	Sampling 4	End
Stage 1	Stage 2								
Extended endogenous respiration→	Anaerobic digestion	AIR→AN	Day 0	Day 12	Day 22	Day 42	Day 55	Day 68	Day 89
Endogenous respiration→	Anaerobic digestion	ER→AN	Day 0	-	-	Day 7	Day 19	Day 28	Day 49
Worm predated digestion→	Anaerobic digestion	WP→AN	Day 0	-	-	Day 7	Day 19	Day 28	Day 49
Anaerobic digestion→	Extended endogenous respiration	AN→AIR	Day 0	Day 12	Day 22	Day 42	Day 55	Day 68	Day 89
Anaerobic digestion→	Endogenous respiration	AN→ER	Day 0	Day 12	Day 22	Day 42	-	-	Day 49
Anaerobic digestion→	Worm predates digestion	AN→WP	Day 0	Day 12	Day 22	Day 42	-	-	Day 49

Table 3 Experimental tracks and actual timelines

Two tracks, AN→ ER and AN→ WP, failed due to the *Tubifex Tubifex* worms' high death rate, where, by the end of the 2nd stage, no worms where found back in any of the triplicate batches. Considering oxygen, temperature and pH level where in the required range, it is likely the lethality was cause by considerable amount of ammonia following the 1st stage anaerobic digestion.

2.2. Excess activated sludge characteristics

For the start of the experiment, waste activated sludge ("WAS") was freshly collected from the Municipal Waste Water Treatment Plant of Harnaschpolder, Den Hoorn, The Netherlands. The WAS, displaying concentrations of 2.53±0.09 g VS/L and 4.03±0.14 g TS/L, was used as an initial substrate for all first stages of the experimental tracks. The digestates resulting from the first stages were used as a feed for the second stages according to the predetermined experimental tracks.

2.3. Tubifex Tubifex worms characteristics

The worms from the specie *Tubifex tubifex* were procured from a local wholesale (Aquadip b.v., The Netherlands). Before use, the worms were allowed an adaptation time to the experimental conditions and substrate content. To this aim the worms were cultivated in a special 18L tank with an airlift system and were fed WAS from the same source as the substrate used in the experiment. Oxygen levels where maintained above 5 mg/L and pH was in the range of 7-7.5. Before the experiments, the worms were not starved, nor purged. Immediately before use, the worms were thoroughly rinsed of any debris and

contaminants with demiwater. The worms were dried of excess water and weighted. 150.14±0.1484 g of wet worms were used per 3.5 L substrate for the WP stage.

2.4. Experimental batches

Each triplicate from the aerobic stage consisted of 3.5L sample. The bottles were aerated at levels above 5 mg/L using air stones, which provided additional suspension and mixing of solids. The aerobic stages were tested at room temperature, which was estimated at around 18- 20°C.

Each triplicate from the anaerobic stage consisted of bottles of: 1L sample of WAS used for sampling and 2L sample used for gas production measurements. The remaining volume required for the second stages was digested in large 5L bottles. In addition to the WAS substrate, the sample was inoculated with 1 mL inoculum/L WAS with 19.64±0.05mg VS/L and 31.68±0.09 mg TS/L. The bottles were flushed for 2 minutes with N_2 gas, isolated with air tight sealing caps and connected to a water sealed gas pressure release system or an AMPTS II system (Bio-Proccess Control, Sweden) with a CO_2 trap. The samples were placed in a thermal shaker at 35°C and 120rpm.

All samples were marked with the initial sample volumes, as well as new sample volume after each sampling, in order to prevent measuring errors due to evaporation. If evaporation occurred, demiwater was added up to the marked level and allowed a sufficient mixing time before sampling.

3. Materials and methods -Analysis

3.1. Parameters

Table 4 presents the measurements performed at different sampling points:

Measurement	Points	VS/TS	Total Ca^{2+}, Mg^{2+}, Fe^{3+}	Soluble Ca^{2+}, Mg^{2+}, Fe^{3+}	Soluble PO_4^{3-}, NO_3^-, NO_2^-, SO_4^{2-}	Floc carbohydrates and proteins	Soluble carbohydrates and proteins	Total N	COD	NH_4^+
Complete measurements	Start, Switch point, End	✓	✓	✓	✓	✓	✓	✓	✓	✓
Partial measurement	Intermediary sampling points	✓	×	✓	✓	×	✓	×	×	✓

Table 4 Parameters

Soluble components are referred in the case of measurements made on samples previously filtered over a 0.45 µm polyether sulfone filter (VWR International, USA). Floc components are referred to for measurements made on the remaining solids after separation and discharge of the supernatant. Total components are referred to for measurements made on the unaltered sample, including floc and soluble components.

3.2. Extracellular polymeric substances extraction

The EPS extraction method was a modified approach from [Frølund et al.,1996]. A 2mM Na_3PO_4, 4mM NaH_2PO_4, 9mM NaCl and 1mM KCl buffer was created and pH was adjusted to pH 7 using 1M NaOH solution. 70g resin/g VS cation exchange resin Dowex Marathon C Na^+ form (Sigma Aldrich- Missouri, USA) were used for the extraction. The resin was washed twice with 50ml buffer for 30 min at a constant mixing speed. 0.5g VS were extracted from the sludge by centrifuging the required sludge volume at 12 000 rpm for 15 minutes. The resulting pellet was then twice resuspended to initial volume using the buffer and centrifuged at 12 000 rpm for 15 minutes. The resulting 0.5g pellet was added to the 35g resin and the glassware was filled up with buffer to the 150-ml mark. The extraction procedure required 17h or mixing the suspension at 800 rpm and 4°C. Finally, the resin was allowed to settle and the supernatant was centrifuged at 12 000g and 4°C for 30min. The resulting supernatant was filtered over 0.45µm polyether sulfone filter (VWR International, USA) and stored at low temperature until measurements.

[Park & Novak, 2007] Cation exchange resin extraction method extracts predominantly Ca^{2+} and Mg^{2+} bound EPS, presumingly the lectin like proteins which are commonly associated with divalent cations and have a high affinity for binding polysaccharides. However, Fe^{3+} and Al^{3+} bound proteins are not extracted. So, the CER extraction method is more suitable for detecting changes following AIR digestion and not AN digestion and this should be considered in the interpretation of the results.

3.3. Carbohydrates measurement

The carbohydrates measurement was adapted from [Dubois et al., 1956]. 1ml of 5% phenol solution was added to the 2ml sample and the blend was mixed and allowed to react for 10 min. 5ml of 97% sulphuric acid were added in spouts and the mix was left for rest for 10 min. The mixture was then powerfully mixed and allowed to fully develop its color for 30 min at room temperature. The absorbance value was measured in 4cm cuvettes at a 487nm wavelength with a spectrophotometer Genesys 10S UV-VIS Thermo Scientific machine (Thermo Fisher-Bleiswijk, Netherlands), against a demiwater blank.

The calibration curve between absorbance and carbohydrates concentration was made using glucose and the results are presented in Table 5 and Figure 1:

Point	Carbohydrates		
	Glucose concentration [mg/l]	Abs 487nm	Function calibration curve
Reference	0	0.088	c%= 97.655*(Abs)
1	8	0.15	
2	16	0.162	
3	20	0.262	
4	32	0.314	
5	40	0.365	
6	48	0.431	
7	60	0.648	

Table 5 Carbohydrates calibration curve

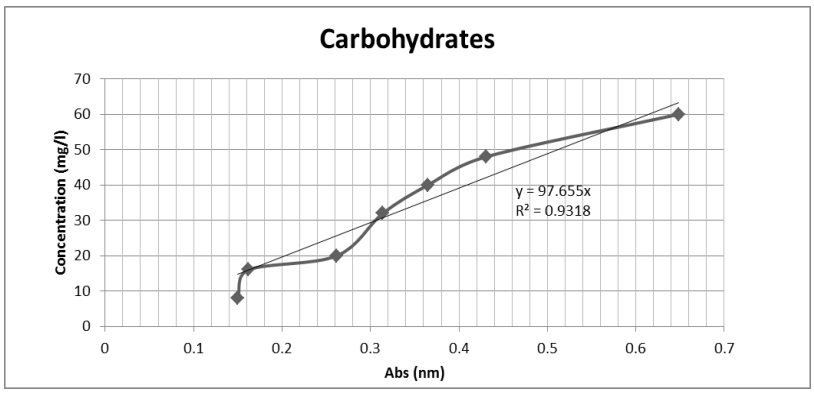

Figure 1 Carbohydrates calibration curve

Due to the very high concentrations of nitrate leading to no soluble polysaccharides being measured in samples, the carbohydrates measurements where not corrected for the interference of nitrate as suggested by [Rosenberger et al., 2005] with the formula:

$$Real\ conc._{Carbs} = \ Measured\ conc._{Carbs} - 0.19 * Conc._{NO3-}$$ (Eq.1)

3.4. Proteins and humics measurement

The proteins measurement was adapted from [Lowry et al., 1951] and corrected according to [Frølund et al.,1996] to include the adjustment for the interference of humics.

Solutions A, B, C and E were previously prepared, where:

- A: 143 mM NaOH and 270 mM Na_2CO_3
- B: 57 mM CuSO4
- C: 124 mM $C_4H_4Na_2O_6$
- E: 1: 2 ratio Folin-Ciocalteu phenol: demiwater

Solution D was prepared on the measurement day, where:

- For proteins measurement, solution D was a 100:1:1 ratio of A: B: C solutions
- For humics measurement, solution D was a 100:1:1 ratio of A: Demiwater: C solutions

3.5ml of solution D were added to the 2.5ml sample and the blend was mixed and allowed to react for 10 min. 0.5ml of solution E were added and the mixture was vortexed and allowed to fully develop its color for 45 min at room temperature. The absorbance value was measured in 4cm cuvettes at a 750nm wavelength with a spectrophotometer Genesys 10S UV-VIS Thermo Scientific machine (Thermo Fisher-Bleiswijk, Netherlands), against a demiwater blank.

The calibration curve between absorbance and proteins concentration was made using Bovine albumin serum ("BSA") and the results are presented in Table 6 and Figure 2:

Point	Proteins		
	BSA concentration [mg/l]	Abs 750nm	Function calibration curve
Reference	0	0.063	c%= 168.06*(Abs)
1	13	0.15	
2	26	0.211	
3	39	0.279	
4	52	0.335	
5	65	0.41	
6	130	0.696	
7			

Table 6 Proteins calibration curves

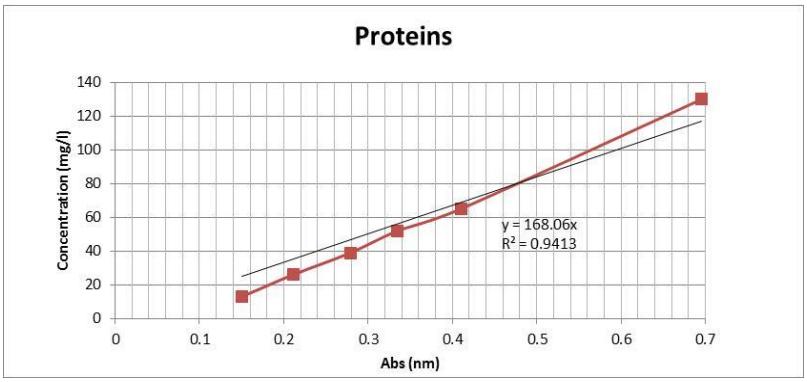

Figure 2 Proteins calibration curves

Even though this adjustment is neglected in all reviewed scientific paper, all proteins measurements were corrected for the interference of humics, in order to avoid overestimations of 40% higher proteins concentration, according to [Frølund et al.,1996], with the formula:

$$Real\ abs._{Proteins} = 1.25 * (Measured\ abs._{Proteins} - Measured\ abs._{Humics}) \quad \text{(Eq.2)}$$

3.5. Biochemical methane potential measurement

For each of the 4 studied tracks, in the anaerobic stage a Biochemical methane potential measurement was made, using the sample itself as a substrate, which was inoculate with 1 mL inoculum/L WAS with a 19.64±0.05mg VS/L and 31.68±0.09mg TS/L, stripped of oxygen and placed in a thermal shaker at 35°C and 120rpm. An automatic machine AMPTS II (Bio-Proccess Control, Sweden) with a in line CO_2 trap was used for this purpose.

3.6. Other parameters measurements

Volatile solids and total solids were measured according to Standard Methods [Eaton et al., 1998].

Total and soluble Ca^{2+}, Mg^{2+}, Fe^{3+} were prepared using a modified version of the [Langerak et al. 1998] digestion method. According to [Langerak et al. 1998] for soluble cations concentrations the samples should be filtered over a 0.45 µm filter, after which the sample should be acidified up to a of pH 2 with HNO3 (65%). For analysis of total cations concentrations, to a 20ml sample, 2.5 ml HNO3 (65%), 7.5 ml HCl (37%) and 10 ml demiwater water should be added. The mixture should be heat digested for 2h at 80°C, subsequently cooled, and diluted with demineralized water in a volumetric flask of 100 ml. Prior to analysis, all samples should be diluted with 0.2% lanthan nitrate solution. The laboratory, where soluble and total cations were analysed, digested the samples by adding 4 ml of HNO3 (80%) to 20 ml of sample in a digestion tube and heating it for 30 minutes until it reached 103°C, and then for another 2 hours at the same temperature. After the digestion was stopped and the sample was cooled, the solution was diluted to a 50ml mixture with demineralized water. The pH of these samples was below pH 1, indicating a full digestion. The measurements where then made using inductively coupled plasma mass spectrometry with an ICP-MS Xseries II machine (Thermo Fisher Scientific Carlsbad – California, USA).

Soluble PO_4^{2-}, NO_3^-, NO_2^-, SO_4^{2-} were measured using ion chromatography with an 881 Compact IC Pro machine (Metrohm- Herisau, Switzerland).

COD and NH_4^+ were measured using photometric cell tests (Hach- Düsseldorf, Germany) on a Hach DR 3900 machine (Hach- Düsseldorf, Germany). Total N was measured using a photometric cell test (Merck Millipore- Darmstadt, Germany) on a Spectroquant NOVA60 machine (Merck Millipore- Darmstadt, Germany).

4. Results

A differentiation between the pure worm predation process and the modified worm predation process due to the concomitant natural sludge breakdown is important. The single worm predation phenomenon excluding endogenous respiration ("(WP-ER)→AN") gives insight into the sludge reduction capabilities of this micro fauna; whilst the combined worm predation process including endogenous respiration ("WP→AN") provides a realistic assessment of the capacity of such a system where both digestion processes inevitably occur simultaneous. By subtracting the performance of the "ER" blanc from the "WP" in the 1st stage, it is likely that the capabilities of the "WP-ER" are underestimated, as it is not possible to assert that components consumed by the "ER" blanc, would not have otherwise been available for the "WP".

The results of stages that include worm predation are not relativized to the weight of the worm population and, thus, are representative solely for a fixed F/M ratio. Reporting results as a function of worm weight would not allow for further comparison with the other stages/tracks that do not include worm populations due to the difference in units.

4.1. Total and volatile solids

Volatile solids ("VS")							
	VS reduction per stage [g/L]		VS reduction per stage [%]			VS reduction rate [g/L,d]	
	Stage 1	Stage 2	Stage 1	Stage 2	Rest	Stage 1	Stage 2
ER→AN	0.1321	0.9081	5.2	35.8	59.0	0.0165	0.0211
WP→AN	0.7314	0.5918	28.8	23.3	47.9	0.0914	0.0138
AIR→AN	0.8804	0.3507	34.7	13.8	51.5	0.0205	0.0073
AN→AIR	1.0660	0.1064	42.0	4.2	53.8	0.0248	0.0022
(WP-ER)→AN	0.5993	0.5918	23.6	23.3	53.1	0.0749	0.0138

Table 7 Volatile solids reduction

The 1st stage of the WP→AN, clearly shows the potential of the worm predated digestion by displaying a VS reduction capability 5.5x of that of the 1st stage of the ER→AN in a similar amount of time, and of 83.1% and 68.6% of that of the 1st stage of the AIR→ AN and AN→AIR, respectively, in 1/6 of the time (Table 7 and Figure 3). This translates into a reduction rate of 0.09 g/L, d, which is 4.5x higher than the 1st stage of AIR→ AN and 3.7x higher than the 1st stage of AN→AIR. By assuming that in 7 days the worm predation process finalized, it can be suggested that WP resembles the AIR digestion from the viewpoint of the extent of VS removal, but outperforms the latter from the viewpoint of the rate of VS removal.

The 2nd stage of the worm predation reduces 0.24 g VS/L more than the extended aeration track and 0.49 g VS/L more than the anaerobic track, likely due to the higher amount of residual substrate undigested in 1st stage. Moreover, in the 2nd stage of the WP→AN, it is noticeable that the digestion process ends before the batch experiment stops, as there is no solids destruction from the last sampling point to the end of the batch (Figure 3). Hence the anaerobic digestion process following worm predation presents a higher digestion rate than the anaerobic digestion process following aerobic digestion. It can be hypothesis that worm predation strongly participates in the solubilization and

breakdown of complex compounds, making then more rapidly available for the second stage of digestion.

The WP→AN track results in the highest overall VS decrease, with 52.1% of the initial VS concentration being digested. This is with 3.6 percentage points more than the AIR→ AN, and with 5.9 percentage points more than the AN→AIR (Figure 5). Assuming that the maximum achievable degradation through consecutive combined anaerobic and aerobic digestions is reached due to duration of the experiment, and that the readily biodegradable VS can be degraded part only aerobically, part only anaerobically and part by both digestion processes, it goes to show that the worm predation process possibly hydrolyzes slowly biodegradable compounds similar to those degraded in the extended aeration, allowing thus for a more extensive digestion.

In the WP→AN tracks, the worm predation is responsible for 81.9% of sludge reduction and endogenous respiration of the remaining 18.1% for the first stage. Looking solely at the effect of worm predation on sludge reduction, the (WP-ER)→AN displays a reduction of 0.60 g VS/L in the 1st stage, representing 68.1% and 56.1% of that of the 1st stage of the AIR→ AN and AN→AIR respectively in 1/6 of the time. A reduction rate of 0.07 g/L, d which is 3.7x and 3.0x times higher than the reduction rate of AIR→ AN and AN→AIR respectively, proves the rapidity of the worm predation process.

The AIR→AN and AN→AIR tracks present higher VS reduction capabilities in the 1st stage, probably due to the availability of both digestion process specific and generally available compounds, and then much lower ones in the 2nd stage, when only digestion process specific substrates remain (Figure 4). However, both tracks reach similar final VS elimination values, supporting the findings of [Park et al., 2006]. By approximating that only 13.8% is digested specifically anaerobically (2nd stage AIR→AN) and only 4.2% specifically aerobically (2nd stage AN → AIR), then the majority of the substrate, 28.2-30.5%, can be degraded both aerobically and anaerobically.

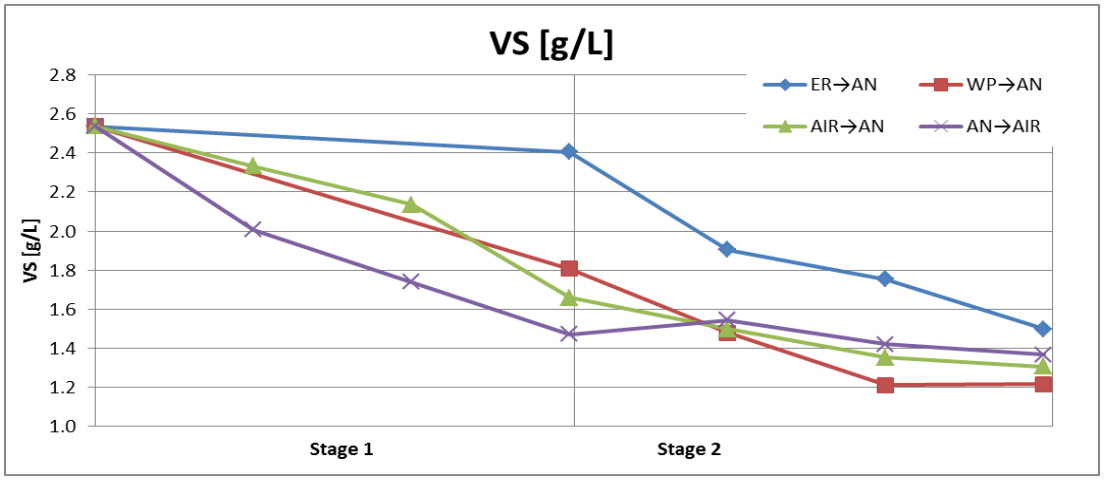

Figure 3 Volatile solids reduction

The slight increase in VS concentration in 2nd stage of AN→AIR (Figure 3) could be explained by the fact that all sample recipients (sampling, AMPTS and storage sample bottles) of anaerobic digestate were mixed together before being dosed as substrate for the aerobic digestion (Figure 3), whilst only the sampling sample recipients were used for measurements. This might have resulted in a false, higher final VS concentration reading.

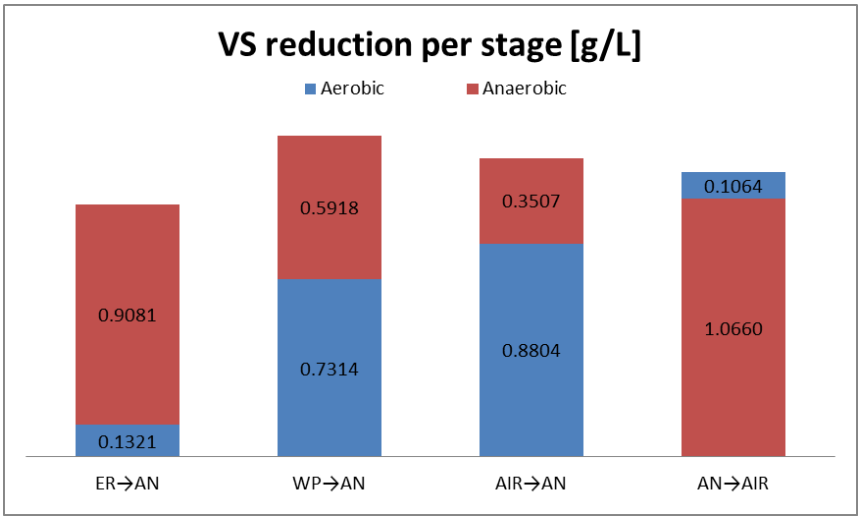

Figure 4 Volatile solids reduction per stage

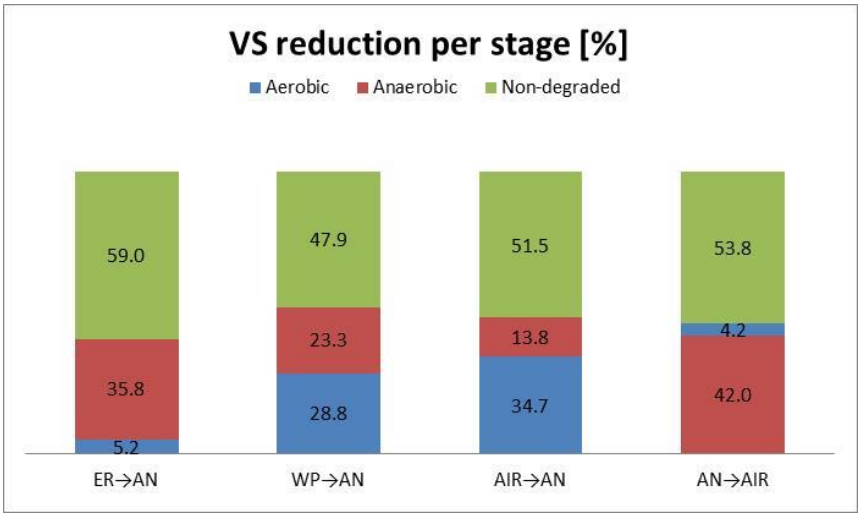

Figure 5 Volatile solids percentages degraded per stage

Full values for VS and TS reduction can be found in Annex Table 1. All values for TS reduction can be found in Annex Table 2 and Figures 1, 2 and 3.

4.2. Total Chemical oxygen demand

Total COD							
	COD reduction per		COD reduction per stage			COD reduction rate	
	Stage 1	Stage 2	Stage 1	Stage 2	Rest	Stage 1	Stage 2
ER→AN	0.9123	1.0833	21.2	25.2	53.5	0.1140	0.0252
WP→AN	1.8810	0.5840	43.8	13.6	42.6	0.2351	0.0136
AIR→AN	2.2760	0.4053	53.0	9.4	37.6	0.0529	0.0084
AN→AIR	1.9027	0.5167	44.3	12.0	43.7	0.0442	0.0108
(WP-ER)→AN	0.9687	0.5840	22.6	13.6	63.8	0.1211	0.0136

Table 8 Total COD reduction

The 1st stage of the WP→AN, reasserts the potential of the worm predated digestion by displaying a COD reduction capability 2x of that from the 1st stage of the ER→AN in a similar amount of time, and of 83% of that of the 1st stage of the AIR→ AN and 101% of that the 1st stage of the AN→AIR in much shorter time (Table 8 and Figure 6). This translates into a reduction rate of 0.24 g/L, d, which is 4.4x times higher than the AIR→ AN and 5.3x times higher than the AN→AIR.

Moreover, in the 2nd stage the WP→AN has the ability to reduce an extra 0.18 g/L COD compared to the same stage from the AIR→AN track and an additional 0.07g/L from the AN→AIR track.

The overall Total COD reduction of worm digestion is in this case comparable to that of the AN→AIR track, with only 1.1 percental points higher, but inferior to that of the AIR→AN track, with 5 percental units lower.

In the WP→AN worm predation is responsible for 51.5% of COD removal and natural breakdown of the remaining 48.5% for the first stage. Looking solely at the effect of worm predation on sludge reduction, the (WP-ER)→AN displays a reduction 0.97 g COD/L in the 1st stage, representing 42.6% and 50.9% of that of the 1st stage of the AIR→ AN and AN→AIR respectively in 1/6 of the time. The reduction rate of 0.12 g/L, d which is 2.3x and 2.7x times higher than the reduction rate of AIR→ AN and AN→AIR respectively, proves the high rate of the worm predation.

The AIR→AN track presents a higher reduction in the aerobic phase than the anaerobic phase of the AN→AIR track. The 2nd stage of both tracks showed a similar reduction capability.

The difference between the overall performance in the VS and COD reduction may be due to the Total COD measurement method, which does not completely oxidize specific organic compounds, does not differentiate between biodegradable and inert organic matter and can be interfered with by the oxidation of inorganic compounds such as sulfate and iron. So, Total COD can be used solely as broad indicator of the reduction processes. Approximating soluble COD by estimating the mg COD/mg VS and subtracting the floc COD is not possible due to the unquantified reduction on COD in the floc during the different processes.

Full values for Total COD reduction can be found in Annex Table 3.

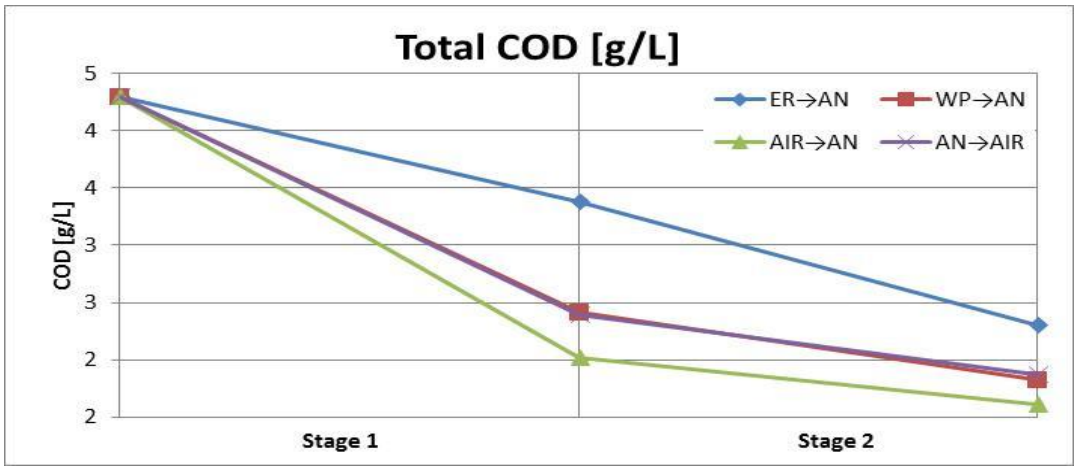

Figure 6 Total COD reduction

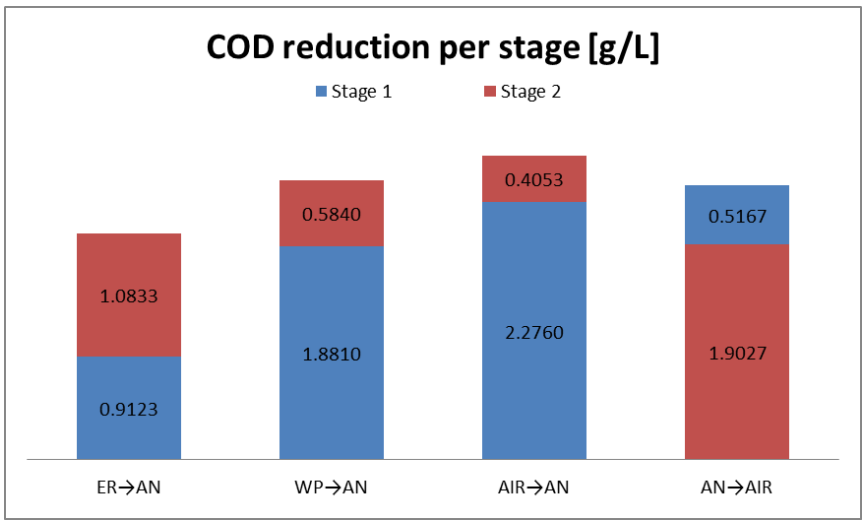

Figure 7 Total COD reduction per stage

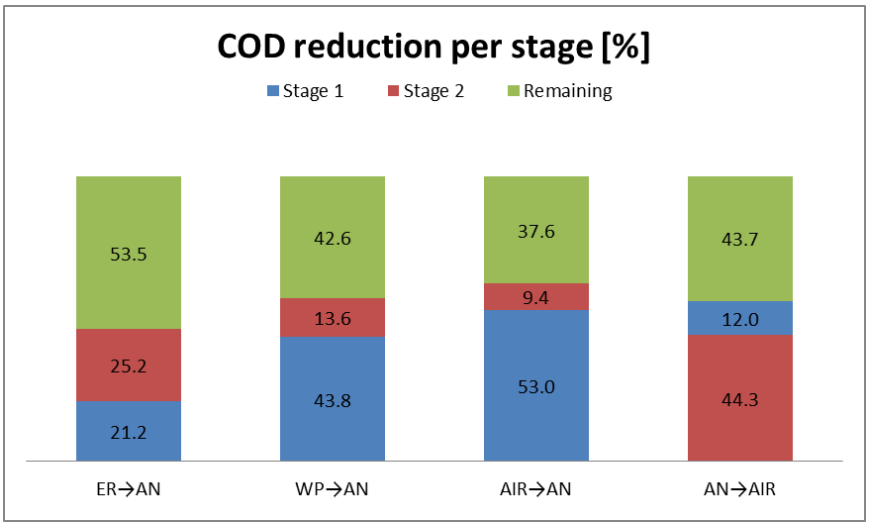

Figure 8 Total COD percentage reduction per stage

4.3. *Tubifex tubifex growth rate and yield*

Worms growth in the WP→AN was observed at a worm growth rate of 0.14 d-1, and with a yield of 1.87 g wet worm/g COD digested,d or 3.02 wet worms/g VS digested,d considering the single worm predation, so excluding the effects of the endogenous respiration. The T.Tubifex independently displayed a reduction capacity of 0.77 mg COD/g wet worms,d and 0.48 mg VS/g wet worms,d. Additional values are presented in Annex Table 4.

4.4. *Soluble sulfate, phosphate, nitrate, nitrite*

Nitrate and nitrite Variations in nitrate and nitrite level result from the processes of nitrification and denitrification. During aerobic phases, the accumulation of nitrate (Eq.3) as a result of nitrification, as well as that of the intermediary nitrite (Eq.4), occurs. During the anaerobic phases, assuming acetate as electron donor, the nitrate reduces to the intermediary nitrite compound (Eq.5) before being converted to N_2 gas (Eq.6). The first step of nitrification and denitrification is the rate limiting one, so accumulation of nitrite is not usual.

$2NH_4^+ + O_2 \leftrightarrow 2NO_2^- + 4H^+ + H_2O$ (Eq.3)

$2NO_2^- + O_2 \leftrightarrow 2NO_3^-$ (Eq.4)

$0.25CH_3COO^- + NO_3^- \leftrightarrow 0.5NO_2^- + 0.25CO_2 + 0.5H_2O + 0.25HCO_3^-$ (Eq.5)

$0.375CH_3COO^- + NO_2^- + 0.625H^+ \leftrightarrow 0.5N_2 + 0.5H_2O + 0.75HCO_3^-$ (Eq.6)

Expectedly, there is an accumulation of NO_3^- in the aerobic phases of ER→ AN, WP→ AN and AIR→ AN. For the initial stage, the worm predation NO_3^- concentration increase was 1.61x the amount of nitrate released in the control and 0.57x of the one released from the extended aeration. Probably due to the short duration of the ER→ AN and WP→ AN, the amount of amassed nitrate is lower than in the case of the extended aeration. However, the increased amount of NO_3^- occurring in the worm predated phase compared to the short endogenous respiration track may indicate a higher ammonium concentration in the batches, and implicitly a higher protein degradation. In the anaerobic phase, the ER→ AN and WP→ AN fully denitrifies even before the first intermediary measurement. However, the presence of nitrate in the last intermediary measurement of the anaerobic phase of AIR→ AN could show that the samples remained anoxic for a very long time and that the normal anaerobic solids digestion and methane production was derailed. This could be due to the high concentration of nitrate accompanied by a low carbon source presence. For the AN→AIR process, in the 1st stage an immediate reduction of any existing nitrate can be observed. However, the lack of nitrate accumulation combined with a decrease in NH_4^+ concentration (Figure 14) during the 2nd stage is unusual, as the NH_4^+ was expected to have nitrified. After a 40d anaerobic digestion it is possible that the entire nitrifying biomass was inactivated or eradicated. Moreover, it is possible NH_4^+ was used in aerobic bacterial growth, as N represents 12% of the typical bacterial composition [Metcalf & Eddy, 2014], or precipitated. However, a distinction between biomass growth and VS removal is not possible.

Nitrite concentrations were considered insignificant and due to their variability in time as intermediary compounds, will not be used for interpretation of processes but only for completing in mass balances.

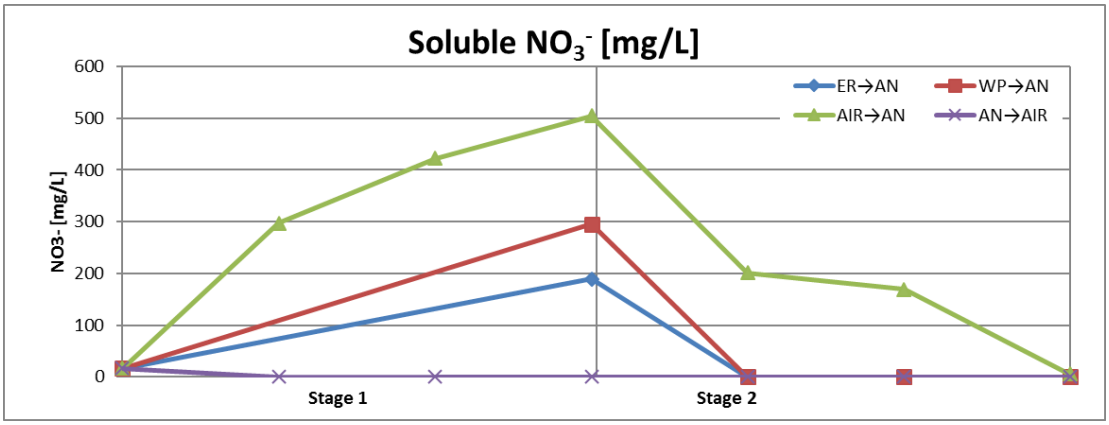

Figure 9 Soluble nitrate

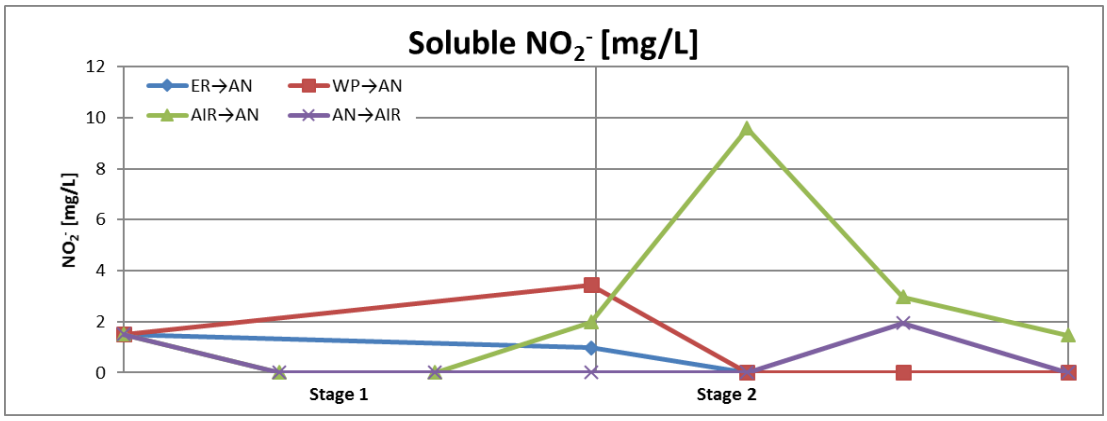

Figure 10 Soluble nitrite

Phosphate Evaluating the variations in soluble phosphate is problematic due to the simultaneity of the processes of: orthophosphate release as a result of degradation of general and PAO biomass, orthophosphate release from PAOs' anaerobic activity, orthophosphate release due to resolubilization from inorganic particles, orthophosphate uptake from PAOs' aerobic activity, orthophosphate uptake for biomass growth and precipitation with different cations. Organic phosphorus is present microbial cells in quantities of 20 mg P/g VS, or 2% of bacteria dry weight, [Metcalf & Eddy, 2014] and is released as orthophosphate as a result of cell degradation. Orthophosphate accumulating bacteria integrate 200-300 mg P/g VSS [Metcalf & Eddy, 2014] under aerobic conditions, which are then releases under anaerobic conditions. The Harnashpolder WWTP, which was the source for the activated sludge used within this experiment, contains a biological phosphorus removal step and PAO organisms.

In the worm predated track, the orthophosphate release reaches 48.1% of the production of the AIR AN and 276% of that of the ER AN.

However, the quantities released cannot only be explained from digestion of typical biomass. Assuming orthophosphate represents 2% of the cell composition [Metcalf & Eddy, 2014], and considering the measured VS removal, only 17.60, 14.63 and 2.64 mg/L increase for the AIR→AN, WP→AN and ER→AN can be accounted to cell death and lysis. The remaining may be the result of digestion of PAO organisms or resolubilization of orthophosphates from the inorganic particles. Additional absorption of phosphate by PAO is not expected as, even if maybe active, the sludge was freshly collected from the aerobic zone and may be already saturated.

In the beginning of the 2nd stage, WP→AN and ER→AN continue to release PO43-maybe as a result of continued degradation of biomass (incl. PAO) or maybe due to the release of PO43- from PAO (probable to a lower extent due to lack of cyclicity and readily available carbon as ATP source). This is until the removal of orthophosphate by precipitation with cations released from floc appears to takes over. The removal of orthophosphate only in anaerobic phases means PO43- formed salts with iron or aluminum, usually released from floc at higher rates in anaerobic stages. In the case of AIR→AN the soluble phosphate decreases immediately, since the major part of the degradation process already took place in Stage 1, leaving only the fraction of VS degradable specifically by anaerobic digestion to be removed. Contrary, for the WP and ER tracks, a higher quantity of VS remained to be digested and release PO43- , thus the rate of phosphate release surpassing the one of phosphate precipitation. The AN→AIR releases any existing PAO orthophosphate immediately, together with the one provided by cell degradation, after which precipitation occurs. For its 2nd stage no phosphate release was detected, which coincides with the low additional VS removal and can also be the result of Fe^{2+} becoming Fe^{3+} in aerobic conditions and having an increased binding power and capability to form precipitates.

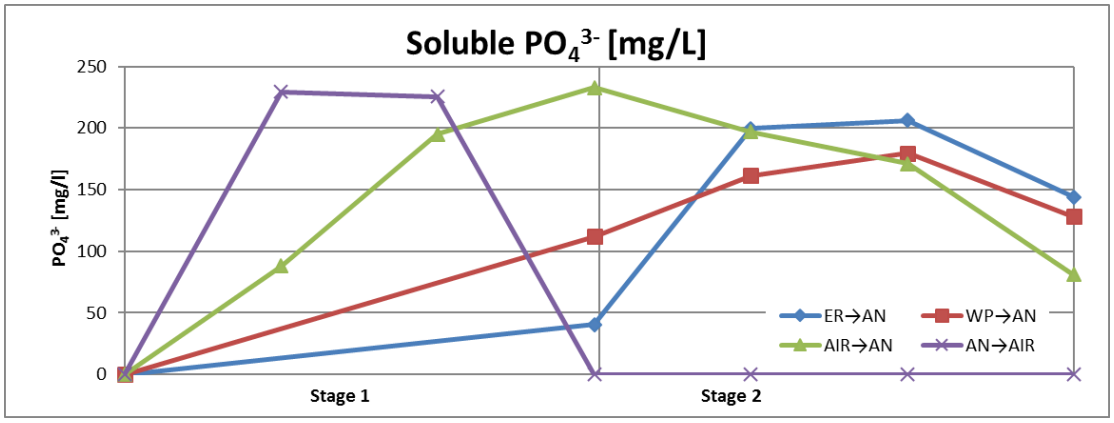

Figure 11 Soluble phosphate

Sulphate Sulfur represents 10mg P/g VS, or 1% of bacteria dry weight, [Metcalf & Eddy, 2014] of the typical biomass composition and is released as sulfate as a result of degradation of organic matter. Under anaerobic condition sulfates are reduced (Eq.7), using acetate as an electron donor, to S^- and further form hydrogen sulfide gas(Eq.8). Otherwise, the S^{2-} anion form ferric and ferrous sulfide components or other compounds.

$CH_3COO^- + {SO_4}^{2-} \leftrightarrow S^- + CO_2 + H_2O + {HCO_3}^-$ (Eq.7)

$S^- + 2H^+ \leftrightarrow H_2S$ (Eq.8)

WP displays a release of 78.6% of the AIR→AN and 172% of the ER→AN. The solubilization of sulphate as the result of cell degradation only accounts for 1% of VS degraded and does not cover the whole measured solubilized amount, the rest originating possibly from the sediments. In the anaerobic stage of these 3 tracks, sulphate is reduced to gas at different rates. The slowest process takes place for the AIR→AN track possibly due to the lack of readily available carbon source, due to an extensive and lengthy VS/COD reduction in Stage 1. The AN→AIR immediately reduces any existing sulphate in the beginning of the anaerobic phase and remains zero, as the rate of gasification is possibly higher than the one of solubilization. In the 2nd stage the amount of VS reduced is unimportant and so no significant release of sulphate can be observed.

Interestingly, the release of soluble anions matches well between the anions themselves, as well as with the VS and Total COD reduction.

The T.Tubifex resulting in an increase in soluble nitrate suggests protein being degraded. However, given the similar behavior with the control and extended aeration tracks as well as the fact that phosphate did not precipitate from the solution in the aerobic phase and only did so in anaerobic phase, it could be assumed the proteins consumed by the worms are not iron nor aluminum bound proteins, but may be lectin like proteins. The later are usually bound by calcium and magnesium, which have a weaker binding power with the PO_4^{3-}.

All data for soluble anions are presented in Annex Table 5.

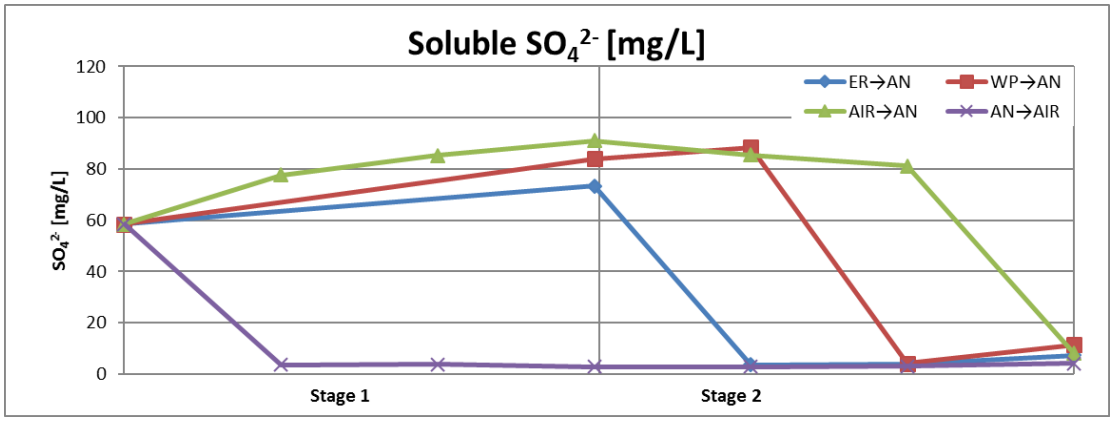

Figure 12 Soluble sulphate

4.5. Gas production and Chemical oxygen demand balance

	ER-AN	WP-AN	AIR-AN	AN-AIR
VS reduced in this stage [g/L]	0.9081	0.5918	0.3507	1.0660
COD reduced in this stage [g/L]	1.0833	0.5840	0.4053	1.9027
CH4 prod. [Nml/L]	257.3	123.3	82.6	419.9
CH4 prod./VS reduced [Nml/g VS]	283.3	208.3	235.5	393.9
CH4 prod./COD reduced [Nml/g COD]	237.5	211.1	203.8	220.7
CH4 prod. rate [Nml/L,d]	6.0	2.9	1.7	9.8
CH4 prod. rate/VS reduced [Nml/d, g VS]	6.6	4.8	4.9	9.2
CH4 prod. rate/CODreduced [Nml/d, g COD]	5.5	4.9	4.2	5.1

Table 9 Methane production

Methane production and production rates are presented in Table 9 and Figure13. Expectedly the AN→AIR presents the highest biogas production as the easily biodegradable COD is fully available for anaerobic digestion. It is followed by ER→AN, as due to the short duration of the first stage, rbCOD remains present in large quantities. The least biogas is produced by the AIR→AN track, where the 1st aerobic stage resulted in the higher COD and VS reduction.

The WP→AN performance is situated between the control and extended endogenous respiration tracks, reflecting clearly the VS and COD reduction from Stage 1. Ideally the predatory grazing step would have transformed organic compounds to a more readily biodegradable form so that the anaerobic digestion step would have produced more methane, but this idea is invalidated by the results.

Daily cumulative gas production values as well as the COD balance are presented in Annex Table 6 and Table 7, respectively.

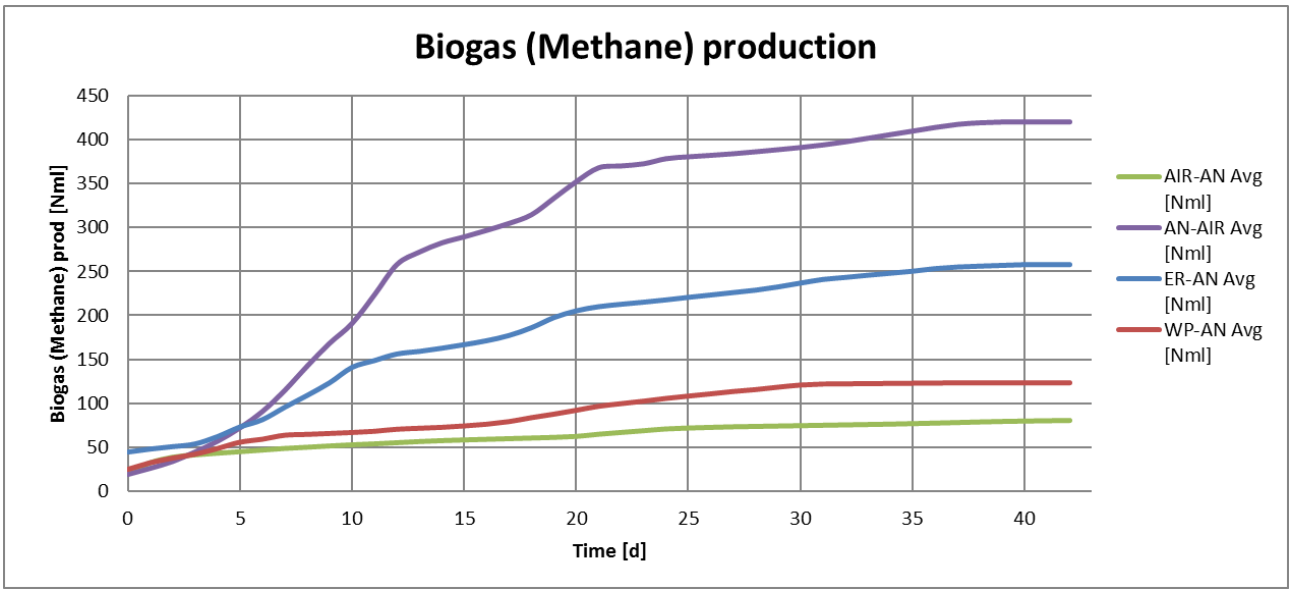

Figure 13 Biogas (Methane) production

4.6. Soluble ammonium

Due to the nitrogen content of all proteins and formation of ammonia during digestion both for anaerobic (Eq.9) and aerobic digestion (Eq.10), formed ammonium (Eq.11) can be used as an indicator of protein digestion.

$$C_nH_aO_bN_d + \left(n - \frac{a}{4} + \frac{b}{2} + \frac{3d}{4}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} + \frac{b}{3} - \frac{3d}{8}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} - \frac{3d}{8}\right) CO_2 + dNH_3 \text{ (Eq.9)}$$

$$C_nH_aO_bN_d + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3d}{4}\right) O_2 \rightarrow \left(\frac{a}{2} - \frac{3d}{2}\right) H_2O + nCO_2 + dNH_3 \text{ (Eq.10)}$$

$$NH_3 + H_2O \leftrightarrow {NH_4}^+ + OH^- + pH \uparrow \text{ (Eq.11)}$$

For a given protein, the anaerobic and aerobic protease results in the same quantity of ammonium, so the two processes can be compared form this viewpoint. However, the following is by no means an accurate quantitative assessment. For the anaerobic phases of all the tracks the NH_4^+ accumulation directly reflects the protein degradation. However, for the aerobic phases of all tracks an almost simultaneous production of NH_4^+ and a nitrification to NO_3^- takes place. Due to lack of knowledge on the typical protein formula, a backward correlation from NH_4^+ to proteins cannot be done. Thus, the ammonium production in only indicative of the protease activity, and an estimation on protein degradation must be made by looking both at NH4+ and NO_3^- (and NO_2^-).

Another interference could be provided for the T. Tubifex predation by the worm growth, which requires high quantities of N, as their mass is high in proteins. However, it is unclear which source of nitrogen they use. Although, in all likelihood, they utilize the organic N resulting from proteins.

For Figure 14: all stages show measured soluble ammonium concentrations. For Figure 15: in the anaerobic stages the produced ammonium equaled the soluble ammonium; in the aerobic stage the stages the produced ammonium equaled the sum of soluble ammonium, nitrite and nitrate considering that 2.55g NO_2^-/ g NH_4^+ and 3.44g NO_3^-/g NH_4^+; in-between stages, ammonium is calculated in both ways in order to have a better visualization of the ammonium variation per stage.

By comparing Figure 14 and Figure 15, it is very interesting to remark the important influence of nitrification in the aerobic phases.

A similar behavior can be observed for the worm predated, control and extended aeration tracks albeit in different extents. The 1st stage of the WP→AN, produces 2.1x more ammonium than the 1st stage of the ER→AN in a similar amount of time, and of 55.1% of that of the 1st stage of the AIR→ AN and 65.46% of the 1st stage of the AIR→ AN in a much shorter time. The 2nd stage results in a further increase, in ascending order, of 27.67 mg NH_4^+ /L, 41.08 mg NH_4^+ /L, 123.67 mg NH_4^+ /L for the AIR→ AN, WP→AN and ER→AN. For all 3 tracks, both their stages correlate very well with the proportion of VS and Total COD digestion. The 1st stage of the AN→AIR did not display a higher NH_4^+ concentration than the AIR→AN, as expected from literature due to the preference of anaerobic digestion for the proteins. As mentioned before, denitrification or further accumulation of NH_4^+ did not occur in Stage 2.

Measurements are presented in Annex Table 8.

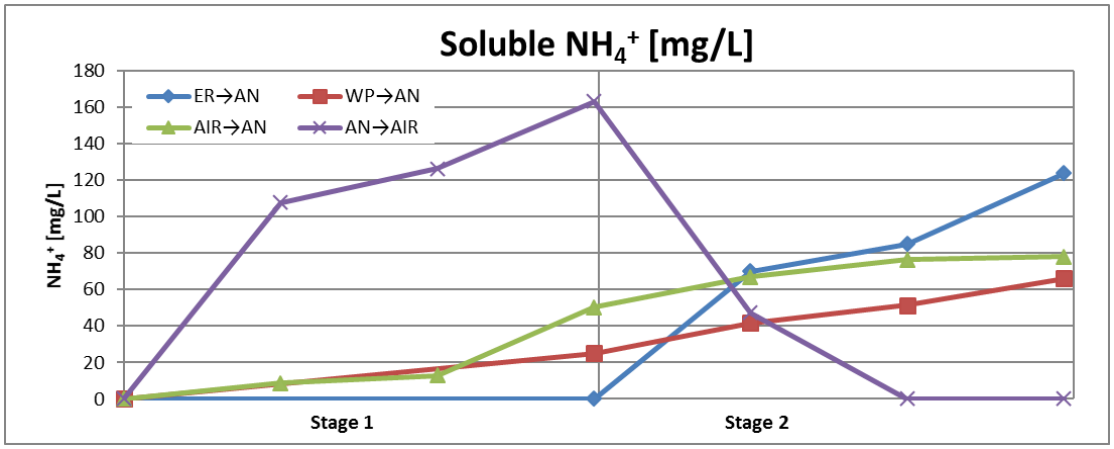

Figure 14 Soluble ammonium

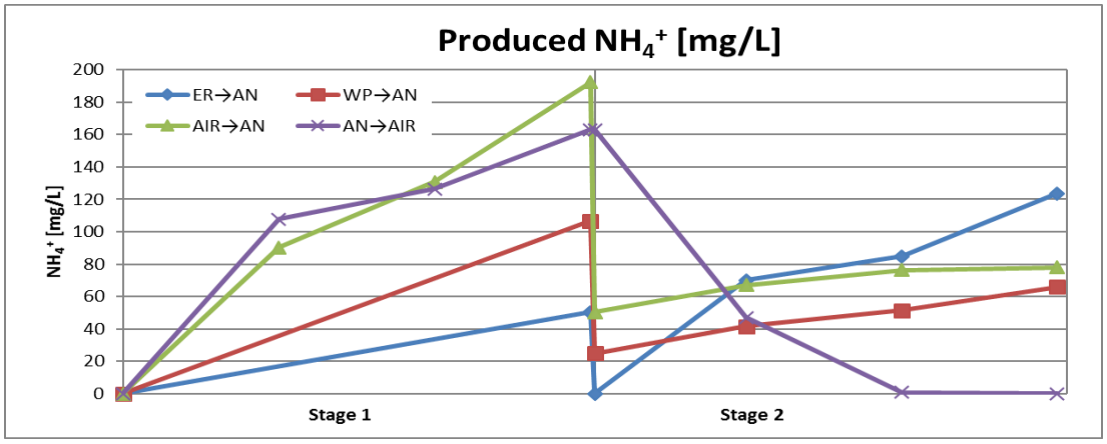

Figure 15 Produced ammonium (including ammonium nitrified to nitrate and nitrite)

4.7. Total nitrogen and organic bound nitrogen

Results for Total nitrogen and organic nitrogen are presented in Annex Table 9, Figure 4 and 5.

4.8. Soluble polysaccharides

Soluble polysaccharides (Figure 16) are the result of simultaneous solubilization from floc and digestion, so values do not absolutely quantify removal from floc or digestion capacity. The method does not differentiate between distinct types of polysaccharides.

The AIR→AN track shows expected results: in the aerobic stage as a clear increase in the release of soluble polysaccharides; followed by a decrease in the anaerobic stage, which could be explained by lower rate of further polysaccharides release accompanied by a constant glucosidase activity.

Interestingly, the WP→AN solubilizes 5.18x the amount of polysaccharides released in the ER→AN and 1.30x the quantity released in the AIR→AN. The worm predated track was then subjected to the

anaerobic stage, where the digestion is highly performing, managing to digest all previously unbounded polysaccharides. This may indicate a low or zero floc polysaccharides release into solution, accompanied by a high glucosidase activity. Furthermore, T. Tubifex may break down the harder biodegradable carbohydrates based compounds into simpler molecules in the aerobic step, as the glucosidase rate in its anaerobic step appears to be accelerated compared to the other tracks. For soluble polysaccharides, the trends in the WP→AN tracks are similar to that of the AIR→AN tracks.

Interestingly, the (WP-ER)→AN only accumulates soluble polysaccharides to a level of 27.64 mg/L compared to the 43.26 mg/L of WP→AN, making the contribution of the endogenous respiration to the worm predation mentionable.

The AN→AIR track displays for both stages an initial increase in soluble polysaccharides followed by their digestion. It may be that only part of proteins bound to polysaccharides could be digested anaerobically, releasing polysaccharides which were then reduced by enzymatic activity. In the aerobic stage, the unprocessed proteins bound to polysaccharides from Stage 1 are digested, releasing the remaining polysaccharides, which were also then digested.

Further results are presented in Annex Table 10.

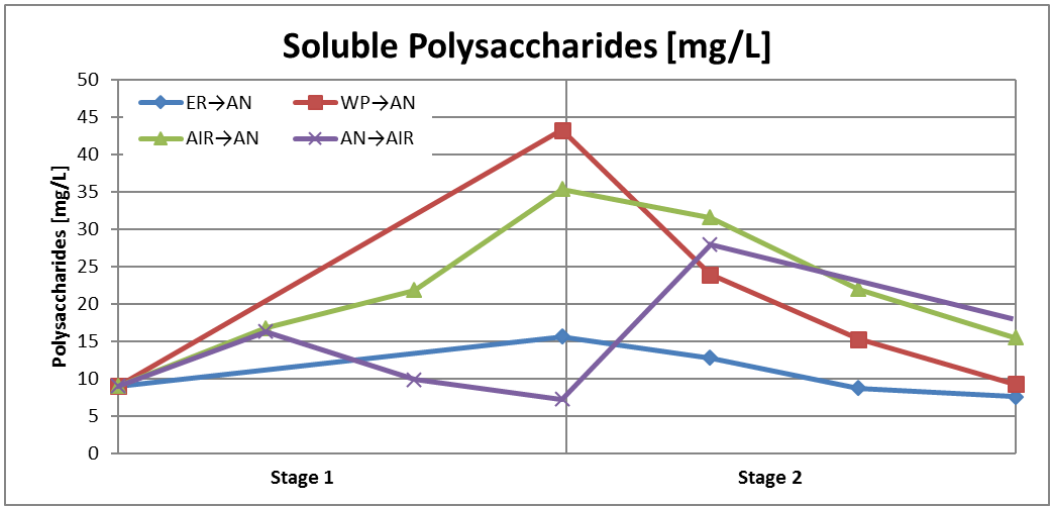

Figure 16 Soluble polysaccharides

4.9. Floc polysaccharides

The floc polysaccharides (Figure 17) concentration [mg/L] is a function of the floc polysaccharides content [mg/ gVS] and the VS concentration [g VS/L] and is used for visualization purpose.

In the aerobic stage, the WP→AN track decreases the polysaccharides floc concentration to a 24.30 mg/L from the initial 51.27 mg/L, a reduction of 142% of that of the control and of 76% of that of the extended aeration. The increased amount of polysaccharides released in Stage 1, coupled with the preexisting knowledge that polysaccharides are bound through lectic like proteins which need to be digested in order to solubilize the polysaccharides, could mean that floc polysaccharides reduction can

be partially associated with the production of soluble proteins and NH_4^+.However a quantification is not possible. WP performed best in the increase in soluble polysaccharides, and second best to AIR in the decrease in floc polysaccharides. The reason for this difference may be that for a constant glucosidase, the rate of polysaccharides solubilization is much higher for the WP that in the case of AIR or AN, allowing for a higher accumulation. Worm predation gives a higher rate, but a lower extent, of polysaccharides solubilization compared to the extended aeration and anaerobic digestion. However the T.Tubifex solubilization of polysaccharides as substrate is an important observation. The removal of polysaccharides from floc in the anaerobic phase of the WP→AN ceased.

The (WP-ER) itself, only removes from floc 10.95 mg/L of the 26.97 mg/L removed, displaying a significant, but not majority, participation in the process compared to the ER.

For the AN→AIR track, the removal of polysaccharides from floc was slightly lower in its 1st stage compared to the 1st stage of the AIR→AN, but managed to slightly surpass the later at overall solubilization after the aerobic phase. This confirms that aerobic digestion has a strong correlation with the removal of floc polysaccharides, but also that anaerobic digestion is capable almost to the same extent as aerobic digestion to solubilize polysaccharides.

Further results are presented in Annex Table 11 and Figure 6.

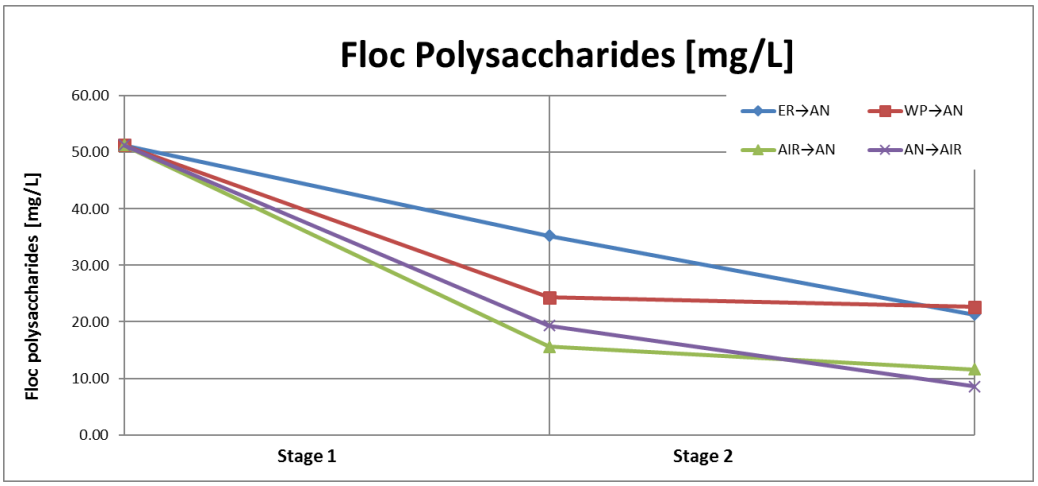

Figure 17 Floc polysaccharides

4.10. Soluble proteins

Soluble proteins are the result of simultaneous removal from floc and digestion, so values do not absolutely quantify removal from floc capacity or digestion capacity. The method does not differentiate between distinct types of proteins based on their complexity. For this reason, a typical formula for the proteins could not be decided on, and, so, making the inverse conversion from produced NH_4^+ to soluble proteins was not possible. Moreover, the correction for humics like compounds includes humics and fluvic acid with no distinction based on type of the compound. An increase in floc humics concentration can be the result of organic matter degradation. However, due to their resistant nature to solubilization and degradation, a decrease in soluble humics can most likely be the result of complexation with

divalent and trivalent cations. Fulvic acids are, on the other side, more soluble and easier degradable. The presence of proteins without (Figure 18) and with humics (Figure 19) correction will be discussed.

Proteins including humics. In Stage 1, the WP→AN solubilizes 8.02x the amount of proteins released in the control ER→AN and 2.74x the quantity released in the track AIR→AN. Similarities between the overall behaviors of the worm predated and extended aeration tracks can again be found. This accumulation can be, as in the case of soluble polysaccharides, the result of a higher rate of solubilization for WP, compared to AIR or AN, with a constant rate of protease and does not necessarily reflect the extent of the solubilization. This is supported by the lower concentration of ammonium displayed in worm predated batch compared to the extended aeration one. Considering both soluble proteins and ammonium together it is shown that the extent of proteins solubilization and degradation by micro fauna predation is more reduced than suggested by Figure 18. In the 2nd stage the WP→AN and AIR→AN remained fairly constant. Keeping in mind the slight increase in produced ammonium for both these tracks, it may show that the plateau is the result of a slower rate of solubilization equaling the rate of protease. The low extent of the soluble protein release may also be the result of a prolonged anoxic environment due to the large quantities of nitrate and low COD (as electron donor for denitrification), accompanied by the slow development of the anaerobic biomass. The second stage of the ER→AN produces a much higher quantity of both soluble proteins and produced ammonium, due to low nitrate quantities which are immediately denitrified and higher availability of rapidly biodegradable compounds remaining from Stage 1. In the 1st stage AN→AIR track releases an important concentration of soluble proteins and produced ammonium, as expected. However, the second stage cannot be evaluated properly due to the nitrate measurement issues. Either no additional solubilization or digestion took place or the rates of both where equal.

In this case, the contribution of (WP-ER) is substantial for the increase in soluble proteins, 88.3%, and half for the increase in produced ammonium, 50.42%, showing the high rate of solubilization and average digestion capabilities of T.Tubifex in the case of proteins.

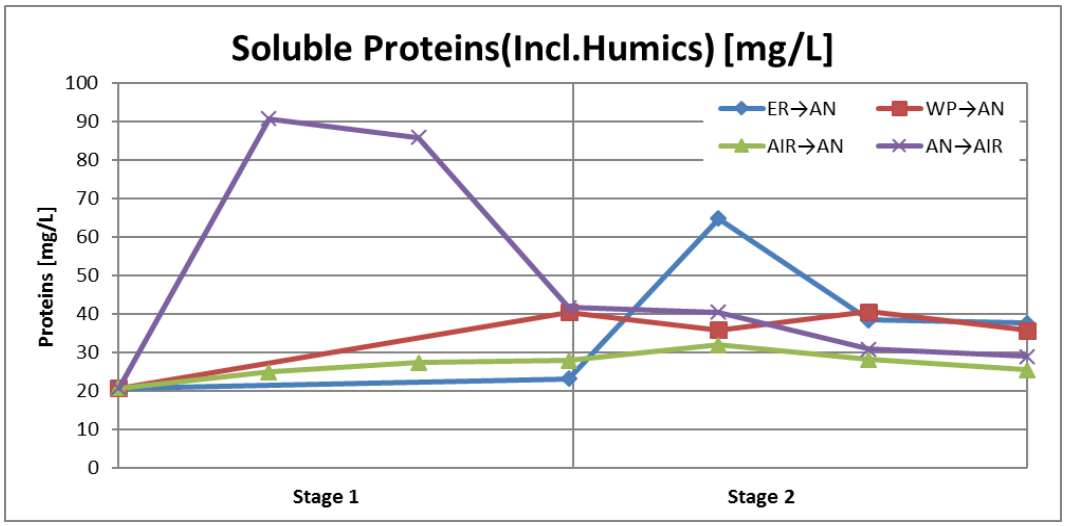

Figure 18 Soluble proteins including humic acids

Proteins excluding humics. For all tracks the values for soluble proteins concentrations are severely diminished and fluctuating to levels not typically mentioned in literature, showing the high interference of humics in the process. The WP→ AN track displays the highest increase in Stage 1 with a release of 6.77 mg/ L, compared to the release of 1.73 mg/L for the ER→AN and the full disappearance of soluble proteins for the AIR→AN. It may be the case that the extended aeration has solubilized and digested all proteins, resulting in a high ammonium concentration; whilst the worm predated and the endogenous respiration have solubilized but only to a smaller extent digested proteins, so an accumulation is noticeable.In WP stage this can be the result of a high rate of solubilization with a lower constant rate of protease. In the ER the results for soluble proteins or ammonia cannot be explained, as there is theoretically no release of floc proteins. In the anaerobic phase of all these tracks, they appear to digest proteins at a higher rate than that of removal from floc. The AN→AIR shows an unusual pattern, as if there is a high release of soluble proteins and ammonium accompanied by a fluctuating protease.

The contrbution of (WP-ER) is substantial for the increase in soluble proteins, 74.4%, and half for the increase in produced ammonium, 50.42%, showing the high rate of solubilization and average digestion capabilities of T.Tubifex in the case of proteins.

Further results are presented in Annex Table 10.

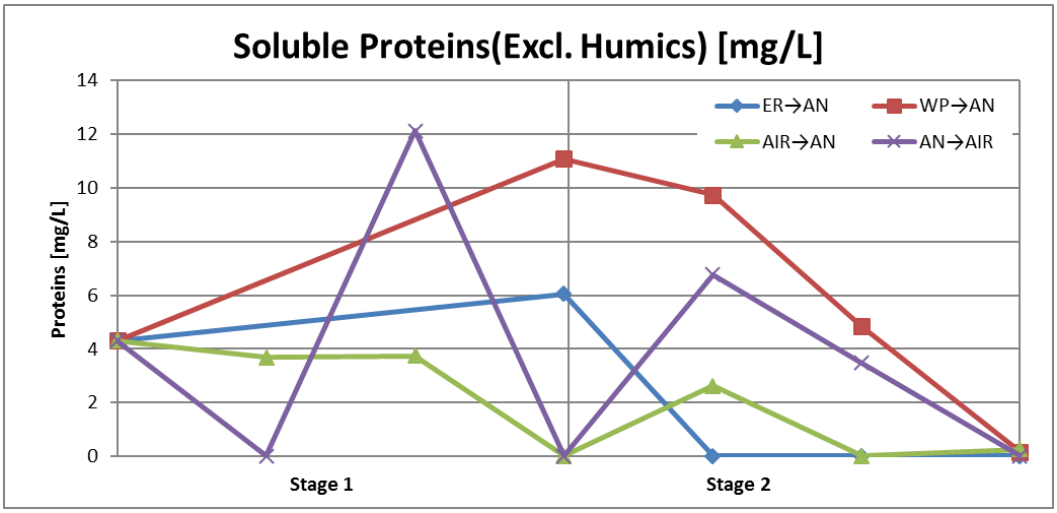

Figure 19 Soluble proteins excluding humic acids

4.11. Floc proteins

The floc proteins concentrations [mg/L] including (Figure 20) and excluding humics (Figure 21) are a function of the floc proteins content [mg/ gVS] and the VS concentration [g VS/L] and are used for visualization purpose.

Proteins including humics. Stage 1 of the WP→AN solubilizes from the floc 2.29x the amount of proteins released in the control ER→AN and 0.81x and 0.84x the quantity released from floc in the tracks AIR→AN and AN→AIR, respectively (Figure 20). For the 2nd stage, the release rate decreases for WP→AN and AIR→ AN, showing a great deal of the proteins where already solubilized and even

degraded in the 1st stage. This is not the case for Stage 2 of the control, where the rate of protein solubilization from floc increases, as the 1st stage did not perform exceedingly well due to time constraints and low rates of solubilization. The AN→AIR and AIR→AN tracks behave almost similarly, showing possibly that from the viewpoint of the capacity of removing proteins from floc, there is not much difference between the two. The differences may occur in the protein type being removed. For all tracks, the reduction in floc protein matches with the measured increase in soluble proteins plus ammonium production.

For the (WP-ER) the contribution of control is almost 43.7% of the floc protein reduction capabilities.

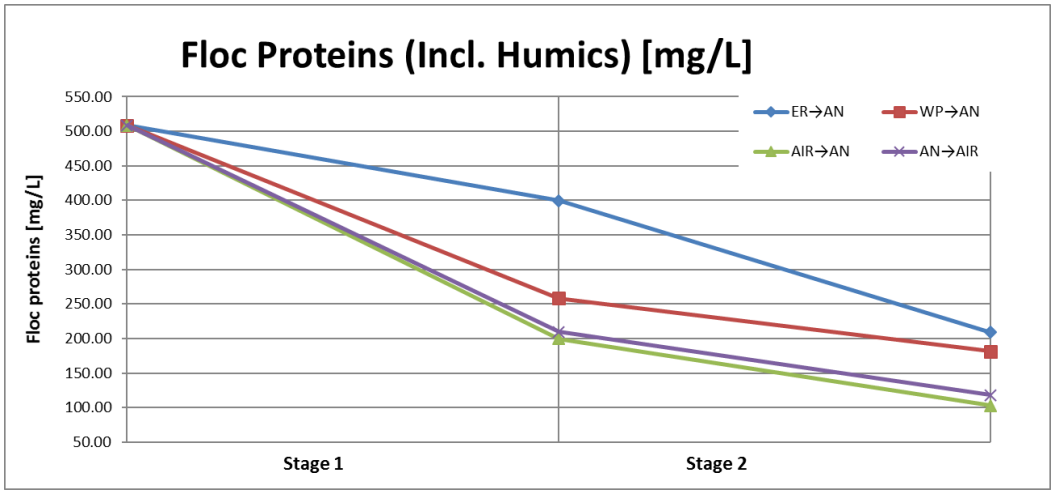

Figure 20 Floc proteins including humics

Proteins excluding humics. The floc proteins concentrations are sharplydiminished and there is a clearer differentiation between the behavior of the tracks. The WP→AN track removes from floc 43.63 mg/L more than the ER→AN, which shows no removal, and 49.22g/L less than the AIR→AN track. In the anaerobic phases, the WP→AN showed a lower reduction rate than the control, but both attained zero floc protein by the end of the batch. The 2nd stage of the AIR→AN displayed a lower rate of reduction compared to the ER and WP anaerobic stages, and did not manage to reduce all floc proteins, possibly due to anoxic conditions. The AN→AIR reduced all proteins in the 1st stage at an increased rate, and an increased in floc protein is observed in the 2nd stage, maybe as a result of aerobic biomass growth and reformation of floc EPS from the reoxidation of iron to Fe^{3+}.

The contribution of (WP-ER) is actually equal to that of the WP, as the control ER had not contribution.

Further results are presented in Annex Table 11 and Figure 7 and 8.

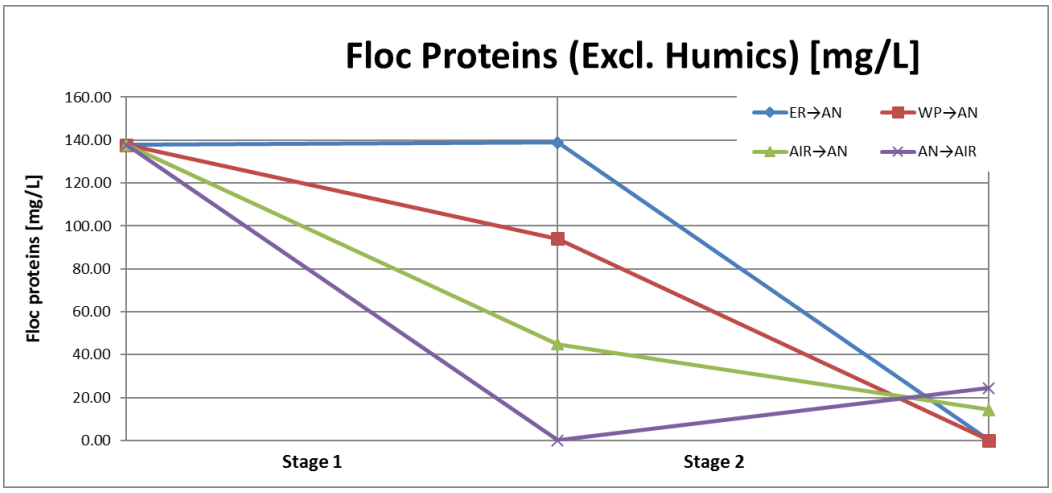

Figure 21 Floc proteins excluding humics

4.12. Soluble calcium, magnesium, iron

Calcium, magnesium (Figure 22-24. and iron (Figure 25) were analyzed for soluble cations content. Soluble cations measurements are a result of solubilization, as well as of: precipitation with other compounds (phosphate, sulphate), complexation with humics or building material for new bacterial or worm biomass. For these reasons, they cannot be used as a precise indication.

Calcium and magnesium. As seen, the influence of divalent Ca^{2+} compared Mg^{2+} is more substantial and sets the trend in the case of divalent cations (Figure 22 and 23). In the aerobic phase of the AIR→AN, the track displays the highest release of both divalent cations, which correlates well with the release of polysaccharides from floc (Figure 24). The decrease in the following anaerobic stage can be the result of precipitation of calcium, with magnesium remaining stable in the meanwhile. Polysaccharides solubilization was also insignificant in this second stage, so not much release of lectin like proteins were expected. The 1st stage of the WP→AN also showed a release of Mg^{2+}, but a reduction of Ca^{2+}. Considering the release of polysaccharides in this stage and assuming the two divalent cations are released in the same ration, Ca^{2+} could have formed complexes with other elements and precipitated or Mg^{2+} was the main cation binding the proteins degraded in order to release the polysaccharides. Little to no changes where observed in the anaerobic stage, where actually little polysaccharides where solubilized. The AN→AIR track released cations in the anaerobic stage as polysaccharides where removed from floc. For the aerobic stage the results are inconclusive as, even though a small amount of polysaccharides where still released, it appears part of the divalent cations salted out in the beginning.

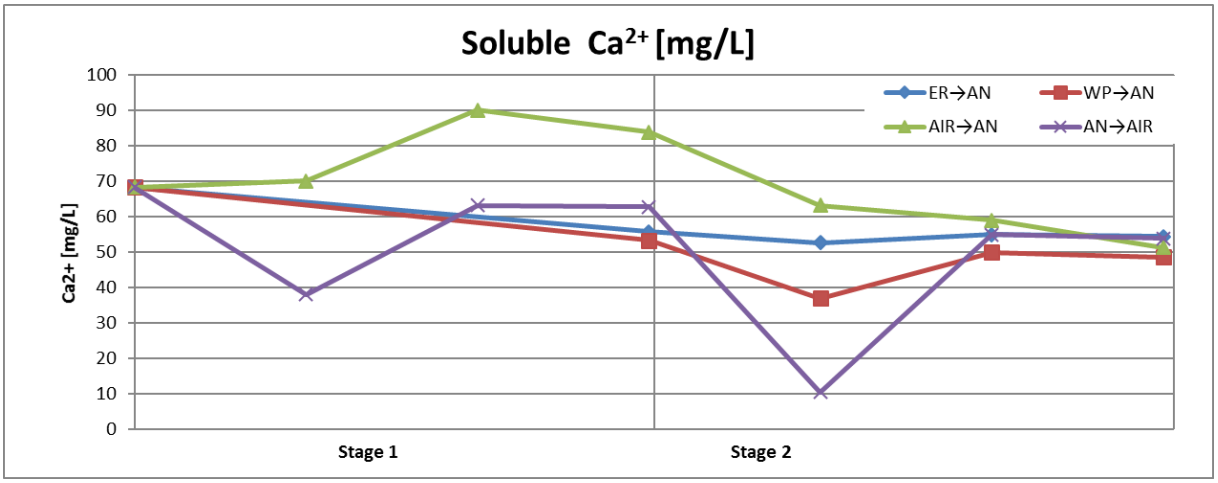

Figure 22 Soluble calcium

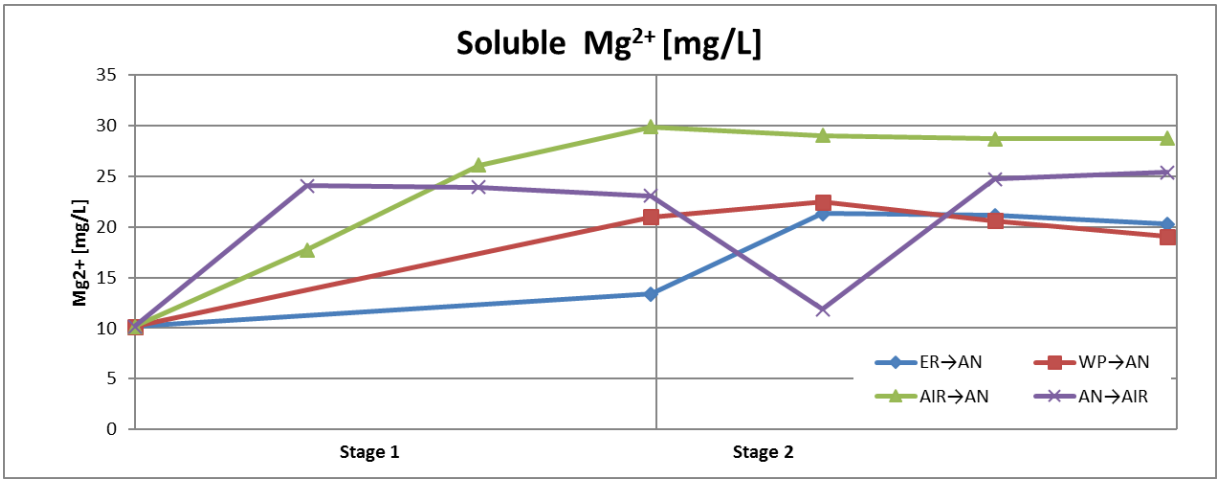

Figure 17 Soluble magnesium

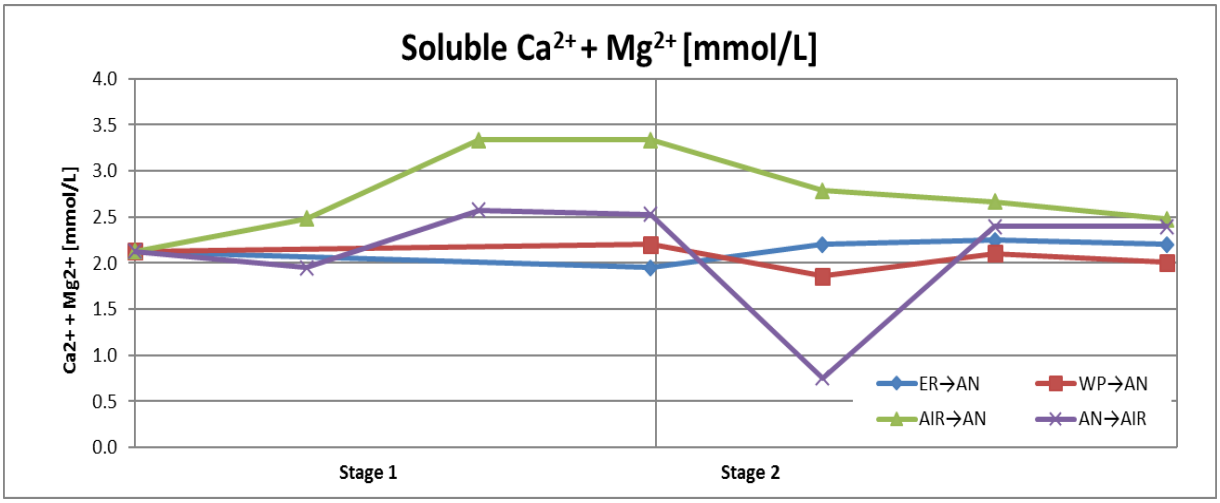

Figure 18 Soluble divalent cations

Iron. The aerobic stage of AIR→AN shows a minor increase in iron, which, together with the release of calcium and magnesium, may suggest that the proteins released from floc were both lectin like proteins and regular proteins. The anaerobic stage resulted in a further solubilization of iron which would correlate well with the solubilization of regular proteins. The WP→AN track displayed an iron release higher to that of the extended aeration, which may mean that more iron bound proteins were released from floc than in the extended aeration. It can asserted that T.Tubifex makes use of both regular, and lectin like proteins and polysaccharides. In the anaerobic phase of the worm predated track it appears a lot of iron compounds are solubilized, corresponding with the removal from floc of regular proteins and lack of removal from floc of polysaccharides an implicitly lectin like proteins and calcium and magnesium. The anaerobic stage of the AN→AIR tracks shows the highest solubilization of iron, which corresponds to it good removal capacity of regular proteins from floc. In the aerobic stage, a short insignificant release takes place, whilst in the end some precipitation of iron compounds seems to occur.

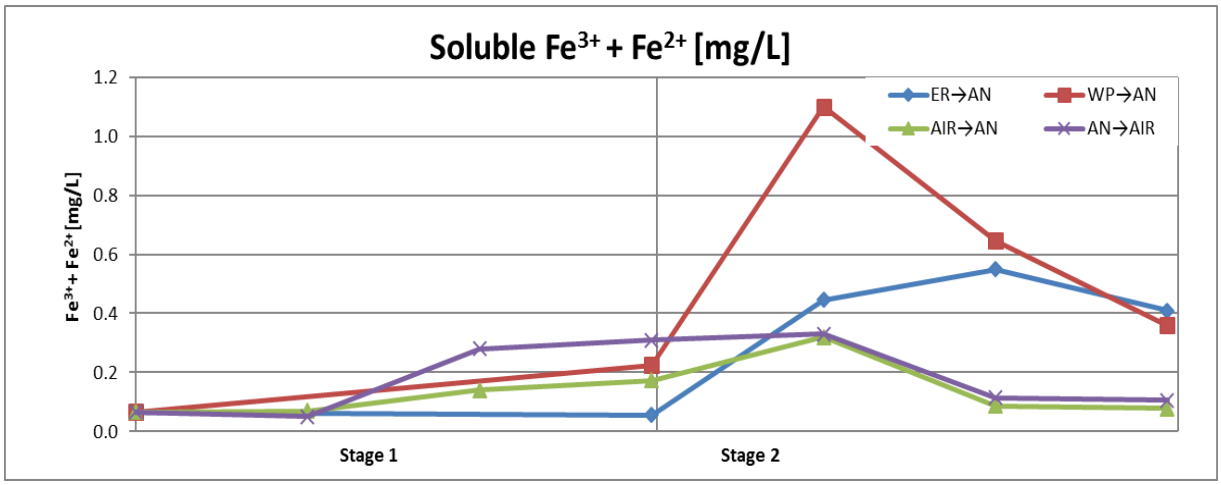

Figure 19 Soluble iron

4.13. Floc calcium, magnesium, iron

Table 12 in Annex presents additional data. The Annex Table 13 and Figures 9 to 16 present Total cations and floc cations concentrations.

5. Conclusions and observations

- The 1st stage of the WP→AN has the highest rate of VS reduction. The track results in the highest overall VS decrease, with 52.1% of the initial VS concentration being digested, showing that the process breaks down flocs and digests slowly bioavailable organic compounds to a higher extent than that of typical aerobic and anaerobic digestion.
- The participation of (WP-ER) compared to the ER from in the worm predation process is high for VS removal and moderate for Total COD, polysaccharides and protein removal. This may show that the combination of both processes yields the high efficiency of the method. Furthermore, the T.Tubifex appears to participate more in the sludge breakdown, solubilization and simplification of complex compounds than in the complete digestion.
- The T.Tubifex independently displayed a reduction capacity of 0.77 mg COD/g wet worms,d and 0.48 mg VS/g wet worms,d.
- Methane production after worm predation was lower than the one of the anaerobic digestion. Worm predation followed by anaerobic digestion will not offer the combined advantage of high solids removal and enhanced methane production.
- The high accumulation of soluble polysaccharides accompanied by the average reduction of floc polysaccharides in the worm predated track indicates a high rate of solubilization, but not a high extent of solubilization or a high rate of glucosidase. Similar results are seen for proteins.
- Worm predation seems to consume both lectin like and regular proteins, as well as polysaccharides.
- Worm predation appears to be closer as a process to the extended aeration.
- Soluble polysaccharides and protein variations are not reliable measurements due to concomitant solubilization and digestion. Soluble cations variations are not reliable measurements due to concomitant precipitation or complexation.
- It appears a clear distinction between aerobic digestion and anaerobic digestion regarding the removal from floc capabilities of both polysaccharides and proteins cannot be made, as they perform similarly. The differences seen in literature may stem from the fact that researchers study the soluble fraction which reflects both solubilization and digestion. The actual reason may be that it appears that glucosidase is more active in anaerobic conditions and protease performs better in aerobic conditions. This gives the false impression that less polysaccharides are removed from floc in anaerobic condition and less proteins are solubilized in aerobic conditions.
- AN→ ER and AN→ WP, failed due to the *Tubifex Tubifex* worms' high death rate. This lethality was likely caused by the considerable amount of soluble ammonia following the 1st stage of anaerobic digestion. As the solution of washing the sludge from ammonia seems unpractical, the feasibility of a worm predation stage following an anaerobic digestion stage seems very low.

6. References

An, K. J., & Chen, G. H. (2008). Chemical oxygen demand and the mechanism of excess sludge reduction in an oxic-settling-anaerobic activated sludge process. *Journal of Environmental Engineering*, 134(6), 469-477.

Buys, B. R., Klapwijk, A., Elissen, H., & Rulkens, W. H. (2008). Development of a test method to assess the sludge reduction potential of aquatic organisms in activated sludge. *Bioresource Technology*, *99*(17), 8360-8366.

Chapman, P. M., Farrel, M. A., & Brinkhurst, R. O. (1982). Relative tolerances of selected aquatic oligochaetes to combinations of pollutants and environmental factors. *Aquatic Toxicology*, *2*(1), 69-78.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28, 350-356.

Eaton, A. D., Clesceri, L. S., Greenberg, A. E., Franson, M. A. H., American Public Health Association, American Water Works Association, & Water Environment Federation. (1998). Standard methods for the examination of water and wastewater (20th ed. 1998.). Washington, DC: American Public Health Association.

Elissen, H. J. H., Mulder, W. J., Hendrickx, T. L. G., Elbersen, H. W., Beelen, B., Temmink, H., & Buisman, C. J. N. (2010). Aquatic worms grown on biosolids: Biomass composition and potential applications. *Bioresource technology*, 101(2), 804-811.

Foladori, P., Velho, V. F., Costa, R. H. R., Bruni, L., Quaranta, A., & Andreottola, G. (2015). Concerning the role of cell lysis-cryptic growth in anaerobic side-stream reactors: the single-cell analysis of viable, dead and lysed bacteria. *Water research*, *74*, 132-142.

Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water research, 30(8), 1749-1758.

Hendrickx, T. L. G., Temmink, H., Elissen, H. J. H., & Buisman, C. J. N. (2009a). Aquatic worms eating waste sludge in a continuous system. *Bioresource Technology*, 100(20), 4642-4648.

Hendrickx, T. L. G., Temmink, H., Elissen, H. J. H., & Buisman, C. J. N. (2009b). The effect of operating conditions on aquatic worms eating waste sludge. *Water Research*, 43(4), 943-950.

Hendrickx, T. L. G., Temmink, H., Elissen, H. J. H., & Buisman, C. J. N. (2010). Aquatic worms eat sludge: mass balances and processing of worm faeces. *Journal of hazardous materials*, 177(1), 633-638.

Hendrickx, T. L., Elissen, H. H., Temmink, H., & Buisman, C. J. (2011). Operation of an aquatic worm reactor suitable for sludge reduction at large scale. *Water research*, *45*(16), 4923-4929.

Huang, X., Liang, P., & Qian, Y. (2007). Excess sludge reduction induced by Tubifex tubifex in a recycled sludge reactor. *Journal of Biotechnology*, *127*(3), 443-451.

Van Langerak, E. P. A., Gonzalez-Gil, G., Van Aelst, A., Van Lier, J. B., Hamelers, H. V. M., & Lettinga, G. (1998). Effects of high calcium concentrations on the development of methanogenic sludge in upflow anaerobic sludge bed (UASB) reactors. *Water Research*, 32(4), 1255-1263.

Liang, P., Huang, X., Qian, Y., & Ding, G. (2003). Influence of environmental factors on the growth of Aeolosoma hemprichi. *Zhongguo Huanjing Kexue*, 24(5), 610-613.

Liang, P., Huang, X., Qian, Y., Wei, Y., & Ding, G. (2006a). Determination and comparison of sludge reduction rates caused by microfaunas' predation. *Bioresource Technology*, 97(6), 854-861.

Liang, P., Huang, X., & Qian, Y. (2006b). Excess sludge reduction in activated sludge process through predation of Aeolosoma hemprichi. *Biochemical Engineering Journal*, 28(2), 117-122.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, 193(1), 265-275.

Metcalf & Eddy (2014) Wastewater Engineering: Treatment and Resource Recovery. 5th Edition, *McGraw-Hill*, New York.

Novak, J. T., Sadler, M. E., & Murthy, S. N. (2003). Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Water Research*, *37*(13), 3136-3144.

Park, C., Abu-Orf, M. M., & Novak, J. T. (2006). The digestibility of waste activated sludges. *Water environment research*, *78*(1), 59-68.

Park, C., & Novak, J. T. (2007). Characterization of activated sludge exocellular polymers using several cation-associated extraction methods. *Water research*, *41*(8), 1679-1688.

Rathore, R. S., & Khangarot, B. S. (2002). Effects of temperature on the sensitivity of sludge worm Tubifex tubifex Müller to selected heavy metals. *Ecotoxicology and environmental safety*, 53(1), 27-36.

Ratsak, C. H., & Verkuijlen, J. (2006). Sludge reduction by predatory activity of aquatic oligochaetes in wastewater treatment plants: science or fiction? A review. *In Aquatic Oligochaete Biology IX* (pp. 197-211). Springer Netherlands.

Rosenberger, S., Evenblij, H., Poele, S.T., Wintgens, T. and Laabs, C. (2005) The importance of liquid phase analyses to understand fouling in membrane assisted activated sludge processes - six case studies of different European research groups. *Journal of Membrane Science* 263(1-2), 113-126.

Serrano, A., Hendrickx, T. L., Elissen, H. H., Laarhoven, B., Buisman, C. J., & Temmink, H. (2016). Can aquatic worms enhance methane production from waste activated sludge?. *Bioresource technology*, 211, 51-57.

Tamis, J., Van Schouwenburg, G., Kleerebezem, R., & van Loosdrecht, M. C. M. (2011). A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant. *Water research*, 45(18), 5916-5924.

de Valk, S., Khadem, A. F., Foreman, C. M., van Lier, J. B., & de Kreuk, M. K. (2016). Physical and biochemical changes in sludge upon Tubifex tubifex predation. *Environmental Technology*, 1-15.

Wei, Y., van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003). Comparison performances of membrane bioreactor and conventional activated sludge processes on sludge reduction induced by Oligochaete. *Environmental science & technology*, 37(14), 3171-3180.

Wei, Y., & Liu, J. (2005). The discharged excess sludge treated by Oligochaeta. *Water Science and Technology*, *52*(10-11), 265-272.

Wei, Y., Zhu, H., Wang, Y., Li, J., Zhang, P., Hu, J., & Liu, J. (2009). Nutrients release and phosphorus distribution during oligochaetes predation on activated sludge. *Biochemical Engineering Journal*, 43(3), 239-245.

Zhang, X., Tian, Y., Wang, Q., & Lin, H. (2013). Waste sludge reduction using Limnodrilus hoffmeisteri: Growth, development and sludge predation potential of aquatic worm correlate with process conditions. *Ecological engineering*, 58, 406-413.

Table 1 Volatile and total solids reduction

	Days [day]	Sampling	Volatile solids ("VS")					Total solids ("TS")				
			Average [g/L]	σ [g/L]	VS reduction per stage [%]	VS reduction per stage [g/L]	VS reduction rate [g/L,d]	Average [g/L]	σ [g/L]	TS reduction per stage [%]	TS reduction per stage [g/L]	TS reduction rate [g/L,d]
ER→AN	0	Start	2.5376	0.0949	5.2064	0.1321	0.0165	4.0310	0.1418	3.3955	0.1369	0.0171
	7	Switch	2.4055	0.0339				3.8942	0.0561			
	19	1M	1.9052	0.0264	35.7851	0.9081	0.0211	3.3916	0.0266	23.7161	0.9560	0.0222
	28	2M	1.7527	0.0321				3.2134	0.0285			
	49	End	1.4974	0.0123				2.9382	0.0226			
WP→AN	0	Start	2.5376	0.0949	28.8224	0.7314	0.0914	4.0310	0.1418	19.8294	0.7993	0.0999
	7	Switch	1.8062	0.0515				3.2317	0.8539			
	19	1M	1.4793	0.0802	23.3216	0.5918	0.0138	2.8575	0.1280	16.4691	0.6639	0.0154
	28	2M	1.2115	0.0379				2.6606	0.0597			
	49	End	1.2144	0.0406				2.5678	0.0575			
AIR→AN	0	Start	2.5376	0.0949	34.6938	0.8804	0.0205	4.0310	0.1418	23.2723	0.9381	0.0218
	12	1M	2.3333	0.0187				3.8051	0.0480			
	22	2M	2.1346	0.0147				3.5921	0.0324			
	42	Switch	1.6572	0.0184				3.0929	0.0300			
	55	3M	1.4974	0.0203	13.8214	0.3507	0.0073	2.9326	0.0386	8.2994	0.3346	0.0070
	68	4M	1.3514	0.0227				2.8106	0.0111			
	89	End	1.3065	0.0187				2.7584	0.0102			
AN→AIR	0	Start	2.5376	0.0949	42.0099	1.0660	0.0248	4.0310	0.1418	26.2193	1.0569	0.0246
	12	1M	2.0072	0.0468				3.5803	0.0031			
	22	2M	1.7382	0.0431				3.2836	0.0300			
	42	Switch	1.4716	0.0238				2.9741	0.0182			
	55	3M	1.5439	0.0332	4.1913	0.1064	0.0022	2.9357	0.0491	5.8815	0.2371	0.0049
	68	4M	1.4209	0.0400				2.8262	0.0519			
	89	End	1.3652	0.0183				2.7370	0.0267			

Total solids ("TS")							
	TS reduction per stage [g/l]		TS reduction per stage [%]			TS reduction rate [g/L,d]	
	Stage 1	Stage 2	Stage 1	Stage 2	Rest	Stage 1	Stage 2
ER→AN	0.1369	0.9560	3.4	23.7	72.9	0.0171	0.0222
WP→AN	0.7993	0.6639	19.8	16.5	63.7	0.0999	0.0154
AIR→AN	0.9381	0.3346	23.3	8.3	68.4	0.0218	0.0070
AN→AIR	1.0569	0.2371	26.2	5.9	67.9	0.0246	0.0049
(WP-ER)→AN	0.6625	0.6639	16.4	16.5	67.1	0.0828	0.0154

Table 2 Overview total solids reduction

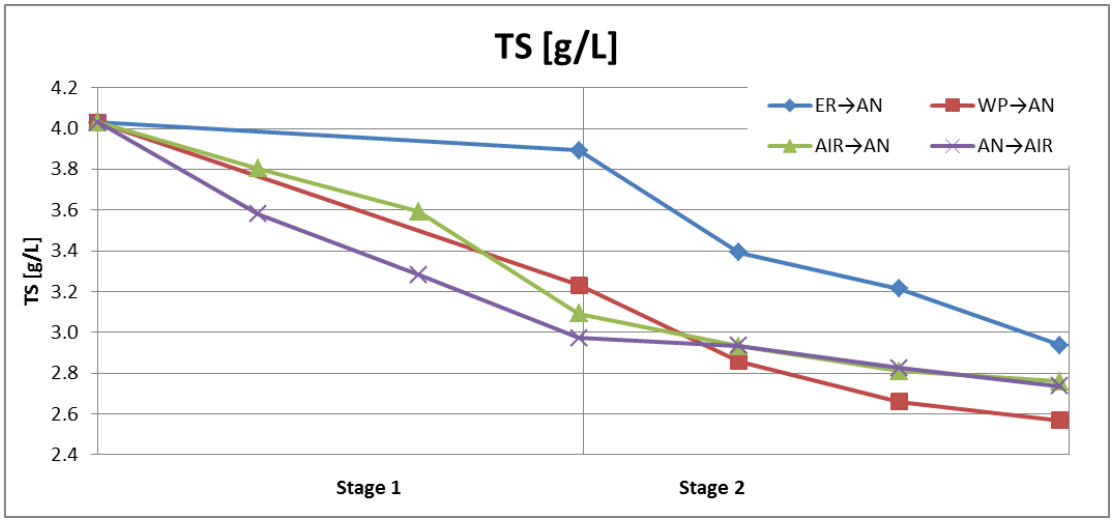

Figure 1 Total solids reduction in time

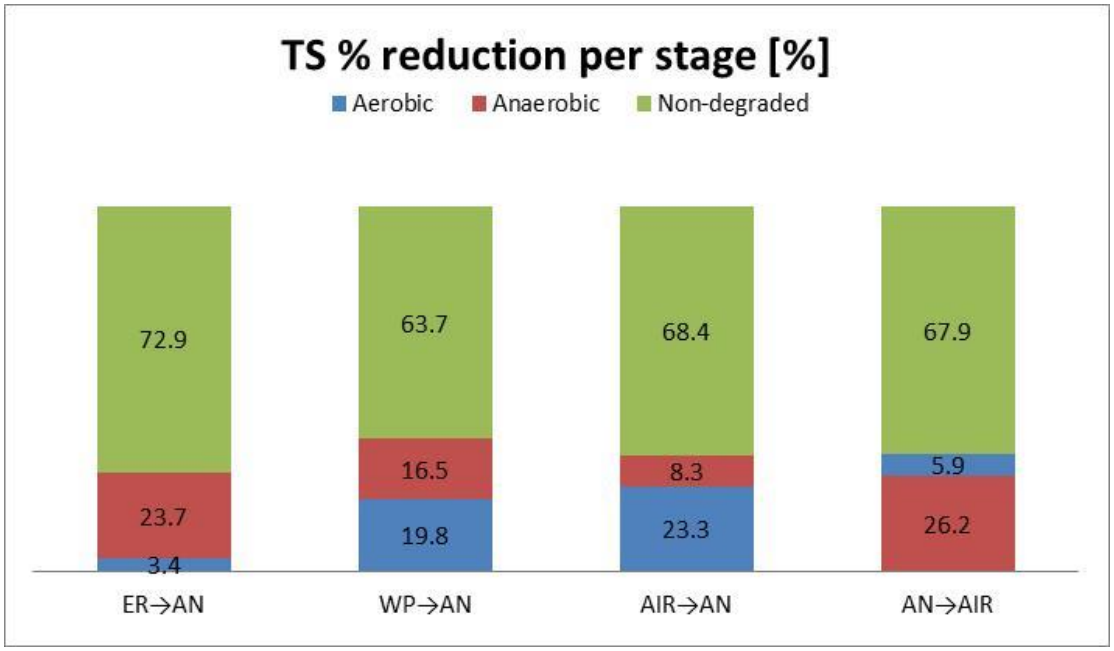

Figure 2 Total solids reduction percentage per stage

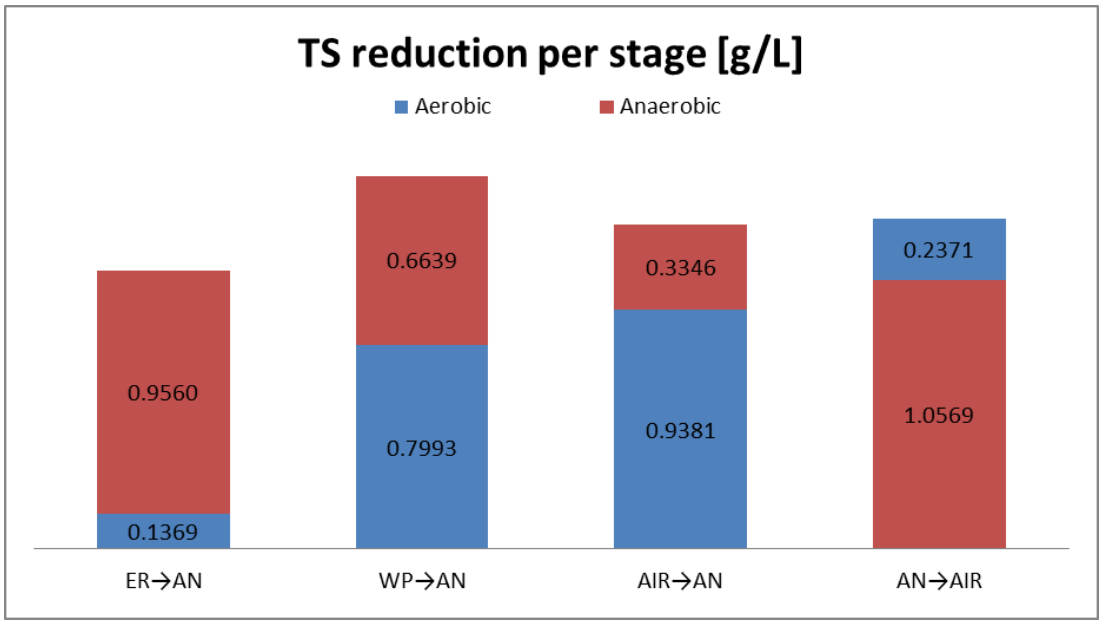

Figure 3 Total solids reduction per stage

	Days	Sampling	Total COD				
			Avg. conc. [g/L]	σ [g/L]	COD reduction per stage [%]	COD reduction per stage [g/L]	COD reduction rate [g/L,d]
ER→AN	0	Start	4.2947	0.0422	-	-	-
	7	Switch	3.3823	0.1656	21.24	0.9123	0.1140
	49	End	2.2990	0.0420	25.23	1.0833	0.0252
WP→AN	0	Start	4.2947	0.0422	-	-	-
	7	Switch	2.4137	0.0581	43.80	1.8810	0.2351
	49	End	1.8297	0.0552	13.60	0.5840	0.0136
AIR→AN	0	Start	4.2947	0.0422	-	-	-
	42	Switch	2.0187	0.0190	53.00	2.2760	0.0529
	89	End	1.6133	0.0663	9.44	0.4053	0.0084
AN→AIR	0	Start	4.2947	0.0422	-	-	-
	42	Switch	2.3920	0.0082	44.30	1.9027	0.0442
	89	End	1.8753	0.0440	12.03	0.5167	0.0108

Table 3 Total COD reduction

	Triplicate	Wet worms weight [g]	Average WW [g]	σ WW [g]	Average weight wet worms [g]	Wet worms weight increase [g]
WP→AN Start	1	150.1	150.14	0.1484	157.39	14.50
	2	150.01				
	3	150.3				
WP→AN Switch	1	153.6	164.63	9.9158		
	2	167.5				
	3	172.8				

T.Tubifex yield				T.Tubifex removal capabilities			
WP [g WW/g VS dig, d]	(WP-ER) [g WW/g VS dig, d]	WP [g WW/g COD, d]	(WP-ER) [g WW/g COD, d]	WP [mg VS/g WW, d]	(WP-ER) [mg VS/g WW, d]	WP [mg COD/g WW, d]	(WP-ER) [mg COD/g WW, d]
2.4776	3.0238	0.9634	1.8707	6.3066	0.4760	16.2192	0.7693

Table 4 Worm growth, yield and removal capabilities

			Soluble Anions							
			Soluble NO^{2-}		Soluble NO^{3-}		Soluble PO_4^{3-}		Soluble SO_4^{2-}	
	Days	Sampling	Avg. conc. [mg/L]	σ or Min/Max [mg/L]	Avg. conc. [mg/L]	σ or Min/Max [mg/L]	Avg. conc. [mg/L]	σ or Min/Max [mg/L]	Avg. conc. [mg/L]	σ or Min/Max [mg/L]
ER→AN	0	Start	1.50	0.00	16.25	0.04	0.00	0.00	58.48	0.32
	7	Switch	0.98	0.85	189.26	4.95	40.62	1.14	73.28	0.53
	19	1M	0.00	0.00	0.00	0.00	199.47	1.39	**3.60**	**3.30/3.89**
	28	2M	0.00	0.00	0.00	0.00	206.09	2.81	**3.68**	**3.61/3.75**
	49	End	0.00	0.00	0.00	0.00	143.92	10.93	**7.39**	**6.91/7.86**
WP→AN	0	Start	1.50	0.00	16.25	0.04	0.00	0.00	58.48	0.32
	7	Switch	**3.44**	**3.26/3.61**	**294.86**	**291.69/298.03**	**111.97**	**109.43/115.51**	83.94	2.17
	19	1M	0.00	0.00	0.00	0.00	161.48	4.72	**88.40**	**87.75/89.05**
	28	2M	0.00	0.00	0.00	0.00	179.58	9.46	**4.10**	**4.09/4.10**
	49	End	0.00	0.00	0.00	0.00	**128.06**	**121.36/134.76**	**11.35**	**10.20/12.49**
AIR→AN	0	Start	1.50	0.00	16.25	0.04	0.00	0.00	58.48	0.32
	12	1M	0.00	0.00	297.17	19.77	**87.81**	**85.56/90.06**	77.55	1.69
	22	2M	0.00	0.00	421.79	28.74	194.83	17.90	85.21	1.07
	42	Switch	1.98	1.72	504.34	32.51	232.63	15.26	90.95	1.11
	55	3M	**9.58**	**0.00/19.16**	**201.15**	**199.66/202.64**	196.88	17.52	85.39	5.99
	68	4M	**2.97**	**2.91/3.02**	**169.25**	**128.17/210.33**	**170.94**	**170.84/171.03**	**81.17**	**76.47/85.86**
	89	End	**1.48**	**0.00/2.95**	**5.73**	**5.27/6.19**	**81.17**	**13.17/17.55**	**8.30**	**7.04/9.56**
AN→AIR	0	Start	1.50	0.00	16.25	0.04	0.00	0.00	58.48	0.32
	12	1M	0.00	0.00	0.00	0.00	229.52	16.56	**3.68**	**3.57/3.78**
	22	2M	0.00	0.00	0.00	0.00	225.62	5.66	3.75	0.29
	42	Switch	0.00	0.00	0.00	0.00	**0.00**	**0.00/0.00**	2.81	0.17
	55	3M	0.00	0.00	0.00	0.00	0.00	0.00	2.83	0.08
	68	4M	1.95	1.69	0.00	0.00	**0.00**	**0.00/0.00**	**3.04**	**2.59/3.48**
	89	End	0.00	0.00	0.00	0.00	0.00	0.00	**4.09**	**3.71/4.47**

Table 5 Soluble anions

Processes involving the sulfate, phosphate and nitrate anions are separate and will be treated as such. For the biological triplicates for which the standard deviation was higher than 10% of the average, the outlier was eliminated in order to give a better approximation of the process. The nitrite was adjusted solely if the nitrate was modified.

Biogas production								
Day	ER-AN Avg [Nml]	ER-AN Stdev [Nml]	WP-AN Avg [Nml]	WP-AN Stdev [Nml]	AIR-AN Avg [Nml]	AIR-AN Stdev [Nml]	AN-AIR Avg [Nml]	AN-AIR Stdev [Nml]
0.0	44.3	1.85	24.7	4.31	19.7	0.16	18.7	1.46
1.0	47.7	1.45	32.3	5.53	32.0	1.20	25.7	1.59
2.0	50.5	1.71	37.6	6.51	38.5	1.17	33.6	1.83
3.0	53.5	1.47	42.0	6.65	41.3	1.54	44.3	2.82
4.0	61.8	1.52	48.5	5.66	43.2	1.41	56.5	3.97
5.0	72.8	1.30	55.6	5.17	45.0	1.63	71.8	4.52
6.0	81.1	1.23	59.1	6.27	46.9	1.94	90.3	5.41
7.0	95.2	1.11	63.6	8.12	48.7	2.24	114.3	7.41
8.0	108.6	0.86	64.5	7.73	50.2	2.15	142.1	10.28
9.0	123.0	1.14	65.6	7.21	51.6	2.08	168.0	11.70
10.0	140.5	2.04	66.8	6.79	53.0	2.16	190.6	14.81
11.0	148.3	2.77	68.1	6.50	54.1	2.28	223.0	13.16
12.0	155.7	3.12	70.5	6.99	55.4	2.52	257.6	8.06
13.0	158.7	3.01	71.5	7.39	56.7	2.81	271.8	5.73
14.0	162.3	3.22	72.6	7.95	57.8	2.93	282.2	6.12
15.0	166.4	3.32	74.2	9.22	58.5	2.86	289.1	6.51
16.0	170.9	3.52	76.1	11.05	59.3	2.82	296.5	7.13
17.0	176.8	3.81	79.1	13.91	60.0	2.78	304.4	8.85
18.0	185.6	3.80	83.6	17.42	60.8	2.74	314.3	11.42
19.0	197.2	3.98	87.6	19.91	61.5	2.71	333.0	16.59
20.0	204.9	4.38	92.0	21.55	62.7	3.23	352.0	16.86
21.0	209.5	5.22	96.6	22.33	65.1	2.85	367.8	11.13
22.0	212.3	5.20	99.6	22.71	67.3	2.96	369.7	10.96
23.0	214.7	5.70	102.4	22.87	69.3	3.19	372.2	10.94
24.0	217.3	6.26	105.5	22.61	71.3	4.45	378.0	14.93
25.0	220.2	6.92	108.2	21.09	72.3	4.66	380.2	13.97
26.0	222.9	7.54	110.8	18.98	73.1	4.41	381.9	13.31
27.0	225.6	8.35	113.4	16.75	73.7	4.27	383.6	12.58
28.0	228.4	9.08	115.6	15.28	74.2	4.21	386.0	11.49
29.0	232.0	10.78	118.3	12.98	74.6	4.14	388.3	10.39
30.0	236.4	12.09	120.8	11.46	75.0	4.09	390.8	9.13
31.0	240.5	13.54	121.9	10.88	75.5	4.04	393.7	7.28
32.0	242.8	14.17	122.1	10.58	75.9	4.00	397.3	5.28
33.0	245.3	14.27	122.4	10.26	76.4	3.97	401.3	3.33
34.0	247.5	13.81	122.6	9.94	76.8	3.94	405.4	2.50
35.0	249.9	13.51	122.8	9.66	77.4	3.93	409.5	4.57
36.0	252.8	13.67	123.0	9.35	78.1	3.78	413.6	7.69
37.0	254.6	13.18	123.2	9.05	78.7	3.66	417.1	10.15
38.0	255.6	13.45	123.3	8.91	79.4	3.56	418.9	11.84
39.0	256.4	13.75	123.3	8.91	79.9	3.41	419.8	13.22
40.0	257.3	14.07	123.3	8.91	80.4	3.15	419.9	13.35
41.0	257.3	14.07	123.3	8.91	80.8	2.99	419.9	13.35
42.0	257.3	14.07	123.3	8.91	81.1	3.02	419.9	13.35
43.0					81.4	3.07		
44.0					81.7	3.15		
45.0					82.0	3.26		
46.0					82.3	3.38		
47.0					82.6	3.53		

Table 6 Methane production

	COD reduced [mg/L]	NO_3^- reduced [mg/L]	SO_4^{2-} reduced [mg/L]	CH_4 gas production [Nml/L]	COD consumed [mg/L]
ER→AN	1083.33	189.26	65.89	257.28	818.10
WP→AN	584.00	294.86	72.59	123.30	552.25
AIR→AN	405.33	498.61	90.65	82.61	593.82
AN→AIR	1902.67	16.25	55.67	419.87	1110.13

Table 7 COD balance

Results in Table 7 are given considering 0.65g COD/g NO_3^-, 0.67 g COD/ g SO_4^{2-} , 2.53 g COD/ ml CH_4 at atmospheric pressure and 35°C.

	Days	Sampling	Soluble NH_4^+		Produced NH_4^+
			Avg. conc. [mg/L]	σ or Min/Max [mg/L]	Avg. conc. [mg/L]
ER→AN	0	Start	0.00	0.00	0.00
	7	Switch	0.00	0.00	50.29
	19	1M	69.93	2.80	69.93
	28	2M	84.93	2.06	84.93
	49	End	123.67	3.06	123.67
WP→AN	0	Start	0.00	0.00	0.00
	7	Switch	**24.95**	**24.2/25.7**	106.70
	19	1M	41.63	1.52	41.63
	28	2M	51.50	1.91	51.50
	49	End	66.03	3.79	66.03
AIR→AN	0	Start	0.00	0.00	0.00
	12	1M	**8.62**	**8.28/8.96**	90.28
	22	2M	**13.00**	**12.2/13.8**	130.89
	42	Switch	50.33	1.21	192.23
	55	3M	66.97	0.49	66.97
	68	4M	76.40	0.61	76.40
	89	End	**78.00**	**77.7/78.3**	78.00
AN→AIR	0	Start	0.00	0.00	0.00
	12	1M	107.67	1.53	107.67
	22	2M	126.33	5.86	126.33
	42	Switch	163.00	1.00	163.00
	55	3M	**47.20**	**47.1/47.3**	47.20
	68	4M	0.00	0.00	0.76
	89	End	0.00	0.00	0.00

Table 8 Measured soluble and calculated produced ammonium

	Days	Sampling	Total N (measured as NO3-)		Org bound N
			Avg. conc. [mg/L]	σ [mg/L]	Avg. conc. [mg/L]
ER→AN	0	Start	910.00	36.06	202.52
	7	Switch	1176.67	51.32	223.95
	49	End	1693.33	50.33	287.95
WP→AN	0	Start	910.00	36.06	202.52
	7	Switch	1260.00	124.90	198.65
	49	End	1000.00	20.00	175.52
AIR→AN	0	Start	910.00	36.06	202.52
	42	Switch	2213.33	94.52	348.20
	89	End	856.67	217.79	131.86
AN→AIR	0	Start	910.00	36.06	202.52
	42	Switch	1370.00	112.69	183.79
	89	End	646.67	70.24	146.86

Table 9 Total Nitrogen and Organic Nitrogen

The method for total nitrogen measures ammonia, organic nitrogen, nitrate and nitrite, after reduction of all these compounds to NO_3^- (Figure 4). The Total N was expected to remain stable in all aerobic phases (as shifts between types of N components occur, but no absorption or consumption) and to decrease in all anaerobic stages (as transformation to N_2 and release as gas takes place). The increase in Total N in all 1st stages of all tracks, as well as the 2nd stages of the ER→AN and AN→AIR, cannot be reasonably explained through a known process, but can be the result of measurement errors, interference with other compounds or unknow processes taking place. Results are not considered reliable and, for this reason, are not discusses or interpreted.

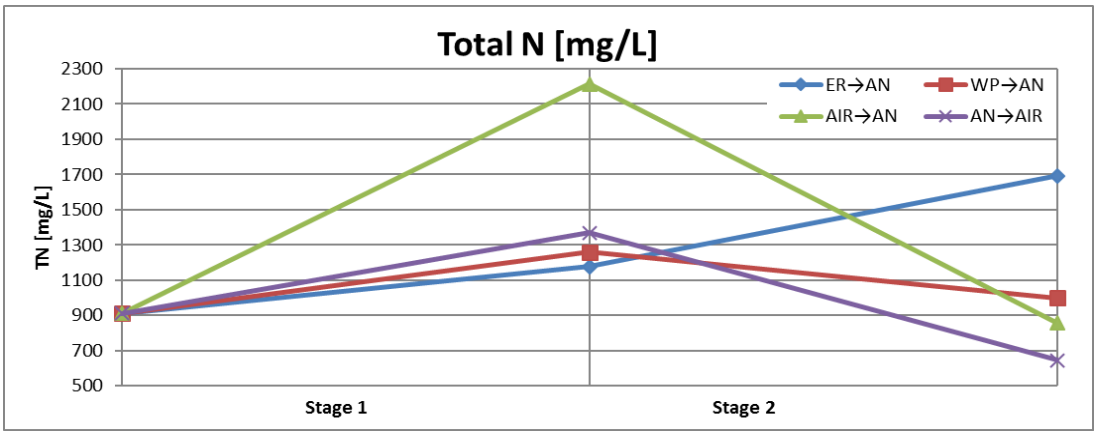

Figure 4 Total nitrogen

The organic nitrogen was calculated as the difference between Total nitrogen and the sum of soluble ammonium, nitrite and nitrate considering the following transformation factors that 2.55g NO_2^-/ g NH_4^+ and 3.44g NO_3^-/g $NH_4^+$and 1.28 g NH_4^+ /g N (Figure 5). The organic bound N is an approximation due to the errors introduced through the different

behavior of the processes involved and predominantly due to faulty Total nitrogen measurements. Results are not considered reliable and, for this reason, are not discusses or interpreted.

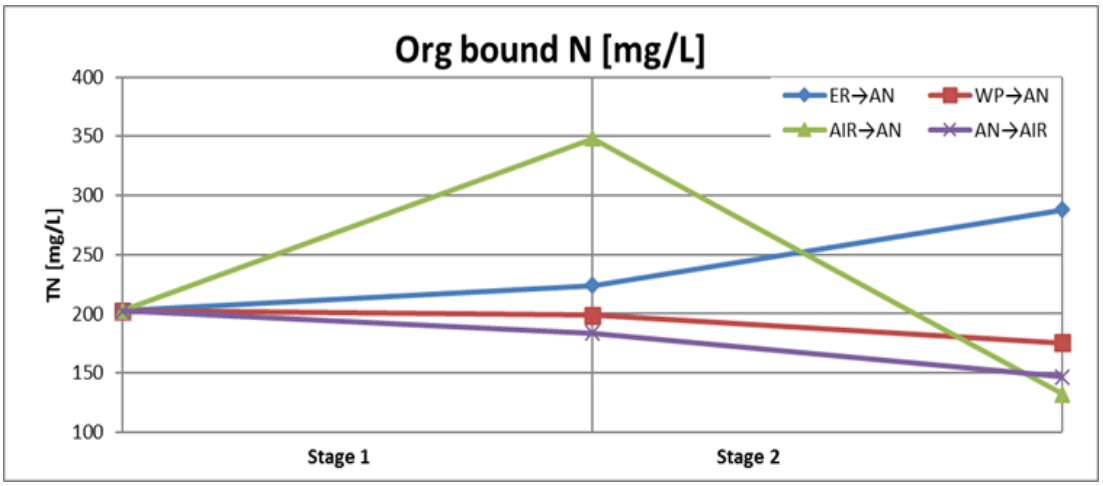

Figure 5 Organic nitrogen

			SOLUBLE EPS					
	Days	Sampling	Polysachharides [mg/L]		Proteins(Excluding Humics) [mg/L]		Proteins (Including Humics) [mg/L]	
			Avg.conc.	Stdev.	Avg.conc.	Stdev.	Avg.conc.	Stdev.
ER→AN	0	Start	9.02	0.15	**4.31**	**3.78/4.83**	20.73	1.14
	7	Switch	15.62	1.03	**6.04**	**5.67/6.41**	23.19	0.34
	19	1M	12.76	1.18	0.00	0.00	64.82	2.39
	28	2M	8.76	0.62	0.00	0.00	38.51	1.81
	49	End	7.58	0.60	0.00	0.00	37.65	1.02
WP→AN	0	Start	9.02	0.15	**4.31**	**3.78/4.83**	20.73	1.14
	7	Switch	**43.26**	**42.28/44.24**	**11.08**	**10.29/11.87**	40.50	3.97
	19	1M	**23.93**	**22.66/25.19**	9.73	0.37	**35.84**	**35.71/35.96**
	28	2M	**15.28**	**14.94/15.62**	**4.83**	**3.78/5.88**	40.61	1.96
	49	End	9.26	0.89	**0.16**	**0.00/0.32**	35.77	0.42
AIR→AN	0	Start	9.02	0.15	**4.31**	**3.78/4.83**	20.73	1.14
	12	1M	16.76	0.78	**3.68**	**3.26/4.10**	24.96	0.81
	22	2M	**21.87**	**20.80/22.95**	**3.73**	**3.68/3.78**	27.34	1.84
	42	Switch	35.35	1.29	**0.00**	**0.00/0.00**	27.93	0.64
	55	3M	**31.62**	**31.01/32.23**	**2.63**	**2.52/2.73**	31.99	1.15
	68	4M	**22.00**	**21.53/22.46**	**0.00**	**0.00/0.00**	28.23	1.01
	89	End	**15.53**	**13.96/17.09**	**0.26**	**0.00/0.53**	25.46	2.36
AN→AIR	0	Start	9.02	0.15	**4.31**	**3.78/4.83**	20.73	1.14
	12	Meas 1	16.28	0.88	0.00	0.00	90.75	0.69
	22	Meas 2	**9.86**	**9.08/10.64**	**12.13**	**9.98/14.29**	**85.96**	**84.20/106.05**
	42	Switch	7.26	0.56	0.00	0.00	41.71	0.53
	55	Meas 3	**27.93**	**27.64/28.22**	**6.77**	**6.41/7.14**	40.53	1.30
	68	Meas 4	-	-	**3.47**	**3.05/3.89**	30.84	2.18
	89	End	18.70	18.16/19.24	0.00	0.00	28.93	1.15

Table 10 Soluble polysaccharides and proteins

	Days	Sampling	FLOC EPS						FLOC EPS		
			Polysacchrides [mg/ gVS]		Proteins (Excluding Humics) [mg/gVS]		Proteins (Including Humics) [mg/g VS]		Polysacchrides [mg/ L]	Proteins (Excluding Humics) [mg/L]	Proteins (Including Humics) [mg/L]
			Avg.conc.	Stdev.	Avg.conc.	Stdev.	Avg.conc.	Stdev.			
ER→AN	0	Start	**20.20**	**19.78/20.62**	**54.25**	**53.67/54.83**	200.44	11.86	51.27	137.67	508.64
	7	Switch	14.66	1.21	**57.77**	**57.56/57.98**	166.04	6.75	35.25	138.97	399.42
	49	End	14.21	0.83	0.00	0.00	139.35	5.30	21.27	0.00	208.66
WP→AN	0	Start	**20.20**	**19.78/20.62**	**54.25**	**53.67/54.83**	200.44	11.86	51.27	137.67	508.64
	7	Switch	**13.45**	**13.24/13.66**	52.06	2.33	142.94	14.16	24.30	94.04	258.17
	49	End	**18.63**	**17.93/19.34**	**0.05**	**0.00/0.11**	149.27	4.60	22.63	0.06	181.27
AIR→AN	0	Start	**20.20**	**19.78/20.62**	**54.25**	**53.67/54.83**	200.44	11.86	51.27	137.67	508.64
	42	Switch	**9.43**	**9.40/9.47**	**27.05**	**25.21/28.89**	120.56	8.69	15.63	44.82	199.79
	89	End	8.88	0.24	**10.92**	**9.45/12.39**	78.79	6.98	11.61	14.27	102.94
AN→AIR	0	Start	**20.20**	**19.78/20.62**	**54.25**	**53.67/54.83**	200.44	11.86	51.27	137.67	508.64
	42	Switch	**13.11**	**13.10/13.12**	**0.00**	**0.00/0.00**	142.51	8.32	19.30	0.00	209.72
	89	End	**6.29**	**6.23/6.35**	17.96	0.95	86.69	5.46	8.59	24.52	118.35

Table 11 Floc polysaccharides and proteins

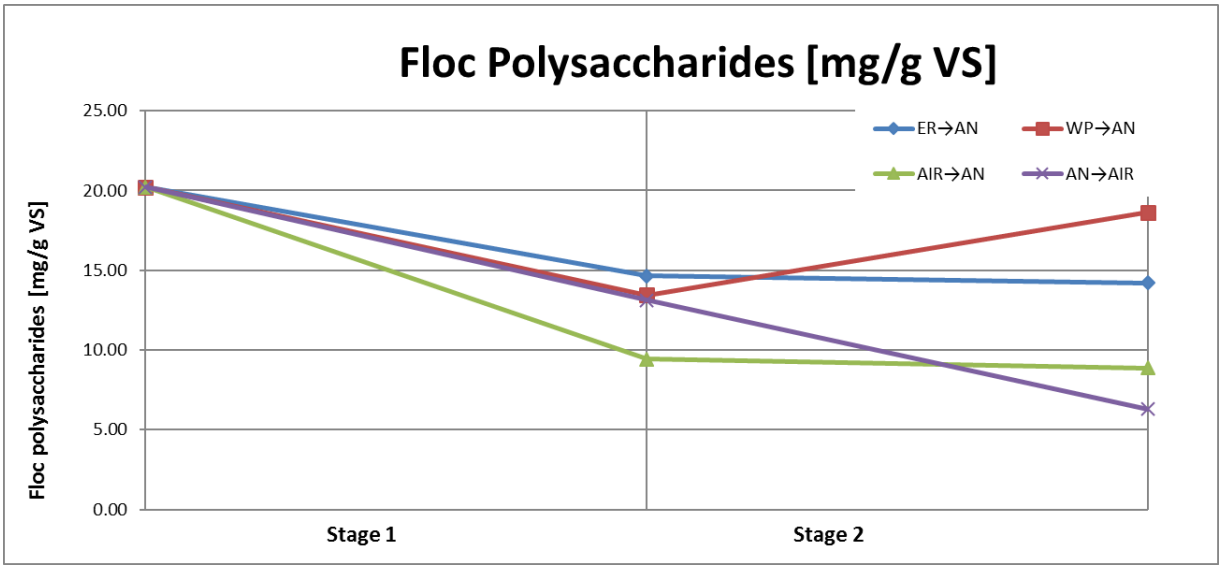

Figure 6 Floc polysaccharides

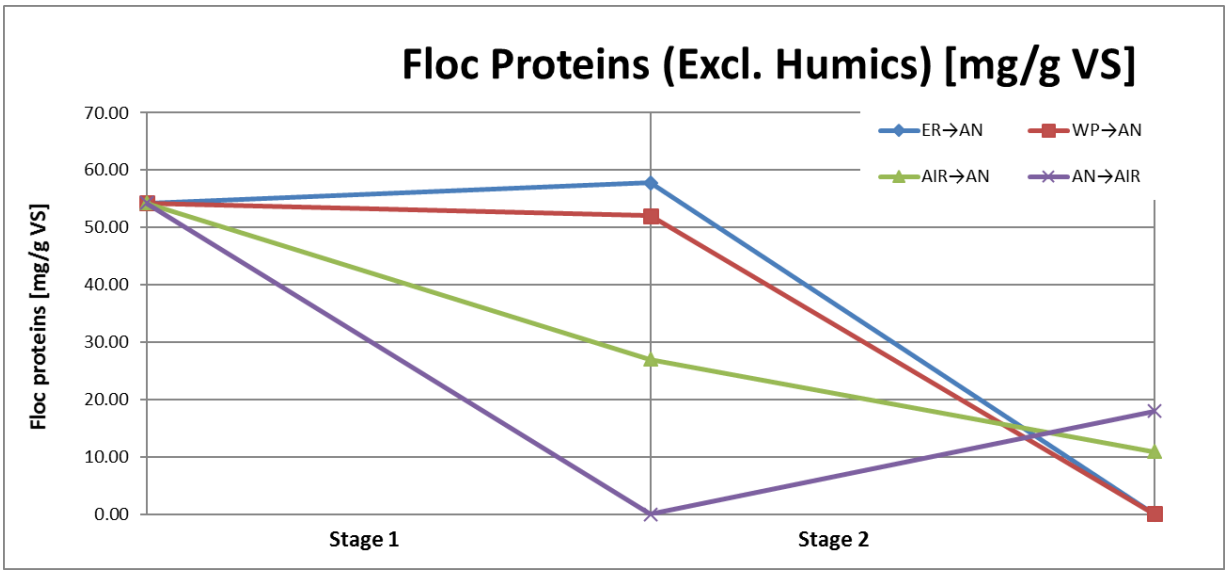

Figure 7 Floc proteins excluding humics

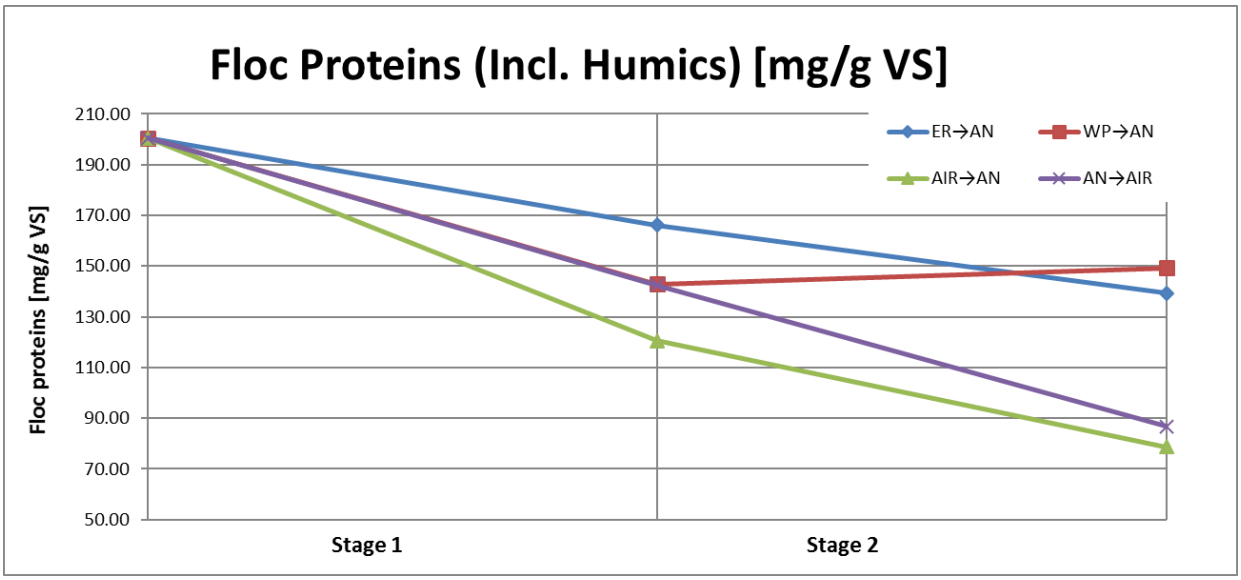

Figure 8 Floc proteins including humics

	Days	Sampling	Soluble salts						
			Ca^{2+} [mg/L]		Mg^{2+} [mg/L]		$Ca^{2+} + Mg^{2+}$ [mmol/l]	$Fe^{2+} + Fe^{3+}$ [mg/L]	
			Avg.	Stdev.	Avg.	Stdev.	Avg.	Avg.	Stdev.
ER→AN	0	Start	68.33	0.25	10.13	0.06	2.13	0.07	0.00
	7	Switch	55.77	0.84	13.37	0.12	1.95	0.06	0.00
	19	1M	52.67	0.15	21.33	0.31	2.20	**0.45**	**0.38/0.44**
	28	2M	54.97	0.76	21.13	0.06	2.25	**0.55**	**0.54/0.56**
	49	End	54.47	1.39	20.30	0.17	2.20	**0.41**	**0.38/0.44**
WP→AN	0	Start	68.33	0.25	10.13	0.06	2.13	0.07	0.00
	7	Switch	**53.35**	**53/53.7**	**21.00**	**20.7/21.3**	2.20	**0.23**	**0.21/0.24**
	19	1M	**37.05**	**36.2/37.9**	22.43	2.11	1.86	**1.10**	**1.10/1.10**
	28	2M	49.93	2.78	20.63	1.12	2.10	**0.65**	**0.60/0.69**
	49	End	48.67	0.67	19.07	0.60	2.01	**0.36**	**0.35/0.37**
AIR→AN	0	Start	68.33	0.25	10.13	0.06	2.13	0.07	0.00
	12	1M	70.10	1.87	17.70	1.13	2.49	**0.07**	**0.068/0.070**
	22	2M	90.13	5.16	26.10	2.12	3.33	**0.14**	**0.13/0.15**
	42	Switch	83.93	7.61	29.90	1.22	3.34	0.17	0.01
	55	3M	63.27	2.68	29.03	0.55	2.79	**0.32**	**0.31/0.33**
	68	4M	59.07	5.45	28.70	0.79	2.67	**0.09**	**0.088/0.087**
	89	End	**51.35**	**50.6/52.1**	28.77	1.85	2.48	**0.08**	**0.05/0.081**
AN→AIR	0	Start	68.33	0.25	10.13	0.06	2.13	0.07	0.00
	12	Meas 1	**38.10**	**37.5/38.7**	**24.05**	**23.0/25.1**	1.95	**0.05**	**0.05/0.052**
	22	Meas 2	**63.20**	**63.2/63.2**	23.93	0.46	2.57	**0.28**	**0.27/0.29**
	42	Switch	**62.80**	**60.3/65.3**	23.07	1.35	2.53	**0.31**	**0.27/0.35**
	55	Meas 3	10.43	0.12	11.87	0.61	0.75	0.33	0.03
	68	Meas 4	54.93	2.06	24.73	0.50	2.40	**0.12**	**0.11/0.12**
	89	End	53.83	2.99	25.37	0.64	2.40	**0.11**	**0.10/0.11**

Table 12 Soluble cations

			Total salts							
			Ca^{2+}		Mg^{2+}		$Ca^{2+} + Mg^{2+}$		$Fe^{2+} + Fe^{3+}$	
			[mg/ L]	[mg/ L]	[mg/ L]	[mg/ L]	[mmol/ L]	[mmol/ L]	[mg/ L]	[mg/ L]
			Avg.	Stdev.	Avg.	Stdev.	Avg.	Stdev.	Avg.	Stdev.
			105.23	14.73	37.03	2.37	4.17	0.38	95.00	23.64
			Floc salts							
			Ca^{2+}		Mg^{2+}		$Ca^{2+} + Mg^{2+}$		$Fe^{2+} + Fe^{3+}$	
	Days	Sampling	[mg/ L]	[mg/ g TS]	[mg/ L]	[mg/ g TS]	[mmol/ L]	[mmol/ g TS]	[mg/ L]	[mg/ g TS]
			Avg.	Avg.	Avg.	Avg.	Avg.	Avg.	Avg.	Avg.
ER→AN	0	Start	36.90	9.15	26.90	6.67	2.04	0.51	94.93	23.55
	7	Switch	49.47	12.70	23.67	6.08	2.22	0.57	94.94	24.38
	49	End	50.77	17.28	16.73	5.70	1.96	0.67	94.59	32.19
WP→AN	0	Start	36.90	9.15	26.90	6.67	2.04	0.51	94.93	23.55
	7	Switch	51.88	16.05	16.03	4.96	1.96	0.61	94.78	29.33
	49	End	56.57	22.03	17.97	7.00	2.16	0.84	94.64	36.86
AIR→AN	0	Start	36.90	9.15	26.90	6.67	2.04	0.51	94.93	23.55
	42	Switch	21.30	6.89	7.13	2.31	0.83	0.27	94.83	30.66
	89	End	53.88	19.53	8.27	3.00	1.69	0.61	94.92	34.41
AN→AIR	0	Start	36.90	9.15	26.90	6.67	2.04	0.51	94.93	23.55
	42	Switch	49.47	12.70	13.97	4.70	1.82	0.51	94.69	31.84
	89	End	50.77	17.28	11.67	4.26	1.75	0.61	94.90	34.67

Table 13 Total and floc cations

Initially a measurement for Total cations was made in all start of tracks, switch of stages and end of tracks. However, the results for all samples showed an increasing Total Ca^{2+}, $Mg^{2+}$and Fe^{3+} concentrations in time for all tracks, which is impossible. It is believed the method was inappropriate and insufficiently aggressive to properly solubilize floc cations. For this reason, only the measurement from the start of the experiment was used in calculations.

The floc cations values are calculated by subtracting the soluble cations fraction from the total cations concentration. As it is not an independent measurement and the interpretation for soluble cations fraction has been already carried out, these results do not bring further information and are not further discussed.

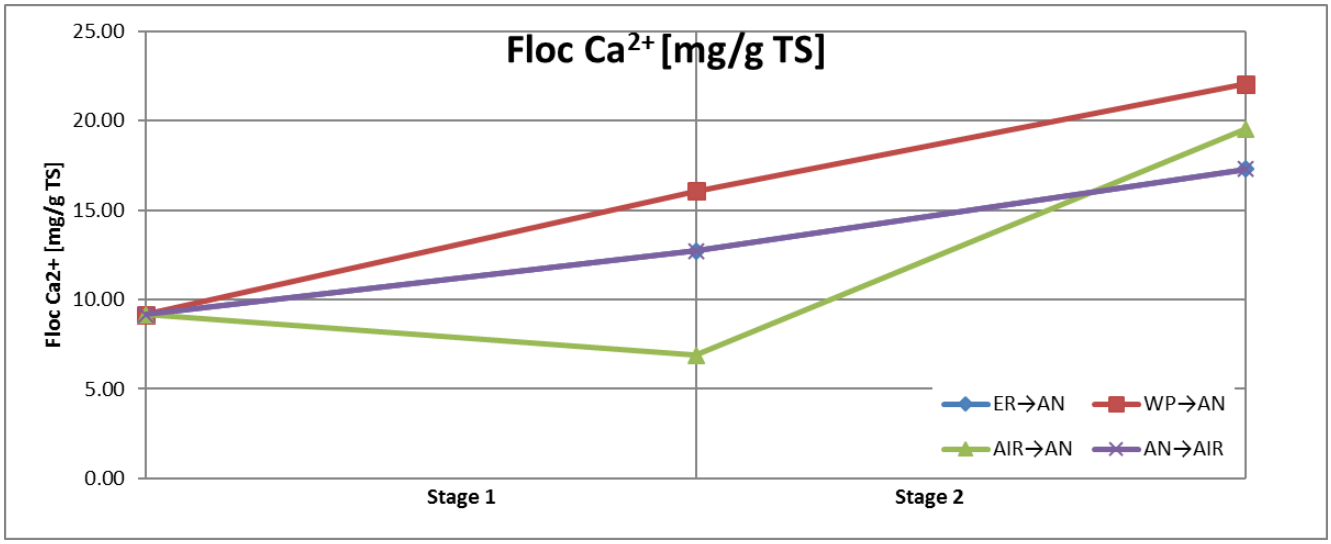

Figure 9 Floc Calcium

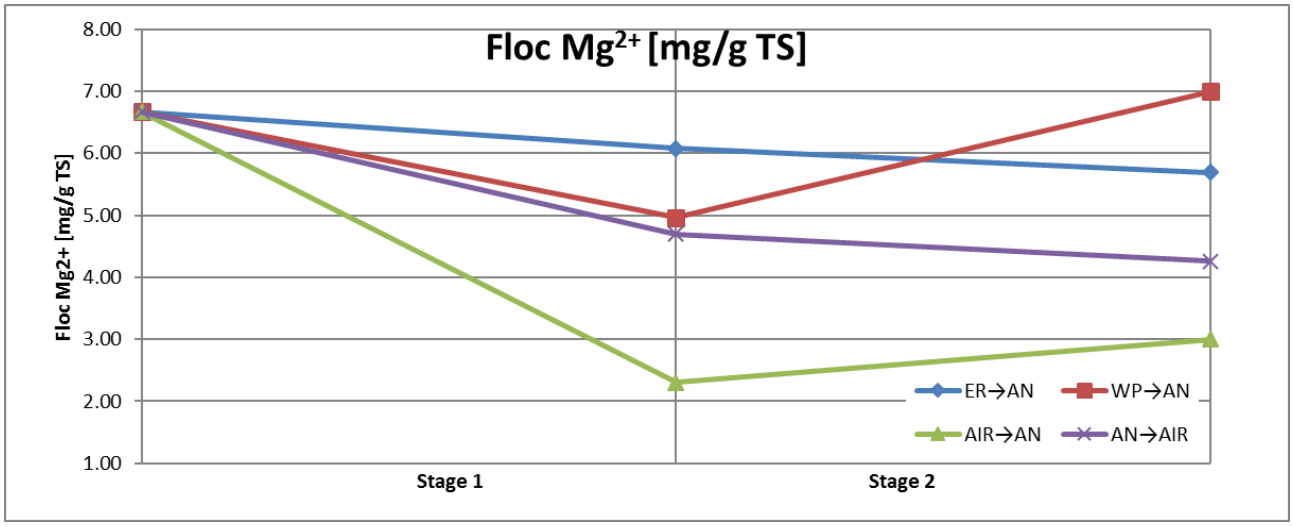

Figure 10 Floc Magnesium

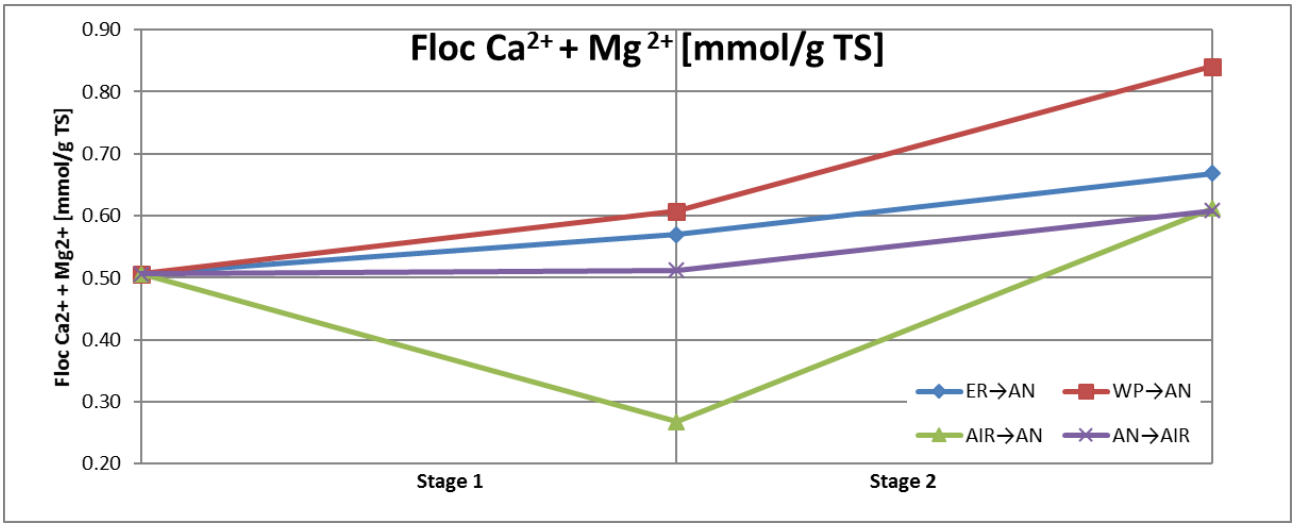

Figure 5 Floc divalent cations

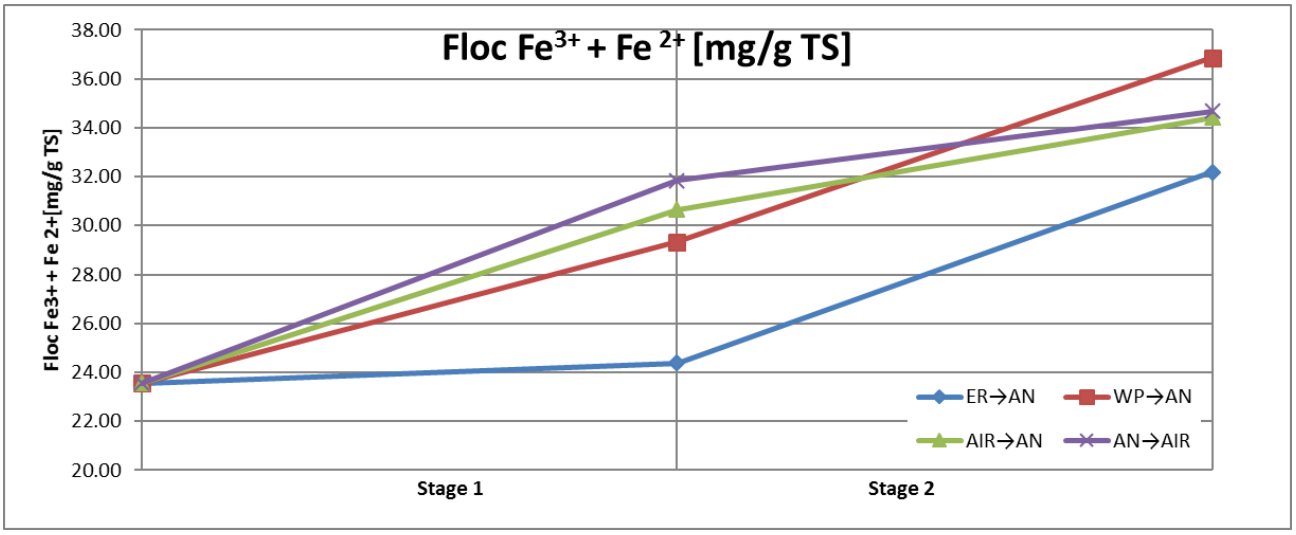

Figure 12 Floc Iron

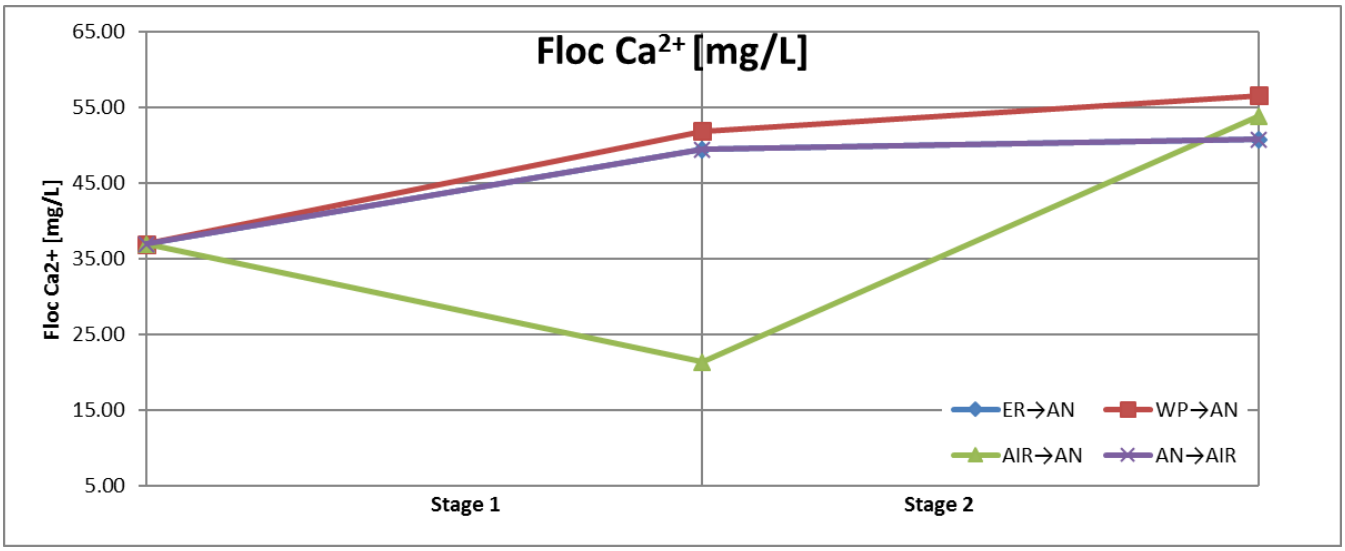

Figure 13 Floc Calcium

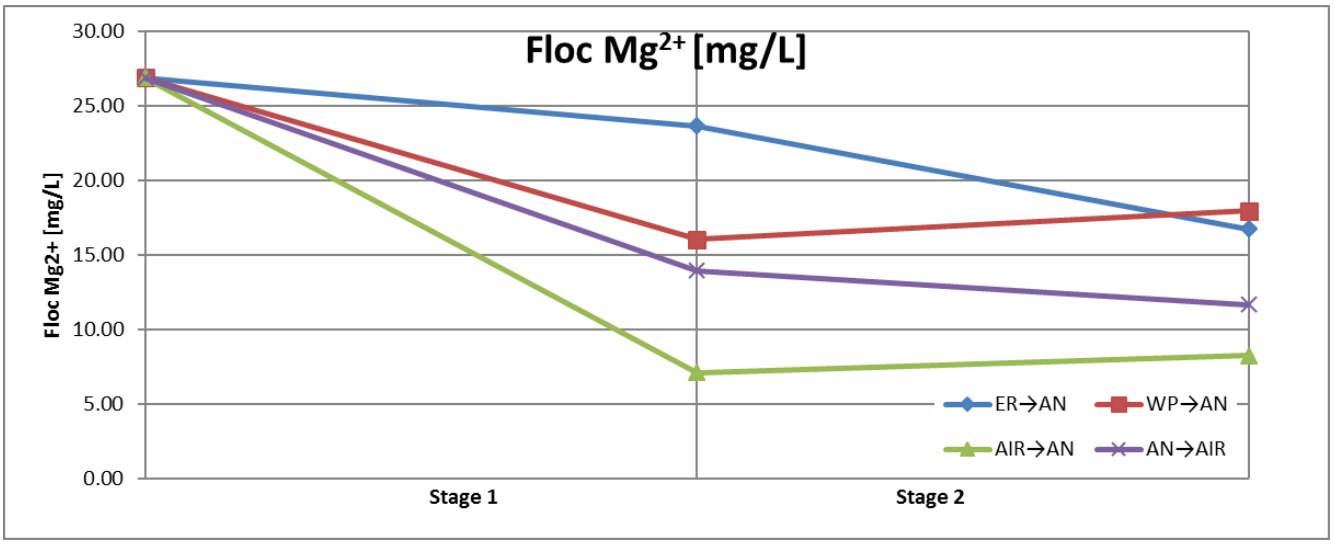

Figure 6 Floc Magnesium

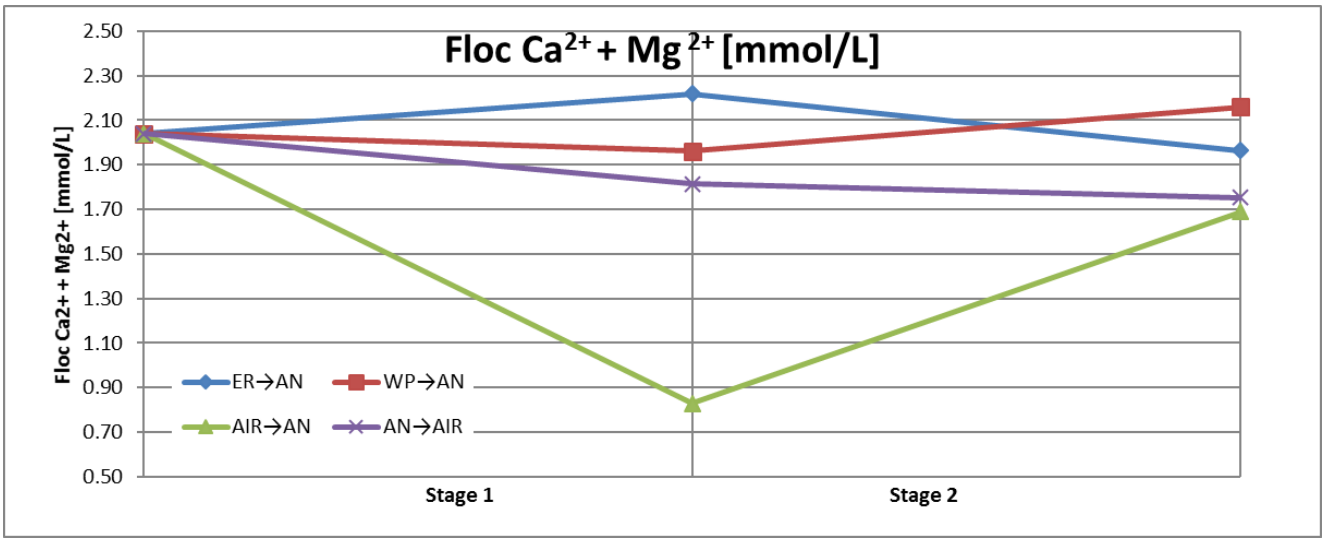

Figure 7 Floc divalent cations

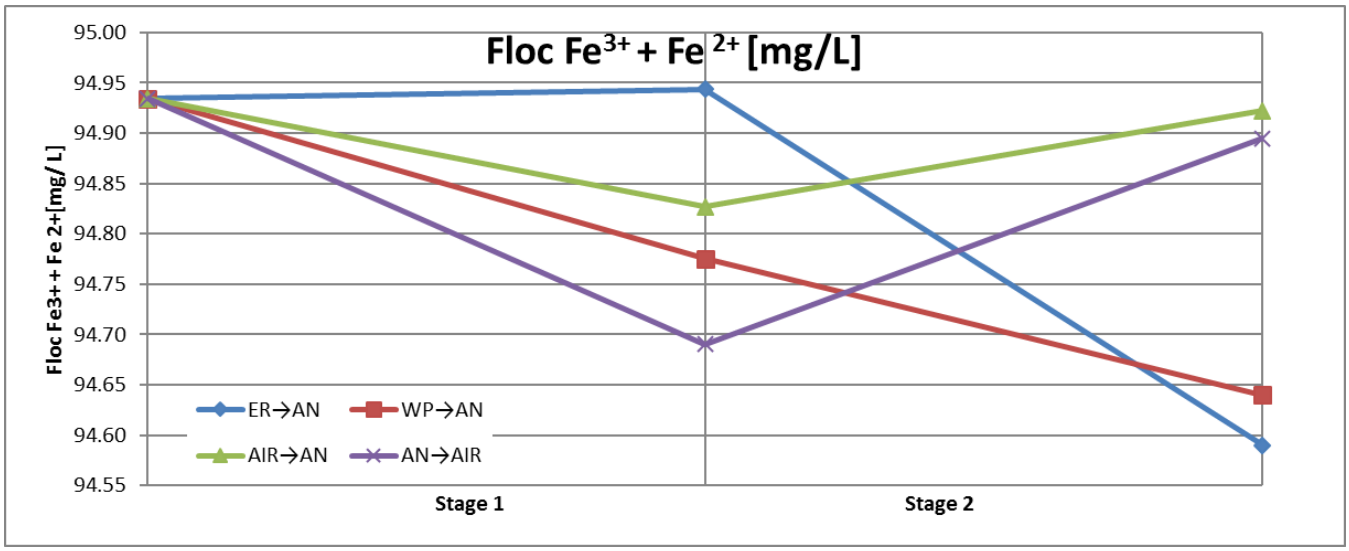

Figure 16 Floc Iron