

# Measuring and Modelling the Impact of Oligochaete Predation Based Activated Sludge Reduction on Wastewater Treatment Plant Operation





# **Measuring and Modelling the Impact of Oligochaete Predation Based Activated Sludge Reduction on Wastewater Treatment Plant Operation**

Marisa Buyers-Basso

for the degree of:  
***Master of Science in Civil Engineering***

Date of Submission: 21, January 2014  
Date of Defence: 28, January 2014

Committee:

Prof.dr.ir. J.B. van Lier

Dr.ir. M.K. de Kreuk

Ph.D.c S.L. de Valk

Ir. J. Tamis MSc

Delft University of Technology  
Sanitary Engineering Section  
Delft University of Technology  
Sanitary Engineering Section  
Delft University of Technology  
Sanitary Engineering Section  
Delft University of Technology  
Environmental Biotechnology Section

Sanitary Engineering Section, Department of Water Management  
Faculty of Civil Engineering and Geosciences  
Delft University of Technology, Delft



## Abstract

Recent Studies on oligochaete predation have shown that large reductions in Activated Sludge (AS) volume can be achieved in a relatively short amount of time and have also indicated that the predation may increase the overall biodegradability of the sludge when paired with anaerobic digestion. The objective of this MSc thesis was to investigate how the implementation of worm predation, or a technology developed to mimic its results, could affect the traditional sludge treatment process and overall Wastewater Treatment Plant (WWTP) operations.

Four main research questions were developed to guide the process of meeting the thesis objective: (1) How does worm predation change the properties of AS? (2) How do the changes in sludge properties affect the solids processing behaviour of AS? (3) If the changes in sludge properties were applied at a full scale WWTP, how would the performance of the plant be affected? (4) Do any modifications need be made to the treatment process to accommodate changes in performance? The scope of this MSc thesis included a literature review, laboratory experimentation and computer modelling to answer the research questions.

Laboratory experimentation was used to determine impact of oligochaete predation on AS characteristics such as solids content, oxygen demand and nutrients, and on its solids processing behaviour including thickening, digestion and dewatering characteristics.

Three-Day batch experiments in a bench scale worm reactor showed that worm predation reduced the total solids (TS) of the Feed-AS by an average of 36%, volatile solids (VS) by 41% and total COD by 53%. The organic content of the sludge was reduced 15 time faster using worm predation than through endogenous respiration. Predation also increased the soluble COD (sCOD) of the sludge by an average of 141%, nitrogen (sN) by 591%, and phosphorus (sP) by 4057%. The worm predated AS (WP-AS) has better settling properties than typical AS with SVIs on average 51% lower and zone settling velocities 10 time faster.

Biochemical Methane Potential (BMP) tests were used to compare the digestibility of the VS in the Feed-AS with the VS remaining in the WP-AS produced in the batch experiments. One BMP test indicated that, in the absence of nitrate inhibition, anaerobic digestion of WP-AS would have Methane potential 18% larger than the Feed-AS while the other showed that methane potential of the WP-AS was 47% lower than the Feed-AS.

The cake solid percentages achieved by dewatering the undigested and digested sludge in a laboratory centrifuge indicated that no difference in performance should be expected from mechanical thickening of WP-AS and dewatering of digested WP-AS rather than typical AS, except for higher concentrations of nutrients and COD in the centrate after thickening. The filterability of digested worm predated sludge is higher than that of AS, which could reduce the chemical addition required in its processing.

The WWTP simulation software, BioWin, was used to model a Baseline WWTP with AS treatment process including nutrient removal. The info information gathered in the laboratory about solids processing of worm predated AS was used as the basis for creating a "worm reactor" which was added to the Baseline WWTP model to identify how the changes to sludge properties would impact the behaviour of the overall treatment performance.

Implementation of the "worm reactor" for waste AS reduction in the model showed that the quality of the WWTP effluent would not be affected while total sludge production could be reduced by approximately 33% while only reducing methane production by 7% due to an increase in digester capacity from the reduction in waste secondary solids. The reduction in the total dewatered sludge resulting from the addition in worm predation would reduce disposal costs and increased digester capacity presents opportunity for Co-digestion with other waste streams which could generate revenue. Future work should focus on improved methods of measuring methane potential of the worm predate sludge, on more efficient ways of modelling of the effects of adding a "worm reactor", and on a complete economic evaluation of the implementation of full scale worm reactors.



## **Preface**

This MSc thesis document is the result of approximately one year of my work, and the culmination of two and a half years of an MSc program here in the Delft University of Technology Sanitary Engineering Department of the Civil Engineering Faculty. This last year has had its ups and downs both related and unrelated to the progress of my MSc thesis. I know that its successful completion would not have been possible without a great deal of help and support from those around me; therefore I would like to take the opportunity to acknowledge and thank the following:

My MSc supervisor, **Steef de Valk**, for welcoming me into his project and supporting the direction my research took even when it veered away from his original course, for working side by side with me in the lab day after day to make sure that I was able to have sufficient data to work with, for brainstorming and problem solving when things did not go the way we expected and of course for tending to, and caring for, our Worms at the Harnaschpolder WWTP, without whom this project would also not have been possible.

My supervising professor, **Merle de Kreuk**, for helping me find a topic which was both interesting and challenging, for always making time to listen to and sympathise with the difficulties I encountered and for helping to find solutions to them... most particularly for identifying the source of a certain BMP related problem which could have continued to cause frustration and delays if she had not recognized it!

The chairman of my thesis committee **Jules van Lier**, and the external member of the committee **Jelmer Tamis**, for taking the time from their busy schedules to really participate and help me throughout my thesis process, and for always pushing me to improve my work with their insightful questions and suggestions.

**Paul Weij** of Delfluent Services for providing me with all of the data I requested about the Harnaschpolder WWTP in the most thorough and prompt way imaginable and for taking the time to meet with me in person to explain the specific aspects of their treatment process.

**Henri Spanjers** for providing guidance on the use of BioWin Software.

The PhD students of the Anaerobic Digestion Group, and the Staff of the Sanitary Engineering Laboratory, for taking the time to teach and assist me with my experiments. It would have been impossible to do without you and I wish you all the success possible in your work!

My friends and family at home in the USA for always being there for me no matter how far I am from you or how long I have been away.

And finally, all of the amazing friends I have made during my time here in the Netherlands. Meeting you has truly been the highlight of the last two and a half years, and I would not have survived without you!



<b>Content</b>	
<b>Abstract</b> .....	<b>I</b>
<b>Preface</b> .....	<b>III</b>
<b>List of Figures</b> .....	<b>IX</b>
<b>List of Tables</b> .....	<b>XI</b>
<b>1 Introduction</b> .....	<b>1-1</b>
1.1 Objective.....	1-1
1.2 Research Questions .....	1-1
1.3 Scope .....	1-1
1.4 Outline.....	1-1
<b>2 Background and Literature Review</b> .....	<b>2-1</b>
2.1 Activated Sludge Production .....	2-1
2.1.1 Activated Sludge Characteristics .....	2-1
2.1.2 Treatment and Disposal of Waste Activated Sludge .....	2-2
2.2 Anaerobic Digestion .....	2-3
2.2.1 Hydrolysis of Waste Activated Sludge .....	2-3
2.2.2 Methane Production.....	2-4
2.3 Dewatering .....	2-5
2.3.1 Mechanical Dewatering .....	2-5
2.3.2 Sludge Characteristics and Dewatering .....	2-6
2.3.3 Settling.....	2-6
2.3.4 Cake Solids Concentration.....	2-7
2.3.5 Dewatering Rate and Filterability .....	2-8
2.3.6 Dewatering Lab Tests vs Pilot Tests.....	2-10
2.4 Excess Sludge Reduction .....	2-11
2.5 Oligochaete Predation .....	2-12
2.5.1 <i>Tubifex tubifex</i> ( <i>T. tubifex</i> ) .....	2-12
2.5.2 Predation on Activated Sludge as a Reduction Technique.....	2-13
2.5.3 Impact on Solids Processing.....	2-13
2.6 Computer Modeling of Wastewater Treatment.....	2-14
2.6.1 Activated Sludge Model (ASM).....	2-14
2.6.2 Wastewater Treatment Model .....	2-16
2.6.3 Plant Wide Model .....	2-17
2.6.4 Simulators.....	2-18
<b>3 Materials and Methods</b> .....	<b>3-1</b>
3.1 Worm Reactor .....	3-1
3.2 Experimental Program.....	3-2
3.2.1 Batch Experiments.....	3-2
3.2.2 Dewatering of Sludge Samples .....	3-2

3.2.3	Digestibility .....	3-2
3.2.4	Dewatering of Digested Samples .....	3-2
3.3	Analyses .....	3-3
3.3.1	Biological Methane Potential (BMP).....	3-3
3.3.2	Sludge Characterization .....	3-5
3.3.3	Solid-Liquid Separation .....	3-5
3.4	Computer Model .....	3-6
<b>4</b>	<b>Laboratory Results .....</b>	<b>4-1</b>
4.1	Batch Experiments .....	4-1
4.2	Dewatering of Sludge Samples .....	4-8
4.2.1	Gravity Thickening .....	4-8
4.2.2	Centrifugation .....	4-9
4.3	Digestion.....	4-11
4.3.1	Methane Potential .....	4-11
4.3.2	Change in Sludge Properties .....	4-17
4.3.3	Combined Worm, Aeration and Digestion Performance .....	4-20
4.4	Dewatering of Digested Samples .....	4-21
4.4.1	Centrifugation .....	4-21
4.4.2	Capillary Suction Time and Specific Resistance to Filtration .....	4-22
<b>5</b>	<b>Discussion Laboratory Results .....</b>	<b>5-1</b>
5.1	Worm Reactor Batch Experiments.....	5-1
5.1.1	Appearance of Distinct Worm Predated Sludge.....	5-1
5.1.2	Effect of Worm Predation on Activated Sludge Properties.....	5-3
5.1.3	Variability in Batch Results .....	5-4
5.2	Thickening of Sludge Samples .....	5-5
5.3	Digestion.....	5-6
5.3.1	Predicted vs Actual Methane Production and Volatile Solids Reduction .....	5-6
5.3.2	Impact of Denitrification .....	5-7
5.3.3	BMP 1 vs BMP 2 .....	5-8
5.3.4	COD Balance .....	5-8
5.3.5	Change in Sludge Properties .....	5-8
5.4	Dewatering of Digested Sludge.....	5-10
<b>6</b>	<b>Computer Model .....</b>	<b>6-1</b>
6.1	Baseline Wastewater Treatment Model.....	6-1
6.1.1	Influent .....	6-1
6.1.2	Treatment Configuration .....	6-2
6.1.3	Baseline Performance .....	6-5
6.2	Worm Predation Model.....	6-8

6.2.1	TSS, VSS and tCOD Reduction.....	6-9
6.2.2	Soluble COD, Nitrogen and Phosphate Increase .....	6-9
6.2.3	Solids Processing Changes .....	6-11
6.3	Modeled Impacts of Worm Predation .....	6-12
6.3.1	Wastewater Treatment .....	6-12
6.3.2	Solids Processing.....	6-12
6.4	Model Reflection .....	6-14
<b>7</b>	<b>Conclusions and Recommendations.....</b>	<b>7-1</b>
7.1	Worm Predation of Activated Sludge .....	7-1
7.2	Solids Processing of Worm Predated Sludge .....	7-1
7.3	Impacts of Worm Predation Based Sludge Reduction on WWTP Performance .....	7-2
7.4	Costs and Benefits of Full Scale Implementation .....	7-3
7.5	Recommendations for Further Work.....	7-4
	<b>Works Cited .....</b>	<b>WC-1</b>
	<b>Appendix A – Oligochaete Predation Literature Review Table .....</b>	<b>A-1</b>
	<b>Appendix B – BMP 1 Individual Data.....</b>	<b>B-1</b>
	<b>Appendix C – BMP 2 Raw Data .....</b>	<b>C-1</b>
	<b>Appendix D – Harnaspolder Influent Data 2012-2013.....</b>	<b>D-1</b>
	<b>Appendix E – Delfluent Data.....</b>	<b>E-1</b>



## List of Figures

Figure 2-1 Typical Activated Sludge Wastewater Treatment Process .....	2-1
Figure 2-2 Typical Waste Activated Sludge Treatment Process .....	2-2
Figure 2-3 Steps of Anaerobic Digestion (Appels, Baeyens, Degreve, & Dewil, 2008) .....	2-3
Figure 2-4 Two Phases of Sludge Dewatering: Filtration, and Expression .....	2-5
Figure 2-5 Comparison of cake solids by gravity thickening and selected dewatering processes (Huang, 1979) .....	2-7
Figure 2-6 Dewatering data showing the cake solids achieved by a variety of dewatering processes (USEPA).....	2-7
Figure 2-7 Capillary Suction Time Apparatus (to be replaced with original pic of device in lab) .....	2-9
Figure 2-8 Upper block of CST apparatus (Vesilind, 1988).....	2-9
Figure 2-9 Tubifex tubifex .....	2-12
Figure 2-10 Wastewater Treatment Process Model Schematic (van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008) .....	2-17
Figure 3-1 Worm Reactor.....	3-1
Figure 3-2 AMPTS BMP 1 Setup.....	3-3
Figure 4-1 Results of Batch 1 Comparing Endogenous Worm Predation to Endogenous Respiration .	4-4
Figure 4-2 Results of Batch 2 Comparing Worm Predation to Endogenous Respiration .....	4-5
Figure 4-3 Settled Sludge Volume Comparisons for Batch 4.....	4-8
Figure 4-4 Settled Sludge Volume Comparisons for Batch 5.....	4-8
Figure 4-5 Picture of EA45-AS SVI test at 30 min.....	4-9
Figure 4-6 Average Cumulative Methane Production BMP 1 .....	4-12
Figure 4-7 Average Specific Cumulative Methane Production BMP 1 .....	4-12
Figure 4-8 Average Cumulative Methane Production BMP 2 .....	4-15
Figure 4-9 Average Specific Cumulative Methane Production BMP 2 .....	4-15
Figure 4-10 Average Cumulative Methane Production BMP 2a .....	4-16
Figure 4-11 Change in Reactor Contents during BMP 1 .....	4-18
Figure 4-12 Change in Reactor Contents during BMP 2. ....	4-20
Figure 4-13 SRF Results.....	4-22
Figure 4-14 Linear portion of SRF Results .....	4-23
Figure 6-1 Diurnal Influent Patterns (Adapted from Figure 3-6 in Metcalf & Eddy, 2004).....	6-2
Figure 6-2 Biowin Process flow Diagram .....	6-3
Figure 6-3 BASELINE MODEL Effluent Quality.....	6-6
Figure 6-4 Location of "Worm Reactor" in BioWin Model .....	6-8
Figure 6-5 Worm Reactor added to the Waste Secondary Sludge Line .....	6-10
Figure 6-6 WORM PREDATION MODEL Effluent Quality.....	6-12



## List of Tables

Table 2-1 Sludge Reduction Technologies (adapted from Perez-Elvira et al 2006).....	2-11
Table 3-1 Macro Nutrient Solution .....	3-4
Table 3-2 Trace Element Solution .....	3-4
Table 4-1 Batch Experiment Overview .....	4-1
Table 4-2 Change in Total Suspended Solids Between Feed-AS, WP-AS and EA-AS for all Batch Experiments.....	4-6
Table 4-3 Change in Volatile Suspended Solids Between Feed-AS, WP-AS and EA-AS for all Batch Experiments.....	4-6
Table 4-4 Change in Total Chemical Oxygen Demand Between Feed-AS, WP-AS and EA-AS for all Batch Experiments.....	4-6
Table 4-5 Change in Soluble Chemical Oxygen Demand Between Feed-AS, WP-AS and EA-AS for all Batch Experiments.....	4-6
Table 4-6 Change in Soluble Nitrogen Between Feed-AS, WP-AS and EA-AS for all Batch Experiments .....	4-6
Table 4-7 Change in Soluble Phosphate Between Feed-AS, WP-AS and EA-AS for all Batch Experiments .....	4-7
Table 4-8 Change in Sludge Volume Index Between Feed-AS, WP-AS and EA-AS for all Batch Experiments.....	4-7
Table 4-9 Change in Batch 5 Sludge Properties Between Feed-AS, WP-AS and EA45-AS .....	4-7
Table 4-10 Zone Settling Velocities for Batch 4 and 5 Sludge .....	4-9
Table 4-11 Centrifuge Cake Solids Percentage for Batch 4 and 5 Sludges.....	4-9
Table 4-12 Centrate Properties for Batch 4 Sludges .....	4-10
Table 4-13 Centrate Properties for Batch 5 Sludges .....	4-10
Table 4-14 BMP 1 Reactor Contents (Total Volume 525 ml/Bottle).....	4-11
Table 4-15 BMP 1 Values .....	4-13
Table 4-16 BMP 1 Value Range .....	4-13
Table 4-17 COD Balance BMP 1.....	4-13
Table 4-18 BMP 2 Reactor Contents .....	4-14
Table 4-19 BMP 2 Values .....	4-16
Table 4-20 VFA Analysis.....	4-16
Table 4-21 COD Balance BMP 2.....	4-17
Table 4-22 Properties of Reactor Contents Pre - BMP 1.....	4-17
Table 4-23 Properties of Reactor Contents Post - BMP 1 .....	4-17
Table 4-24 Percent Change in Sludge Properties during BMP 1.....	4-18
Table 4-25 Properties of Reactor Contents Pre – BMP 2 .....	4-19
Table 4-26 Properties of Reactor Contents Post – BMP 2.....	4-19
Table 4-27 Average Change in Sludge Properties during BMP 2.....	4-20
Table 4-28 Average Overall Reduction in TS, VS and COD for Sludges .....	4-20
Table 4-29 Centrifuge Cake Solids Percentage for BMP 1 and BMP 2 Sludges .....	4-21
Table 4-30 Centrate Property Concentrations Post BMP 1.....	4-21
Table 4-31 Centrate Property Concentrations Post BMP 2.....	4-21
Table 4-32 Difference in Average Centrate Properties from Digestion in BMP 1 .....	4-21
Table 4-33 Difference in Average Centrate Properties from Digestion in BMP 2 .....	4-22
Table 4-34 CST Results Post BMP 1 .....	4-22
Table 4-35 CST Results Post BMP 2 .....	4-22
Table 5-1 Theoretical Methane Production for BMP 1 .....	5-6
Table 5-2 Theoretical Methane Production for BMP 2 .....	5-6
Table 5-3 Average Gas Production and VS Reduction in BMP 2.....	5-6
Table 5-4 BMP 1 Values Corrected for Effect of Denitrification .....	5-7
Table 5-5 BMP 2 Values Corrected for Effect of Denitrification .....	5-7
Table 6-1 2013 Dry Weather Influent Characteristics Model Assumptions.....	6-1
Table 6-2 Primary Clarifier Model Information .....	6-4
Table 6-3 Biological Tank Model Information.....	6-4
Table 6-4 Secondary Clarifier Model Information .....	6-4
Table 6-5 Primary Sludge Gravity Thickening Model Information .....	6-4
Table 6-6 Secondary Sludge Centrifuge Thickening Model Information .....	6-4

Table 6-7 Anaerobic Digestion Model Information.....	6-5
Table 6-8 Digested Sludge Centrifuge Dewatering Model Information .....	6-5
Table 6-9 Average Daily Baseline WWTP Performance .....	6-6
Table 6-10 Average Daily Baseline Thickener Performance.....	6-6
Table 6-11 Average Daily Baseline Digester Performance .....	6-6
Table 6-12 Average Daily Baseline Dewatering Performance .....	6-7
Table 6-13 Average Concentration of Properties in Baseline Activated Sludge and Targets Concentrations for Worm Predated Sludge .....	6-9
Table 6-14 Comparison of Aerobically Digested Soluble Sludge Properties with Post Worm Target Values .....	6-9
Table 6-15 Extrapolated VFA values in Sludge Samples.....	6-10
Table 6-16 sCOD Targets and Model Concentrations for Calculation.....	6-11
Table 6-17 Average Daily Worm Predation WWTP Performance.....	6-12
Table 6-18 Average Daily Worm Predation Thickener Performance .....	6-13
Table 6-19 Average Daily Worm Predation Digester Performance .....	6-13
Table 6-20 Average Daily Worm Predation Dewatering Performance.....	6-14
Table 7-1 Overview of Expected Costs and Benefits of Full Scale Implementation.....	7-3
Table B-1 AS Reactors Cumulative Methane Production Raw Data .....	B-1
Table B-2 WP Reactors Cumulative Methane Production Raw Data .....	B-2
Table B-3 EA Reactors Cumulative Methane Production Raw Data .....	B-3
Table B-4 Blank Reactors Cumulative Methane Production Raw Data .....	B-4
Table C-1 AS Reactors Raw Data .....	C-1
Table C-2 WP Reactors Raw Data .....	C-1
Table C-3 EA Reactors Raw Data .....	C-2
Table C-4 Blank Reactors Raw Data.....	C-2

## 1 Introduction

Methods of secondary wastewater treatment plant activated sludge (AS) minimization are currently receiving a lot of attention due to the high costs of sludge disposal and the projected increase in sludge production as populations grow and connections to secondary wastewater treatment systems increase. Studies on oligochaete predation have shown that large reductions in AS volume can be achieved in a relatively short amount of time and have also indicated that the predation may increase the overall biodegradability of the sludge when paired with anaerobic digestion.

This MSc thesis was undertaken in conjunction with the PhD work of Steef de Valk on oligochaete predation as a waste AS minimization technique. De Valk's work will focus on identifying the mechanisms by which oligochaetes process AS to reduce sludge volume and potentially increase its degradability. The goal of his work is to translate the obtained knowledge into a novel technology for AS treatment.

### 1.1 Objective

The objective of this MSc thesis was to investigate how the implementation of oligochaete predation, or a technology developed to mimic its results, could affect the traditional sludge treatment process and overall Wastewater Treatment Plant (WWTP) operations. By identifying the changes which could be expected from the implementation of the new technology, the goal was to have early insight into changes that might occur in the scaling up process. This insight could then allow for anticipation of steps to be taken during the technology development to ensure that any issues do not become a hindrance to the eventual acceptance and adoption of the full scale technology.

### 1.2 Research Questions

Four main research questions were developed to guide the process of meeting the thesis objective:

1. How does worm predation change the properties of activated sludge?
2. How do the changes in sludge properties affect the solids processing behaviour of activated sludge?
3. If the changes in sludge properties were applied at a full scale wastewater treatment plant, how would the performance of the plant be affected?
4. Do any modifications need be made to the treatment process to accommodate changes in performance?

### 1.3 Scope

The scope of this MSc thesis included a literature review, laboratory experimentation and computer modelling aimed at answering the research questions and achieving the objective described above. The laboratory experimental program was used to determine impact that oligochaete predation of activated sludge has on the sludge's characteristics and solids processing behaviour. Wastewater treatment plant simulation software was then used to model the wastewater treatment process at the Harnaspolder wastewater treatment plant when there activated sludge used in the lab experiments was collected from. The information gathered in the laboratory about solids processing of worm predated AS was used as the basis for creating a "worm reactor" in the wastewater treatment plant model to identify how the changes to sludge properties would impact the behaviour of the overall treatment performance and what modifications, if any, might be made to accommodate the changes.

### 1.4 Outline

The background and literature review in Chapter 2 provides relevant information on the activated sludge process, treatment and disposal of waste AS, oligochaete predation and computer modelling of wastewater treatment. Chapter 3 explains the methodology used for this MSc thesis to answer the research questions. The methodology includes both laboratory experimentation to answer research questions 1 and 2 and computer modelling to address questions 3 and 4. In Chapter 4 the results of the laboratory testing are presented and in Chapter 5 the implications they have for the research are discussed. Chapter 6 describes the computer modelling process and how it was used. Chapter 7 will present the conclusions of this thesis and include recommendations for further study on the subject.



## 2 Background and Literature Review

### 2.1 Activated Sludge Production

The activated sludge process is the most commonly applied method of biological wastewater treatment and is depicted in Figure 2-1.

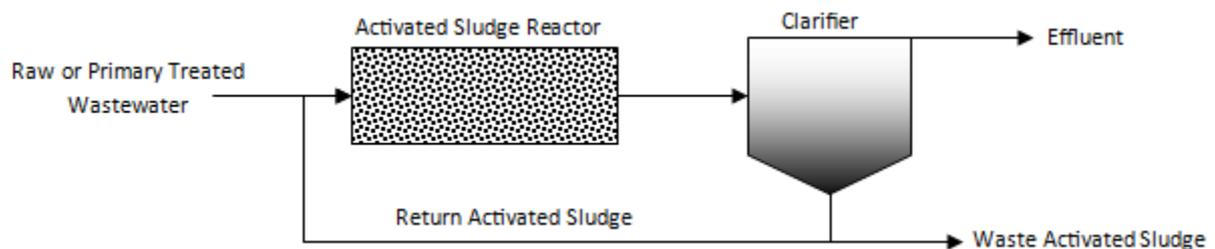


Figure 2-1 Typical Activated Sludge Wastewater Treatment Process

In a traditional activated sludge process raw or primary treated wastewater is aerated in an aeration basin to stimulate growth of heterotrophic microorganisms which consume the organic material present in the wastewater. Process modification such as anaerobic and/or anoxic zones and internal recycles can be incorporated into the activated sludge reactor to stimulate the growth of additional microorganisms used to reduce nutrient concentrations. The microorganisms grow and accumulate in sludge flocs which are separated from the treated water in a clarifier. A portion of the separated sludge is returned to the aeration basin in order to maintain a stable activated sludge concentration in the reactor, while the excess growth is removed from the system as excess or waste activated sludge (AS) which must be treated and disposed of.

Implementation of the Urban Waste Water Treatment Directive 91/271/EEC requiring biological wastewater treatment in Member States within the European Union increased the amount of waste AS requiring treatment from 5.5 million tonnes of dry matter in 1992 (Europa, 2012) to 10.13 million tonnes in 2010 (Milieu Ltd, WRc and RPA for the European Commission, DG Environment under Study Contract DG ENV.G.4/ETU/2008/0076r, 2010).

#### 2.1.1 Activated Sludge Characteristics

Waste AS is composed of the settled total suspended solids (TSS) flocs present in the activated sludge reactor and water. Settled waste AS is typically 99% water when it is pumped from the clarifier. The remaining "solid" flocs which form in the reactor contain microorganisms, organic matter and inorganics. The inorganic or fixed suspended solids (FSS) fraction is made up of flocculated influent inorganics such as clay or silt, and precipitated salts. Depending on the treatment steps prior to the activated sludge reactor the inorganic fraction can range from 20% to 35% of the total solids concentration. The organic, or volatile suspended solids (VSS) fraction is comprised of the active microorganisms which metabolize incoming organic material, the extracellular polymeric substances (EPS) produced by microorganisms to form the biofilms which connect them in flocs, unconverted influent organics, inert influent organic material which cannot be metabolized but adsorbs to sludge flocs, and inert endogenous residue from cell decay and endogenous respiration. Depending on the type of treatment the proportions of these fractions can vary greatly. (van Handaal & van der Lubbe, 2007)

The biofilm of microorganisms and EPS forms the biodegradable fraction of the organic material which can be further reduced during anaerobic digestion. In most cases, 10% or less of the biofilms organic matter is made up of the microorganisms while the EPS matrix is over 90 percent. (Flemming & Wingender, 2010)

### 2.1.1.1 EPS

Extracellular polymeric substances have several functions, are comprised of various organic constituents and are produced in a variety of ways. While the exact composition of EPS is still unclear, and is thought to vary greatly between different biofilms and bacterial communities, its composition is predominantly proteins with polysaccharides, lipids and additional components such as humic acids and DNA. These components are the product of active cell secretion, cell decay and lysis and sorption from the surrounding environment. (Flemming & Wingender, 2010)

Carbohydrates and proteins are the two EPS constituents most commonly analyzed giving measure of  $EPS_c$  and  $EPS_p$  respectively. Measure of the  $EPS_p:EPS_c$  ratio in activated sludges shows the majority have a value greater than 2.  $EPS_p$  is considered to increase hydrophobicity while  $EPS_c$  is hydrophilic. Hydrophobicity is considered to increase floc formation. EPS and sludge flocs are negatively charged. (Liu & Fang, 2003)

EPS is generated by the microorganisms to form protective barriers, retain water, adsorb organic compounds and allows for enzymatic activities, exchange of genetic information and aggregation of cells into cohesive biofilms among other things. (Flemming & Wingender, 2010) As EPS is the main component of activated sludge and the means of floc formation within the activated sludge reactor it will influence how the AS behaves during treatment.

### 2.1.2 Treatment and Disposal of Waste Activated Sludge

A typical sludge treatment process consists of sludge thickening, anaerobic digestion, and dewatering as shown in Figure 2-2. The thickened waste AS, often combined with thickened primary settled sludge, is stabilized by anaerobic digestion to reduce its volume, produce energy-rich biogas and reduce the pathogen content. Dewatered digested sludge is transported to off-site disposal in landfills, as fertilizer or by incineration, depending on the pertinent regulations. Reject waters from the thickening and dewatering processes contain concentrated amounts of nutrients and other pollutants and must receive further treatment, typically by recycle to the head of the plant, which increases overall plant loading and treatment cost.

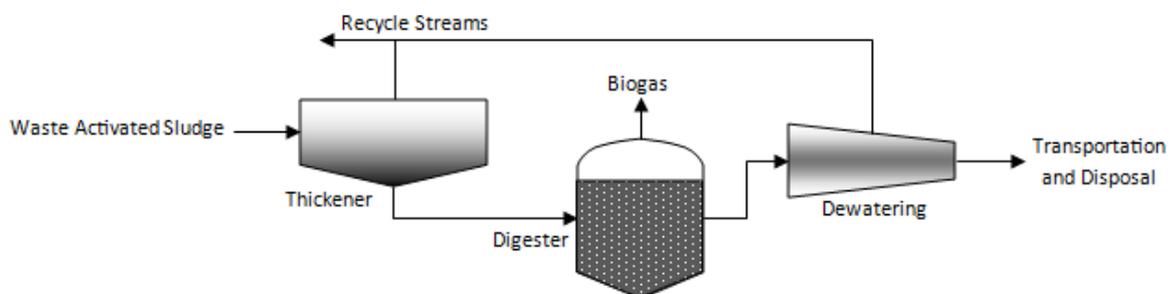


Figure 2-2 Typical Waste Activated Sludge Treatment Process

Sludge treatment, particularly the transportation and disposal costs of the final dewatered material, make up a large percentage of a treatment plants overall operating costs (Ratsak & Verkuijlen, 2006).

## 2.2 Anaerobic Digestion

Anaerobic digestion is a complex process composed of 4 steps in which a consortium of different microorganisms convert organic matter to methane and carbon dioxide as shown in Figure 2-3.

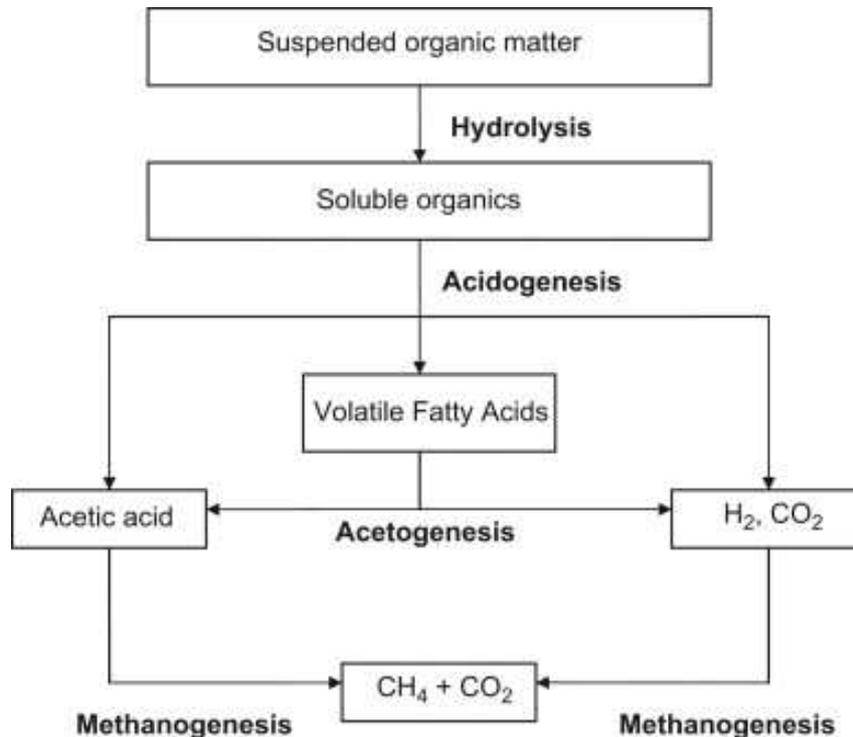


Figure 2-3 Steps of Anaerobic Digestion (Appels, Baeyens, Degreve, & Dewil, 2008)

The first step, hydrolysis, is the breakdown of insoluble and complex organic substances such as proteins, carbohydrates and lipids into soluble compounds like amino acids, monosaccharides, and long chain fatty acids. Acidogenesis, the second step, is the further breakdown of these soluble compounds into volatile fatty acids (VFAs). Third, the VFA's are converted to acetic acid or hydrogen and carbon dioxide during acetogenesis. And finally, there are two paths for methanogenic bacteria to create methane from the acetogenesis products, degrading acetate into methane and carbon dioxide or using hydrogen as an electron donor and carbon dioxide as an acceptor to produce methane. (Appels, Baeyens, Degreve, & Dewil, 2008)

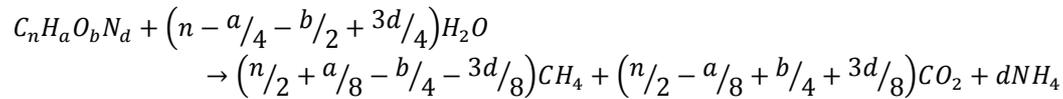
### 2.2.1 Hydrolysis of Waste Activated Sludge

Hydrolysis is critical to biological waste treatment since in order for the organic substrates to be degraded they must be able to transfer into the bacterial cells where metabolic reactions take place, and in order to do so must be small enough to cross bacterial membranes. For this reason, microorganisms excrete extracellular enzymes known as hydrolases which degrade the complex organic matter into their soluble by-products so it can be utilized for energy and growth. (Burgess & Pletschke, 2008)

Hydrolysis is generally considered the rate limiting step in the anaerobic digestion of AS. (Vavilin, Fernandez, Palatsi, & Flotats, 2008) In the hydrolysis of waste AS, rupture of cell walls and degradation of EPS release the biologically available organic matter needed for acidogenesis. The organic component of waste AS is primarily the cells generated during the aerobic process. Cells are less than optimal as a substrate for microbial degradation due to the semi-rigid structure of cell walls containing glycan strands cross-linked by peptide chains intended to prevent osmotic cell lysis. Limitations in the anaerobic digestion process such as the requirement for long retention times and the low degradation efficiency of the organic sludge content are often attributed to difficulties with the hydrolysis of waste AS (Appels, Baeyens, Degreve, & Dewil, 2008)

### 2.2.2 Methane Production

One of the benefits of anaerobic digestion is the production of Methane which can be burned as fuel to produce energy which can be used on site to reduce the amount of electricity that must be purchased to run the treatment plant. The following general stoichiometry for the anaerobic conversion of organic matter can be used to predict the amount of methane that will be produced:



This method of calculation requires a general knowledge of the composition of the organic matter being degraded. In the case of digestion of WAS it could be assumed that the volatile solids in the sludge follows the general formula for biomass which is  $C_5H_7O_2N$  and has a molecular weight of 133g/mol. Using this as substrate, the stoichiometric conversion becomes:



Thus 1 mol of biomass digested anaerobically is expected to yield 2.5 mol of methane. If the VS of the WAS being digested is measured then the methane production can be predicted with the following calculation:

$$CH_4(mol) = \frac{VS(g) \cdot 2.5}{113}$$

Another method of predicting the methane production is by making a COD balance. The only two compounds in the reaction above that have COD value are the organic matter and the methane, thus the amount of COD digested as organic matter must equal the amount of COD produced as methane. Methane has a COD content of 4g COD per g  $CH_4$  and a molar mass of 16, thus if the COD of the WAS being digested is measured then the methane production can be predicted with the following calculation:

$$CH_4(mol) = \frac{COD(g)}{4 \cdot 16}$$

Given the pressure and temperature conditions the predicted volume of methane can be calculated from the moles of methane using the ideal gas law.

## 2.3 Dewatering

As discussed, waste AS from the clarifier is approximately 99% water, thus reducing the water content of the sludge is critical to limiting volume and weight and thus the disposal and transportation costs for the excess sludge. Prior to anaerobic digestion, waste AS is thickened to reduce the volumetric and heating requirements of the digestion process. Thickening is achieved through a number of methods including gravity settling, flotation, and mechanical methods like centrifugation, gravity belt and rotary drum thickening. The thickening process usually increases the solids percentage to between 4 and 6%. Post digestion, sludge is dewatered to improve ease of handling, reduce transportation costs and meet requirements for various disposal options. The aim of dewatering is to increase the solids content as much as possible; typically between 20 and 40% solids can be achieved. (Metcalf & Eddy, 2004)

Dewatering of sludge occurs in two distinct steps, filtration and expression. Initially at low solids concentration water drains or is pressed from the sludge without the solids particles contacting on another in the filtration phase. As water is removed, the solids percentage increases and the particles consolidate into a cake structure. At the point when the cake solids begin to bear a portion of the applied dewatering pressure, and the water pressure drops below the applied pressure, the expression phase begins. In this second phase particles deform and water is squeezed from pore spaces and from within the sludge particles to further reduce the water content of the cake. The dewatering behavior described is illustrated in Figure 2-4. (Novak, Agerbæk, Sørensen, & Hansen, 1999)

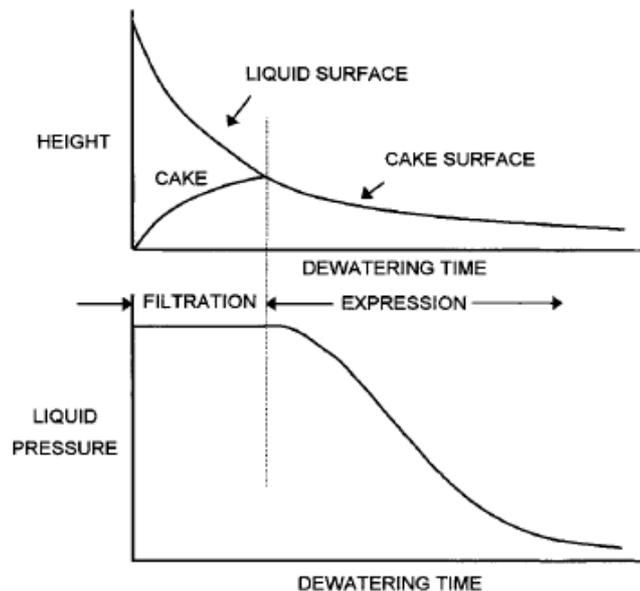


Figure 2-4 Two Phases of Sludge Dewatering: Filtration, and Expression (Novak, Agerbæk, Sørensen, & Hansen, 1999)

In order to achieve cake solids percentages desired for wastewater treatment sludge, filtration alone is not sufficient, thus the expression phase an important target for mechanical dewatering equipment. (Novak, 2006)

### 2.3.1 Mechanical Dewatering

Mechanical dewatering equipment is used for most dewatering applications, often with chemical conditioning to increase the sludge dewaterability.

For sewage sludge, belt filter presses and centrifuges are the most common dewatering equipment applied. (Novak, 2006) Both are continuous feed dewatering devices. Belt filter presses use gravity drainage followed by porous belts to apply pressure for filtration and high pressure rollers remove additional quantities of water with expression. The dewatered filter cake is removed from belts with scraper blades and the filtrate is recycled for further treatment.

Centrifuges, used both for thickening of WAS and for dewatering of digested sludge, make use of centrifugal forces in a spinning bowl which concentrates solids on the periphery. An internal helical

scroll is used to move accumulated solids toward a tapered end or “beach” of the bowl and provides pressure on the solids for expression to remove additional water from the cake. Cake is discharged from the bowl by a screw conveyor while centrate is recycled for further treatment. (Metcalf & Eddy, 2004)

Chemical conditioners such as synthetic organic polymers or metal ion salts are used with most sludge to increase dewatering rates. These conditioners are used to promote coagulation of colloidal material within the sludge, but do not affect the density of the sludge flocs, thus primarily increase the rate of dewatering which can be achieved in the filtration step, but not the final dewatered cake solids concentration. (Novak, 2006)

### 2.3.2 Sludge Characteristics and Dewatering

The sludge characteristics greatly influence the dewatering rate and the achievable solids concentration of different types of dewatering equipment since each sludge will have a distinct ability to retain water which will influence the amount of water that can be removed in spite of the dewatering device that is used. (Novak, 2006) Water within sludge (waste activated or digested) is generally classified as:

- **Free:** Bulk water which does not interact with sludge particles which can be separated by gravity settling
- **Interstitial:** Water which is held between particles, within the EPS floc structure and within cells, the majority of which can become free water if the containing structure is destroyed and thus can be removed through squeezing in mechanical dewatering
- **Vicinal:** Layers of water molecules held to particle surfaces by hydrogen bonding which has lower density and higher viscosity than water in bulk. Vicinal water can still not move freely after mechanical manipulation and thus is very difficult to remove without drying.
- **Chemically Bound:** Water that is chemically bound to sludge particles and only removable through thermal drying

Free and interstitial waters are those most easily targeted for removal in sludge thickening and dewatering, thus the vicinal and chemically bound water content is often considered the limit of dewaterability. (Vesilind, 1994) The lower limit of bound water (vicinal and chemically bound) content has been observed as approximately 1 g bound water/g dry solids or a solids content of 50% for dewatered cake. (Novak, 2006)

### 2.3.3 Settling

The settling characteristics of activated sludge play a large role in gravity separation of sludge particles from the free water. Two of the most common ways to characterize a sludge’s settling characteristics are the sludge volume index (SVI) and the zone settling velocity (ZSV). The SVI is defined as the volume of 1 g of sludge after 30 minutes of settling, and is measured by putting well mixed sludge of a known solids concentration into a cylinder of a given volume ( $V_0$ ) and then allowing the sludge to settle for 30 minutes and recording the volume of the sludge blanket ( $V_{30}$ ) at that time. The SVI of the Sludge is calculated as follows:

$$SVI (ml/g) = \frac{V_{30}(ml) \cdot 10^3}{V_0(ml) * TSS(g/L)}$$

Well settling sludges are typically considered those with an SVI less than 100 ml/g, while those with values of greater than 150-200 ml/g are considered bulking sludges typically associated with filamentous bacteria growth.

The ZSV represents the maximum rate of sedimentation of the sludge. It is obtained from the slope of the linear part of a sedimentation curve such as those produced by a settled sludge volume test. The settled sludge volume test is performed in a similar manner to the SVI test; however instead of only measuring the sludge volume at 30 minutes, it is measured at 5, 10, 15, 20, 30, 45, and 60 min and plotted over the time. (Metcalf & Eddy, 2004)

### 2.3.4 Cake Solids Concentration

The achievable solids concentration of sludge samples can be compared using laboratory tests such as centrifugation at a standardized time and centrifugal (g) force in a laboratory centrifuge. Although different cake solids may be achieved using other mechanical dewatering devices, the relative cake solids that can be achieved with different sludges can be compared because it is the intrinsic sludge properties that influence the degree of dewatering that can take place as was discussed in Novak 2006 based on the data of Huang 1979 and the USEPA Dewatering Municipal Wastewater Sludges: Design Manual that are presented in Figure 2-6 and Figure 2-6 respectively.

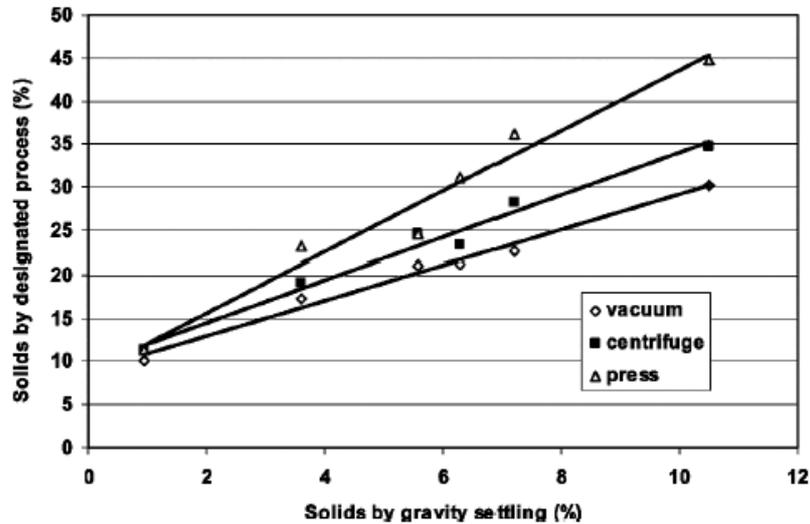
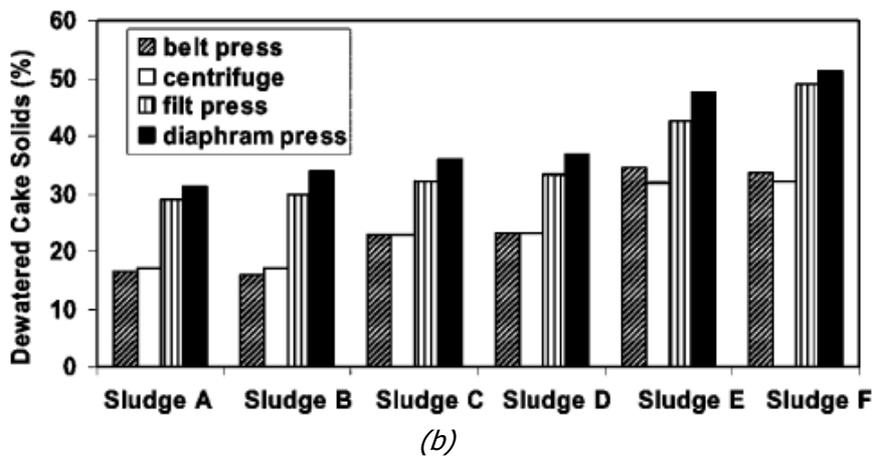


Figure 2-5 Comparison of cake solids by gravity thickening and selected dewatering processes (Huang, 1979)



(b) Figure 2-6 Dewatering data showing the cake solids achieved by a variety of dewatering processes (USEPA)

### 2.3.5 Dewatering Rate and Filterability

The rate of dewaterability as an indication of the ease with which a sludge will release its water is typically measured by specific resistance to filtration (SRF) or capillary suction time (CST). The SRF is more prevalent in dewatering analysis because it is based on physical theory; however the CST test is simpler and more time effective. Both are frequently used for determining the effect of chemical conditioners on the dewatering rate and in finding optimal dosages. (Novak, 2006)

#### 2.3.5.1 Specific Resistance to Filtration

The SRF is based on Darcy's law describing flow through a porous medium. This law has been applied by many researchers to describe the rate of water removal from sludge by filtration using the following formula:

$$dV/dt = \frac{PA}{\mu R_T}$$

Where:

$dV/dt$  = change of filtrate volume over time

A = Filter surface area

P = Pressure

$R_t$  = Total filtration resistance

$\mu$  = Filtrate viscosity

The total filtration resistance is the sum of the resistance of the filter cake,  $R_c$ , and the resistance of the filter media,  $R_m$ . The specific resistance of the filter cake,  $r$ , (i.e. the resistance per unit of dry mass per unit area) can be isolated from the filter cake resistance by dividing  $R_c$  by the product of the sludge solids concentration,  $C$ , and volume,  $V$ , over the filter area,  $A$ . Using these relationships the equation above is converted to the following:

$$dV/dt = \frac{PA}{\mu \left( \frac{rCV}{A} + R_m \right)}$$

Integration of the above formula results in the following:

$$t/V = \frac{\mu r CV}{2PA^2} + \frac{R_m \mu}{PA}$$

The formula above can be used to describe the relationship between  $t/V$  and  $V$  in slope-intercept form

$$t/V = bV + a$$

Thus by measuring the volume of filtrate produced over time and plotting  $t/V$  vs  $V$ , the slope of this line will be  $b$ , which represents:

$$b = \frac{\mu r CV}{2PA^2}$$

By determining the slope of the line in the  $t/V$  vs  $V$  plot the specific resistance to filtration,  $r$ , of the filter cake can be calculated.

$$r = \frac{2PA^2 b}{\mu C}$$

SRF values are typically in the range of  $10^{12} - 10^{13}$  m/kg. Sludge that is easier to dewater may have values in the range of  $10^{10} - 10^{11}$  m/kg while difficult to dewater sludge may have values as high as  $10^{14} - 10^{15}$  m/kg. (Sanin, Clarkson, & Vesilind, 2011)

### 2.3.5.2 Capillary Suction Time

The CST or "time to dewater" in seconds is measured using a simple apparatus consisting of a plastic block base which is topped with a filter paper and then another upper plastic block which is connected to an electrical timing device.



Figure 2-7 Capillary Suction Time Apparatus (to be replaced with original pic of device in lab)

As shown in Figure 2-7, a cylindrical metal collar is fitted through the upper block into which a given volume of sludge is placed. As water in the sludge drains through the filter paper it starts the timer when reaching the inner circumferential sensor imbedded in the upper block, and the timer is stopped when the water reaches the outer sensor. A schematic of the underside of the upper block is shown in Figure 2-8. (Vesilind, 1988)

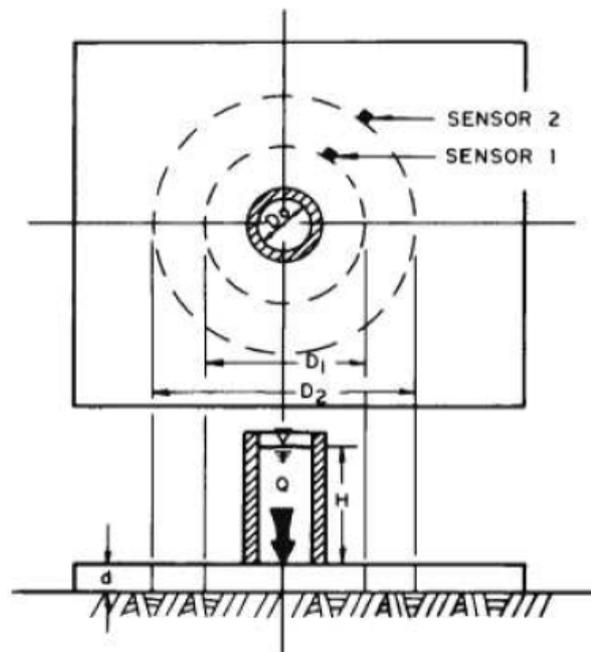


Figure 2-8 Upper block of CST apparatus (Vesilind, 1988)

Criticism of the CST test as a measure of dewaterability is its empirical nature, the dependence of the time results on the apparatus, filter and physical sludge properties, and the difficulty of correlating its results with other measurements of dewaterability such as the SRF. Vesilind 1988 worked to remedy these issues by developing a theoretical model of the CST test. This model identifies the fundamental measurement of dewaterability not at the CST but as a sludge filterability constant,  $\chi$ , which is found from the CST, an instrument constant,  $\phi$ , based on the geometry of the CST device and properties of the filter paper, and the physical sludge properties which influence the CST reading namely the solids content,  $C$ , and viscosity,  $\mu$ , as shown below:

$$\chi = \phi \left[ \frac{\mu C}{CST} \right]$$

when

$$\phi = (D_2^2 - D_1^2) \left( \frac{\pi d}{AP} \right)$$

where

$D_1$  = Inner sensor diameter

$D_2$  = Outer sensor diameter

$d$  = Filter paper thickness

$A$  = Area of the collar

$P$  = Capillary suction of the filter paper

The higher the filterability constant, the easier a sludge is to dewater. A major benefit of this model is that if the instrument constant is known for different studies, CST data can be compared. Another benefit is that the filterability constant, like the SRF is based on physical theory, and the inverse of the filterability correlates well with the more difficult to obtain SRF, thus correlations between studies using the different methods can also be made. (Vesilind, 1988)

### 2.3.6 Dewatering Lab Tests vs Pilot Tests

It should be noted that neither the laboratory cake solids test nor the filterability tests discussed above are sufficient to predict the actual performance of full scale dewatering in a treatment plant or to base a detailed design upon. Both are used as quick and simple comparisons of different sludge types or conditioning methods. In order to adequately understand how a dewatering process will work full scale, extensive pilot testing is often required. (US EPA, 1987)

## 2.4 Excess Sludge Reduction

Due to the costs of sludge disposal and the projected increase in sludge production as populations grow and connection to secondary wastewater treatment systems increases, methods of sludge minimization are receiving a lot of attention (Wei, van Houten, Borger, Eikelboom, & Fan, 2003). Technologies developed for sludge minimization aim to either reduce production of sludge in the water line during wastewater treatment or increase degradation potential and dewaterability of the sludge in the sludge line during sludge treatment. (Perez-Elvira, Diez, & Fdz-Polanco, 2006) The types of treatment processes and technologies are categorized in Table 2-1.

Table 2-1 Sludge Reduction Technologies (adapted from Perez-Elvira et al 2006)

Location	Approach	Mechanism	Method
<b>Water Line</b>	Reduce Yield Coefficient	Natural Cell Lysis	Extended Aeration
		Lysis-Cryptic Growth	Chemical Oxidation
			Integrated Chemical and Heat Treatment
		Maintenance Metabolism	High Purity Oxygen
			Enzymatic Reaction
	Intrinsically Low Yield Coefficient	Uncoupling Metabolism	Membrane Bioreactor
		Predation on Bacteria	Chemical Uncoupler
			Oxic-settling-anaerobic Process
		<b>Oligochaetes</b>	Two-Stage System
		Anaerobic/Aerobic Systems	
<b>Sludge Line</b>	Pre-treatment prior to Anaerobic Digestion	Physical	Cavitation
			Thermal
			Mechanical
		Chemical	Radiation
			Acid or Alkaline Hydrolysis
			Ozonation
	Biological	Enzyme Treatment	
		Fungal Treatment	
		<b>Microfauna Predation</b>	
	Modified Anaerobic Digestion	Combined	Thermal/Mechanical
			Chemical/Thermal
		Two Stage	Temperature Phased
			Anoxic Gas Flotation

## 2.5 Oligochaete Predation

Predation is noted in both categories of sludge minimization. As predators consume bacterial sludge for maintenance, growth, and reproduction, there is a transfer to a higher trophic level, thus a large amount of the potential energy is lost as heat and a lower amount of biomass is the result. The micro fauna deemed to have the most potential for sludge minimization are oligochaete worms as determined by a comparison of 4 different microfauna *species* (class/subclass) commonly found in WWTP: *Aeolosoma hemprichi* (Clitellata/ Oligochaeta), *Daphnia magna* (Crustacea/Branchiopoda), *Tubifex tubifex* (Clitellata/ Oligochaeta) and *Physa acuta* (Gastropoda/Heterobranchia). In this comparison, the oligochaete species, *Aeolosoma hemprichi* and *Tubifex tubifex*, showed the most promise as they had the highest rates of sludge reduction and *Tubifex tubifex* was not significantly affected by different sludge types. (Liang, Huang, Qian, Wei, & Ding, 2006)

Oligochaete worms are terrestrial, aquatic or semi aquatic and can be found in both fresh and saline environments. A number of oligochaete species will feed on waste organic matter such as the sludge produced by wastewater treatment plants. In fact both attached growth (sessile) and free swimming oligochaetes have been observed living in WWTPs. (Ratsak & Verkuiljen, 2006)

Oligochaete species are commonly found in wastewater treatment activated sludge processes and are used for experiments with oligochaete predation. These species typically are from the free swimming family Aeolosomatidae, and the sessile family Tubificidae. A third family Naididae is also often reported in literature; however these have been reclassified as Naidinae a free-swimming subfamily in the otherwise sessile Tubificidae family. Another worm family Lumbriculidae, particularly the species *Lumbriculus variegatus*, has also been used for many predation experiments, although it does not commonly occur in wastewater treatment plants. The choice of *Lumbriculus variegatus* is because it is similar in size to the larger Tubificidae specie (namely *Tubifex tubifex*) but reproduces through fragmentation rather than sexual reproduction thus a shorter time is required for growth. (Elissen, 2007)

### 2.5.1 *Tubifex tubifex* (*T. tubifex*)

*T. Tubifex* is also more commonly known as the sludge worm or sewage worm since it had been found to naturally exist in wastewater treatment plants and exhibits an extreme tolerance to pollution (Ratsak & Verkuiljen, 2006). *T. tubifex* is recognized by its red color which also gives it another nickname, the bloodworm, and denotes its relatively large supply of hemoglobin, which stores available dissolved oxygen (Microscopy Resource Center, 2012). This is considered the reason that *T. tubifex* has been observed to survive for extended periods in anoxic conditions. It is a slender segmented hermaphroditic oligochaete of the Naididae/Tubificinae family typically ranging in length from 1 to 8.5 centimeters, but known to grow up to 20 cm.



Figure 2-9 *Tubifex tubifex*

*T. tubifex* selectively feeds on organically rich particles in the size range of fine silt and clay, mainly ingesting particles <63  $\mu\text{m}$  (75% by volume). The fecal pellets it produces mainly have a mean diameter of <25  $\mu\text{m}$  and mode of <10  $\mu\text{m}$ . Conflicting studies show preferred natural environment being fine grained nutrient rich sediment and course grained nutrient poor sediment, however it was shown that specific growth rate and rapidity of maturation are both increase when *T. tubifex* feeds on highly caloric AS compared as opposed to organically poor natural silt. Tubificidae in general show affinity for heterotrophic aerobic bacteria and *T. tubifex* is predominant where there are great quantities of readily biodegradable organic material (such as human waste) and has a high tolerance for pollution all of which make it a good candidate for activated sludge reduction. *T. tubifex* also have

some market value as they are often sold as fish feed, so if growth is an objective it is important to note that their optimal temperature for reproduction is between 20 and 25 degrees Celsius and that the population density inversely affects the growth rate and fecundity. (Ratsak & Verkuiljen, 2006)

### **2.5.2 Predation on Activated Sludge as a Reduction Technique**

Using oligochaete worms as a method of sludge reduction is a concept which is gaining attention. Wei et al. 2003 summarizes results of several studies showing significant sludge reduction due to the presence of oligochaetes in conventional activated sludge reactors, but notes the practical difficulties of cultivating such organisms within the wastewater treatment process without understanding how their growth is linked with operational parameters.

To avoid the difficulties of cultivating worms in the wastewater treatment train, researchers have focused on sludge reduction in separate worm reactors with either free-swimming, sessile or a combination of worms. (Hendrickx, Elissen, Temmink, & Buisman, 2011) The table in Appendix A summarizes the most noteworthy findings from recently published worm predation studies. Common aspects of worm predation of activated sludge reported in the literature include:

- Large increase in solids reduction rate with worms over extended aeration
- Improved settling characteristics of worm feces over WAS
- Increase in soluble COD (sCOD) due to worm presence
- Release of nutrients due to worm presence
- Relative increase in heavy metals concentration<sup>1</sup>

Worm predation of waste activated sludge prior to anaerobic storage also showed an increase in overall solids reduction over conventional anaerobic digestion thereby indicating that the worm predation process might increase the biodegradability of the sludge (Tamis, van Schouwenburg, Kleerebezem, & van Loosdrecht, 2011).

### **2.5.3 Impact on Solids Processing**

Few studies have been carried out on how the implementation of a worm reactor for AS reduction would impact the solids processing of a wastewater treatment plant. Hendrickx, et al. 2009b reported the difference between waste AS and feces of the sessile worm species *Lumbriculus variegatus* with respect to the difference observed in their solids processing characteristics.

The study found that sludge settling characteristics are greatly improved through worm predation with the SVI of worm feces being over 50% lower than that of waste AS, and TSS concentrations in the gravity settled worm feces over two times higher than in the activated sludge. Further dewatering of the settled sludge showed that specific resistance to filtration (SRF) was approximately 30% higher in worm feces than in waste AS, but that a 10% improvement in cake solids percentages could be achieved through centrifugation of worm feces than with waste AS. Forty percent less time was also required to achieve a TSS of 3% with vacuum filtration of worm feces than was needed for waste AS, however the worm feces showed a greater SRF to further dewatering. Reject water from gravity settling and dewatering of worm feces will have higher dissolved COD and nutrient content than that from waste AS, although the dewatering process does not increase this concentration significantly from what it is after gravity settling. (Hendrickx, Temmink, Elissen, & Buisman, 2009b)

Anaerobic digestion of worm feces showed a lower potential for biogas production than waste activated sludge. The observed reduction in VS due to worm predation of activated sludge and the decrease in biogas potential of the worm feces would decrease the methane production of the sludge by approximately 40%. Worm predation of digested activated sludge proved possible, and showed a higher biomass yield than predation of aerobic sludge, although the sludge had to be washed first to reduce ammonia toxicity. The worms themselves also proved to be digestible with a high biogas yield, thus showing they would not inhibit performance if conveyed to the digester unintentionally with the worm feces. (Hendrickx, Temmink, Elissen, & Buisman, 2009b)

---

<sup>1</sup> Heavy metals are not directly affected by worm predation however since the organics are reduced the relative concentration increases

## 2.6 Computer Modeling of Wastewater Treatment

In wastewater treatment, models are used to gain insight into plant performance, evaluate plant designs and upgrades, determine performance under high risk situation, and develop or evaluate alternative control strategies.

Mechanistic rather than empirical models have the most potential for application to wastewater treatment plants as they are based on conceptualization of the biological and physical mechanisms operating in the system rather than simply previously observed relationships. First a conceptual model which identifies the compounds within a system and the processes operating on these compounds is developed by describing the interactions between processes and compounds as well as between the processes themselves. The mechanistic model is developed from the conceptual model by choosing the relevant compounds and processes for the objectives of the model and mathematically formulating these process rates and stoichiometric interactions. Modeling of wastewater treatment systems can be done under steady state or dynamic conditions. Steady state models have constant flow and load inputs and outputs. Dynamic models have flows and loads which vary over time. As municipal wastewater systems have large variations in flow and concentrations, both throughout the day and throughout the year, dynamic modeling is typically the most appropriate means of getting realistic output data. (van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

There are three levels of modeling associated with wastewater treatment: activated sludge models, wastewater treatment models and plant wide models. An activated sludge model is used to describe the behavior of organisms and substrates within an activated sludge system based on its state variables, dynamic processes and parameters. A wastewater treatment model is a combination of the activated sludge model, hydraulic model, oxygen transfer model and settling model required to describe the whole activated sludge treatment process (see section 2.1). A plant wide model includes both the wastewater treatment model and a sludge treatment model including thickening, digestion and dewatering when applicable. (van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

### 2.6.1 Activated Sludge Model (ASM)

The micro kinetics of system (biological and chemical conversions) processes are dependent only on the concentrations in a reactor at the point at which the reaction take place since microorganisms and chemicals do not know what type of reactor they are in. In this way laboratory derived values for chemical and microbial reactions can be considered valid for describing the processes in full scale treatment since their behavior is independent of scale. (van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

There is a huge variety of microorganisms in activated sludge systems, which with laborious identification and enumeration techniques could be distinguished. However for the purposes of an activated sludge model this information is more than what is typically required and thus all microorganisms which perform a specific function in the system are grouped as surrogate organisms which are assigned characteristics which represent the macroscopic behavior overall group but not necessarily the particular characteristics of an individual species included within it. (van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

Examples of surrogate organisms and the functions they fulfill in activated sludge models include:

- Ordinary Heterotrophic Organisms (OHOs) - COD Removal, Denitrification, Hydrolysis, Adsorption, Ammonification
- Ammonia Oxidizing Biomass (AOBs) – Nitrification
- Nitrite Oxidizing Biomass (NOBs) – Nitrification
- Anaerobic Ammonia Oxidizers (ANAMMOX) – Nitrification
- Phosphorus Accumulating Organisms (PAOs) – COD Removal , Biological Phosphorus Removal, Hydrolysis, Adsorption, Ammonification
- Methylophils – Denitrification using Methanol

(EnviroSim Associates Ltd)

The biological processes carried out by the groups of microorganisms are defined by their stoichiometry and kinetics.

The stoichiometry of the reaction is determined based on the relevant compounds involved, the use of conservation balances (carbon, phosphorus, nitrogen, COD, charge etc.) and experimentally determined relationships (See Example 2-1a).

*Example 2-1 ASM Stoichiometry*

**a.** The most basic function of activated sludge is the aerobic conversion from organic substrate to ordinary heterotrophic biomass. The general equation for growth of OHOs (typical composition  $C_5H_7O_2N$ ) on a generic organic substrate is:



From this general equation the following balances can be derived:

Carbon Balance:  $a(w) + d(1) = e(5) + f(1)$

Hydrogen Balance:  $a(x) + c(4) + d(1) = e(7) + g(2)$

Oxygen Balance:  $a(y) + b(2) + d(3) = e(2) + f(2) + g(1)$

Nitrogen Balance:  $a(z) + c(1) = e(1)$

Charge Balance:  $c(+1) + d(-1) = 0$

Assuming the composition of the organic substrate and thus the values of  $w$ ,  $x$ ,  $y$ , and  $z$  are known, there is a system of 5 equations and 7 unknown stoichiometric coefficients. By setting the substrate coefficient,  $a$ , equal to 1 and the biomass coefficient,  $e$ , equal to the heterotrophic yield value ( $Y_H$ ) in moles biomass/mole substrate (standard or experimentally determined value for model input) the number of unknowns become 5 and the 5 balance equations are sufficient to determine the overall stoichiometry of the reaction.

**b.** This procedure can be further simplified by using the COD values of each of the compounds in the reaction\*. If  $Y_H$ , this time in gBiomassCOD/gSubstrateCOD, and the fraction of nitrogen in biomass ( $f_N$ ) in gN/gBiomassCOD are established for model input through standard values or experimental data, then using a COD balance the reaction can be simplified and stoichiometric coefficients determined as follows:

\*Note the COD values of both  $CO_2$  and  $H_2O$  are 0 and are thus omitted. As these compounds are typically not of interest in wastewater treatment systems this omission is suitable.

The use of conservation balances is one of the main reasons for the switch from biochemical oxygen demand (BOD) to chemical oxygen demand (COD) as the measure of the organic content of wastewater. This is because COD is a measure of the amount of electrons which are transferred to oxygen to oxidize ALL the organic material, and thus is conservative (See Example 2-1b), while the BOD measurement of oxygen required is dependent on various experimental factors and thus is not conserved.

Each reaction has its own rate reaction which describes the rate of conversion of the compound whose stoichiometric coefficient has been set as 1 (See Example 2-1). This is typically substrate based (substrate coefficient is 1) or growth based (biomass coefficient is 1). The conversion rates of the other compounds are simply calculated by multiplying their stoichiometric coefficient with the reaction rate equation. Typically for ASM, Monod kinetics is the standard rate equation. The Monod equation is defined by 2 constants (model inputs), the maximum rate ( $\mu_{max}$ ) and the affinity constant ( $K$ ) which is defined as the concentration ( $S$ ) at which the rate ( $\mu$ ) is half the maximum rate.

$$\mu = \mu_{max} \frac{S}{K + S}$$

The affinity term  $(S/(K+S))$  has a value between 0 and 1 which describes how the maximum growth rate is affected by concentrations present in the reactor. The most important term for this in microbial growth is often the substrate affinity term  $(S_s/(K_s+S_s))$  however other compounds in the system can have an effect on the rate of reaction. Other affinity terms are therefore added to the rate equation to account for these effects. Again take the example the aerobic conversion from organic substrate to ordinary heterotrophic biomass. As both oxygen and ammonia are required for growth the concentrations of each will impact the rate. In the case of oxygen, the affinity constant is the affinity term  $(S_o/(K_o+S_o))$  which is an observed parameter, whereas for ammonia since the affinity constant is so low that it's not practically measurable, the affinity term  $(S_{NH}/(K_{NH}+S_{NH}))$  is simply used as a switching function to ensure that the reaction does not proceed once the ammonia concentration has reached 0. Affinity terms can be used to describe the effects of inhibitory compounds on the rate. In practice the maximum growth rate is often multiplied by each of the relevant affinity terms, however a more accurate approach would be to use only the lowest relevant affinity term as this will be limiting.

(van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

### 2.6.2 Wastewater Treatment Model

The behavior of the ASM and other chemical reactions within a reactor are driven by the constituent concentration present in the system. In order to approximate this concentration the transport mechanisms within the wastewater treatment plant must be modeled.

The model input is defined by an influent vector of the wastewater flow and concentrations of relevant compounds being transported into the system. The wastewater treatment process is described using a hydraulic model which characterizes the hydraulics of the full scale system including the physical features of each process unit (size, mixing etc.) and the flow pathways between them. Each process unit is modeled as an individual reactor to account for its unique mixing, mass transfer characteristics and internal conversions. For example, in a biological reactor model an ASM will be used to describe the internal bioconversions of relevant compounds and an aeration/ gas transfer model will be used to describe oxygen transfer and/or off-gassing of nitrogen; For a settler reactor model, a particle settling model (typically a layered 1-dimensional flux model) can be used to determine distribution of solids concentrations between the locations of the overflow and underflow. Over each individual reactor a mass balance is applied for each of the relevant compounds. The interactions between process units are characterized by the flow rates and compound concentrations of the flow pathways between them either as inputs or outputs of the connected reactor's mass balance. The output of the final unit in the process scheme is the model output. A schematic of the components of a wastewater treatment model are given in Figure 2-10.

The overall process model (i.e. the hydraulic model, process unit models and the interactions between them) is solved numerically to determine the concentrations of all relevant compounds included in the model.

(van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

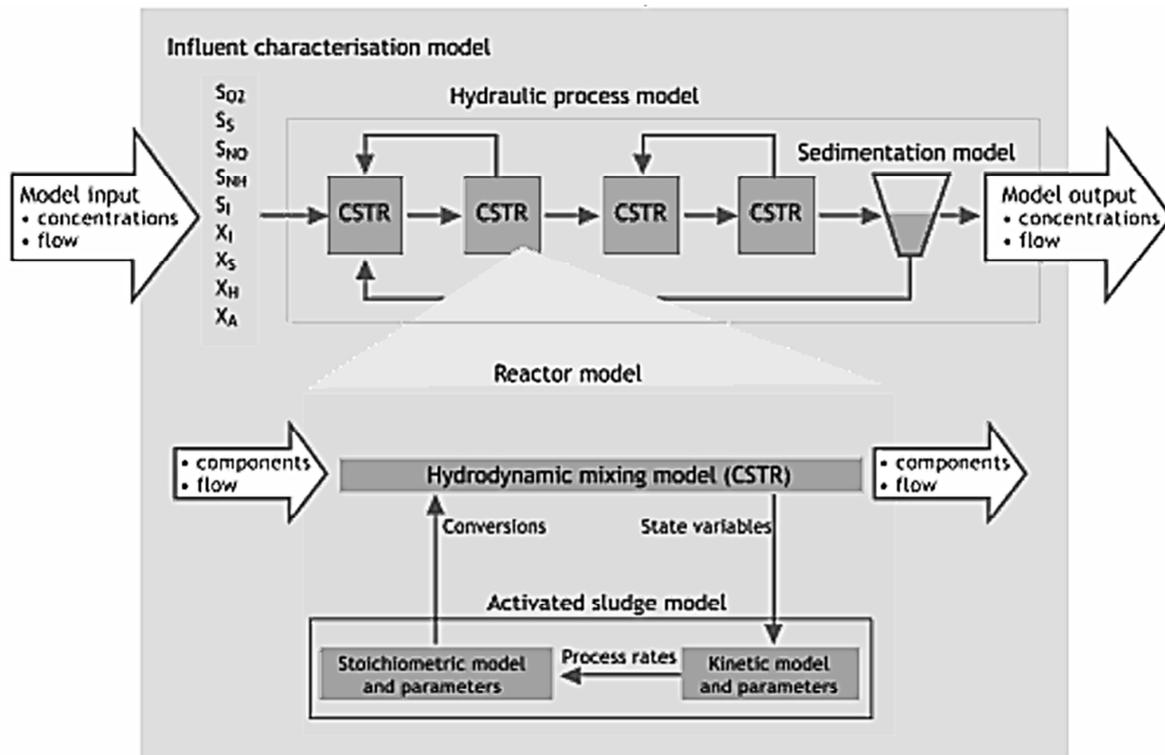


Figure 2-10 Wastewater Treatment Process Model Schematic  
(van Loosdrecht, Ekama, Wentzelm, Brdjanovic, & Hooijmans, 2008)

### 2.6.3 Plant Wide Model

A plant wide model includes the wastewater treatment model and the sludge treatment model to give an overall picture of the how the treatment plant functions and the interactions between water and sludge treatment (recycle flows, solids concentration etc.).

The general idea of a sludge treatment model is the same as that introduced for the wastewater treatment model in the previous section. In a plant wide model, the input for the sludge treatment process will come from the sludge output of the wastewater treatment model. The process units of sludge treatment were introduced at the start of this chapter as thickening, anaerobic digestion, and dewatering and will be incorporated into the plant wide hydraulic model.

Mechanical thickening and dewatering processes are commonly approximated using Point separation models which perform a simple mass balance calculation to split the incoming solids into two streams where the percentage removal is specified on a mass basis for the thickened stream while the remaining solids pass to the clarified stream. Gravity thickeners can be modeled similarly to settlers with 1-Dimensional flux models as mentioned above, or can be approximated more basically with Ideal solid / liquid separation models or point separation models. (EnviroSim Associates Ltd) The clarified streams from these solids liquid separation units are connected to the wastewater treatment model as recycle flows.

Anaerobic digestion like the activated sludge process involves biological and chemical conversions and thus a similar style of model, an Anaerobic Digestion Model (ADM), which describes the stoichiometry and kinetics of the anaerobic digestion process, is used to model performance of an aerobic digestion unit.

Examples of surrogate organisms and the functions they fulfill in anaerobic digestion models include:

- Ordinary Heterotrophic Organisms (OHOs) - fermentation of readily biodegradable (complex) substrate to acetate, propionate, carbon dioxide and hydrogen
- Propionic Acetogens – converting propionate to acetate, CO<sub>2</sub> and hydrogen
- Methanogens - converting acetate to methane and CO<sub>2</sub> and converting CO<sub>2</sub> and hydrogen to methane and water

(EnviroSim Associates Ltd)

The ADM is then incorporated into the anaerobic digestion reactor model to account for the bioconversions in the mass balance.

#### **2.6.4 Simulators**

Software that allows a modeler to simulate the wastewater treatment plant configuration is known as a simulator. General purpose simulators such as MATLAB™ can be applied for this purpose however this is both time consuming and required a highly skilled programmer. Specific wastewater treatment simulators have therefore been developed which include predefined process unit models which can be easily connected into a process configuration by connecting process unit blocks. One such specific simulator is the BioWin 3.1 software produced commercially by EnviroSim Associates Ltd. which was used for modeling component of this thesis and will be discussed further in subsequent chapters.

### 3 Materials and Methods

The purpose of this study was to (1) determine how worm predation affects the biodegradation potential of AS, (2) determine how worm predation affects the solids liquid separation behavior of AS and how sludge components are distributed between each phase, (3) identify and model potential impacts, modifications and improvements to the traditional sludge treatment process and overall WWTP operations that might arise from worm predation of WAS or a sludge reduction technology developed to mimic its results.

To achieve the aforementioned objectives a combination of laboratory analyses of sludge produced by a lab scale worm reactor and computer modeling were carried out. The following sections of this chapter describe the materials and methods used.

#### 3.1 Worm Reactor

In the WWTP Harnaspolder, a lab scale worm reactor is currently operated as pictured in Figure 3-1.

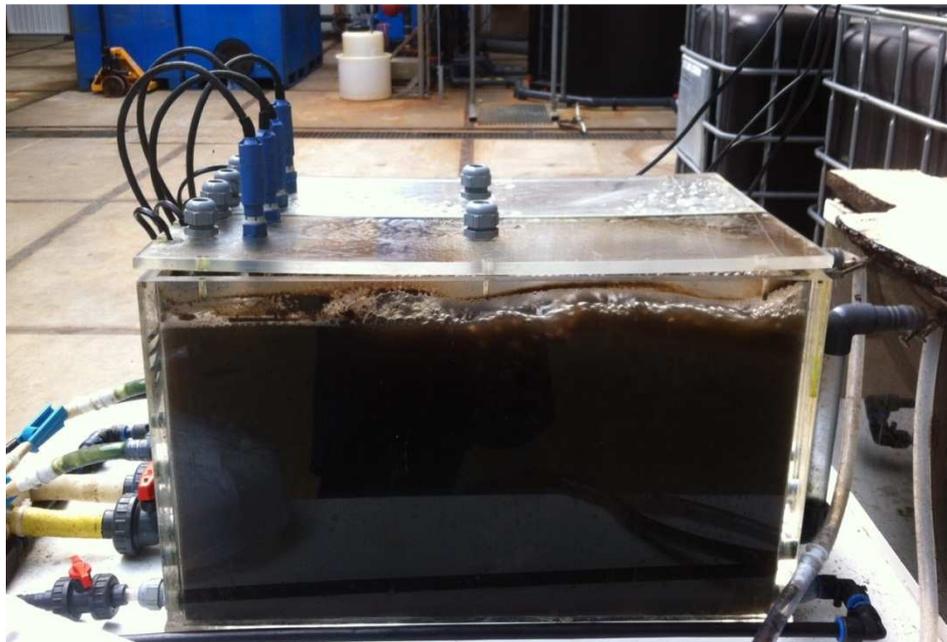


Figure 3-1 Worm Reactor

The reactor has two compartments (approx. 20 L) side by side one of which was outfitted with nets to act as carrier material for the sessile worm *Tubifex tubifex*. This worm compartment was inoculated with *T. tubifex* from the pilot worm reactor at the WWTP Wol Vega. The other compartment is used as a control and is kept free of worms. Both are aerated through perforated PVC headers along the bottom of the tank to maintain an average DO greater than 5 mg/l. Mixing in the compartments is achieved by an airlift system. The temperature of the reactor is maintained at  $20 \pm 1^\circ\text{C}$ . The pH of both reactors is monitored to ensure it stays in the neutral range. Activated sludge is collected from the WWTP as feedstock for the two compartments of the reactor. The reactor is currently operated as a batch process. In the first 2 batches described later in this thesis, carrier nets were included in the worm reactor for the *T. tubifex* to attach to. It was observed that the worms were not using the nets but instead remaining at the bottom of the reactor therefore the nets were removed for subsequent batches.

## 3.2 Experimental Program

In order to assess the impact of a worm reactor on WWTP processes, the following experimental program was developed. Results of the experimental program were used to estimate how worm predation of activated sludge at a wastewater treatment plant would impact sludge properties and model how those changes would then impact the overall wastewater treatment process.

### 3.2.1 Batch Experiments

First, batch experiments in the worm reactor were used to distinguish the impact of worm predation on sludge properties including SVI, TS & VS, TSS & VSS, COD, soluble COD (sCOD), and soluble nutrients ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$ ) from the effects of endogenous respiration. At startup both compartments receive the same amount (approximately 17 L) of AS feedstock (Feed-AS). In the worm compartment sludge reduction occurs through predation by the *T. tubefex* (see Section 2.5) and conversion to worm feces or worm predated activated sludge (WP-AS). In the control compartment sludge reduction occurs through endogenic respiration producing extended aeration activated sludge (EA-AS). The properties of the AS feed were measured, and the properties of the WP-AS and EA-AS were compared daily throughout the batch to determine at which point a significant distinction can be made between the worm predation and endogenic respiration and the mass of worms necessary to see a difference between the two sludges. Once the timing for producing distinct WP-AS and EA-AS samples was established, batch experiments were used to produce sludge for further analysis.

### 3.2.2 Dewatering of Sludge Samples

The gravity settling behavior of the Feed-AS, WP-AS and EA-AS sludge samples were compared using settled sludge volume and SVI. Due to the fact that the WWTP Harnaspolder uses centrifuge thickening for WAS, the centrifuged cake solids concentration and centrate properties of the sludge samples were also compared.

### 3.2.3 Digestibility

Biological Methane Potential (BMP) tests were used to determine the digestibility of the sludge samples. In addition to the AS feed, WP-AS and EA-AS, two other sludge samples were assessed. In the first round of BMP testing, a mixed sludge of 50% WP-AS and 50% AS (by volume) was used to determine if the mixture might increase degradability. In the second set of BMP tests the extended aeration compartment was run for a longer period until it had reached the same amount of volatile solids reduction as the worm predated sludge to determine if the worms increased the biodegradability of the remaining volatile solids. Properties of the BMP mixtures of sludge and inoculum including TS & VS, COD, soluble COD (sCOD), soluble nutrients ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$ ) and volatile fatty acids (VFA) were analyzed before and after digestion to assess the changes resulting from the digestion process.

### 3.2.4 Dewatering of Digested Samples

After the BMP test, the solids liquid separation properties of the digested samples were compared using specific resistance to filtration (SRF) and the capillary suction time (CST) analysis along with viscosity measurements and a laboratory centrifuge to measure cake solids percentage and centrate properties.

### 3.3 Analyses

#### 3.3.1 Biological Methane Potential (BMP)

Biological methane potential was measured at 35°C using anaerobic sludge from the mesophilic digesters at WWTP Harnaspolder as inoculum, and the Feed-AS and WP-AS and EA-AS sludge samples produced by the worm reactor as substrates. Each sludge substrate and an inoculum Blank were assessed in triplicate.

Volatile solids (VS) concentration of the inoculum and substrates was measured prior to startup. The maximum fill volume for the BMP bottle ( $V_M$ ) was selected and the desired ratio ( $r$ ) of inoculum VS to substrate VS was set at 2. Using these values and the lowest VS concentration among the substrates, the volume of inoculum ( $V_{inoc}$ ) needed for each BMP bottle was calculated with the following:

$$V_{inoc} = \frac{r \cdot V_M \cdot VS_{subs}}{(VS_{inoc} + r \cdot VS_{subs})}$$

The volume of the lowest VS concentration substrate was calculated with the following:

$$V_{subs} = V_M - V_{inoc}$$

As the volume of inoculum added to the BMP bottles and the ratio,  $r$ , must be the same for each substrate, the volumes required for the remaining substrates were calculated with the following:

$$V_{subs} = \frac{V_{inoc} \cdot VS_{inoc}}{r \cdot VS_{subs}}$$

For the inoculum Blank no substrate is added, thus the amount of gas production which can be attributed to the degradation of the inoculum used can be distinguished from the gas production attributed to the digestion of the substrate.

Due to laboratory logistics, two different protocols had to be used to perform the two BMP tests that were carried out to completion during the experimental program.

##### 3.3.1.1 BMP Protocol 1

The first protocol used an Automatic Methane Potential Test System (AMPTS) system produced by Bioprocess Control AB as shown in Figure 3-2. The AMPTS system used glass bottles as reactors. Using the VS contents of the substrates and inoculum and setting the target fill volume ( $V_M$ ) at 400 ml the required volumes of substrate and inoculum to be added to the reactor bottles were calculated as described above. In the first protocol, solutions of macro nutrients and trace elements conducive to anaerobic growth were also added to the reactors. These solutions were prepared according to the proportions shown in Table 3-1 Macro Nutrient Solution and Table 3-2 respectively, adapted from those proposed in Owen et al. 1979.

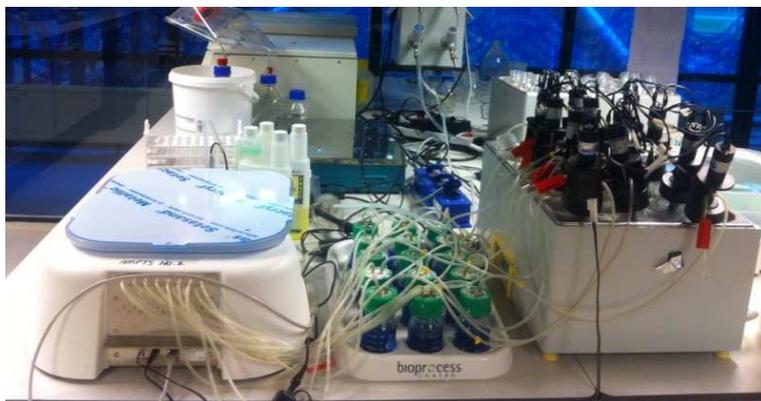


Figure 3-2 AMPTS BMP 1 Setup

Trisaminomethane buffer was also used to ensure no pH inhibition. The required volumes of buffer (10mM), macro nutrient solution (0.6 ml stock /100 ml  $V_{\text{inoc+subs}}$ ) and trace element solution ((0.06 ml stock /100 ml  $V_{\text{inoc+subs}}$ ) were added to each BMP bottle based on the inoc+subs mixture with the lowest VS content. The fill volume of each reactor bottles volume was kept consistent at 525 ml by adding distilled water to the combinations of substrate, inoculum, buffer, and stock solutions. With the same total volume in each bottle, the concentration of stock, trace and buffer solutions were consistent for all substrates.

Reactor bottles were kept in the mesophilic temperature range of 35°C using a water bath incubator. Mechanical mixers with adjustable interval and speed were used at 112 RPM for 30 minutes on and 1 minute off to ensure mixing within the bottles. The liquid and headspace were flushed with nitrogen gas before sealing to ensure anaerobic conditions. Gas produced in the reactor bottles was directed through glass bottles filled with 3M NaOH for carbon dioxide sequestration such that only the methane production would be measured. Methane production was continuously measured using a wet gas flow measuring device with a multi-flow cell arrangement that works according to the principle of liquid displacement & buoyancy. A digital pulse is generated when a defined volume of gas flows through the device. An integrated embedded data acquisition system was used to record and display the data. (Bioprocess Control AB, 2013)

*Table 3-1 Macro Nutrient Solution*

<b>Nutrient</b>	<b>Concentration</b>	<b>Units</b>
<b>NH<sub>4</sub>Cl</b>	170	g/L
<b>CaCl<sub>2</sub>.2H<sub>2</sub>O</b>	8	g/L
<b>MgSO<sub>4</sub>.7H<sub>2</sub>O</b>	8	g/L

*Table 3-2 Trace Element Solution*

<b>Nutrient</b>	<b>Concentration</b>	<b>Units</b>
<b>FeCl<sub>3</sub>.4H<sub>2</sub>O</b>	2	g/L
<b>CoCl<sub>2</sub>.6H<sub>2</sub>O</b>	2	g/L
<b>MnCl<sub>2</sub>.4H<sub>2</sub>O</b>	0.5	g/L
<b>CuCl<sub>2</sub>.2H<sub>2</sub>O</b>	30	mg/l
<b>ZnCl<sub>2</sub></b>	50	mg/l
<b>HBO<sub>3</sub></b>	50	mg/l
<b>(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>2</sub>.4H<sub>2</sub>O</b>	90	mg/l
<b>Na<sub>2</sub>SeO<sub>3</sub>.5H<sub>2</sub>O</b>	100	mg/l
<b>NiCl<sub>2</sub>.6H<sub>2</sub>O</b>	50	mg/l
<b>EDTA</b>	1	g/L
<b>HCl 36%</b>	1	ml/L

### **3.3.1.2 BMP Protocol 2**

The second protocol used for BMP testing was conducted with glass reactor bottles with total volume ( $V_T$ ) of 304 ml. The maximum target fill volume ( $V_M$ ) was set at 150 ml. No nutrient, trace element or buffer solutions were used in the second protocol; instead the pH of the inoculum and substrate mixtures was adjusted to between 7 and 7.5 using a NaOH solution. No distilled water was added to the mixtures in the second protocol, thus the headspaces ( $V_T - V_{\text{subs+inoc}}$ ) varied for the different substrates. A rubber septum was used to seal the bottles and allow for pressure measurements and gas and liquid sample abstraction. The liquid and headspace were flushed with nitrogen gas to ensure anaerobic conditions. The bottles were then placed in a temperature controlled shaker at 35°C and 130 rpm to achieve mesophilic conditions and continuous mixing.

Pressure measurements were taken using a Greisinger Electronic GMH 3151 Digital Pressure Meter at regular intervals throughout the test (daily at the start and every 2 to 3 days near the end) to determine the gas production.

Methane and carbon dioxide percentage was measured by extracting a sample from the headspace of the bottle, storing it in VACUETTE® vacuum vials and analyzing them using an Agilent Technologies

7890A Gas Chromatograph calibrated specifically to measure carbon dioxide and methane. Data was processed using OpenLAB Chromatography Data System (CDS) ChemStation Edition software.

### **3.3.2 Sludge Characterization**

#### **3.3.2.1 Solids**

The total solids (TS), VS, total suspended solids (TSS) and volatile suspended solids (VSS) were measured in triplicate according to Standard Methods 2540. Suspended solids measurements were conducted using 0.7  $\mu\text{m}$  filters (APHA, 2012)

#### **3.3.2.2 Organics and Nutrients**

Total COD, soluble COD,  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  were measured in duplicate according to standard methods (APHA, 2012) using Spectroquant® photometric test kits. Samples for nutrients and soluble COD were filtered with a 0.7  $\mu\text{m}$  membrane filter prior to analysis. Dilution of the samples was used where necessary to ensure concentrations in the test kits range.

#### **3.3.2.3 Volatile Fatty Acids**

Volatile Fatty Acid concentrations were measured using an Agilent Technologies 7890A Gas Chromatograph. Data was processed using OpenLAB Chromatography Data System (CDS) ChemStation Edition software.

### **3.3.3 Solid-Liquid Separation**

#### **3.3.3.1 Settled Sludge Volume Sludge Volume Index (SVI)**

The Settled Sludge Volume and SVI of sludge samples were measured according to standard methods 2710 C and D (APHA, 2012) using a calibrated 250 ml cylinder in lieu of a 1 L cylinder. On the cylinder used each ml equated to a height difference of 1mm.

#### **3.3.3.2 Capillary Suction Time (CST)**

The CST of digested samples were determined according to standard method 2710G (APHA, 2012) using a 304M CST-apparatus and Whatmen no. 17 filter paper from Triton Electronics Ltd. The instrument constant,  $\phi$ , (See section 2.3.5.2) of the CST setup was determined to be 0.047. Viscosity of the digested sludge samples was measured using a Paar Physica UDS 200 rotational rheometer at controlled shear rates varying from 5 to 1000  $\text{s}^{-1}$  and taking the average value over this range.

The temperature of the digested sludge samples for CST and viscosity measurements, and the sample reservoir in the rheometer, were maintained at 35°C using an external water bath and cylindrical temperature system.

#### **3.3.3.3 Specific Resistance to Filtration**

The SRF of digested samples was measured using a laboratory pressure filter and Whatman no. 1 filter paper. 100 ml of digested sludge was added to the pressure filter above the filter paper. A pressure of 1 bar was applied in the filter. Filtrate was collected in a graduated cylinder on a mechanical balance to record the mass of filtrate produced at regular intervals over the time of filtration. The viscosity of the filtrate was assumed to be 0.01 poise, that of water at 20 deg C.

#### **3.3.3.4 Centrifugation**

Cake solids percentage of digested sludge was measured using a Thermo Scientific™ Sorvall ST 16 centrifuge at specific rpm for 15 minutes. Centrate was decanted and cake collected and measured according to standard method 2710 (APHA, 2012). Centrate was also analyzed for organics and nutrients with methods mentioned in section 3.3.2.2 however only total concentrations were measured as samples were not filtered prior to analysis.

### **3.4 Computer Model**

Computer modeling was conducted using the program BioWin 3.1 which is a commercial wastewater treatment process simulator that ties together biological, chemical, and physical process models. BioWin incorporates a proprietary biological model (activated sludge and anaerobic digestion) supplemented by other process models (water chemistry models for calculation of pH and chemical precipitation, mass transfer models for oxygen modeling and other gas-liquid interactions, hydraulic models for mixing etc.) which together simulate the many facets of the wastewater treatment process and their interactions with one another. (EnviroSim Associates Ltd) As the purpose of this thesis is not a detailed description of the basis of BioWin Simulator's models, they will not be detailed further, however the key parameters default values and basis for these models can be found in the from the BioWin manual available at <http://www.envirosim.com/downloads/BWManual.pdf>.

Biowin was used to model both a baseline conventional wastewater treatment process, and a wastewater treatment process incorporating a WAS reduction technique based on worm predation. Detailed information on the model setup and assumptions is presented in Chapter 6.

## 4 Laboratory Results

This section presents the results of the laboratory experimental program described in Chapter 3. First the results of the batch experiments comparing worm predation to endogenous respiration are presented. Second the differences between the dewatering properties of the sludge samples are presented. Next the differences resulting from digesting the sludge samples and their respective biodegradation potentials are reported and finally the dewatering properties of the digested samples are compared.

### 4.1 Batch Experiments

Five batch experiments were carried out during the experimental program. The differences between the batches are shown in Table 4-1.

*Table 4-1 Batch Experiment Overview*

Batch	Feed Sludge	Volume Sludge/ Compartment (L)	Mass Worms (g)	Avg Worm Density (g/L)	Worm to Feed Solids ratio (g/g)
1	2x diluted AS	17	101-123	7	3
2	2x diluted AS	17	150-225	11	4
3	Undiluted AS	17	755	44	14
4	Undiluted AS	17	800	47	11
5	Undiluted AS	17	754	44	11

The first 2 Batches were used to show how the sludge properties in each of the worm reactor compartments changed during the batch process and to find out at which point a clear distinction could be made between the effects of worm predation and endogenous respiration. For these first 2 batches, sludge properties were measured daily. Results of Batches 1 and 2 are presented graphically

in

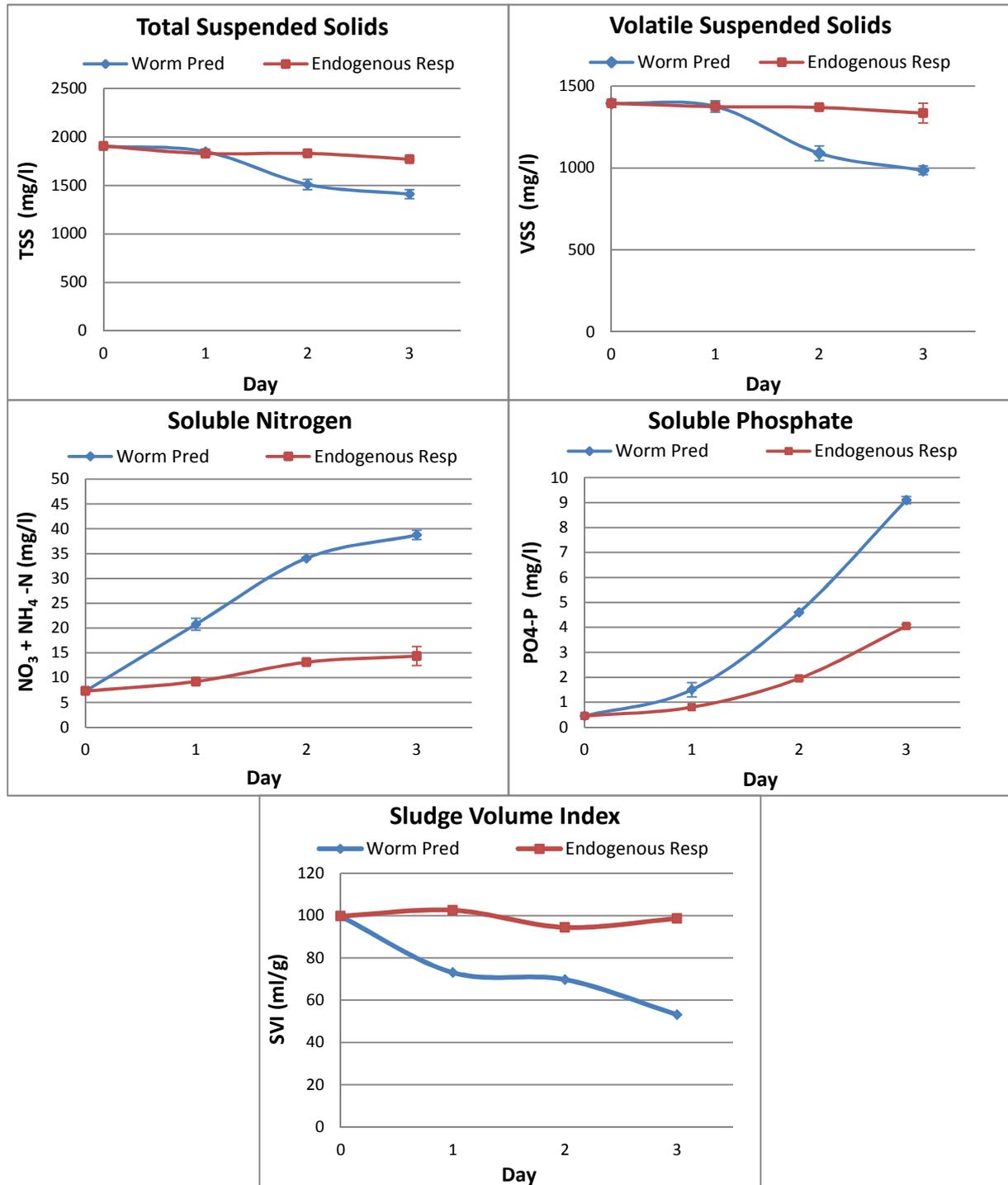


Figure 4-1 and

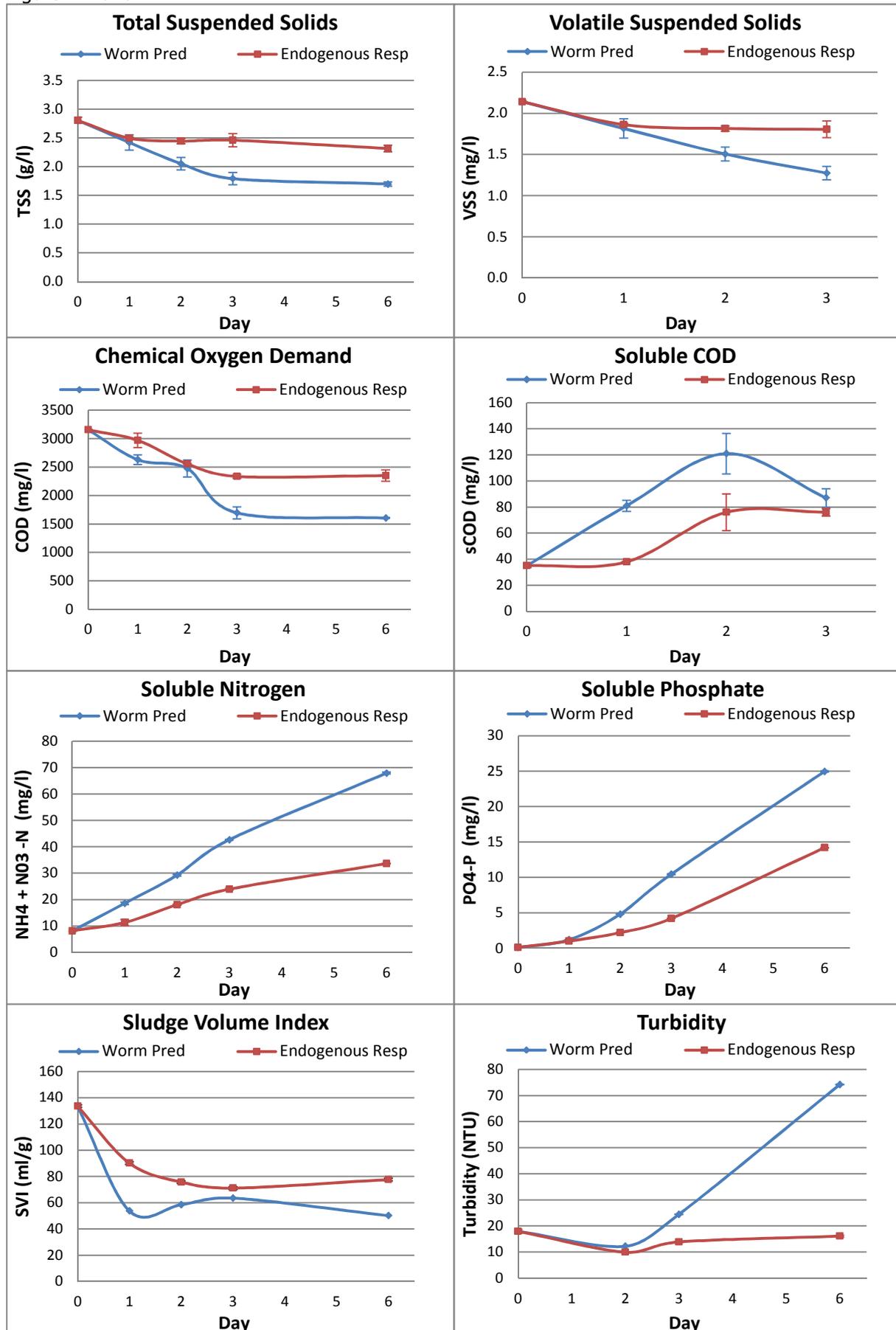


Figure 4-2 respectively. Subsequent batches were used to prepare WP-AS and EPAS for further experimentation thus properties were only measured on the day of feeding (Day 0) and on Day 3 when sufficient differentiation could be made between worm predation and endogenous respiration as inferred from Batches 1 and 2. The differences in sludge properties between the Day 0 Feed-AS and the Day 3 WP-AS and EA-AS for all of the Batches are shown in Table 4-2 through Table 4-8.

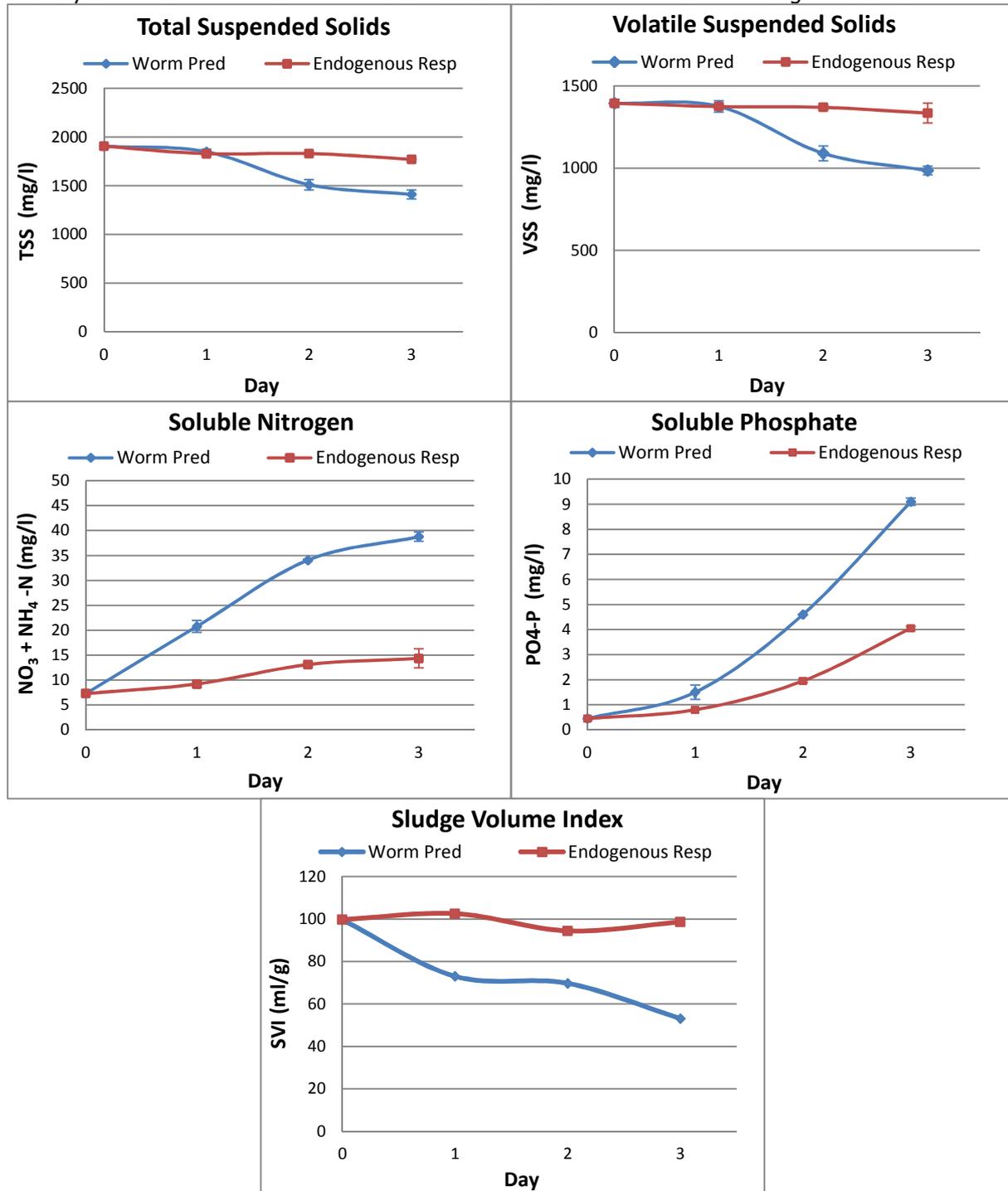


Figure 4-1 Results of Batch 1 Comparing Endogenous Worm Predation to Endogenous Respiration

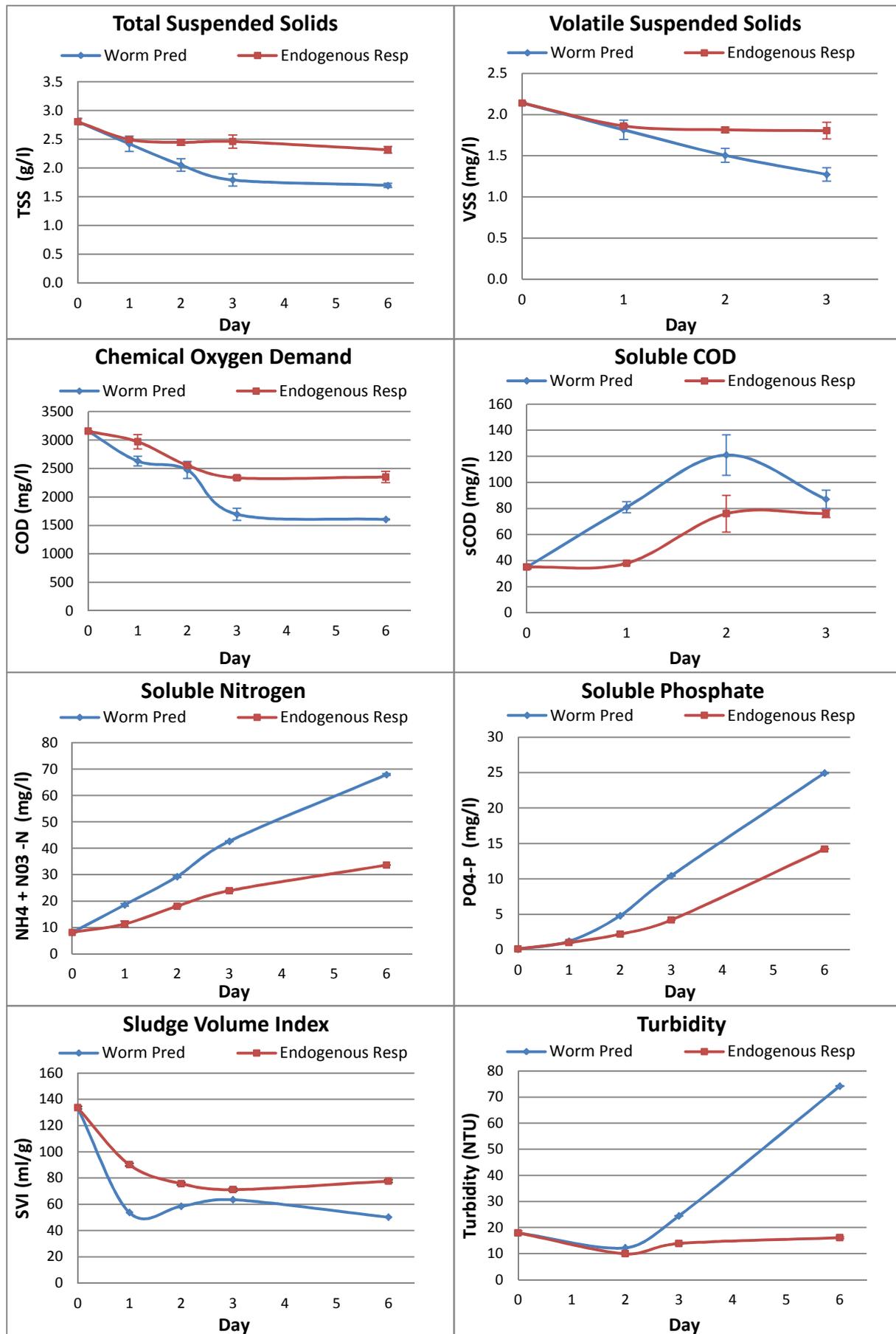


Figure 4-2 Results of Batch 2 Comparing Worm Predation to Endogenous Respiration

Table 4-2 Change in Total Suspended Solids Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

TSS (g/l)					
Batch	AS Day 0	WP Day 3	% Change	EA Day 3	% Change
1	1.905	1.410	-26%	1.770	-7%
2	2.807	1.792	-36%	2.460	-12%
3	3.256	2.772	-15%	3.119	-4%
4	4.096	1.627	-60%	3.575	-13%
5	3.902	2.145	-45%	4.357	12%

Table 4-3 Change in Volatile Suspended Solids Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

VSS (g/l)					
Batch	AS Day 0	WP Day 3	% Change	EA Day 3	% Change
1	1.395	0.985	-29%	1.309	-6%
2	2.141	1.273	-41%	1.805	-16%
3	2.372	1.825	-23%	2.232	-6%
4	2.957	1.104	-63%	2.57	-13%
5	2.805	1.409	-50%	3.000	7%

Table 4-4 Change in Total Chemical Oxygen Demand Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

tCOD (mg/l)					
Batch	AS Day 0	WP Day 3	% Change	EA Day 3	% Change
2	3155	1695	-46%	2335	-26%
3	3390	5737	69%*	3225	-5%
4	4217	1670	-60%	3588	-15%
5	4285	2012	-53%	4145	-3%

\* Worm Predated samples contained worm biomass which skewed COD result

Table 4-5 Change in Soluble Chemical Oxygen Demand Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

sCOD (mg/l)					
Batch	AS Day 0	WP Day 3	% Change	EA Day 3	% Change
2	35	87	149%	76	117%
3	32	149	373%	54	71%
4	48	120	150%	47	-2%
5	72	161	124%	80	11%

Table 4-6 Change in Soluble Nitrogen Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

NH <sub>4</sub> + NO <sub>3</sub> - N (mg/l)					
Batch	AS Day 0	WP Day 3	% Change	EA Day 3	% Change
1	7.3	38.75	431%	14.35	97%
2	8.15	42.7	424%	23.95	194%
3	3.75	41.45	1005%	23.4	524%
4	7.5	48.75	550%	29.25	290%
5	8.5	55.0	547%	26.2	208%

Table 4-7 Change in Soluble Phosphate Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

<b>PO<sub>4</sub>-P (mg/l)</b>					
<b>Batch</b>	<b>AS Day 0</b>	<b>WP Day 3</b>	<b>% Change</b>	<b>EA Day 3</b>	<b>% Change</b>
<b>1</b>	0.45	9.1	1922%	4.05	800%
<b>2</b>	0.1	10.45	10350%	4.2	4100%
<b>3</b>	1.05	18.5	1662%	8.3	690%
<b>4</b>	0.7	16.8	2300%	12.95	1750%
<b>5</b>	0.2	8.3	4050%	4.9	2350%

Table 4-8 Change in Sludge Volume Index Between Feed-AS, WP-AS and EA-AS for all Batch Experiments

<b>SVI (ml/g)</b>					
<b>Batch</b>	<b>AS Day 0</b>	<b>WP Day 3</b>	<b>% Change</b>	<b>EA Day 3</b>	<b>% Change</b>
<b>1</b>	100	53	-47%	99	-1%
<b>2</b>	134	63.5	-53%	71.1	-47%
<b>3</b>	97	45	-53%	84.96	-12%
<b>4</b>	127	71	-44%	119	-6%
<b>5</b>	141	60	-57%	97	-31%

In Batch 5, aeration of sludge without worms was carried out for an additional 6 weeks to achieve approximately the same level of VS and COD reduction as the worm predated sludge. The properties of this 45 day Extended Aeration Activated Sludge (EA45-AS) are compared to the properties of Feed-AS and WP activated sludge in Table 4-9.

Table 4-9 Change in Batch 5 Sludge Properties Between Feed-AS, WP-AS and EA45-AS

<b>Batch 5</b>					
<b>Property</b>	<b>AS Day 0</b>	<b>WP Day 3</b>	<b>% Change</b>	<b>EA Day 45</b>	<b>% Change</b>
<b>TS (g/l)</b>	4.58	2.58	-44%	3.38	-26%
<b>VS (g/l)</b>	2.86	1.27	-56%	1.84	-36%
<b>COD (mg/l)</b>	4285	2012	-53%	1923	-55%
<b>sCOD (mg/l)</b>	72	161	124%	958	1269%
<b>NH<sub>4</sub> + NO<sub>3</sub> - N (mg/l)</b>	8.5	55	547%	169	1888%
<b>PO<sub>4</sub>-P (mg/l)</b>	0.2	8.3	4050%	23.9	11850%

## 4.2 Dewatering of Sludge Samples

### 4.2.1 Gravity Thickening

In addition to the standard 30 minute SVI reported in the previous section, in Batches 4 and 5 the settled sludge volume test was also performed over 60 minutes to compare the rates at which the settled volumes were achieved. The results of the settled sludge volume tests for Batches 4 and 5 are shown in Figure 4-3 and Figure 4-4 respectively.

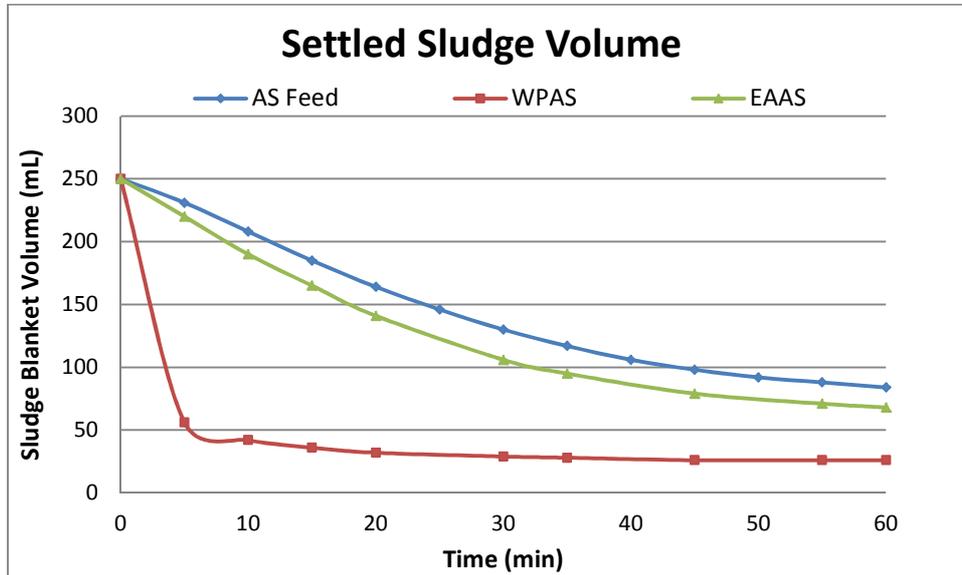


Figure 4-3 Settled Sludge Volume Comparisons for Batch 4

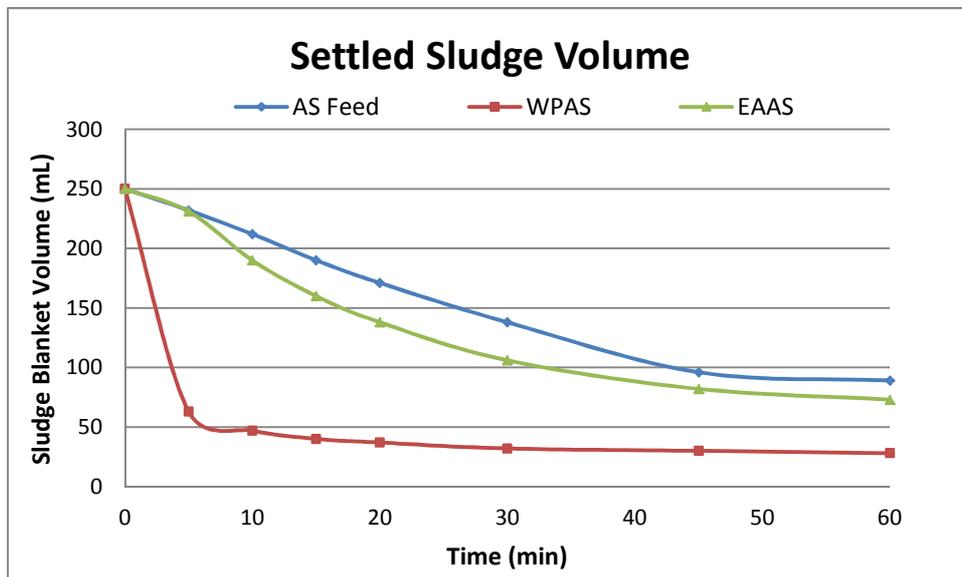


Figure 4-4 Settled Sludge Volume Comparisons for Batch 5

The sludge blanket volume is in this instance equal to the sludge blanket height in mm, since the cylinder used had a gradation of 1 ml/mm. Thus the zone settling velocity ZSV can be calculated for the initial linear part of each curve. For the WP-AS samples this portion is between 0 and 5 minutes, while for the Feed-AS and EA-AS it appears to be between 0 and 20 minutes. The calculated ZSV of each sludge from the two Batches is presented in Table 4-10.

*Table 4-10 Zone Settling Velocities for Batch 4 and 5 Sludge*

	<b>Batch 4 ZSV (m/h)</b>	<b>Batch 5 ZSV (m/h)</b>
<b>Feed-AS</b>	0.258	0.237
<b>WP-AS</b>	2.328	2.244
<b>EA-AS</b>	0.327	0.336

It was not possible to measure the SVI and ZSV of the EA45-AS. This was due to the fact that the entire measuring cylinder remained cloudy. It did appear that there was a slightly denser section at the very bottom of the cylinder which accumulated in the first 5 minutes but it was impossible to distinguish a distinct sludge blanket as is shown in Figure 4-5 which was taken after 30 minutes of settling.



*Figure 4-5 Picture of EA45-AS SVI test at 30 min*

#### 4.2.2 Centrifugation

At the WWTP Harnaspolder waste activated sludge is thickened using a centrifuge, and the centrate, or reject water, is returned to the head of the liquid treatment process. In order to assess how worm predation might affect the thickening performance, a laboratory centrifuge was used to determine the cake solids concentrations of the sludges and the properties of the centrates. Feed-AS, and WP-AS and EA-AS produced by Batch 4 were centrifuged at 3500 RPM for 15 min and by Batch 5 were centrifuged at 4500 rpm for 15 minutes. The resulting average cake solids percentages are shown in Table 4-11.

*Table 4-11 Centrifuge Cake Solids Percentage for Batch 4 and 5 Sludges*

<b>Sample</b>	<b>Batch 4 Cake Solids</b>	<b>Batch 5 Cake Solids</b>
<b>Feed-AS</b>	4.99%±0.32%	4.35%±0.15%
<b>WP-AS</b>	4.91%±0.14%	4.65%±0.02%
<b>EA-AS</b>	4.73%±0.32%	5.48%±0.08%

The differences in concentration between the Feed-AS and the WP-AS and EA-AS centrate properties for the Batch 4 sludges and Batch 5 sludges are shown in Table 4-12 and Table 4-13 respectively.

*Table 4-12 Centrate Properties for Batch 4 Sludges*

<b>Property</b>	<b>Feed-AS (mg/L)</b>	<b>WP-AS (mg/L)</b>	<b>% Difference</b>	<b>EA-AS (mg/L)</b>	<b>% Difference</b>
<b>COD</b>	68 ± 7	165 ± 6	142%	100 ± 2	46%
<b>NH<sub>4</sub>-N</b>	1.3 ± 0.1	11.5	820%	0.3 ± 0.1	-76%
<b>NO<sub>3</sub>-N</b>	4.4 ± 0.2	35.3 ± 1.8	710%	28.5 ± 0.7	555%
<b>PO<sub>4</sub>-P</b>	6.3	18.4 ± 0.4	191%	11.1	76%

*Table 4-13 Centrate Properties for Batch 5 Sludges*

<b>Property</b>	<b>Feed-AS (mg/L)</b>	<b>WP-AS (mg/L)</b>	<b>% Difference</b>	<b>EA-AS (mg/L)</b>	<b>% Difference</b>
<b>COD</b>	85 ± 18	243 ± 10	186%	116 ± 37	36%
<b>NH<sub>4</sub>-N</b>	1.6 ± 0.1	15.2 ± 0.8	877%	0.2	-87%
<b>NO<sub>3</sub>-N</b>	5.0 ± 0.3	39.4 ± 1.9	687%	24.2 ± 0.6	383%
<b>PO<sub>4</sub>-P</b>	4.45 ± 0.4	11.4 ± 0.1	155%	5.3 ± 0.1	18%

### 4.3 Digestion

#### 4.3.1 Methane Potential

Two Biological Methane Potential tests were carried out to completion during the experimental program to compare the digestibility of the Feed-AS sludge with the WP-AS and EA-AS produced by the worm reactor.

##### 4.3.1.1 BMP 1

The first BMP test was done according to BMP protocol 1 described in *Section 3.3.1.1*. The substrate used in the first BMP was that described for Batch 2. In addition to the Feed-AS, WP-AS and EA-AS, a mix of 50% AS and 50% WP-AS by volume was also used as a substrate. This MIX substrate was included because it was hypothesized at that point in the research process that enzymes which break down sludge might be excreted by the worms with their feces and enhance the breakdown of the non-predated sludge when mixed.

The measured volatile solids concentrations of the 4 substrates and the anaerobic inoculum are shown in Table 4-14 along with the resulting calculated volumes of inoculum, substrate, buffer, macronutrient and trace element stock added to each of the reactors. It should be noted that the WP-AS substrate had the lowest VS concentration of the 4, therefore the calculations of inoculum, buffer and stock solution volumes were based upon it as described in section 3.3.1

Table 4-14 BMP 1 Reactor Contents (Total Volume 525 ml/Bottle)

	VS (g/l)	Vol Inoc (ml/Bottle)	Vol Subs (ml/Bottle)	Vol Buffer (ml/Bottle)	Vol Macro (ml/Bottle)	Vol Trace (ml/Bottle)	Vol Comb (ml/bottle)	Vol H <sub>2</sub> O (ml/Bottle)
<b>AS</b>	3.17	45.5	142	89	2.4	0.24	279	246
<b>WP-AS</b>	1.27	45.5	355	89	2.4	0.24	492	33
<b>EA-AS</b>	1.81	45.5	249	89	2.4	0.24	386	139
<b>MIX</b>	2.22	45.5	203	89	2.4	0.24	340	185
<b>Inoc Blank</b>	19.8	45.5	0	89	2.4	0.24	137	388

The data from BMP 1 is presented graphically in Figure 4-6 which shows the cumulative methane production average volumes (normalized for STP conditions) of the 3 reactor bottles used for each substrate and the cumulative standard deviations. Raw data and graphs of the cumulative gas production data for the individual reactor bottles can be seen in Appendix B. The test was carried out for 30 days, however as can be seen from looking at the raw data, the data-logging for some of the individual reactors stopped prior to the 30 day mark. For this reason some of the averages stop prior to, or show a notable change during the, last days of the test period and thus only the results up to 25 days are presented as they represent the averages of the majority of the reactors.

In order to distinguish between the gas production from substrate addition and that inherent to the inoculum, the specific methane production for the 4 substrates was calculated by subtracting the inoculum methane production from the total methane production. The specific methane production data is presented in Figure 4-7.

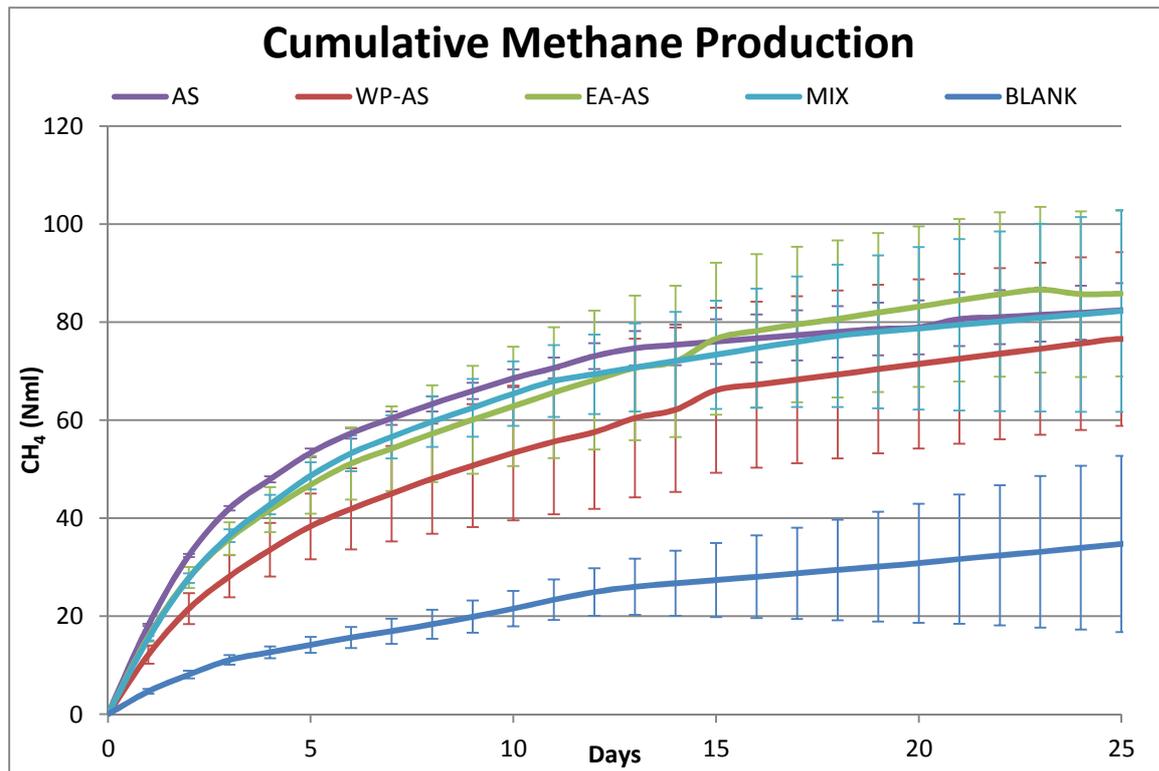


Figure 4-6 Average Cumulative Methane Production BMP 1

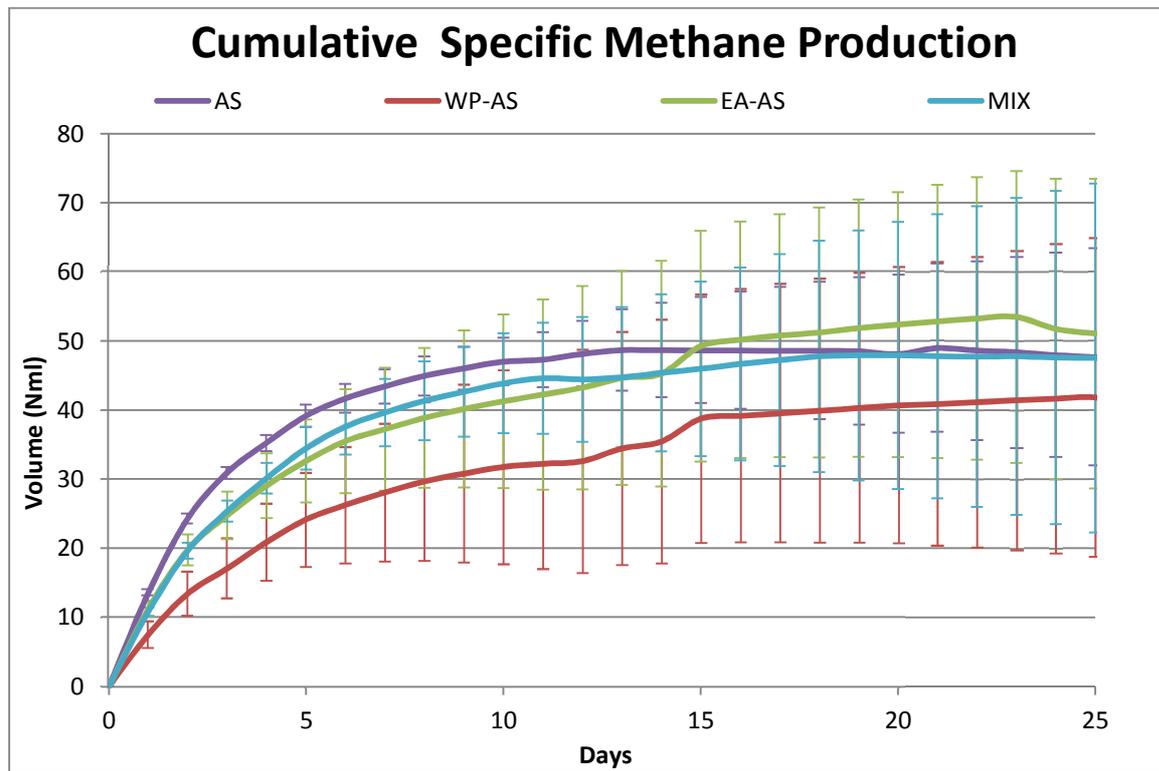


Figure 4-7 Average Specific Cumulative Methane Production BMP 1

Based on the information presented in Figure 4-7 a maximum value was selected for the total methane production from each substrate. This was divided by the mass of substrate volatile solids added to the reactor to determine the BMP of each substrate. The methane, volatile solids and BMP values are shown in Table 4-15.

Table 4-15 BMP 1 Values

Substrate	Specific CH <sub>4</sub> Produced (Nml)	Substrate VS Added (mg)	BMP (ml/g)
AS	47	450	104
WP-AS	42	450	94
EA-AS	51	450	113
MIX	47	450	104

The table shows that identical amounts of volatile solids were added to each reactor for each substrate. This is due to the fact that properties of the mixtures described in Table 4-14 were not measured after mixing. Due to this lapse, the properties of the reactor contents had to be calculated from the properties of their respective components. In order to assess the impact of the calculated mass of substrate volatile solids added to the BMP value, a sensitivity analysis was used. The sensitivity analysis generated a range for the mass of substrate VS based on the standard deviation of the inoculum and substrate VS measurements and an assumed divergence of  $\pm 2$  ml for each volume added to the mixture (excepting the small volumes of macronutrients and trace elements). The maximum and minimum values were used to determine the range for the BMP results which are shown in Table 4-16.

Table 4-16 BMP 1 Value Range

Substrate	Specific CH <sub>4</sub> Produced (Nml)	Substrate VS Added (mg)	BMP (ml/g)
AS	47	306-593	79-154
WP-AS	42	285-618	68-148
EA-AS	51	286-613	83-179
MIX	47	301-599	78-156

A COD Balance was performed for BMP 1 using the calculated COD concentration ranges in the reactor bottles before digestion, the measured COD concentration after digestion, the COD content of the methane produced and the COD content which would be consumed for denitrification of the nitrate present in the bottle prior to digestion. Given that the oxygen equivalent of nitrate is 2.85 mg O<sub>2</sub>/mg NO<sub>3</sub>-N, and assuming a heterotrophic yield of 0.67, it can be calculated that 8.5 mg COD will be consumed per 1 mg of NO<sub>3</sub>-N converted to N<sub>2</sub> gas. (Henze, van Loosdrecht, Ekama, & Brdjanovic, 2008) The COD consumed for denitrification would not be available for conversion to methane as the heterotrophic biomass responsible has a faster growth rate than those responsible for anaerobic digestion. (Henze, van Loosdrecht, Ekama, & Brdjanovic, 2008) The mass of COD into and out of the reactor and the ratio between the two are shown in the COD balance in Table 4-17.

Table 4-17 COD Balance BMP 1

	COD IN (mg)	COD OUT (mg)			OUT/IN
		Methane	Denitrification	COD End	
AS	2238 - 2438	117	0.7	2124	0.92 - 1.00
WP-AS	2286-2483	51	18.7	4666	1.91 - 2.07
EA-AS	2403 - 2485	123	7.1	2080	0.89 - 0.92
Mix	2408 - 2631	117	3.2	2170	0.87 - 0.95
Inoculum	1614 - 1764	50	1.6	1777	1.04 - 1.13

As the MIX sample did not show significantly different behavior than the AS sample which the majority of the MIX volatile solids were made up of, it was assumed that the interaction between the AS and WP-AS in the sample did not alter the sludge behavior enough for it to be of interest in further research<sup>2</sup>. For this reason the following results on the change in sludge properties during digestion in BMP 1 and the post processing dewaterability analysis of the digested sludge produced by BMP 1 will not include information about the behavior of the MIX sample.

<sup>2</sup> de Valks work with enzyme extraction showed no significant difference in levels found in worm predated and Feed-AS (Steeff de Valk, Personal Communication, June 2013)

### 4.3.1.2 BMP 2

The second BMP test was done according to BMP protocol 2 described in *Section 3.3.1.2*. The substrate used in the first BMP was that described for Batch 5. In addition to the Feed-AS, WP-AS and EA-AS, BMP 2 included the EA45-AS produced from the additional extended aeration of sludge without worms for 45 days after feeding. The additional extended aeration was carried out with the goal of reducing the VS and COD content of the sludge to the extent achieved in 3 days of worm predation. This EA45-AS substrate was included to determine if there was any increase in the remaining biodegradable fraction due to worm presence. Due to the amount of time that was required to reduce the organics content of the EA45-AS sludge to an extent similar to the WP-AS, a separate BMP was done for the EA45-AS sludge (BMP 2a) which used a different stock of inoculum collected from the WWTP Harnachpolder.

The measured volatile solids concentrations of the 4 substrates and 2 anaerobic inoculum blanks are shown in Table 4-18 along with the resulting calculated volumes of inoculum and substrate added to each of the reactors, and the headspace remaining in the reactor bottles.

Table 4-18 BMP 2 Reactor Contents

<b>BMP 2</b>	<b>VS (g/L)</b>	<b>V<sub>inoc</sub> (ml/Bottle)</b>	<b>V<sub>subs</sub> (ml/Bottle)</b>	<b>V<sub>comb</sub> (ml/Bottle)</b>	<b>V<sub>head</sub> (ml/ Bottle)</b>
<b>AS</b>	2.86	15	60	75	229
<b>WP-AS</b>	1.27	15	135	150	154
<b>EA-AS</b>	2.82	15	61	76	228
<b>Inoc Blank 1</b>	23.4	15	0	15	289
<b>BMP 2a</b>	<b>VS (g/L)</b>	<b>V<sub>inoc</sub> (ml/Bottle)</b>	<b>V<sub>subs</sub> (ml/Bottle)</b>	<b>V<sub>comb</sub> (ml/Bottle)</b>	<b>V<sub>head</sub> (ml/ Bottle)</b>
<b>EA45-AS</b>	1.84	21	129	150	154
<b>Inoc Blank 2</b>	22.3	21	0	21	129

The pressure increase the bottles was measured over the duration of the tests to determine the gas production. The gas composition was measured by taking gas samples from the headspace and measuring the relative amounts of carbon dioxide and methane with a gas chromatograph - mass spectrometer. The measured gas compositions were used to calculate what amount of gas produced during the BMP was methane. It was assumed that all gas produced was primarily made up of these two components with other gasses present being negligible, thus the relative percentage of methane was assumed to be the percentage of methane in the gas produced and measured by the pressure increase. Methane percentages for AS, WP-AS, and EA-AS substrates were consistently between 75% and 80%, with averages of 78.7, 79.1% and 77.7% respectively while the percentage of methane in the inoculum blank was between 55% and 75% with an average of 68.5%.

The results from BMP 2 are presented graphically in Figure 4-8 which shows the cumulative methane production average volumes (Normalized for STP conditions) of the 3 reactor bottles used for each substrate. Raw data of the gas production and methane content data for the individual reactor bottles can be seen in Appendix C. In order to distinguish between the methane production from substrate addition and that inherent to the inoculum, the specific methane production for the substrates was calculated by subtracting the inoculum methane production from the total methane production. The specific methane production data is presented in Figure 4-9.

The cumulative specific methane production value for each substrate was divided by the mass of substrate volatile solids added to the reactor to determine the BMP of each substrate. The methane, volatile solids and BMP values are shown in Table 4-19.

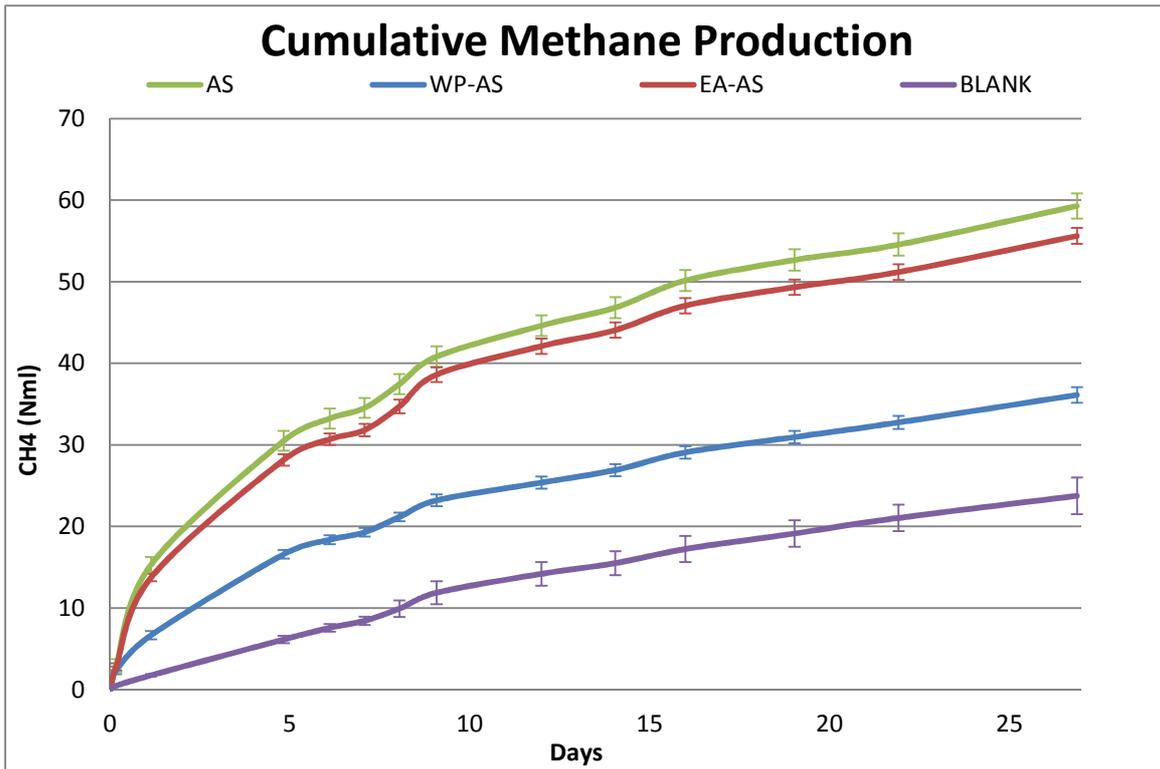


Figure 4-8 Average Cumulative Methane Production BMP 2

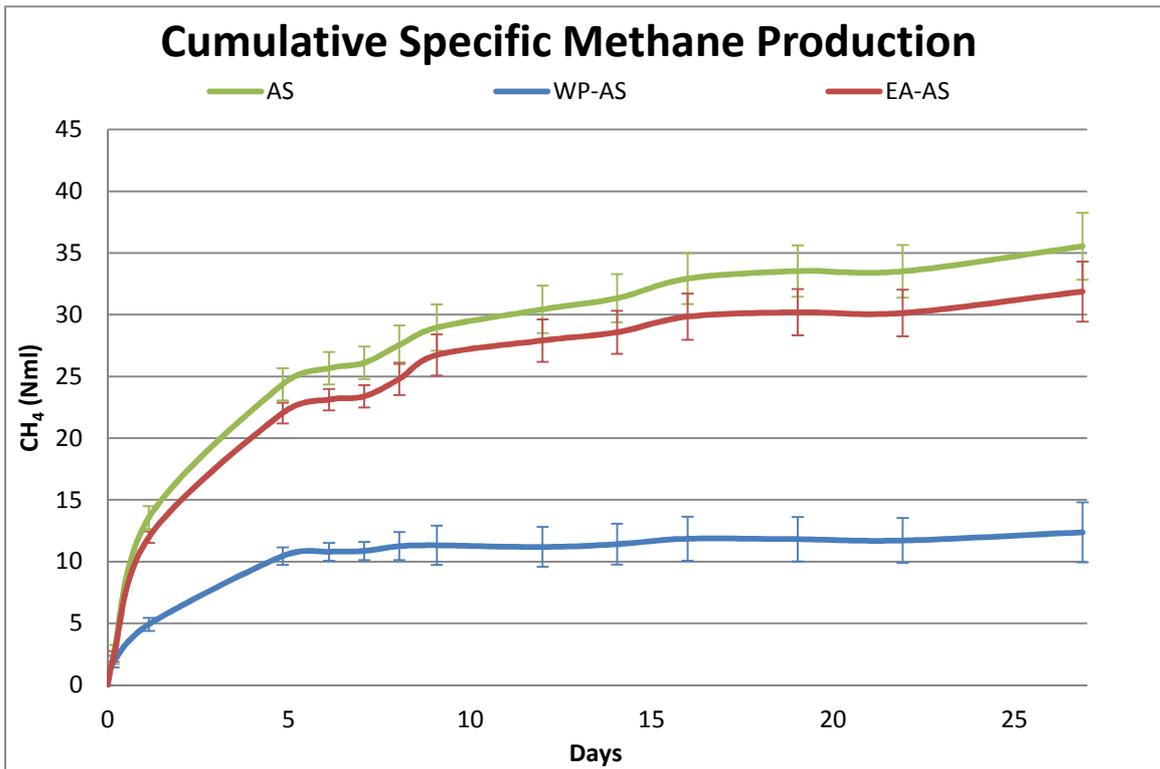


Figure 4-9 Average Specific Cumulative Methane Production BMP 2

Table 4-19 BMP 2 Values

Substrate	Specific CH <sub>4</sub> Produced (Nml)	Substrate VS Added (mg)	BMP (ml/g)
AS	35.5 ± 2.7	176 ± 1.2	202 ± 15
WP-AS	12.4 ± 2.4	203 ± 1.2	61 ± 12
EA-AS	31.9 ± 2.4	198 ± 1.2	161 ± 12

The VFA contents of the mixtures in BMP 2 were measured prior to digestion and after 1 week to determine if there was any VFA accumulation due to methanogen inhibition. The results of this analysis are presented in Table 4-20.

Table 4-20 VFA Analysis

	Pre BMP 2				Day 8			% Difference		
	AS	WP	EA	Blank	AS	WP	EA	AS	WP	EA
Acetic Acid (mg/l)	9.3	27	14	14	8.8	29	4.1	-5%	5%	-69%
Propionic Acid (mg/l)	0.0	3.0	3.3	0.0	0.0	0.0	0.0	0%	-100%	-100%
i-C6 (mg/l)	7.4	1.8	5.5	16	11.0	1.9	8.2	49%	4%	50%
Total (mg/l)	17	32	22	30	20	30	12	19%	-5%	-45%

The results from BMP 2a are presented graphically in Figure 4-10 which shows the cumulative methane production averages of the 3 reactor bottles used for the EA45-AS substrate and Inoculum Blank.

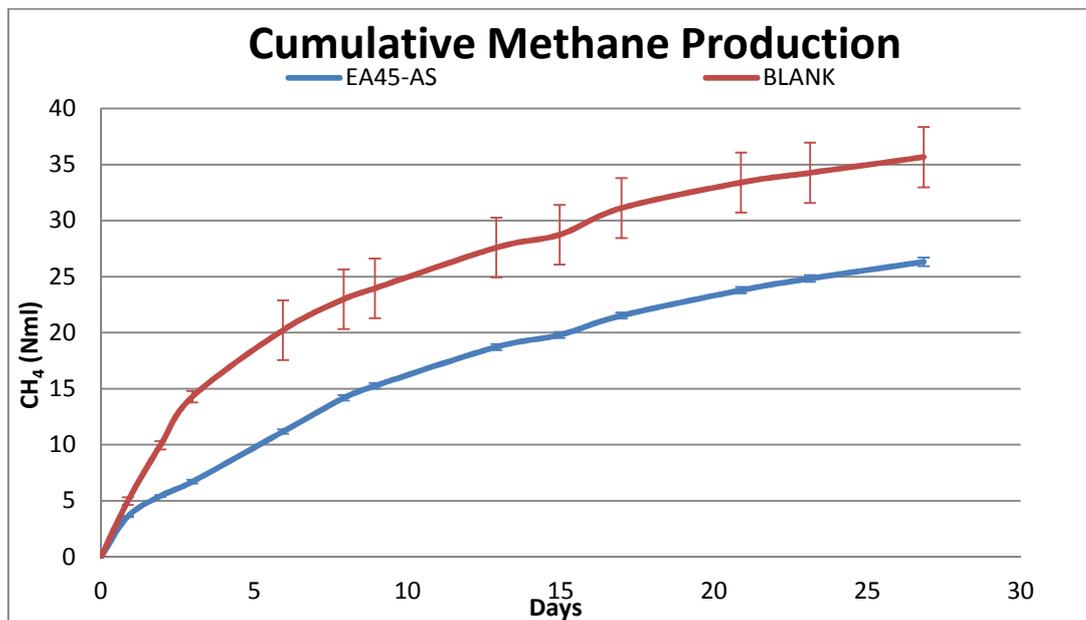


Figure 4-10 Average Cumulative Methane Production BMP 2a

Figure 4-10 shows that the inoculum Blank produced more methane than the EA45-AS. This indicates some inhibition of the conversion of COD to methane in the EA45-AS reactors. As the BMP of the EA45-AS was performed only to compare the biodegradability of it and the WP-AS, the following results on the change in sludge properties during digestion in BMP 2 and the post processing dewaterability analysis of the digested sludge produced by BMP 1 will not include information about the behavior of the EA45-AS sample.

A COD Balance was performed for BMP 2 and 2a using the COD concentrations in the reactor bottles before and after digestion, the COD content of the methane produced, and the COD content which would be consumed for denitrification of the nitrate present in the bottle. Given that the oxygen

equivalent of nitrate is 2.85 mg O<sub>2</sub>/mg NO<sub>3</sub>-N, and assuming a heterotrophic yield of 0.67, it can be calculated that 8.5 mg COD will be consumed per 1 mg of NO<sub>3</sub>-N converted to N<sub>2</sub> gas. The COD consumed for denitrification would not be available for conversion to methane as the heterotrophic biomass responsible has a faster growth rate than those responsible for anaerobic digestion. (Henze, van Loosdrecht, Ekama, & Brdjanovic, 2008) The mass of COD in and out of the reactor and the ratio between the two are shown in the COD balance in Table 4-21.

Table 4-21 COD Balance BMP 2

	COD IN (mg)		COD OUT (mg)		OUT/IN
		Methane	Denitrification	COD End	
<b>AS</b>	763 ± 1	169 ± 5	0.2 ± 0.0	609 ± 20	1.02 ± 0.03
<b>WP-AS</b>	763 ± 8	103 ± 3	33.7 ± 2.5	665 ± 15	1.05 ± 0.02
<b>EA-AS</b>	765 ± 2	159 ± 3	3.7 ± 0.05	617 ± 17	1.02 ± 0.02
<b>Inoc</b>	374 ± 1	68 ± 16	0	372 ± 2	1.17 ± 0.02
<b>EA45-AS</b>	943 ± 63	75 ± 1	128 ± 3	809 ± 4	1.07 ± 0.07
<b>Inoc 2</b>	476 ± 21	102 ± 8	1.3 ± 0.0	412 ± 138	1.08 ± 0.29

### 4.3.2 Change in Sludge Properties

In addition to the methane production potential, the change in properties of the reactor contents over the digestion period were also measured.

#### 4.3.2.1 BMP 1

Since the mixture properties in BMP 1 were not directly measured the concentrations in the reactors had to be calculated from the concentrations and volumes of the mixture components. Due to the uncertainty of this type of calculation a range for the concentration was prepared based on the standard deviation of the property measurements and an assumed divergence of ±2 ml for each volume added to the mixture (excepting the small volumes of macronutrients and trace elements). The values calculated for the sludge property concentrations at the start of BMP 1 are shown in Table 4-22.

Table 4-22 Properties of Reactor Contents Pre - BMP 1

	TS (g/l)	VS (g/l)	COD (mg/l)	sCOD (mg/l)	sP (mg/l)
<b>AS</b>	3.16-3.80	2.42-2.73	4184-4733	859-977	12.2-13.3
<b>WP-AS</b>	3.17-3.90	2.38-2.78	4273-4822	969-1073	21.4-23.4
<b>EA-AS</b>	3.15-3.84	2.38-2.77	4492-4824	922-1005	17-19.7
<b>Blank</b>	1.97-2.68	1.52-1.92	3016-3424	315-360	4.8-7

The properties of the reactor contents were measured after digestion in BMP 1. These results are presented in Table 4-23.

Table 4-23 Properties of Reactor Contents Post - BMP 1

	TS (g/l)	VS (g/l)	COD (mg/l)	sCOD (mg/l)	sP (mg/L)
<b>AS</b>	3.33 ± 0.08	2.38 ± 0.06	4045 ± 21	1014 ± 14	24.4 ± 0.9
<b>WP-AS</b>	3.51 ± 0.07	2.61 ± 0.12	8888 ± 1354	1013 ± 4	25.3
<b>EA-AS</b>	3.04 ± 0.04	2.26 ± 0.08	3963 ± 18	987 ± 4	24.0 ± 1.1
<b>Blank</b>	2.37 ± 0.05	1.91 ± 0.11	3385 ± 21	988 ± 37	23.6 ± 1.5

Due to the different volumes and concentration of the different unthickened substrates added to the inoculated reactors, and the dilution with distilled water to achieve the same volume in each reactor, the change in sludge property concentrations alone does not provide a good enough reference point for comparison of how the use of different substrates during digestion would change the digested sludge properties. As digester loading rates are often based on the volatile content of the sludge, a comparison was made of the mass of each property in the reactor before and after digestion relative to the amount of substrate volatile solid added to the reactor. The results of this comparison are

shown in Figure 4-11. Error bars for the Pre BMP 1 series show the range of values due to the uncertainty of calculating rather than directly measuring the mixture properties prior to digestion.

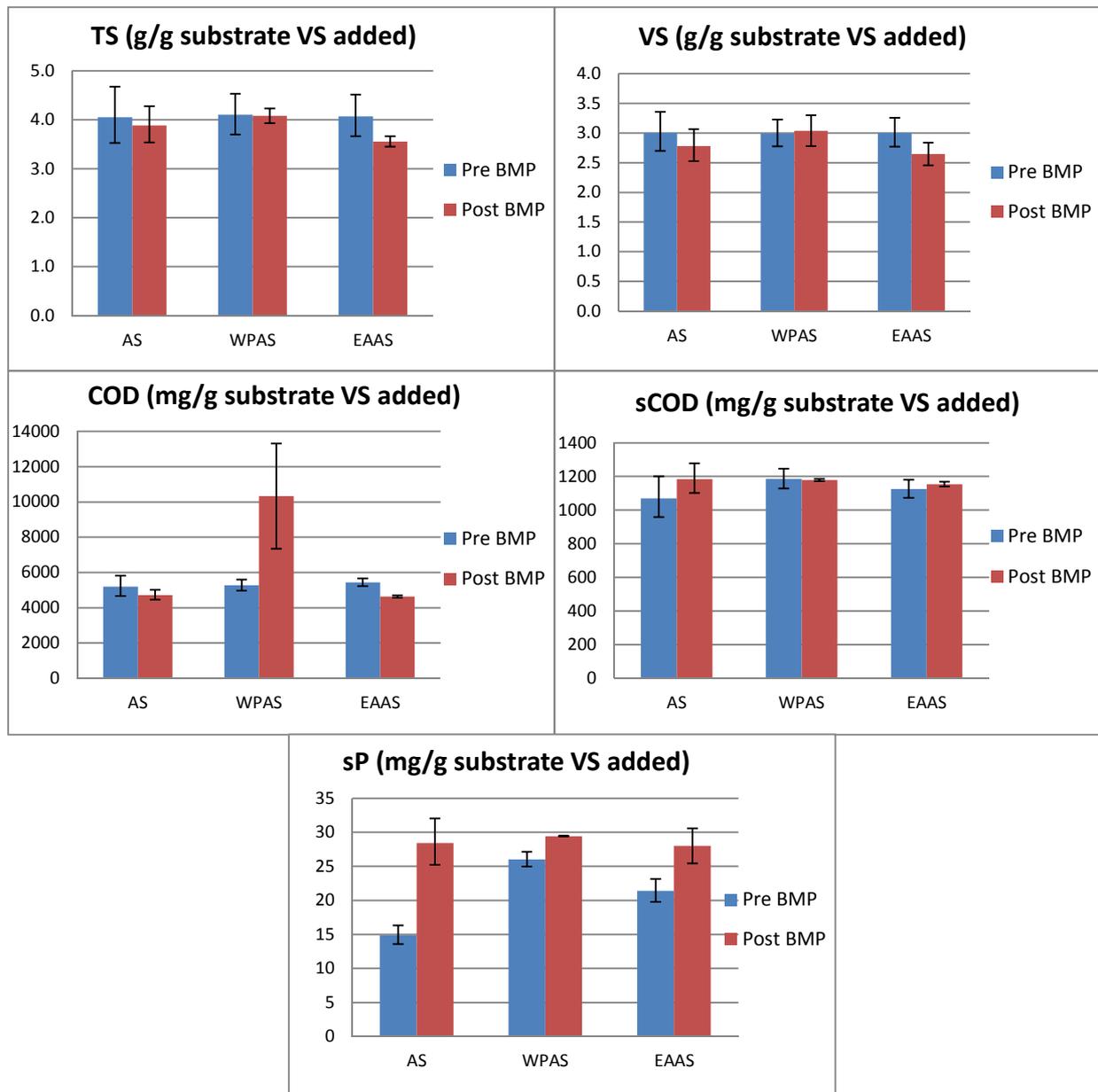


Figure 4-11 Change in Reactor Contents during BMP 1

The percentage of change seen in each reactor due to digestion is the same regardless of the units used to characterize the concentration. The average observed changes for each of the sludge properties in each of the reactor sets of BMP 1 are shown in Table 4-24. A range is shown to account for the error incurred by calculating the pre BMP 1 values.

Table 4-24 Percent Change in Sludge Properties during BMP 1

	TS	VS	COD	sCOD	sP
<b>AS</b>	-13% to 5.3%	-13% to -1.6%	-15% to -3.3%	3.7% to 18%	83% to 100%
<b>WP-AS</b>	-10% to 10.5%	-6.0% to 9.8%	84% to 108%	-5.6% to 4.5%	8.3% to 18%
<b>EA-AS</b>	-21% to -3.4%	-18% to -4.9%	-18% to -12%	-1.8% to 7.1%	2% to 41%
<b>Inoculum</b>	-12% to 20%	-0.5% to 26%	-1.2 to 12.2	175% to 214%	237% to 388%

### 4.3.2.2 BMP 2

The mixture properties for BMP 2 were measured directly before and after digestion. The pre and post digestion measurements are presented in Table 4-25 and Table 4-26 respectively.

Table 4-25 Properties of Reactor Contents Pre – BMP 2

	TS (g/L)	VS (g/L)	COD (mg/L)	sCOD (mg/L)	sN (mg/L)	PO <sub>4</sub> -P (mg/L)
<b>AS</b>	10.57 ± 0.09	6.54 ± 0.07	10170 ± 14	281 ± 1.4	195 ± 19	20.9 ± 1.8
<b>WP-AS</b>	5.75 ± 0.03	3.45 ± 0.02	5085 ± 57	267 ± 1.4	135 ± 1.9	20.7 ± 0.8
<b>EA-AS</b>	10.73 ± 0.07	6.77 ± 0.02	10068 ± 32	270 ± 8.5	186 ± 23	21.3 ± 1.6
<b>Blank 1</b>	33.97 ± 2.10	21.36 ± 1.20	24965 ± 64	644 ± 8.5	840 ± 37	44.7 ± 0.7

Table 4-26 Properties of Reactor Contents Post – BMP 2

Sample	TS (g/L)	VS (g/L)	COD (mg/L)	sCOD (mg/L)	sN (mg/L)	PO <sub>4</sub> -P (mg/L)
<b>AS</b>	9.19 ± 0.15	5.35 ± 0.05	8120 ± 269	378 ± 2.8	313 ± 7	44.6 ± 0.8
<b>WP-AS</b>	5.06 ± 0.08	2.67 ± 0.03	4365 ± 99	208 ± 2.8	146	44.2
<b>EA-AS</b>	9.21 ± 0.27	5.41 ± 0.10	8123 ± 230	322 ± 40	302 ± 6	49.2 ± 0.08
<b>Blank 1</b>	33.4 ± 0.48	20.1 ± 0.14	24975 ± 149	1470 ± 26	1238 ± 283	85.6 ± 0.6

Due to the different volumes and concentration of the different unthickened substrates added to the inoculated reactors, the change in sludge property concentrations alone does not provide a good enough reference point for comparison of how the use of different substrates during digestion would change the digested sludge properties. As digester loading rates are often based on the volatile content of the sludge, a comparison was made of the mass of each property in the reactor before and after digestion relative to the amount of substrate volatile solid added to the reactor. The results of this comparison are shown in Figure 4-12. The percentage of change seen in each reactor due to digestion is the same regardless of the units used to characterize the concentration. The average observed changes for each of the sludge properties in each of the reactor sets of BMP 2 are shown in Table 4-27.

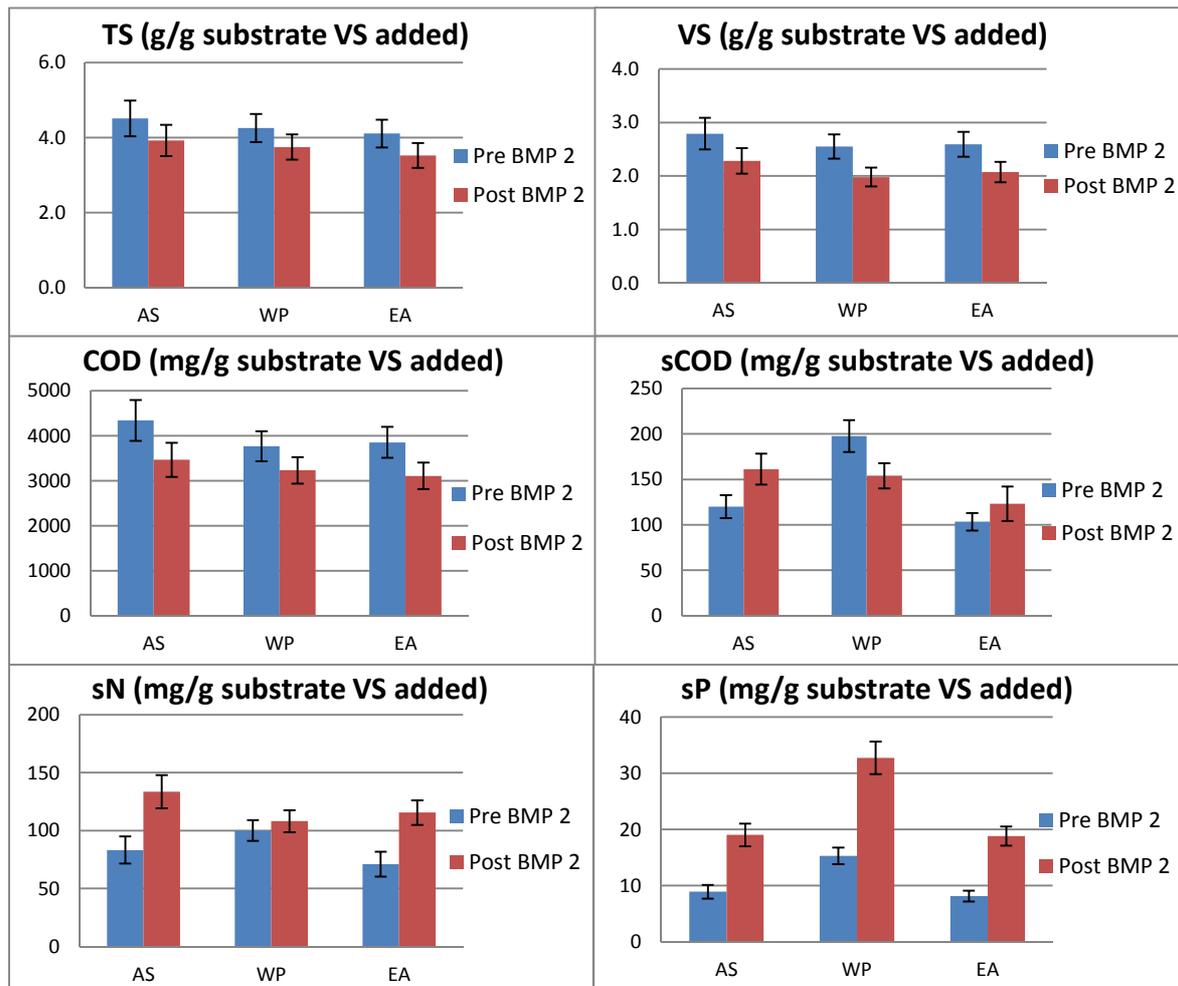


Figure 4-12 Change in Reactor Contents during BMP 2.

Table 4-27 Average Change in Sludge Properties during BMP 2

	TS	VS	COD	sCOD	sN	sP
<b>AS</b>	-13%	-18%	-20%	35%	61%	113%
<b>WP</b>	-12%	-23%	-14%	-22%	8%	114%
<b>EA</b>	-14%	-20%	-19%	19%	63%	131%
<b>Blank</b>	-2%	-6%	0%	128%	47%	91%

### 4.3.3 Combined Worm, Aeration and Digestion Performance

The total reductions in TS, VS and COD combining the reduction in the worm reactor and the reductions in the digester were calculated for the Feed-AS, WP-AS and EA-AS were calculated using the average reduction seen in the Batch comparisons and the reduction in the BMP 2 reactors minus the reduction attributed to the inoculum. For the WP-AS reduction during the Batch phase occurred through worm predation in the worm reactor. For the EA-AS, and EA45-AS reduction during the batch phase occurred through endogenous respiration in the control compartment of the worm reactor during the 3 days, and 45 days respectively. For the Feed-AS no reduction occurred in the worm reactor. The overall reductions for the three sludges are shown in Table 5-6.

Table 4-28 Average Overall Reduction in TS, VS and COD for Sludges

	TS	VS	COD
<b>Feed-AS</b>	33%	40%	39%
<b>WP-AS</b>	53%	69%	66%
<b>EA-AS</b>	37%	46%	45%
<b>EA45-AS</b>	30%	48%	62%

#### 4.4 Dewatering of Digested Samples

Post processing of the digested sludge from BMP 1 and BMP 2 was performed to determine and compare the dewaterability of the different sludge substrates after digestion.

##### 4.4.1 Centrifugation

At the WWTP Harnaschpolder digested sludge is dewatered using a centrifuge, and the centrate, or reject water, is returned to the head of the liquid treatment process. In order to assess how worm predation might affect the dewatering performance, a laboratory centrifuge was used to determine the cake solids concentrations of the digested sludge and the properties of the centrate. The digested contents of the AS, WP-AS, EA-AS and MIX reactors from BMP 1 were centrifuged at 3000 RPM for 15 min and from BMP 2 were centrifuged at 9000 RPM for 15 min. The resulting average cake solids percentages are shown in Table 4-28.

Table 4-29 Centrifuge Cake Solids Percentage for BMP 1 and BMP 2 Sludges

Substrate	BMP 1 Cake Solids	BMP 2 Cake Solids
Feed-AS	5.53% ± 0.03%	6.77% ± 0.07%
WP-AS	5.68% ± 0.11%	6.01 ± 0.67%
EA-AS	6.20% ± 0.38%	6.94% ± 0.05%

The differences in average concentration between the digested AS, WP-AS and EA-AS centrate properties for the BMP 1 and BMP 2 sludges are shown in Table 4-30 and Table 4-30 respectively.

Table 4-30 Centrate Property Concentrations Post BMP 1

	COD (mg/L)	NH <sub>4</sub> -N (mg/L)	PO <sub>4</sub> -P (mg/L)
AS	1160 ± 6	564 ± 22.1	22.5 ± 0.4
WP-AS	1206 ± 6	530 ± 11.9	23.6 ± 0.7
EA-AS	1279 ± 1	538 ± 10.7	22.6 ± 0.5

Table 4-31 Centrate Property Concentrations Post BMP 2

	COD (mg/L)	NH <sub>4</sub> -N (mg/L)	PO <sub>4</sub> -P (mg/L)
AS	355 ± 1.4	353 ± 4.9	40.5 ± 0.4
WP-AS	201 ± 1.4	159 ± 3.5	45.1 ± 2.1
EA-AS	346	319 ± 7.8	47.2 ± 3.1

Again it should be noted that different volumes and concentrations of substrate (and water in the case of BMP 1) were added to the same volume of inoculum in each of the reactors. For this reason the centrate concentrations themselves do not provide an adequate basis for the comparison of the change in centrate properties that can be expected by digesting worm predated sludge rather than activated sludge. As before to get an accurate idea of the change in centrate properties resulting from using worm predation as a substrate instead of activate sludge, a comparison is made based on the mass of property in the centrate per mass of substrate VS added. These results for BMP 1 and BMP 2 are shown in Table 4-32 and Table 4-32 respectively.

Table 4-32 Difference in Average Centrate Properties from Digestion in BMP 1

	AS	WP-AS	% Difference	EA-AS	% Difference
COD (mg/g VS added)	1353	1404	4%	1489	10%
NH <sub>4</sub> -N (mg/g VS added)	658	617	-6%	626	-5%
PO <sub>4</sub> -P (mg/g VS added)	26	27	5%	26	0%

Table 4-33 Difference in Average Centrate Properties from Digestion in BMP 2

	AS	WP-AS	% Difference	EA-AS	% Difference
<b>COD (mg/g VS added)</b>	155	176	14%	153	-1%
<b>NH4-N (mg/g VS added)</b>	154	139	-10%	141	-8%
<b>PO4-P (mg/g VS added)</b>	17.7	39.6	124%	20.9	18%

#### 4.4.2 Capillary Suction Time and Specific Resistance to Filtration

Capillary suction time and Specific resistance to filtration were measured to determine the ease of dewaterability of the digested sludge.

The CST test was performed on the digested sludges from BMP 1 and BMP 2. In order to calculate the filterability constant described in section 2.3.5.2 the viscosity of the digested sludge was also measured. Table 4-34 and Table 4-35 show the average CST, viscosity and calculated filterability constant results from BMP 1 and BMP 2 respectively.

Table 4-34 CST Results Post BMP 1

	CST (sec)	Viscosity (cP)	TS (g/l)	filterability constant (kg <sup>2</sup> /sec <sup>2</sup> /m <sup>4</sup> )
<b>AS</b>	44.2 ± 10.4	3.42	3.33 ± 0.08	1.20 ± 0.3 E-05
<b>WP-AS</b>	21.2 ± 2.3	3.21	3.51 ± 0.07	2.48 ± 0.3 E-05
<b>EA-AS</b>	13.9 ± 1.5	3.35	3.04 ± 0.04	3.42 ± 0.4 E-05

Table 4-35 CST Results Post BMP 2

	CST (sec)	Viscosity (cP)	TS (g/l)	filterability constant (kg <sup>2</sup> /sec <sup>2</sup> /m <sup>4</sup> )
<b>AS</b>	40.9 ± 4.1	5.05	9.19 ± 0.15	5.28 ± 0.5 E-05
<b>WP-AS</b>	13.0 ± 0.7	3.41	5.06 ± 0.08	6.16 ± 0.3 E-05
<b>EA-AS</b>	26.8 ± 2.9	5.23	9.21 ± 0.27	8.36 ± 0.9 E-05

Specific resistance to filtration of the digested sludge was measured only after BMP 2. The resulting plots of  $t/V$  vs  $V$  for the three substrates are shown in Figure 4-13.

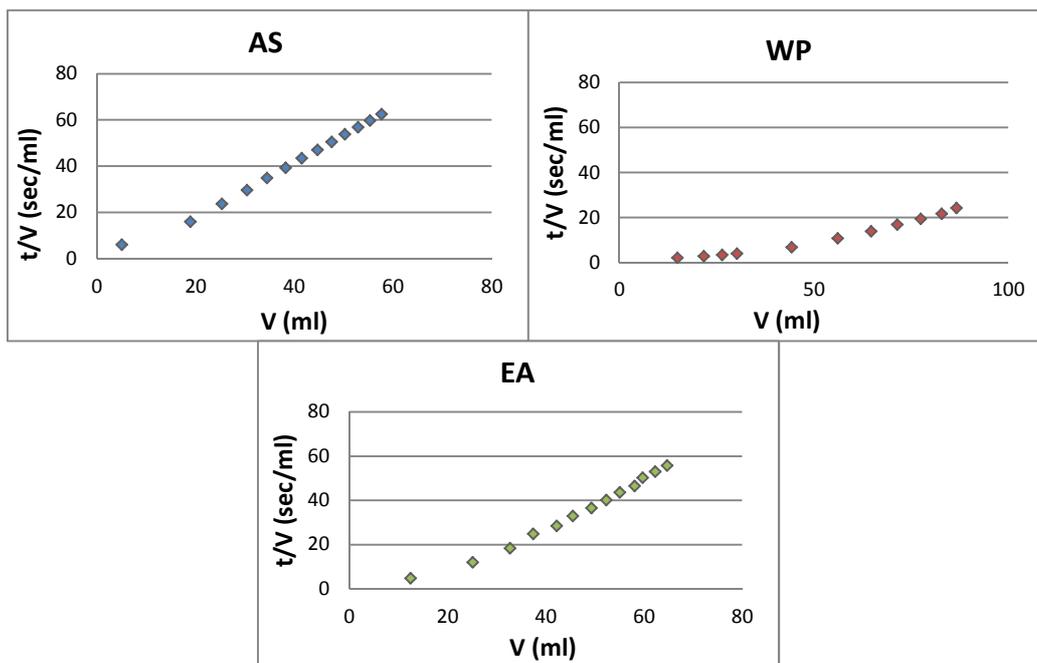


Figure 4-13 SRF Results

As the figures show the first point(s) in each of the graphs do not display the linear behavior that is seen in the remainder of the graphs. During the completion of the test it was observed that for the first few minutes the filtrate flowed from the samples at a higher pace than was exhibited in the following time. This is likely due to the fact that the substrates were unthickened prior to digestion and thus the remaining free water in the digested sludge passed through the filter early in the test before a cake layer was fully formed and its influence limited the flow. For this reason data points from the first 5 minutes of the tests were removed before fitting the trend lines for the SFR calculation which is consistent with the methods of other researchers. The revised graphs and the linear equations of their trend lines are shown in Figure 4-14. The resulting SRF values calculated from these slopes were  $3.95 \times 10^{11}$  for digested AS,  $2.43 \times 10^{11}$  for digested WP-AS, and  $3.68 \times 10^{11}$  for digested EA-AS. The lower the SRF value the greater the higher rate of dewatering achievable.

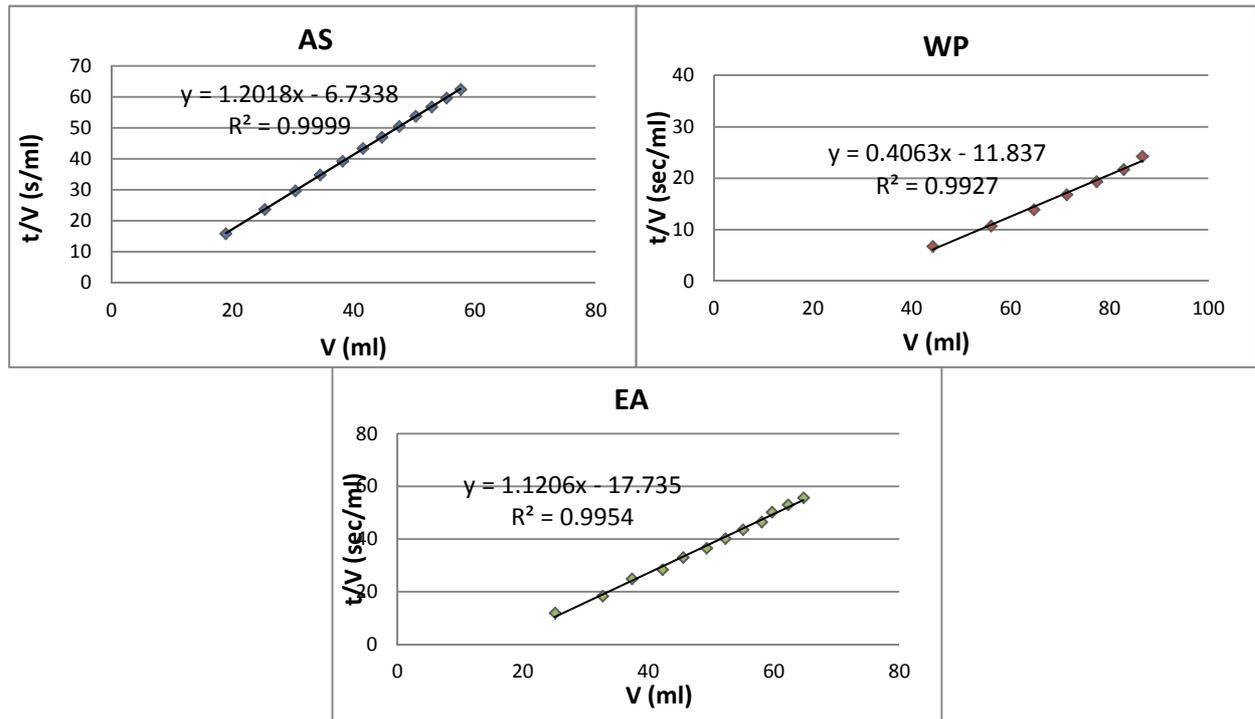


Figure 4-14 Linear portion of SRF Results



## 5 Discussion Laboratory Results

### 5.1 Worm Reactor Batch Experiments

#### 5.1.1 Appearance of Distinct Worm Predated Sludge

The first goal of the Worm Reactor Batch experiments was to find the point in time in the batch at which the sludge properties in the worm chamber differed from those in the aerated chamber. This was considered the point at which the effects of worm predation were more significant than those of endogenous respiration. To that effect, the sludge properties of each compartment in Batches 1 and 2 were measured daily and the results were presented in

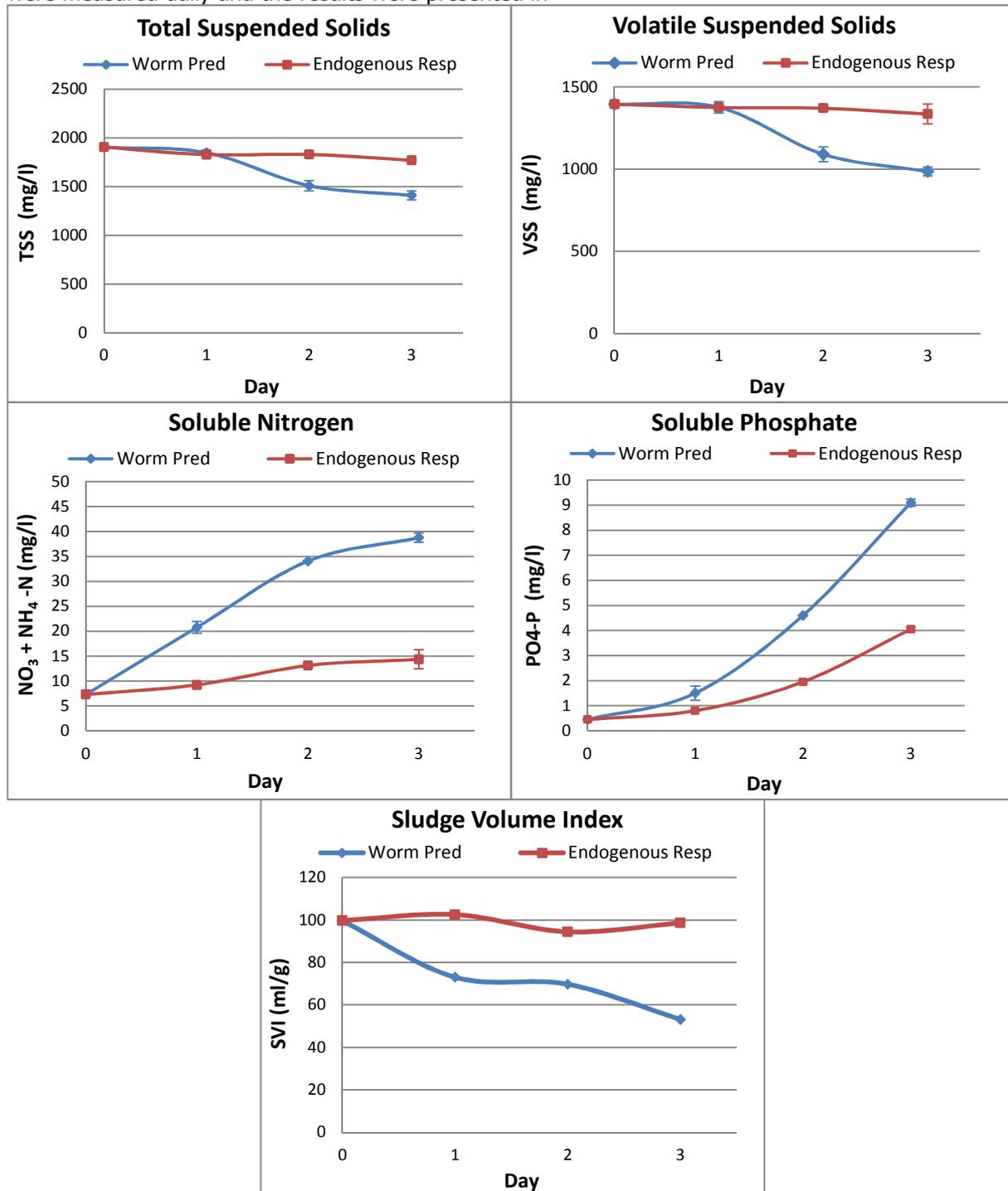


Figure 4-1 and

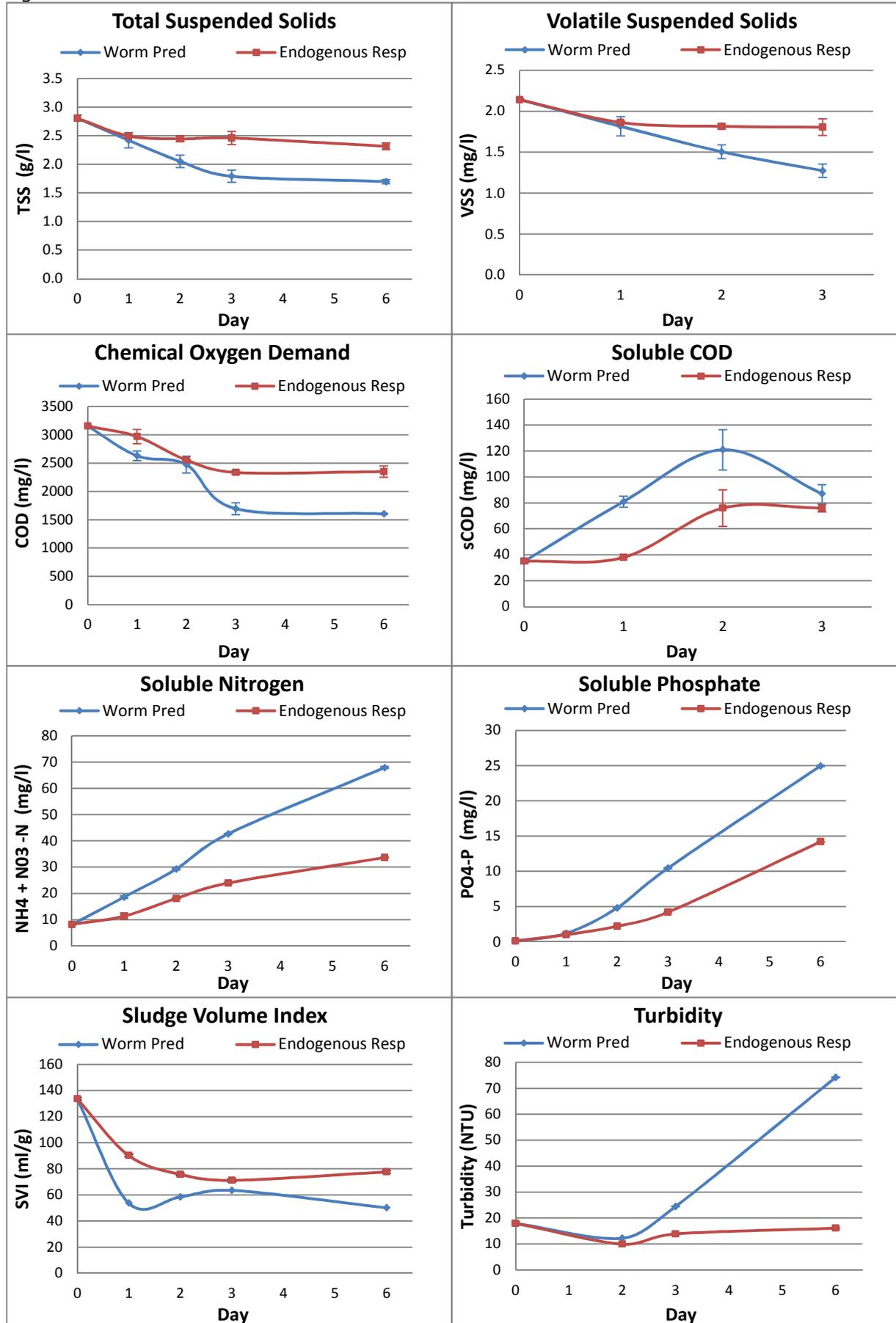


Figure 4-2. From these results it was determined that on Day 3 of a batch the sludge in the worm reactor could be considered to be worm predated activated sludge (WP-AS) that had notably different properties than the sludge from the aerated basin which could be considered extended aerated activated sludge (EA-AS). Therefore for further experimentation on WP-AS and EA-AS, sludge could be harvested on Day 3 of a batch.

It is interesting to note that a perceptible difference between the worm predation and endogenous respiration was not apparent until day 2 of the batch for TSS, VSS, while for the soluble components and SVI, differences were noticeable on day 1. The earlier presence of soluble components and good settling sludge is considered to be due to the influence of worm faeces which was within the worms' digestive tracts prior to their introduction into the batch. The effects of the partially digested material which was evacuated once the worms were in the batch process would likely appear more quickly than the changes in Feed sludge solids content. As the worms were also feeding on activated sludge from the Harnaspolder WWTP prior to their introduction to the batch it is unlikely that the partially digested faeces had significant impact on the final sludge properties.

### 5.1.2 Effect of Worm Predation on Activated Sludge Properties

The second goal of the batch experiments was to understand the effect that worm predation had on the characteristics of the activated sludge. Five Batches in total were conducted which provided a set of data which is presented in Table 4-2 through Table 4-8. The impact of worm predation is distinguished from the impact of endogenous respiration by subtracting the percent change between Feed-AS and EA-AS from the percent change from between Feed-AS and WP-AS. The average effect of aerated worm predation on activated sludge during this experimental program is listed below. The percent changes seen in the worm predated sludge are further broken down into the percentage that was due to worm presence and the percentage that would have been seen from aeration alone.

- 36% ± 17% Reduction in TSS
  - 5% aeration
  - 31% predation
- 41% ± 16% Reduction in VSS
  - 7% aeration
  - 34% predation
- 53% ± 7% Reduction in total COD
  - 12% aeration
  - 41% predation
- 141% ± 117% Increase in soluble COD
  - 41% aeration
  - 100% predation
- 591% ± 239% Increase in Soluble Ammonia and Nitrate
  - 263% aeration
  - 328% predation
- 4057% ± 3693% Increase in Soluble Phosphate
  - 1938% aeration
  - 2119% predation
- 51% ± 5% Reduction in SVI
  - 20% aeration
  - 31% predation

The changes in sludge properties due to worm predation observed in the batch experiments coincide with those reported in literature as described in Chapter 2. The observed decrease in VS and total COD coupled with the increase in soluble COD during worm predation indicate that while degrading the organic material in the activated sludge, the worms reduce the particulate organics to smaller soluble compounds. It is inferred that the increase in the soluble nitrogen and phosphate also indicate the reduction of particulate or bound forms of the nutrients to more soluble components, however as COD is more vital to worm or biomass growth the amount remaining in solution is less than that of the nutrients.

The EA45-AS produced by continued aeration of non-worm predated sludge in Batch 5 shows that with aeration alone it took 15 times as long to reduce the organic content (VS and COD) of the sludge as it took with aerated worm predation. Also the concentrations of soluble nutrients that were in the sludge were dramatically higher in the EA45-AS samples than in the WP-AS. Based on the amount of solids which were observed to remain in suspension during the SVI test of the EA45-AS (Figure , it is concluded that these particles were small enough to pass through the 0.7  $\mu\text{m}$  filter used to separate soluble from suspended material and thus contributed to the amount of soluble material measured in the EA45-AS. It is also possible that a greater amount of soluble nutrients are utilized by the worms during the reduction of the organic fraction of the sludge than was utilized during the extended aeration.

### **5.1.3 Variability in Batch Results**

There was a good deal of variability between the batches with respect to the degree of the change in sludge properties due to worm predation and extended aeration that was achieved. Particularly for worm predation, the variability in the percentage of increase of soluble COD, Nitrogen and Phosphorus had high standard deviations. For the worm results, various worm-related factors such as the state of maturation, could contribute to the lack of consistency, however this does not explain the variations seen in the EA-AS day 3 properties which would be expected to show more consistent behaviour. It is concluded that the variation in behaviour seen between the batches in the sludge produced by both compartments is likely due to differences in the Feed Sludge itself, thus leading to differences in how it was broken down by the worms or behaved during endogenous respiration. Differences in the feed sludge could arise from changes in the wastewater treatment process at the WWTP Harnaschpolder based on the time of year or amount of chemical addition for phosphorus removal.

## 5.2 Thickening of Sludge Samples

The results from the batch experiments show that the SVI of the WP-AS is approximately 50% lower than the Feed-AS, and that worm predation converts the feed sludge from a moderately settling sludge (SVI 100-150 ml/g) to a good settling sludge (SVI < 100 ml/g). The zone settling velocity of the WP-AS is also approximately 10 times greater than that of the Feed-AS, showing that not only does the WP-AS settle better than normal AS but it also does so much faster.

In order to assess the effect of predation on the centrifuge thickening of WAS at the Harnaschpolder WWTP, a laboratory centrifuge was used to determine and compare the cake solids percentage achievable for the Batch 4 and 5 sludge samples, as well as the centrate properties which would represent the concentration in the recycle flow being returned to the water treatment process. The Batch 4 sludge was centrifuged at 3500 rpm for 15 minutes but the differences in the cake solids percentages found for Feed-AS, WP-AS and EA-AS were not significant, all falling within the range of 4-6% solids normal for a thickening process and with standard deviations that did not make them distinguishable from one another. The Batch 5 sludge was centrifuged at 4500 rpm also for 15 minutes, this time the standard deviations were smaller, however the percent solids content for Feed-AS and WP-AS were very close to each other with the WP-AS having a slightly higher solids percentage. The EA-AS achieved almost 1% higher solids content than the other two samples. Based on this data it is not anticipated that worm predation will significantly affect the cake solids percentage achieved by centrifuge thickening.

The difference in the concentrations of constituents in the centrate of the Feed-AS and the WP-AS were fairly consistent between the two centrifuge tests. The worm predation increased the concentration in the centrate by an average of:

- 164% COD
- 849% Ammonia
- 699% Nitrate
- 173% Phosphate

These increases in the concentration of the centrate will increase the load returned with the reject water from centrifuge dewatering and thus the overall load to the wastewater treatment process.

### 5.3 Digestion

#### 5.3.1 Predicted vs Actual Methane Production and Volatile Solids Reduction

The theoretical Methane production for the reactors in BMP 1 and BMP 2 were calculated according to the two methods described in Section 2.2.2 using a VS basis and a COD basis. The predicted values were based on the addition of "theoretically biodegradable" VS and COD which was calculated by subtracting the VS and COD contributed by the inoculum from the total VS and COD in the reactor. The theoretical values for the different substrate in BMP 1 and BMP 2 are presented in Table 5-1 and Table 5-2 respectively. As discussed previously, the concentrations of the reactor contents in BMP 1 were calculated rather than measured; therefore a range of predicted values is shown for the BMP 1 substrates, which takes into account the additional error incurred through the calculation.

Table 5-1 Theoretical Methane Production for BMP 1

	Based on VS			Based on COD		
	VS (mg)	CH <sub>4</sub> (mmol)	CH <sub>4</sub> (Nml)	COD (mg)	CH <sub>4</sub> (mmol)	CH <sub>4</sub> (Nml)
AS	262 - 636	5.8 - 14.1	130 - 315	399 - 901	6.2 - 14.1	140 - 315
WP	242 - 661	5.4 - 14.6	120 - 327	446 - 948	7.0 - 14.8	156 - 332
EA	242 - 655	5.4 - 14.5	120 - 325	560 - 949	8.8 - 14.8	196 - 332
MIX	257 - 642	5.7 - 14.2	127 - 318	565 - 1098	8.8 - 17.2	198 - 384

Table 5-2 Theoretical Methane Production for BMP 2

	Based on VS			Based on COD		
	VS (mg)	CH <sub>4</sub> (mmol)	CH <sub>4</sub> (Nml)	COD (mg)	CH <sub>4</sub> (mmol)	CH <sub>4</sub> (Nml)
AS	176	3.9	87	394	6.2	138
WP	203	4.5	100	395	6.2	138
EA	198	4.4	98	394	6.2	138
EA45	178	3.9	88	462	7.2	162

The observed methane production was lower than the theoretical methane production. This is not surprising since it is not expected that in activated sludge all the volatile material present will be biodegradable. This is particularly true when the activated sludge is from a secondary treatment process including biological nutrient removal (such as at the Harnaschpolder WWTP) where as a consequence of solids retention times greater than 10 days, partial sludge stabilization occurs in the activated sludge process (Bolzonella, Pavan, Battistoni, & Battistoni, 2005).

VSS removal in the range 20–35%, and gas production from 0.6 and 0.8 m<sup>3</sup>/kgVSS destroyed are generally found for mesophilic anaerobic digestion of waste activated sludge from secondary and BNR processes (Bolzonella, Innocenti, & Cecchi, 2002). Due to the uncertainty associated with the calculated pre-digestion sludge properties in BMP 1, and the nature of the AMPTS data which reports only methane and not total gas production, it is difficult to compare its results with these typical values. For BMP 2 however, the VS reduction percentages and gas production per gram of VS destroyed in BMP 2 were calculated and are presented in Table 5-3.

Table 5-3 Average Gas Production and VS Reduction in BMP 2

Substrate	VS Pre BMP 2 (g)	VS Post BMP 2 (g)	VS Reduction	Total Gas Production (ml)	m <sup>3</sup> /kgVS destroyed
Feed-AS	0.49	0.40	18%	75.2	0.8
WP-AS	0.52	0.40	23%	45.6	0.4
EA-AS	0.51	0.41	20%	71.5	0.7

The values for VS reduction and gas production per gram of VS destroyed in BMP 2 are very similar to the ranges reported as typical for activated sludge in the literature, indicating that the behaviour reported in BMP 2 is consistent with performance in other studies.

### 5.3.2 Impact of Denitrification

Both the BMP 1 and BMP 2 results reported in Table 4-15 and Table 4-16 indicate that the methane potential of the worm predated sludge appears to be lower than the methane potential of the typical activated and extended aerated sludges, with the WP-AS reactors producing 30% to 85% of the methane volume per mass of VS than the AS reactors. This disparity in Methane potential becomes less significant when the influence of denitrification on the readily biodegradable COD in the BMP reactors is taken into consideration. Table 4-17 and Table 4-21 show that, based on the nitrate concentration in the reactors prior to digestion, the COD consumed for denitrification in the WP-AS reactors was 27% (BMP 1) and 169% (BMP 2) greater than that consumed for denitrification in the AS reactors. If the substrates had been thickened prior to digestion, as would be common at a full scale WWTP, the majority of the nitrate would have been removed with the reject water and more COD would have been available for conversion to methane.

It was also not possible to calculate a BMP value for the EA45-AS sludge from the methane production in the bottles since the production by the inoculum blank reactors was higher than the production of the EA45-AS reactors. This observation is attributed to inhibition of the conversion of substrate COD to methane. One of the notable characteristics of the EA45-AS sludge is the high concentration of nitrate. For this reason it is conjectured that the reason for the limited methane production was the uptake of COD for denitrification and production of nitrogen gas.

In order to determine how the BMP results would be affected if the additional COD used for denitrification in the reactors had instead been converted to methane, the calculated amount of nitrogen gas which was produced and counted as methane was subtracted from the total methane production and the calculated amount of methane which would have been produced if the COD had been converted anaerobically was added to the total methane production. Given that denitrification of 1 g of  $\text{NO}_3\text{-N}$  will consume 8.6 gCOD (Henze, van Loosdrecht, Ekama, & Brdjanovic, 2008) and that the COD content of Methane is 4 gCOD/g $\text{CH}_4$ , denitrification of 1 g of COD will produce approximately 90 ml of nitrogen gas (at STP) while anaerobic digestion of that same gram of COD would produce 350 ml of  $\text{CH}_4$ . Due to the different procedures, in BMP 1 all nitrogen gas produced would have been counted as Methane, while in BMP 2 the nitrogen gas produced would have been attributed to  $\text{CO}_2$  and  $\text{CH}_4$  based on the ratios at which they were present in the gas. The corrected methane production and BMP values assuming anaerobic COD conversion to methane rather than anoxic denitrification are presented in Table 5-4 and Table 5-5 for BMP 1 and BMP 2 respectively.

Table 5-4 BMP 1 Values Corrected for Effect of Denitrification

Substrate	Original Methane Prod (ml)	COD Consumed for Denit. (mg)	$\text{N}_2$ counted as $\text{CH}_4$ (ml)	COD Consumed for Denit. as $\text{CH}_4$ (ml)	Corrected Methane Production (ml)	Corrected BMP (ml $\text{CH}_4$ /gVS added)
Feed-AS	82.0	2.0	0.2	0.7	82.5	103
WP-AS	77.2	53.5	4.8	18.7	91.1	122
EA-AS	86.0	20.4	1.8	7.1	91.3	122
Mix	82.0	9.2	0.8	3.2	84.4	107

Table 5-5 BMP 2 Values Corrected for Effect of Denitrification

Substrate	Original Methane Prod. (ml)	COD Consumed for Denit. (mg)	$\text{N}_2$ Counted as $\text{CH}_4$ (ml)	COD Consumed for Denit. as $\text{CH}_4$ (ml)	Corrected Methane Production (ml)	Corrected BMP (ml $\text{CH}_4$ /gVS added)
Feed-AS	59.3	1.3	0.1	0.5	59.6	204
WP-AS	36.1	33.7	2.4	11.8	45.6	107
EA-AS	55.6	3.7	0.3	1.3	56.6	166
EA45-AS	26.3	127.7	7.4	44.7	63.6	152

Due to the higher corrected BMP of WP-AS in BMP 1 and the higher corrected BMP of Feed-AS in BMP 2, it is difficult to conclude from these results whether or not worm predation will increase or decrease the methane potential of the remaining volatile solids relative to the methane potential of volatile solids in typical activated sludge. For the purposes of this thesis it is concluded that the volatile solids remaining after worm predation will have approximately the same methane potential as those found in activated sludge. In BMP 2, the EA45-AS substrate was included to see if worms increased the biodegradation potential of the VS remaining after worm predation. From the results presented above it does not appear that worm predation increases the biodegradability of the remaining volatile solids however as there is only one result which is based on the corrected BMP values it is difficult to make a concrete conclusion. Further BMP testing, preferably comparing thickened Feed-AS, WP-AS and EA45-AS to negate the influence of nitrate, should be conducted to confirm the conclusions drawn.

### **5.3.3 BMP 1 vs BMP 2**

The reason for the difference in behaviour in BMP 1 and BMP 2 is difficult to pinpoint due to the many differences in the methods of the two. First, BMP 1 used feed sludge from Batch 2 that had been diluted prior to worm predation while the worm reactor feed in BMP 2 was from Batch 5 and undiluted. Second, the effects of worm predation on the properties of the sludge substrates differ from batch to batch as can be seen in the results of the batch comparisons; and finally, the analytical methods of the two BMP tests were completely different as described in Section 3.3.1. These discrepancies are another reason that further BMP testing with consistent methodology is essential to increase the pool of data used to identify the effect of worm predation on the biodegradability of activated sludge.

### **5.3.4 COD Balance**

The COD balances presented in Table 4-17 and Table 4-21 show that the ratio of COD OUT to COD IN for most samples is close to 1, meaning that the COD entering and leaving the system is approximately equal. This is as it should be, since COD is a conservative property, and indicates that no other conversions are taking place that are not being accounted for. The one main exception is the extremely high ratio seen for the WP-AS sample in BMP 1, caused by the extremely high value of COD in the reactor post digestion. It is concluded that this measurement is not correct due to some error in the experimental procedure and should thus be ignored.

### **5.3.5 Change in Sludge Properties**

In order to compare how digestion of worm predated sludge affected the digested sludge properties, a comparison was made of the property masses per mass of substrate VS added, which will be referred to as the specific property content. The reason for looking at the specific property content was twofold. First it allowed for comparison on an equal footing of reactor contents where different volumes and concentration of substrate were added to the same amount of inoculum. Secondly because digester loading rates are based on the VS content of the sludge it allows for assessment of property changes per amount of VS added.

The specific Total Solids content of all three sludges in BMP 1 decreased after digestion, although a relatively small decrease was observed for the WP sample. It is considered likely that the lesser decrease for WP in BMP 1 is due to the uncertainty in the pre BMP solids content as in BMP 2 all sludges show a similar decrease in specific total solids.

The specific Volatile Solids content of the WP sludge in BMP 1 showed a slight increase during digestion whereas the AS and EA samples showed a decrease. It is again believed that the observed increase was due to the uncertainty in the pre BMP number due to its calculation and that instead the actual pre BMP value was in the higher range of the standard deviations. This theory is supported by the data from BMP 2 which shows a decrease in specific volatile solids for all three sludge samples with the highest reduction being seen in the WP sample.

The specific COD of the BMP 1 sludge showed a decrease for the AS and EA sludge, and a very large increase for the WP sludge. The observed increase is considered to result from a significant error in the measurement of the post BMP 1 WP COD concentration. Since methane was produced by the WP reactors and COD is a conservative property it is impossible that the concentration of COD in the reactors could increase at all let alone to the extent that is shown in figure 4-7. The probability that

the COD measurement for the WP sample was incorrect is supported by the data from BMP 2 which shows a decrease in the COD of all the sludges, although the decrease for the WP sample is slightly less than that of the others.

The specific soluble COD in both BMP 1 and BMP 2 showed an increase in the AS and EA sludges during digestion and a decrease in the WP sludge, although the changes were more pronounced in BMP 2. The trend appears to show a levelling out of the sCOD values after digestion which would indicate that irrespective of the sludge being digested, the level of sCOD remaining after digestion will be the same assuming the same VS loading rate. A potential reason for this levelling out trend of sCOD is that some hydrolysis occurs within the worms which increases the amount of specific soluble COD in the WP-AS substrate compared to the Feed-AS and EA-AS. The soluble COD made available by the worms in the WP-AS is will also be hydrolysed in the Feed-AS and EA-AS, however not until the anaerobic digestion process has begun. The soluble COD remaining in all 3 reactors after the BMP is at a similar level because it was the fraction which cannot be converted to methane.

The specific soluble nitrogen content of the three sludges in BMP 2 also shows this tendency toward levelling out. Their nitrogen content in all three sludge increased to a similar level, however the increase in the WP sludge was much lower since it started at a higher level than the AS and EA sludge. This indicates that irrespective of the sludge being digested, the level of sN remaining after digestion will be the same assuming the same VS loading rate. It is possible that this levelling out seen in the nitrogen content is due to the greater amount of nitrogen in the form of nitrate that is in the WP-AS substrate. This nitrate, as discussed before, is converted to nitrogen gas in the anaerobic reactors and thus removed from the sludge. However the levelling out process occurs, it does not change the expectation that nitrogen levels post digestion will be similar regardless of the substrate used.

The specific soluble phosphate in BMP 1 shows a general increase in all three sludges, but as the increase in the WP sample is lower than that of the other two, the levelling out effect appears again. This is different than what was observed in BMP 2 where again all sludges showed an increase in the soluble phosphate content, however no levelling out appeared but the increase was approximately 100% of the initial starting value for all the sludges.

Due to the greater reliability of the data obtained from BMP 2, the expected changes of the properties in the different sludges during digestion are based more heavily on this data. Of course the behaviour observed in the BMP bottles cannot be directly applied to the behaviour that will be expected in a full scale digester since many components of the full scale digestion (pre thickening, mixing with primary sludge, etc.) have not been incorporated. Instead the behaviour is used to indicate what alterations might occur from digesting WP-AS rather than WAS.

It is predicted from the data that in the digestion of WP sludge rather than AS, the percentage of VS destruction will remain relatively unchanged while the TS concentration after digestion will be slightly higher for worm predated sludge due to the lower initial VS content of the worm predated sludge. Similarly the COD reduction during digestion of WP sludge will be slightly lower than that of AS since a portion of the Biodegradable COD has already been consumed by the worms. The concentration of soluble COD and soluble Nitrogen after digestion are expected to be the same regardless of the sludge digested due to the levelling out of the specific contents described above. The sP concentration after digestion is expected to be higher if WP sludge is used rather than AS, however the degree to which it will increase is unclear.

#### 5.4 Dewatering of Digested Sludge

In order to determine if a worm predation based waste activated sludge reduction technology would impact the dewatering performance of the digested sludge, various dewatering analyses were performed on the digested sludge produced by BMP 1 and BMP 2. It should be noted that buffer, macronutrients, trace elements and water were added to the BMP 1 mixtures of substrate and inoculum to achieve the same volume in all reactors, while in BMP 2 the substrate and inoculum mixtures were left undiluted and thus are expected to better represent the dewatering behavior that could be expected at the WWTP. However, the substrate samples for both BMP 1 and BMP 2 were not thickened prior to digestion thus the solids percentage of the digested sludge in both cases was lower than what is typical for anaerobic sludge. Additionally at the WWTP Harnaschpolder, as at many WWTP with anaerobic digestion facilities, thickened waste activated sludge is mixed with thickened primary settled sludge prior to digestion. None of the BMP tests incorporated primary sludge, thus there was additional deviation from the conditions that would be seen at the wastewater treatment plant.

First, the digested sludge was centrifuged to determine if the relative cake solids percentage was affected by the digestion of worm predated or extended aerated sludge rather than typical waste activated sludge. Two different speeds were used to dewater the sludge. After BMP 1, and at the lower speed, the digested WP-AS achieved a slightly higher cake solids percentage than the digested AS, while after BMP 2 the digested AS had the higher percentage. In both cases, the digested EA-AS had the highest cake solids percentage. Due to the similarity of the values found for all samples and their standard deviations, it does not appear that there was any significant difference found in the achieved cake solids percentages between the three digested sludges from either of the BMPs. While acknowledging that conditions for centrifuging the digested samples in the lab vary greatly from those that would be experienced at the wastewater treatment plant, the data obtained suggests that no difference in dewatering cake solids percentage would be expected from dewatering digested sludge that included worm predated sludge rather than typical activated sludge.

The centrate from the laboratory centrifuge tests was also compared to determine if the recycle streams would be affected by the digestion of worm predated sludge rather than AS. This comparison was again done on a mass per gram of substrate VS added basis in order to account for the different amounts and concentrations of substrate added to the reactors prior to digestion. Although the magnitude of the percent changes between the Digested Feed-AS centrate and the digested WP-AS centrate properties are not consistent between the 2 BMP tests, the trends of increase or decrease are. It appears that by using worm predated substrate rather than activated sludge, the concentration of both COD and phosphate in the centrate will increase while the concentration of ammonia will decrease. It should be noted once more however that the substrates used in the BMPs had not been thickened, thus the amount of soluble nutrients which went into the reactors was much higher than would be typical.

The ease with which a sludge will release water is important for the rate of dewatering that can be achieved and is directly related to the sludge's filterability or conversely related to its resistance to filtration. The SFR and CST measurements were used to compare the filterability of the digested AS, WP-AS and EA-AS samples to determine how the use of worm predated sludge would affect dewatering. Based on the CST analysis the sludge with the highest filterability was the digested EA sludge followed by the WP and then the AS. The SRF gave slightly different results. While all 3 sludges were found to fall into the more easily dewatered sludge range, the SRF test indicated that the digested WP sludge had the lowest resistance to filtration followed by the EA and then the AS. In either case the results indicate that digested WP sludge will have a higher rate of dewatering than the AS.

Based on the centrifugation and filterability testing it appears that using digested worm predated rather than typical digested activated sludge will not increase the percentage of cake solids achieved but will improve the ease of dewaterability. This means that dewatering of digested worm predated sludge is likely to require less conditioning than AS to achieve the same percent solids in the same amount of time.

## 6 Computer Model

The modeling component of this thesis was aimed at determining what impact the implementation of worm predation, or a worm predation based technology, for waste secondary sludge reduction would have on the overall operation of a wastewater treatment plant. This was done by first setting up a BASELINE MODEL in BioWin of a wastewater treatment plant operating without any sludge reduction technology. The laboratory results were then used to predict what differences in sludge quality could be expected between worm predated sludge rather and simple activated sludge. The BASELINE MODEL was then adjusted using additional BioWin process units to mimic the effects of worm predation on the waste secondary sludge in a WORM PREDATION MODEL. The changes in performance seen between the BASELINE MODEL and the WORM PREDATION MODEL were then compared with respect to effluent quality and solids treatment performance solid treatment performance and compared to the behavior predicted by the laboratory results.

### 6.1 Baseline Wastewater Treatment Model

The sludge used in the lab scale worm reactor, and the anaerobic sludge used for the BMP analysis, came from the WWTP Harnaschpolder, therefore the baseline wastewater treatment process was modeled with a similar design and configuration. For the sake of simplicity, only one train of the treatment process handling  $\frac{1}{4}$  of the plants inflow was included in the model.

#### 6.1.1 Influent

The dry weather flow was calculated from daily 2012-2013 influent data obtained from the WWTP Harnaschpolder which is included in Appendix D. The cutoff for dry weather flow was recommended as 150,000 m<sup>3</sup>/day combined flow from the 2 influent mains. (Paul Weij, personal communication, August 29, 2013) The averages for all flow and loads data on days with inflow equal to or less than this figure were used to determine the average dry weather flow and are shown in Table 6-1. Note that the flow shown represents  $\frac{1}{4}$  of the total flow to the plant as it has been adjusted for the 1 train represented by the model.

*Table 6-1 2013 Dry Weather Influent Characteristics Model Assumptions*

<b>Annual Daily Flow</b>	36609	m <sup>3</sup> /day
<b>Total COD</b>	625	g/m <sup>3</sup>
<b>Total Kjeldhal Nitrogen</b>	64	g/m <sup>3</sup>
<b>Total Phosphorus</b>	9	g/m <sup>3</sup>
<b>Temp</b>	17	deg C

As the Harnaschpolder WWTP is a municipal system it is assumed that the daily variations in flow and organics concentration to the treatment plant tend to follow the diurnal patten typically observed at such facilities. The multiplication factors of the diurnal flow and concentration patterns shown in Figure 6-1 were applied to the average values assumed above to create a 24 hour dynamic influent.

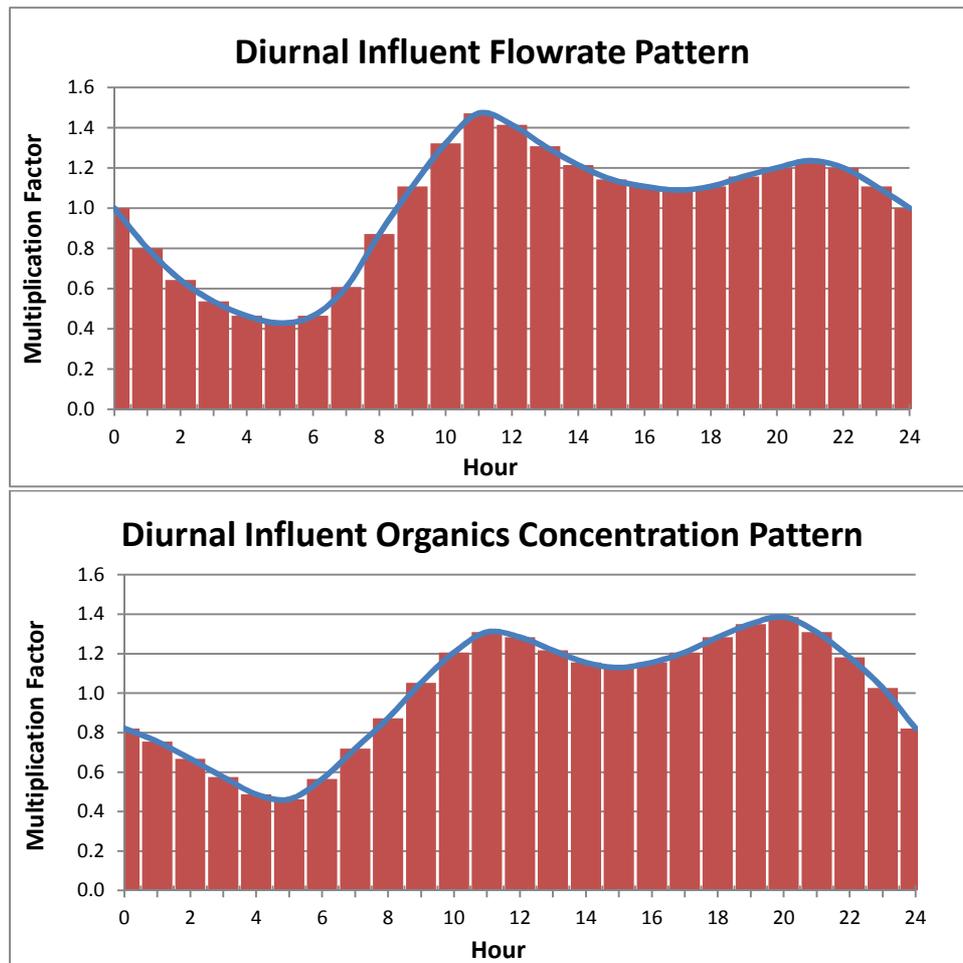


Figure 6-1 Diurnal Influent Patterns (Adapted from Figure 3-6 in Metcalf & Eddy, 2004)

### 6.1.2 Treatment Configuration

The wastewater treatment train receiving  $\frac{1}{4}$  of the total influent to the plant includes one (1) primary clarifier, two (2) biological processes tanks and four (4) secondary clarifiers.

The biological process tanks at Harnaspolder are circular tanks divided up into 4 zones. The first zone is a selector used to produce sludge with good settling properties by providing high levels of organics and nutrients to prevent growth of the filamentous bacteria which leads to bulking sludges. The second zone is for pre-denitrification of the return sludge. The third zone is anaerobic to stimulate biological phosphate removal. Ferric Chloride is also added for chemical phosphorus removal just upstream of the biological process and accounts for approximately 40% of the phosphorus removal in the plant (Paul Weij, personal communication, September 9, 2013). Finally there is a combination aerobic/anoxic zone, where alternating periods of aeration create aerobic and anoxic conditions. During the aerobic periods, organic matter is reduced and ammonia and organic nitrogen is oxidized to nitrate. In the following anoxic periods the nitrate is converted to nitrogen gas which returns to the atmosphere (Nickels, 2009). Aeration is executed based on an online  $\text{NH}_4\text{-N}$  concentration measurement. The aeration starts with  $\text{NH}_4\text{-N}$  higher than 1 mg N/l. The oxygen set-point during this aeration is 0.5 mg  $\text{O}_2$ /l. Aeration stops with a concentration in the aeration/anoxic tank  $\text{NH}_4\text{-N} < 0.1$  mg N/l. The typical aeration time of one cycle is around 75 minutes, anoxic time around 30 minutes per cycle. After the biological tanks the wastewater is directed to a degassing chamber before being distributed between the secondary clarifiers. The  $\text{NH}_4\text{-N}$  online measurement is taken in this degassing chamber. (Paul Weij, personal communication, August 26, 2013) It is also from this tank that the Feed-AS for the worm reactor was obtained.

The solids treatment process consists of a gravity thickener for primary sludge, a centrifuge thickener for secondary sludge, an anaerobic digester for stabilization of thickened primary and secondary

sludge and a centrifuge for dewatering of digested sludge. Recycle flows from the thickening and dewatering are returned to the primary clarifier.

The process flow diagram of the modeled section of the Harnaschpolder as it looks in the BioWin simulator is shown in Figure 6-2.

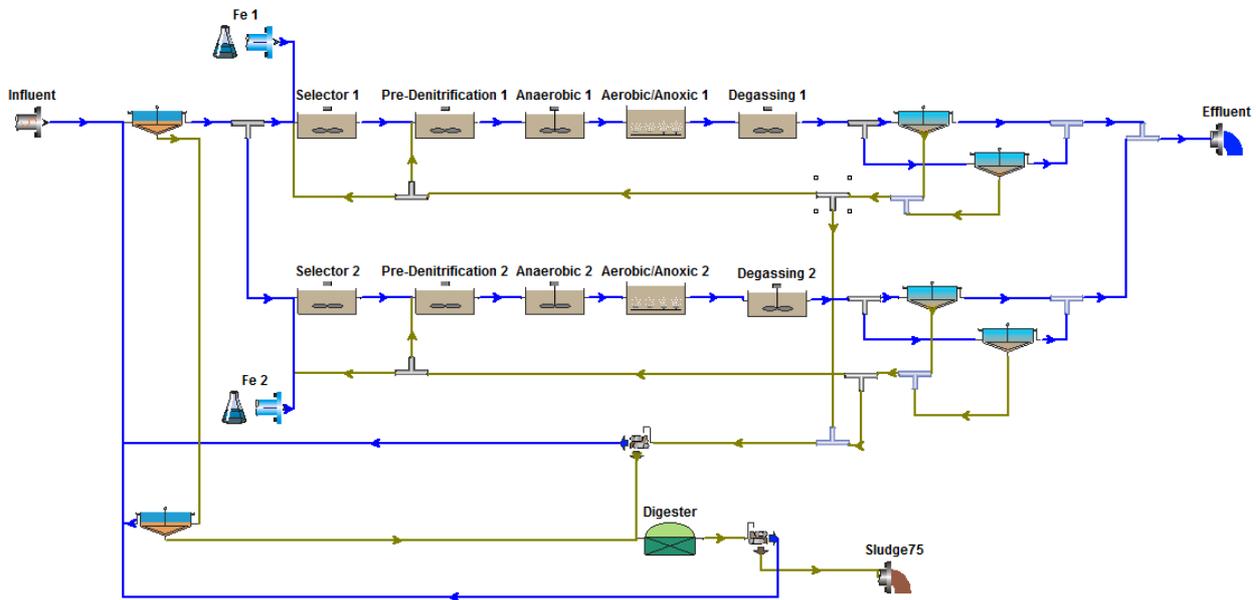


Figure 6-2 Biowin Process flow Diagram

The tanks in the model were sized based on information published by Delfluent Services (See Appendix E) and performance based on information obtained from Metcalf & Eddy 2004. It was not possible in the basic BioWin 3 simulator<sup>3</sup> used to set the aeration pattern in the Aerobic/Anoxic zone based on the ammonia content of the degassing tank as is done at Harnaschpolder. Instead the schedule for aeration was entered manually. This scheduled aeration pattern along with the underflow percentages, return sludge percentage, and ferric chloride addition were adjusted to ensure the effluent would meet the targets set by the following discharge requirements of WWTP Harnaschpolder:

- Nitrogen 10 mg N/l (yearly average)
- Phosphate 1 mg P/l (10-days rolling average)
- Chemical Oxygen Demand (COD) 125 mg/l (daily average)
- Biochemical Oxygen Demand (BOD) 20 mg/l (daily average)
- Total Suspended Solids (TSS) 30 mg/l (daily average)

(Delfluent Services bv, 2013)

Although the nitrogen and phosphorus limits are both long term averages, since the influent data was based on a 1-day cycle the goal was to achieve the given limits as a daily average.

The dimensions and input values for each process unit of the base model are summarized in Table 6-2 to Table 6-8 along with their source and/or any additional relevant information. Parameters not specified were left as the default values of the proprietary BioWin model.

<sup>3</sup> BW Controller is an additional module produced by EnviroSim Associates Ltd. which augments BioWin models with a range of process control simulation capabilities commonly employed in wastewater treatment systems such as the ability to base aeration on ammonia concentration. (envirosim.com)

Table 6-2 Primary Clarifier Model Information

<b>Number</b>	1	
<b>BioWin Unit Type</b>	Ideal Clarifier	
<b>Surface Area</b>	1735 m <sup>2</sup>	Delfluent Services
<b>Depth</b>	3.5 m	Delfluent Services
<b>Solids Removal Efficiency</b>	50%	Adjusted to meet targets (initially 65% M&E Figure 5-46)
<b>Target Underflow Percent Solids</b>	2-6%	M&E Table 14-19
<b>Underflow (% of Influent)</b>	0.4%	Adjusted to meet targets

Table 6-3 Biological Tank Model Information

<b>Number</b>	2 trains	
<b>BioWin Unit Type</b>	Bioreactor	Separate bioreactor for each zone
<b>Selector Zone Volume</b>	625 m <sup>3</sup>	Delfluent Services
<b>Pre-Denitrification Zone Volume</b>	610 m <sup>3</sup>	Delfluent Services
<b>Anaerobic Zone Volume</b>	3865 m <sup>3</sup>	Delfluent Services
<b>Aerobic/Anoxic Zone Volume</b>	20700 m <sup>3</sup>	Delfluent Services
<b>Target MLSS</b>	3500-4000 mg/l	Delfluent Services
<b>DO Setpoint during Aeration</b>	0.5 mg/l	Delfluent Services
<b>Oxygen Modeling</b>	Used	
<b>RAS (% of Influent)</b>	46 - 47%	Adjusted to meet targets
<b>WAS (% of Influent)</b>	1.4 - 1.5%	Adjusted to meet targets
<b>Aerobic/Anoxic Zone Aeration Pattern</b>	3 hours aerated 2 hours unaerated	Adjusted to meet targets
<b>Ferric Chloride Addition (Mass Fe)</b>	Paced at 95% influent tP	Adjusted to meet targets

Table 6-4 Secondary Clarifier Model Information

<b>Number</b>	4	
<b>BioWin Unit Type</b>	Model Clarifier	
<b>Surface Area</b>	2507 m <sup>2</sup>	Delfluent Services
<b>Depth</b>	3.5 m	Delfluent Services
<b>Underflow (% of Influent)</b>	12%	Adjusted to meet targets

Table 6-5 Primary Sludge Gravity Thickening Model Information

<b>Number</b>	1	
<b>BioWin Unit Type</b>	Ideal Clarifier	
<b>Surface Area</b>	354 m <sup>2</sup>	Delfluent Services
<b>Depth</b>	5.3 m	Delfluent Services
<b>Solids Removal Efficiency</b>	90%	M&E Table 14-48
<b>Target Underflow Percent Solids</b>	5-10%	M&E Table 14-19
<b>Underflow (% of Influent)</b>	0.2%	Adjusted to meet targets

Table 6-6 Secondary Sludge Centrifuge Thickening Model Information

<b>Number</b>	1	
<b>BioWin Unit Type</b>	Dewatering Unit	
<b>Solids Removal Efficiency</b>	95%	M&E Table 14-48 (assume chemical addition)
<b>Target Underflow Percent Solids</b>	4-8%	M&E Table 14-48
<b>Underflow (% of Influent)</b>	0.3%	Adjusted to meet targets

*Table 6-7 Anaerobic Digestion Model Information*

<b>Number</b>	1	
<b>BioWin Unit Type</b>	Anaerobic Digester	
<b>Volume</b>	5800 m <sup>3</sup>	Delfluent Services (1 quarter total digestion volume)
<b>Depth</b>	4.5 m	Biowin Default

*Table 6-8 Digested Sludge Centrifuge Dewatering Model Information*

<b>Number</b>	1	
<b>BioWin Unit Type</b>	Dewatering Unit	
<b>Solids Removal Efficiency</b>	92%	M&E Table 14-48 (assume chemical addition)
<b>Target Underflow Percent Solids</b>	22%	M&E Table 14-48
<b>Underflow (% of Influent)</b>	0.07%	Adjusted to meet targets

Due to the on-off aeration pattern used in the aerobic/anoxic zone it was not possible to do a steady state simulation of the system. Therefore a dynamic simulation had to be run each time model parameters were changed. To get to a stable situation this meant running a simulation from seed for duration of approximately 150 days. This created the rather unrealistic situation where many dry weather flow days were being simulated back to back which caused the COD removal in the primary clarifier to be much higher than would typically be seen at a Dutch WWTP when a solids removal percentage of 65% (typical for the conditions) was applied. The high COD removal in the primaries limited the ability to denitrify downstream in the aerobic/anoxic zone. In order to remedy this issue and achieve effluent TN levels below the limit of 10 mg/l, the set removal in the primary clarifier was reduced to 50%.

### 6.1.3 Baseline Performance

The focus of the modeling was on how the addition of a worm predation based sludge reduction technology would affect the performance of a WWTP. In order to do this it was important to first have a frame of reference. To this effect the baseline wastewater treatment plant model (BASELINE MODEL) was used to establish the "typical" performance of the Harnaschpolder WWTP.

The overall performance of the baseline plant is shown in Figure 6-3 which shows the effluent concentrations of regulated parameters over 30 days of stable operation. The average effluent values for the baseline operation are presented in Table 6-9. The baseline solids treatment performance is summarized by process unit in Table 6-10 to Table 6-12.

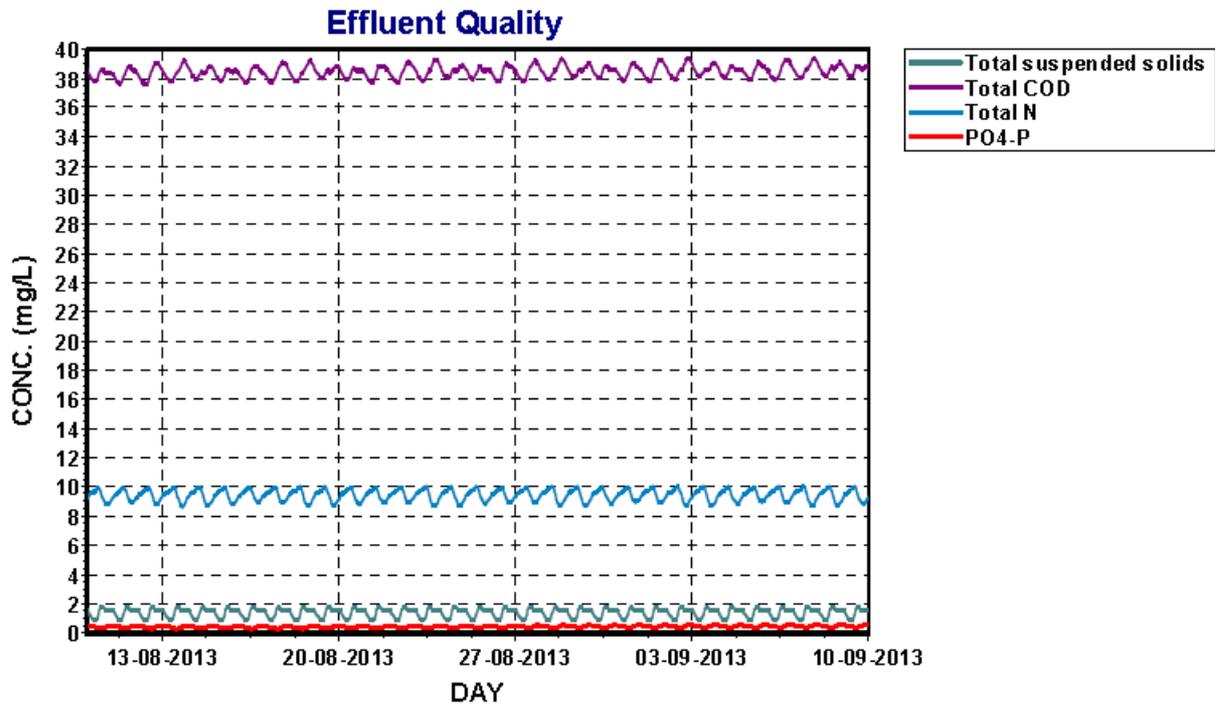


Figure 6-3 BASELINE MODEL Effluent Quality

Table 6-9 Average Daily Baseline WWTP Performance

<b>Effluent TSS</b>	1.4 mg/l
<b>Effluent tCOD</b>	39 mg/l
<b>Effluent tN</b>	9.4 mg/l
<b>Effluent PO<sub>4</sub>-P</b>	0.55 mg/l

Table 6-10 Average Daily Baseline Thickener Performance

<b>Thickened Flow</b>	110 m <sup>3</sup> /d
<b>Thickened Total Solids</b>	5.7%
<b>Thickened Volatile Solids</b>	2.8%
<b>Centrate Flow</b>	417 m <sup>3</sup> /d
<b>Centrate TSS</b>	790 mg/l
<b>Centrate VSS</b>	390 mg/l
<b>Centrate COD</b>	620 mg/l
<b>Centrate TN</b>	42 mg/l
<b>Centrate sPO<sub>4</sub>-P</b>	0.5 mg/l

Table 6-11 Average Daily Baseline Digester Performance

<b>Solids Loading Rate</b>	2.1 kgVSS/m <sup>3</sup> .day
<b>Hydraulic Retention Time</b>	33
<b>VSS Destruction</b>	48%
<b>Biogas (Dry) Flow Rate</b>	3630 m <sup>3</sup> /day
<b>Methane Content of Biogas</b>	63%
<b>Methane Production</b>	2286 m <sup>3</sup> /day

*Table 6-12 Average Daily Baseline Dewatering Performance*

<b>Dewatered Sludge Flow</b>	26 m <sup>3</sup> /d
<b>Dewatered Total Solids</b>	29.8%
<b>Dry Solids</b>	7.75 tonnes/day
<b>Centrate Flow</b>	157 m <sup>3</sup> /d
<b>Centrate TSS</b>	4200 mg/l
<b>Centrate VSS</b>	2100 mg/l
<b>Centrate COD</b>	3400 mg/l
<b>Centrate TN</b>	1100 mg/l
<b>Centrate sPO<sub>4</sub>-P</b>	420 mg/l

## 6.2 Worm Predation Model

The laboratory results presented in the previous two chapters provided the foundation for determining what changes needed to be made to the BASELINE MODEL to represent a system that includes worm predation, or a worm predation based technology as a sludge minimization technique, in a WORM PREDATION MODEL.

While the sludge used in the experimental program was unsettled activated sludge taken from the degassing tank in order not to limit the aeration in the worm reactor, it was assumed that any sludge minimization technology would be implemented after settling of activated sludge in the secondary clarifiers in the waste secondary sludge stream to reduce tankage requirements. For this reason the unthickened waste sludge stream is the location in which it was assumed that worm predation is implemented (see Figure 6-4), and thus where modifications to the BASELINE MODEL were incorporated to mimic the conversion of conversion of waste AS to WP-AS.

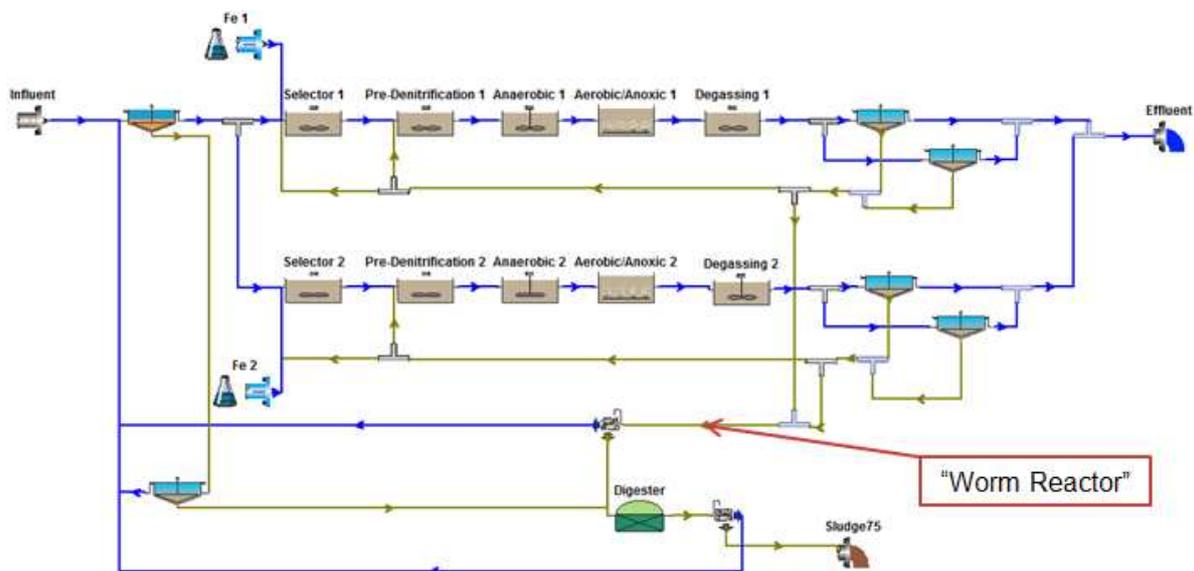


Figure 6-4 Location of "Worm Reactor" in BioWin Model

The laboratory batch experiments indicate that during worm predation of activated sludge there will be an average reduction of 36% TSS, 41% VSS and 53% total COD, and an increase of 141% soluble COD, 591% soluble nitrogen, and 4057% soluble phosphate. The conversion of AS to WP-AS in the unthickened waste sludge stream therefore needed to be incorporated into the BASELINE MODEL to develop the new WORM PREDATION MODEL. Because the standard deviations for the average changes were relatively large, target ranges for the reduction or increase in worm predated sludge properties were selected as targets for the Worm Predation Model:

- 25-45% TSS Reduction
- 30-50% VSS Reduction
- 45-60% COD Reduction
- 50-250% sCOD Increase
- 350-850% sN Increase
- 1000-7000% sP Increase

The BASELINE MODEL was used to determine what the concentrations of these components would be in the waste secondary sludge stream prior to worm predation and the target ranges for the percent changes were used to determine what range the concentration should fall within after worm predation. The following description of how worm predation was incorporated into the model will be discussed in terms of the average values, however in the actual model calculations were made for each of the 24 hours to account for the varying flows and concentrations of waste sludge resulting from the variable daily influent described in Section 6.1.1.

These average Pre Worm concentrations found from the BASELINE MODEL and the target ranges for Post Worm Predation are reported in Table 6-13.

*Table 6-13 Average Concentration of Properties in Baseline Activated Sludge and Targets Concentrations for Worm Predated Sludge*

Sludge Component	Pre Worm Concentration From BASELINE MODEL (mg/l)	Post Worm Target Concentration Range for WORM PREDATION MODEL (mg/l)
TSS	12,500	6875 – 9375
VSS	6,200	3100 – 4340
tCOD	9,300	3720 – 5115
sCOD	38	57 – 133
sN*	7.2	32.4 – 68.4
sPO <sub>4</sub> -P	0.55	5.5 – 38.5

\*sN is the sum of Ammonia-N, Nitrate-N and Nitrite-N

### 6.2.1 TSS, VSS and tCOD Reduction

The reduction of TSS, VSS and COD was the first consideration when determining how to mimic worm predation with a BioWin Model. Table 6-13 shows that the majority of the COD in the waste sludge is particulate while only a small fraction is soluble. This meant that the content of all three components could be reduced to approximately the same degree by setting a fixed solids removal percentage using a Dewatering Unit element where soluble components are not removed. The Dewatering Unit alone however was not sufficient to comprise the total removal of the three compounds since the target removals for all three constituents were not the same. With worm predation, the removal of VSS and COD is higher than the removal of TSS because the worms will utilize a portion of the biodegradable organic content. This meant that a biological element was also needed in the model to facilitate additional VSS and COD destruction. The Aerobic Digester element was chosen for this purpose in BioWin due to the ease of monitoring the percentage of VSS destruction (an element specific variable) which is also a good indicator of COD destruction in activated sludge. Since using a biological process will effect on all of the sludge properties, when combining the Aerobic Digester with the dewatering unit to simulate the worm predation it was decided that the digester should be first in the process line followed by the more easily adjusted dewatering unit. The target VSS destruction in the Aerobic Digester was chosen as 10%. The dewatering unit was set to remove 35% solids with an underflow of 1 m<sup>3</sup>/d. The addition of the Aerobic digester as part of the model to mimic worm predation is also appropriate as it creates an aerobic environment in the secondary sludge as an aerated worm reactor would.

### 6.2.2 Soluble COD, Nitrogen and Phosphate Increase

The second consideration when determining how to mimic worm predation with BioWin Model was the increase in the concentrations of soluble COD, nitrogen and phosphate. Due to the implementation of the Aerobic Digester for COD and VSS removal the concentration of these soluble properties changed from those in the waste secondary sludge. The Post Aerobic Digestion Averages of the soluble components are shown in Table 6-14.

*Table 6-14 Comparison of Aerobically Digested Soluble Sludge Properties with Post Worm Target Values*

Sludge Component	Post Worm Target Concentration Range for WORM PREDATION MODEL	Post Aerobic Digestion Concentrations (mg/l)
sCOD	57 – 133	41
sN	32.4 – 68.4	55.4
sPO <sub>4</sub> -P	5.5 – 38.5	39.8

The values of the soluble components after aerobic digestion showed that only the soluble COD still needed to be increased to the levels expected after worm predation. The required sCOD increase was accomplished by the addition of a State Variable Influent Stream with a constant flow of 1 m<sup>3</sup>/d to the Worm Predation Model between the Aerobic Digester Element (Worm organic removal) and the Dewatering Unit Element (worm solids removal) added to the waste secondary sludge stream prior to thickening. These three components for the model "Worm Rector" as pictured in Figure 6-5.

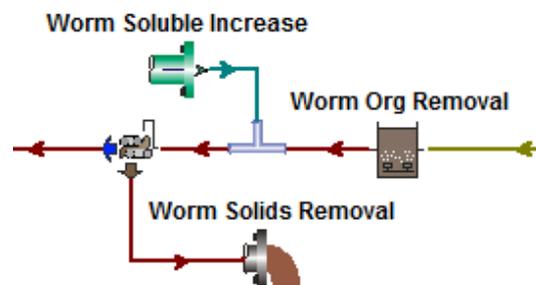


Figure 6-5 Worm Reactor added to the Waste Secondary Sludge Line

It should be noted that the total volumetric flow through the "Worm Reactor" remains consistent since the flow rate of the Dewatering Unit Underflow is equal to the Flowrate of the State Variable Influent.

In BioWin sCOD, or "filtered COD", is a combined variable which is calculated as the sum of the following soluble state variables that are expressed in COD:

- Dissolved  $H_2$  ( $S_{H_2}$ ),
- Methanol ( $S_{bMeth}$ ),
- Acetate ( $S_{bsa}$ ),
- Propionate ( $S_{bsp}$ ),
- Readily Biodegradable Complex COD ( $S_{bsc}$ ),
- Soluble Inert COD ( $S_{us}$ )
- Slowly Biodegradable Colloidal COD ( $X_{sc}$ )

(EnviroSim Associates Ltd).

This meant that the remaining increase in sCOD had to be distributed among the individual state variables which make it up. The fractionation of the soluble COD in the worm predated samples was not determined experimentally, thus assumptions had to be made to determine how the total sCOD increase would be distributed:

- **$S_{H_2}$**  – No increase in the dissolved hydrogen gas concentration due to worm predation was assumed as it is primarily a function of water temperature and partial pressure.
- **$S_{bMeth}$**  – No increase in methanol concentration was assumed that due to the aerated environment of the worm reactor.
- **$S_{bsa}$  and  $S_{bsp}$**  – The VFA analysis of the BMP 2 reactor contents prior to digestion shown in Table 4-20 and the proportions of the BMP 2 reactor contents shown in Table 4-18 made it possible to extrapolate the concentrations of acetic acid and propionic acid in the Batch 5 Feed-AS, and WP-AS samples. The extrapolated concentrations are presented in Table 6-15.

Table 6-15 Extrapolated VFA values in Sludge Samples.

	Acetic Acid subs (mg/l)	Propionic Acid subs (mg/l)
Feed-AS	8.1	0.0
WP-AS	28	3.4

A 250% increase in acetate concentration due to worm predation was observed. An increase in propionate was also observed however since the initial value in the Feed-AS was 0 mg/l, a percentage increase could not be determined from the data. It is assumed that both of these components will increase due to worm predation and thus needs to be accounted for in the model.

- **$S_{bsc}$ ,  $S_{us}$  and  $X_{sc}$**  – Due to the expected disturbance to the sludge particles during aeration and worm predation it is considered likely that worm predation facilitates the conversion of particulate COD to slowly biodegradable colloidal COD, readily biodegradable complex COD and soluble inert COD ( $S_{us}$ ) therefore an increase in these compounds is also assumed.

Since both the Nitrogen and Phosphate increases were on the higher end of their target ranges it was assumed that the sCOD increase would be as well. A blanket increase of 250% was therefore applied to the Pre Worm concentrations of Acetate, Propionate, Readily Biodegradable Complex COD, Soluble Inert COD, and Slowly Biodegradable Colloidal COD to determine the target Post worm concentration. The BASELINE MODEL was used to determine the fractionation of sCOD before Worm Predation. The average values are presented in Table 6-16 along with the calculated target values post worm predation, and the values post aerobic digestion which the State Variable Influent needed to adjust them from.

*Table 6-16 sCOD Targets and Model Concentrations for Calculation*

sCOD Component	Pre Worm Concentration From BASELINE MODEL (mg/l)	Post Worm Target Concentration for WORM PREDATION MODEL (mg/l)	Post Aerobic Digestion Concentrations (mg/l)
$S_{H2}$	0.27	0.27	0
$S_{pMeth}$	0	0	0
$S_{bsa}$	0.6	2.1	0
$S_{bsp}$	0.65	2.3	0
$S_{bsc}$	1.49	5.2	0.75
$S_{us}$	35	123	34
$X_{sc}$	0	0	0

The mass of each sCOD state variable that needed to be added was calculated using the concentrations presented in Table 6-16 and the unthickened secondary waste sludge flow rate. The mass was then distributed to the set State Variable Influent flowrate of 1 m<sup>3</sup>/d. As noted above, the average values were presented in the description for simplicities sake, while in the model, the variable waste sludge flow, and property concentration were used to determine the amount and type of sCOD that needed to be added for each hour in the 24 hour dynamic flow cycle.

### 6.2.3 Solids Processing Changes

Due to the removal of solids in the worm reactor, the operation of the Secondary Sludge Centrifuge Thickener had to be adjusted. The target percent solids for thickened worm predated sludge did not change from those reported Table 6-6 because the results of laboratory centrifuge tests indicated that the percent solids achieved by the thickening would be unaffected by worm predation. An initial trial of the WORM PREDATION MODEL showed that after the secondary sludge thickener instead of an increase in the centrate COD due to worm predation as occurred in the lab, there was a decrease of 30% in the model. Since the soluble component of COD is unaffected by the dewatering unit the decrease must be due to more particulates remaining in the worm predated supernatant. The solids removal percentage of the thickener was therefore adjusted to 80%. The underflow of the thickener was reduced from 0.3% to 0.15% of the influent flow to achieve approximately 5% solids in the thickened secondary sludge.

No adjustment to the operation of the Anaerobic Digester were required since the Laboratory results indicated that the methane potential of the volatile solids remaining after worm predation was similar to that of typical activated sludge, and that sludge properties in the digester are not expected to be significantly different than those which would be expected from the digestion of activated sludge.

Due to the reduction in thickened sludge, and consequently digested sludge flow, the Digested Sludge Centrifuge Dewatering needed to be adjusted to achieve the target percent solids shown in Table 6-8. The target percent solids for dewatered digested worm predated sludge did not change from those expected from typical digested waste activated sludge because the results of laboratory centrifuge tests indicated that the percent solids achieved by the dewatering would be unaffected by worm predation. The underflow of the dewatering unit was reduced from 0.07% to 0.05% of the influent flow to achieve approximately 22% solids in the dewatered sludge.

### 6.3 Modeled Impacts of Worm Predation

The WORM PREDATION MODEL was used to determine how the performance of the plant would be affected by the implementation of worm predation or a worm predation based WAS reduction technology.

#### 6.3.1 Wastewater Treatment

The overall performance of the treatment plant with worm predation is presented in Figure 6-6 which shows the effluent concentrations of regulated parameters over 30 days of stable operation. The average effluent values for the baseline operation are presented in Table 6-17.

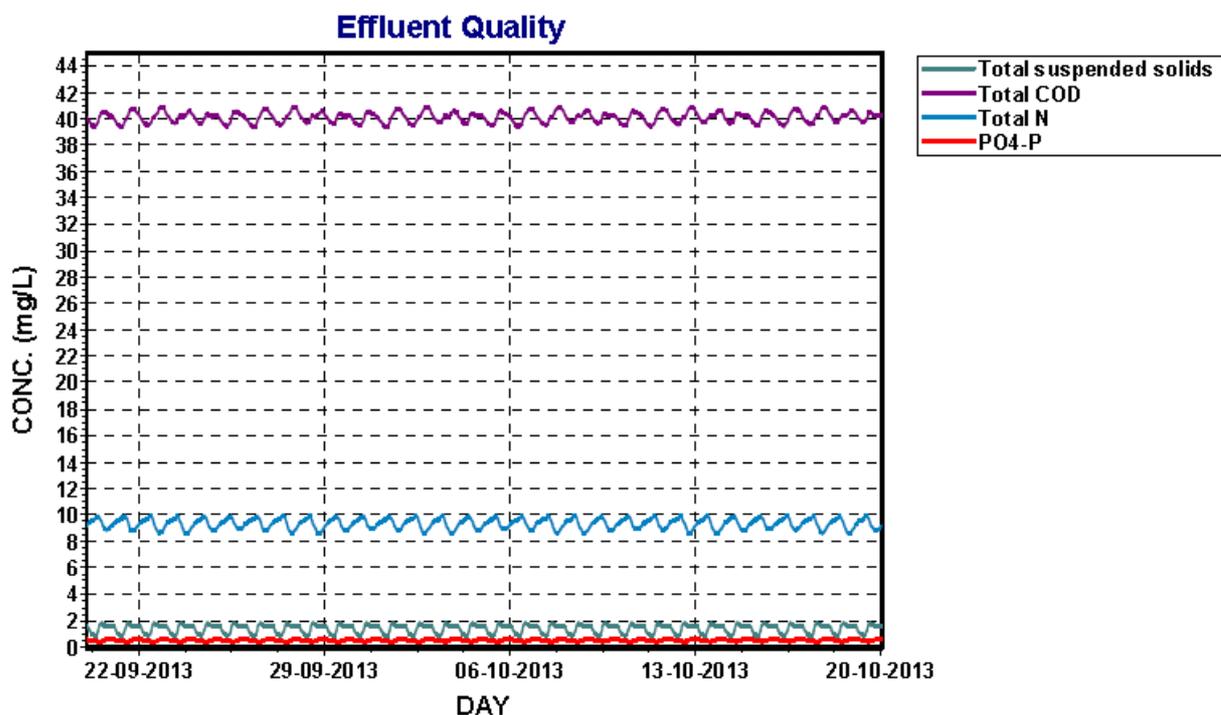


Figure 6-6 WORM PREDATION MODEL Effluent Quality

Table 6-17 Average Daily Worm Predation WWTP Performance

Effluent TSS	1.4 mg/l
Effluent tCOD	40 mg/l
Effluent tN	9.2 mg/l
Effluent PO <sub>4</sub> -P	0.50 mg/l

Comparison of Figure 6-6 and Table 6-17 with Figure 6-3 and Table 6-9 shows that implementing Worm Predation does not significantly influence the effluent quality of the modeled wastewater treatment plant. This indicates that using worm predation or a worm predation based technique for WAS reduction would not cause operational issues for the overall process.

#### 6.3.2 Solids Processing

The solids treatment performance in the WORM PREDATION MODEL is summarized by process unit in Table 6-18 to Table 6-20. In order to determine how well the WORM PREDATION MODEL correlates with the experimentally determined effects of worm predation on solids processing, the model results were compared with the observations made in the laboratory.

Table 6-18 Average Daily Worm Predation Thickener Performance

<b>Thickened Flow</b>	55 m <sup>3</sup> /d
<b>Thickened Total Solids</b>	5.9%
<b>Thickened Volatile Solids</b>	2.8%
<b>Centrate Flow</b>	470 m <sup>3</sup> /d
<b>Centrate TSS</b>	1730 mg/l
<b>Centrate VSS</b>	810 mg/l
<b>Centrate COD</b>	1350 mg/l
<b>Centrate TN</b>	125 mg/l
<b>Centrate sPO<sub>4</sub>-P</b>	40 mg/l

In the WORM PREDATION MODEL, the average thickened secondary sludge flow is 50% less than the BASELINE MODEL and the centrate flow is 13% higher in order to maintain the same percent solids in the thickened sludge. This is to be expected since the total amount of solids being thickened decreased due to the worm predation. The concentration of nutrients in the centrate from the secondary sludge thickener increased by 198% for TN and by 7900% for soluble phosphate, and the concentration of COD increased by 118%. The modeled total nitrogen increase is fairly consistent with the laboratory results as the increase in ammonia and nitrate accounts for the majority of the total nitrogen increase. The increase in phosphate is higher than what was predicted with the laboratory centrifuge however it corresponds well with the increase seen in the sludge properties. It is possible that the changes in the laboratory centrate were less pronounced due to particulates from the cake entering the supernatant samples when they were decanted.

Table 6-19 Average Daily Worm Predation Digester Performance

<b>Solids Loading Rate</b>	1.6 kgVSS/m <sup>3</sup> .day
<b>Hydraulic Retention Time</b>	47
<b>VSS Destruction</b>	55%
<b>Biogas (Dry) Flow Rate</b>	3420 m <sup>3</sup> /day
<b>Methane Content of Biogas</b>	62%
<b>Methane Production</b>	2120 m <sup>3</sup> /day

In the WORM PREDATION MODEL, the overall volatile solids loading rate to the anaerobic digester decreased with respect to the BASELINE MODEL due to the reduction in thickened secondary sludge achieved by the "worm reactor". The lower loading resulted in an increase of 42% in the hydraulic retention time and thus greater volatile solids reduction in the digester. Although the amount of volatile solids being fed to the digester was 23% less in the WORM PREDATION MODEL, the increased VS reduction resulted in only a slight reduction of 6% in biogas production. There WORM PREDATION MODEL also showed slight reduction in the methane content of the biogas leading to an overall reduction of 7% in methane production.

It is difficult to directly compare the modeled behavior of the anaerobic digester to the observations made during the BMP in the laboratory since in the model the waste secondary sludge is being digested along with the primary sludge and in both systems the majority of volatile solids to the digester originate in the primary sludge stream. The organic content of primary sludge is highly biodegradable since it has undergone essentially no biological conversions prior to anaerobic digestion, thus the expected VSS destruction and methane potential of the primary volatiles solids is higher than activated sludge which will be reflected in the modeled results.

*Table 6-20 Average Daily Worm Predation Dewatering Performance*

<b>Dewatered Sludge Flow</b>	17.3 m <sup>3</sup> /d
<b>Dewatered Total Solids</b>	29.8%
<b>Dry Solids</b>	5.15 tonnes/day
<b>Centrate Flow</b>	110 m <sup>3</sup> /d
<b>Centrate TSS</b>	4100 mg/l
<b>Centrate VSS</b>	2000 mg/l
<b>Centrate COD</b>	3300 mg/l
<b>Centrate TN</b>	1200 mg/l
<b>Centrate sPO<sub>4</sub>-P</b>	540 mg/l

The primary goal of worm predation was solids reduction. Its effectiveness can be seen in the reduction of dewatered solids which require disposal. The BASELINE MODEL showed a sludge production of approximately 7.75 tonnes dry solids per day while in the WORM PREDATION MODEL this value was reduced by 33.5% to a value of 5.15 tonnes dry solids per day. The changes in the properties of the centrate recycle due to dewatering of digested worm predated sludge rather than digested activated sludge were minimal. This is to be expected as the differences between the digested sludge properties were fairly low. The projected increases in the centrate concentrations seen in the laboratory were likely due to the fact that unthickened sludge was digested in the two BMPs. Reduction in the dewatering rates due to worm predation could not be modeled as the dewater unit in the model is based on a set removal percentage and does not reflect the dewatering properties of the sludge.

#### 6.4 Model Reflection

The BioWin model created for this MSc thesis accomplished its objective of providing a method of comparison between operation of an activated sludge treatment plant with and without worm predation as a method of waste activated sludge reduction. However, through the experience gained in building and using the model to compare Baseline and Worm Predation performance it was possible to see in retrospect that endeavoring to create a model based on the Harnaschpolder WWTP process may not have been the most suitable means of making the comparison.

The first difficulty with the model as it is described above was the excessive time required to achieve stable operating conditions in the dynamic simulations which were required by on-off aeration pattern in the biological tanks. The inability to use simple steady state simulations to quickly determine changes in operating conditions slowed the process of adjusting the model and interpreting its results. A simpler model representing a more typical activated sludge process with continuously aerated or unaerated tanks would have in all probability been sufficient for comparing how the implementation of worm predation affected the plant's baseline performance.

The second difficulty was the inability to recreate Harnaschpolder's ammonia based control system for the aeration in the aerobic/anoxic zone in the standard BioWin 3 Simulator. Having to schedule a time-based aeration pattern through trial and error to achieve the required effluent quality was not only time consuming, it limited the flexibility of the model to accommodate different loading and temperature conditions since the optimum aeration pattern changed depending on the process temperature.

## 7 Conclusions and Recommendations

The preceding chapters have presented work performed to determine how worm predation of activated sludge when used as a sludge reduction technique will affect the properties of the waste secondary sludge and how those changes in properties might impact the performance of a full scale wastewater treatment plant. The work was separated into two main components. Laboratory experiments were used to assess the changes in sludge properties which resulted from worm predation and how worm predated solids processing behaviour differed from that of typical activated sludge. Computer modelling was subsequently used to compare the performance of a Baseline wastewater treatment plant with the performance with one that incorporated worm predation for waste secondary sludge reduction. This chapter will present the conclusions drawn from the work presented with respect to worm predation of activated sludge, solids processing of worm predated sludge, their impact on wastewater treatment plant performance, and a brief discussion respecting the costs and benefits which would be expected from full scale implementation. Finally recommendations will be made respecting further study of this topic.

### 7.1 Worm Predation of Activated Sludge

The following conclusions have been made about the impact of worm predation of activated sludge on Sludge Properties:

- When using worm predation with *T. tubifex* treating activated sludge in a batch process, it takes approximately 3 days to have worm predated sludge with characteristics distinguishable from the effects of endogenous respiration.
- Worm predation increases the rate at which total solids, volatile solids and COD are reduced in an aerated batch process.
- Worm predation of activated sludge decreases TS, VS and COD content of the sludge and increases the concentration of soluble nitrogen, soluble phosphate and soluble COD. Soluble COD increase seems to be in part due to an increase in the concentration of the volatile fatty acids acetate and propionate, which may indicate that anaerobic processes are occurring within the worm's digestive tracts.

### 7.2 Solids Processing of Worm Predated Sludge

The following conclusions have been made about the solids processing behaviour of worm predated sludge compared with that of typical activated sludge:

- Worm predated sludge has improved settling characteristics when compared to activated sludge or extended aeration sludge, with lower 30 min SVIs and zone settling velocities 10 times greater than those of activated sludge.
- Worm predation does not appear to impact the achievable cake solids percentage from centrifuges thickening, however due to the increases in soluble sludge properties described in conclusion 3, the concentrations of nitrogen phosphate and COD in the centrate will increase.
- The methane potential of the volatile solids remaining after worm predation is expected to be the same as the methane potential of the volatile solids in activated sludge as long as there is no nitrate in the sludge to induce COD consumption for denitrification.
- After anaerobic digestion of Activated Sludge or Worm Predated Sludge, the digested sludge properties are expected to be similar regardless of the type of substrate used, assuming that the same concentration of sludge and the same volatile solids loading rate is applied.
- No difference is expected in the cake solid percentage achieved through centrifuge dewatering of digested activated sludge or digested worm predated sludge, however digested WP-AS has less resistance to filtration than digested AS.

### 7.3 Impacts of Worm Predation Based Sludge Reduction on WWTP Performance

The following conclusions have been made about the impacts that implementation of a worm predation based WAS reduction process would have on WWTP operations:

- The overall effluent quality from the wastewater treatment plant does not appear to be affected by the addition of worm predation as a sludge reduction technique, thus implementation of such a technology should be possible with little to no modification to the water treatment process.
- While the achievable thickened cake solids percentage from centrifugation of waste secondary sludge is not expected to change due to worm predation, the improved settling characteristics of the worm predated sludge may lead to improved thickening performance in facilities which instead employ gravity thickening for this purpose.
- The decrease in total and volatile solids through worm predation translates to a decrease in the amount of sludge being sent to anaerobic digestion.
  - At treatment plants with their own anaerobic digestion and cogeneration facilities the reduction in VS loading will increase the retention time in the digester leading to an increase in VS reduction and thus only a slight reduction in methane production. Alternatively, the unused capacity in the existing digester resulting from the reduction in loading can be used to accommodate other waste streams with greater methane potential (e.g. fats oils and grease, primary or mixed sludge from other facilities, surplus worms from the reactor, etc) which could increase the overall methane and energy production at the plant as well as generate a source of income from tipping fees.
  - Treatment plants without their own anaerobic digestion facilities will benefit from the reduction in the total amount of solids which must be shipped elsewhere for processing and the costs associated with it.
- The reduced resistance to filtration seen in the digested WP-AS indicates that dewatering of this sludge will likely require less conditioning than digested AS to achieve the same dewatering rates, which could be a means of cost saving for the treatment facility. It should be noted that the implications of the digestion with primary sludge on the dewaterability were not investigated. The overall reduction in the amount of solids being thickened and dewatered with the implementation of worm predation would ultimately reduce the amount of conditioning chemicals required for both thickening and dewatering.

#### 7.4 Costs and Benefits of Full Scale Implementation

While a full scale cost benefit analysis regarding the implementation of a worm reactor for waste secondary sludge reduction was not included in the scope of this thesis, a brief analysis can be made of the potential sources of costs and benefits indicated by the results presented above. The list of items identified, their influence as a cost or a benefit, and the additional information required to perform a detailed economic evaluation are given in Table 7-1.

Table 7-1 Overview of Expected Costs and Benefits of Full Scale Implementation

Item	Cost/Benefit	Additional Information Required
<b>Worm Reactor Construction</b>	Cost	Worm Reactor configuration and components
		Retention time in continuous Worm Reactor process
		Optimal worm density at startup
<b>Worm Reactor Operation</b>	Cost	Oxygen requirements. Hendrix 2010 indicates 0.5-1 mg O <sub>2</sub> per g VSS digested by <i>Lumbriculus variegatus</i>
		Energy requirements of aeration blowers
		Electricity prices
<b>Reduction in Waste Secondary Sludge</b>	Cost	Energy cost of reduced Methane production
		Electricity prices
	Benefit	Additional capacity available for co-digestion of additional waste streams
		Tipping fees for accepting additional waste streams for co-digestion
		Energy saved by additional Methane production from co-digestion of additional waste streams
		Electricity prices
		Chemical conditioner required per mass of secondary sludge thickened
		Chemical conditioner prices
<b>Reduction in Total Dewatered Sludge Production</b>	Benefit	Disposal prices per mass of dewatered sludge
<b>Improved Filterability of WP-AS</b>	Benefit	Chemical conditioner required per mass digested WP-AS dewatered
		Chemical conditioner required per mass digested typical AS dewatered
		Chemical conditioner prices

### **7.5 Recommendations for Further Work**

The following recommendations respecting future work on measuring and modelling the effects of worm predation on wastewater treatment plant operation are based on the experience gained during this MSc Thesis:

- Include detailed fractionation of total and soluble COD pre and post worm digestion to better understand the conversions taking place inside the worms
- Investigate the change in sludge properties under conditions which are more representative of those expected at a full scale WWTP including but not limited to:
  - Worm predation of settled activated sludge
  - BMPs with thickened WP-AS and Feed-AS
  - Including co-digestion with primary sludge in BMPs
  - Dewatering studies of sludge produced in the aforementioned BMPs
- Conduct all BMPs with a standardized protocol
- In future modelling to assess impacts of worm predation on an activated sludge process it would be advisable to use a more conventional process which does not include the on-off aeration pattern of Harnaschpolder to allow for the less time-consuming steady state analysis to find stable performance conditions.
- In future modelling of Harnaschpolder in BioWin use of the BW Controller module could allow for modelling aeration based on ammonia concentration as it is at the WWTP.
- Detailed economic analysis of the costs and benefits associated with the addition of

## Works Cited

- APHA. (2012). *Standard Methods for the Examination of Water and Wastewater* (22nd ed.). Washington DC: American Public Health Association.
- Appels, L., Baeyens, J., Degreve, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, 755-781.
- Bioprocess Control AB. (2013). *Automatic Methane Potential Test System Operation and Maintenance Manual*. Retrieved October 2013, 15, from Bioprocess Control: <http://www.bioprocesscontrol.com/media/45898/Manual-AMPTS-II-version-1.6.pdf>
- Burgess, J. E., & Pletschke, B. I. (2008). Hydrolytic enzymes in sewage sludge treatment: A mini-review. *Water SA*, 343-349.
- Elissen, H. J. (2007). *Sludge reduction by aquatic worms in wastewater treatment with emphasis on the potential application of Lumbriculus variegatus*. Wageningen: PhD thesis Wageningen University.
- Elissen, H. J., Hendrickx, T. L., Temmink, H., & Buisman, C. J. (2006). A new reactor concept for sludge reduction using aquatic worms. *Water Research*, 3713-3718.
- EnviroSim Associates Ltd. (n.d.). User Manual for Biowin 3.
- Europa. (2012, September 18). *Sewage Sludge: Introduction*. Retrieved March 26, 2013, from Europa: <http://ec.europa.eu/environment//waste/sludge/index.htm>
- Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews - Microbiology*, 623-633.
- Guo, X.-s., Liu, J.-x., Wei, Y.-s., & Li, L. (2007). Sludge reduction with Tubificidae and the impact on the performance of the wastewater treatment process. *Journal of Environmental Sciences*, 257-263.
- 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*, 4923-4929.
- Hendrickx, T. L., Temmink, H., Elissen, H. J., & Buisman, C. J. (2008). The effect of operating conditions on aquatic worms eating waste sludge. *Water Research*, 943-950.
- Hendrickx, T. L., Temmink, H., Elissen, H. J., & Buisman, C. J. (2009a). Aquatic worms eating waste sludge in a continuous system. *Bioresource Technology*, 4642-4648.
- Hendrickx, T. L., Temmink, H., Elissen, H. J., & Buisman, C. J. (2009b). Aquatic worms eat sludge: Mass balances and processing of worm faeces. *Journal of Hazardous Materials*, 633-638.
- Hendrickx, T. L., Temmink, H., Elissen, H. J., & Buisman, C. J. (2010). Design parameters for sludge reduction in an aquatic worm reactor. *water research*, 1017-1023.
- Henze, M., van Loosdrecht, M. C., Ekama, G. A., & Brdjanovic, D. (2008). *Biological Wastewater Treatment: Principles Modelling and Design*. London: IWA Publishing.
- Huang, X., Liang, P., & Qian, Y. (2007). Excess sludge reduction induced by Tubifex tubifex in a recycled sludge reactor. *Journal of Biotechnology*, 443-451.

## Works Cited

- Liang, P., Huang, X., Qian, Y., Wei, Y., & Ding, G. (2006). Determination and comparison of sludge reduction rates caused by microfaunas' predation. *Bioresource Technology*, 854–861.
- Liu, Y., & Fang, H. H. (2003). Influences of Extracellular Polymeric Substances (EPS) on Flocculation, Settling, and Dewatering of Activated Sludge. *Critical Reviews in Environmental Science and Technology*, 237-273.
- Metcalf & Eddy. (2004). *Wastewater Engineering: Treatment and Reuse* (International ed. 4th ed.). Singapore: McGraw-Hill.
- Microscopy Resource Center. (2012). *Tubifex (Annelida)*. Retrieved April 8, 2013, from Microscopy Resource Center:  
<http://www.olympusmicro.com/micd/galleries/moviegallery/pondscum/annelida/tubifex/>
- Milieu Ltd, WRc and RPA for the European Commission, DG Environment under Study Contract DG ENV.G.4/ETU/2008/0076r. (2010). *Environmental, economic and social impacts of the use of sewage sludge on land*. Brussels: Milieu Ltd.
- Novak, J. T. (2006). Dewatering of Sewage Sludge. *Drying Technology*, 1257-1262.
- Novak, J. T., Agerbæk, M. L., Sørensen, B. L., & Hansen, J. A. (1999). Conditioning, Filtering, and Expressing Waste Activated Sludge. *Journal of Environmental Engineering*, 816-824.
- Perez-Elvira, S. I., Diez, P. N., & Fdz-Polanco, F. (2006). Sludge minimisation technologies. *Reviews in Environmental Science and Bio/Technology*, 375-398.
- Ratsak, C. H., & Verkuijlen, J. (2006). Sludge reduction by predatory activity of aquatic oligochaetes in wastewater treatment plants: science or fiction? *Hydrobiologia*, 197-211.
- Sanin, F. D., Clarkson, W. W., & Vesilind, P. A. (2011). *Sludge Engineering: The Treatment and Disposal of Wastewater Sludges*. Lancaster: DEStech Publications Inc.
- Tamis, J., van Schouwenburg, G., Kleerebezem, R., & van Loosdrecht, M. C. (2011). A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant. *Water Research*, 5916-5924.
- US EPA. (1987, September). *Design Manual: Dewatering Municipal Wastewater Sludges*. Retrieved May 25, 2013, from National Service Center for Environmental Publications:  
<http://nepis.epa.gov/Exe/ZyNET.exe/300045WA.txt?ZyActionD=ZyDocument&Client=EPA&Index=1986%20Thru%201990&Docs=&Query=%28pilot%29%20OR%20FNAME%3D%22300045WA.txt%22%20AND%20FNAME%3D%22300045WA.txt%22&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry>
- van Handaal, A., & van der Lubbe, J. (2007). *Handbook Biological Wastewater Treatment - Design of Activated Sludge Systems*. Leidschendam: Quist Publishing.
- van Loosdrecht, M. C., Ekama, G. A., Wentzelm, M. C., Brdjanovic, D., & Hooijmans, C. M. (2008). Modelling Activated Sludge Processes. In M. Henze, M. C. van Loosdrecht, G. A. Ekama, & D. Brdjanovic, *Biological Wastewater Treatment: Principals, Modelling and Design* (pp. 361-392). London: IWA Publishing.
- Vavilin, V. A., Fernandez, B., Palatsi, J., & Flotats, X. (2008). Hydrolysis kinetics in an aerobic degradation of particulate organic material: an overview. *Waste Management*, 939-951.

## Works Cited

- Vesilind, A. P. (1988). Capillary Suction Time as a Fundamental Measure of Sludge Dewaterability. *Journal (Water Pollution Control Federation)*, 215-220.
- Vesilind, A. P. (1994). Sludge dewatering : why the water will win every time. *Proceedings of the 49th Industrial Waste Conference* (pp. 1-8). West Lafayette, Indiana: CRC Press.
- Wei, Y., & Liu, J. (2005). The discharged excess sludge treated by Oligochaeta. *Water Research Technology*, 265-272.
- Wei, Y., Van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003). Minimization of excess sludge production for biological. *Water Research*, 4453-4467.
- Wei, Y., van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003). Minimization of excess sludge production for biological wastewater treatment. *Water Research*, 4453-4467.
- Wei, Y., Wang, Y., Guo, X., & Liu, J. (2009b). Sludge reduction potential of the activated sludge process by integrating an oligochaete reactor. *Journal of Hazardous Materials*, 87-91.
- Wei, Y., Zhu, H., Wang, Y., Li, J., Zhang, P., Hu, J., et al. (2009a). Nutrients release and phosphorus distribution during oligochaetes predation on activated sludge. *Biochemical Engineering Journal*, 239-245.

Works Cited

## Appendix A – Oligochaete Predation Literature Review Table

Study	Primary Worm(s)	Experimental Setup	Findings of Note									
<b>(Wei &amp; Liu, 2005)</b>	<i>Tubifex tubifex</i>	Continuous plug flow reactors treating waste AS. 1 control and 1 inoculated with the sessile worm <i>T. tubifex</i>	<ul style="list-style-type: none"> <li>Average sludge reduction based on TSS: 59% in worm reactor; 14% in control</li> <li>Small free-swimming worms from activated sludge process colonized both reactors after 35 days</li> <li>Presence of free-swimming worms increased sludge reduction % in control and reduced it in worm reactor</li> <li>Presence of both sessile and free-swimming worms improved settling characteristics of sludge</li> </ul>									
<b>(Elissen, Hendrickx, Temmink, &amp; Buisman, 2006)</b>	<i>Lumbriculus variegatus</i>	Sequencing batch treating nitrifying waste AS. 1 control and 1 with sessile worm <i>L. variegatus</i> immobilized on carrier material and separated compartment for worm feces collection	<ul style="list-style-type: none"> <li>Presence of worm increased solids reduction rate by 49 mg TSS/d</li> <li>75% of solids consumed by worms was digested while remaining 25% converted to faeces</li> <li>Worm feces settles faster and better than initial waste AS. SVI in ml/g</li> </ul> <table border="1"> <thead> <tr> <th></th> <th>Feces</th> <th>AS</th> </tr> </thead> <tbody> <tr> <td>SVI<sub>5min</sub></td> <td>80</td> <td>220</td> </tr> <tr> <td>SVI<sub>30min</sub></td> <td>61</td> <td>113</td> </tr> </tbody> </table>		Feces	AS	SVI <sub>5min</sub>	80	220	SVI <sub>30min</sub>	61	113
	Feces	AS										
SVI <sub>5min</sub>	80	220										
SVI <sub>30min</sub>	61	113										
<b>(Elissen, 2007)</b>	<i>Lumbriculus variegatus</i>	Batch experiments in non-aerated Petri dishes or Erlenmeyer flasks with forced aeration. Control experiments and experiments with <i>L. variegatus</i> in settled sludge layer	<ul style="list-style-type: none"> <li>Presence of worms increases sludge reduction rate by ~ 2 times but does not change final reduction percentage achieved by both worm and control of 54%</li> <li>Minimum worm/sludge ratio of 0.4 (based on dry solids) to see difference in rate. Likely specific to <i>L. variegatus</i></li> <li>20-40% of the AS VSS reduction converted to worm biomass</li> </ul>									
<b>(Guo, Liu, Wei, &amp; Li, 2007)</b>	<i>Branchinria Sowerbyi</i> and <i>Limnodrilus</i> sp.	Continuous feed worm reactor treating waste (stage 1) and return (stage 2) sludge from integrated oxidation ditch with vertical circle	<ul style="list-style-type: none"> <li>Average sludge reduction rate 46.4% in stage 1</li> <li>Sludge yield in stage 2 a magnitude of 10<sup>4</sup> times lower than recommended values for conventional oxidation ditch treatment</li> <li>Worm predation caused an increase in soluble COD, TN and TP in the worm reactor</li> </ul>									
<b>(Huang, Liang, &amp; Qian, 2007)</b>	<i>Tubifex tubifex</i>	<ol style="list-style-type: none"> <li>Continuous worm reactor w/ varying worm density treating return AS for a conventional activated sludge process treating synthetic waste</li> <li>24 hr. batch tests with different worm densities</li> </ol>	<ul style="list-style-type: none"> <li>Optimal worm density for sludge reduction ability was 2500 mg/l based on dry weight</li> <li>Increase in dissolved COD, ammonia and phosphorus rates in sludge clearly correlate with an increase in worm density</li> <li>Although dissolved components increased in the return sludge</li> </ul>									
<b>(Hendrickx, Temmink, Elissen, &amp; Buisman, 2008)</b>	<i>Lumbriculus variegatus</i>	Sequencing batch treating nitrifying waste AS. 1 control and 1 with sessile worm <i>L. variegatus</i> immobilized on carrier material and separated compartment for worm feces	<ul style="list-style-type: none"> <li>DO &gt; 8 mg/l leads to highest sludge consumption rate but TSS reduction of only 36%. DO &lt; 2.5 mg/l leads to sludge consumption rate 4 times lower but high TSS reduction of 77%</li> <li>Ammonia release by worms of 0.02</li> </ul>									

Study	Primary Worm(s)	Experimental Setup	Findings of Note
		collection	<p>mg NH<sub>3</sub>/mg TSS digested</p> <ul style="list-style-type: none"> <li>• Increase in ammonia concentrations slowed sludge consumption rates. pH dependent as only unionized ammonia is toxic</li> <li>• Optimum reactor temp 15°C. Maximum reactor temp 25°C.</li> <li>• Light does not impact worm activity</li> <li>• Worm reactor estimated to increase WWTF's O<sub>2</sub> consumption 15-20%, ammonia load 5% and hydraulic load 5-15%</li> </ul>
<b>(Hendrickx, Temmink, Elissen, &amp; Buisman, 2009a)</b>	<i>Lumbriculus variegatus</i>	Continuous reactor treating waste activated sludge with <i>L. variegatus</i> immobilized on carrier material and separated compartment for worm feces collection	<ul style="list-style-type: none"> <li>• TSS reduction 16-26%, sludge consumption rate 39 - 92 mg TSS/(g ww d)</li> <li>• Organic content of sludge appears to have a direct correlation with TSS reduction % and an indirect correlation with sludge consumption rate</li> <li>• Worms contributed to 41-71% of VSS reduction while rest was from natural sludge breakdown</li> <li>• Worm growth is necessary in continuous operation to counteract losses of worm biomass in effluent</li> <li>• Growth rate of worms was limited by immobilization on carrier. No growth at mesh size of 300 µm. Growth rate of 0.013 /d at mesh size of 350 µm still less than 0.05-0.11 reported for immobilized worms</li> </ul>
<b>(Wei, et al., 2009a)</b>	<i>Lumbriculida hoffmeisteri</i>	Batch tests with sterilized sludge, activated sludge and deionized water feed to worms and control reactors	<ul style="list-style-type: none"> <li>• Nitrogen release in both sterilized and activated sludge was higher in sludge with worms than sludge without worms or worms without sludge</li> <li>• Phosphorus release in sterilized sludge was higher in sludge with worms than sludge without worms or worms without sludge</li> <li>• Phosphorus release in activated sludge was higher in sludge with worms and sludge without worms than worms without sludge</li> </ul>
<b>(Wei, Wang, Guo, &amp; Liu, 2009b)</b>	<i>Limnodrilus hoffmeisteri</i>	Continuous worm reactor treating discharged excess sludge from integrated oxidation ditch with vertical cycle (IODVC) and then returning it to the process	<ul style="list-style-type: none"> <li>• Worm reactor and oligochaete presence had little effect on sludge yield</li> <li>• Worm reactor improved sludge settling characteristics</li> <li>• Phosphorus release in effluent of IODVC observed</li> </ul>

Study	Primary Worm(s)	Experimental Setup	Findings of Note
<b>(Hendrickx, Temmink, Elissen, &amp; Buisman, 2009b)</b>	<i>Lumbriculus variegatus</i>	Sequencing batch treating bioP waste AS. 1 control and 1 with sessile worm <i>L. variegatus</i> immobilized on carrier material and separated compartment for worm feces collection	<ul style="list-style-type: none"> <li>• 21% TSS reduction and 26%vss reduction with worms</li> <li>• SVI 84% lower for worm feces then WAS and settled solids concentration was 84% higher</li> <li>• SRF 30% higher for worm feces</li> <li>• 48% less time needed with vacuum filtration of worm feces to achieve 3% solids then with WAS</li> <li>• Solids concentration achieved with centrifugation of worm feces was 9% higher than with WAS</li> <li>• Increase of 5% for nitrogen and 10% for phosphorus to internal WWTF load from processing worm feces expected</li> <li>• 40% less methane production from digesting worm feces rather than WAS</li> <li>• Worm predation of anaerobically digested sludge possible if first washed to remove ammonia</li> <li>• Highest overall solids reduction from combining worm predation with digestion (50%)</li> </ul>
<b>(Hendrickx, Temmink, Elissen, &amp; Buisman, 2010)</b>	<i>Lumbriculus variegatus</i>	Sequencing batch experiments of worm reactor with sessile worm <i>L. variegatus</i> immobilized on carrier material treating waste sludge where either the design variables or the origin of sludge treated was altered	<ul style="list-style-type: none"> <li>• Decrease in worm growth rate with increase in worm density</li> <li>• Worm density above 2 kgww/m<sup>2</sup> for maximum sludge consumption rate</li> <li>• Sludge load over 100 mg TSS/g ww.day should be maintained to prevent substrate limitation</li> <li>• Specific oxygen uptake rate of 4.9 mg O<sub>2</sub>/g ww.day or 0.5-1 mg O<sub>2</sub>/ mg VSS digested</li> <li>• Carrier mesh size of 350 μm optimal for maintaining stable worm behavior</li> </ul>
<b>(Hendrickx, Elissen, Temmink, &amp; Buisman, 2011)</b>	<i>Lumbriculus variegatus</i>	Lab scale sequencing batch worm reactor and large scale continuous worm reactor with <i>L. variegatus</i> immobilized on carrier material treating WAS from lab scale activated sludge system	<ul style="list-style-type: none"> <li>• Large scale continuous reactor feasible</li> <li>• Net growth of worm biomass at a rate of 0.014 d</li> <li>• Release of nutrients by the worms was 17 mg PO<sub>4</sub>-P/g TSS digested and 55 mg (NH<sub>4</sub>-N β NO<sub>3</sub>-N)/g TSS digested</li> </ul>
<b>(Tamis, van Schouwenburg, Kleerebezem, &amp; van Loosdrecht, 2011)</b>	<i>Aulophorus furcatus</i>	Pilot scale worm reactor treating waste activated sludge prior to anaerobic sludge holding tank	<ul style="list-style-type: none"> <li>• Sludge reduction in worm reactor 30-40% on TSS basis</li> <li>• Additional reduction of sludge observed in anaerobic holding tank observed for an estimated 65% total reduction</li> </ul>



## Appendix B – BMP 1 Individual Data

Table B-1 AS Reactors Cumulative Methane Production Raw Data

Day	AS 1 CH <sub>4</sub> Volume [Nml]	AS 2 CH <sub>4</sub> Volume [Nml]	AS 3 CH <sub>4</sub> Volume [Nml]
0	0	0	0
1	18.5	18.1	18
2	32.7	32.4	32.1
3	42.4	42	41.6
4	48.5	47.7	47.5
5	54.2	53	52.8
6	58.2	57	56.7
7	61.5	59.9	59.7
8	64.3	63	62.6
9	66.6	66.1	65.1
10	68.9	69.2	67.5
11	70.6	72	69.3
12	71.5	74.6	
13	72.3	77	
14	73.2	77.5	
15	74.1	77.9	
16	74.9	78.4	
17	75.8	78.8	
18	76.7	79.3	
19	77.5	79.7	
20	77.7	80.1	
21		80.6	
22		81	
23		81.5	
24		81.9	
25		82.4	
26		82.8	
27		83.2	
28		83.7	
29		84.1	
30		84.5	

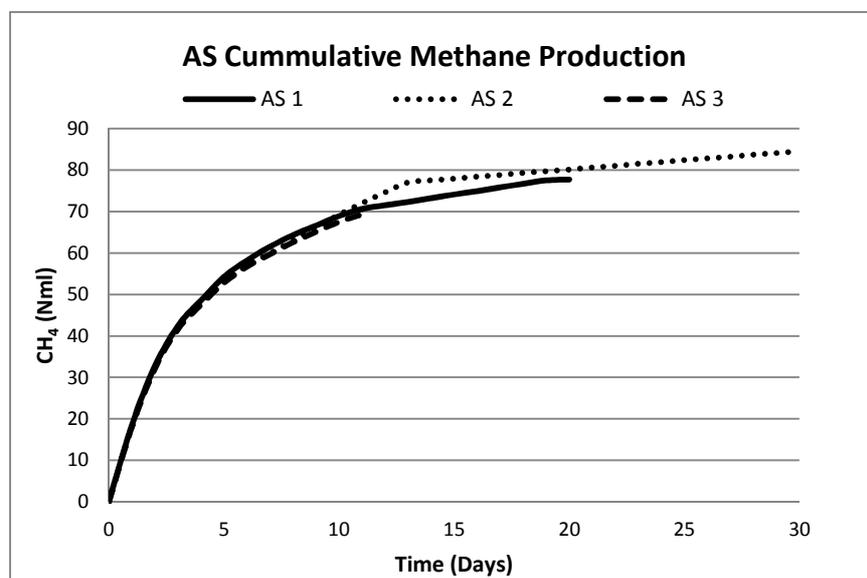


Table B-2 WP Reactors Cumulative Methane Production Raw Data

Day	WP 1 CH <sub>4</sub> Volume [Nml]	WP 2 CH <sub>4</sub> Volume [Nml]	WP 3 CH <sub>4</sub> Volume [Nml]
0	0	0	0
1	10.6	11.1	14.8
2	19.6	19.9	25.1
3	25.9	26.2	32.3
4	31	31.3	38.3
5	35.5	35.7	43.8
6	38.3	38.7	48.7
7	41	41.7	52.3
8	43.7	44.5	55.9
9	46.2	47.3	58.6
10	48.8	49.9	61.2
11	51.1	52.2	63.5
12	53.4	54.6	64.6
13	55.8	59.8	65.7
14	56.3	63.3	66.8
15		64.4	67.8
16		65.5	68.9
17		66.5	70
18		67.6	71
19		68.7	72.1
20		69.8	73.1
21		70.8	74.2
22		71.8	75.3
23		72.8	76.3
24		73.8	77.4
25		74.8	78.3
26		75.8	
27		76.8	
28		77.2	
29			
30			

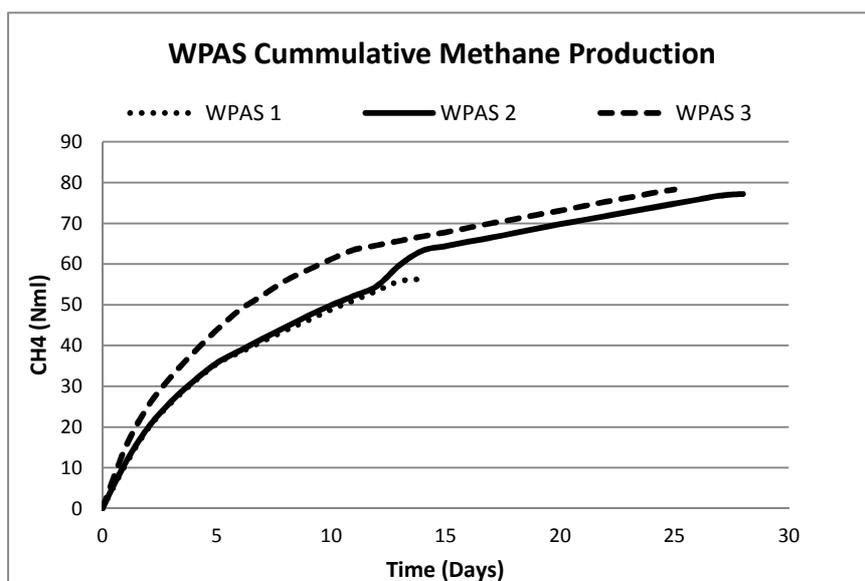


Table B-3 EA Reactors Cumulative Methane Production Raw Data

Day	EA 1 CH <sub>4</sub> Volume [Nml]	EA 2 CH <sub>4</sub> Volume [Nml]	EA 3 CH <sub>4</sub> Volume [Nml]
0	0	0	0
1	15.1	15.1	17.5
2	27.1	26.1	30.4
3	35.5	32.9	39.1
4	41.4	38	45.7
5	46.8	42.3	51.3
6	51.7	45.5	56.2
7	55.1	48.2	59.2
8	58.5	50.9	62.3
9	61.8	53.3	65.1
10	65	55.6	67.8
11	68.3	58	70.5
12	71.5	61.4	71.6
13	74.2	65	72.7
14	76.3	65.8	73.8
15	78.3		74.9
16	80.4		76
17	81.9		77.1
18	83.1		78.2
19	84.4		79.5
20	85.6		80.7
21	86.9		82
22	88.1		83.2
23	88.8		84.4
24			85.7
25			85.8
26			
27			
28			
29			
30			

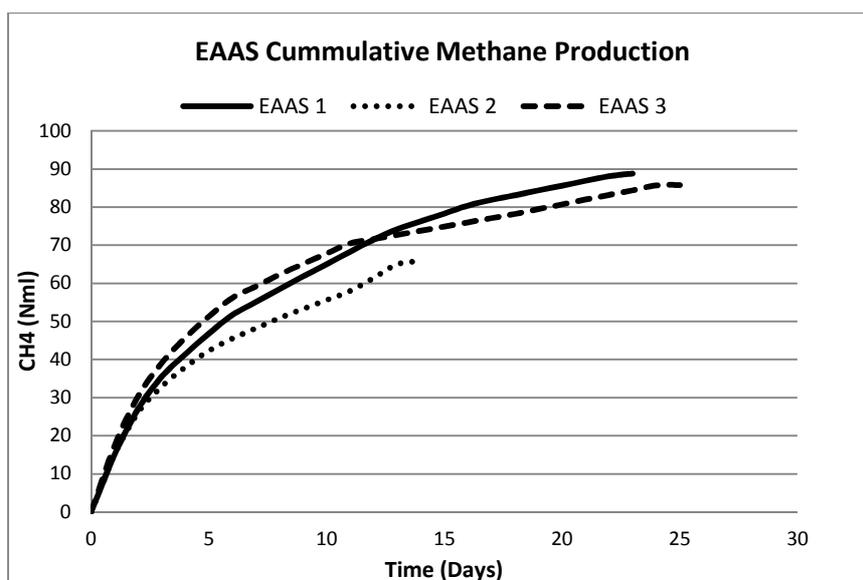
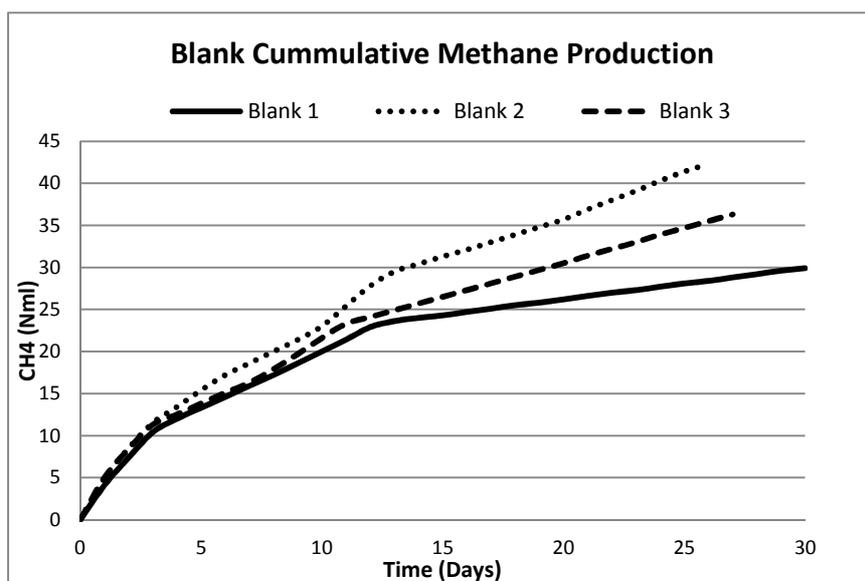


Table B-4 Blank Reactors Cumulative Methane Production Raw Data

Day	Blank 1 CH <sub>4</sub> Volume [Nml]	Blank 2 CH <sub>4</sub> Volume [Nml]	Blank 3 CH <sub>4</sub> Volume [Nml]
0	0	0	0
1	4.1	4.9	5
2	7.4	8.5	8.4
3	10.4	11.5	11.3
4	12	13.4	12.5
5	13.3	15.4	13.8
6	14.6	17.2	15.1
7	15.9	18.6	16.3
8	17.2	20	17.9
9	18.6	21.4	19.7
10	20	23	21.6
11	21.4	25.4	23.3
12	22.9	27.8	24.1
13	23.6	29.5	24.9
14	24	30.4	25.7
15	24.3	31.3	26.5
16	24.7	32.1	27.3
17	25.1	33	28.1
18	25.5	33.9	28.9
19	25.8	34.8	29.7
20	26.2	35.7	30.5
21	26.6	36.9	31.4
22	27	38	32.2
23	27.3	39.1	33
24	27.7	40.3	33.9
25	28.1	41.4	34.7
26	28.4	42.3	35.5
27	28.8		36.3
28	29.2		
29	29.6		
30	29.9		



**Appendix C – BMP 2 Raw Data***Table C-1 AS Reactors Raw Data*

Time	Date	Gas Pressure Increase (bar)			% Methane		
		AS 1	AS 2	AS 3	AS 1	AS 2	AS 3
<b>13:40</b>	2-Oct-13						
<b>17:40</b>	2-Oct-13	0.013	0.022	0.021			
<b>17:00</b>	3-Oct-13	0.075	0.08	0.081			
<b>9:30</b>	7-Oct-13	0.091	0.099	0.1			
<b>16:15</b>	8-Oct-13	0.017	0.017	0.018			
<b>15:30</b>	9-Oct-13	0.008	0.009	0.008	78.9%	79.1%	78.9%
<b>14:45</b>	10-Oct-13	0.019	0.019	0.018			
<b>15:30</b>	11-Oct-13	0.023	0.021	0.02			
<b>13:30</b>	14-Oct-13	0.022	0.025	0.025			
<b>14:45</b>	16-Oct-13	0.013	0.015	0.014	78.3%	78.9%	79.0%
<b>13:30</b>	18-Oct-13	0.022	0.02	0.021			
<b>14:15</b>	21-Oct-13	0.015	0.017	0.016			
<b>11:45</b>	24-Oct-13	0.011	0.015	0.01	78.6%	78.5%	78.5%
<b>10:45</b>	29-Oct-13	0.026	0.035	0.029			

*Table C-2 WP Reactors Raw Data*

Time	Date	Gas Pressure Increase (bar)			% Methane		
		WP 1	WP 2	WP 3	WP 1	WP 2	WP 3
<b>13:40</b>	2-Oct-13						
<b>17:40</b>	2-Oct-13	0.017	0.025	0.024			
<b>17:00</b>	3-Oct-13	0.042	0.038	0.041			
<b>9:30</b>	7-Oct-13	0.094	0.092	0.093			
<b>16:15</b>	8-Oct-13	0.017	0.016	0.017			
<b>15:30</b>	9-Oct-13	0.009	0.009	0.008	79.6%	79.5%	80.1%
<b>14:45</b>	10-Oct-13	0.017	0.018	0.018			
<b>15:30</b>	11-Oct-13	0.023	0.02	0.014			
<b>13:30</b>	14-Oct-13	0.021	0.02	0.02			
<b>14:45</b>	16-Oct-13	0.015	0.015	0.013	78.7%	78.8%	79.8%
<b>13:30</b>	18-Oct-13	0.022	0.019	0.02			
<b>14:15</b>	21-Oct-13	0.018	0.018	0.017			
<b>11:45</b>	24-Oct-13	0.019	0.018	0.014	78.9%	76.4%	79.8%
<b>10:45</b>	29-Oct-13	0.037	0.028	0.03			

Table C-3 EA Reactors Raw Data

Time	Date	Gas Pressure Increase (bar)			% Methane		
		EA1	EA2	EA3	EA1	EA2	EA3
<b>13:40</b>	2-Oct-13						
<b>17:40</b>	2-Oct-13	0.015	0.018	0.021			
<b>17:00</b>	3-Oct-13	0.071	0.071	0.071			
<b>9:30</b>	7-Oct-13	0.094	0.096	0.091			
<b>16:15</b>	8-Oct-13	0.016	0.017	0.016			
<b>15:30</b>	9-Oct-13	0.007	0.009	0.006	78.0%	78.5%	76.9%
<b>14:45</b>	10-Oct-13	0.016	0.02	0.02			
<b>15:30</b>	11-Oct-13	0.025	0.027	0.023			
<b>13:30</b>	14-Oct-13	0.022	0.024	0.021			
<b>14:45</b>	16-Oct-13	0.012	0.013	0.013	78.0%	78.4%	77.9%
<b>13:30</b>	18-Oct-13	0.02	0.019	0.019			
<b>14:15</b>	21-Oct-13	0.014	0.015	0.015			
<b>11:45</b>	24-Oct-13	0.012	0.013	0.011	77.3%	77.4%	77.3%
<b>10:45</b>	29-Oct-13	0.03	0.028	0.028			

Table C-4 Blank Reactors Raw Data

Time	Date	Gas Pressure Increase (bar)			% Methane		
		Inoc 1	Inoc 2	Inoc 3	Inoc 1	Inoc 2	Inoc 3
<b>13:40</b>	2-Oct-13						
<b>17:40</b>	2-Oct-13	0.003	0.003	0.003			
<b>17:00</b>	3-Oct-13	0.009	0.008	0.008			
<b>9:30</b>	7-Oct-13	0.03	0.027	0.029			
<b>16:15</b>	8-Oct-13	0.01	0.009	0.009			
<b>15:30</b>	9-Oct-13	0.005	0.006	0.006	61.8%	57.1%	63.8%
<b>14:45</b>	10-Oct-13	0.006	0.014	0.005			
<b>15:30</b>	11-Oct-13	0.012	0.005	0.016			
<b>13:30</b>	14-Oct-13	0.014	0.014	0.011			
<b>14:45</b>	16-Oct-13	0.009	0.006	0.007	71.9%	70.5%	70.4%
<b>13:30</b>	18-Oct-13	0.006	0.013	0.009			
<b>14:15</b>	21-Oct-13	0.011	0.01	0.01			
<b>11:45</b>	24-Oct-13	0.01	0.011	0.01	74.4%	74.3%	72.1%
<b>10:45</b>	29-Oct-13	0.02	0.005	0.019			

**Appendix D – Harnaspolder Influent Data 2012-2013**

Note that Values presented in italics have been interpolated as actual data was no available.

<b>Date</b>	<b>T (°C)</b>	<b>Flow (m<sup>3</sup>/d)</b>	<b>COD (mg/l)</b>	<b>Total Nitrogen (mg/l)</b>	<b>Total Phosphorus (mg/l)</b>
<b>3/1/2012</b>	13.72	147054	705.34	62.33	8.97
<b>3/2/2012</b>	13.78	147134	635.99	64	8.83
<b>3/3/2012</b>	13.86	151550	690.06	64.01	8.93
<b>3/4/2012</b>	14.14	189471	701.51	63.29	8.73
<b>3/5/2012</b>	12.81	275456	570.97	39.89	6.02
<b>3/6/2012</b>	13.79	153166	547.23	43.6	8.27
<b>3/7/2012</b>	13.14	336912	569.52	47.15	7.08
<b>3/8/2012</b>	12.07	206758	367.85	35.81	4.99
<b>3/9/2012</b>	13.42	162309	541.23	56.32	7.9
<b>3/10/2012</b>	13.64	165017	564.63	59.67	8.33
<b>3/11/2012</b>	13.99	164613	593.22	59.69	8.14
<b>3/12/2012</b>	14.21	157149	550.21	58.33	8
<b>3/13/2012</b>	14.17	153835	706.8	65	9.27
<b>3/14/2012</b>	14.24	160980	584.84	64.32	9.06
<b>3/15/2012</b>	14.25	163565	590.3	63.69	8.6
<b>3/16/2012</b>	14.52	152627	608.05	63.67	8.63
<b>3/17/2012</b>	14.57	157499	707.77	66.01	9.03
<b>3/18/2012</b>	14.74	156644	685.57	64.02	8.4
<b>3/19/2012</b>	14.91	148272	657.53	66.68	9.1
<b>3/20/2012</b>	14.81	147039	693.3	69.67	9.73
<b>3/21/2012</b>	14.82	152596	643.35	68.01	9.67
<b>3/22/2012</b>	14.8	147743	616.35	67.67	9.37
<b>3/23/2012</b>	14.77	147662	715.66	67.34	9.67
<b>3/24/2012</b>	14.98	149458	716.25	67.03	9.63
<b>3/25/2012</b>	15.25	145184	710.05	69.69	9.3
<b>3/26/2012</b>	15.04	144793	695.83	64.35	9.4
<b>3/27/2012</b>	15.3	145529	719	69.32	9.76
<b>3/28/2012</b>	15.33	156537	806.52	71.4	9.84
<b>3/29/2012</b>	15.47	143728	736.89	74.61	11.04
<b>3/30/2012</b>	15.53	143703	707.15	70.66	10.56
<b>3/31/2012</b>	15.66	149635	896.04	70	10.62
<b>4/1/2012</b>	15.8	150700	886.6	69.95	9.79
<b>4/2/2012</b>	15.71	144641	681.07	66.34	9.5
<b>4/3/2012</b>	15.55	144594	753.12	72.32	9.86
<b>4/4/2012</b>	15.45	165148	796.71	70.95	9.74
<b>4/5/2012</b>	15.43	153975	673.87	65.47	9.7
<b>4/6/2012</b>	15.58	146904	611.04	69.43	9.47
<b>4/7/2012</b>	15.72	152382	685.25	68.4	9.47
<b>4/8/2012</b>	15.75	140663	639.82	70	9.14
<b>4/9/2012</b>	14.67	254328	723	61.64	8.82

## Appendix D

<b>4/10/2012</b>	14.58	204410	419.01	40.29	5.56
<b>4/11/2012</b>	15.26	149932	670.96	61.34	8.53
<b>4/12/2012</b>	15.44	145320	596.44	64.67	9.1
<b>4/13/2012</b>	15.55	142814	614.06	67	9.86
<b>4/14/2012</b>	15.65	149341	632.48	67	9.4
<b>4/15/2012</b>	15.77	153203	678.38	66.7	9.3
<b>4/16/2012</b>	15.79	145201	659.26	68.67	9.6
<b>4/17/2012</b>	15.68	180529	747.24	70.35	10.55
<b>4/18/2012</b>	14.8	156214	780.42	59.99	9.43
<b>4/19/2012</b>	15.46	163885	595.88	66.65	9.5
<b>4/20/2012</b>	15.32	164538	693.55	61.32	9.04
<b>4/21/2012</b>	15.39	170355	572.65	59.69	8.57
<b>4/22/2012</b>	15.69	150596	676.46	63.03	8.7
<b>4/23/2012</b>	15.06	185870	621.1	56.31	8.07
<b>4/24/2012</b>	15.08	202736	535.93	54	7.84
<b>4/25/2012</b>	15.37	170664	684.41	62.56	9.26
<b>4/26/2012</b>	15.52	183626	486.21	58.25	8.17
<b>4/27/2012</b>	15.57	148852	594.25	57.62	8.5
<b>4/28/2012</b>	14.78	391613	487.24	36.55	5.67
<b>4/29/2012</b>	14.9	168759	492.96	45.01	6.4
<b>4/30/2012</b>	15.5	142570	549.07	57.05	7.4
<b>5/1/2012</b>	15.1	295780	430.7	41.64	5.6
<b>5/2/2012</b>	15.58	160703	492.65	51.66	7.07
<b>5/3/2012</b>	14.94	381575	375.89	32.74	5.06
<b>5/4/2012</b>	15.84	173328	479.54	49.34	7.2
<b>5/5/2012</b>	16.03	167472	543.6	55.35	8.33
<b>5/6/2012</b>	16.14	166913	596.35	58.29	8.33
<b>5/7/2012</b>	16.2	156797	580.47	59.21	8.15
<b>5/8/2012</b>	16.01	182668	557.86	57.97	8.66
<b>5/9/2012</b>	15.56	243344	490.86	42.67	6.93
<b>5/10/2012</b>	15.99	230807	419.43	42.64	6.38
<b>5/11/2012</b>	16.39	163069	472.72	51.34	7.27
<b>5/12/2012</b>	16.61	160022	613.81	57	8.44
<b>5/13/2012</b>	16.65	159952	573.56	58.71	7.79
<b>5/14/2012</b>	16.69	158107	558.96	60.7	8.37
<b>5/15/2012</b>	16.13	214743	498	50.56	7.62
<b>5/16/2012</b>	16.55	159871	483.42	56.31	8.16
<b>5/17/2012</b>	16.7	150301	522.68	59.7	8.27
<b>5/18/2012</b>	16.73	152933	537.65	58.05	8.14
<b>5/19/2012</b>	16.77	150533	563.5	59	8.57
<b>5/20/2012</b>	16.76	183200	588.86	53.93	7.93
<b>5/21/2012</b>	17.07	152085	538.34	55.33	7.4
<b>5/22/2012</b>	17.14	149073	580.43	60.34	8.83
<b>5/23/2012</b>	17.24	151857	548.28	68.28	9.22
<b>5/24/2012</b>	17.35	155882	519.16	60.67	8.2
<b>5/25/2012</b>	17.53	151033	573.92	62.33	8.33

## Appendix D

<b>5/26/2012</b>	17.64	147894	605.13	62.07	8.55
<b>5/27/2012</b>	17.8	142158	568.68	60.77	8.15
<b>5/28/2012</b>	18.11	143814	573.81	62	8.13
<b>5/29/2012</b>	18.35	153547	604.76	63.94	8.36
<b>5/30/2012</b>	18.25	147878	556.53	62.96	8.99
<b>5/31/2012</b>	18.41	224180	609.55	60.85	8.56
<b>6/1/2012</b>	18.18	159136	487.44	45	6.43
<b>6/2/2012</b>	18.55	149093	508.57	57.64	7.76
<b>6/3/2012</b>	18.03	232256	462.05	46.16	6.42
<b>6/4/2012</b>	17.39	295708	388.48	35.91	5.15
<b>6/5/2012</b>	17.52	157604	387.18	45.7	6.37
<b>6/6/2012</b>	17.59	246706	441.44	45.56	6.33
<b>6/7/2012</b>	17.72	191033	356.18	44	6.53
<b>6/8/2012</b>	18.07	158173	441.24	52.35	7.49
<b>6/9/2012</b>	18.3	162375	464.03	55.66	7.67
<b>6/10/2012</b>	18.27	158112	522.1	58.38	7.84
<b>6/11/2012</b>	18.34	255318	536.01	45.25	7.38
<b>6/12/2012</b>	18.02	197068	340.33	38.3	5.8
<b>6/13/2012</b>	18.4	154655	501.79	57	7.83
<b>6/14/2012</b>	18.39	150375	445.89	56.89	8.43
<b>6/15/2012</b>	18.05	235459	568.57	52.67	8.1
<b>6/16/2012</b>	18.07	188551	433.3	46	6.49
<b>6/17/2012</b>	18.46	148827	491.11	55.74	7.37
<b>6/18/2012</b>	17.79	337560	414.82	38.37	5.59
<b>6/19/2012</b>	18.07	157257	434.28	50.27	6.97
<b>6/20/2012</b>	18.43	154252	479.02	56.34	8.07
<b>6/21/2012</b>	18.6	230059	537.81	55.79	7.47
<b>6/22/2012</b>	18.27	241674	377.35	34.33	5.27
<b>6/23/2012</b>	18.67	154447	501.61	53.03	7.37
<b>6/24/2012</b>	18.38	287672	454.31	40.56	5.76
<b>6/25/2012</b>	18.27	180091	407.78	43.91	6.02
<b>6/26/2012</b>	18.69	154121	535.18	57	8.1
<b>6/27/2012</b>	18.5	151467	495.58	58.66	8.3
<b>6/28/2012</b>	18.82	151522	504.61	59.35	8.3
<b>6/29/2012</b>	19	147281	551.79	61.65	8.88
<b>6/30/2012</b>	19.17	149819	585.42	61.65	8.78
<b>7/1/2012</b>	19.42	148192	561.49	59.72	8.14
<b>7/2/2012</b>	19.47	143058	580.67	60.69	8.57
<b>7/3/2012</b>	19.31	144966	564.36	62.33	9.07
<b>7/4/2012</b>	19.41	148961	504.79	59.01	8.9
<b>7/5/2012</b>	19.49	161687	495.37	46.47	7.16
<b>7/6/2012</b>	19.01	448202	342.88	27.67	4.42
<b>7/7/2012</b>	19.66	180799	500.7	45.95	6.75
<b>7/8/2012</b>	19.92	261558	518.92	42.97	6.37
<b>7/9/2012</b>	19.69	195983	367.59	37.72	4.9
<b>7/10/2012</b>	19.8	157953	383.62	46.91	6.95

## Appendix D

<b>7/11/2012</b>	18.46	480221	272.96	24.61	3.94
<b>7/12/2012</b>	17.78	361981	278.39	23.6	3.78
<b>7/13/2012</b>	18.63	275539	284.32	29.03	4.62
<b>7/14/2012</b>	19.17	252754	371.19	35.11	5.4
<b>7/15/2012</b>	19.34	182696	327.93	36.71	5.24
<b>7/16/2012</b>	19.2	357791	307.35	30.68	4.69
<b>7/17/2012</b>	18.33	343200	136.34	18.33	2.57
<b>7/18/2012</b>	19.02	204585	334.79	38	5.6
<b>7/19/2012</b>	18.83	274806	258.12	29.34	4.62
<b>7/20/2012</b>	19.12	172598	329.68	38.24	5.67
<b>7/21/2012</b>	19.32	163044	381.43	43.62	6.43
<b>7/22/2012</b>	19.33	156184	396.11	45.22	6.49
<b>7/23/2012</b>	19.31	155694	417.74	47.45	6.95
<b>7/24/2012</b>	19.2	152248	417.63	49.88	7.38
<b>7/25/2012</b>	19.45	153468	438.73	50.27	7.27
<b>7/26/2012</b>	19.67	146970	441.3	50.68	7.2
<b>7/27/2012</b>	19.86	183534	517.86	53.34	7.78
<b>7/28/2012</b>	20.01	200076	496.58	40.88	6.44
<b>7/29/2012</b>	20.18	180716	471.02	49.37	6.66
<b>7/30/2012</b>	18.24	331512	352.76	25.55	4.09
<b>7/31/2012</b>	19.14	240240	396.49	34.03	5.18
<b>8/1/2012</b>	19.58	189094	336.21	37.78	5.61
<b>8/2/2012</b>	19.86	211714	352.67	35.62	5.32
<b>8/3/2012</b>	20.02	180154	378.3	42.37	6.3
<b>8/4/2012</b>	20.19	157668	418.18	43.32	6.59
<b>8/5/2012</b>	20.26	208344	454.08	42.86	6.68
<b>8/6/2012</b>	19.75	294520	328.03	28.43	4.5
<b>8/7/2012</b>	19.91	243810	429.39	35.27	5.34
<b>8/8/2012</b>	20.01	182392	349.56	39.45	5.37
<b>8/9/2012</b>	20.1	156900	328.1	43.53	6.01
<b>8/10/2012</b>	20.18	153472	404.91	48.05	6.86
<b>8/11/2012</b>	20.21	152720	<i>349.798</i>	<i>47.14133</i>	<i>6.339333</i>
<b>8/12/2012</b>	20.07	151668	485.51	55.5	7.4
<b>8/13/2012</b>	20.11	152238	482.89	54.51	7.5
<b>8/14/2012</b>	20.17	155400	542.76	55.03	7.86
<b>8/15/2012</b>	20.29	154694	491.48	44.49	7.9
<b>8/16/2012</b>	20.57	145296	476.35	55.54	7.81
<b>8/17/2012</b>	20.54	150182	507.81	57.52	8.46
<b>8/18/2012</b>	20.57	150756	544.24	57.59	8.57
<b>8/19/2012</b>	20.76	151424	558.01	56.11	7.77
<b>8/20/2012</b>	21.06	153248	508.97	57.09	7.72
<b>8/21/2012</b>	21.23	155560	589.21	58.53	9.11
<b>8/22/2012</b>	21.34	153286	602.43	57.89	8.48
<b>8/23/2012</b>	21.26	150266	625.25	60.04	8.66
<b>8/24/2012</b>	21.2	156766	552.34	59.96	8.94
<b>8/25/2012</b>	20.86	316540	587.48	45.04	6.87

## Appendix D

<b>8/26/2012</b>	19.65	494936	259.8	20.69	3.2
<b>8/27/2012</b>	20.7	164338	398.16	45	6.12
<b>8/28/2012</b>	20.81	165122	460.28	51.61	7.38
<b>8/29/2012</b>	20.91	158576	493.12	52.58	7.74
<b>8/30/2012</b>	20.84	257894	530.12	48.78	7.32
<b>8/31/2012</b>	18.61	574064	210.66	17.24	2.6
<b>9/1/2012</b>	19.9	184668	414.06	41.56	5.66
<b>9/2/2012</b>	20.39	170164	458.91	48.58	6.42
<b>9/3/2012</b>	20.4	166538	471.31	50.57	6.96
<b>9/4/2012</b>	20.4	164948	469.49	53.06	7.41
<b>9/5/2012</b>	20.51	164804	454.19	53.61	7.62
<b>9/6/2012</b>	20.54	160616	464.14	55.13	7.62
<b>9/7/2012</b>	20.47	160658	521.38	57.14	7.62
<b>9/8/2012</b>	20.53	162518	552.03	57.68	7.58
<b>9/9/2012</b>	20.59	159852	535.07	51.49	7.41
<b>9/10/2012</b>	20.61	157210	482.08	54.54	7.35
<b>9/11/2012</b>	20.51	187858	575.63	54.53	7.45
<b>9/12/2012</b>	20.67	174806	554.48	55.4	7.78
<b>9/13/2012</b>	19.73	208142	440.56	43.56	6.43
<b>9/14/2012</b>	20.37	157340	515.2	55.48	7.8
<b>9/15/2012</b>	20.36	153614	562.58	58.64	8.12
<b>9/16/2012</b>	20.46	153380	529.96	58.59	7.71
<b>9/17/2012</b>	20.34	150116	501.93	57.6	7.47
<b>9/18/2012</b>	19.99	216988	525.49	50.83	7.12
<b>9/19/2012</b>	19.99	190410	488.96	48.96	6.98
<b>9/20/2012</b>	19.91	175796	439.38	51.05	6.56
<b>9/21/2012</b>	19.93	150510	466.64	54.58	7.52
<b>9/22/2012</b>	19.51	205256	507.43	49.34	6.73
<b>9/23/2012</b>	19.78	200480	594.69	53.46	7.49
<b>9/24/2012</b>	17.22	421812	395.26	30.23	4.65
<b>9/25/2012</b>	18.33	206206	290.44	33	4.47
<b>9/26/2012</b>	19.2	157914	429.1	51.54	6.98
<b>9/27/2012</b>	19.34	174460	458.69	56.51	7.2
<b>9/28/2012</b>	18.9	182618	479.75	50.65	6.97
<b>9/29/2012</b>	19.13	181516	507.6	52.06	7.26
<b>9/30/2012</b>	19.54	151404	541.74	58.57	7.66
<b>10/1/2012</b>	19.58	158100	538.97	59.68	7.83
<b>10/2/2012</b>	19.34	166932	498.56	44.57	7.84
<b>10/3/2012</b>	18.63	293240	472.47	43.32	6.52
<b>10/4/2012</b>	17.43	351706	326.74	26.84	4.02
<b>10/5/2012</b>	17.7	319090	<i>326.746</i>	<i>22.65</i>	<i>4.197</i>
<b>10/6/2012</b>	16.59	502792	253.63	22.82	3.27
<b>10/7/2012</b>	18.03	199790	386.6	41.16	5.32
<b>10/8/2012</b>	18.67	179930	446.2	50	6.56
<b>10/9/2012</b>	18.67	168728	444.08	54.62	7.34
<b>10/10/2012</b>	18.68	155994	481.98	56.65	7.88

## Appendix D

<b>10/11/2012</b>	18.66	170516	526.99	58.24	7.94
<b>10/12/2012</b>	17.63	300006	454	41.89	6.18
<b>10/13/2012</b>	15.99	463446	273.17	25.51	3.63
<b>10/14/2012</b>	15.05	540350	165.32	18.37	2.43
<b>10/15/2012</b>	15.87	316460	217.43	24.58	3.05
<b>10/16/2012</b>	17.12	227492	320.87	39.57	5.51
<b>10/17/2012</b>	17.53	201798	401.26	44.53	6.23
<b>10/18/2012</b>	17.65	210376	471.29	46.17	6.43
<b>10/19/2012</b>	17.06	318282	322.11	33.02	4.85
<b>10/20/2012</b>	17.86	184542	411.75	45	6.01
<b>10/21/2012</b>	17.37	271996	365.75	36.64	5.01
<b>10/22/2012</b>	17.97	176926	411.72	46.06	5.91
<b>10/23/2012</b>	18.14	166266	476.47	50.6	6.83
<b>10/24/2012</b>	18.44	168290	501.27	53.13	7.69
<b>10/25/2012</b>	18.46	161574	480.4	54.12	7.69
<b>10/26/2012</b>	18.49	160508	523.88	56.74	7.69
<b>10/27/2012</b>	18.43	168654	566.82	59.21	7.85
<b>10/28/2012</b>	18.23	172090	526	56.16	7.38
<b>10/29/2012</b>	16.34	416210	434.96	37.74	5.31
<b>10/30/2012</b>	14.72	498992	182.66	17.94	2.89
<b>10/31/2012</b>	16.58	209122	341.81	38.43	5.59
<b>11/1/2012</b>	16.96	222080	396.67	45	6.31
<b>11/2/2012</b>	16.6	234900	410.38	41.63	5.83
<b>11/3/2012</b>	16.89	209474	434.44	46.06	6.36
<b>11/4/2012</b>	16.07	343112	410.13	36.46	5.24
<b>11/5/2012</b>	15.22	271014	293.54	31.54	4.36
<b>11/6/2012</b>	15.99	292202	362.11	38.51	5.4
<b>11/7/2012</b>	15.21	252586	307.58	30.21	4.23
<b>11/8/2012</b>	16.39	186054	403.33	45.46	6.55
<b>11/9/2012</b>	16.71	178538	507.27	52.07	7.84
<b>11/10/2012</b>	16.83	199710	544	52.11	7.67
<b>11/11/2012</b>	15.67	273352	412.79	38.88	5.63
<b>11/12/2012</b>	16.88	172738	451.07	49.93	6.72
<b>11/13/2012</b>	16.74	176078	489.58	53.14	7.68
<b>11/14/2012</b>	16.86	166696	515.39	56.66	7.82
<b>11/15/2012</b>	17.12	165102	571.63	58.67	6.89
<b>11/16/2012</b>	17.03	162254	543.71	61.14	8.43
<b>11/17/2012</b>	16.99	162730	557.88	62.65	8.36
<b>11/18/2012</b>	16.42	208640	535.44	54.15	7.22
<b>11/19/2012</b>	16.78	159316	534.95	55.53	8.05
<b>11/20/2012</b>	16.52	157972	540	60.61	8.33
<b>11/21/2012</b>	16.61	162464	585.22	61.1	8.39
<b>11/22/2012</b>	16.26	162584	525.55	58.1	8.17
<b>11/23/2012</b>	15.59	221744	599.78	52.32	7.98
<b>11/24/2012</b>	15.71	230194	536.54	51.46	7.39
<b>11/25/2012</b>	15.07	194112	399.84	42.64	5.98

## Appendix D

<b>11/26/2012</b>	15.79	170700	494.61	52.05	7.01
<b>11/27/2012</b>	15.82	164592	530.55	58.05	7.96
<b>11/28/2012</b>	15.88	186130	623.64	61.94	8.77
<b>11/29/2012</b>	15.57	227602	514.3	51.83	7.83
<b>11/30/2012</b>	15.48	180578	481.37	51.38	7.73
<b>12/1/2012</b>	13.82	358954	492.64	38.69	6.11
<b>12/2/2012</b>	12.89	329128	328.73	26.44	3.95
<b>12/3/2012</b>	13.27	300094	356.83	30.46	4.5
<b>12/4/2012</b>	13.62	286902	367.16	37.96	5.44
<b>12/5/2012</b>	13.29	245944	352.59	37.13	5.12
<b>12/6/2012</b>	14.08	207792	361.12	44.58	6.26
<b>12/7/2012</b>	12.77	356808	390.13	37.35	5.47
<b>12/8/2012</b>	13.24	214678	386.15	40.95	5.81
<b>12/9/2012</b>	12.85	341778	388.91	35.63	4.97
<b>12/10/2012</b>	12.97	260632	331.26	33.52	4.66
<b>12/11/2012</b>	13.68	194020	429.04	45.57	6.41
<b>12/12/2012</b>	13.73	204870	461.14	47.71	6.06
<b>12/13/2012</b>	13.85	184344	435.51	50.07	7.11
<b>12/14/2012</b>	13.12	331468	576.46	44.13	6.64
<b>12/15/2012</b>	<i>13.285</i>	<i>269767</i>	<i>363.57</i>	<i>33.545</i>	<i>5.195</i>
<b>12/16/2012</b>	13.36	244626	402.08	36.37	5.74
<b>12/17/2012</b>	13.47	238566	407.92	42.59	5.76
<b>12/18/2012</b>	13.1	240806	443.11	40.13	6.28
<b>12/19/2012</b>	13.62	188284	484.94	48.24	6.85
<b>12/20/2012</b>	13.71	248358	449.68	45.38	6.63
<b>12/21/2012</b>	12.56	241412	322.54	34.01	2.85
<b>12/22/2012</b>	13.05	359606	439.21	36.68	3.27
<b>12/23/2012</b>	11.99	582520	203.29	16.34	1.62
<b>12/24/2012</b>	13.2	253248	334.08	32.92	4.39
<b>12/25/2012</b>	12.65	442412	262.5	24.84	3.53
<b>12/26/2012</b>	13.09	272880	328.55	31.57	4.2
<b>12/27/2012</b>	13.07	279480	340	34.03	4.5
<b>12/28/2012</b>	13.29	245364	359.23	35.96	5.19
<b>12/29/2012</b>	13.56	204644	397.65	41.06	5.61
<b>12/30/2012</b>	13.98	196652	462.31	48.08	6.27
<b>12/31/2012</b>	14.15	214056	560.8	51.58	6.86
<b>1/1/2013</b>	12.77	395076	345.52	29	4.3
<b>1/2/2013</b>	13.58	198996	371.12	41.47	4.89
<b>1/3/2013</b>	13.85	192544	411.93	46.61	5.97
<b>1/4/2013</b>	14.15	183196	496.11	50.12	6.62
<b>1/5/2013</b>	14.31	181340	472.49	50.55	6.71
<b>1/6/2013</b>	14.43	185980	446.95	52.59	6.97
<b>1/7/2013</b>	14.59	178108	439.78	52.55	6.81
<b>1/8/2013</b>	14.54	178024	478.49	55.14	7.57
<b>1/9/2013</b>	14.18	247116	463.28	47.3	6.78
<b>1/10/2013</b>	14.39	189340	441.88	43.48	6.57

## Appendix D

<b>1/11/2013</b>	14.1	200428	477.73	49.17	6.73
<b>1/12/2013</b>	14.36	175560	498.58	56.09	7.31
<b>1/13/2013</b>	14.35	177832	549.19	56.71	7.37
<b>1/14/2013</b>	14.17	167980	625.55	56.57	8.11
<b>1/15/2013</b>	13.79	173068	547.67	59.05	8.31
<b>1/16/2013</b>	13.65	173572	513.65	57.6	7.86
<b>1/17/2013</b>	13.56	162072	524.81	59.05	8.36
<b>1/18/2013</b>	13.4	167496	529.27	59.55	8.36
<b>1/19/2013</b>	13.35	168864	584.42	60.11	8.42
<b>1/20/2013</b>	13.29	171804	616.71	59.61	7.67
<b>1/21/2013</b>	13.11	166092	591.63	58.52	7.85
<b>1/22/2013</b>	12.96	166544	516.66	61.3	8.04
<b>1/23/2013</b>	12.9	<i>166757.2</i>	<i>562.335</i>	<i>60.391</i>	<i>7.705</i>
<b>1/24/2013</b>	12.79	<i>182194.4</i>	<i>570.801</i>	<i>63.161</i>	<i>9.049</i>
<b>1/25/2013</b>	12.74	158532	525.89	58.56	8.41
<b>1/26/2013</b>	12.74	166512	508.5	58.59	8.31
<b>1/27/2013</b>	9.52	548340	443.71	32.15	4.8
<b>1/28/2013</b>	9.99	288720	336.71	31.96	4.5
<b>1/29/2013</b>	11.18	271708	398.08	40.54	5.86
<b>1/30/2013</b>	11.49	402028	292.57	26.1	3.68
<b>1/31/2013</b>	11.89	222219	336.16	37.76	5.02
<b>2/1/2013</b>	11.95	282735	436.47	38.58	5.86
<b>2/2/2013</b>	11.87	242614	374.27	35.12	5.17
<b>2/3/2013</b>	12.46	209973	449.16	44.12	6.21
<b>2/4/2013</b>	12.4	206456	417.97	43.64	6.32
<b>2/5/2013</b>	11.91	246715	468.7	42.63	6.17
<b>2/6/2013</b>	11.03	317992	314.53	34.29	4.72
<b>2/7/2013</b>	11.97	225546	411.44	44.06	5.97
<b>2/8/2013</b>	11.65	232072	392.16	41.56	5.81
<b>2/9/2013</b>	12.36	196242	451.61	49.18	6.68
<b>2/10/2013</b>	12.3	222996	463.51	47.07	6.36
<b>2/11/2013</b>	12.21	185705	<i>441.7491</i>	<i>47.02636</i>	<i>6.380182</i>
<b>2/12/2013</b>	12.12	177967	<i>435.5149</i>	<i>48.06959</i>	<i>6.380066</i>
<b>2/13/2013</b>	12.16	177573	<i>444.9641</i>	<i>49.18054</i>	<i>6.504262</i>
<b>2/14/2013</b>	11.96	208514	<i>443.1995</i>	<i>49.36005</i>	<i>6.482162</i>
<b>2/15/2013</b>	11.6	201647	<i>483.6599</i>	<i>62.27711</i>	<i>8.162915</i>
<b>2/16/2013</b>	11.95	199017	<i>484.0879</i>	<i>61.08188</i>	<i>7.99757</i>
<b>2/17/2013</b>	12.37	180468	<i>494.5382</i>	<i>60.14018</i>	<i>7.895818</i>
<b>2/18/2013</b>	12.46	172143	495.93	57.63	7.57
<b>2/19/2013</b>	12.33	171489	525.56	65.48	8.71
<b>2/20/2013</b>	12.4	171477	501.42	60.75	7.99
<b>2/21/2013</b>	12.37	169513	481.14	57.73	7.78
<b>2/22/2013</b>	12.29	166616	521.32	58.17	7.88
<b>2/23/2013</b>	12.31	166140	542.63	58.61	7.61
<b>2/24/2013</b>	12.24	169507	553.85	56.64	7.36
<b>2/25/2013</b>	12.11	166206	512.88	55.6	7.12

Appendix D

<b>2/26/2013</b>	<i>12.085</i>	157536	532.33	56.25	7.87
<b>2/27/2013</b>	<i>12.024</i>	163632	577.95	60.51	8.25
<b>2/28/2013</b>	12.13	157209	526.95	61.11	8.21



**Appendix E – Delfluent Data**

Construction period	2004 / 2006
Start-up period	September - December 2006
Site area	25 hectares
Type of treatment	Activated sludge, circulation system
Treatment capacity (design)	1.26 million p.e. (at 150g Total Oxygen Demand)
Maximum dry weather flow	10,950 m <sup>3</sup> /hour
Maximum rain weather flow	35,800 m <sup>3</sup> /hour
Fine bar screens	5, bar distance 6 mm
Pre-sedimentation tanks	Quantity: 4, diameter 47 m, depth 3.5 m, total surface area 6,940 m <sup>2</sup>

**Biological tanks**

Quantity	8
Diameter	64 m
Depth	8,2 m
Total volume	206.600 m <sup>3</sup>
- Selector	Total volume 5,200 m <sup>3</sup>
- Pre-denitrification	Total volume 4,880 m <sup>3</sup>
- Anaerobic	Total volume 30,920 m <sup>3</sup>
- Aerobic/Anoxic	Total volume 165,600 m <sup>3</sup>
Aeration system	Fine bubble aeration

**Final clarifier / sedimentation tanks**

Quantity	16
Diameter	56,5 m
Depth	3,5 m
Total surface area	40.110 m <sup>2</sup>
Nitrogen removal	Pre-denitrification + Intermittent nitrification/denitrification
Phosphate removal	Bio-P with optionally additional chemicals

## Discharge requirements

- Nitrogen	10 mg N/l (yearly average)
- Phosphate	1 mg P/l (10-days rolling average)
- Chemical Oxygen Demand (COD)	125 mg/l (daily average)
- Biochemical Oxygen Demand (BOD)	20 mg/l (daily average)
- Total Suspended Solids (TSS)	30 mg/l (daily average)
Effluent pumps	3+2, capacity 13,000 m <sup>3</sup> /hour, 1,600 kW
Primary sludge thickeners	2, depth 5.3 m, total surface area 1,414 m <sup>2</sup>
Sludge thickening centrifuges	3+1, capacity 100 m <sup>3</sup> /hour
Sludge digestion tanks	2 tanks, total volume 23,200 m <sup>3</sup>
Digested sludge dewatering centrifuges	3+1, capacity 30 m <sup>3</sup> /hour
Biogas production	17,500 Nm <sup>3</sup> /day
Gas generators (biogas / natural gas)	2+1, power 1,330 kW (electrical)
Diesel generators	2, power 1,330 kW (electrical)
Air treatment - bio filters	6, total capacity 160,000 Nm <sup>3</sup> /hour
- Biological filters	Quantity: 6, total volume: 360 m <sup>3</sup>
- Active carbon filters	Quantity: 6, total volume: 80 m <sup>3</sup>

Source: <http://delfluent.nl/en/plant/wwtp-harnaschpolder/ratios/>