Sludge reduction by Oligochaetes in a full scale reactor

Reactor characteristics and worm biomass dynamics

June 2010
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Thesis nr: 003/2010

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Preface

This thesis report is about an ingenious system using ecology for a practical solution. For me, doing a double specialization in both aquatic ecology and environmental technology, it was the ideal subject to graduate on. I could not have done this research by myself and therefore I would like to thank ir. Gerrit van Schouwenburg, Hans and Reinier from SR Technology and Johan from RWZI Wolvega for their help at the waste water treatment plant. The dataset I used for the statistical analysis were the result of 3 years measuring and needed a lot of preparation. For this I am thanking Steven, Brett and Peter of the Environmental Biotechnology department of Technical University Delft. Structuring the data is important and dr. ir. Piet Verdonschot from Alterra helped a lot in this. The whole research and especially data analysis and data interpretation would not have been possible without a smart way of ecological thinking, so dr. ir. Edwin Peeters thanks a lot! And last but not least my direct supervisor ir. Jelmer Tamis. He really made me do stuff which I never expected to be able to. Because of his “really get things done” mentality we were able to solve a lot of difficult problems and he helped a lot whenever I tented to get lost in details by guiding me back on track.

Photograph titelpage: Hans Bronkhorst

Abstract

Sludge disposal has become a major cost for waste water treatment plants without anaerobic digestion and with an organic matter load of less than 100000 population equivalents. One approach to degrade the amount of sludge is culturing Oligochaetes on carrier material in separate reactors.

In this study, population dynamics of a full scale worm reactor were investigated. The first part of the research consisted of characterizing performances of a full scale reactor and comparing performances with continuous lab scale reactors reported in the literature. A sludge degrading full scale reactor was realized with the potential to reduce sludge processing cost with 40%. Experiments showed that sludge consumption by Oligochaetes resulted in increased compactness of sludge. Sludge degradation rates (0.8 kg TSS/m^3/d) were in the same order of magnitude compared to values reported in literature.

The second part of this research consisted of gaining more insight into worm biomass dynamics within the full scale reactor using statistical analysis. A dataset of three years collection was investigated, multilinear regression was used to determine influence on reduction and multivariate analysis was used to determine influence on a complex biomass variable.

Multilinear regression analysis showed a positive correlation between biomass density and sludge degradation (R^2=35%). Multivariate analysis showed a positive correlation between growth rate and sludge degradation (R^2=31%). Size of worms was also investigated, and multivariate analysis showed that small worms (< 3 mm) were correlated with high biomass concentrations . Larger worms (> 4 mm) were more sensitive for intense turbulence and fluctuations such as malfunctions (R^2=36%).

In conclusion a three year dataset of a worm reactor system was investigated. The variables degradation and biomass density varied highly over time. Using multilinear regression and multivariate analysis, small fraction of this variance could be explained.
Both analysis indicated that the parameter influent sludge concentration was an important factor and indicated that the more sludge enters the reactor the more is being degraded.

Samenvatting

Het verwerken van slib op afval waterzuivering is een grote kosten post voor zuiveringen zonder anaërobe vergisting en een capaciteit om afvalwater te zuiveren voor minder dan 100000 populatie eenheden. Een manier om slib af te breken is door het toepassen van een natuurlijk proces: het consumeren van bacterieresten door aquatische Oligochaeten.

In deze studie is een proces onderzocht waarin Oligocheaten onder gecontroleerde omstandigheden worden gevoed met bacterieresten uit een afvalwaterzuivering. Dit onderzoek vond plaats met behulp van een experimentele schaal wormen reactor gelokaliseerd op de afvalwaterzuivering in Wolvega. Het eerste deel van het onderzoek bestond uit het karakteriseren van de prestaties van de reactor en vergelijken met andere continu systemen. Uit dit onderzoek is gekomen dat consumptie door wormen de structuur van slib verandert wat positieve gevolgen heeft voor de ontwatering. Tevens wordt het volume gereduceerd waardoor slib verwerkingskosten met 40% kunnen worden verlaagd. De consumptie snelheden van de wormen in de grote schaal reactor (0.8 kg TSS/m^3/d) waren in dezelfde orde van grote als waarden gerapporteerd door andere onderzoekers.

Het tweede deel van het onderzoek bestond uit het creëren van inzicht in verandering in de wormen biomassa door middel van statistische analyses. Een dataset met gegevens verzameld over drie jaar was geanalyseerd. Multilineaire regressie analyse is gebruikt om de invloed van milieuvariabelen op slib afbraak te bepalen. Een multivariate analyse is gebruikt om de invloed van milieuvariabelen op wormen biomassa te bepalen.

Uit multilineaire regressie analyse bleek dat er een positieve correlatie is tussen biomassa dichtheid en slib afbraak (R^2=35%, i.e. 35% van de variatie afbraak is verklaart door biomassa). Uit de multivariate analyse bleek dat eren positieve correlatie is tussen groei en slib afbraak (R^2=31%). De grootte van wormen was ook onderzocht en hieruit kwam dat kleine wormen (>3mm) gecorreleerd waren met hoge biomassa concentraties. Grotere wormen(> 4mm) waren gevoeliger voor turbulentie in de wormen reactor.

In conclusie is er een dataset van drie jaar data verzamelen tot stand gekomen. De variabelen slib afbraak en wormen biomassa varieerden erg over tijd. Met statistische analyse technieken kon een klein deel van de variatie verklaard worden. Beide analyses toonden aan dat de ingaande slib hoeveelheid een belangrijke parameter is en geven de veronderstelling dat des te meer slib wordt aangeboden aan de reactor, des te meer slib wordt afgebroken.

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1. Introduction

Since 1995 it has been prohibited to use waste water treatment sludge as fertilizer in the Netherlands. Sludge disposal has become a major cost for waste water treatment plants (WWTP) without anaerobic digestion and a load of less than 100000 population equivalents (up to 50% of total operation costs) (Wei, 2003). There are three major strategies to reduce sludge in conventional activated sludge systems, as presented in Table 1.

Table 1. Strategies to reduce sludge in conventional activated sludge system

Enhanced pre-treatment	Reduce the organic matter load before entering waste water treatment process
Yield reduction	Retain sludge in waste water treatment process for a longer period; less food (organic matter) is available per microorganism and sludge has more time to be mineralized. This results in less kg of sludge per kg of organic matter.
Sludge disintegration	A higher portion of the sludge becomes more biodegradable so that more can be mineralized and assimilated by the biomass.

A biological way of sludge disintegration is through consumption by organisms. During transfer to a higher trophic level in the food chain, potential energy is lost as heat due to maintenance processes, respiration and reproduction (Ratsak and Verkuijlen, 2006). Different types of sessile and free-swimming aquatic Oligochaetes have been found in Aerobic Tanks (AT) of WWTP (Janssen et al., 2002, Elissen et al., 2008). It has been found that population peaks of *Nais* sp. and *Aeolosoma hemprichi* coincided with a reduction of waste sludge (measured as total suspended solids, TSS) and lower sludge volume index (SVI) values (Ratsak, 2001; Wei, 2003). Worms in AT reduce sludge and correlations between occurrence of worms and various process conditions have been found (Table 2).

Table 2 Worm growth correlations with process conditions (positive + and negative -)

Environment	Author	Species	Influence on biomass growth	
			Environmental factor	Correlation
Natural	Armendariz, 2008	*Dero costatus*	Temp	+
			Plant biomass	+
AT	Janssen et al., 2002	*Tubifex tubifex*	FE dosage in AT	+
			Dissolved Oxygen	+
			Shear	-
	Wei, 2003	*Aeolosoma hemprichi*[*]	Temp	+
			pH	-
		Nais elinguis[*]	TSS	-
			Temp	+
			Dissolved Oxygen	+
			Food/Microorganisms	-
Cultivation	Leppänen and Kukkonen, 1998	*Lumbriculus variegates*	Size worms	-
	Elissen et al., 2006	*Lumbriculus variegates*	Worms / Sludge (limiting)	-
	Wei, 2005	*Aeolosoma. hemprichici*	Temp	+
		Nais elinguis	HRT	+
	Huang et al., 2007	*Tubifex tubifex*	Temp	+

[*] during species blooms in AT

Despite these correlations, attempts to control growth and maintain high densities inside waste water treatment processes have been unsuccessful (Ratsak and Verkuijlen, 2006). Trend analysis of a 2,5 years period monitoring dataset confirmed that growth of free swimming Oligochaetes is unlikely to be controllable (Elissen et al., 2008). Another approach is culturing Oligochaetes on carrier material in separate reactors, in which sludge reducing Oligochaete populations can be cultivated under optimal conditions. Continuous worm growth systems that were operated on lab scale for more than 50 days have been reported and are presented in Table 3.

Table 3 Continuously operated worm reactors under laboratory conditions

Researcher	Species family	Reactor volume L	Degradation efficiency %TSS/TSS	Worms contribution %	Operating time days
Wei and Liu, 2006	Tubifex	11	48	nd*	50
Guo et al., 2007	Tubifex	92	47	nd*	100
Huang et al., 2007	Tubifex	20	33	57	150
Hendrickx et al., 2009a	Lumbriculidae	4	18	71	80

* not determined

A requirement for continuous reactors is steady-state of biomass, meaning that worm decay rate equals worm growth rate. Since sludge degradation is coupled to presence of worm biomass, application of this technology would require control of worm biomass dynamics. However, stable population growth in full scale reactors has so far not been reported.

In this study biomass dynamics of a full scale worm reactor are investigated. The research questions are:

- What is the degradation efficiency of a full scale worm reactor?
- What is the effect of worm consumption on structure of sludge?
- What is the influence of worm biomass on sludge reduction in a full scale worm reactor?
- Which physiological/biological variables influence worm biomass in a full scale reactor?
- What is the influence of worm population length distribution on worm biomass in a full scale reactor?
- What is the influence of nutrients on worm biomass in a full scale reactor?

The hypotheses are:
- Sludge is consumed by worms and thereby compacted.
- Sludge reduction can be explained by worm biomass.
- Worm biomass dynamics in the full scale reactor can be controlled by process parameters|.

The first part of the research is about characteristics of the full scale reactor and is to investigate the benefits of the system. The second part is to gain insight into biomass dynamics and how changes in biomass density influence sludge degradation. Here fore a three year dataset is analysed with multilinear regression and multivariate analysis.

2. Material en method

2.1. Reactor setup and operational parameters

A full scale worm reactor was operated at WWTP Wolvega, the Netherlands in the period November 2006 – April 2010. WWTP Wolvega is a conventional activated sludge system; more details on the WWTP specifications are presented in Appendix I.

The water processing chain and integration of sludge degrading worm reactor are presented in Figure 1. This research focuses on the full scale worm reactor, which has been designed to process one third of the daily sludge production (500 kg TSS/d). Return sludge was withdrawn from the settling tank and pumped through a drum filter (mesh size: 500 µm) which was mixed with disposable effluent water, resulting in a diluted sludge stream entering the worm reactor. The relative flows of the return sludge and the water were controllable, making it possible to feed the reactor with a sludge concentration of choice (usually between 1 -3 g TSS/l).

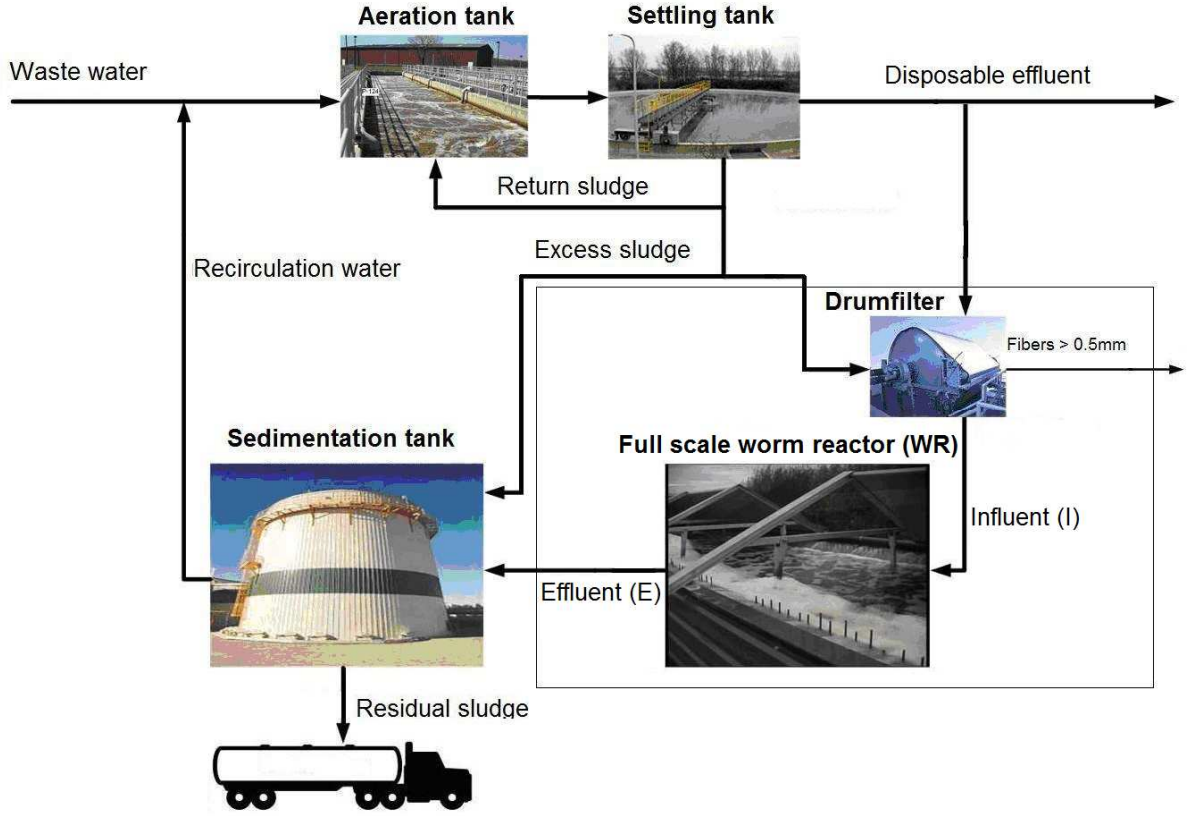

Figure 1 Process overview of full scale worm reactor in WWTP

Sludge was filtered before entering the worm reactor, in order to prevent blockage of the heat exchanger which has 2mm channels. Temperature was controlled by a heat exchanger and a thermometer coupled to a heat pump. A blower (Aerzener, Delta blower G4-004 LXT) injected oxygen into the reactor from the bottom, causing an airlift with two functions: maintaining the dissolved oxygen concentration and mixing, which enhances transport of food and oxygen. Oxygen input was controlled manually by an operator. If dissolved oxygen (DO) concentration became lower than 2 mg/l, the blower power was increased. Worm density on the nets was controlled by a biomass regulation mechanism, causing increased turbulence thereby removing parts of the biomass layer. The operator inspected the worm density on the

nets and activated the mechanism if needed. The worm reactor compartments were controlled by Priva Top Control 6.2 software and online measurements were stored in Microsoft Access. Table 4 shows the applied operational parameters.

Table 4 Overview of operational parameters

Parameter	Unit	
Volume	m^3	125
Surface carrier material	m^2	1100
Flow(sludge + water)	m^3/d	200 (350)*
Hydraulic retention time	h	15 (9)*
Temperature	^oC	25
Dissolved oxygen	mg/L	>2
Sludge concentration influent	g/L	1-3

* Value in brackets is during increased throughput for a period of 3 months

The system effluent TSS concentration was set, i.e. the amount of sludge entering the reactor depended on the reduction activity. The aimed species to prevail in the reactor under the above mentioned parameters was *Dero (Aulophorus) furcatus.*

2.2. Measured variables

Table 5 shows the measured variables and their average frequency of measurement. The frequency of the measurement varied over time with more intense measurement frequencies in 2009.

Table 5 Measurement scheme

Variable	Symbol	Unit	Sample point **	Sample frequency (/week)
TSS concentration	S	kg/m^3	I,E*	online***(2)
Volumetric flow*	F	m^3/h	I,E	online***(1)
Temperature*	T	°C	WR	online
Dissolved oxygen*	DO	mg/L	WR	online***(1)
Ammonium	NH_4^+	mg/L	I,E	1
Nitrite	NO_2	mg/L	I,E	1
Nitrate	NO_3	mg/L	I,E	1
Phosphate	PO_4	mg/L	I,E	1
Water level*	W	cm	WR	5
Worm biomass on carrier material	X_{wn}	kg tss/m^3	WR	2
Worm population length distribution	X_{wp}	-	WR	5
Worms in water phase	X_{ww}	/L	WR	2
Sludge volume index	SVI	mL/g	I,E	****

* Variable is regulated
** I=Influent, WR=Worm Reactor, E=Effluent
*** Online = interval at two seconds, (calibration frequency is between brackets)
**** Measurement used for separate experiment (fourfold measurements per sample point)

Environmental variables

Physiological variables

Volume flow of the full scale reactor was measured with magnetic flow meter (Flowmag FM 20). Shear in the system varies and was determined by the airlift and water level.

Four variables for the biomass regulation were used to define the influence of this mechanism.

- Cleaning intensity, low- and high flow ($CL_{FL,}$ CL_{FH})
- Frequency of cleaning (CL_N)
- Biomass removed by cleaning (CL_{KG}).

The latter variable was determined by analysing TSS peaks from the online meter of the effluent stream.

Malfunctions

The worm reactor system was subjected to different types of technical malfunctions. Each malfunctions was categorised into one of the following:

1. Oxygen malfunctions
2. Flow malfunctions
3. Feed malfunctions
4. Temperature malfunctions

Each of this variable was measured online (Table 5) thus fluctuations in these measurements indicated the type of malfunction. However, the degree of disturbance of malfunctions had to be determined. Therefore, each malfunction was rated in the scale 1-10 (1- lowest disturbance; 10- highest disturbance). This was determined by analysing TSS graphs from the online meter of the effluent stream.

Sludge reduction

Online TSS concentration (Lange Solitax SC) was used for measurement of sludge reduction. TSS concentration meters were calibrated with a manually determined dry weight sample. This was performed by first dewatering with filter (black ribbon filters; 25µm, Schleicher and Schuell) and then drying further for two hours at 105°C.

As mentioned above, the system was subjected to malfunctions which are causing fluctuations in actual load to the reactor. However, online TSS meters measured the amount of sludge entering continuously. By integrating the measured values and combining them with sludge flow, the mass balance over the system was determined. Sludge reduction was defined as the difference between the influent and effluent mass flow (kg TSS / day):

Sludge reduction $$R = \int F_{IN} \cdot S_{IN} - \int F_{UIT} \cdot S_{UIT}$$ kg/d

Sludge characteristics

Sludge volume index (SVI) was determined to investigate the ability of worms to compact sludge. SVI is defined as the volume in millilitres occupied by 1 g of a suspension after 30 min settling, and is calculated as follows:

$$SVI = \frac{settled\_sludge\_volume(mL/L) \cdot 1000}{suspended\_solids(mg/L)}$$ ml/g

Chemical variables

An online oxygen meter (Lange LDO sc100) was used and calibrated using a manual oxygen meter (O2 WTW 315i).

Nutrient variables NH_4-N, NO_2-N, NO_3-N and PO_4-P were measured with Dr Lange ® kits LCK-305, LCK-339, LCK-341 and LCK-348 respectively.

Biological variables
Worm biomass was determined by measuring quantities attached to carrier material and by counting worms in liquid phase. Growth was calculated as the change in biomass attached to carrier material. Worm population length distribution was measured for a period of two months.

Worms on carrier material
Matter attached to carrier material consisted of worms and sludge. The ratio was determined by sieving worm flocs from nets through a strainer and flushing with ethanol (70%). The residual consisted of pure worms and dry weight was determined by drying samples overnight at 105°C. The filtrate consisted of sludge, dry weight was determined by first dewatering with filter (black ribbon filters; 25µm, Schleicher and Schuell) and then drying further for two hours at 105°C.

The worm/sludge mixture was removed from an area of 0,25 m^2 of the carrier material with a water vacuum cleaner (Kärcher AZ204). Four samples were taken from different locations within the reactor after which volume and dry weight was determined by drying samples overnight at 105°C . The carrier material worm biomass was calculated as follows:

$$X_{wn} = \frac{V_{sample} \cdot C_x}{A_{sample}} \cdot \%w \cdot \frac{A_R}{V_R} \qquad \text{g TSS / m}^3$$

X_{wn}	worm biomass on carrier material	[g TSS worm/m^3]
V_{sample}	volume of sample	[L]
C_X	concentration dry weight sample	[g TSS/L]
A_{sample}	sampled surface	[m^2]
%w	percentage worms (m/m)	[%]
A_R/V_R	suface/volume ratio of reactor	[m^2/m^3]

Specific growth of worms was calculated as the change in amount attached to carrier material:

$$\mu = \frac{1}{X_{wn}} \frac{dX_{wn}}{dt} \qquad [d^{-1}]$$

Worms in water phase
The number of worms in the water phase was determined by taking a 100 ml sample of worm reactor liquid and counting worms.

Worm population length distribution
Samples were discarded from nets and worms were categorized in lengths of <2, 3, 4, 5 and >6. For every sample, the number of worms in each category was determined. This method only determines distribution of worms and does not take into account that longer worms weigh more than short worms. Therefore, relative worm length values were converted to an interaction term with X_{wn}:

$X_{wnp<2} = X_{wn} * X_{wp<2} * 1/6$
$X_{wnp23} = X_{wn} * X_{wp23} * 2/6$
etc.

2.3. Data analysis

A dataset was collected containing the measured values of all above mentioned variables with daily averages from online meters and non-daily measurements for the period of September 2007 to November 2009.

Data was assessed for normality by examining Q-Q plots and calculating skewness, kurtosis and performing Kolmogorov-Smirnov test using SPSS 17. Skewness indicates asymmetry, kurtosis incidates peaks relative to a normal distribution, Kolmogorov-Smirnov is a 'goodness of fit' test of a dataset compared with a reference normal distribution. Normality is important to prevent data analysis based on outliers and it is an condition necessary for certain statistical tests.

If data was not distributed normally, Spearman's rho correlations were determined instead of the Pearson correlation. For analyses of multiple independent variables on one dependent variable, a multi linear regression analysis was applied. This analysis assumes causality of independent variables on the dependent variable, requires normality of residuals and is based on the following equation.

$$y_i = \beta_0 + \beta_1 \cdot X_1 + \beta_2 \cdot X_2 + ...\beta_p \cdot X_p + \varepsilon \sim\sim\sim \varepsilon = N(0, \sigma_2)$$

when:

y	dependent variable
β	regression coefficient
x	independent variable
ε	residuals
$N(0, \sigma_2)$	condition of normal distribution

For analysis of multiple independent variables on multiple dependent variables, a multivariate analysis was applied. The worm biomass consists of multiple dependent variables and is described by the different variables (worm on carrier material, worms in waterphase and growth). Environmental variables were correlated to this complex variable with multivariate analysis. This type of analysis can recognize latent patterns in large datasets and can be performed with Canonical correspondence analysis (CANOCO) software (Version 4.5, written by Ter Braak, 1989).

A preliminary test was done to determine the correct response model (linear or unimodal) with detrended correspondence analysis (DECORANA) software (part of CANOCO v4.5). This analysis is a measure for the amount of variability within a dataset and results in a gradient length; for gradients larger than 3.5, an unimodal model is required. Direct gradient analysis (DCA) of datasets used in this study showed that none of the gradient lengths was longer than 3.5 (Appendix IX), indicating that the linear response model (Redundancy analysis) was the most appropriate to analyse both datasets. By using forward selection with a Monte Carlo Permutation test (part of CANOCO v4.5) statistical significant variables ($P < 0.05$) were selected

.

Biomass variables were not determined daily and nutrient variables weekly which resulted in blank measuring points in the dataset. Since CANOCO can not deal with blank samples, two strategies were applied to achieve datasets without empty lines (For example see Table 6, Table 7 and Table 8).

Table 6 Example raw dataset

Day	R	X_{wn}	NH_{UIT}
0	108	0,29	1,5
1	102		
2	51		
3	-85		
4	59		
5	137	0,21	
6	106	0,25	
7	51	0,25	2,1
8	73		
9	81		
10	59	0,28	
11	60		
12	28	0,30	
13	54	0,34	
14	97	0,34	13,7

Strategy to achieve complete dataset

Averaging of 5 days

Interpolation + sludge accumulation

- Biomass variables: interpolated
- It is expected that sludge accumulates in the reactor, possibly resulting in a delayed observed effect. An reduction term is added with an average of three days before

-Nutrient variables: empty lines removed

Table 7 Example dataset with averaging

Day	R	X_{wn}	NH_{uit}
0	47	0,29	1,5
5	89	0,23	2,1
10	59	0,32	13,7

Table 8 Data set with interpolation

Day	R	$R_{average}$	$X_{wn,\ interpolated}$	NH_{UIT}
2	51	87	0,26	
3	-85	22	0,24	
4	59	8	0,22	
5	137	37	0,21	
0	108	110	0,29	1,5
7	51	98	0,25	2,1
14	97	60	0,34	13,7

As a result of these adapted datasets different influences of variables on biomass dynamics were investigated. Variables used in CANOCO are shown in Table 9.

Table 9 Dataset description and entered variables in CANOCO

Dataset	Influence	N	Variables		
			Dependant**	Independent***	Omitted
Average (5 days)	Environmental variables on X_w	48	X_{wn}, X_{ww}*, u	R, S_{in}, S_{uit}, M, DO, BLW, F, T, CL	X_{wp}, Nutrient, W
Average (5 days)	Environmental variables on X_w	48	X_{wn}, X_{ww}, u	R, S_{in}, S_{uit} , M, DO, BLW, F, T, CL	X_{wp}, Nutrient, W
Interpolation	Environmental variables on X_w	259	X_{wn}, X_{ww}*, u	R, R_a, S_{in}, S_{uit} , M, DO, BLW, F, T, CL	X_{wp}, Nutrient, W
Interpolation	Length of ratio worms on X_w	86	X_{wn}, X_{ww}*, u	X_{wp}, R, R_a, S_{in}, S_{uit} , M, DO, BLW, F, T, CL	Nutrient, W
Interpolation	Length of ratio worms on X_w	86	X_{wn}, X_{ww}*, u	X_{wp}*, R, R_a, S_{in}, S_{uit} , M, DO, BLW, F, T, CL	Nutrient, W
Interpolation	Interaction term Length and X_{wn} (X_{wnp}) on X_w	86	X_{wn}, X_{ww}*, u	X_{wnp}*, R, R_a, S_{in}, S_{uit} , M, DO, BLW, F, T, CL	Nutrient, W
Interpolation	Environmental variables on X_{wnp}	86	X_{wnp}*	R, R_a, S_{in}, S_{uit} , M, DO, BLW, F, T, CL, W	X_{wn}, X_{ww}, u, Nutrient
Interpolation	Nutrient on X_w****	35	X_{wn}, X_{ww}*, u	Nutrient, R, R_a, S_{in}, S_{uit} , M, DO, BLW, F, T, CL	X_{wp}, W

* Variable was LN transformed ** In CANOCO showed as 'Species' *** In CANOCO showed as 'Environment data' **** Empty lines removed

Partitioning of variance within multivariate analysis was applied to investigate influence of a single variable and still taking into account other variables. This was done by setting the variable of interest as independent and other variables as covariables.

Statistical significant difference of SVI before and after consumption was analysed by an independent sample t-test. An assumption for this test is that variance of compared variables is equal, this was tested with a Levene's test (Levene's test P-value >0.05).

3. Results and discussion

3.1. Characterisation: Growth of worms and sludge reduction in full scale reactor

Throughout operation of the full scale worm reactor, the amount of worms on the carrier material (X_{wn}) varied over time. A yearly average of X_{wn} 1.9 ± 0.8 kg TSS worm/m^3 was observed. Fluctuations in biomass are shown in Figure 2.

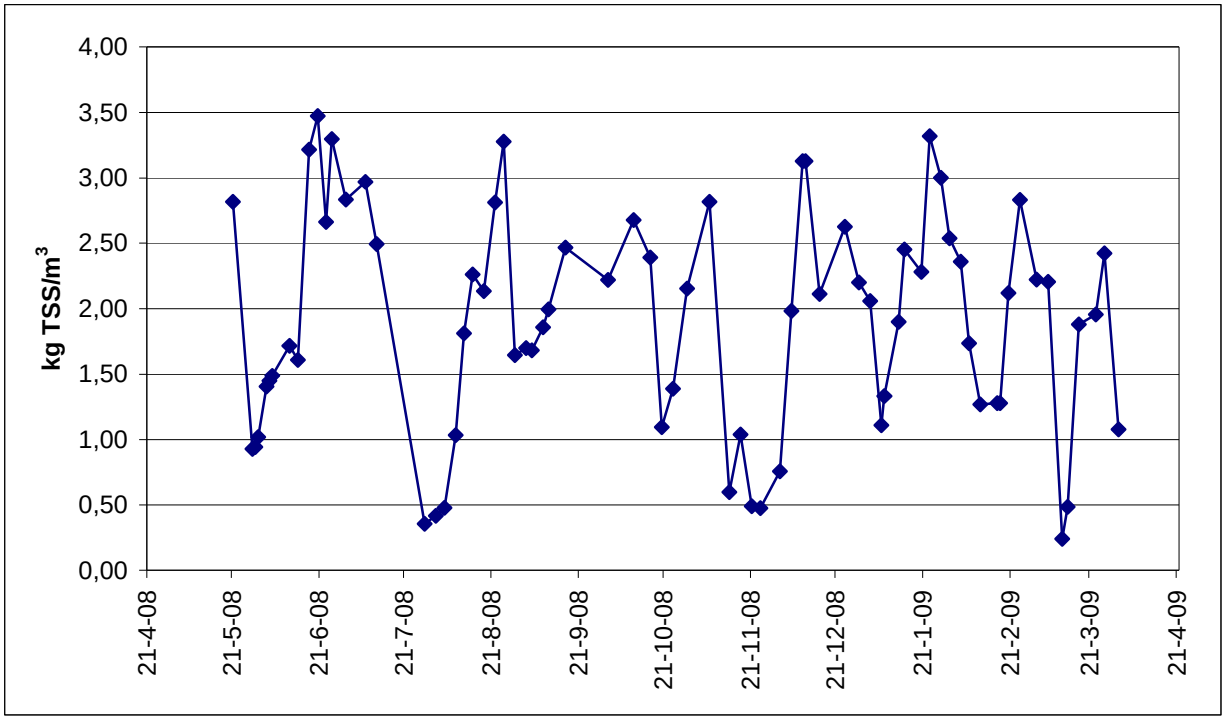

Figure 2. Worm biomass on carrier material

The worm reactor is equipped with an biomass regulation system which is expected to be responsible for the following mechanisms:

1. Prevention of over population which causes swimming away of worms.
2. Removal of thick flocs of worms resulting in more food availability per worm.
3. Removal of older worms. Younger worms might have a firmer attachment to nets and are associated with higher growth- and consumption rates.
4. Removal of unwanted species which cannot withstand harsh shear conditions.

During operation, several exponential growth phases could be identified and were used to determine growth (Appendix III) . Growth and consumption rates of aquatic Oligochaetes were calculated (using formulas in Appendix IV) and compared with continuous systems from literature (Table 10).

Table 10 Growth and sludge consumption of aquatic worms in continuous systems

Researcher	Species family	Reactor size	Volume specific sludge degradation	Biomass specific sludge degradation	Worm biomass	Biomass specific growth rate	Biomass yield
			r_s	q_s	X_{wn}	μ_s	Y_{ws}
		m^3	kg tss/m^3/d	kg tss/kg tss worm/d	kg tss worm/m^3	/d	kg tss worm/ kg tss sludge
Hendrickx et al., 2009a	Lumbriculidae	0.004	0.10*	0.36*	0.29**	0.01	0.18***
Huang et al., 2007	Tubifex	0.003	1.08	0.43	2.5	nd****	nd****
This work – Full scale	Dero (Aulophorus)	125	0.81	0.52	1.89	0.11	0.22

* calculation with $r_s = q_s * X_{wn}$ (seeAppendix IV)
** adopted from Hendrickx et al., 2009a, A_R/V_R=1.4, worm dw/ww ratio= 0.14
*** adopted from Hendrickx et al., 2009a, sludge VSS/TSS ratio = 0.73
**** nd = not determined

Worm specific growth

The full scale reactor in this study was designed to favour the free swimming *Dero (Aulophorus) furcatus.* This species is desirable because of its high specific consumption rate (q_s = 0.5 kg TSS / kg TSS / d) under the conditions of interest. Specific growth of this species is higher than Lumbriculidae species (Table 10), however batch experiments with Lumbriculidae species showed a growth rate of 0.08 days (Buys et al., 2008). The final aim of a worm reactor is to degrade sludge at high rate. This can be accomplished either by growing worms at high rate or by worms which use sludge inefficient, i.e. need high amounts of sludge to grow.

The *D. furcatus* thrives in this worm reactor because of selective pressure. One mechanism behind this could be that a high growth rate gives an advantage in situations were biomass is frequently removed from the carrier material. Another mechanism could be that *D. furcatus* will suffer less from shear stress than other species and has an advantage at short hydraulic retention times and increased turbulence. This was also found for *Dero* spp. by Inamori et al., 1983.

The observed growth yield (Y_{ws} in Table 10) of worms was in the same order of magnitude as observed for other Lumbriculidae worm species (Table 10) and for protozoa growing on activated sludge; 0.16 - 0.54 kg TSS/ kg sludge TSS (Ratsak et al., 1996).

Sludge degradation

Sludge degradation was observed in the full scale reactor and is shown in Table 11 (for details see Appendix V).

Table 11. Average annual sludge degradation in full scale reactor

Year	Sludge degradation
2007	32%
2008	21%
2009	14%

The observed degradation is similar to other systems (33%-48% degradation, Table 3). Hendrickx reported 18% degradation, though in these experiments worms were limited in growth. Lower degradation in 2008 is due to an invasion of a Lumbriculus species. In 2009, the amount of technical malfunctions increased.

The volume specific sludge reduction is an important parameter for the design of a reactor. The value for the full scale design (0.8 ± 0.4 kg TSS / m^3 /d) is in the same order of magnitude as the 1.1 kg TSS / m^3 /d that was reported for a lab-scale reactor by Huang (Table 10). However, the worm biomass concentration on carrier material was subjected to high variation (Figure 2). This was mainly due to technical malfunctions and fluctuations in operational parameters and worm dynamics. Instead, a dynamic system was operated with worm biomass density constantly increasing and decreasing between 0.5 kg TSS/m^3 and 4 kg TSS/m^3. The variation in biomass density is further investigated with statistical analysis (chapter 3.2).

Structural change of sludge

Comparison of sludge volume index (SVI) before and after worm sludge consumption in the full scale reactor is shown in Figure 3.

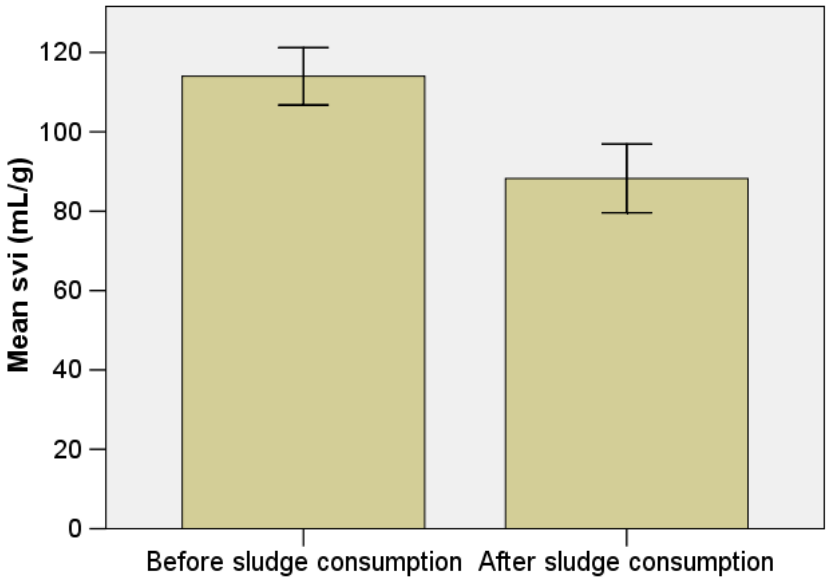

Figure 3. Sludge volume index of sludge before and after worm consumption (N=4, Error bars: 95% confidence intervals)

Figure 3 indicates that the SVI is lower after sludge consumption. This was confirmed by an independent sample t-Test including Levene's test. Sludge volume index was significantly different before and after sludge consumption ($P<0.05$, Appendix VI). Another process influencing the SVI could be the heat exchanger through which the sludge is pumped with 4 bar through 2mm channels. This possibility was tested by measuring SVI before and after the heat exchanger. An independent sample t-Test indicated no significant difference before and after the heat exchanger (N=4, P=0.80). Sample sizes in these experiments were small ; however, the results are similar to those of previous studies which found a correlation between presence of worms and decreased SVI values (Wei, 2003; Ratsak and Verkuijlen, 2006; Elissen et al., 2008). It is expected that retention of sludge in worms guts decreases particle size of sludge flocs and thereby compacting the sludge.

Practical outcomes for a waste water treatment plant.

Sludge consumption by aquatic worms results in a volume reduction of approximately 30% and worms are compacting the sludge from a SVI of 114 ± 7 mL/g to 88 ± 8 mL/g. This means the following for a WWTP. The compacting positively influences dewatering properties of sludge, however this will not directly impact the WWTP economically. The volume reduction effect has the potential to reduce operating costs on the WWTP.

Conventional sludge processing costs on a WWTP in the Netherlands are 600 €/tonne TSS sludge (Wiegant et al., 2005). Application of a worm reactor would reduce the amount of sludge to be processed however an additional investment is needed for the worm reactor. A worm reactor to treat 1000 tonne TSS/year including operational costs and maintenance is estimated to cost an additional 140 €/tonne TSS sludge (Tamis et al., 2010) . Eventually application of a worm reactor can change the costs to process a tonne of sludge from €600 to (140 + 600 * 0.7)= €560. This is a minor difference, however further degradation of sludge has been observed in the sedimentation tank (Figure 1). This was probably due to anaerobic digestion and is currently being investigated but out of scope for this research. It is expected that a combination of a worm reactor and digestion can reduce the volume with 65%, which has the potential to reduce the costs to (140 + 600 * 0.35)= € 350 per tonne TSS sludge.

3.2. Worm biomass dynamics: Factors influencing worm biomass

Since not all data were normal distributed (Descriptive statistics of all variables are shown in Appendix VII), Spearman's rho coefficients using bivariate correlations were determined for the biomass variables X_{wn} (N=93), growth (N=286), worms in water phase (N=262), worm population length distribution (N=42) and chemical and physical variables. Significant (P<0.05) correlations which explain more than 15% are displayed in Table 12 (for all significant correlations see Appendix VIII).

Table 12 Significant Spearman's rho correlations (P<0.05) regarding biomass (2-tailed)

Variable 1	Variable 2	Spearman's rho correlation
Worms on carrier material (X_{wn})	Cleaning frequency (CL_N)	0.40
	Cleaning flow low intensity (CL_{Fl})	0.43
	Dissolved oxygen (DO)	-0.37
Worms in water phase (X_{ww}) *	Blower power (BLW)	0.38
	Average dissolved oxygen (DO_a)	-0.43
Worm population length distribution <2mm (X_{wp_2}) *	Sludge influent (S_{in})	0.44
	Flow (F)	0.41
Worm population length distribution 2-3 mm (X_{wp23}) *	Flow deviation (F_{stda})	0.40
Worm population length distribution 4-5 mm (X_{wp45}) *	Sludge influent (S_{in})	-0.38
	Flow deviation (F_{stda})	-0.41

* LN transformed data

No correlations were found with notable high influence, only small influences on variations in worm biomass of environmental variables were found. This is probably due to the complexity of the worm biomass dynamics. For this reason multivariate analysis was needed.

Influence of environmental variables on worm biomass

Influence of environmental variables on biomass (X_{wn}, X_{ww} and μ = growth) was determined with a redundancy analysis (RDA) of two datasets. The resulting ordination diagrams are presented in Figure 4. The length of the arrow is a measure of the importance of the variable, while the arrowhead points in the direction of increasing influence.

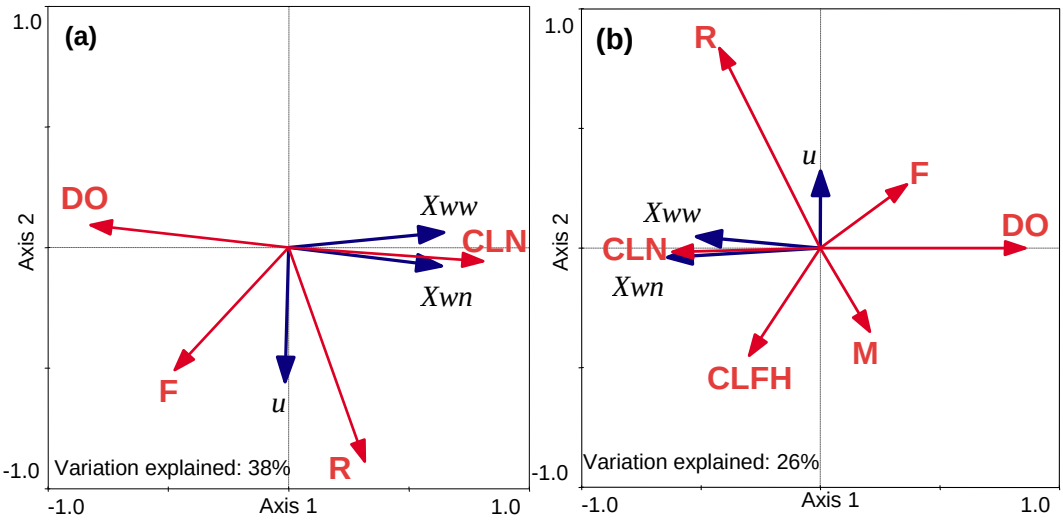

Figure 4 Direct ordination of biomass (bold blue) related to significant environmental variables (thin red P<0.05) a: dataset 1 with 5 day averages (N=48) b: dataset 2 with interpolations (N=259). Abbreviations used Xwn = worms on carrier material, Xww = worms in water phase, *u* = growth, DO= dissolved oxygen, F=Flow, R=reduction, CLN= Cleaning frequency, M=malfunction, CLFH= cleaning flow high intensity.

Analysis results showed that 38% of the variation in biomass (X_{wn}, X_{ww}, μ) was explained by sludge reduction (R), flow (F), dissolved oxygen (DO) and cleaning frequency (CLN). Interpolations were considered to be acceptable since both datasets resulted in the same variables. With dataset using interpolation, the extra variables malfunction(M) and high flow intensity cleaning (CLFH) were correlated.

Multivariate analysis indicated that variables X_{wn} and X_{ww} were of high importance since they were on the first axis. Multivariate analysis (Figure 4, direction of arrow) and bivariate analysis (Table 12) indicated that X_{wn} is negatively correlated with DO. This can be explained by oxygen uptake of the worms. Low biomass concentrations on carrier material correlates with low oxygen uptake thus oxygen concentration in the liquid phase increased. However, since DO concentrations are assumed not to be limiting, it is not an explanatory variable for biomass. When DO was left out of the analysis, no other variables were included.

Growth rate was mainly correlated with degradation of sludge, R has relatively high importance (Figure 4, length of arrow) in relation to biomass . This correlation seems to be influenced by other parameters, since bivariate correlations showed small Spearmans rho coefficient (μ and R: 0.27, Appendix VIII).

Since the variable sludge influent (S_{in}) can be controlled, it was interesting to relate this variable to biomass. Redundancy analysis with selection of S_{in} showed that 32% of the variation in biomass was explained by S_{in}, CL_N and DO, which contributions were significant (Figure 5). Partitioning of variance showed that S_{in} explains 6% of the variability. When this variable was included sludge reduction and flow were not included due to the high correlation of these variables with sludge influent.

Multivariate analysis (Figure 4) and bivariate analysis (Table 12) show that X_{wn} has a positive correlation with CL_N. This is because the regulation system for worm biomass frequency control was intensified by operator at high biomass concentrations. Short hydraulic times (high F) seems associated with more growth, possibly because of high ‘refreshment’ (removal rate of inhibiting compounds) of biomass layer. On the contrary, other researchers found that long hydraulic retention times are correlated with growth (Wei, 2005, Table 2) . Long lasting cleaning steps (CL_{FH}) create periods of extreme shear and may influence ‘growth’ negatively.

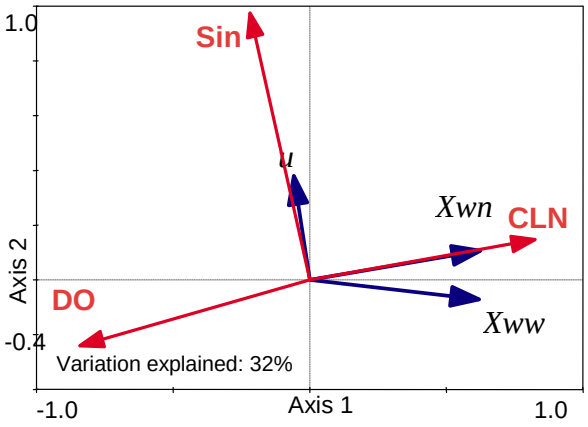

Figure 5 Direct ordination of biomass (bold blue) related to significant process parameter (thin red P<0.05) Dataset 1with 5 day averages (N-48) Abbreviations used Xwn = worms on carrier material, Xww = worms in water phase, *u* = growth, DO= dissolved oxygen, CLN= Cleaning frequency, Sin= Sludge influent

Influence of worm population distribution on worm biomass

Influence of worm population length distribution was analysed with an interpolated dataset (N=86). Analysis of the dataset with untransformed X_{wp} data resulted in no significant (P= 0.22) X_{wp} variables. After transformation of $X_{wp,}$(natural log), worm population length distribution variables could be included but showed small importance.

By combining length distribution with worm on carrier material as described above, worm population length distribution variables could be included. Redundancy analysis showed that 36% of the variation in biomass was explained by sludge concentration influent, daily temperature maximum, X_{wnp23} and X_{wnp56}, these variables contributed significantly. Partitioning showed that X_{wnp23} and X_{wnp56} could be related to 5% of the variability. Figure 6 shows the ordination diagram of this analysis.

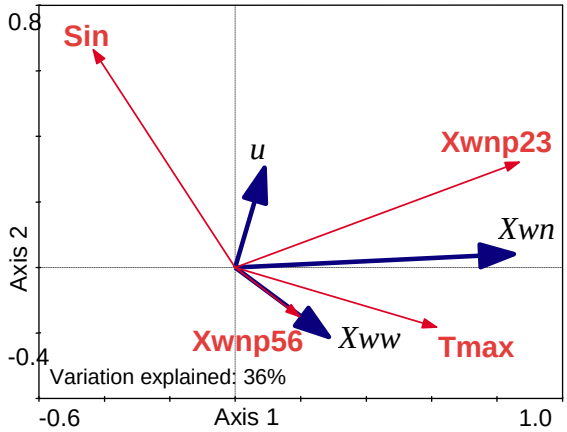

Figure 6 Direct ordination of biomass (bold blue) related to significant process parameters (thin red P<0.05) dataset 2 with interpolations (N=86). Abbreviations used Xwn = worms on carrier material, Xww = worms in water phase, *u* = growth, Sin= sludge influent, Tmax= daily maximum temperature, Xwnp = LN transformed Xwn*Xwp worm length value, 2-3 mm and 5-6 mm.

Figure 6 indicates that presence of smaller worms is positively correlated with X_{wn}. This was probably caused by the increased cleaning which removed longer worms. No correlation between worm length and growth rate was observed. This is in contrast to previous research which reported that smaller worms grow faster (Leppänen and Kukkonen, 1998, Table 2).

Influence of environmental variables on worm population distribution.
Redundancy analysis showed that 21% of the variation in worm population length distribution was explained by daily maximum temperature , malfunction, flow and water level. These variables contributed significantly. Figure 7 shows the ordination diagram of this analysis.

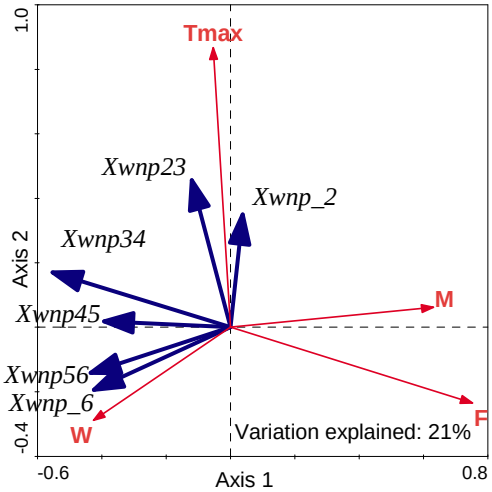

Figure 7 Direct ordination of worm length distribution (bold blue) related to significant process parameters (thin red P<0.05) dataset 2 with interpolations (N=86). Abbreviations used Xwnp = LN transformed Xwn*Xwp worm length value Tmax= daily maximum temperature, M=malfunction, F=flow, W=water level

This figure shows that shear is an important factor influencing worm length distribution. High flow and malfunctions probably influence larger worms negatively. Low water levels are associated with irregular flow and high water levels are favourable for large worms. Small worms are probably more resistant against high turbulence conditions.

Influence of nutrients on worm biomass
Redundancy analysis resulted in 35% of the variance in biomass explained by DO, CL_N, R and NH_{4uit}, this contribution was significant. Partitioning showed that 5% was related to NH_{4uit}. This indicates small influence of ammonium on biomass. Literature reported that ionised ammonia is toxic (96h EC_{50}) to aquatic Oligochaetes at concentrations of 0.7 mg NH_3-N /L (Hickey and Vickers, 1994). Ionised (NH_3) and unionised (NH_4^+) ammonia are in equilibrium and its distribution is a function of pH and temperature (Emerson et al., 1975). The highest ionised ammonia value included in this analysis was 0.25 mg NH_3-N / L (calculated from 24.9 mg NH_4^+-N /L at pH 7.5, T= 25°C). Hendrickx et al., 2009b found that ionised ammonia concentrations of 0.4 mg NH_3-N /L inhibited sludge consumption of *L. variegatus* (calculated from total ammonium 12 mg N / L at pH 7.9, T= 18.3°C).

3.3. Worm biomass dynamics: Influence of worm biomass on sludge reduction

Bivariate correlations were performed and significant ($P<0.05$) Spearman rho coefficients with average sludge reduction for three days and environmental variables are presented in Table 13.

Table 13 Significant Spearman's rho correlations (P<0.05) regarding reduction (2-tailed)

Variable	Spearman's rho correlation
Sluge influent (S_{in})	0.59
Worms on carrier material (X_{wn})	0.34
Malfunctions (M)	-0.30
Temperate, daily minimum (T_{min})	0.31
Cleaning frequency (CL_N)	0.29
Cleaning, amount removed (CL_{KG})	0.25
Cleaning, low flow intensity (CL_{Fl})	0.30
Dissolved nitrite effluent (N_{2UIT})	0.32

Variables which can control reduction and possible interactions between those variables were included in a multi linear regression analysis (stepwise). Normality of residuals (ε) was tested as described above. Results of multi linear regression analysis are shown in Table 14.

Table 14 Multi linear regression analysis of RA, stepwise selected variables are significant (P<0.001), ε is N(0,σ2) and collinearity variables were removed (Tolerance <0.2, VIF>5)

Description	N	Constant	S_{IN}	X_{wn}	X_{wnclkg}	T_{min}	F-test	R^2 (adj)
Xwn	91	-46.42	0.11	291.90			16.16	0.25
Xwn with interactions	53	-75.72	0.16	409.37	-6.18		10.42	0.35
Xwn interpolated	285	-56.17	0.09	136.63		2.55	22.64	0.19

These results show that there was a significant influence on reduction(R) of biomass on carrier material (X_{wn}) when corrected for sludge influent (S_{in}). The linear model including S_{in} and X_{wn} could explain 25% of the variation in R which is more than 12% (R^2 of spearman rho coefficient, Table 13) of X_{wn} on R. A correlation between abundance of worms and reduction of sludge was observed by other researchers (Ratsak, 2001; Wei, 2003).

Model comparison of observed and predicted values is shown in Figure 8.

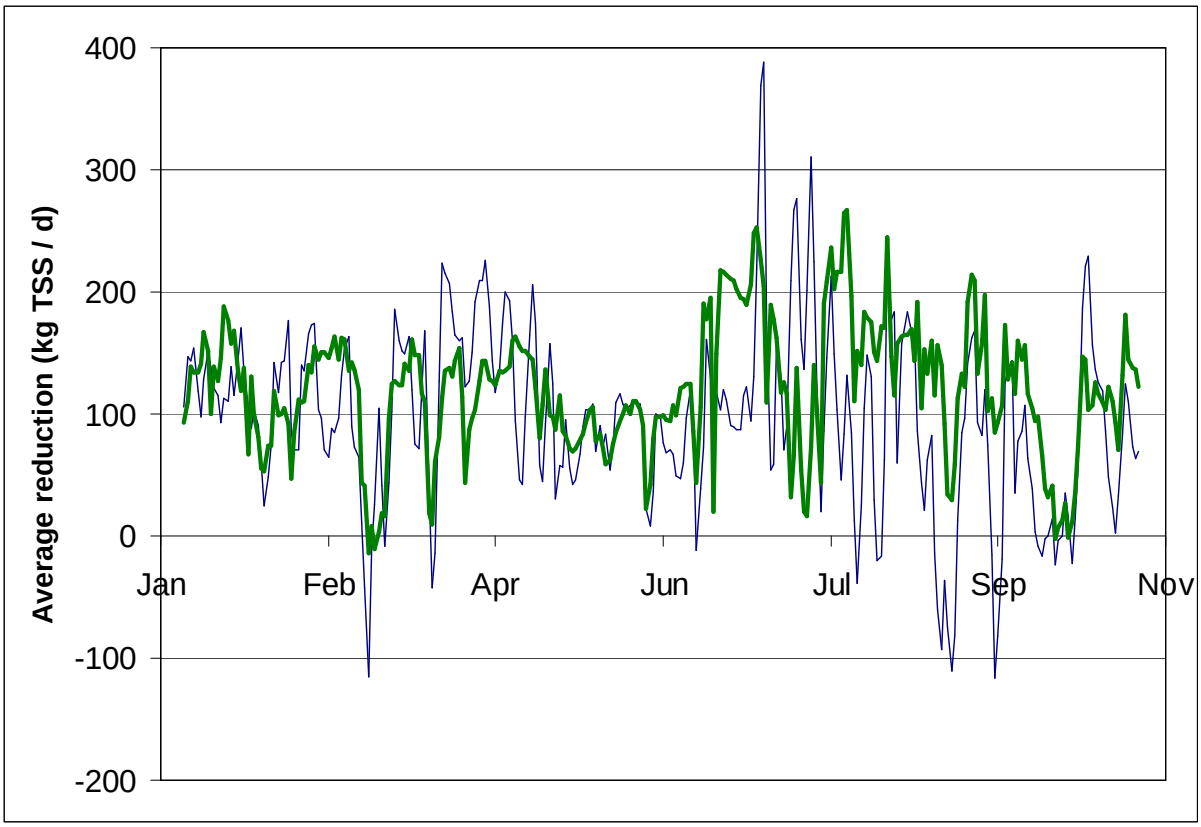

Figure 8 Observed sludge reduction (blue thin line) and predicted sludge reduction including S_{in}, X_{wn}, $X_{wn*clkg}$ (green thick line)

This figure shows that that high peaks could not be predicted. Observed negative peaks are probably caused by the cleaning mechanism, because this will cause release of accumulated worm sludge. However, the included variables in this dataset could not describe this process (see multivariate analysis of biomass). With a multi regression analysis including the interaction term X_{wn}*CL_{kg} (second row Table 14) this interaction showed a significant difference, however negative peaks could not be completely predicted and positive peaks neither.

Observed positive peaks are probably caused by accumulation of sludge in the reactor. Averaging over three days was applied based on practical experience and was expected to reduce accumulation effects, however these effects are still visible, probably longer averaging periods are needed.

Another explanation for the high upper and lower peaks might be deviation in the measurement method. The optical meters showed to be sensitive for variation in types of sludge. However, the continuous optical TSS meters were calibrated up to five times a week. An average correction factor of 10% was applied to calculate actual TSS influent and effluent streams.

3.4. Synthesis: Effect on sludge degradation by aquatic oligochaetes in a full scale reactor

Full scale worm characteristics

The full scale worm reactor in combination with the sedimentation tank has the potential to degrade the amount of sludge on a waste water treatment plant an can reduce the operational costs. However the contribution of sludge degradation of the worm reactor fluctuates largely over time and a larger amount of the degradation occurs in the sedimentation tank. The mechanisms of this process should be further investigated.

Worm biomass dynamics in full scale reactor

Fluctuation in sludge degradation where investigated with statistical analysis. Influence of environmental variables on sludge reduction were investigated with multilineair regression and showed that sludge influent concentration and worms on carrier material were important parameters. Influence of the following three conditions on worm biomass dynamics was investigated with multivariate analysis:

- environmental variables
- worm population length distribution
- nutrients

For the first two analyses, sufficient sample points (>80) were available and one third of variance in worm biomass dynamics was explained. Variables with large influence were dissolved oxygen concentration and cleaning frequency, which could be accounted by operator responses to biomass changes. A correlation between growth rate and sludge reduction was found and a higher concentration of TSS in the influent stream seems to influence worm biomass positively. Partitioning showed that sludge influent explained 6% of the variance. Taking into account the large variance in biomass on carrier material (Figure 2) it is likely that only a small fraction could be explained. Small worms (< 3 mm) are desirable for high biomass concentrations. Larger worms (> 4 mm) are probably more sensitive for intense turbulence and fluctuations such as malfunctions. For the analysis an adapted dataset with interpolations was used which showed to be acceptable since the other dataset using averaging showed similar results (Figure 4). The interpolation dataset has the advantage that short term effects are more likely to be noticed, an example of such a “short term” effect is the malfunction variable (Figure 4) which was not visible in an five day averaging dataset. Since interpolations are acceptable the frequency of measuring could be decreased from five days a week till three days a week. However it has to be taken into account that averaging is a “safer” way to deal with data than interpolation.

For the nutrient influence analysis, sample number (N=35) was too low for proper analysis. An effect of ammonium on worm biomass was found, however an effect is more likely to be noticed when higher ammonia concentrations occur in the worm reactor. In practise this means that for further investigation of ammonium, this variable should be monitored more intensively for a certain period, i.e. higher sampling frequency. In summary, on third of the variation in biomass could be explained, possible with ammonium included as a variable a larger amount of the variation can be explained.

4. Conclusion

Several conclusions can be drawn from the results this study consisting of a characterisation part and an exploration of worm biomass dynamics.

Characterisation reactor:

- A full scale worm reactor was operated which produced an average biomass on carrier material of 1.9 ± 0.8 kg TSS worm/m^3. Sludge degradation was observed in a full scale worm reactor with annual averages of 37%, 21% and 14% TSS/TSS for a three year period. A volume specific sludge reduction of 0.8 ± 0.4 kg TSS/m^3/d was observed.

- Sludge volume index before and after consumption by worms changed from 114 ± 7 mL/g to 88 ± 8 mL/g respectively.

In conclusion, sludge consumption by aquatic worms results in a volume reduction and worms are compacting the sludge. The volume reduction has the potential to result in a cost reduction for the waste water treatment plant of 40%.

Exploration of worm biomass dynamics

- A multi linear model showed that 25% of the variation in sludge degradation (average over 3 days) could be explained by sludge influent and worms on carrier material.

- Influence of the following three conditions on worm biomass dynamics were investigated with multivariate analysis (Redundancy analysis):

1. Environmental variables:
   Redundancy analysis showed that 32% of the variation in biomass (Worms on carrier material X_{wn}, worms in waterphase X_{ww} and growth μ) could be explained by sludge influent, cleaning frequency, and dissolved oxygen. Partitioning of variance showed that sludge influent could be related to 6% of the variability.

2. Worm population length distribution:
   Redundancy analysis showed that 36% of the variation in biomass was explained by sludge influent, temperature dialy maximum, worms with length 2-3 and worms with length 5-6 mm. Partitioning of variance showed that worm the length values could be related to 5% of the variability

3. Nutrients
   Influence of nutrients could not be determined due to insufficient sample number.

In conclusion a three year dataset of a worm reactor system was investigated. The variables degradation and biomass density varied highly over time. Using multilinear regression and multivariate analysis, one third of this variance could be explained.
A correlation between biomass and reduction was found. Both analysis indicated that the parameter influent sludge concentration was an important factor and indicated that the more sludge enters the reactor the more is being degraded.

5. Recommendations

Based on the results of this study, for following recommendations were formulated:

- To determine influence of nutrients on biomass, there were insufficient sample number (N=35) and concentrations did not exceed toxic values reported in literature. When high dissolved ammonia concentrations might be measured, frequent measuring of ammonia should be carried out.

- For population length distribution measurement amounts worms of different lengths were determined. However it was not taken into account that larger more weight more and consume more. In this study an assumption of weight was done based on worms on carrier material. For more accurate data when counting and categorizing worms the samples should be measured for dry weight as well.

- Influent sludge concentration turned out to be an important controlling parameter. One possibility could be that the more feed is available the more worms consume. Another possibility could be that compostition of feed changes and influences consumption behaviour. These possibilities could be tested in lab scale experiments with varying environmental variables, such as:
    - Sludge influent concentrations
    - Sludge influent composition

- Worm length distribution can be analysed more accurate by conducting controlled lab experiments where a certain length of worm is retained and other lengths are removed.

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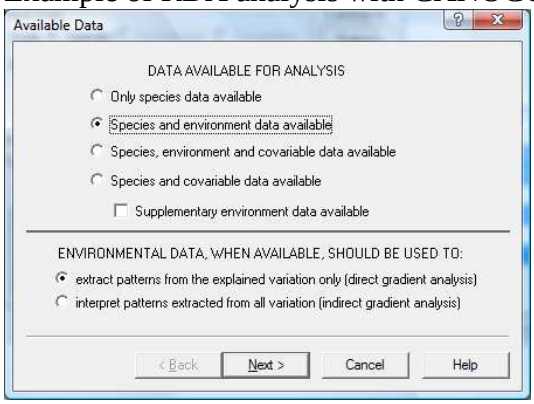
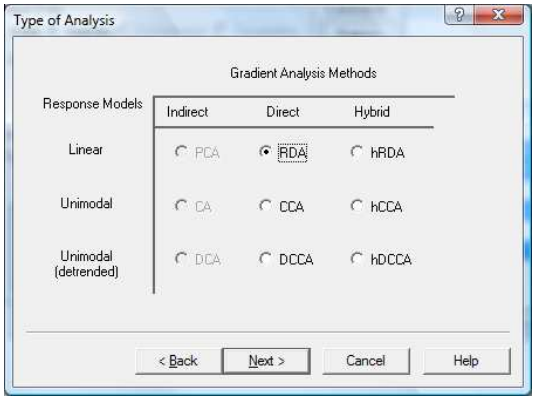
Appendix I. Specifications WWTP Wolvega

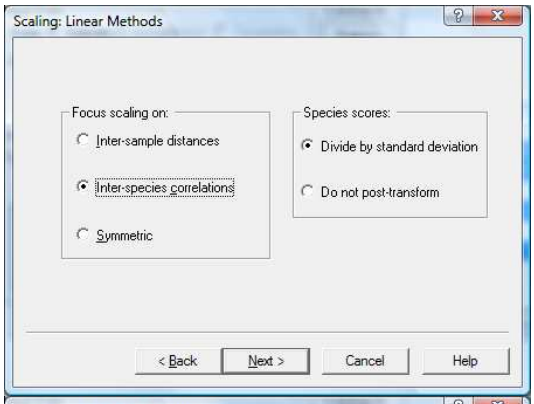
Capacity	35.000	ie_{136}
Industrial share	20	%
Type of treatment plant	Activated sludge	-
Pre-settling	No	-
Type of P Treatment (Chem/Bio)	Chem	-
Chemicals	$FeSO_4$	-
Me/P	0,41	mol/mol
N effluent	6,2	mg N/l
P effluent	1,3	mg P/l
BOD/N ratio influent	4,4	-
BOD/P ratio influent	28	-
Sludge load	0,026	kg BZV/kg ds.d
Sludge age	30	d
Excess sludge production	1.600	kg ds/d
Ash ratio active sludge	30	%
SVI	109	ml/g

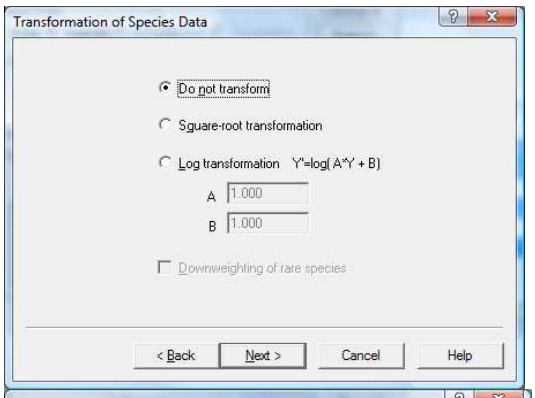
Appendix II. Multivariate analysis: Canoco settings example

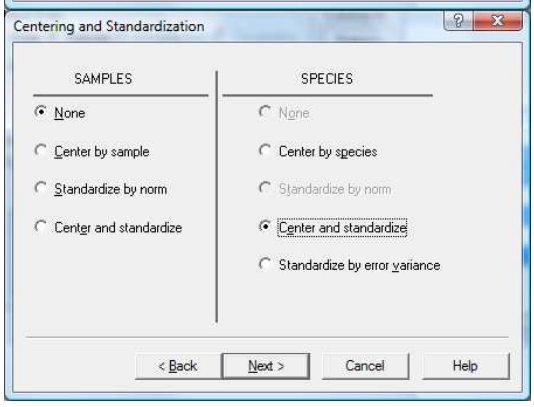
Example of RDA analysis with CANOCO v4.5

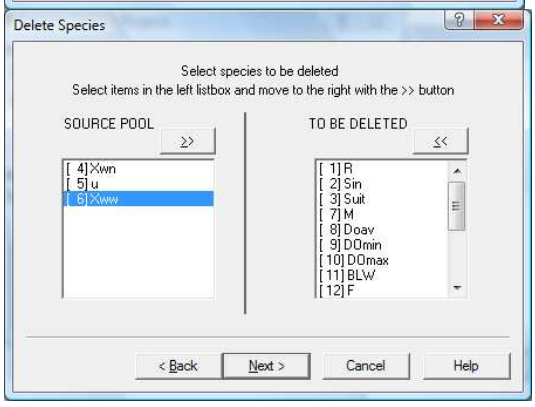


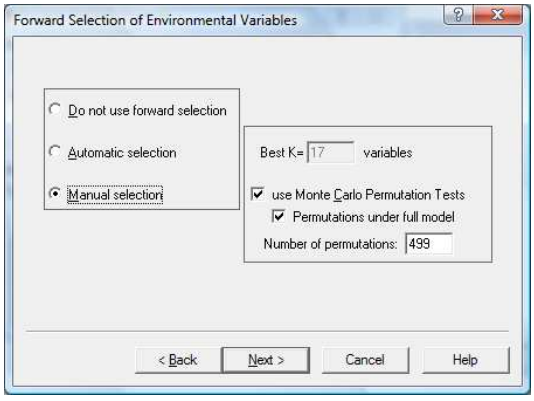


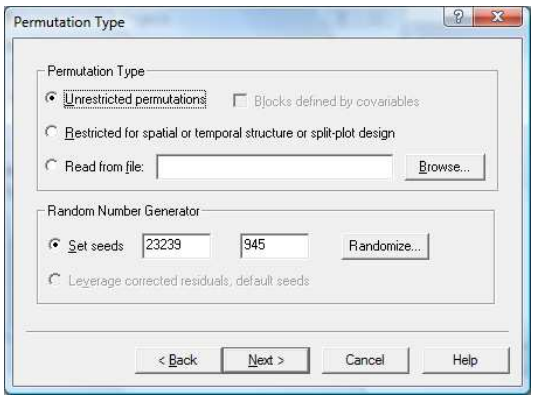






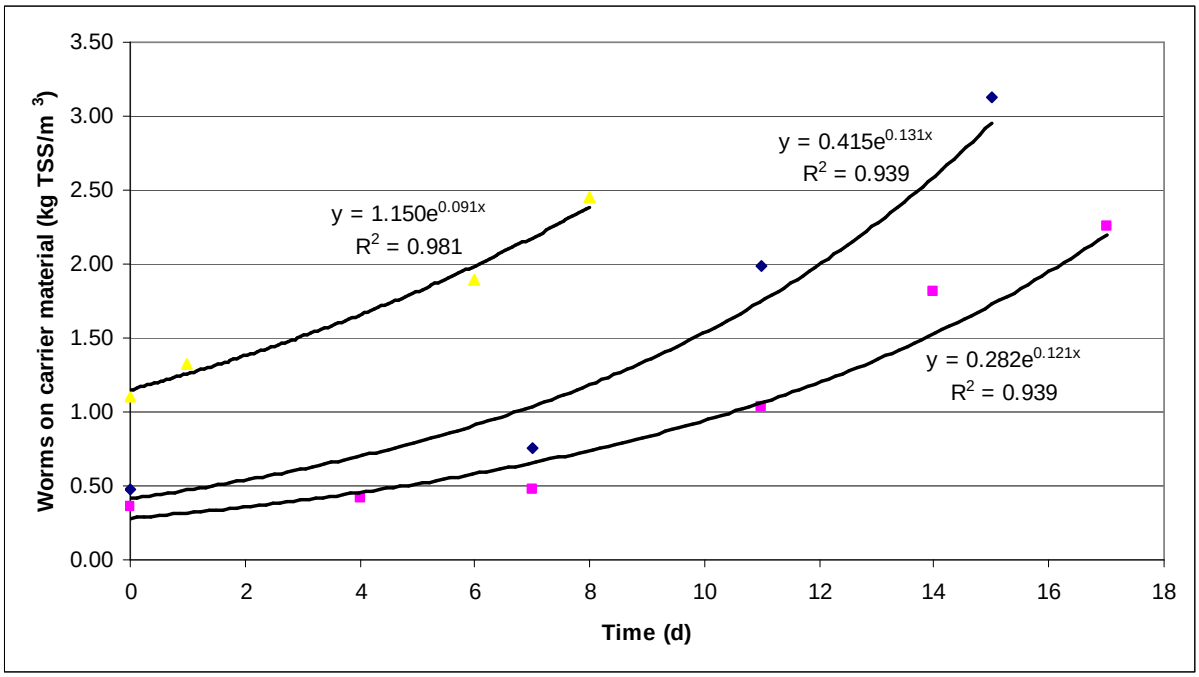






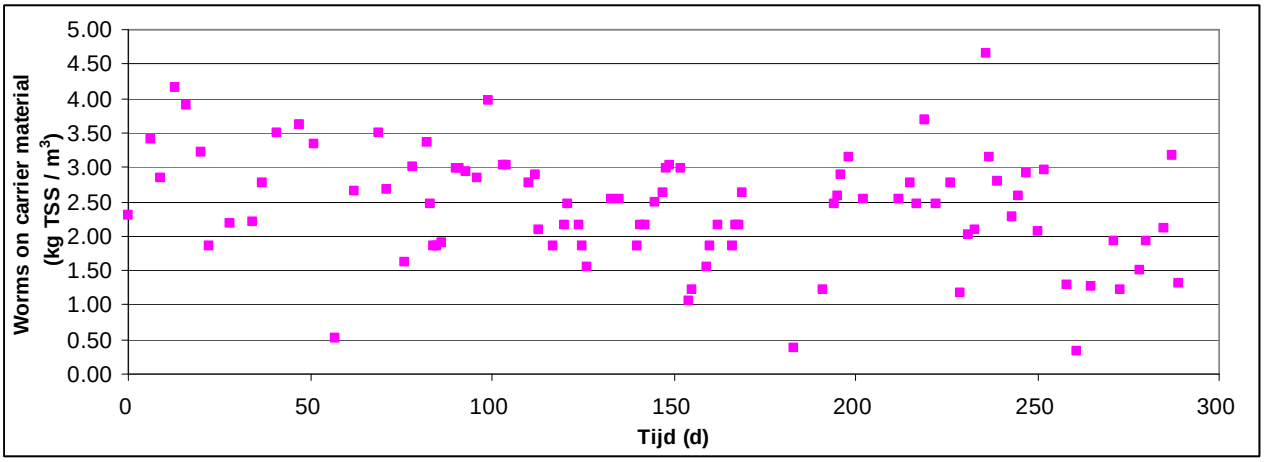
Appendix III. Biomass curves

Figure 1: Biomass curve used to determine growth



For accurate growth rate determinations exponential curves were fitted assuming these are best for representing biological growth. The curves were selected from the overview of worms on carrier (Figure 2). The average growth rate of these worms is (0,12 + 0,13 + 0,09) / 3 = 0,11 kg TSS / m^3 / d

Figure 2: Overview of worms on carrier material



Appendix IV. Formulas reactor characteristics

$$r_S = \frac{F \cdot \left(X_{IN} - X_{UIT} \right)}{V_R}$$

$$q_S = \frac{r_S}{X_{wn}}$$

$$\mu = \frac{1}{X_{wn}} \frac{dX_{wn}}{dt}$$

$$Y = \frac{qs}{\mu}$$

r_s: Volume specific reduction rate (kg TSS sludge/m^3.d)c

q_s: Biomass specific reduction rate (kg TSS sludge /kg TSS worms.d)

X_{wn}: Active worm biomass (kg TSS worms/m^3)

μ: Biomass specific growth rate (/d)

Y: Growth yield of worms on sludge (kg TSS worms/kg TSS sludge)

F: Volumetric flow (m^3/d)

X_{IN}: Total suspended solids influent (kg/m^3)

X_{UIT}: Total suspended solids iffluent (kg/m^3)

V_R: Reactorvolume (m^3)

t: Time (d)

Appendix V. Annual degradation in full scale worm reactor

Period			Days	IN	OUT	Degradation	
	Start	End		tonne tss	tonne tss	tonne tss	%
Summer 2007	21/03/2007	21/09/2007	184	42	24	19	44%
Winter 2007	21/09/2007	21/03/2008	182	54	42	12	22%
			366	96	66	30	32%
Summer 2008	21/03/2008	21/09/2008	184	76	60	16	21%
Winter 2008	21/09/2008	21/03/2009	181	85	67	18	21%
			365	161	127	34	21%
Summer 2009	21/03/2009	21/09/2009	184	121	102	19	16%
Winter 2009	21/09/2009	21/03/2010	181	76	67	8	11%
			365	197	169	27	14%

Appendix VI. SVI change by worm consumption

Difference between: before and after consumption by worms

Independent Samples Test

day			Levene's Test for Equality of Variances		t-test for Equality of Means						
										95% Confidence Interval of the Difference	
			F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
1	svi	Equal variances assumed	.610	.464	4.024	6	.007	29.500	7.331	11.561	47.439
		Equal variances not assumed			4.024	5.420	.009	29.500	7.331	11.085	47.915
2	svi	Equal variances assumed	.016	.902	7.265	6	.000	25.750	3.544	17.077	34.423
		Equal variances not assumed			7.265	5.817	.000	25.750	3.544	17.011	34.489

Difference between: before and after heat exchanger

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SVI	Equal variances assumed	3.322	.118	-.263	6	.802	-2.000	7.613	-20.628	16.628
	Equal variances not assumed			-.263	3.859	.806	-2.000	7.613	-23.445	19.445

Appendix VII. Descriptive statistics (Jan 2009 – Oct 2009)

Variable	Mean	Std. Deviation	Minimum	Maximum	Skewness	Kurtosis	Kolmogrov
R	98.57	104.89	-213.00	440.00	-0.082	0.838	.019
RA	97.18	73.79	-117.00	388.00	0.07	1.39	.079
RAF	96.71	74.16	-117.00	388.00	0.07	1.33	.087
Xwn*	0.28	0.09	0.04	0.53	-0.16	0.45	.200*
Xwwremtranf	5.68	0.80	3.22	7.72	-0.10	0.15	.200*
Xwp_2LN	-0.47	0.20	-0.89	-0.13	-0.64	-0.38	.200*
Xwp23LN	-0.48	0.13	-0.86	-0.23	-0.70	1.18	.200*
Xwp34LN	-0.78	0.21	-1.60	-0.42	-1.21	4.56	.200*
Xwp45LN	-1.26	0.39	-2.30	0.00	0.41	2.04	.200*
Xwp56LN	-1.82	0.53	-2.64	0.00	0.87	2.02	.200*
Xwp_6LN	-1.94	0.72	-2.76	0.00	1.76	2.89	.000
M	1.00	1.38	0.00	8.00	1.61	3.11	.000
DOA	4.91	1.37	1.20	10.10	1.23	2.38	.000
DOmin	3.02	1.89	0.00	9.40	0.72	0.51	.000
DOmax	5.90	1.52	1.80	13.60	1.58	3.98	.000
BLW	37.43	6.34	0.00	65.00	-0.44	6.62	.000
F	326.71	137.70	0.00	654.00	0.72	-0.49	.000
Tmin	24.84	3.44	10.50	29.30	-1.63	3.05	.000
Tmax	26.97	1.70	21.40	30.10	0.08	-0.34	.000
W	11.64	1.54	8.00	15.50	0.47	0.14	.000
CLFh	2.83	6.04	0.00	19.00	1.86	1.90	.000
CLN	1.78	1.66	0.00	6.00	0.21	-0.99	.000
CLFl	5.62	4.85	0.00	10.00	-0.28	-1.91	.000
CLKG	11.51	17.98	0.00	122.00	2.62	9.51	.000
PIN	2.72	3.20	0.80	19.00	4.82	25.07	.000
NHIN	3.36	6.17	0.10	32.90	3.99	18.21	.000
N3IN	2.05	2.23	0.30	8.30	1.56	1.74	.000
N2IN	0.38	0.73	0.00	2.90	3.19	9.55	.000
PUIT	3.84	1.41	1.00	7.80	0.39	0.46	.200*
NHUIT	11.04	10.85	0.20	60.00	2.23	8.71	.008
N3UIT	12.20	4.93	2.30	21.20	0.11	-0.41	.200*
N2UIT	2.58	2.51	0.00	9.60	1.14	0.34	.000

* in kg TSS / m^2 (A:V = 8.8)

Appendix VIII. Significant Spearman rho coefficients

Correlations are significant at the 0.05 level (2-tailed)

U	RA	0.27
	RAF	0.25
	Xwn	0.37
	Xwp45LN	-0.30
Xwn	R	0.25
	RA	0.34
	u	0.37
	Tmax	0.31
	DOA	-0.37
	DOmin	-0.35
	DOmax	-0.32
	CLFh	0.24
	CLN	0.40
	CLFl	0.43
	CLKG	0.28
	Xwp45LN	-0.39
Xww	R	0.24
	BLW	0.38
	Tmin	0.29
	Tmax	0.34
	PUIT	0.32
	DOA	-0.43
	DOmin	-0.34
	DOmax	-0.40
	Xwp56LN	0.38
Xwp_2LN	SIN	0.44
	SUIT	0.30
	F	0.41
	N3IN	0.56
	Xwp23LN	-0.38
	Xwp34LN	-0.75
	Xwp45LN	-0.42
	Xwp56LN	-0.45
Xwp23LN	Fstda	0.40
	TFLA	0.44
	NHIN	0.61
	N2IN	0.64
	Xwp_2LN	-0.38
	Xwp45LN	-0.46
	Xwp56LN	-0.31
	Xwp_6LN	-0.34
	F	-0.31
	W	-0.35
Xwp34LN	F	-0.34
	Xwp_2LN	-0.75
	Xwp45LN	0.49
	Xwp56LN	0.47
	Xwp_6LN	0.30
Xwp45LN	SIN	-0.38
	Fstd	-0.32
	Fstda	-0.41
	TFLA	-0.32
	PIN	-0.65
	Xwn	-0.39
	Xwp_2LN	-0.42
	Xwp23LN	-0.46
	Xwp34LN	0.49
	Xwp56LN	0.85
	Xwp_6LN	0.62
Xwp56LN	u	-0.30
	Xwwremtranf	0.38
	Fstd	-0.31
	Fstda	-0.34
	PIN	-0.61
	Xwp34LN	0.47
	Xwp45LN	0.85
	Xwp_6LN	0.51
	Xwp_2LN	-0.45
	Xwp23LN	-0.31
Xwp_6LN	BLW	0.32
	Fstda	-0.32
	TFLA	-0.35
	Xwp23LN	-0.34
	Xwp34LN	0.30
	Xwp45LN	0.62
	Xwp56LN	0.51

Appendix IX. Multivariate analysis: DECORANA analysis of dataset

Dataset	Description	N	Indirect gradient analysis (DCA)	
			1st ax gradient	2nd ax gradient
1	Average 5 days	48	0.64	0.55
	Average 5 days	17	0.47	0.25
	Average 7 days	23	0.44	0.51
	Average 7 days	12	0.46	0.38
2	Interpolated biological	259	1.08	0.99
	Interpolated biological	86	1.16	0.91
	Interpolated biological, Empty lines removed	30	0.59	0.52
	Interpolated biological, Empty lines removed	13	0.64	0.28