



**Aquatic worm reactor for improved
sludge processing and resource recovery**

Tim L.G. Hendrickx

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Aquatic worm reactor for improved sludge processing and resource recovery

Tim L.G. Hendrickx

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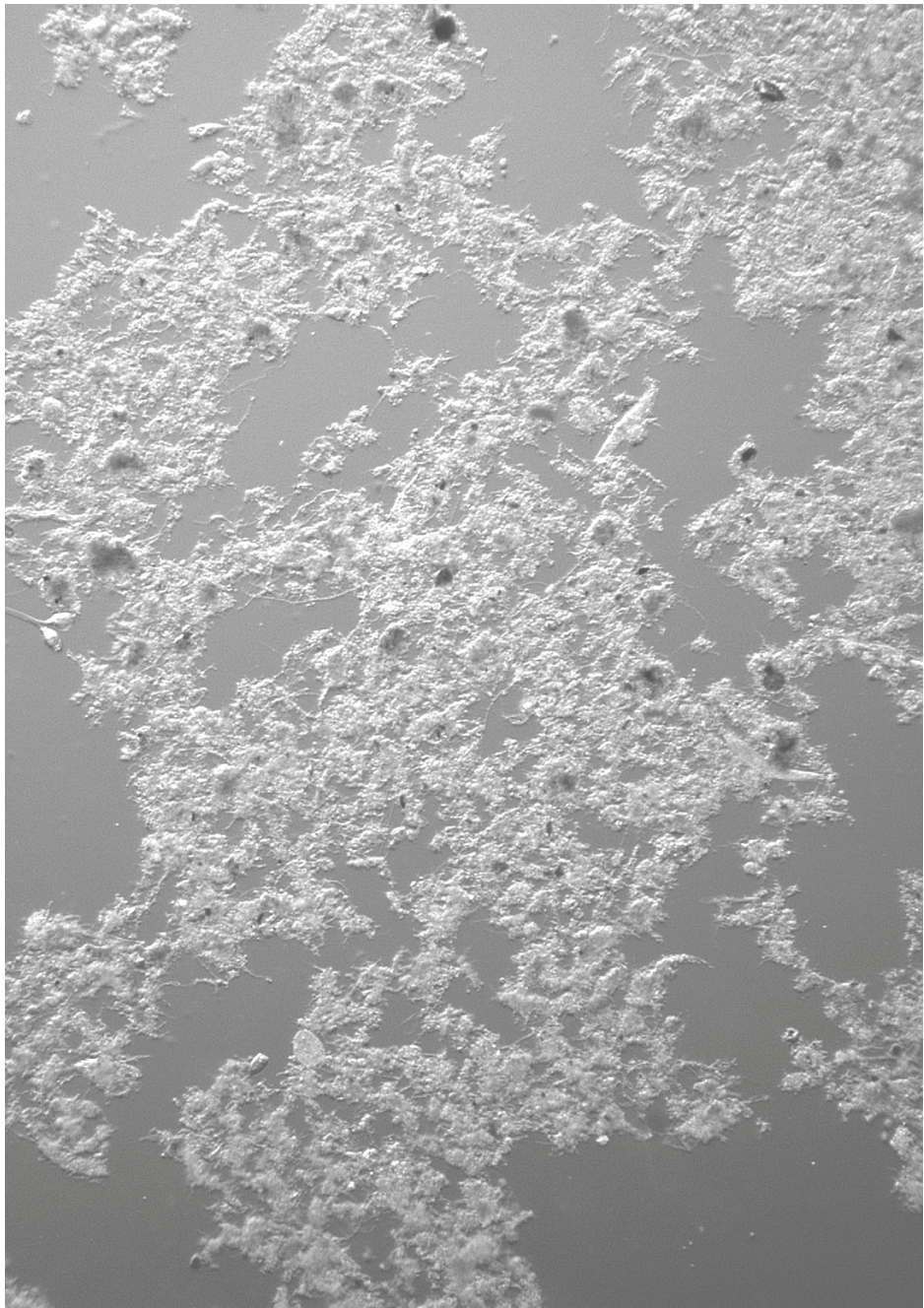
Aquatic worm reactor for improved sludge processing and resource recovery

Abstract

Waste water treatment plants (WWTPs) produce enormous amounts of biological waste sludge. In the Netherlands alone, 350,000 tons of dry solids are produced per year. Due to the contaminants it contains, such as heavy metals, pathogens and organic micropollutants, incineration is the main disposal route. Sludge processing may account for as much as 50 % of the operational costs of a WWTP. A biological approach to reduce the amount of waste sludge, and therefore sludge processing costs, is the use of the aquatic worm *Lumbriculus variegatus*. A new reactor concept was introduced in which the worms are immobilised in a carrier material. This carrier material also separates the worm faeces from the initial waste sludge, which is beneficial for further processing. To reach the objective of a full-scale application, the design parameters and the effect of operational conditions were determined in sequencing batch experiments. Using a carrier material with a mesh size of 350 μm , a worm biomass density of 1.1 kg ww/m² of carrier material was achieved, with a corresponding surface sludge consumption rate of 58 mg TSS/(m²·d). A maximum net worm biomass growth rate of 0.014-0.015 d⁻¹ was found for worms immobilised in a carrier material. To maintain a maximum sludge consumption rate by the worms, the following conditions were required, a dissolved oxygen concentration above 8.1 mg/L, a temperature around 15°C and an unionised ammonia concentration below 0.1 mg NH₃-N/L. The implications of a worm reactor at a WWTP were assessed by determining the release of nutrients by the worms and the consequences on further processing of worm faeces. Nutrients were released at a rate of 58.0 mg NH₄-N/g TSS digested and 25.8 mg PO₄-P/g TSS digested by the worms. A much higher solids concentration was achieved in thickening of worm faeces when compared to the waste sludge, which could result in a 67 % reduction in transportation costs. The potential applications for re-use of the protein-rich worm biomass were also discussed. A new reactor that is suitable for full-scale application was introduced and successfully operated for two months. Installation of a worm reactor has most potential at smaller WWTPs, without an on-site digester and dewatering equipment and therefore high sludge processing costs. Further technological optimisation (including a method to harvest the worms) of the worm reactor should be achieved by operating a pilot reactor. Another interesting option could be to apply a worm reactor to sludges cleaner than those from municipal WWTPs and focus on resource recovery by producing more valuable worm biomass.

Keywords: waste water treatment, sludge reduction, aquatic worm, *Lumbriculus variegatus*, worm reactor, operational conditions, design parameters, resource recovery.

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Chapter 1

General introduction

1

Hendrickx, T.L.G., Temmink, H., Elissen, H.J.H. and Buisman, C.J.N.

1.1 Sludge production by waste water treatment plants

The activated sludge process is the most common biological treatment technology for municipal and industrial waste waters. Although efficient in the removal of organic material and nutrients, it produces large amounts of waste sludge that need to be disposed of. This sludge contains nitrogen, phosphorus, (dead) bacterial biomass and adsorbed (in)organic material, which potentially makes it a suitable fertiliser in agriculture. However, sludge also contains heavy metals, organic micropollutants and pathogens, which has led to stringent legislation on the use of sludge. Until it became prohibited in 1995, the application of sludge in agriculture was a significant disposal route in the Netherlands (Figure 1.1). Table 1.1 compares the legal limits for application in agriculture with the metal content of sludge from Dutch waste water treatment plants (WWTPs), showing that for most metals these limits are exceeded. However, these legal limits vary per country. For example, according to the EU limits, application of sludge in agriculture is (still) allowed. As a result, in countries like Germany, Spain and England 36-60 % of their yearly sludge production by WWTPs is still applied in agriculture (Eurostat, 2009). In addition to legal constraints, also public acceptance plays a role in the use of sludge (Spinosa, 2001).

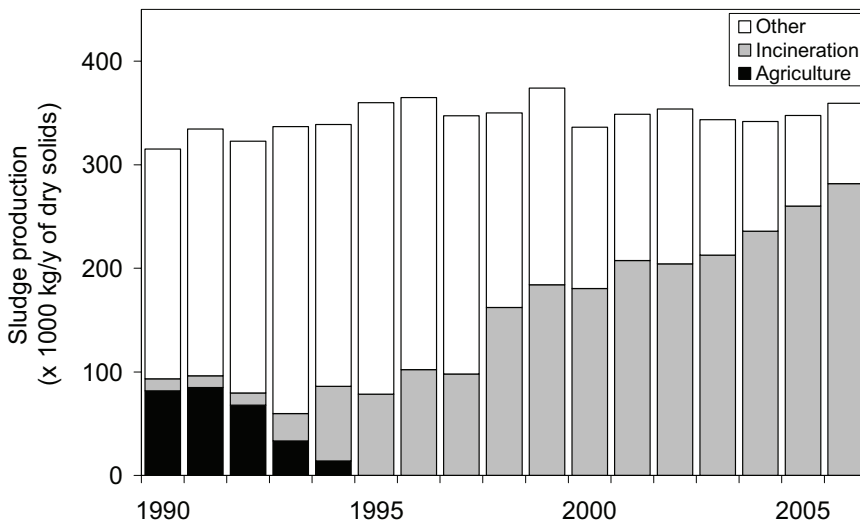


Figure 1.1 Total sludge production by municipal waste water treatment plants and disposal routes in the Netherlands (CBS, 2009). "Other" include composting, landfill and wet oxidation.

Total sludge production in the EU in 2003 was around 8 million tons of dry solids (ds) per year. This is expected to increase due to an increased connection of the population to sewerage systems and more stringent requirements on effluent quality (IWA, 2007). In the Netherlands alone, municipal WWTPs produce approximately 350,000 tons of dry solids per year (CBS, 2009). Incineration is now the main disposal route for sludge (Figure 1.1) and is also expected to become the main disposal route in other EU countries (IWA, 2007), as incineration of sludge solves the problem of hazardous organic compounds and pathogens in the sludge and immobilises most of the heavy metals in the remaining ashes. These ashes are mainly used as replacements for filler materials in e.g. the asphalt and cement industries.

Table 1.1 Metals content in biological sludge produced by Dutch municipal waste water treatment plants (CBS, 2009), compared with the Dutch and the EU limits for agricultural use (STOWA, 2005a).

Metal	Sludge in the Netherlands (mg/kg ds)	Dutch limit (mg/kg ds)	EU limit (mg/kg ds)
Cd	1.5	1.25	20 - 40
Cr	44	75	-
Cu	390	75	1000 - 1750
Hg	1.1	0.75	16 - 25
Ni	30	30	300 - 400
Pb	143	100	750 - 1250
Zn	985	300	2500 - 4000
As	9.3	-	-

1.2 Sludge processing

Figure 1.2 shows the most common sludge processing chain in which incineration is the final treatment, and which will be used as the base case in this thesis. Sludge that is wasted from WWTPs consists mainly of water with only a small percentage of solids (0.5-1 %). As a result, not only the amount of waste solids is an issue, but also its volume. The first step in sludge treatment is therefore thickening which increases the solids concentration to ~3 % (Figure 1.2), thereby already resulting in an initial volume reduction of 30-60 %. Anaerobic digestion of the thickened sludge recovers energy in the form of methane. The digested sludge is then further dewatered to a dry solids content of 20-30%. Finally, the sludge is further dried and (co)incinerated, at the very high costs of 250-400 €/ton of dry solids (Cartmell *et al.*, 2006 and STOWA, 2005b). These costs are mainly related to drying the sludge and the costs that come with incineration, such as flue gas treatment and disposal of the ashes. The revenue from energy generation by sludge incineration only partially compensates for these costs.

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Depending on the size of a WWTP, treatment of the thickened sludge may take place on-site for larger WWTPs, or at a centralised location for sludge from smaller WWTPs (< 50,000 p.e.). For the latter, this means that the thickened sludge needs to be transported to this centralised location, which is costly and has an environmental impact. Table 1.2 shows the capacity distribution of WWTPs in The Netherlands and the large number of smaller WWTPs.

The total costs for sludge processing are high and may represent as much as 40-60% of the operational costs of a WWTP (Kroiss, 2004, Wei *et al.*, 2003). Beneficial use of components in the sludge is therefore interesting to (partially) offset the high sludge processing costs. Secondly, a reduction in both the amount and the volume of waste sludge that needs to be processed is economically and environmentally desirable.

Table 1.2 Capacity distribution and their sludge production of waste water treatment plants in the Netherlands (CBS, 2009). p.e. = person equivalent.

Capacity (p.e.)	Average design capacity (p.e.)	# WWTPs	% of total sludge production
< 5,000	2,500	66	< 1
5,000 – 10,000	7,000	62	< 1
10,000 – 25,000	15,700	87	1
25,000 – 50,000	35,000	71	4
50,000 – 100,000	70,200	71	14
100,000 – 250,000	142,500	47	39
> 250,000	434,800	19	42

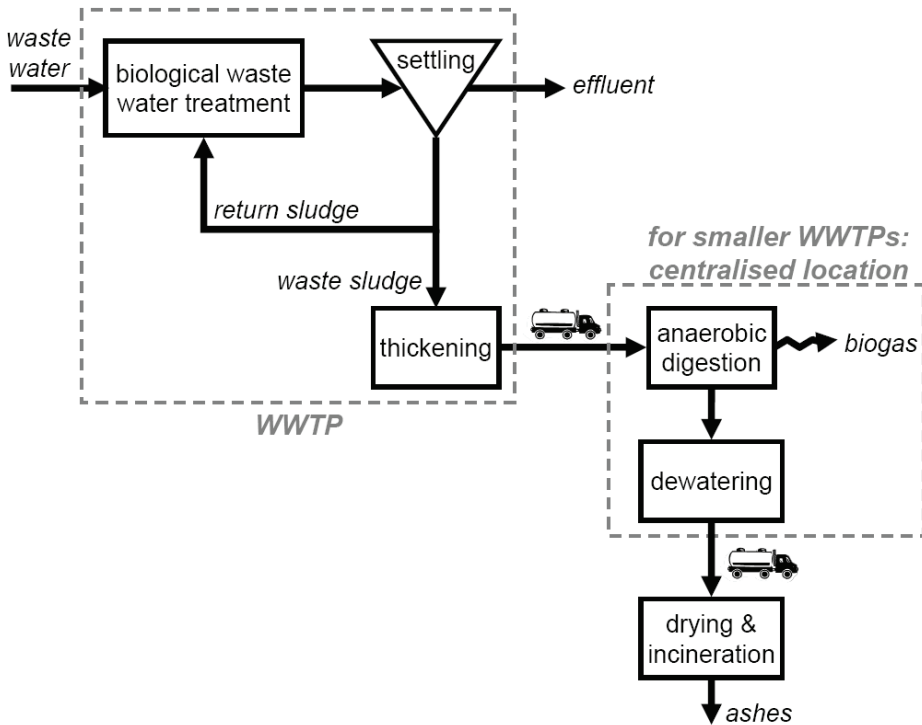


Figure 1.2 Schematic overview of processing of biological waste sludge with incineration as the final treatment.

1.3 Beneficial use of sludge / sludge as a resource

The use of WWTP sludges as a resource is currently mainly limited to energy recovery (Kroiss, 2004, Spinosa, 2004). However, recovery of phosphorus is gaining interest, as its current global phosphorus reserves are expected to be depleted in the next 50-100 years (Cordell *et al.*, 2009), thereby making recovery from sludge an attractive option. Phosphorus can be removed from the sludge by acid treatment, although this also releases the heavy metals from the sludge. Alternatively, phosphorus can be recovered from the ashes after incineration of waste sludge. Thermochemical treatment removes the heavy metals and the recovered phosphorus can be used as a resource for fertilisers (Adam *et al.*, 2009; SUSAN, 2009).

The energy content of sludge can be used in several ways. First of all, energy can be recovered in the form of methane by anaerobic digestion, which is commonly applied at larger WWTPs (Lier *et al.*, 2001). However, this still leaves a residual stream of digested

1. General introduction

sludge, which may still have a heating value of ~15 MJ/kg of dry solids (Cartmell *et al.*, 2006). To recover this energy or that from undigested sludge, further drying takes place, followed by mono-incineration, co-incineration with municipal solid waste or co-incineration in power plants. The remaining ashes are landfilled or used as a filling material in e.g. old mines (STOWA, 2005b). In the production of e.g. Portland cement, waste sludge is used for its heating value, but at the same time the ashes replace part of the filling materials that are used in the process (Rulkens, 2008).

The recovery of energy and phosphorus takes place at the end of the sludge processing chain. Consequently, the costs for sludge thickening, transportation and dewatering remain high, which drives the need for sludge minimization techniques.

1.4 Reducing the amount of sludge

A multitude of sludge reduction technologies are available. Several mechanical, physical and chemical techniques can be applied to disintegrate the sludge, after which the lysis products are returned to the WWTP resulting in (partial) mineralization of the (disintegrated) biomass (Ødegaard, 2004 and Wei *et al.*, 2003). These techniques require a considerable input of energy and / or chemicals and will still require a certain amount of sludge to be wasted from the process to remove compounds that cannot be mineralised, such as phosphorus and heavy metals. Sludge reduction may also be applied within the activated sludge process itself, by increasing the sludge age, by uncoupling the microbial metabolism (Low *et al.*, 2000) or by grazing on the bacterial population by higher organisms (Ratsak and Verkuilen, 2008). The latter has been extensively investigated over the past decades. Extending the food chain in the activated sludge with higher organisms is accompanied by a decrease in the production of excess sludge. Several aquatic worms have been investigated, such as the Aeolosomatidae and Tubificidae that naturally occur in aeration tanks or in the settlers at WWTPs (Elissen *et al.*, 2008, Elissen, 2007, Ratsak and Verkuilen, 2006). However, promoting the growth of these aquatic worms and maintaining high densities within the activated sludge process proved unpredictable and impossible to control (Elissen *et al.*, 2008; Wei *et al.*, 2003). Reducing the amount of wasted sludge in a separate worm reactor may have more potential, as it allows for separate optimisation of the conditions for the worms, independent of the activated sludge process.

1.5 Separate worm reactor for sludge reduction

Recent research on separate worm reactors with aquatic worms has essentially focussed on the introduction of a second aeration tank inoculated with worms. In such a reactor, the aeration provides oxygen to the aquatic worms, but also to the sludge itself. Consequently, the contribution of natural sludge breakdown is significant, particularly if long retention times in the worm reactor are applied. Natural sludge breakdown includes processes such as

maintenance and endogenous respiration (Loosdrecht and Henze, 1999). Wei and Liu (2006) used a reactor suitable for free swimming as well as sessile worms. Plastic carriers were placed in the worm reactor for the sessile species. Although an average sludge reduction of 59 % was reported, a large part could be contributed to natural breakdown of the sludge. Later research with the same reactor (Wei *et al.*, 2009) indicated that there was no significant correlation between worm density and net sludge production, i.e. sludge reduction could not be attributed to the worms. However, an improvement in the sludge settling characteristics was reported. Guo *et al.* (2007) introduced a similar system with vessels for sessile worms (Tubificidae) in a worm reactor, in which the sludge was mixed by aeration. On average, the contribution of natural breakdown of the sludge was estimated at 7 %, but it could be as high as 50 %. Huang *et al.* (2007) introduced a reactor for sessile worms (Tubificidae) and showed clear sludge reduction at higher worm densities, but they did not account for the contributions of natural breakdown and accumulation. Also, a higher sludge flow rate through their worm reactor, and therefore potentially a smaller worm reactor, resulted in significant worm losses with the outflow of the worm reactor. Furthermore, the stability of the worm populations was unclear in each of the abovementioned worm reactors and worm biomass was not harvested. Instead, the worm population was expected to reach equilibrium between growth, decay and wash-out from the reactor. Summarizing, sludge reduction by worms has, so far, not led to a reactor concept that is applicable at full scale. This requires a stable worm population, a clear sludge reduction caused by the worms themselves and a potential for upscale.

Buyts *et al.* (2008) introduced sludge reduction with *Lumbriculus variegatus* (Figure 1.3), an aquatic worm which is not commonly found in WWTPs. Compared to other aquatic worms, the main advantages of *L. variegatus* are growth through division (which eliminates the need of a breeding stage), easy separation from the sludge due to its size and a clear reduction in the amount of sludge. Additionally, its high protein content of more than 60 % (Hansen *et al.*, 2004) offers an opportunity for (partial) recovery of nitrogen from the sludge. Finally, an initial concept was introduced to retain these worms in the reactor by immobilisation in a mesh carrier material (Elissen, 2007). This reactor concept, which will be introduced and discussed in detail in Chapter 2 of this thesis, uses a carrier material to immobilise the worms, and separates the compact worm faeces from the sludge. This concept will be the basis for the research presented in this thesis.

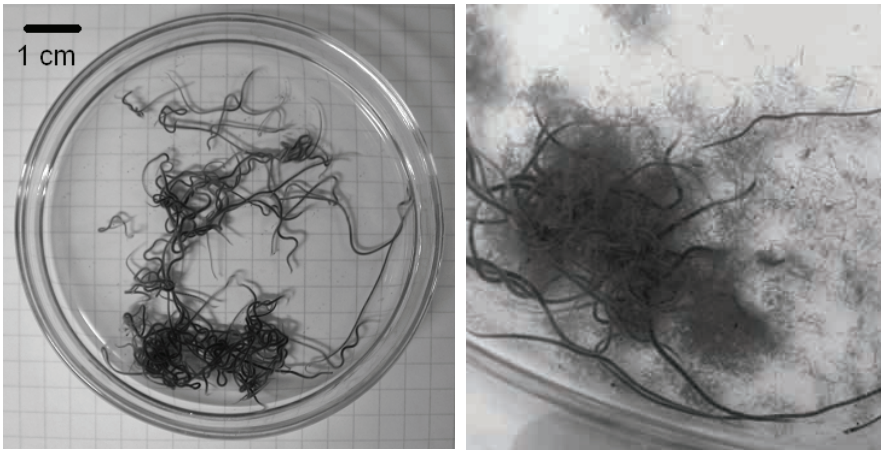


Figure 1.3 *Lumbricus variegatus* in a glass dish (left) and with sludge (right).

1.6 Objective and outline of thesis

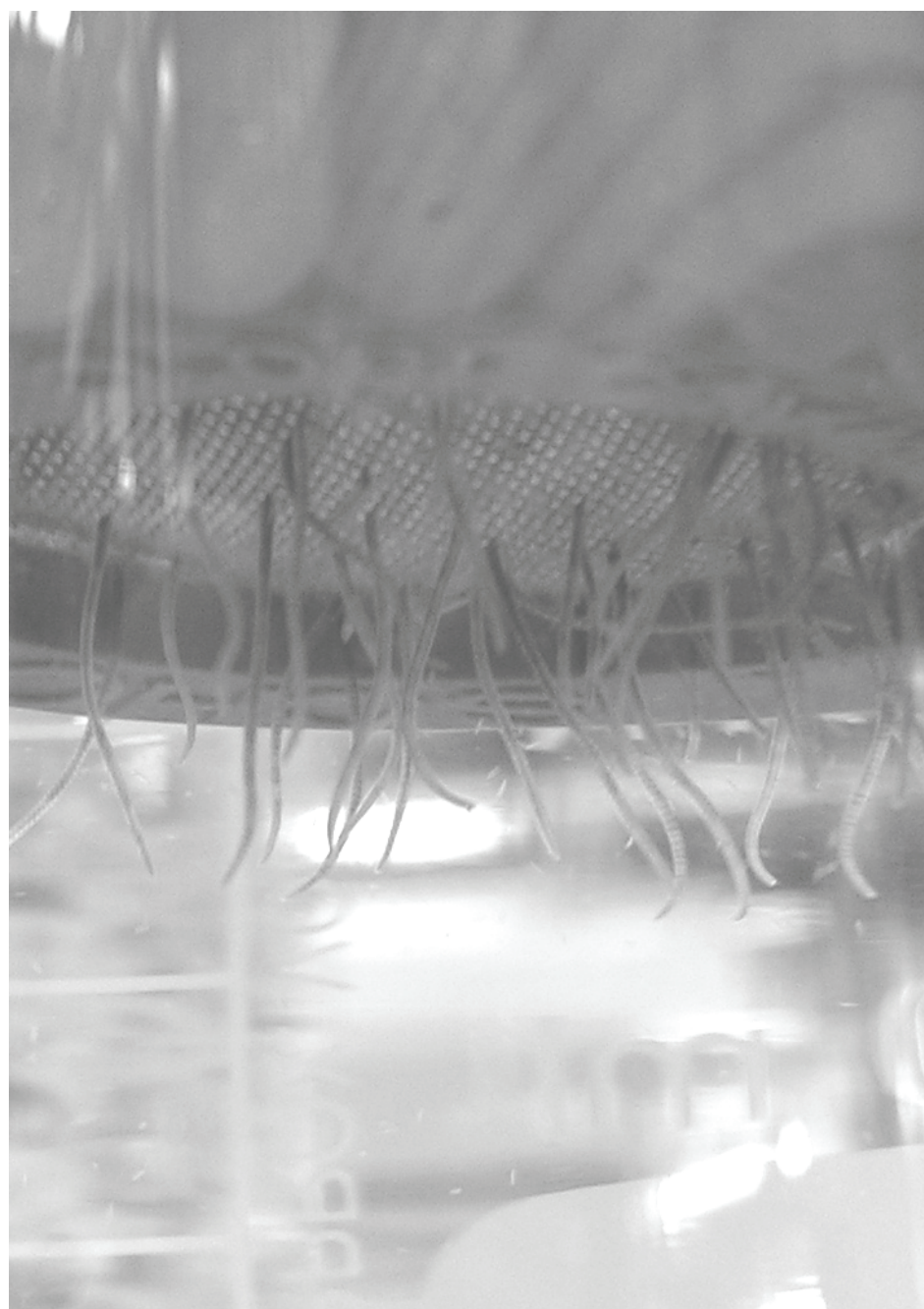
The objective of this thesis is to develop a reactor that is suitable for reduction with *L. variegatus* of sludge produced at municipal WWTPs. The reactor concept that is used for this purpose, is introduced in **Chapter 2**. Initial experiments that prove the potential of the concept are also described in this chapter. For continuous operation at large scale, a stable worm population is essential. Therefore, in **Chapter 3** the feasibility of a continuous reactor is tested in experiments of several weeks. Detailed mass balances for the sludge will also be given, with a distinction between sludge breakdown by the worms, natural sludge breakdown and accumulation. The effect of the conditions under which the reactor can be operated, such as temperature, dissolved oxygen concentration and ammonia concentration, is described in **Chapter 4**. In addition to sludge breakdown, the worms compact the sludge into worm faeces and release nutrients. The impact these will have on a WWTP are discussed in **Chapter 5**, which deals with the mass balances over the worm reactor and further processing of the worm faeces. To correctly design a worm reactor, the most important parameter is the amount of sludge that can be processed per unit of surface area, which will be addressed in **Chapter 6**. Efficient use of reactor volume is essential to maintain a low footprint of a worm reactor. For this purpose, a new configuration of the reactor concept is introduced in **Chapter 7** and tested for its suitability for scaling up of the process. In addition to the engineering and operational details of the worm reactor, the potential for an application of the excess worm biomass is given in **Chapter 8**. Finally, the current economic perspective for a worm reactor at a small 35,000 p.e. WWTP and recommendations for further improvement are discussed in **Chapter 9**.

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Chapter 2

A new reactor concept for sludge reduction using aquatic worms

2

Abstract

Biological waste water treatment results in the production of waste sludge. The final treatment option in the Netherlands for this waste sludge is usually incineration. A biological approach to reduce the amount of waste sludge is by aquatic worms. In this chapter we test the applicability of a new reactor concept for sludge reduction by the aquatic worm *Lumbriculus variegatus*. In this reactor concept the worms are immobilised in a carrier material. In sequencing batch experiments the sludge breakdown by the worms is compared to sludge breakdown in a blank reactor (i.e. without worms). Sludge digestion by the worms results in a distinct sludge reduction, which is almost three times higher than in the blank experiment. The worm faeces that are produced in the worm reactor have a sludge volume index (SVI) that is approximately half that of the initial waste sludge. Due to the configuration of the worm reactor, waste sludge, worm faeces and worms are separated, which is beneficial to further processing. The obtained results show that the proposed reactor concept has a high potential for use in large scale sludge processing.

Keywords: activated sludge reduction; worm reactor; aquatic worms; *Lumbriculus variegatus*

The experimental results presented in this chapter were obtained by the author of this thesis (T.L.G. Hendrickx). He also wrote the (published) paper based on these results. As this was the first publication on the described reactor concept, its inventor was chosen as first author (H.J.H. Elissen).

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2. Reactor concept

2

2.1 Introduction

Both municipal and industrial waste waters are often treated by the (aerobic) activated sludge process. This results in the production of large amounts of waste sludge, consisting of biomass and (in)organic material. This waste sludge needs to be processed and disposed of. Regulations for its disposal are becoming more stringent, as it often contains contaminants such as heavy metals and organic micropollutants. Usually incineration is the final option for sludge treatment in the Netherlands. Since sludge consists mainly of water with only a small percentage of solids, incineration is preceded by thickening and dewatering. In particular at small waste water treatment plants (WWTPs), transport of the thickened sludge to central sludge processing installations is required. This increases both the environmental burden and the total sludge processing costs. The latter may be as high as 50 - 60 % of the total operational costs of WWTPs (Wei *et al.*, 2003). A reduction in the amount of waste sludge is therefore attractive from both an environmental and an economical point of view. This can be accomplished by mechanical, chemical, physical and biological methods (Ødegaard, 2004). The main disadvantage of most of these techniques is a high energy input and / or the use of chemicals. A biological approach is consumption (predation) of waste sludge by higher organisms, such as protozoa and metazoa. The idea is to extend the food chain, which is accompanied by a decrease in the total amount of biomass. Several researchers have proposed to apply predators that naturally occur in waste water treatment processes (Wei *et al.*, 2003). In particular aquatic “bristle worms” (Oligochaeta and Aphanoneura) have received a lot of attention – such as the free-swimming species *Aeolosoma* sp. and *Nais* sp. and crawling species like Tubificidae. These worms can appear in high densities – during so-called worm blooms – in the aeration tanks or sludge basins of WWTPs. The worm blooms are reported to be accompanied by lower sludge production rates. However, Wei *et al.* (2003) mention that a practical application is still uncontrollable as there is no clear relationship between process conditions (e.g. retention times, temperature, sludge loading rates and shear forces) and worm growth. They state that one of the challenges is to maintain high densities of worms for a long time, in particular in full scale applications. However, conditions beneficial to predator growth may not be optimal for bacterial processes and overall treatment efficiency. To overcome this problem, Lee and Welander (1996) applied a two-stage system in which the first reactor favoured bacterial growth, whilst the second step was optimised for predator growth. Although Protozoa were used for predation, the same principle could also be applied with aquatic worms. The introduction of the predation step resulted in lower apparent sludge yields compared to systems without predation.

Elissen (2007) investigated several aquatic worm species for their sludge reduction ability and concluded that the crawling species *Lumbriculus variegatus* (Oligochaeta; Lumbriculidae), has most potential for waste sludge reduction in a separate worm reactor.

L. variegatus rarely occurs in waste water treatment processes, but is found widely throughout Europe and North-America in natural water bodies. Individuals can be up to 10 cm long and 1.5 mm thick. In its natural habitat *L. variegatus* uses its head to forage in sediments and debris, while its tail end – specialized for gas exchange – typically projects upwards (Drewes and Fourtner, 1989). As reproduction takes place through fragmentation (autotomy), *L. variegatus* has a clear advantage over sexually reproducing Oligochaeta such as Tubificidae, which need a “breeding” stage. It has been shown in batch experiments that *L. variegatus* can strongly enhance the breakdown rate of activated sludge (Buys *et al.*, 2008). This breakdown is the sum of sludge consumption by the worms and natural sludge breakdown by several microbial processes that take place in activated sludge, such as maintenance and endogenous respiration (Loosdrecht and Henze, 1999). Initial experiments also showed that separation of waste sludge and worm faeces is possible with a new reactor concept in which *L. variegatus* is immobilized in a carrier material. This also eliminates the need to separate the worms from the sludge. This chapter describes the results of a sequencing batch experiment in which the feasibility of this reactor concept for sludge reduction was investigated.

2.2 Materials and methods

2.2.1 Reactor concept

The reactor concept is schematically presented in Figure 2.1. It consists of a beaker (sludge compartment) containing both waste sludge and worms. The open side of the beaker is covered with a carrier material, through which the worms can protrude their tails. The beaker is placed in the water compartment (partially submerged) with the carrier material facing downwards. By aerating the water compartment, the worms position themselves in the carrier material since *L. variegatus* feeds with its head, but respire and defecates via its tail. As a result, the worms keep their heads in the sludge compartment and protrude their tails into the water compartment. The carrier material therefore acts as both a support material for the worms and a separation layer between the waste sludge and the worm faeces. The feasibility of this reactor concept was investigated with a sequencing batch experiment.

2. Reactor concept

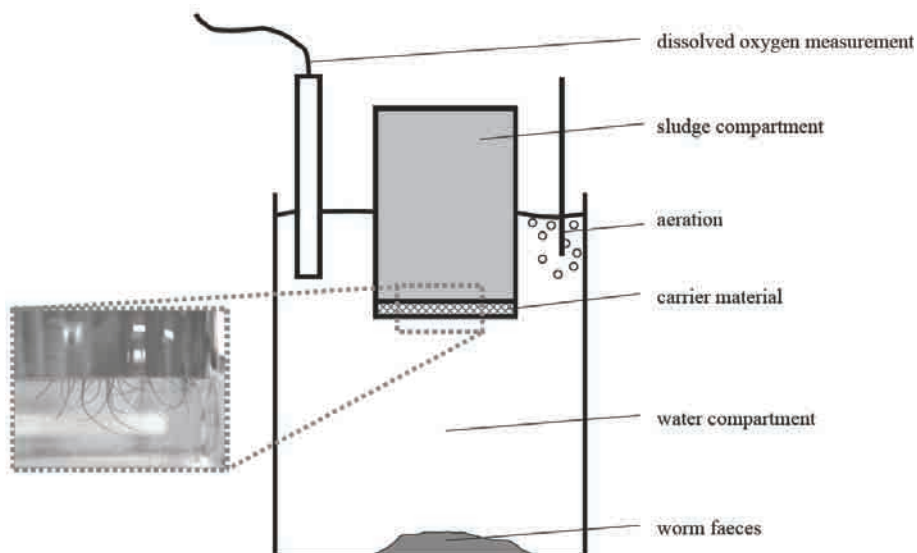


Figure 2.1 Experimental set-up for the sequencing batch experiments.

2.2.2 General

Total suspended solids (TSS) of sludge and worm faeces in all experiments were determined according to Standard Methods (APHA, 1998) using Schleicher & Schuell 589/1 black ribbon filters (pore size 12 - 25 μm). Possible errors, as a result of sample handling, were checked by filling the sludge compartment and then immediately emptying it for TSS analysis. On average 99 % of the TSS was recovered, demonstrating the accuracy of the applied method. The settleability of the original waste sludge and of the worm faeces was assessed by determining the sludge volume index (SVI) according to Standard Methods (APHA, 1998) at 20°C. In addition to the final SVI after 30 minutes of settling, values were also recorded at intermediate times. The wet weight (ww) of the worms was determined by placing the worms on a perforated piece of aluminium foil. Adhering water was removed by pushing the back of the foil against dry paper tissue and gently squeezing the worms. Dry weight (dw) of *Lumbriculus variegatus* is 13 % of its wet weight (Buys *et al.*, 2008).

2.2.3 Sequencing batch experiment

The set-up shown in Figure 2.1 was used for the sequencing batch experiments. Daily, the contents of the water and the sludge compartment were replaced. The sludge compartment was filled with 100 mL of activated sludge (nitrifying sludge, TSS = ± 4 g/L) from the

municipal WWTP of the city of Leeuwarden, the Netherlands. Sludge was provided in excess to the worms, to ensure that sludge availability was not a limiting factor. To remove coarse material from the sludge, it was first sieved using a 1 mm mesh. The water compartment was filled with effluent from the same treatment plant. This effluent was first filtered using black ribbon filters (Schleicher & Schuell 589/1, pore size 12-25 μm) to remove any suspended material that could interfere with the accuracy of the TSS measurements. At the end of each step (24 hours) in the batch sequence, the sludge compartment was taken from the water compartment. The worms were separated from the remaining sludge, counted, weighed and used in the next step in the batch sequence. TSS of the remaining sludge in the sludge compartment and of the worm faeces in the water compartment was determined. As a carrier material a polyamide mesh (300 μm ; SEFAR) with a surface area of 7.5 cm^2 was used.

The water compartment was aerated to maintain the dissolved oxygen (DO) concentration between 8 and 9 mg/L, which was checked using a Hach[®] Luminescent Dissolved Oxygen (LDO) meter. This ensured that the process was not limited by oxygen availability. Hendrickx *et al.* (2006) showed that a lower DO (~ 2.5 mg/L) indeed results in a lower sludge consumption rate.

Together with the sequencing batch experiment with worms, a blank sequencing batch experiment without worms was run under the same conditions. In these blank tests, only the TSS of the sludge in the sludge compartment was determined.

2.3 Results

Within a few minutes from the start of each step in the batch sequence the worms protruded their tails through the carrier material (as shown in Figure 2.1). During the experiment a maximum of 5 % of the worms fell from the carrier material into the water compartment. The sludge within the sludge compartment settled onto the carrier material, forming a sludge blanket that does not settle through the mesh openings.

2.3.1. Sequencing batch experiments

Figure 2.2 compares the cumulative sludge breakdown in the worm experiment and the blank experiment. As sludge had been provided in excess, the sludge was never completely consumed at the end of each step. The sludge breakdown rates were approximately constant, with 77 mg TSS/d in the worm experiment and 28 mg TSS/d in the blank experiment. If we assume that the natural sludge breakdown takes place to the same extent in both experiments, the difference of 49 mg TSS/d can be attributed to consumption by the worms.

2. Reactor concept

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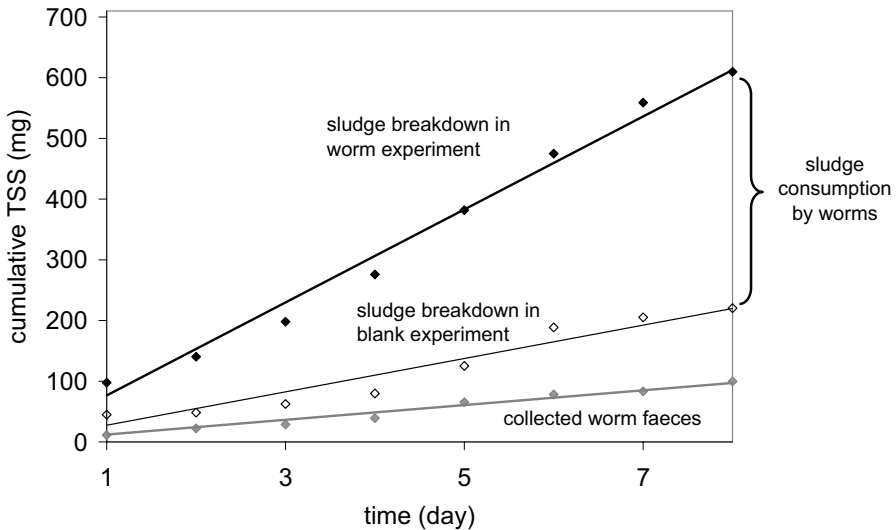


Figure 2.2 Cumulative sludge breakdown in the sludge compartments from the blank and worm sequencing batch experiments and faeces production in the worm sequencing batch experiment. $T = 22.9 \pm 1.2$ °C. DO concentration in the water phase = 8.4 ± 0.4 mg O₂/L. Initial worm weight of 77 worms: 0.79 ± 0.04 g ww (~ 0.10 g dw).

Also shown in Figure 2.2 is the amount of produced worm faeces in the worm experiment. Comparing sludge consumption by the worms with produced worm faeces shows that only 25 % of the consumed sludge was converted into worm faeces (based on TSS). Under the conditions of this experiment, this means that the worms have digested 75 % of the consumed sludge. Figure 2.3 shows a TSS-based mass balance for the sludge that was consumed by the worms.

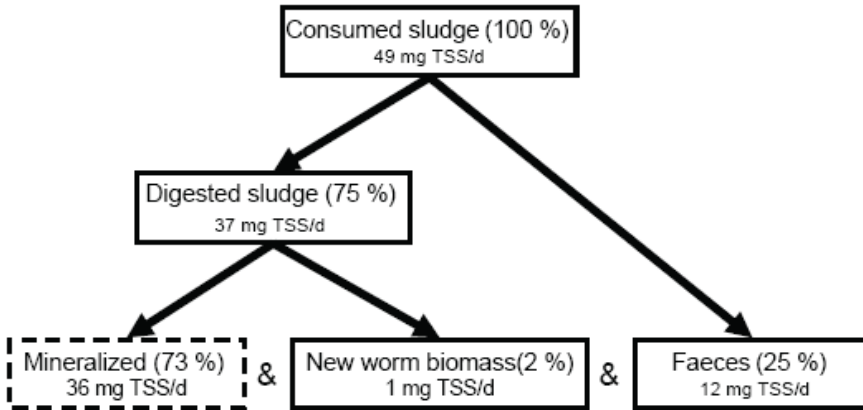


Figure 2.3 TSS-based mass balance for the sludge that was consumed by the worms.

During the experiments worm growth varied between -8 and 7 mg dw per day, with an average of 1 mg dw per day (equal to 8 mg ww per day), which results in an average worm biomass yield of 0.03 g dw/g digested TSS. However, it should be noted that the daily worm growth rates are in the same order as the experimental error of the wet weight determination.

2.3.2 Settleability of worm faeces

As mentioned earlier, the proposed reactor concept makes it possible to separate the waste sludge from the worm faeces. The distinct compact structure of the collected worm faeces is shown in Figure 2.4, where it is compared to the sludge flocs of the initial waste sludge.

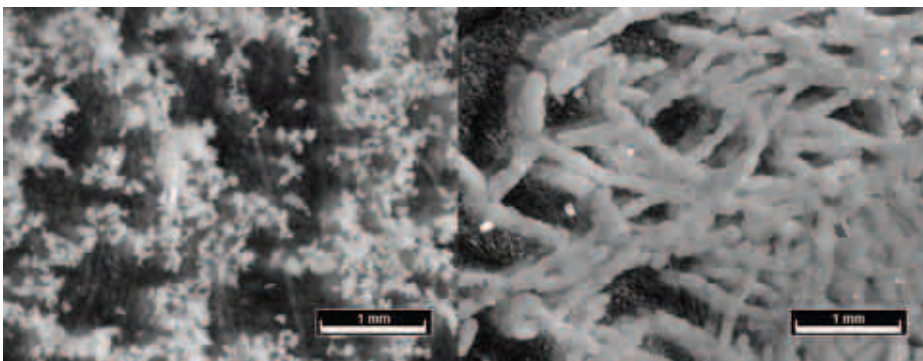


Figure 2.4 Waste sludge (left) versus worm faeces (right).

2. Reactor concept

To assess the effect of the cylindrical morphology of the worm faeces on settling properties, the sludge volume index (SVI) curves of these faeces and of the initial waste sludge were compared. Figure 2.5 shows these two SVI curves.

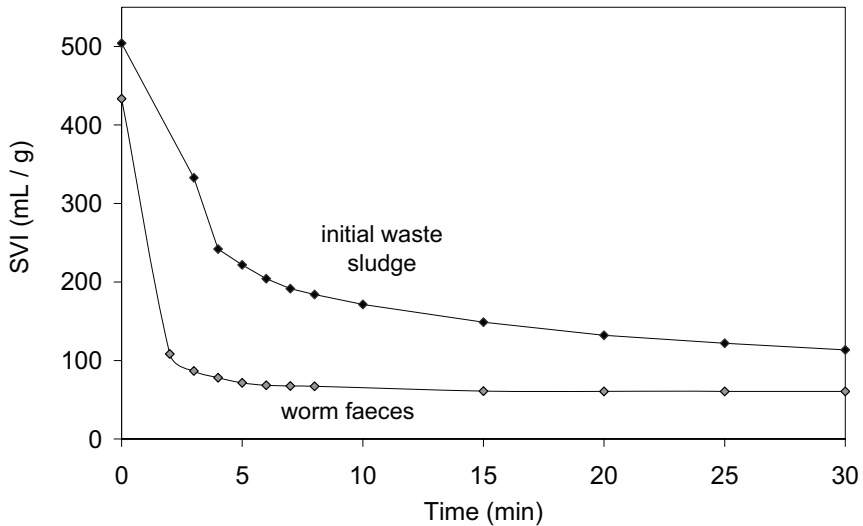


Figure 2.5 Development of the sludge volume index (SVI) in time for waste sludge and worm faeces at 20 °C.

Clearly, the worm faeces settle much faster than the initial waste sludge and within the first 5 minutes most of the faeces have settled. The SVI values after 30 minutes respectively were 113 and 61 mL / g for the initial sludge and the faeces, showing that the faeces have settled into a more compact sludge.

2.4. Discussion

2.4.1 Sludge breakdown rate

The rate of sludge breakdown in the worm experiment is significantly higher than the sludge breakdown rate in the absence of worms. Under the conditions described in this chapter, a single-layer surface area of $61 \cdot 10^3 \text{ m}^2$ would be required to deal with a waste sludge production of 4000 kg TSS/d (from a 100,000 population equivalent WWTP). However, we used a worm density of 1 kg ww/m² ($\sim 10^5$ worms/m²), which was not yet optimised. In practice, much higher worm densities with a higher sludge consumption rate may be obtained. This is determined by the available sludge and the maximum possible worm density per surface area. In particular the latter factor will determine the economic feasibility of the reactor concept.

2.4.2 Sludge reduction efficiency

A 75 % decrease in the amount of TSS of consumed waste sludge was observed in addition to the natural sludge breakdown. Not only would this reduce the amount of waste sludge that needs to be disposed of, but it also leads to a decrease in the associated sludge processing costs and environmental burden. However, in previous batch experiments without carrier material, lower reduction percentages were found, typically 10 - 50 % (Winters, 2004). This indicates that the performance of the worm is strongly dependent on process operation and conditions, such as its immobilization, the type of sludge and oxygen concentration. Another explanation for the much higher sludge reduction percentage found in the sequencing batch experiment could be that some of the worms defecated in the sludge compartment. This means that not all worm faeces were collected in the water compartment and accounted for and therefore a higher apparent sludge reduction efficiency was observed.

2.4.3 Worm faeces

Worm faeces and waste sludge were separated by the carrier material. As was shown, the worm faeces settled much faster than the initial waste sludge. These improved settling characteristics of the final waste product will contribute towards a decrease in sludge processing costs, as it can be expected that dewaterability characteristics will improve accordingly. This should be investigated further, preferably on a large scale.

2.4.4 Worm biomass

Buys *et al.* (2008) found that 20 - 40 % of the sludge digested by the worms was converted into worm biomass (based on dry matter) in mixed aerobic batch experiments (i.e. without immobilizing the worms in a carrier material). The worm yield of 3 % per day in the sequencing batch experiments was lower. This could be due to the immobilization and

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inverted positioning of the worms in the carrier material, which could restrain the worms in their feeding behaviour. Additionally, the daily worm growth was in the same order as the experimental error and only small in relation to the average total ww of 790 mg. To accurately determine the growth rate of the worms in the sequencing batch experiments, long term experiments with larger amounts of sludge and worms will have to be carried out. It will be important to consider the fate of the worm biomass, as we have partially converted the waste sludge into worm biomass. The high protein content of the worms, 60 % of their dry weight (Hansen *et al.*, 2004), makes re-use an attractive option, for example as live fish food or as slow fertiliser in agriculture (Winters, 2004). However, care should be taken regarding the fate of heavy metals and organic micropollutants originating from the waste sludge, as these possibly accumulate in the worms. This should be further investigated.

2

2.5 Conclusions

The presented reactor concept for sludge consumption by *L. variegatus* has potential for decreasing the environmental burden and costs of sludge processing at WWTPs. This was proven with a sequencing batch worm experiment in which the following was achieved:

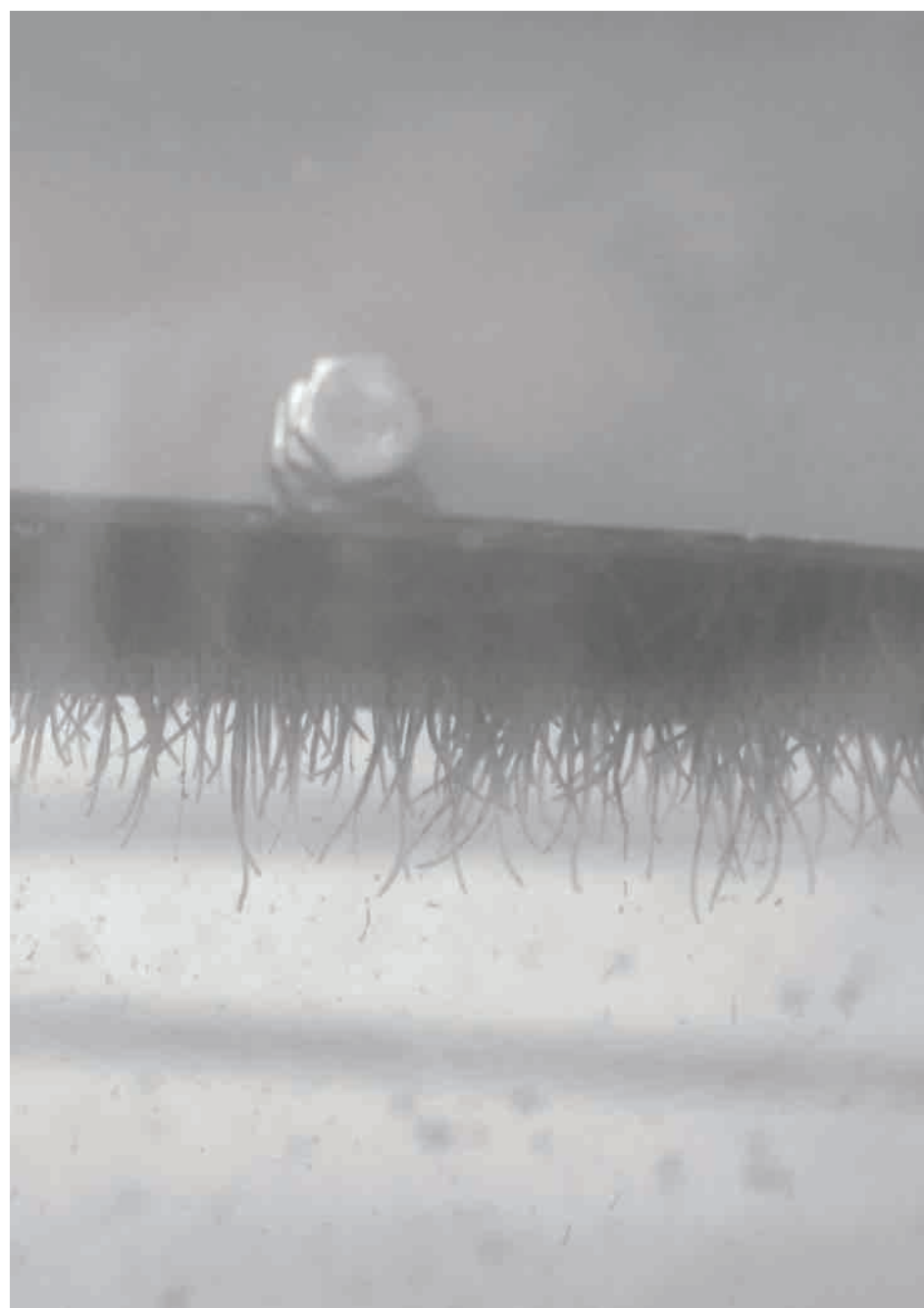
- A distinct decrease in the amount of sludge, as the sum of worm faeces and produced worm biomass was much lower than the amount of waste sludge that the worms consumed.
- Worm faeces with a sludge volume index that was approximately half that of the initial waste sludge. Additionally, the worm faeces settled much faster than the initial waste sludge.
- A separation between waste sludge, worms and worm faeces, which is beneficial to further processing.

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Chapter 3

Aquatic worms eating waste sludge in a continuous system

Abstract

Aquatic worms are a biological approach to decrease the amount of biological waste sludge produced at waste water treatment plants. A new reactor concept was introduced (Chapter 2) in which the aquatic oligochaete *Lumbriculus variegatus* is immobilised in a carrier material. The current chapter describes the experiments that were performed to test whether this concept could also be applied in continuous operation. In a first experiment waste sludge from a lab scale activated sludge system (treating pre-settled domestic sewage) was fed directly to the worm reactor. The return of non-consumed sludge from this worm reactor to the activated sludge system did not affect performance of the latter. In a second experiment, mass balances for total and volatile suspended solids (TSS and VSS) over two runs of the worm reactor showed a clear distinction between accumulation of sludge, natural sludge breakdown and sludge digestion by worms. The importance of correcting for natural sludge breakdown was shown, as the contribution of the worms to total VSS reduction was 41 and 72 %, respectively. TSS and VSS reduction by the worms were 16-26 % and 22-30 %, respectively, for sludge from the lab scale activated sludge system. The mesh size of the carrier material had a large effect on growth of worms in the reactor. By increasing the mesh size from 300 to 350 μm , worm biomass growth was possible in the reactor at a rate of 0.013 d^{-1} and with a yield of 0.13 g dw/g VSS digested by the worms. No significant release of soluble organic material and ammonia were observed in the worm reactor.

Keywords: *Lumbriculus variegatus*, sludge reduction, continuous operation, mass balance, worm reactor.

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3. Continuous reactor

3.1 Introduction

Domestic sewage and industrial waste waters are commonly treated by aerobic biological processes. This results in the production of large amounts of biological excess sludge consisting mainly of (in)organic material and (dead) bacteria. In particular for small waste water treatment plants (WWTPs) the costs for sludge disposal are high as the sludge usually needs to be transported to central sludge treatment facilities. Waste sludge management may thus represent up to 50 % of the WWTP's operational costs (Wei *et al.*, 2003). As a resource, the use of waste sludge is currently mainly limited to biogas production by anaerobic digestion and (co)incineration with use of the ashes in e.g. the production of Portland cement (Rulkens, 2008). The beneficial application of waste sludge as a fertiliser is becoming more and more restricted due to the heavy metals and organic micropollutants it contains. As a result, landfill and in particular incineration are becoming the more common disposal routes. Waste sludge is thus more and more considered a waste product, which has led to an increased interest in techniques to reduce the amount of waste sludge produced by waste water treatment processes. One approach is to disintegrate the solids and return the mineralization products to the aeration tank of the WWTP, thereby decreasing the overall sludge yield. Several mechanical, physical and chemical methods are available for sludge disintegration, such as ultrasonic and thermal treatment and ozonation (Ødegaard, 2004). An important drawback of these techniques is the input of energy and/or chemicals, which also makes the techniques very costly. Biological approaches to reduce the production of waste sludge include operation at long sludge retention times (for instance in extended aeration processes) and stimulation of the growth of higher organisms that feed on the waste sludge (Wei *et al.*, 2003). By extending the food chain with large amounts of aquatic worms that naturally occur in WWTPs, the net biomass production and observed sludge yield are expected to decrease (Ratsak and Verkuijlen, 2006). Free swimming species of aquatic worms are occasionally observed at very high densities in the aeration tanks of WWTPs. Despite the numerous attempts to control their growth and maintain high densities of these worm species within the sludge, the conclusion was that this cannot be done for worms inside the waste water treatment process (Wei *et al.*, 2003). Recently, extensive monitoring of worm densities and operation conditions at four full scale WWTPs in the Netherlands confirmed this conclusion (Elissen *et al.*, 2008). Further attempts have been made by trying to promote worm growth in a separate reactor, treating the sludge after it has been wasted from the waste water treatment process. This allows optimum operating conditions for the worms. Recent attempts using such a reactor with Tubificidae or Aeolosomatidae have been reported by Wei and Liu (2006), Huang *et al.* (2007) and Guo *et al.* (2007). In these reactors the worms were mixed with the sludge and the air supplied to the reactor was used by both the worms and the sludge itself. Their results indicated high sludge reduction percentages (45 – 60 %) over long operating times (53 – 235 days). It

remained unclear, however, what the exact contributions of worms, natural sludge breakdown and particularly accumulation were towards total sludge breakdown. Furthermore, no special attention was given to growth of the worm biomass and worms and sludge were not separated, as there was no intention to recover and re-use the worm biomass e.g. as a protein source.

The aquatic oligochaete *Lumbriculus variegatus*, a sessile species that is not commonly present in WWTPs, can also be used to reduce the amount of waste sludge (Buys *et al.*, 2008). We proposed a concept for a separate reactor in which the worms are immobilised in a carrier material (Elissen *et al.*, 2006), as shown in Figure 3.1.

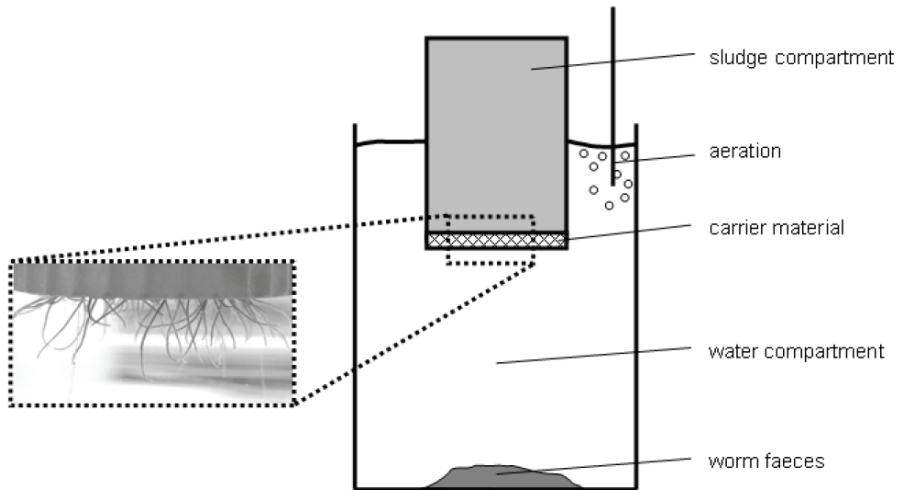


Figure 3.1 Reactor concept in which the aquatic worm *Lumbriculus variegatus* can feed on waste sludge (Elissen *et al.*, 2006).

The worms feed from the waste sludge on one side of the carrier material, whilst their tails protrude through the carrier so that they defecate on the other side. The worms position themselves this way as they use their tails to take up oxygen, which is provided on that side of the carrier. In this configuration, oxygen can efficiently be supplied to the worms, without the waste sludge consuming a considerable part of the supplied oxygen. Additionally, the intention is to harvest the worms from the system, thus obtaining a valuable protein-rich product from the waste sludge. The initial results from batch experiments with this concept were very promising, with a total suspended solids (TSS) reduction of 75 %, worm faeces with a sludge volume index half that of the waste sludge

3. Continuous reactor

and growth of the worm biomass (Elissen *et al.*, 2006). Later experiments, with the same sludge and under similar conditions, showed a lower, but still substantial, TSS reduction of 36 % (Hendrickx *et al.*, 2009)

To allow this concept to be applied at full scale, it first needs to be tested in continuous operation. For this purpose, a worm reactor was operated with waste sludge from a lab scale activated sludge system. The worm reactor was first operated with a direct feed of waste sludge and with return of non-consumed sludge and possible metabolites to the activated sludge system. In this way the effects on the performance of the activated sludge system could be determined.

Secondly, the worm reactor was operated without return of non-consumed sludge. With mass balances for sludge over the worm reactor and a parallel operated blank reactor, a clear distinction was made between the contributions of the worms, natural breakdown and accumulation to total sludge breakdown. As the worms mainly digest the organic fraction of the waste sludge that they consume (Elissen, 2007), detailed mass balances for volatile suspended solids (VSS) are given. Special attention was given to growth of worm biomass. An increase in the amount of worm biomass is a prerequisite for practical application, as it eliminates the need of frequent inoculation of the reactor with fresh worms.

3.2 Materials and Methods

3.2.1 General

Sludge and effluent from a lab scale activated sludge system were used in the experiments. The activated sludge system treated pre-settled domestic sewage in a completely mixed aeration tank followed by a settler. The hydraulic retention time (HRT) was 0.50 - 0.77 days and the sludge retention time (SRT) was 25 days in a first experiment and 16 days in a second experiment. The average organic loading rate was 0.17 g COD/(g TSS·d). Filtered effluent (using black ribbon filters; 12-25 µm, Schleicher and Schuell) from the activated sludge system was used in the water compartment of the worm reactor. A second activated sludge system was operated under the same conditions and with the same waste water. This parallel reactor served to check whether changes in the activated sludge system can be attributed to connecting the worm reactor or to other factors, such as changes in waste water composition or temperature.

The size of the worm reactor required to process the daily waste sludge production from the activated sludge system was calculated based on the results from batch experiments. These were performed according to the method described in Elissen *et al.* (2006). Digestion of sludge by the worms is defined as the amount of sludge consumed (ingested) by the worms minus the amount of faeces (egested). Sludge reduction by the worms is defined as

digestion divided by the consumption. An estimate for TSS reduction was obtained using the organic fractions of sludge and faeces: $\text{TSS reduction} = (\text{organic fraction sludge} - \text{organic fraction faeces}) / (1 - \text{organic fraction faeces})$. Wet weight (ww) of the worms was determined by placing the worms on a perforated piece of aluminium foil. By gently pressing paper towelling against the back of the foil, adhering water was removed from the worms. Dry weight (dw) was determined by drying the worms overnight at 105°C. The average dw/ww ratio was 0.14.

3.2.2 Worm reactor experiments

The reactor was divided into two equal sections, each with a sludge compartment with a volume of 143 cm³ and a carrier material surface area of 55 cm². One section of the (empty) worm reactor is shown in Figure 3.2. For the carrier material we used a 300 µm polyamide mesh (SEFAR). Each water compartment had a volume of 4 L. The sludge that was wasted from the activated sludge system was pumped into the sludge compartment of the worm reactor. Sludge that was not consumed by the worms, and did not accumulate in the worm reactor, left the sludge compartment via an overflow. Some of the effluent in the water compartment was replaced each time the worm faeces were removed manually (daily). The water compartments were aerated using small air diffusers.

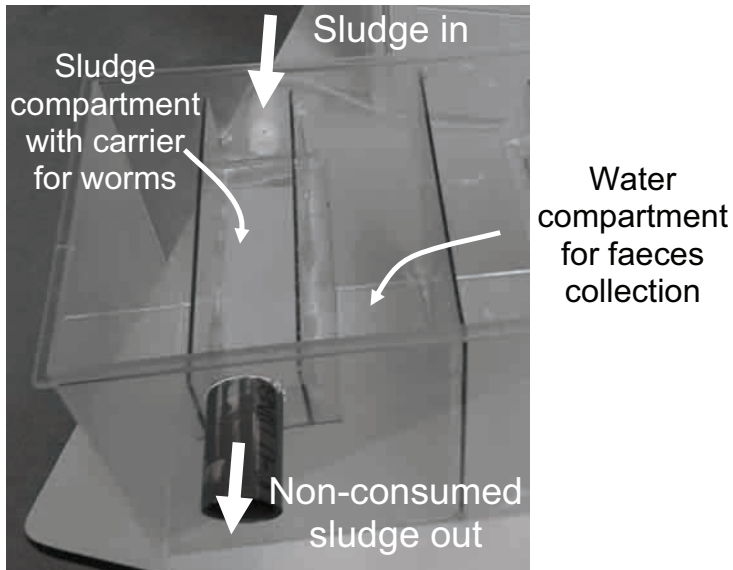


Figure 3.2 Photo of one of the two sections of the empty worm reactor.

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Two experiments were performed with this worm reactor as schematically shown in Figure 3.3. In a first experiment, waste sludge (0.36 L/d) was daily fed directly from the aeration tank of the activated sludge system, while non-consumed sludge was returned to the aeration tank. Sludge concentrations in the activated sludge systems (aeration tank + settler) were measured frequently. Effluents from the activated sludge systems were analysed frequently for chemical oxygen demand (COD) and ammonia concentrations. To allow quantification of natural breakdown of total suspended solids (TSS) and volatile suspended solids (VSS) in the worm reactor, a second experiment was performed. In this second experiment, one section of the worm reactor was operated with worms, whilst the other (blank) was operated under the same conditions and with the same sludge, but without worms. The blank reactor served to quantify natural sludge breakdown in the sludge compartment. 0.2 L of sludge was fed daily to recycle containers of both the worm reactor and the blank reactor, with recirculation of sludge over the sludge compartment at a flow rate of 28 L/d. In this way a constant flow was maintained in the sludge compartment. Worm faeces were collected manually every day. The second experiment was performed twice, first with a 300 μm mesh carrier material, later with a 350 μm mesh carrier material. At the end of all the experiments, the TSS and VSS of the sludge that accumulated in the worm reactor were determined after the worms were separated from the sludge and weighed.

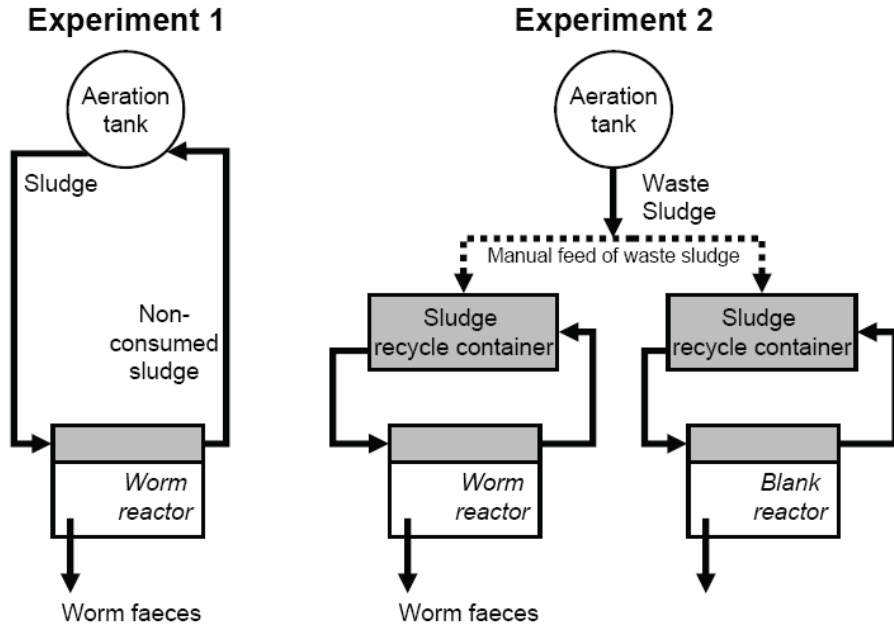


Figure 3.3 Scheme of the set-up of the two experiments performed with the worm reactor. In the first experiment, both (equal) sections were used with worms in it. For the second experiment, one section was used with worms, whilst the other served as a blank reactor without worms.

3.2.2 Analytical

TSS and VSS were determined according to Standard Methods (APHA, 1998) using black ribbon filters (12-25 μm , Schleicher and Schuell). Total ammonium nitrogen concentration ($\text{NH}_3\text{-N} + \text{NH}_4^+\text{-N}$) was determined according to Standard Methods (APHA, 1998), using Dr Lange[®] test kits. Prior to analysis, samples were filtered (0.45 μm) to remove solids and diluted 10 times to exclude the possible effect of interfering ions. Chemical Oxygen Demand (COD) was measured according to Standard Methods (APHA, 1998) using Dr Lange[®] test kits.

Temperature and dissolved oxygen concentration were measured occasionally using a HACH[®] Luminescent Dissolved Oxygen (LDO) meter. The pH was measured using the WTW pH/Cond 340i.

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3.3 Results

The observed sludge yield in the activated sludge system was 0.33 g TSS/g COD removed. Batch experiments with the waste sludge from this system showed that the worms consumed it at an average rate of 50 mg TSS/(g ww·d). This was achieved with a worm density of 0.15 g ww per cm² of carrier material. The average reduction in TSS was 19 %, which is significantly lower than what we found in previous batch experiments with sludge from a municipal WWTP (Elissen *et al.*, 2006, Hendrickx *et al.*, 2009).

3.3.1 Worm reactor with return of non-consumed sludge

In experiment 1, the waste sludge from the activated sludge system was fed directly to the sludge compartment of the worm reactor (as shown in Figure 3.3). Part of the sludge (with possible products from the worms' metabolism) was returned from the worm reactor to the aeration tank of the activated sludge system. During the 50 days of operation, no effect on the performance of the activated sludge system was observed with respect to COD removal and nitrification (Table 3.1). The small changes that were observed after connecting the worm reactor, also occurred in the parallel operated activated sludge reactor (blank). These changes could, therefore, not be attributed to the return of non-consumed sludge from the worm reactor to the first activated sludge system.

Table 3.1 Performance and characteristics of the two activated sludge systems before and during operation of the worm reactor. The first system was connected with the worm reactor, whilst the second system operated as a blank, i.e. without a worm reactor connected to it. The values are given as: average (standard deviation).

		Before (day 1-14)	During (day 15-64)
<i>System with worm reactor</i>			
COD removal	%	84 (4)	88 (3)
Effluent ammonia	mg N/L	0.23 (0.08)	0.25 (0.09)
pH	-	7.5 (0.2)	7.5 (0.3)
T	°C	24.3 (0.7)	21.4 (1.1)
<i>Blank system</i>			
COD removal	%	85 (5)	87 (3)
Effluent ammonia	mg N/L	0.21 (0.06)	0.22 (0.10)
pH	-	7.5 (0.1)	7.5 (0.2)
T	°C	24.3 (0.7)	21.3 (1.0)

Over the 50 days of operating the worm reactor, the amount of worm biomass drastically decreased from 11.08 g ww to 5.96 g ww. This would make it impossible to run a worm reactor for a long period of time without frequent addition of fresh worms. Visual observation of the collected worms at the end of the experiment showed that the worms were still lively and active. They still expressed their natural escape reflex, which is commonly used as an indicator for the vitality of *L. variegatus* (Drewes, 1997). This shows that the sludge compartment of the worm reactor is a suitable environment for the worms. Loss of worms via the outlet of the sludge compartment and into the water compartment was observed, but not quantified. This was likely the main cause for the drastic decrease of worm biomass in the reactor.

Throughout operation of the worm reactor worm faeces were collected with a visibly more compact structure than the original sludge, showing that the sludge was consumed by the worms. On average, this resulted in worm faeces with a lower organic fraction (0.71 ± 0.07 g VSS/g TSS) than the waste sludge (0.76 ± 0.02). Based on the decrease in organic fraction, the average TSS reduction was calculated to be 17 %. A complete TSS balance with a distinction between sludge consumption by worms, natural breakdown and accumulation could not be made with this reactor. Therefore, a second experiment was performed with the same reactor, but without returning the non-consumed sludge to the aeration tank. The second experiment also included the operation of one of the sections of the worm reactor without worms, to quantify the natural breakdown of sludge.

3.3.2 Recirculation of sludge over the worm reactor

Experiment 2 with sludge recirculation over the worm reactor (and a parallel operated blank reactor, see Figure 3.3) was first performed with a 300 μm mesh carrier material. Figure 3.4 shows the VSS balances for both the blank and the worm reactor. The average natural sludge breakdown rate in the blank reactor was estimated at 0.026 g VSS/(g VSS-d) and assumed to be the same in the worm reactor. The worms consumed 40 % of the sludge fed to the worm reactor. Of this consumed sludge, 30 % was digested by the worms and 70 % was mineralised. The total VSS reduction in the worm reactor was the sum of sludge digestion by the worms and natural sludge breakdown, 41 % and 59 % respectively.

3. Continuous reactor

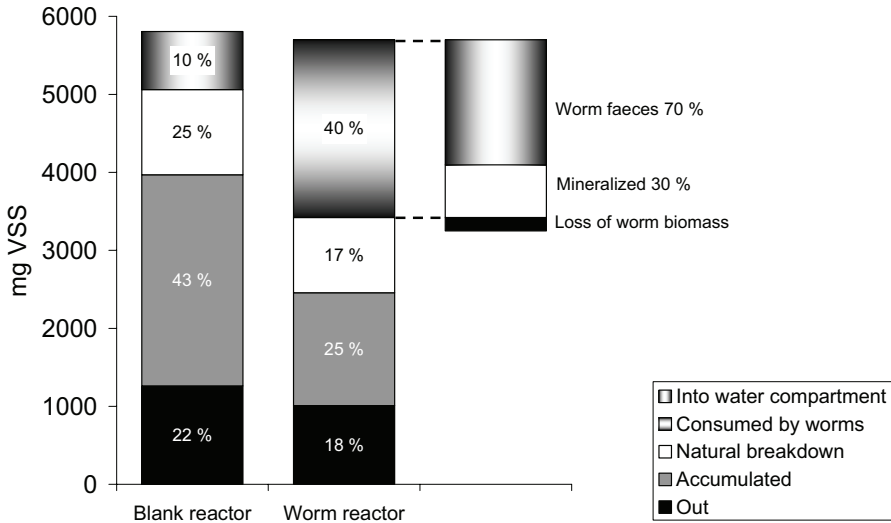


Figure 3.4 VSS balance for the total amount of sludge fed to the worm reactor and the blank reactor. Total TSS fed to each reactor during the 23 days of operation was 7.0 g TSS. Mesh size of the carrier material was 300 μm .

From the TSS balances over the same reactors (results not shown) it was estimated that sludge consumption by the worms was 2.9 g TSS. In total 2.2 g TSS of worm faeces were collected. As a result, the TSS reduction of the sludge that was consumed by the worms was 26%. The digestion of sludge by the worms was further confirmed by the decrease in organic fraction from sludge (0.80 g VSS/g TSS) to worm faeces (0.73). From this decrease in organic fraction, the estimated TSS reduction was 27%, approximately the same as what was found from the TSS balance.

The reactor was started with 4.55 g ww of worms. At the end, 2.13 g ww was still in the reactor. In the water compartment (with the faeces) and with the remaining sludge (recycle container), a total of 1.09 g ww was collected (and not returned to the reactor). Even when correcting for this loss of worms, the worm biomass still decreased in weight, similar to experiment 1, indicating that long term operation of this reactor would not be possible without frequent inoculation with fresh worms.

To assess the possibility that the growth of worms was limited by the mesh size, the experiment was repeated, using the same reactor, but with a carrier material with a larger

mesh size of 350 μm . Figure 3.5 shows the daily added VSS of the waste sludge and the collected worm faeces during the 27 days of operation of this reactor.

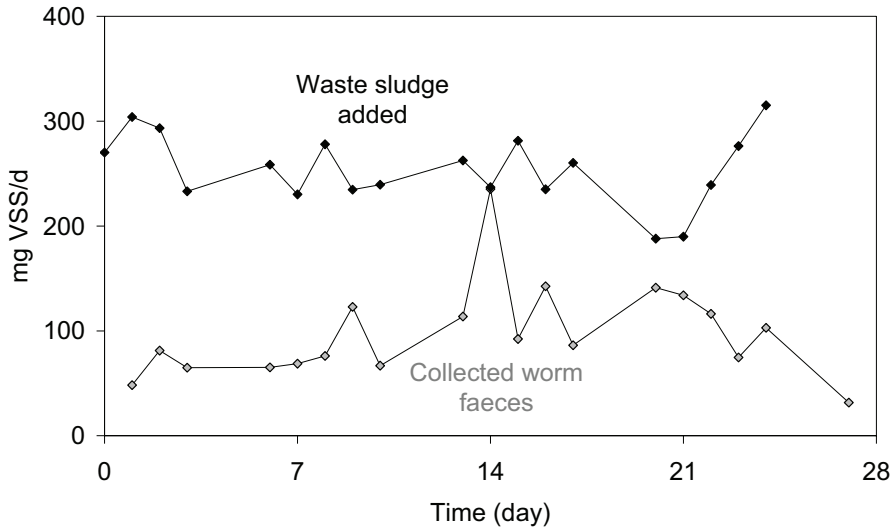


Figure 3.5 VSS fed to the worm reactor and worm faeces collected from the water compartment. A 350 μm mesh was used as a carrier material. In total 5.7 g VSS was fed to the worm reactor.

The worm reactor was started with 1.81 g ww of worms and ended with 2.04 g ww. Throughout operation of the reactor, worms occasionally fell into the water compartment, in total 0.39 g ww. These were not returned to the sludge compartment. Overall, a worm growth of 0.62 g ww was measured and the specific worm biomass growth rate was 0.013 d^{-1} . Figure 3.6 shows the VSS balances for the blank and the worm reactor. The natural breakdown rate was estimated to be $0.016 \text{ g VSS}/(\text{g VSS}\cdot\text{d})$. Of the sludge consumed by the worms, the worms digested 21 %, which was partially (3 %) converted into new worm biomass. This corresponds to a growth yield of 0.13 g dw worm biomass per g VSS digested by the worms. The total VSS reduction in the worm reactor could be attributed to the worms (71 %) and natural sludge breakdown (29 %).

The TSS balances (results not shown) showed a TSS reduction by the worms of 16 %. This matched the 16 % TSS reduction calculated from the decrease in the organic fraction from sludge of 0.69 g VSS/g TSS to worm faeces 0.63 g VSS/g TSS.

3. Continuous reactor

In addition to collecting the worm faeces from the water compartment of the worm reactor, the concentrations of dissolved organic material (COD) and ammonia were measured. These were low and similar to those in the effluent that was used in the water compartment.

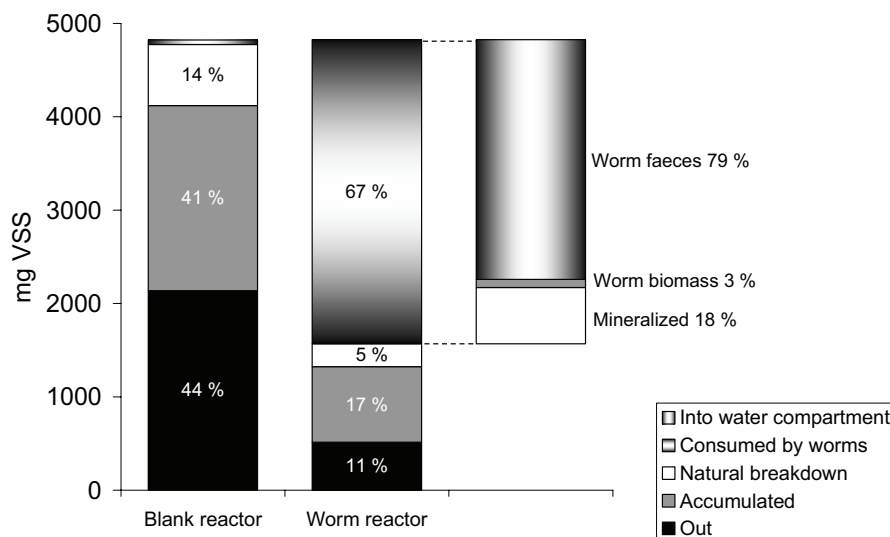


Figure 3.6 Balances for the organic fraction (VSS) of the sludge fed to the blank reactor and the worm reactor. The sludge consumed by the worms is further divided into worm faeces, mineralization and new worm biomass. Mesh size of the carrier material was 350 μm .

3.4 Discussion

In the continuous reactors, the worms produced compact worm faeces from the waste sludge. The average TSS reduction, however, varied from only 16 to 26 %. This is much lower than the 75 % (Elissen *et al.*, 2006) and 36 % (Hendrickx *et al.*, 2009) TSS reduction found in previous batch experiments with sludge from a municipal WWTP, but under otherwise similar experimental conditions. This shows that the origin of the sludge has a large effect on the nutritional value of sludge for the worms. This will be further looked into, as it can be a decisive factor in the applicability of our sludge reduction concept.

TSS reduction also varied in the current experiments carried out with sludge coming from the same system, but during different months of the year. This could be due to a change in the nutritional value of sludge for the worms (e.g. due to changes in waste water

composition). When the organic fraction of the sludge is used as a measure for the nutritional value, our results indeed showed a higher TSS reduction by the worms (26 %) when the organic fraction of the sludge was high (0.80) compared to only 16 % TSS reduction for the sludge with a lower organic fraction (0.69). The average sludge consumption rate, estimated at 39 and 92 mg TSS/(g ww·d), respectively, was higher for the lower organic fraction. This is in agreement with the results from Leppänen and Kukkonen (1998a) for *L. variegatus* feeding on several sediments. With a lower organic fraction of the sediment, the faeces production rate (as a measure for sediment consumption rate) increased. They postulated that the feeding rate of the worms is dominated by net energy gain, i.e. less needs to be consumed of a food source with a high nutritional value. It should, however, also be noted that between the two experiments the mesh size of the carrier material was changed, which may also have affected the TSS reduction.

A robust worm population is crucial for long term operation of a worm reactor. Loss of worm biomass from the reactor (with the faeces, the sludge overflow or by death of worms) needs to be compensated with growth of new worms or with the (undesired) inoculation of the reactor with fresh worms. For non-immobilised worms feeding on waste sludges, Buys *et al.* (2008) reported biomass growth rates of 0.05-0.11 d⁻¹, whereas the growth rate in our continuous reactor with a 350 µm mesh carrier material was only 0.013 d⁻¹. Clearly growth is limited by immobilising the worms in a carrier material, although in all experiments the worms in the sludge compartment remained lively and healthy. It has been reported that worms need to grow to a certain size before they can grow in number, i.e. before they divide (Leppänen and Kukkonen, 1998b). The worms in our reactor might physically not be able to grow to this size when a 300 µm mesh is used. With the 350 µm mesh worms can grow larger, which may also allow them to grow in number.

An important factor for the upscale of the reactor concept is the worm density. In combination with the worm specific consumption rate, it will determine the required carrier surface area for a given daily waste sludge production. Whether an equilibrium or optimum worm density is determined by the supply rate of substrate or by the mesh size of the carrier material (or a combination of both) is currently investigated. Another important aspect was the loss of worms that fell from the mesh into the water compartment and were collected with the worm faeces. This could be a natural way of disposal of excess worm biomass. However, when the worm biomass will be used as a useful resource, an alternative way of maintaining a constant worm population is needed, allowing recovery and reuse of the worms.

3. Continuous reactor

As with other sludge destruction methods (such as anaerobic digestion), release of ammonia, soluble organic material and other compounds by (partial) mineralization of sludge by the worms is an important aspect of the process. These are collected in the effluent that is used in the water compartment of the worm reactor. From the worm reactor, this effluent with the mineralization products will be returned to the activated sludge process. The impact of an additional nitrogen and COD load on WWT processes will, therefore, have to be considered. For nitrogen (as ammonia) we estimated (Hendrickx *et al.*, 2009) the release to be approximately 20 mg N/g TSS digested by worms, whereas in for example anaerobic digestion this is higher with around 78 mg N/g TSS (Araújo *et al.*, 1998). This is partially due to the formation of new protein-rich worm biomass, for which nitrogen is required. In the water compartment of this study, no significant increases in dissolved COD or ammonia were found. These could have been converted by the sludge (at the interface between water and sludge compartment) or the worm faeces. Furthermore, a relatively high effluent replacement rate (0.4 L/d) was used in the water compartment of the worm reactor (thus greatly diluting released metabolites), which was in this case dictated by the collection of faeces. In practice this will be determined by the ammonia concentration in the water compartment, which needs to be kept low enough to prevent it from being toxic to the worms, i.e. below 0.1 mg NH₃-N/L (Hendrickx *et al.*, 2009).

The mass balances over the continuous worm reactors showed the importance of correcting for natural sludge breakdown when quantifying the contribution of the worms. In the first mass balance, digestion by worms and natural sludge breakdown contributed with 41 and 59 %, respectively, to total VSS breakdown. For the second mass balance, these contributions were 71 and 29 %, respectively. As noted before, the sludge consumption rate was higher in the worm reactor of the second mass balance experiment, resulting in the observed lower amount of sludge accumulated in the sludge compartment of the worm reactor. As can be expected, this resulted in a lower contribution of natural sludge breakdown.

3.5 Conclusions

Sludge reduction was achieved in a continuously operated reactor with the aquatic worm *Lumbriculus variegatus* immobilised in a carrier material. From mass balances over the worm reactor we found that a total suspended solids (TSS) reduction of 16 to 26 % could be attributed to the worms (22-30 % volatile suspended solids (VSS) reduction). The variation in sludge reduction between the different experiments was most likely related to changes in the nutritional value of the sludge for the worms.

A clear distinction was made between the contributors towards total sludge reduction. For the two VSS balances made over the continuous worm reactor, the worms contributed 41 and 71 %, respectively, towards total VSS reduction. The rest was caused by natural sludge breakdown.

The mesh size of the carrier material in which the worms were immobilised had an effect on worm growth. We observed no growth of worms when using a 300 μm mesh. When using a larger mesh size of 350 μm , a worm growth rate of 0.013 d^{-1} was observed with a yield of 0.13 g dw/g VSS digested. This allows long term operation without the need for frequent inoculation of the reactor with fresh worms.

No significant release of soluble COD and ammonia as metabolic products of the worms was observed in the water compartment of the worm reactor. This may be due to conversion of these compounds inside the worm reactor. Return of non-consumed sludge from the worm reactor to the activated sludge system had no significant effect on the performance (COD removal, nitrification) of the activated sludge system.

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3. Continuous reactor

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Chapter 4

The effect of operating conditions on aquatic worms eating waste sludge

Abstract

Several techniques are available for dealing with the waste sludge produced in biological waste water treatment. A biological approach uses aquatic worms to consume and partially digest the waste sludge. In our concept for a worm reactor, the worms (*Lumbriculus variegatus*) are immobilised in a carrier material. For correct sizing and operation of such a worm reactor, the effect of changes in dissolved oxygen (DO) concentration, ammonia concentration, temperature and light exposure were studied in sequencing batch experiments. DO concentration had an effect on both sludge consumption rate and sludge reduction efficiency. Sludge consumption rate was a factor four higher at DO concentrations above 8.1 mg/L, when compared to DO concentrations below 2.5 mg/L. Sludge reduction was 36 and 77 % at these respective DO concentrations. The effect is most likely the result of a difference in gut residence time. An increase in unionised ammonia concentration drastically decreased the consumption rate. Ammonia is released by the worms at a rate of 0.02 mg N/mg TSS digested; therefore, replacing the effluent in the worm reactor is required to maintain a low ammonia concentration. The highest sludge consumption rates were measured at a temperature around 15°C, whilst the highest TSS reduction was achieved at 10°C. Not exposing the worms to light did not affect consumption or digestion rates. High temperatures (above 25°C) as well as low DO concentrations (below 1 mg/L) in the worm reactor should be avoided as these lead to significant decreases in the number of worms. The main challenges for applying the worm reactor at a larger scale are the supply of oxygen to the worms and maintaining a low ammonia concentration in the worm reactor. Applying a worm reactor at a waste water treatment plant was estimated to increase the oxygen consumption and the ammonia load by 15-20 % and less than 5 % respectively.

Keywords: sludge reduction, aquatic worms, dissolved oxygen, ammonia, light, temperature

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4. Operating conditions

4.1 Introduction

Waste water treatment plants (WWTPs) produce enormous amounts of biological waste sludge. Due to the contaminants it contains (such as heavy metals and organic micropollutants), regulations for the disposal of waste sludge are becoming more stringent, thus limiting the application as, for example, a fertiliser. As a result, (co)incineration is becoming the more common final treatment method, followed by landfill or immobilisation of the ashes in a product (such as Portland cement). The complete sludge disposal route – which typically includes thickening, dewatering, drying and transport to central sludge treatment facilities – may represent up to 50 % of the operational costs at WWTPs (Wei *et al.*, 2003). This has led to an increased interest in techniques to reduce the amount of waste sludge. Mechanical, physical and chemical methods require a substantial input of chemicals and / or energy, which may make them costly. Biological approaches include the use of aquatic worms, which is believed to lead to a lower overall biomass yield through the extension of the food chain (Ratsak and Verkuijlen, 2006). We recently proposed a new reactor concept to reduce the amount of waste sludge (Elissen *et al.*, 2006). This system utilises the aquatic worm *L. variegatus*, immobilised in a carrier material. The worms consume and partly digest waste sludge from one side of the carrier material, whilst their tails protrude through the carrier to take up oxygen from the water compartment (Figure 4.1). This way, the worm faeces are collected in the water compartment.

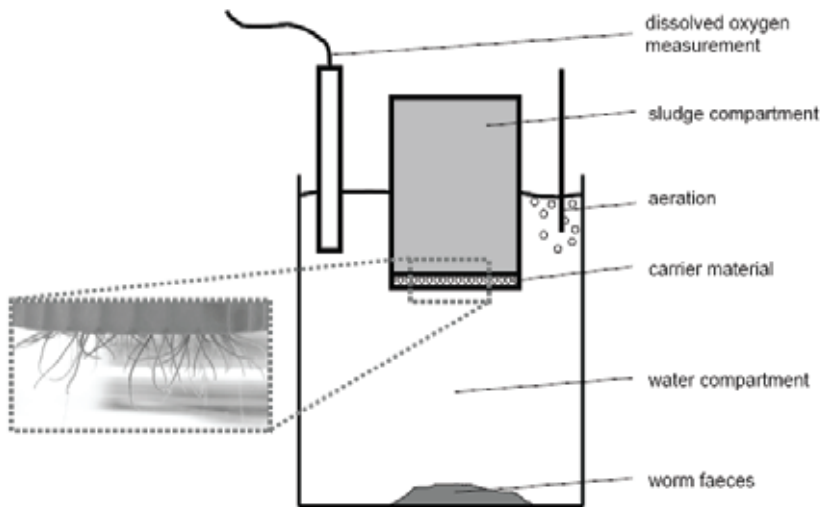


Figure 4.1 Scheme of the reactor concept in which the aquatic worm *Lumbriculus variegatus* feeds on waste sludge (Elissen *et al.*, 2006).

Initial experiments showed that this system has a high potential for reducing the amount of biosolids (up to 75 %). Additionally, the worm faeces showed better settleability compared to the original waste sludge and potentially useful protein-rich worm biomass is produced. To apply this process at a larger scale, it needs to be able to operate under varying conditions of which we identified dissolved oxygen (DO), ammonia, temperature and exposure to light to be the most important. These parameters may be adjustable (such as DO concentration) or imposed on the process (e.g. temperature). The rate at which the worms consume the sludge is the main design parameter for a worm reactor. How this rate is affected by DO, ammonia, temperature and light exposure determines the range within which the worm reactor needs to be operated to maintain a sufficiently high capacity. Apart from the importance of the effect of changes in these parameters on the consumption rate (sub-lethal effect), a healthy worm population should be maintained in the reactor to obtain a robust system. Therefore, the lethal effect of DO, ammonia and temperature should also be known.

As *L. variegatus* is commonly used as a standard test organism for toxicity and bioaccumulation experiments on sediments (e.g. Ankley *et al.*, 1993), information on the effect of these parameters is available. However, since only faeces production is measured in these experiments, the effect on consumption rates and digestion efficiencies is not available. Toxicity and bioaccumulation experiments generally use sediments from rivers or lakes, under standard conditions (e.g. EPA, 2000). DO is an important factor, as it needs to be supplied to the water compartment, from where the worms take it up with their tails. The input of oxygen also represents an increase in energy input and should, therefore, be minimised. Available literature on the effect of DO has mainly focussed on worm survival and on worm activity, as measured by heat production or oxygen consumption (e.g. Gnaiger and Staudigl, 1987). The worms can withstand periods of more than 5 days under anaerobic or very low DO conditions (Putzer *et al.*, 1990; Mattson *et al.*, 2008). However, these experiments were performed with non-fed worms. Gnaiger and Staudigl (1987) report that the worms do consume substrate under anoxic conditions, but give no consumption rate. Furthermore, the effect of ammonia is important, as it is known to be toxic to *L. variegatus* in its unionised form. This is mostly determined by the pH of the effluent (the pKa is 9.41 at 20°C). Ammonia may already be present in the effluent that is used in the water compartment of the worm reactor (Figure 4.1), depending on the extent of nitrification at the WWTP. In addition, ammonia is one of the products of the metabolism of *L. variegatus* (Gardner *et al.*, 1993). Reported LC50 values (Lethal Concentration for 50 % of the worms) range from 0.29 to 1.20 mg NH₃-N/L (Hickey and Vickers, 1994; Schubauer-Berigan and Monson, 1994; Whiteman *et al.*, 1996 and Besser *et al.*, 1998). Temperatures at WWTPs vary over the year and may thus affect the performance of a

4. Operating conditions

worm reactor. Several references report an increase in faeces production rate with temperature (Leppänen and Kukkonen, 1998; Landrum *et al.*, 2004 and Williams, 2005). At higher temperatures, the survival of the worms may become an issue, as the LT50 (Lethal Temperature for 50 % of the worms) in a 4 day exposure experiment was determined to be 24.9 - 28.7°C (Quinn *et al.*, 1994). Finally, exposure of the worms to light may be an important factor in scaling up the worm reactor, as it may not be practical to expose all the worms to light, particularly those in the centre of the reactor. There is, however, no reference to light affecting worm behaviour or metabolism. The characteristic escape reflex (Drewes, 1989) is related to sudden changes in light intensity, rather than a natural day / night rhythm. Preliminary results for the effects of these parameters on *L. variegatus* feeding on waste sludge are available (Elissen, 2007). However, those experiments were performed with free crawling worms. In the current experiments the worms are immobilised in a carrier material, which restricts their movement and access to the surface area between water and air. This may result in different responses by the worms.

4

This chapter describes the experiments that were performed to determine the lethal and sub-lethal effects of changes in dissolved oxygen, ammonia concentration, temperature and light exposure on *L. variegatus*. All experiments were performed in sequencing batch experiments using the reactor concept we proposed for sludge reduction using *L. variegatus* (see Figure 4.1). The implications on the scale-up of the worm reactor will also be discussed.

4.2 Materials and Methods

4.2.1 General

Fresh secondary sludge and effluent from the municipal WWTP of the city of Leeuwarden (the Netherlands) were used. Sludge was sieved over a 1 mm mesh and the effluent was filtered over a black ribbon filter (12-25 µm, Schleicher and Schuell). Sludge (max. 1 day old) and filtered effluent were stored at 4°C. Worms were randomly taken from a large population, which was grown on the same sludge. Wet weight (ww) of the worms was determined by placing them on a perforated piece of aluminium foil. By gently pressing paper towelling against the back of the foil, adhering water was removed.

4.2.2 Sequencing batch experiments

Sequencing batch experiments were performed according to the method described in Elissen *et al.* (2006). Figure 4.1 shows the experimental set-up. One experiment consisted of four consecutive batches which lasted 23 hours each. Thus, results were obtained for worms feeding on sludge for a total of 92 hours. A blank experiment (i.e. without worms) was performed at the same time and under the same conditions to correct for natural sludge

breakdown. Sludge consumption and digestion were calculated as shown in Figure 4.2. For survival experiments the same experimental set-up was used, but only the worms were counted, without quantifying sludge consumption and faeces production.

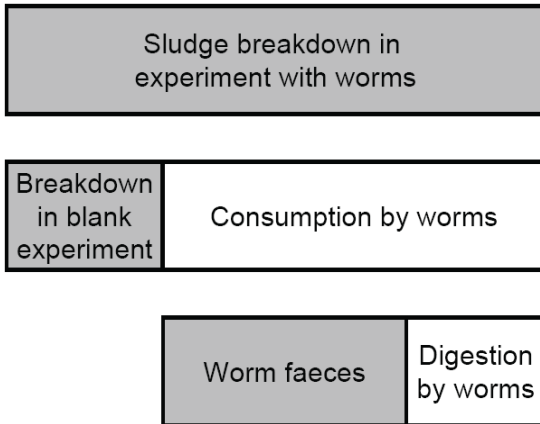


Figure 4.2 Scheme of measured (grey) and calculated (white) amounts of TSS in an experiment.

The results for sludge consumption, sludge digestion and faeces production rates were standardised for worm wet weight (ww) and time and are presented in mg TSS/(g ww·d). Digestion efficiency in terms of TSS reduction (%) is defined as digestion divided by consumption. The errors in the measurements (TSS, wet weight of the worms) were estimated and used to calculate the experimental errors in the consumption, digestion and faeces production rates.

All experiments were performed using a polyamide carrier material (SEFAR) with a mesh size of 300 μm and a surface area of 7.5 cm^2 . The experiments started with 100 mL sludge in the sludge compartment and 900 mL effluent in the water compartment. In each experiment, 163 worms were used with a total wet weight of 1.5-2.2 g, resulting in a worm density of 2-3 kg ww/ m^2 . Worm survival (%) was defined as the number of worms at the end of the 4-day sequence, divided by the number of worms at the start of the experiment. Each experiment was performed in parallel with a reference experiment (no added ammonia, DO > 9 mg/L and T = 19 \pm 2°C).

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The DO concentration in the water compartment was controlled by air and / or nitrogen gas diffusers. The temperature experiments at 10, 15 and 25°C were performed in a temperature controlled cabinet. For the ammonia experiments, the water compartment was spiked with ammonium chloride. The pH of the water compartment was adjusted by adding hydrochloric acid. The resulting increase in chloride concentration (max. 160 mg/L) did not result in toxic concentrations for the worms (e.g. Schubauer-Berigan and Monson, 1995). The experiments with continuous light exposure were performed using a 60 W light, placed at 0.5 m from the set-up. The experiments in the dark were performed in the same room, but the set-up was placed in a fully darkened cabinet.

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To ensure that the measured effects were related to changes in the DO concentration in the water compartment, the DO concentration in the sludge compartment was measured continuously in some of the experiments. This showed that initially some oxygen is present in the sludge compartment, but that the concentration always rapidly decreased to below 1 mg/L within half an hour. These measurements were performed at both high (± 9 mg/L) and low (± 4 mg/L) DO concentrations in the water compartment. The DO effects can, therefore, be attributed to the DO in the water compartment. Additionally, the pH was measured in the sludge compartment in some of the experiments. Throughout an experiment there was only a slight change ($< 5\%$) in pH, which typically was 7.2 at the start.

4.2.3 Calculations

TSS reduction = (sludge digested by the worms / sludge consumed by the worms) · 100 %

Worm survival = (worms at the end / worms at the start) · 100 %

Fitting the experimental data from the DO experiments to a simple approximation was done using the non-linear regression function in SPSS 12.0.1, with the minimum and maximum for both rate and DO concentration as parameters. The worm survival curve as a function of temperature was estimated using SPSS 12.0.1.

Estimating the increases in hydraulic and nitrogen loads and the oxygen consumption used the following assumptions: 1) 100,000 person equivalent WWTP with a daily waste water flow of 34,000 m³/d (with a biological oxygen demand (BOD) of 600 mg/L and a nitrogen content of 50 mg N/L). 2) Secondary sludge production of 3500 kg TSS/d. 3) Maximum allowable ammonia concentration of 0.1 mg NH₃-N/L. 4) Sludge digestion by worms required 1.2 g O₂ / g TSS (= 1.42 g O₂ / g VSS, the theoretical oxygen requirement for oxidation of sludge).

4.2.4 Analytical methods

Total ammonia nitrogen concentrations ($\text{NH}_3 + \text{NH}_4^+$) were determined according to Standard Methods (APHA, 1998), using Dr Lange[®] test kits. Prior to analysis, samples were diluted 10 times to exclude the possible effect of interfering ions. The distribution between $\text{NH}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ as a function of pH and temperature was calculated using the data reported in Emerson *et al.* (1975). Total suspended solids were determined according to Standard Methods (APHA, 1998) using black ribbon filters (12-25 μm , Schleicher and Schuell). Dissolved oxygen concentrations and temperature in the water compartment were measured every 30 minutes using a HACH[®] Luminescent Dissolved Oxygen (LDO) meter. The pH was measured using the WTW pH/Cond 340i.

4.3. Results

4.3.1 Dissolved Oxygen concentration

Figure 4.3 shows that the DO concentration in the water compartment has a clear effect on sludge consumption and faeces production by the worms. It also shows a more efficient digestion of the sludge by the worms with decreasing DO concentration, i.e. a larger difference between the consumption and faeces rates.

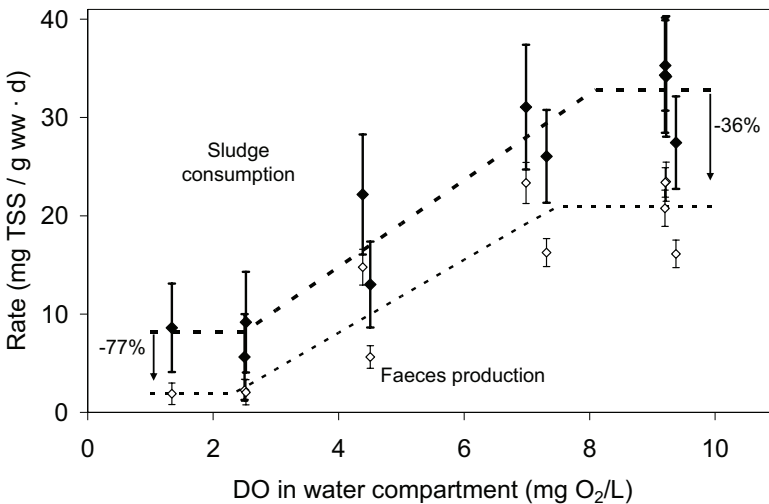


Figure 4.3 Effect of dissolved oxygen concentrations in the water compartment on the sludge consumption rate and faeces production rate by worms in sequencing batch experiments. $T = 18.6 \pm 0.4$ °C, pH was not measured. The error bars indicate the calculated experimental error. The broken lines show simple approximations of the measured results.

4. Operating conditions

The broken lines in Figure 4.3 represent a simple approximation of consumption and faeces production, by using minimum and maximum rates at low and high DO, respectively, with a linear increase in between. The results of fitting the experimental data to this approximation are shown in Table 4.1.

Table 4.1 Results of fitting the data to a simple approximation of the effects of dissolved oxygen concentration on consumption rate, faeces rate and TSS reduction by *L. variegatus* feeding on waste sludge.

		mg TSS/(g ww · d)	at DO (mg O ₂ /L)
Consumption rate	maximum	32.8 ± 2.0	> 8.1 ± 0.9
	minimum	8.1 ± 0.9	< 2.5 ± 1.1
Faeces rate	maximum	21.0 ± 2.0	> 7.5 ± 1.0
	minimum	1.9 ± 4.1	< 2.3 ± 1.3
TSS reduction	maximum	77 %	< 2.3
	minimum	36 %	> 8.1

The observed effect of DO concentration on defecation rate is in agreement with the findings of Gnaiger and Staudigl (1987), who also reported a much lower defecation rate under anoxic conditions (< 0.03 mg O₂/L). They postulated that the worms – at low DO – minimise the amount of energy spent on searching for food whilst simultaneously switching to a (slower) anaerobic metabolism. They do not, however, mention the effect of oxygen on the consumption rate. If *L. variegatus* does indeed minimise its energy spent on feeding, it can be expected that worm gut residence time increases, resulting in a higher digestion percentage. This could not be determined from results in literature for worms feeding on sediments, as only the defecation rates were measured. From our experiments, it became apparent that at low DO concentrations the worms indeed digested a larger part of the sludge they consumed (Figure 4.4). As the worms mainly use the organic part of the sludge (Elissen, 2007), an increase in digestion efficiency should be accompanied by a lower organic fraction of the produced worm faeces. This was confirmed by the results shown in Figure 4.4, where the organic fraction of the waste sludge and the collected faeces are compared for all experiments.

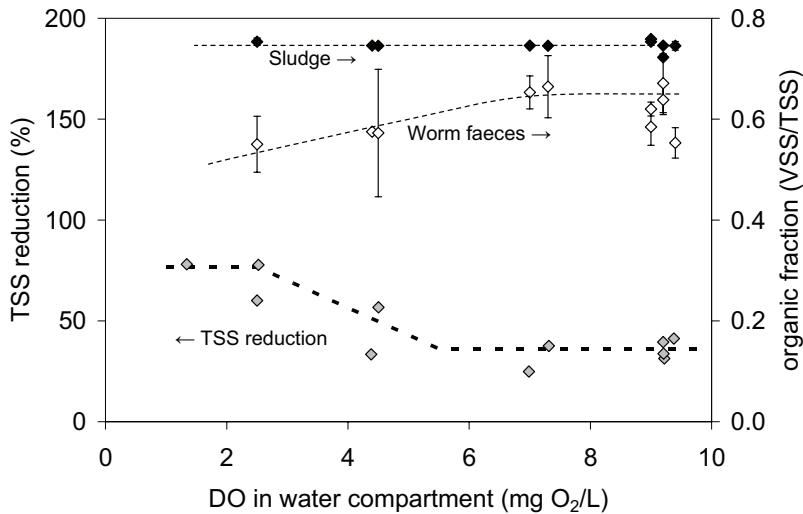


Figure 4.4 Organic fraction of the produced worm faeces at different dissolved oxygen concentrations in the water compartment, compared with organic fraction of the sludge and the measured TSS reduction by the worms. The error bars show the standard deviation in the averages.

Apart from the rate at which the worms consume sludge, survival of the worms is important for the robustness of the worm reactor. As shown in Figure 4.5, survival of the worms became an issue at a DO concentration below 1 mg/L. However, already at a DO concentration below 2.5 mg/L the specific biomass growth rate of the worms was negative (results not shown), thus preventing long term operation at low DO (at the worm density of 2-3 kg ww/m² used in these experiments).

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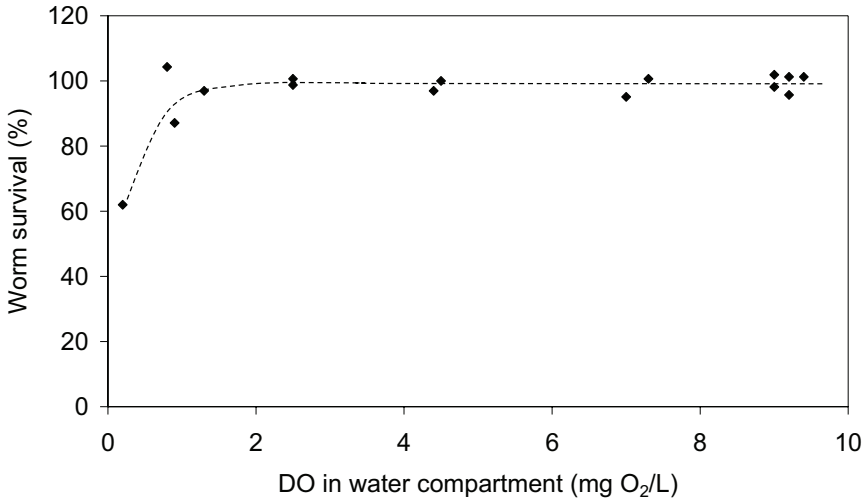


Figure 4.5 Effect of DO concentration on the survival of worms in a 4-day sequencing batch experiment. Each experiment was started with 163 worms. The broken line shows an estimated survival curve.

4.3.2 Ammonia toxicity

Two experiments were performed to determine the effect of ammonia on sludge consumption by *L. variegatus*. The first one was performed at the relatively high pH of the effluent (± 8.6) and in the second experiment, the pH was lowered to ± 7.9 . Figure 4.6 clearly demonstrates that at a lower pH of 7.9, sludge consumption by the worms is less affected by the ammonium concentration than at the higher pH of 8.6. The toxic effect of ammonia has mainly been ascribed to unionised ammonia, NH_3 (Besser *et al.*, 1998 and Schubauer-Berigan *et al.*, 1995). This is confirmed by our results, where the consumption rate decreases linearly with the concentration of unionised ammonia (data not shown): sludge consumption rate (in mg TSS/g ww·d) = $28.0 - 34.5 \cdot \text{ammonia concentration (in mg NH}_3\text{-N/L)}$ with $R^2 = 0.995$. An increase in ammonia concentration did not result in a change in sludge digestion efficiency by the worms (results not shown).

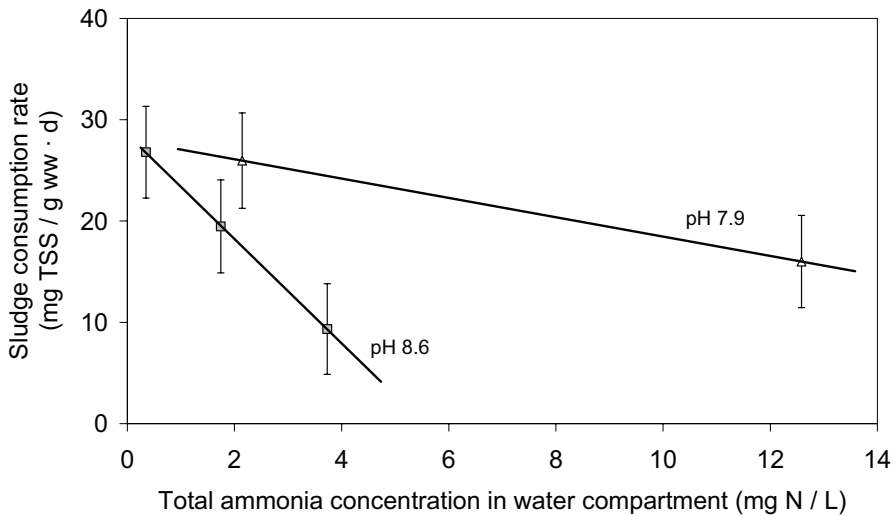


Figure 4.6 Effect of total ammonium on the sludge consumption rate by *L. variegatus*. $T = 18.3 \pm 0.5^\circ\text{C}$, $\text{DO} = 9.3\text{-}10.2 \text{ mg/L}$, low $\text{pH} = 7.9 \pm 0.3$, high $\text{pH} = 8.6 \pm 0.3$. The error bars show the calculated experimental error.

The expected ammonia concentrations in effluents from WWTPs in the Netherlands that apply nitrogen removal (or at least nitrification) would be below 1 mg N/L total ammonia at a pH of 7.8 (STOWA, 2005). According to Figure 4.6 these values would hardly affect the sludge consumption rate. If the nitrification capacity for some reason becomes lower, temporary higher ammonia concentrations in the effluent (which is used in the water compartment of the worm reactor) may lower the sludge consumption rate of the worms. Although the worm will temporarily consume less sludge under those conditions, the effect was shown to be largely reversible when the worms were exposed to lower ammonia concentrations again (data not shown). However, too high ammonia concentrations can lead to mortality of the worms. Several survival experiments were performed to determine at which ammonia concentrations this can be expected to become a problem. Within the range we measured (up to 57.6 mg N/L total ammonia at a pH of 8.6), the worms can survive without significant losses (data not shown). At lower pH values the worms can withstand even higher ammonia concentrations, as was shown by Besser *et al.* (1998), who reported an LC50 of 302 mg N/L total ammonia (at $\text{pH} = 8.0$ and $T = 23^\circ\text{C}$).

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At the highest concentrations the worms protruded their tails less far through the mesh, thus decreasing their exposure to the ammonia in the water compartment. Analysis of the sludge compartment indeed showed a lower concentration of unionised ammonia, when compared to the water compartment.

Ammonia is also produced by the worms themselves, as a product of their metabolism (Gardner *et al.*, 1993) and by mineralising the sludge. Measurement of total ammonia amounts in the water compartment at both the start and the end of the experiment without added ammonia, showed a release of 0.22 mg total ammonia-N/(g ww·d) for worms consuming waste sludge (this is equal to 20 mg total ammonia-N/g TSS digested). This is similar to the results found for worms that were not immobilised in a carrier material (Elissen, 2007).

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4.3.3 Temperature

The effect of temperature on the sludge consumption rate by the worms is shown in Figure 4.7. There seemed to be an optimum around 15°C, although more measurements are required to determine the exact location of this optimum. Available literature on the optimum temperature for *L. variegatus* is not conclusive. It was shown to be between 15 and 20°C (Leppänen *et al.*, 1999; Williams, 2005 and Elissen, 2007), but Chapman *et al.* (1999) stated that it lies between 20 and 25°C. It is clear that lowering the temperature to 10°C decreased the consumption rate significantly when compared to 20°C, which is in agreement with literature data (Landrum *et al.*, 2004; Williams, 2005).

In addition to an effect on sludge consumption rate, we also found a clear effect on TSS reduction. Figure 4.8 shows a decrease in efficiency between 10 and 20°C. At 28°C, however, we found a very high sludge reduction. This was not further investigated, but a possible explanation could be the overlapping effects of worm behaviour (changes in gut residence time) and sludge properties (possible response of the bacterial biomass to the change in temperature). Lower temperatures lead to longer gut residence times in the worm associated with a lower feeding rate, resulting in a higher digestion rate. However, at higher temperatures enzymatic and bacterial processes proceed faster, thus making the sludge more readily available as food for the worms. It should further be remarked that the results merely show the effect of changing the temperature for a given sludge cultivated at one temperature, whilst in reality the composition of the sludge may be different in cold and warm periods.

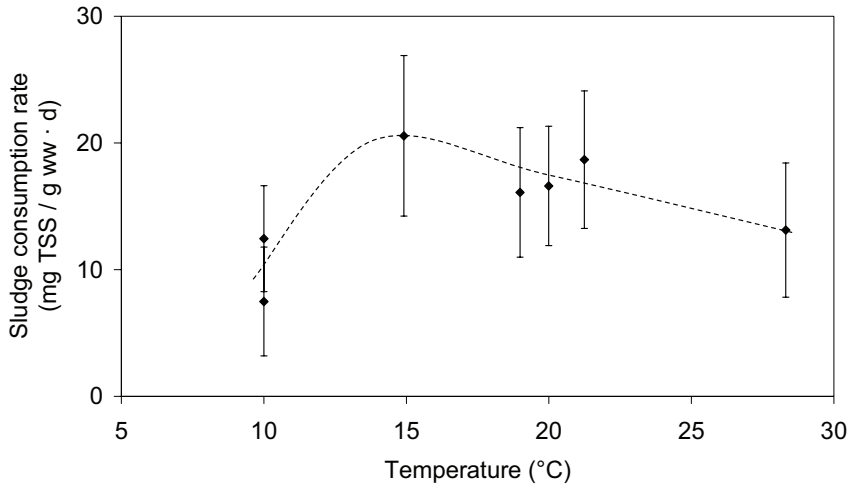


Figure 4.7 Sludge consumption rates at different temperatures, measured in sequencing batch experiments. DO > 9.0 mg/L, pH = 8.7-9.0 and total ammonia below 1.4 mg N/L. The error bars show the experimental error.

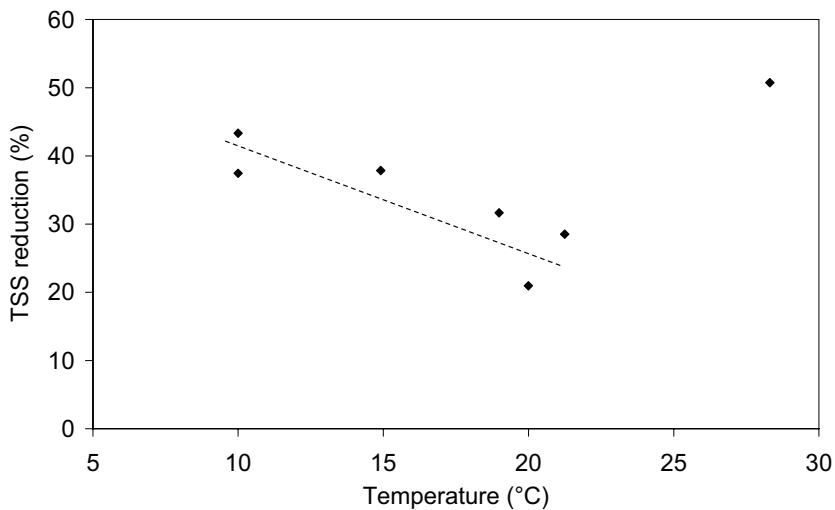


Figure 4.8 Sludge reduction by the worms at different temperatures. DO above 9.0 mg/L, pH = 8.7-9.0 and total ammonia below 1.4 mg N/L.

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Several survival experiments were performed to determine survival in the reactor if the temperature should exceed 20°C. Figure 4.9 shows that this may become problematic above 25°C, which is agreement with the reported LT50 of 24.9 - 28.7°C for non-feeding worms (Quinn *et al.*, 1994).

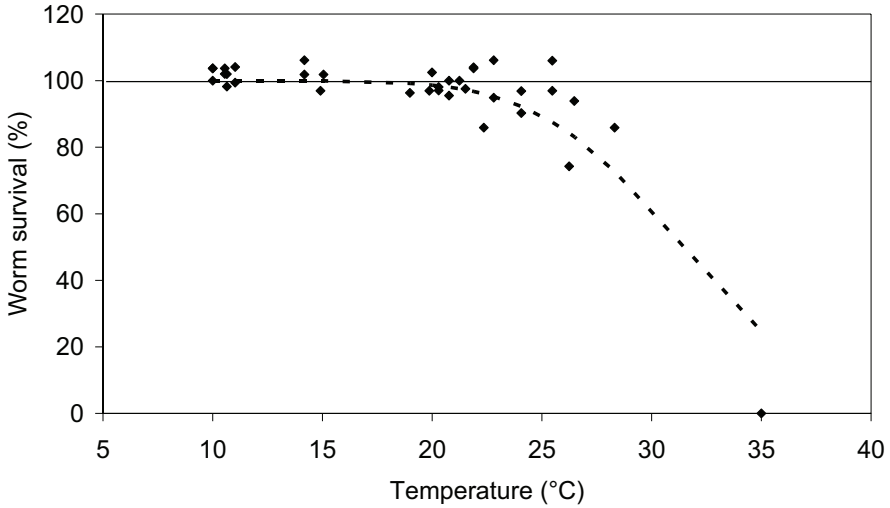


Figure 4.9 Effect of temperature on worm survival. The solid line shows 100 % survival. The broken line shows an estimated survival curve.

4.3.4 Light exposure

The results showed no significant differences between the sludge consumption, sludge digestion and faeces production rates for the experiments performed under either dark or light conditions (see also Hendrickx *et al.*, 2006). The results by Elissen (2007) showed that dark conditions are somewhat beneficial to the worms. Therefore, we do not expect any problems by not exposing all the worms to light in the reactor.

4.4 Discussion

The effects of DO concentration, ammonia concentration, temperature and light exposure described in this chapter, set different constraints on the size and operation of the worm reactor. An important design parameter is the sludge consumption rate per worm, as a lower

rate results in a larger amount of worms required to deal with the daily sludge production of a WWTP. This is likely to increase the size of the reactor.

The DO concentration was shown to have an effect on both sludge consumption rate and reduction efficiency. Operation at a DO concentration above 8.1 mg/L resulted in a TSS reduction of 36 % combined with the highest sludge consumption rate, and would thus require the smallest possible worm reactor. Maintaining such a high DO concentration by aeration would, however, require a substantial input of energy. DO concentrations between 1 and 2.5 mg/L, would make oxygen transfer much more efficient and result in a higher TSS reduction of 77 %. Due to the lower sludge consumption rate this would, however, be at the cost of a four times larger reactor. Moreover, the current worm density of 2-3 kg ww/m² was not sustainable at these low DO concentrations. Though there was no decrease in the number of worms, their weight did decrease. Operating the reactor at intermediate DO concentrations may be more economically favourable, where the lower operational costs for aeration would compensate for the higher investment costs of a larger worm reactor. In any case the oxygen needs to be supplied to the worms, which we estimated to increase the total oxygen demand of the WWTP by 15-20 %. Still, at all DO concentrations, sludge is compacted into worm faeces.

Ammonia is released by the worms into the water compartment of the worm reactor at a rate of 20 mg N/g TSS digested. To prevent the described inhibiting effect of unionised ammonia on sludge consumption rate by the worms, the effluent in the water compartment of the worm reactor needs to be replaced to maintain a sufficiently low concentration. This effluent will have to be returned to the WWTP for nitrogen removal, thereby also increasing the hydraulic load on the WWTP. Depending on the pH (7.3-7.8), we estimated this additional hydraulic load to be 5-15 % of the WWTP influent flow, whilst the nitrogen load would increase by less than 5 %. Both loads are higher when compared to sludge dewatering only, i.e. when dewatering is not preceded by a worm reactor for compacting and reducing the amount of sludge. Particularly the increased nitrogen load will have an impact on the denitrification capacity of the WWTP, for which an additional carbon source may have to be supplied. The nitrogen release is, however, much lower when compared to a process such as anaerobic digestion, where the additional nitrogen load represents 10-15 % (Siegrist *et al.*, 2008). This is most likely explained by the formation of protein-rich worm biomass in our process, rather than mineralization of the sludge as occurs in anaerobic digestion. Due to the high nitrogen content of worm biomass (~10 %) (Hansen *et al.*, 2004) when compared to waste sludge (~5 %), most of the nitrogen in the digested sludge can be expected to be used for worm growth. The exact contribution of the formation of worm

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biomass towards the total nitrogen mass balance will depend on the worm yield and should be determined in longer term experiments.

Temperature does have an effect on consumption and digestion; however, its control would require a considerable energy input. For WWTPs in the Netherlands, average monthly temperatures typically vary between 10 and 21°C, with a year average temperature of 15°C (STOWA, 2006). The results showed that operation of a worm reactor at these temperatures should not pose any problems, as an optimum for the sludge consumption rate was found around 15°C. Nonetheless, it is important to prevent temperatures above 25°C, which result in significant worm mortality. When increasing the temperature from 10 and 20°C, the TSS reduction was found to decrease. Operation at a low temperature (10°C) does result in a considerably lower consumption rate, and thus a larger reactor would be needed. However, the production of waste sludge will also vary with temperature. At lower temperatures in winter time, the overall sludge yield generally is lower, resulting in a lower daily waste sludge production, which compensates for the lower consumption rate of the worms.

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At lab scale, promising results have been achieved with aquatic worms reducing the amount of waste sludge and compacting it into worm faeces. Two challenges for a full-scale application are the supply of oxygen to the worms and removal of ammonia from the water compartment. How to deal with this is currently being tested in continuously operated reactors at a larger scale than the batch experiments described in this chapter. An economic evaluation of these results should reveal whether the costs savings made by the worm process (less waste solids, better settleability of solids, potentially valuable worm biomass) could outweigh the extra costs (construction and operation of the worm reactor, extra input of oxygen, higher ammonia load on the WWTP).

4.5 Conclusions

With our reactor concept, the aquatic worm *L. variegatus* can be used for reducing the amount of biological waste sludge. For correct sizing and operation of the worm reactor, the effects of the following conditions should be taken into account.

- 1) The dissolved oxygen concentration affects both consumption rate and digestion efficiency. Above 8.1 mg/L the highest sludge consumption rate was found with a TSS reduction of 36 %. A four times lower sludge consumption rate was found below 2.5 mg/L, but with a higher TSS reduction of 77 %. The current worm density of 2-3 kg ww/m² was not sustainable at DO concentrations below 2.5 mg/L.
- 2) An increased ammonia concentration in the water compartment resulted in a lower sludge consumption rate by the worms. Since unionised ammonia is the toxic form for

the worms, this effect is strongly pH dependent. Ammonia is released by sludge consuming worms at a rate of 20 mg N per g TSS digested by the worms.

- 3) Temperature had a clear effect on both sludge consumption and sludge digestion efficiency by the worms. The consumption rate seemed to have an optimum around 15°C. Increasing the temperature from 10 to 20°C, resulted in a gradual decrease in sludge digestion efficiency. A temperature below 25°C is required to ensure worm survival.
- 4) Sludge consumption rate, faeces production rate and sludge digestion efficiencies are not affected when the worms are kept in the dark.

Oxygen input to the worm reactor and ammonia removal from the worm reactor are two key factors in determining the economic feasibility of a worm reactor for sludge reduction. The worm reactor is estimated to increase the total oxygen consumption at a WWTP by 15-20 %. The ammonia load on a WWTP is expected to increase by less than 5 %, whereas the hydraulic load was estimated to increase by 5-15 %.

Acknowledgements

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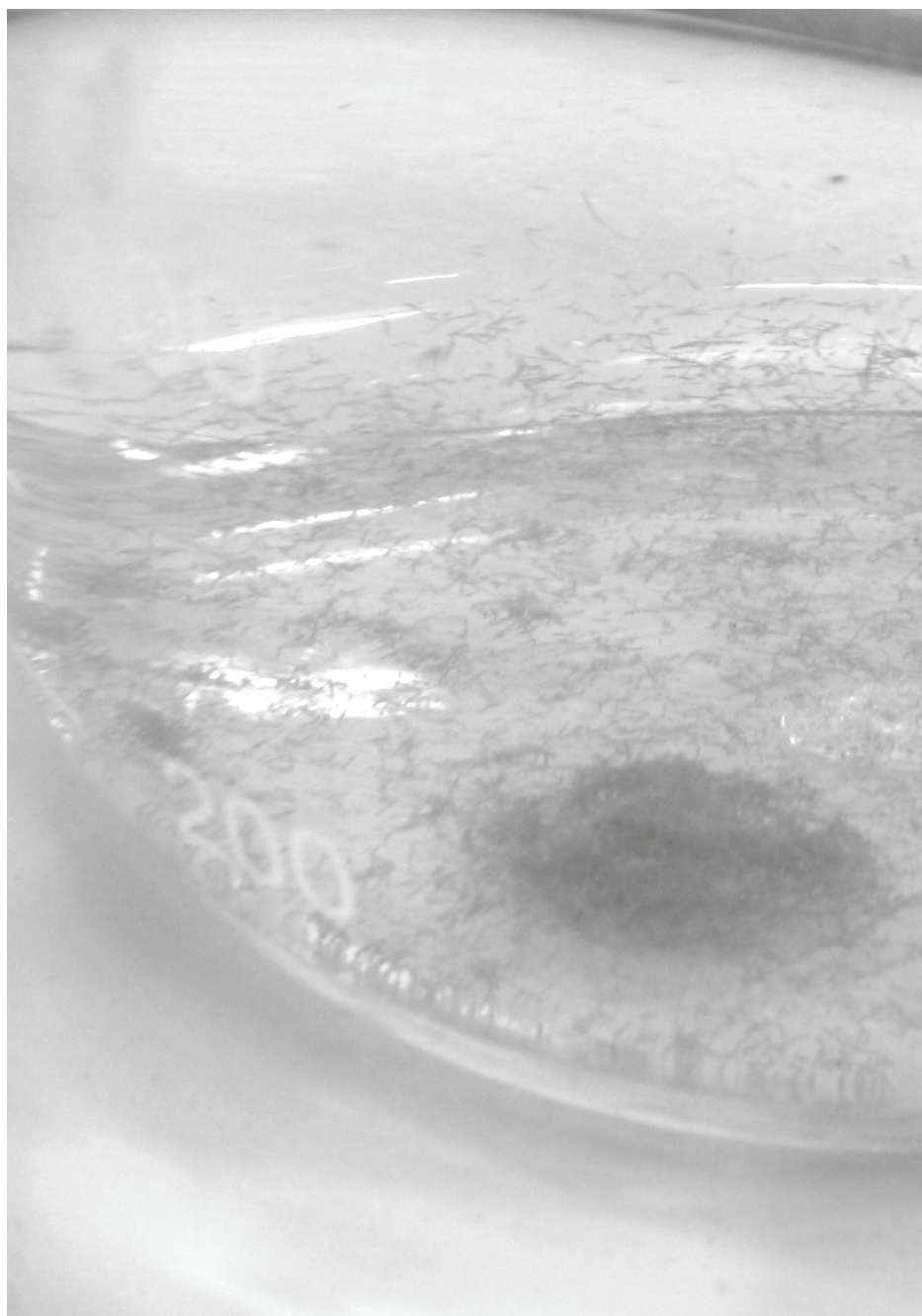
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Chapter 5

Worms eat sludge: mass balances and further processing of worm faeces

Abstract

Reduction of the amount of waste sludge from waste water treatment plants (WWTPs) can be achieved with the aquatic worm *Lumbriculus variegatus* in our reactor concept. In addition to a reduction of waste sludge, further processing of produced worm faeces and released nutrients should also be considered. This chapter gives the mass balances for sludge consumed by *L. variegatus*, showing the fate of the consumed organic material, nutrients (nitrogen and phosphorus) and metals associated with the sludge. A distinction is made between conversion into worm biomass, release as dissolved metabolites and remaining in the worm faeces. The results showed that 39 % of the nitrogen and 12 % of the phosphorus in the sludge digested by the worms are used in the formation of new worm biomass. Release of phosphates by the worms is expected to result in an additional internal phosphorus load on the WWTP of 10 %, for released ammonia this was estimated at 5 %. Heavy metals are not released and can be removed with the worm faeces. For further processing of the worm faeces, experiments showed that settling leads to a factor 2.5 higher solids concentration, compared to settling of waste sludge. This could lead to a 67 % reduction of the volumetric load on thickening equipment. Should worm faeces be used for methane production through anaerobic digestion, then the methane production will be 40 % lower, caused by a VSS reduction by the worms and a lower methane yield on worm faeces. The worm reactor is expected to be most interesting for smaller WWTPs where a decrease on the volumetric load on sludge handling operations will have most impact.

Keywords: worm reactor, *Lumbriculus variegatus*, biological sludge, mass balance, dewatering, methanisation

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An adapted version of this chapter has been submitted to Environmental Science and Technology

5. Mass balances and faeces processing

5.1 Introduction

Municipal waste water treatment is mainly performed by the activated sludge process in which up to 50 % of the organic material in the waste water is converted into biological sludge. As a result, waste water treatment plants (WWTPs) produce enormous amounts of excess sludge, which requires further processing. The associated high costs have led to an increased interest in sludge reduction techniques (Elissen *et al.*, 2006). A biological approach for reducing the amount of waste sludge is the use of the aquatic worm *Lumbriculus variegatus* (Buys *et al.*, 2008 and Elissen *et al.*, 2006). A new reactor concept was recently introduced (Figure 5.1) in which these aquatic worms are immobilised in a carrier material, which also acts as a separator for waste sludge and worm faeces. Initial batch experiments were very promising with up to 36-75 % total suspended solids (TSS) reduction, growth of new worm biomass and collection of worm faeces with a higher settleability than the waste sludge (Elissen *et al.*, 2006 and Hendrickx *et al.*, 2009).

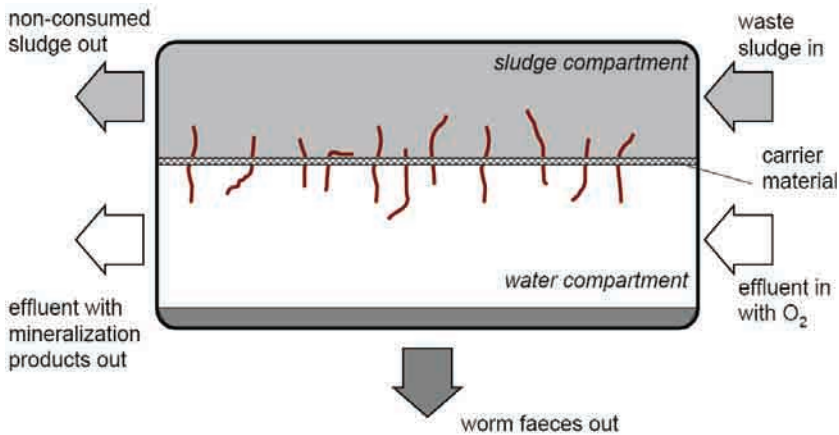


Figure 5.1 Reactor concept for sludge reduction using the aquatic worm *L. variegatus*.

Important for the application of worms is not only the actual TSS reduction that can be achieved, but also the implications on the required further processing steps, including:

- Treatment of released worm metabolites (organic material and nutrients), resulting in an additional (internal) load on the WWTP,
- Processing of worm faeces instead of waste sludge, which affects solids processing as well as the composition of the reject water from solids dewatering,
- Production of excess worm biomass.

Most worm reactors described in literature (e.g. Huang *et al.*, 2007 and Guo *et al.*, 2007) have both sludge and worms in one aerated reactor volume. Sludge is passed over the worm reactor at a certain flow rate, resulting in the return of a mixture of non-consumed sludge, worm faeces and released metabolites to the WWTP. Metabolites released by the worms can therefore be converted already in the worm reactor (Wei *et al.*, 2009), e.g. the conversion of released ammonia into nitrate. Compared to such systems, our reactor concept (see Figure 5.1) allows for a separate collection and characterisation of metabolites, worm faeces and worms.

Separate processing of the worm faeces could have advantages. They have a better settleability compared to waste sludge and can, therefore, be collected at a high TSS concentration (Elissen *et al.*, 2006). This can be expected to lead to lower solids processing costs compared to waste sludge. When considering the entire sludge processing chain, biogas production by anaerobic digestion of waste sludge should also be considered. Our worm reactor is probably most suitable for smaller WWTPs, as these have relatively high sludge processing and transportation costs. For anaerobic digestion of the waste sludge, it is often transported to a digester at a larger WWTP. Compared to waste sludge, worm faeces have a lower organic fraction, which will have an effect on digester performance.

Release of metabolites has been looked at in most research on sludge reduction with aquatic worms. Release of nitrogen compounds by the worms will increase the internal nitrogen load on the WWTP. By nitrification and denitrification processes this could be converted to nitrogen gas, though this may require an external carbon source. Phosphorus, however, is only removed from the WWTP with the excess sludge. Consequently, release of phosphorus compounds from the sludge by the worms would eventually lead to an undesired decrease in overall phosphorus removal efficiency. Also important are the heavy metals that are associated with sludge, which are mainly removed from a WWTP with the excess sludge (Stephenson and Lester, 1987). Should these be released from the sludge by the worms, this would lead to an (undesired) increased metal concentration in the WWTP effluent, which is discharged into the environment.

Finally, excess worm biomass is produced. In contrast to other reports on worm reactors, our intention is to harvest the excess biomass for further use. This worm biomass will contain part of the nitrogen and phosphorus compounds from the sludge, but may also contain some of the heavy metals that are associated with the sludge.

This chapter describes the results of mass balance experiments that were performed to quantify the excretion of metabolites (nitrogen- and phosphorus compounds and soluble organic material) by *L. variegatus* consuming waste sludge in the reactor concept shown in Figure 5.1. The effect of the worms on the heavy metals present in the sludge was also

5. Mass balances and faeces processing

included in the mass balances. For further processing of the solids, comparisons were made between waste sludge and the worm faeces with respect to settleability (sludge volume index), dewaterability (specific resistance to filtration) and dewatering by centrifugation. To assess the effects of worm faeces on the complete sludge processing chain, also the methanisation potential of worm faeces was compared to that of waste sludge. The implications of a worm reactor and its output streams at a full scale WWTP will be discussed.

5.2 Materials and Methods

5.2.1 General

Sludge and effluent from the Leeuwarden municipal WWTP were used, which applies both chemical (iron salts) and biologically enhanced phosphorus removal. For the experiments, sludge was first sieved, removing particles larger than 1 mm. Effluent was filtered over black ribbon filters (12-25 μ m, Schleicher and Schuell), before being used in the experiments.

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5.2.2 Experimental

The mass balances were established in sequencing batch experiments as described in Hendrickx *et al.* (2009). Each experiment consisted of 4 consecutive batches of 23 hours each. Thus, results were obtained for a total of 92 hours. Dissolved oxygen (DO) concentration was 8.6-9.7 mg O₂/L, pH 8.4-8.7 and temperature was 18-20°C. Sludge was supplied in excess to the worms and \pm 2.2 g wet weight (ww) of worms was used in each experiment. Worms were counted and their wet weight was determined using a perforated piece of aluminium foil. By gently pressing paper towelling against the back of the foil, adhering water was removed from the worms. Dry weight (dw) was determined by drying the worms overnight at 105°C. The average dw over ww ratio was 0.15. A 300 μ m polyamide mesh (SEFAR) was used as a carrier material. Effluent, sludge and supernatant of the sludge were analysed for TSS, VSS, COD, total N, total P, ammonia, nitrate and phosphate. The same analyses were performed at the end of each batch experiment for remaining sludge, worm faeces and their supernatants. Blank sequencing batch experiments were performed in parallel under the same conditions and using the same sludge, but without worms. These served to determine the release of organic material and nutrients when no worms were present.

Larger amounts of worm faeces were collected in a larger batch worm reactor. Fresh sludge was regularly fed to this reactor and worm faeces were collected manually every 2 or 3 days.

5.2.3 Analyses

Chemical Oxygen Demand (COD), total nitrogen (total N), total phosphorus (total P), and ammonia ($\text{NH}_4 + \text{NH}_3$) were determined according to Standard Methods (APHA, 1998) using Dr Lange® test kits. Nitrate (NO_3) and phosphate (PO_4) were determined according to Standard Methods (APHA, 1998) using ion chromatography (Metrohm 761 Compact IC). Total, fixed and volatile suspended solids (TSS, FSS and VSS) concentrations were determined according to Standard Methods (APHA, 1998) using black ribbon filters (12-25 μm , Schleicher and Schuell).

Metals were extracted by adding 10 mL of 70 % HNO_3 to samples of sludge, faeces and worms (containing approximately 0.5 g of organic material). A blank sample, containing milliQ water and 10 mL 70 % HNO_3 was also processed. The samples were digested in microwave-assisted destruction step during 15 minutes at 180°C. After the digested samples had cooled down, they were collected in 100 mL flasks which were filled up to the 100 mL mark with milliQ water. These samples were analysed by ICP (Perkin Elmer 5300 DV) for As, Cd, Cr, Cu, Ni, Pb, Zn and Fe. TSS and VSS of the sludge and faeces used in these experiments were determined, as was the dw to ww ratio of the worms.

Sludge volume index (SVI) was measured according to Standard Methods (APHA, 1998) using a 1000, 500 or 250 mL graduated glass cylinder. Experiments with the same sludge and the different graduated cylinders showed good reproducibility (relative standard deviation < 6 %).

Time-to-filtrate (TTF) experiments were performed according to Standard Methods (APHA, 1998) using 50 mL of sample and Whatman grade 2 filter paper ($d = 47$ mm). Filtrate was collected in a 100 mL graduated cylinder at an absolute pressure of 50 kPa. The collected filtrate volume (V) was recorded as a function of time (t) to allow calculation of the specific resistance to filtration (SRF) using Equation 5.1 (Christensen and Dick, 1985). The SRF showed good reproducibility amongst measurements on the same sludge (relative standard deviation < 5%).

$$\text{SRF} = 2 \cdot p \cdot b \cdot A^2 / (\mu \cdot c) \quad \text{Equation 5.1}$$

SRF	specific resistance to filtration	m/kg
p	applied pressure	Pa
b	slope from the graph of t/V versus V	s/m^6
A	filtration surface area	m^2
μ	viscosity of filtrate	Pa·s
c	mass of deposited solids per volume of collected filtrate	kg/m^3

5. Mass balances and faeces processing

Centrifugation tests were performed with 50 mL of sludge or worm faeces in glass centrifuge tubes during 10 minutes at 4500 rpm. Overlying water was decanted from the tube after centrifugation, leaving the wet pellet behind. The dewatered sludge concentration was calculated from the initial sludge concentration and the weight of the pellet: dewatered TSS (g/kg) = start TSS (g/kg) · sample weight (g) / pellet weight (g).

Methanisation potential experiments were performed at 35°C using seed sludge from the full scale anaerobic digester at the Leeuwarden WWTP. Glass bottles with a total volume of 525 mL were used. Each bottle was equipped with an OxiTop[®] pressure head and a gas sampling point sealed with a butyl rubber stopper. Each bottle was filled with ± 30 mL seed sludge (with a COD of 30 g/L). Substrate (sludge, worm faeces or worms) was added so that the COD ratio of seed sludge and substrate was larger than 2. The liquid and the headspace were flushed with nitrogen gas before the bottles were sealed. The bottles were then placed in a climate room (35°C) on a shaking plate (150 rpm). After 30-60 minutes the bottles had adjusted to 35°C after which the pressure measurement was started. The measurements were stopped when gas production reached a plateau, i.e. no additional gas production was measured compared with the blank measurement (which contained seed sludge, but no substrate). With intervals of one week, gas composition was measured using a Shimadzu GC-2010 Gas Chromatograph containing GS-Q (CO₂) and HP molsieve (O₂, N₂ and CH₄) columns. TSS, VSS and COD of the seed sludge, substrate and final sludge were determined.

5

5.3 Results and Discussion

5.3.1 Mass balance

Figure 5.2 summarises the mass balances that were determined for sludge that was consumed by the worms. TSS and VSS reduction in these experiments were 21 and 26 % respectively, which was lower than the 35-75 % TSS reduction in previous experiments (Elissen *et al.*, 2006 and Hendrickx *et al.*, 2009). Considering the solids, i.e. sludge to faeces, total COD reduction was 42 % and total N reduction was similar with 39 %. As worm mainly digest the organic fraction, the VSS reduction (and using the theoretical value of 1.42 g COD/g VSS) should result in a COD reduction of 37 %, which is close to the observed value. The total N reduction was significantly higher than VSS reduction, indicating that the worm specifically target nitrogen compounds in the sludge.

In the water compartment of parallel performed blank experiments (under the same conditions, but without worms), no significant increases in dissolved COD and ammonia were found. For phosphorus however, a clear increase in soluble phosphate concentration in the water compartment was measured. This could be explained by the release of phosphates from the sludge under the anaerobic conditions in the sludge compartment, followed by the

diffusion through the carrier material into the water compartment. The WWTP from where the sludge was obtained applied a combination of biologically enhanced phosphorus removal and chemical phosphorus removal using iron salts. Both could release phosphates again under anaerobic conditions.

As in the blank experiments, no partially degraded organic material (COD) was measured in the water compartment of the worm experiments. It was therefore assumed that the measured 34 % COD reduction was the result of complete mineralization to CO₂. The mineralization products from the sludge digested by the worms, were mainly found as ammonia and phosphate in the water compartment. These were released at 12.2 g NH₄-N/kg TSS consumed and 5.4 g PO₄-P/kg TSS consumed (58.0 g NH₄-N and 25.8 g PO₄-P per kg TSS digested). This ammonia release is higher than what was found in other, less elaborate, experiments (Hendrickx *et al.*, 2009). The effluent from the water compartment with these released nutrients will require treatment. As estimated earlier (Hendrickx *et al.*, 2009), treatment of the released ammonia would increase the nitrogen load on a WWTP with less than 5 %. The released phosphates are estimated to represent an additional phosphorus load of approximately 10 %.

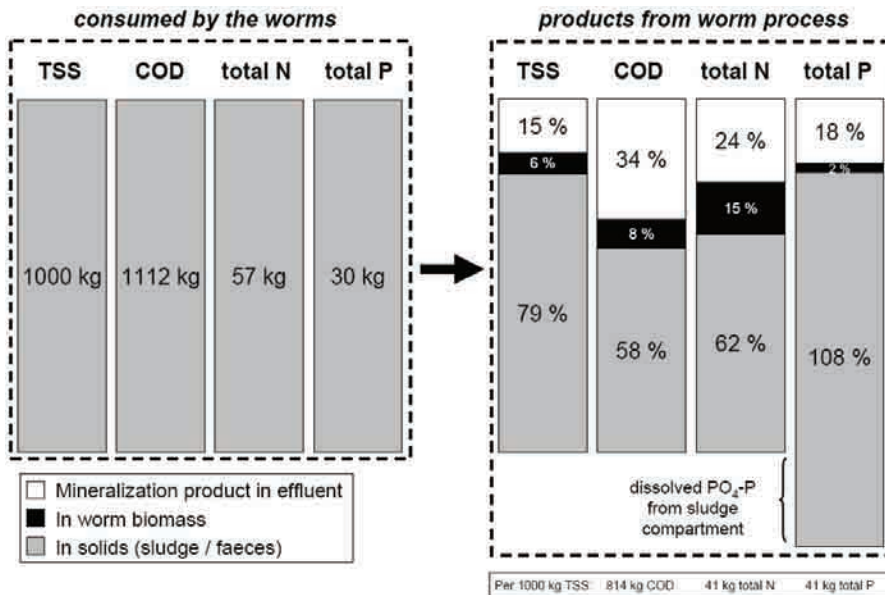


Figure 5.2 Mass balances for COD, total N and total P over the sludge consumed by worms in sequencing batch experiments. Sludge from the Leeuwarden WWTP was used in these experiments. Mineralization products were mainly CO₂, NH₄ and PO₄.

5. Mass balances and faeces processing

Interestingly, the phosphorus content in the solids increased from 30 g total P/kg TSS in sludge to 41 g total P/kg TSS in worm faeces. This was also observed in experiments using a different sludge (results not shown). This could only partially be explained by the fact that worm biomass contains relatively more total N than total P (~ 130 mg N/g dw and ~ 12 mg P/g dw) when compared to sludge. In comparison to nitrogen, sludge contains an excess amount of phosphorus for the worms. Residual phosphorus compounds in the consumed sludge would remain in the worm faeces or be excreted as soluble compounds into the effluent. However, the results clearly showed a transfer of phosphates from the sludge compartment to the water compartment (as was observed in the blank experiments), followed by attachment to the faeces. This attachment could be caused by the reverse of the processes that released the phosphates from the sludge in the sludge compartment, as the water compartment was aerobic. This was not further investigated. However, this made it impossible to distinguish between phosphate originating from the worm metabolism and transfer from the sludge compartment.

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A higher phosphorus content of the worm faeces means that a larger P load can be removed from the WWTP with these solids. However, this was for batch experiments where sludge was available in excess to the worms, i.e. there was transfer of phosphorus from non-consumed sludge to worm faeces. In a continuous reactor this could be completely different, due to less excess sludge and a shorter residence time in the sludge compartment. Additionally, the high phosphorus content in the worm faeces potentially make the (incineration ashes from) worm faeces a more interesting resource for phosphorus recovery (Adam *et al.*, 2009).

Worm biomass production

The average worm biomass yield on the sludge was 0.28 g dw/g TSS digested. The newly formed worm biomass contained 8, 15 and 2 % of the consumed COD, total N and total P respectively. When considering only the sludge digested by the worms (21 % of the consumed sludge) 39 % of the total N was used for the formation of new worm biomass, whilst for total P this was only 12 %.

Metals

Sludge, worm faeces and worms were analysed for metals. The results for Fe, Cu and Zn are shown in Table 5.1, concentrations of the other metals were below detection limits. The metals content of the faeces was clearly higher than that of the sludge (when related to TSS), but roughly the same when expressed per FSS, the inorganic fraction of sludge which passes undigested through the gut of the worm. Heavy metals associated with the sludge are entrapped in the sludge matrix or bound to bacterial extracellular polymeric substances

(EPS) (Stephenson and Lester, 1987; Olivier, 1974). Since the worms digest mainly the organic material in the sludge, this could lead to changes in the sludge matrix. Consequently, it could be expected that metals are released by the worms. From the results it is clear that this did not occur. A worm reactor would thus not result in a change in the metal load that is removed with the solids from the WWTP. Should the worm reactor be operated at lower DO concentrations where the worms digest a larger part of the organic material (Hendrickx *et al.*, 2009), it is possible that a fraction of the metals will be released from the sludge. However, this was not investigated further. Table 5.1 also shows that the metal concentrations in the worms are much lower than in the sludge. It was already shown that the worms do not specifically bioaccumulate the metals from the sludge (Chapter 8). The amount of metals in newly formed worm biomass in the current experiments (assuming the same metals concentrations in existing and new worm biomass) represent less than 0.8 % of the amount of metals in the sludge consumed by the worms.

Table 5.1 Metal concentrations in the waste sludge and the worm faeces. The values for worm biomass are in mg metal/kg dw.

		Waste sludge	Worm faeces	Worm biomass	Metal recovery
Fe	g/kg TSS	24.0	30.7	0.45	97 %
	g/kg FSS	88.6	85.8		
Cu	g/kg TSS	0.34	0.43	0.024	96 %
	g/kg FSS	1.269	1.20		
Zn	g/kg TSS	0.70	0.98	0.11	105 %
	g/kg FSS	0.26	0.27		

5.3.2 Settling and dewatering of sludge and worm faeces

The average sludge volume indices (SVI) for waste sludge and worm faeces were 160 ± 34 and 65 ± 9 mL/g respectively. Settling of the worm faeces resulted in a TSS concentration of 15.4 g/kg, much higher than what could be achieved by settling the waste sludge (6.3 g/kg). The much lower SVI for the worm faeces is similar to the results found earlier (Elissen *et al.*, 2006).

Using the pre-settled solids, the effect on further dewatering was evaluated using the specific resistance to filtration (SRF) and centrifugation. The SRF of $(2.5 \pm 0.7) \cdot 10^{12}$ m/kg for waste sludge was about 30 % lower than the SRF for worm faeces, which was $(3.3 \pm 0.8) \cdot 10^{12}$ m/kg. A possible explanation for this could be the higher sensitivity of the worm faeces to shear (results not shown), resulting in smaller particles that block the pores of the used filter material. Whether this can be prevented by the addition of flocculants (as

5. Mass balances and faeces processing

generally occurs in dewatering of sludge) or the use of different filter materials was not tested. Centrifugation of the worm faeces resulted in a solids concentration of 69 g TSS/kg, somewhat higher than for waste sludge (63 g TSS/kg). This could be due to the more compact structure of the worm faeces, which made it easier to remove the bound water that is trapped within the floc structure of sludge (Jin *et al.*, 2004)

The dewaterability tests also showed (Figure 5.3) that to reach a TSS concentration of 3 % (or 30 g TSS/kg) (common at WWTPs), on average 24 s of vacuum filtration (at 50 kPa) was required for the worm faeces, compared to 39 s for the waste sludge. This implies that less time (and therefore less energy) is required to reach the same TSS or that the same time can be used to achieve a higher TSS.

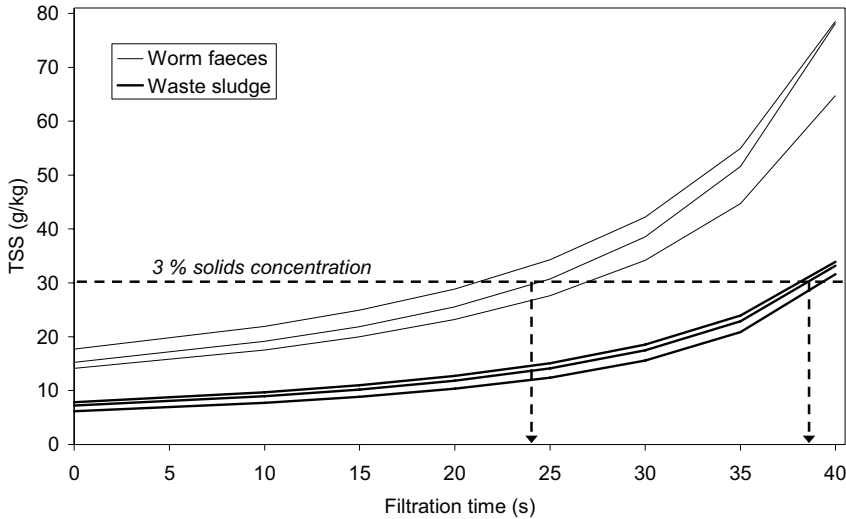


Figure 5.3 Typical curves obtained in the filtration tests with worm faeces and waste sludge. A vacuum pressure of 50 kPa was applied and Whatman grade 2 filter paper was used as filter material. The broken lines indicate the filtration times needed to reach a solids concentration of 3 %.

The combined effect of TSS reduction and collection of the worm faeces at a much higher concentration than the waste sludge, leads to a huge reduction of the volumetric load on the sludge handling equipment. Using the measured TSS reduction of 21 % and the solids

concentration results from the SVI measurements, a 67 % reduction in the volume of waste biosolids (in that case worm faeces) can be achieved. However, the specific resistance to further dewatering was 30 % higher for the worm faeces, using a filter paper. Using different filter materials could give different results, though this was not tested. As mentioned in the introduction, using a worm reactor for waste sludge reduction would be most interesting for smaller WWTPs. As the sludge processing costs for these smaller WWTPs are largely related to transportation, an initial reduction in volume is of most interest, which can be achieved with a worm reactor.

5.3.3 Reject water from settling and dewatering

Figure 5.4 shows the effect of dewatering methods (centrifugation and vacuum filtration) on the release of dissolved COD and nutrients into the reject water. The results are presented as a fraction of the COD and of the nutrients concentrations measured in the supernatant of settled sludge (SVI test). For the waste sludge and worm faeces only small differences were observed for COD and ammonia. For phosphate however, a large increase was observed for waste sludge, whereas there was no further increase from dewatering of worm faeces. However, due to partial mineralization of the sludge by the worms, as discussed earlier, the supernatant after settling the worm faeces already showed a higher concentration of 18.8 mg PO₄-P/L compared to the supernatant of the waste sludge (3.9 mg PO₄-P/L). Dewatering will, however, not result in a further release of phosphorus from the worm faeces, thereby not increasing the load on the WWTP when returning the reject water. Instead, phosphorus is removed with the solids.

5. Mass balances and faeces processing

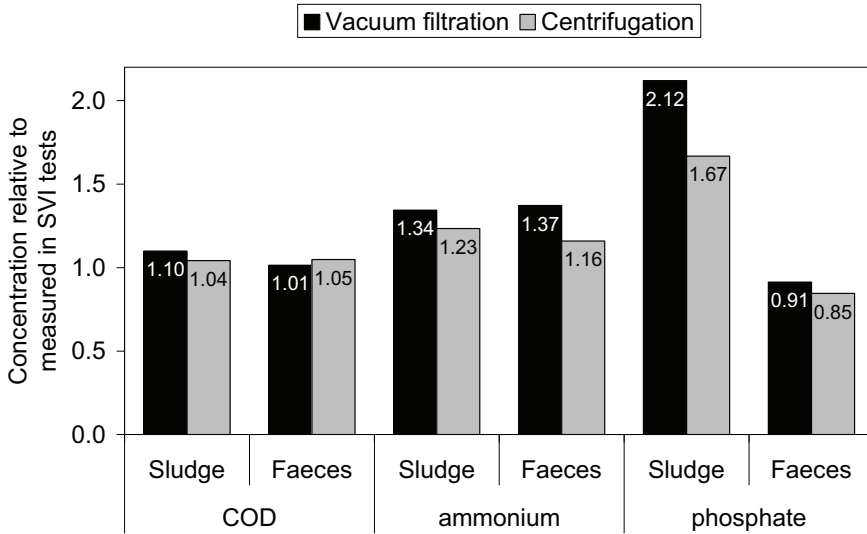


Figure 5.4 Effect of dewatering / thickening method on COD and nutrient “release” from Leeuwarden WWTP sludge and faeces. Bars represent the concentration measured relative to that measured in the supernatant from the SVI tests. A value of 1 means the concentration in the reject water is equal to that in the supernatant after settling.

5.3.4 Anaerobic digestion of sludge and worm faeces (and worms)

The results of the anaerobic digestion experiments with sludge, worm faeces and worms are summarised in Figure 5.5. It is clear that worm faeces have a lower potential for biogas formation than waste sludge, although the combination of a worm reactor and anaerobic digestion of worm faeces would result in the largest overall TSS reduction (~ 50 %). Anaerobic digestion followed by a worm reactor resulted in an overall TSS reduction of ~ 42 %, although it should be noted that the digested sludge had to be washed to remove the large amounts of ammonia, which would otherwise be toxic to the worms (Hendrickx *et al.*, 2009). Nonetheless, the worms could digest the anaerobically digested sludge (9 % of TSS, 21 % of VSS) and grow on it with a yield of 0.38 g dw/g TSS digested, which is significantly higher than the yield on aerobic sludge. This indicates that fractions of the sludge that are digested by the worms had become more readily available, which should be investigated further. Anaerobic digestion of the worm biomass itself was tested as well, resulting in a high biogas yield of 0.72 g CH₄-COD/g worm-COD added. Disintegration of the worm biomass proceeded fast in the anaerobic sludge, most likely due to a combination

of a high temperature (35°C) and an ammonia concentration of ~150 mg N/L, which is toxic to the worms.

Introducing a worm reactor would reduce the potential for methane formation from the sludge with about 40 %, caused by the 26 % VSS reduction achieved by the worms and a lower methane yield on worm faeces. This shows that worms use a part of the sludge, that otherwise would have been available for methane formation. Though it is unlikely that both a worm reactor and an anaerobic digester would be situated at the same WWTP, it can be expected that the waste sludge from smaller WWTPs is transported to the anaerobic digester at a central WWTP. As mentioned before, a worm reactor would be most interesting for smaller WWTPs, which have relatively high sludge handling costs. Despite a reduction in the potential for biogas production, transport costs could be reduced considerably, which could be of greater importance for smaller WWTPs. Methanisation of worm biomass (~95 % organic material) proceeded fast and resulted in a high biogas yield. For anaerobic digestion this means that should worms end up in the faeces and thus in the digester, these will not have a negative impact on the anaerobic process. Furthermore, if no high value application for the excess worms can be found, they can easily be converted to biogas.

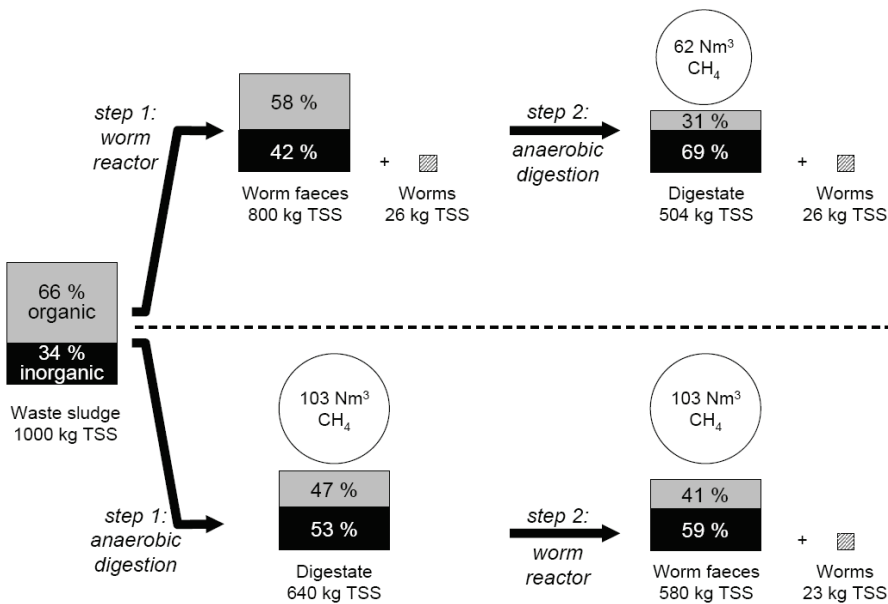


Figure 5.5 Mass balances for two alternatives of combining sludge consumption by worms and biogas production through anaerobic digestion.

5. Mass balances and faeces processing

5.4 Conclusions

Mass balances over the sludge consumed by worms showed:

- A nutrient release of 58.0 mg $\text{NH}_4\text{-N/g}$ TSS digested and 25.8 mg $\text{PO}_4\text{-P/g}$ TSS digested. The required treatment of these nutrients was estimated to result in an additional internal load on the WWTP of 5 and 10 % for nitrogen and phosphorus, respectively.
- The nitrogen in the sludge digested by the worms, was efficiently (39 %) used in the formation of new worm biomass. For phosphorus this was only 12 %.
- Heavy metals in the sludge mostly remained in the worm faeces. Less than 0.8 % of the heavy metal load on the worm reactor is incorporated into new worm biomass.

Dewatering experiments with waste sludge and worm faeces showed that the latter can be collected at much higher TSS concentrations. The combination of TSS reduction and a higher faeces solids concentration can lead to a 67 % decrease in the volumetric load on sludge handling equipment.

5

Methanisation of worm faeces resulted in a 40 % lower methane production when compared to waste sludge, caused by the 26 % VSS reduction by the worms and a lower methane yield. This shows that the worms digest a part of the sludge that otherwise could have been converted to methane. Methanisation of worm biomass is fast and 72 % of the worm can be converted into methane (COD based).

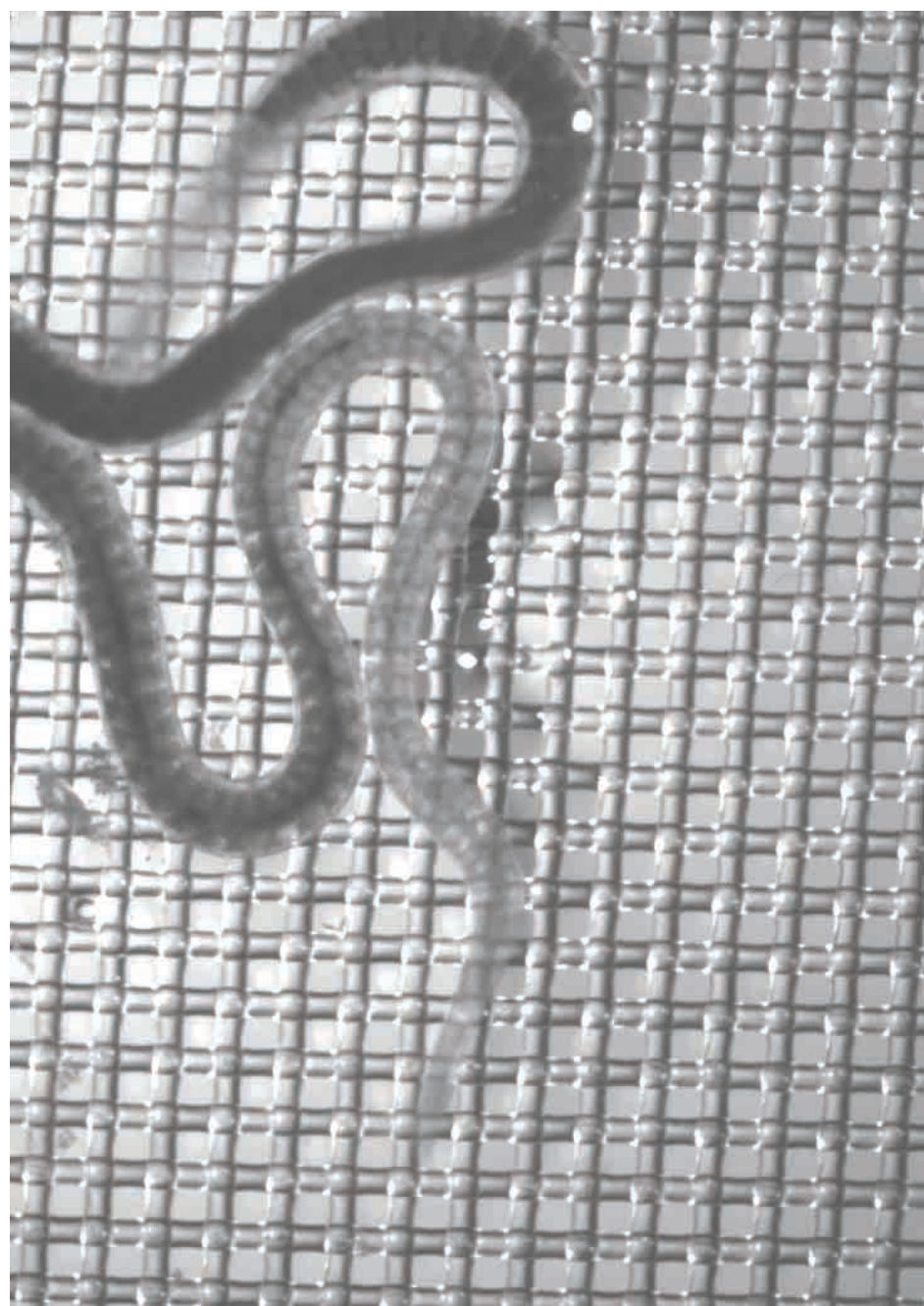
Based on the results presented in this chapter, a worm reactor shows most potential for smaller WWTPs. Here, decreasing the volumetric load on sludge handling and transport operations will have most impact, even at a relatively low TSS reduction (21 %) by the worms.

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Chapter 6

Design parameters for sludge reduction in a worm reactor

Abstract

Reduction and compaction of the amount biological waste sludge from waste water treatment plants (WWTPs) can be achieved with the aquatic worm *Lumbriculus variegatus*. In our reactor concept for a worm reactor, the worms are immobilised in a carrier material. The size of a worm reactor will therefore mainly be determined by the sludge consumption rate per unit of surface area. This design parameter was determined in sequencing batch experiments using sludge from a municipal WWTP. A maximum consumption rate of 70-90 g TSS/(m²·d) was observed, however, this required an unsustainable worm density of at least 2 kg ww/m². Long term experiments with 300 and 350 µm mesh sizes showed stable worm densities of 0.87 and 1.1 kg ww/m², respectively. At these worm densities, the surface specific consumption rates were 45 and 58 g TSS/(m²·d) for the 300 and 350 µm mesh sizes, respectively. Using a 350 µm mesh will therefore result in a 29% smaller reactor compared to using a 300 µm mesh. To maintain maximum sludge consumption rate, a minimum sludge load on the worms of 100 mg TSS/(g ww·d) was determined. Large differences can be expected between different sludge types, although it was not clear what caused these differences. Worm biomass growth and decay rate were determined in sequencing batch experiments. The decay rate of 0.023 d⁻¹ for worms in a carrier material was considerably higher than the decay rate of 0.018 d⁻¹ for free worms. As a result, the net worm biomass growth rate for free worms of 0.026 d⁻¹ was much higher than the 0.009-0.011 d⁻¹ for immobilised worms. Finally, the specific oxygen uptake rate of the worms was determined at 4.9 mg O₂/(g ww·d), which needs to be supplied to the worms by aeration of the water compartment in the worm reactor.

Keywords: Sludge reduction, *Lumbriculus variegatus*, worm density, sludge load, oxygen uptake rate, sludge type

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6. Design parameters

6.1 Introduction

The aquatic worm *Lumbriculus variegatus* can be used to reduce the amount of biological excess sludge produced by waste water treatment plants (WWTP) and compact it into worm faeces (Elissen *et al.*, 2006). In addition to the promising results achieved with batch experiments, continuous operation of a worm reactor was also shown to be feasible (Chapter 3). The reactor concept shown in Figure 6.1 was used for this purpose, where the worms are immobilised in a carrier material. The most important design parameter for such a worm reactor is therefore the sludge consumption rate per unit of surface area. This surface specific rate is determined by the (stable) worm density that can be achieved and the specific sludge consumption rate, of which the latter is probably dependent on the sludge load that is applied. Net growth of worms is an important prerequisite for a stable operation of the worm reactor. Previous experiments indicated that growth of worms was limited by the mesh size of the carrier material (Chapter 3). It has been suggested that the natural increase in worm number (by spontaneous division) is related to their individual weight (Leppänen and Kukkonen, 1998). Placing the worms in a mesh might physically restrict the size and weight to which a worm can grow and can, therefore, be expected to also determine the stable worm biomass density in the carrier material. Furthermore, the type of sludge probably has an impact on sludge consumption and digestion rates, as was already observed in Chapter 3. Consequently, different types of sludge may lead to differences in the surface area that is required. And finally, the worms use oxygen which needs to be supplied to the worm reactor. To calculate the required aeration capacity the specific oxygen uptake rate (SOUR) by the worms needs to be determined.

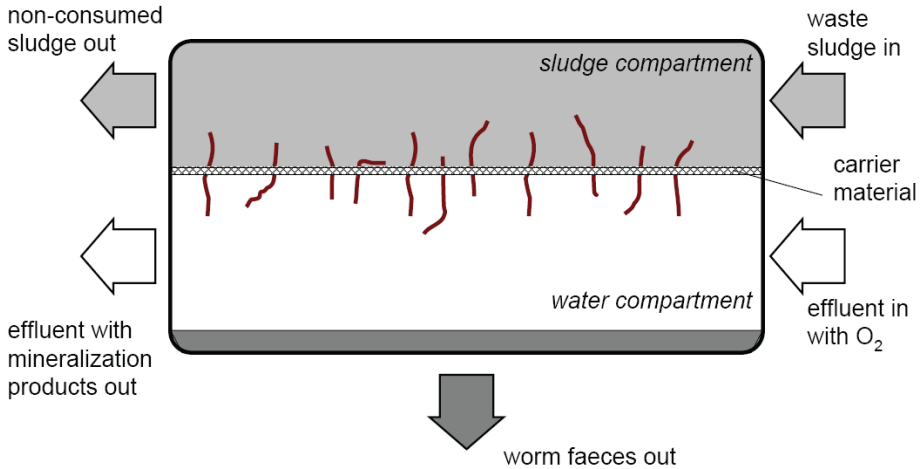


Figure 6.1 Scheme of reactor concept with worms immobilized in a carrier material.

This chapter describes the results of the sequencing batch experiments that were performed with sludge from the Leeuwarden municipal WWTP to determine the abovementioned design parameters for a worm reactor. In these experiments the mesh size of the carrier material, the worm biomass density and the sludge load on the worms were varied. In addition, 5 other types of sludge were assessed for their surface specific sludge consumption rate. Finally the SOUR of the worms was determined. The implications of these parameters on the design of a worm reactor will be discussed.

6.2 Materials and Methods

Sludges from six different WWTPs were used for the experiments: two municipal WWTPs in the Netherlands (Leeuwarden and Bennekom) and four lab-scale systems: a membrane bioreactor (MBR) system operated at a sludge retention time (SRT) of 50 days (Remy *et al.*, 2009) and three conventional activated sludge (CAS) systems operated at SRTs of 5, 15 and 75 days. Table 6.1 shows their characteristics. Effluents were filtered over black ribbon filters (12-25 μm , Schleicher and Schuell) before being used in the experiments. Leeuwarden and Bennekom sludges were first sieved over a 1 mm mesh.

Worms were randomly taken from a larger population grown at least two weeks on the same sludge that was used in the experiments. They were counted and their wet weight (ww) was determined by placing the worms on a piece of polyamide mesh material

6. Design parameters

(150 μm). Adhering water was removed by gently pushing paper towelling against the back of the mesh.

Table 6.1 Details of the installations from where the sludges were obtained. p.e. = COD based person equivalent.

	Lab AS	Lab MBR	Bennekom WWTP	Leeuwarden WWTP
size in p.e.	0.06	0.12	20,000	190,000
denitrification	no	yes	yes	yes
P-removal	no	no	biological	biological + chemical
SRT (d)	5,15,75	50	40	25

Chemical Oxygen Demand (COD), total nitrogen (total N), total phosphorus (total P), and total ammonia ($\text{NH}_4 + \text{NH}_3$) were determined according to Standard Methods (APHA, 1998) using Dr Lange[®] test kits. Total and volatile suspended solids (TSS and VSS) were determined according to Standard Methods (APHA, 1998) using black ribbon filters (12-25 μm , Schleicher and Schuell).

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Sequencing batch experiments were performed as described in detail in Hendrickx *et al.* (2009). Worm growth experiments were performed using the same set-up as the sequencing batch experiments. Sludge and effluent were replaced daily, except during the weekends. Worms were counted and their wet weight (ww) was determined every 2-4 days. The net worm biomass growth rate was calculated by dividing the increase in wet weight by the initial wet weight and the experimental time. Additional experiments were performed in which the worms were not immobilised in a carrier (Figure 6.2). Finally, to assess their decay rate, experiments were performed with both free and immobilised worms without feeding the worms, also using the set-up shown in Figure 6.2.

The specific oxygen uptake rate (SOUR) by the worms was determined using the OxiTop[®] system (WTW). 500 mL bottles were used with a rubber insert containing NaOH pellets (to capture produced CO_2) and an OxiTop[®] screw cap for measuring the pressure in the headspace. Six bottles were used, three containing Leeuwarden WWTP effluent (43.5 mL each) and three containing Leeuwarden WWTP sludge with a TSS of 4.0 g/kg (43.5 mL each). Of each batch of three, one was used as a blank and the other two contained 2.24-2.69 g ww of worms. Oxygen consumption was measured during 24 hours at 20°C. SOUR was also determined by using the sequencing batch set-up. In this case the water compartment was not aerated, but the DO concentration was allowed to decrease over time.

With the known volume of the water compartment and the decrease in DO concentration, the SOUR of the worms used in the experiment could be calculated.

Dissolved oxygen (DO) concentration and temperature were monitored in the water compartment using a HACH® Luminescent Dissolved Oxygen (LDO) meter. Ammonia concentrations and pH were measured at the start and end of an experiment. The pH was measured using the WTW pH/Cond 340i. Experiments were performed at a temperature of about 20 °C, DO concentration above 8.1 mg O₂/L and ammonia concentrations low enough not to inhibit sludge consumption by the worms, i.e. below 0.1 mg NH₃-N/L (Hendrickx *et al.*, 2009).

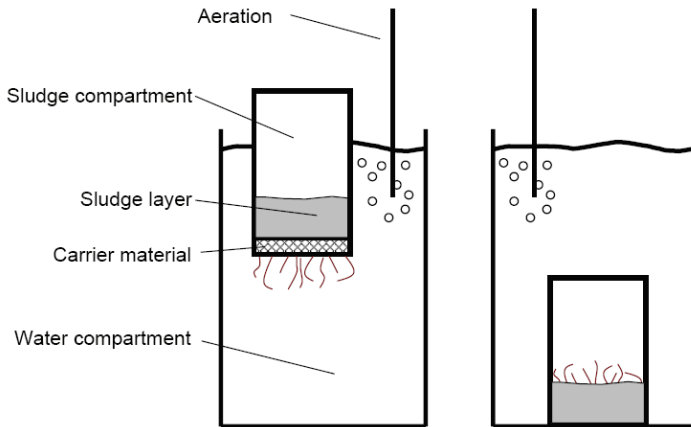


Figure 6.2 Sequencing batch set-ups used for the worm growth and decay (without sludge) experiments.

6.3 Results

6.3.1 Worm biomass growth and decay

Sequencing batch experiments were performed with worms immobilised in a carrier material and with free worms. The resulting biomass decay rates (of non-fed worms) and biomass growth rates are shown in Table 6.2. A comparison of the decay rates for worms in the carrier material and for free worms showed a faster decay for the immobilised worms. This also resulted in a lower net growth rate for the immobilised worms. The net worm biomass growth rates in the carrier material were somewhat lower than the 0.013 d⁻¹ found in a continuous worm reactor (Chapter 3). However, as shown in Figure 6.3, also the worm density had a strong negative effect on net growth rate, which was also observed for free

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worms (data not shown). Even though the error in this calculation of the growth rate was large (due to the relatively large error in the determination of small amounts of wet weight), a trend of decreasing growth rates with higher worm densities could clearly be observed. Later experiments indicated that sludge availability had not been the limiting factor in these experiments, as the growth rate did not increase when the amount of TSS in the sludge compartment was increased (data not shown). The effect of worm density was confirmed by the clear difference observed between the two experiments with free worms. In the petridish experiment the worm density was low (0.15 kg ww/m^2), resulting in a high net growth rate of 0.026 d^{-1} , while a much lower net growth rate of 0.011 d^{-1} was observed at the higher worm density in the bottle (0.90 kg ww/m^2).

Table 6.2 Worm biomass decay and growth rates. n.d. = not determined.

Carrier	decay rate, k_d (d^{-1})	net growth rate (d^{-1})	Sludge
<i>none</i> , in petridish	$0.017^{(1)}$	0.026 ± 0.005	Leeuwarden
<i>none</i> , in bottle	0.019 ± 0.001	0.011 ± 0.001	Leeuwarden
300 μm	0.023 ± 0.001	0.011 ± 0.001	Leeuwarden
350 μm	n.d.	0.009 ± 0.002	Leeuwarden
350 μm	n.d.	0.011	Lab AS

⁽¹⁾ from Elissen (2007)

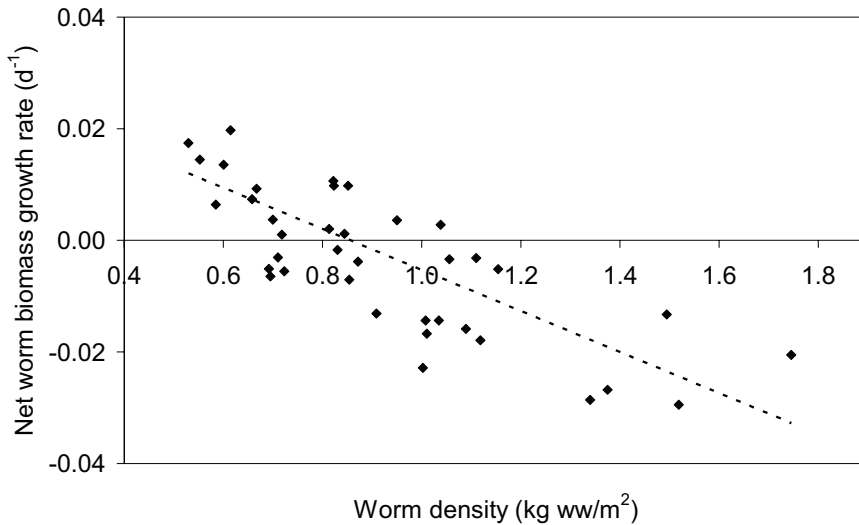


Figure 6.3 Calculated net worm biomass growth rates in a sequencing batch experiment using a 300 μm mesh carrier material and sludge from the Leeuwarden WWTP. The experiment was started with 1.7 g ww of worms. The absolute error in the net growth rate was 0.011 d^{-1} . The dotted line indicates the negative trend.

6.3.2 Mesh size of carrier material

Previous experiments with a continuous reactor demonstrated sustainable worm growth in a long-term experiment when a 350 μm mesh was used, but not when a 300 μm mesh was used (Chapter 3). Worm growth is believed to be related to individual worm weight, meaning that worms need to grow to a certain size before they spontaneously divide (Leppänen and Kukkonen, 1998). Four mesh sizes (250, 300, 350 and 400 μm) were therefore looked at in sequencing batch experiments to determine their effect on individual worm weight. Figure 6.4 shows the average individual worm weight during the experimental period of 7 weeks. The worms could grow in individual weight for the 350 and 400 μm mesh size, but decreased in weight for the 250 and 300 μm mesh sizes. The sudden drop in individual wet weight (week 4) in the 400 μm mesh coincided with a 60 % increase in the number of worms in the carrier, caused by worm division. Even though the largest individual worm size was obtained in the 400 μm mesh, a large fraction of worms continuously fell from this carrier material, as also shown in Figure 6.4. This also occurred with the 350 μm mesh, but to a much lesser extent. For the 250 and 300 μm meshes worm

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losses were negligible. After 7 weeks the average worm weight in the 350 μm mesh was nearly 50 % higher than in the 300 μm mesh.

As a compromise between achievable worm growth and worm losses from the carrier, two mesh sizes (300 and 350 μm) were selected for long-term experiments in which the stable worm density and corresponding specific sludge consumption rate were determined. Figure 6.5 shows that in both cases, a stable amount of worm biomass in the carrier material could be achieved. For 300 μm this was 0.87 kg ww/m² and for 350 μm this was about 25 % higher at 1.1 kg ww/m². The maximum sludge consumption rates determined at these worm densities were similar with 52 and 54 mg TSS/(g ww·d) for the 300 and 350 μm mesh size, respectively. As a result, for the 350 μm mesh a higher surface specific consumption rate of 58 g TSS/(m²·d) was achieved compared to 45 g TSS/(m²·d) for the 300 μm mesh.

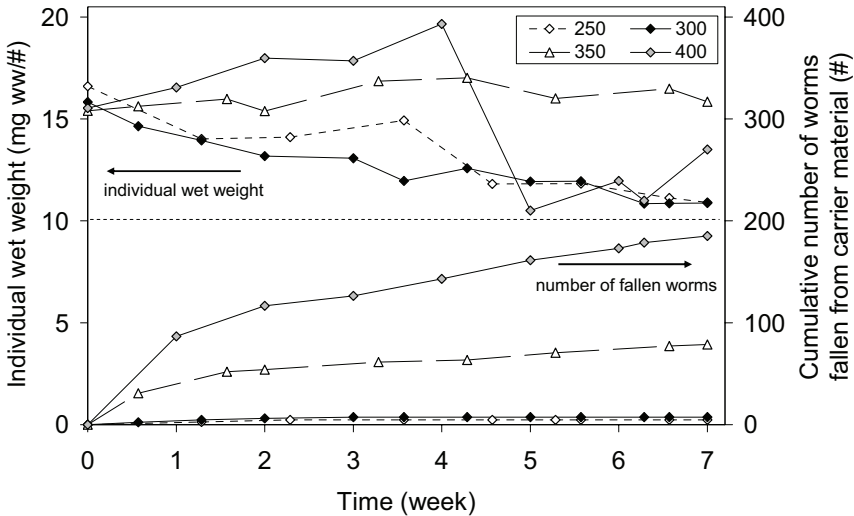


Figure 6.4 Evolution of the average individual worm weight (top) and the cumulative number of worms fallen from the carrier material (bottom) in sequencing batch experiments using different mesh sizes (250, 300, 350 and 400 μm) for the carrier material.

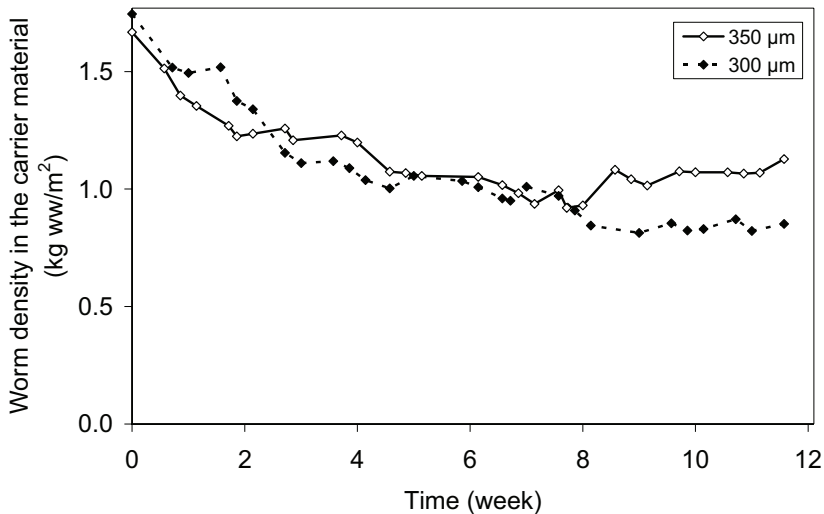


Figure 6.5 Worm density in sequencing batch experiments using 300 and 350 µm mesh carrier materials.

6.3.3 Worm density and sludge load

Two series of experiments were performed to determine the effect of worm density and sludge load on the consumption rate by the worms. In a first experiment the worm density on the carrier material was varied by starting each sequencing batch experiment with a different amount of worms. Figure 6.6 shows that with an increase in worm density, the specific sludge consumption rate decreased. A rough estimate for the maximum sludge consumption rate is 30-50 mg TSS/(g ww·d), reached at a worm density below approximately 2 kg ww/m². This maximum sludge consumption rate agrees with those found in the mesh size experiments. Above a worm density of approximately 2 kg ww/m², due to the large number of worms on a small surface area, apparently competition between the worms for the same sludge decreased the worm specific sludge consumption rate. The constant (maximum) amount of sludge consumed per experiment of 70-90 g TSS/(m²·d) at worm densities above 2 kg ww/m², supported this observation that competition between the worms had taken place.

To assess the effect of the sludge load on the worms, a second experiment was performed in which the amount of sludge was varied whilst starting at a constant worm density of 2.9 kg ww/m². Figure 6.7 shows a the maximum sludge consumption rate was achieved above a sludge load of 100 mg TSS/(g ww·d). A further increase in the sludge load did not result in

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a higher consumption rate. At a lower sludge load the consumption rate by the worms decreased, most likely caused by substrate (sludge) limitation. The maximum total amount of sludge consumed per experiment corresponded to a surface specific consumption rate of 80 mg TSS/(m²-d), which was similar to rates found previously at varying worm density (Figure 6.6).

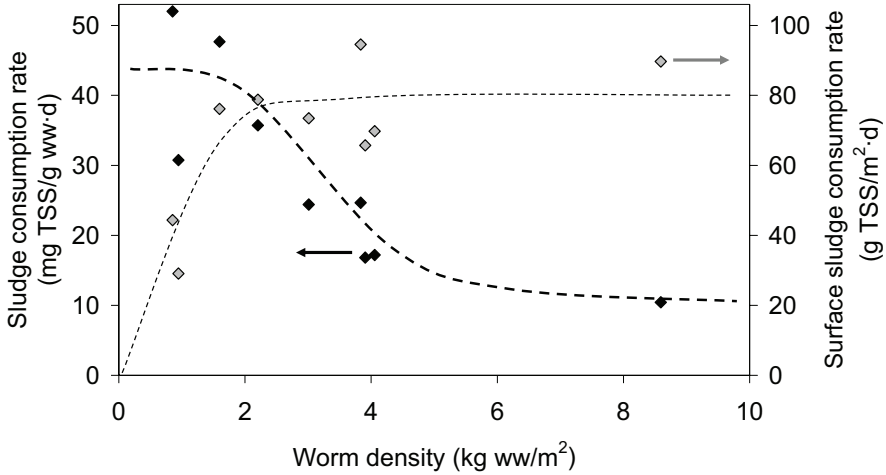


Figure 6.6 Effect of the worm density on sludge consumption rate at a constant amount of TSS (average 433 mg TSS) in the sludge compartment. Worm specific (\blacklozenge) and surface specific (\blacklozenge) sludge consumption rates are shown. Broken line represents an approximation of the experimental results.

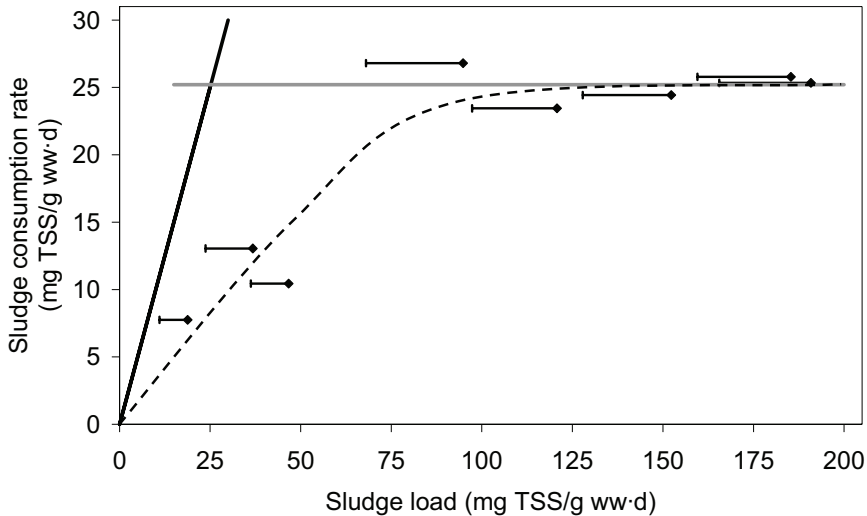


Figure 6.7 Effect of the sludge load on sludge consumption rate by the worms. Worm density was constant at (2.9 ± 0.3) kg ww/m². Left error bar indicates the decrease in sludge load due to sludge being consumed by the worms during the experiment. Solid black and grey lines represent 100 % sludge consumption and the determined maximum sludge consumption rate, respectively. The broken line represents an approximation of the experimental data.

6.3.4 Sludge type

The experiments described so far were all performed with sludge from the Leeuwarden municipal WWTP. The effect of sludge characteristics on the design parameters was assessed using the sludge types given in Table 6.1. The results for the surface consumption and surface digestion rates are shown in Figure 6.8. Despite the large differences in consumption rates between sludge from the lab-scale and full-scale WWTPs, the surface sludge digestion rates were all of the same order of magnitude, with perhaps the exception of CAS 75 (Figure 6.8). As worms digest mainly the organic material in sludge, the organic fraction (VSS/TSS-ratio) of the sludge could be a measure for the nutritious value of sludge to the worms. Also, since worm biomass contains a high fraction of protein, the nitrogen content of the sludge could be indicative for the nutritional value. However, the results in Table 6.3 do not indicate such a correlation between sludge composition and the amount of digested sludge.

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Table 6.3 TSS reduction, worm density, sludge digestion rate and sludge composition for the different sludges.

	TSS reduction %	Worm density kg ww/m ²	Sludge digestion rate mg TSS/(g ww·d)	Organic fraction VSS/TSS	N content mg N/g TSS	P content mg P/g TSS
Leeuwarden	31	2.7	7.2	0.63	60	30
Bennekom	40	2.1	12.1	0.73	87	16
MBR 50	12	1.5	14.4	0.75	65	21
CAS 5	10	1.2	15.1	0.70	46	17
CAS 15	16	1.1	22.4	0.69	62	15
CAS 75	5	0.8	8.6	0.68	51	17

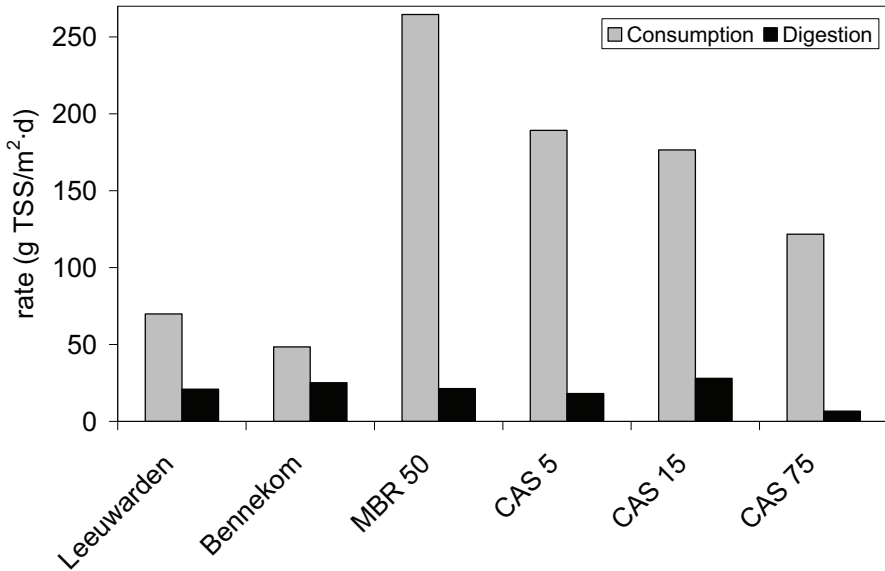


Figure 6.8 Surface specific sludge consumption and digestion rates for different sludge types determined in sequencing batch experiments.

6.3.5 Specific oxygen uptake rate

The specific oxygen uptake rate (SOUR) was determined for non-fed as well as for feeding worms, resulting in 2.68 and 4.89 mg O₂/(g ww·d) respectively (Table 6.4). Using an estimate for the amount of VSS digested by the worms in these experiments (5-10 mg VSS/g ww·d), the oxygen requirement for VSS digested by worms is only 0.5-1.0 mg O₂/mg VSS.

Table 6.4 Specific oxygen consumption rates for non-fed and for worms feeding on Leeuwarden WWTP sludge.

	specific O ₂ uptake rate mg O ₂ /(g ww·d)	method
Non-fed worms, free	2.68 ± 0.20	OxiTop®
Feeding worms, free	4.82 ± 0.26	OxiTop®
Feeding worms, in carrier	4.96 ± 0.05	sequencing batch

6.4 Discussion

6.4.1 Worm growth and decay

The results showed a considerably higher biomass decay rate of 0.023 d⁻¹ for immobilized worms compared to the decay rate of 0.018 d⁻¹ for free worms. As a result, the net worm biomass growth rate was lower for worms in a carrier material. Worm biomass growth rates were also shown to be negatively correlated with the worm density and above a certain stable worm density, net worm biomass growth rates became negative. For start-up of a large scale reactor with immobilised worms the net growth rate will gradually decrease until a stable worm density is reached. A lower worm density, and thus a higher net worm biomass growth rate, can be maintained by harvesting worm biomass from the reactor. This is a good operating strategy, particularly when the aim is to produce worm biomass for re-use applications.

6.4.2 Mesh size of carrier material

The mesh size of the carrier material had an effect on individual worm size. Although the largest mesh size (400 µm) clearly resulted in a higher individual worm weight, an unacceptable large fraction of the worms continuously fell from the carrier material into the water compartment. For long-term operation of a worm reactor, worm growth in the carrier is crucial and worm losses from the carrier should be minimized; the 300 and 350 µm mesh sizes were therefore selected for further experiments. In long-term sequencing batch experiments with the 300 and 350 µm mesh carrier materials stable worm densities of 0.87

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and 1.1 kg ww/m² respectively, could be achieved. The corresponding surface specific sludge consumption rates were 45 and 58 g TSS/(m²·d) respectively. Compared to a 300 µm mesh, a 350 µm mesh can therefore result in a 29 % reduction of the required surface area. Higher surface rates were possible, with a maximum of 70-90 g TSS/(m²·d), but only at higher, unstable, worm densities.

6.4.3 Worm density and sludge load on the worms

The sludge consumption rate by the worms was dependent on both the worm density and the sludge load the worms received. To achieve the maximum sludge consumption rate per surface area (i.e. the smallest reactor size), a worm density above 2 kg ww/m² was required. Above this density, the surface specific sludge consumption rate remained constant at 70-90 g TSS/(m²·d), but the consumption rate per worm weight gradually decreased. This can be explained by competition between the worms for the same sludge, probably caused by an overlap of the feeding areas of the individual worms. To show that this was not caused by substrate (sludge) limitation, the sludge load was varied at a constant worm density. At a sludge load below 100 mg TSS/(g ww·d), sludge availability to the worms appeared to become the limiting factor, probably caused by the restricted position of the worms in the carrier material. When considering the (plug flow) configuration of Figure 6.1, the sludge load on the worms will gradually decrease along the length of the reactor towards a value below a minimum value required to achieve maximum sludge consumption rate. As a result, this part of the carrier material will not be used optimally, which should be taken into account for the design of the reactor.

6

6.4.4 Sludge type

Several sludge types were tested in sequencing batch experiments. The worms consumed all sludges, but surface specific sludge consumption rates ranged from 48-265 g TSS/(m²·d). Although the worm density was not optimised for each sludge type, the results strongly suggest that the maximum achievable surface specific rate varies with the type of sludge. The size of a worm reactor will therefore be site-specific. However, the digestion rates for the different sludge types appeared to be much closer. This indicates that a higher consumption rate could merely be the result of satisfying the need for a certain amount of available and digestible organic material. The current results, however, did not show a direct relation with the organic fraction of the sludge or the nutrient (nitrogen and phosphorus) content of the sludge. A more detailed study into the composition of the sludge and the worm metabolism should provide more information on which fraction of the sludge can be digested by the worms. So far, little information is available on the metabolism of *L. variegatus*, other than that the gut contains digestive fluids (Moore, 1978 and Leppänen and

Kukkonen, 1998). The applicability of the worm process for certain sludge types may then also be predicted.

6.4.5 Specific oxygen uptake rate

The specific oxygen uptake rate (SOUR) by worms feeding on waste sludge was 4.9 mg O₂/(g ww·d). Gnaiger and Staudigl (1987) reported a similar SOUR of 4.6 mg O₂/(g ww·d) for worms feeding on a sediment-like substrate. The same SOUR was found for immobilised and for free worms, indicating that the carrier material does not prevent the worms from taking up a sufficient amount of oxygen. The oxygen uptake expressed per amount of organic material digested by the worms was estimated at 0.5-1.0 mg O₂/mg VSS digested. In a full-scale worm reactor some oxygen can be supplied with the effluent (see Figure 6.1), which will flow through the water compartment at a certain rate. Based on the need to keep the concentration of released ammonia below inhibiting concentrations, this flow rate was estimated to be ± 0.38 L/g TSS digested (Hendrickx *et al.*, 2009). When saturated with oxygen (10 mg O₂/L), the effluent can only supply less than 1% of the required oxygen. Furthermore, the DO concentration should be kept above 8.1 mg/L in the whole reactor, which is required to maintain maximum sludge consumption rate (Hendrickx *et al.*, 2009). (Re)aeration of the effluent within the worm reactor will therefore be required.

6. Design parameters

6.5 Conclusions

Sequencing batch experiments were performed to determine the design parameters for correctly sizing and operating a worm reactor for sludge reduction. From the results it was concluded that:

- The net growth rate of worms is limited by the carrier material we used in the worm reactor, caused by a higher decay rate of 0.023 d^{-1} for the immobilised worms, compared to 0.018 d^{-1} for free worms.
- The mesh size of the carrier material had an effect on worm growth. As a result, a stable worm density of 1.1 kg ww/m^2 can be achieved with a larger mesh size of $350 \text{ }\mu\text{m}$, which is 25 % higher when compared to the $300 \text{ }\mu\text{m}$ mesh size. Using a $350 \text{ }\mu\text{m}$ mesh size will therefore result in a smaller worm reactor.
- Competition between the worms occurred at a higher, but unstable, worm density of 2 kg ww/m^2 .
- To ensure maximum sludge consumption rate by the worms, a sludge load above $100 \text{ mg TSS}/(\text{g ww}\cdot\text{d})$ should be maintained in the worm reactor, thereby preventing substrate (sludge) limitation.
- The size of a worm reactor depends on the type of sludge. It was unclear what determined the nutritious value, and therefore the sludge consumption rate, of sludge for the worms. Digestion rates for the different sludges were similar.
- The specific oxygen uptake rate by the worms was $4.9 \text{ mg O}_2/(\text{g ww}\cdot\text{d})$ or $0.5\text{-}1.0 \text{ mg O}_2/\text{mg VSS digested}$. To supply the worms with sufficient oxygen, aeration of the effluent in the water compartment of the worm reactor is required.

6

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Chapter 7

A reactor configuration for full scale application of sludge reduction with aquatic worms

Abstract

Aquatic worms can be used for reducing the amount of biological waste sludge produced at waste water treatment plants (WWTPs). In our reactor concept for a worm reactor, the aquatic worm *Lumbriculus variegatus* is immobilised in a carrier material and worm faeces can be collected separately from the waste sludge. However, net worm growth in this reactor concept was limited compared to free worms. To overcome this problem, a new configuration was designed with a vertically orientated carrier material. A feasibility experiment showed that the net worm biomass growth rate was significantly higher than in previous experiments with horizontally placed carrier materials. A larger worm reactor of the new configuration was successfully operated for a period of 8 weeks in which it received all the waste sludge from a lab-scale activated sludge reactor. The net worm biomass growth rate was 0.014 d^{-1} , significantly higher than found in a horizontal carrier ($0.009\text{-}0.013 \text{ d}^{-1}$). Nutrient release by the worms into the effluent was $20 \text{ mg PO}_4\text{-P/g TSS digested}$ and $64 \text{ mg (NH}_4\text{-N + NO}_3\text{-N)/g TSS digested}$. A large part of the ammonia released by the worms was nitrified inside the worm reactor, whilst the remaining ammonia load was mainly removed from the worm reactor with the worm faeces. The new reactor configuration is suitable for scaling-up of the worm reactor. The main advantages compared to a horizontal carrier material were a higher net worm biomass growth rate, efficient use of reactor volume (up to 22.5 m^2 of carrier material per m^3 of reactor volume), easy removal of worm faeces and easy aeration of the water compartment to supply the worms with oxygen.

Keywords: sludge reduction, *Lumbriculus variegatus*, worm reactor, reactor configuration, worm growth.

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7. Reactor configuration for upscale

7.1 Introduction

The amount of biological waste sludge produced at municipal waste water treatment plants (WWTPs) can be reduced and compacted using the aquatic worm *Lumbriculus variegatus* (Buys *et al.*, 2008). A new reactor concept was introduced in which these worms are immobilised in a carrier material (Elissen *et al.*, 2006) and which allowed for separate collection of the worm faeces. This concept not only proved to work in batch reactors, but also showed to be feasible in a continuously operated reactor (Chapter 3). Worm growth, however, was limited by the mesh in which the worms are immobilised, caused by a higher biomass decay rate compared to free worms (Chapter 6). This caused the net growth rate of free worms to be more than a factor two higher compared to worms immobilised in a carrier material (Chapter 6). Net growth in the carrier material was also limited by worms occasionally falling from the carrier material into the water compartment and then being collected with the worm faeces. To overcome these limitations on worm growth, a new configuration of the worm reactor was designed, where the same carrier material was placed vertically inside the water compartment (Figure 7.1). This way the worms were positioned horizontally. This may stimulate worm growth as their orientation is closer to their natural position where they burrow their heads into sediments and project their tails upwards. In the configuration we used so far, the worms were in an inverted position, with their heads upwards into the sludge compartment.

This chapter describes the results of the experiments that were performed with this new reactor configuration for sludge reduction with the aquatic worm *L. variegatus*. In a first experiment, the feasibility of worm growth in the new reactor configuration was tested in a small reactor. In a second experiment a larger worm reactor was operated, which received all the waste sludge produced by a lab-scale activated sludge system. The larger reactor was operated for a period of 8 weeks, in which faeces production and nutrients release were measured. Worm growth was determined at the end of the experimental run. Finally, the suitability of this new reactor configuration for scaling-up is discussed.

7.2 Materials and Methods

7.2.1 Analyses

Total, Volatile and Fixed Suspended Solids (TSS, VSS and FSS) were determined according to Standard Methods (APHA, 1998) using black ribbon filters (12-25 μm , Schleicher and Schuell). Chemical Oxygen Demand (COD), total nitrogen (total N), total phosphorus (total P) and total ammonia ($\text{NH}_4 + \text{NH}_3$) were determined according to Standard Methods (APHA, 1998) using Dr Lange[®] test kits. Nitrate (NO_3) and phosphate (PO_4) were determined according to Standard Methods (APHA, 1998) using ion chromatography (Metrohm 761 Compact IC).

7.2.2 Feasibility experiment

A feasibility experiment with the new reactor configuration was performed with sludge and effluent from the Leeuwarden WWTP. Sludge was first sieved (1 mm mesh) and effluent was filtered over black ribbon filters (12-25 μm , Schleicher and Schuell) before being used in the experiments. A small version of the reactor shown in Figure 7.1 was used, with only one mesh cylinder with a diameter of 4 cm and a height of 30 cm. The mesh size of the carrier material was 350 μm . Fresh sludge was added every 1-3 days and was recirculated over the mesh cylinder. The effluent in the water compartment was replaced once a week. At the same time, worms were removed from the mesh to weigh them. Their wet weight (ww) was determined by placing the worms on a polyamide mesh material (150 μm). By pushing paper towelling against the back of the mesh, adhering water was removed from the worms. Worms fallen from the carrier into the water compartment were not accounted for.

7.2.3 Activated sludge system

Sludge and effluent from a lab-scale activated sludge system were used in the experiments. This system treated pre-settled domestic sewage in a completely mixed aeration tank (50 L) followed by a settler. Waste water was frequently analysed for total COD, total N and total P. Every other day, sludge was analysed for TSS and effluent was analysed for total COD, soluble COD, ammonia, nitrate and phosphate. The system was operated at a sludge retention time (SRT) of 18 days by wasting a fixed volume of sludge directly from the aeration tank. The waste water flow rate was 78 L/d with an average total COD of 408 mg/L, which resulted in an average organic loading rate of 0.16 g COD/(g TSS·d).

7.2.4 Sequencing batch experiments

Sequencing batch experiments with a horizontal carrier material were performed as described in detail in Hendrickx *et al.* (2009), using sludge and effluent from the lab-scale activated sludge system. Effluent was first filtered over black ribbon filters (12-25 μm , Schleicher and Schuell). Worms were counted and their wet weight (ww) was determined. The carrier material used in the experiments had a mesh size of 350 μm .

7.2.5 Continuous worm reactor

Characteristics of the larger worm reactor are given in Table 7.1. Worms (29.8 g ww) were introduced in the worm reactor via the open top of the mesh cylinders. Waste sludge from the activated sludge system was directly pumped to the inlet of the sludge compartment, i.e. the bottom of the mesh cylinders (Figure 7.1). Effluent from the activated sludge system was collected in an overflowing bucket, from where it was pumped to the inlet of the water compartment. The effluent flow rate through the water compartment of the worm reactor

7. Reactor configuration for upscale

was decreased stepwise from 43 L/d to 2.8 L/d. Worm faeces were pumped from the bottom of the water compartment at a rate of 1.2 L/d. The water compartment was aerated using a diffuser (with an air flow rate of about 690 mL/min) inside a pipe. This visibly created some mixing of the effluent in the water compartment, which could distribute dissolved oxygen throughout the worm reactor, but allowed worm faeces to settle. The outflow from the worm reactor was collected and analysed for total COD, soluble COD, ammonia, nitrate and phosphate. Sludge that was not consumed by the worms was not found in the worm outlet, but formed a sludge bed inside the mesh cylinders. Collected worm faeces were analysed for TSS, total COD and its supernatant for total COD, ammonia, nitrate and phosphate. Waste sludge and worm faeces were occasionally analysed for total N and total P. At the end of the experimental run, all the worms in the mesh cylinders were collected and their ww was determined.

Temperature and dissolved oxygen (DO) concentration in the water compartment of the worm reactor were measured using an optical dissolved oxygen measurement probe (Oxymax W COS61, Endress and Hauser).

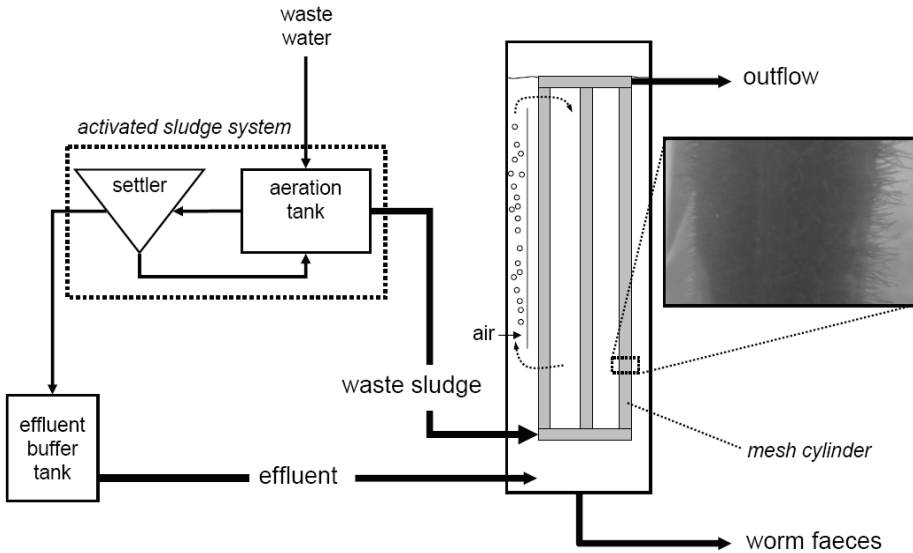


Figure 7.1 Set-up for the experiment with the large continuous worm reactor.

Table 7.1 Dimensions of the large worm reactor

mesh size carrier material	µm	350
mesh cylinders	#	3
surface area	cm ²	1257 (x 3)
height mesh cylinder	cm	100
diameter mesh cylinder	cm	4
volume sludge compartment	L	1.3
volume water compartment	L	31

7.3 Results and discussion

7.3.1 Feasibility experiment

A first experiment was performed with a small version of the new reactor configuration and Leeuwarden WWTP sludge. In a period of 40 days, the worm biomass in the reactor increased from 9.8 to 18 g ww. This showed that net worm growth rate (0.015 d^{-1}) was possible also in this configuration, even higher than in a horizontal carrier ($0.009\text{-}0.013 \text{ d}^{-1}$), but still below rates found for non-immobilised worms (0.026 d^{-1}) (Chapter 6).

7.3.2 Continuous worm reactor

The size of the larger worm reactor was estimated from the results of a sequencing batch experiment with a horizontal carrier material as described in Hendrickx *et al.* (2009). With sludge (and effluent) from the lab-scale activated sludge system, these experiments showed a sludge consumption rate of 138 mg TSS/(g ww·d) when a worm density of 1.2 kg ww/m² was used. TSS reduction in the batch experiments was 11% (16% based on VSS). Based on these results, a worm reactor to treat the waste sludge from the lab-scale activated sludge system would require 58 g ww of worm biomass and a carrier surface area of 485 cm². The large continuous worm reactor we used offered ample surface area (3770 cm²), thereby avoiding worm density to become a limiting factor (Chapter 6). The larger reactor was started with a lower amount of worm biomass (29.8 g ww) to demonstrate that worm growth would be possible.

The larger continuous worm reactor was operated without any problems during the entire experimental period of nearly 8 weeks. The cumulative amounts of waste sludge fed to the worm reactor and collected worm faeces are shown in Figure 7.2. In total 431 g TSS of waste sludge was fed to the worm reactor and 167 g TSS was collected as worm faeces. However, sludge accumulation was observed as a sludge bed in the mesh cylinders, which was expected since the reactor was started with an insufficient amount of worms. The amount of sludge consumed by the worms was therefore estimated from the amount of

7. Reactor configuration for upscale

collected worm faeces and the TSS reduction (11 %) found in the batch experiments. This resulted in an estimated total sludge consumption of 187 g TSS and a total sludge digestion by the worms of 20 g TSS. The sludge consumption rate of 110 mg TSS/(g ww·d) during the last days of operation, was lower than the 138 mg TSS/(g ww·d) in the sequencing batch experiment. This could be explained by the DO concentration of 6.7 mg/L in the water compartment, which was below the optimum concentration (8.1 mg/L) for the worms (Hendrickx *et al.*, 2009).

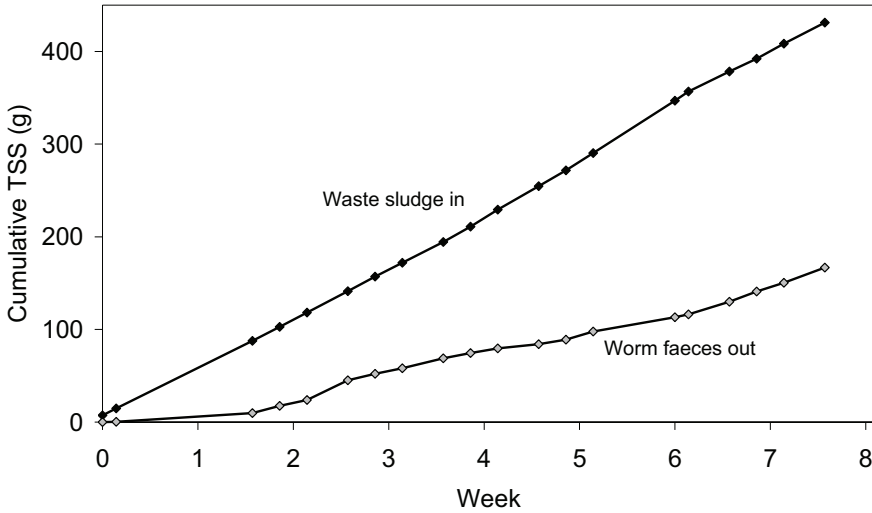


Figure 7.2 Cumulative amounts of added waste sludge and collected worm faeces from a continuous worm reactor, which received all the waste sludge from a lab-scale activated sludge system.

Worm biomass

The worm reactor was started with 29.8 g ww of worms, divided over the three mesh cylinders. At the end of the 8 weeks of operation, 49.5 g ww of worms was found in the mesh cylinders. During operation of the worm reactor a total of 6.7 g ww of worms was collected with the worm faeces (worms that had fallen from the mesh). Thus, a total worm growth of 26.8 g ww was observed, which corresponded with a yield of 0.20 g dw/g TSS digested by the worms. This is higher than the yield of 0.13 g dw/g TSS digested found in the continuous worm reactor with a horizontal carrier material (Chapter 3). The average

worm net biomass growth rate was 0.014 d^{-1} , which is only slightly lower than the growth rate found in the feasibility experiment.

Visual inspection of the mesh cylinders showed that worms were situated along the entire sludge bed inside each mesh cylinder. By the end of the experiment the total sludge bed height in each cylinder had increased to 20–45 cm. However, most of the worms (~ 80 %) were situated in the top ~ 10 cm of the sludge bed. This corresponded to a worm density of 1.1 kg ww/m^2 carrier material, which matched the stable worm density found in sequencing batch experiments with the same carrier material (Chapter 6).

Nutrients

On average the sludge from the activated sludge system contained 48.6 mg total N/g TSS and 14.9 mg total P/g TSS. The collected worm faeces contained 40.4 mg total N/g TSS and 15.7 mg total P/g TSS. Similar to experiments with sludge from a municipal WWTP (Chapter 5), the phosphorus content of the faeces was higher than in the sludge, whereas the nitrogen content was lower. Based on a mass balance over the worm reactor, an ammonia release of 7.8 mg $\text{NH}_4\text{-N/g}$ TSS digested and a phosphate release of 20 mg $\text{PO}_4\text{-P/g}$ TSS digested were calculated. For phosphate this was similar to what was found for sludge from a municipal WWTP (Chapter 5), but for ammonia this was much lower than the 58 mg $\text{NH}_4\text{-N/g}$ TSS digested found in those experiments. However, at the same time nitrate was produced in the worm reactor and the combined release of ammonia and nitrate amounted to 64 mg N/g TSS digested by the worms. This showed that nitrification of the released ammonia had taken place in the worm reactor, which was observed throughout the entire experimental run. Whether this occurred in or near the sludge bed in the mesh cylinders or by nitrifiers in the worm faeces, was not further investigated. Nitrification can also be expected to take place in a full scale worm reactor. Not only will this result in an increase of the oxygen demand of the worm reactor, but also in a decrease of the internal ammonia load on the WWTP. For the worm reactor, an advantage of nitrification in the worm reactor is that less effluent is required to keep the ammonia concentration low enough ($< 0.1 \text{ mg N/L}$ of unionised ammonia) to prevent inhibition of the sludge consumption rate by the worms (Hendrickx *et al.*, 2009).

Analysis of the supernatant of the worm faeces showed that the total ammonia concentration was high, up to 9.2 mg N/L . At the same time, the total ammonia concentration in the outlet of the worm reactor was very low ($< 0.5 \text{ mg N/L}$) and appeared to be independent of the effluent replacement flow rate, which was decreased stepwise from 42 L/d to 2.8 L/d. This showed that the ammonia load released by the worms (and that was not nitrified), was removed from the reactor mainly with the supernatant of the worm faeces

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(Figure 7.3), thus keeping the ammonia concentration in the water compartment low. Possibly ammonia adsorbed to worm faeces, as has also been reported to occur for sludge (Nielsen, 1996), although in our case this was about a factor 10 higher (2.9 mg $\text{NH}_4\text{-N/g}$ TSS of faeces). Another reason for the ammonia release could be some mineralization of the worm faeces after collection from the worm reactor. For phosphate the average concentration in the supernatant of the worm faeces was 7.2 mg $\text{PO}_4\text{-P/L}$, only somewhat higher than in the outlet of the worm reactor (6.0 mg $\text{PO}_4\text{-P/L}$). The phosphate load removed with the faeces remained constant during the entire experimental period (Figure 7.3). The stepwise decrease in effluent replacement rate (with a constant phosphate concentration) caused the decrease in the phosphate load in the outflow from the worm reactor (Figure 7.3).

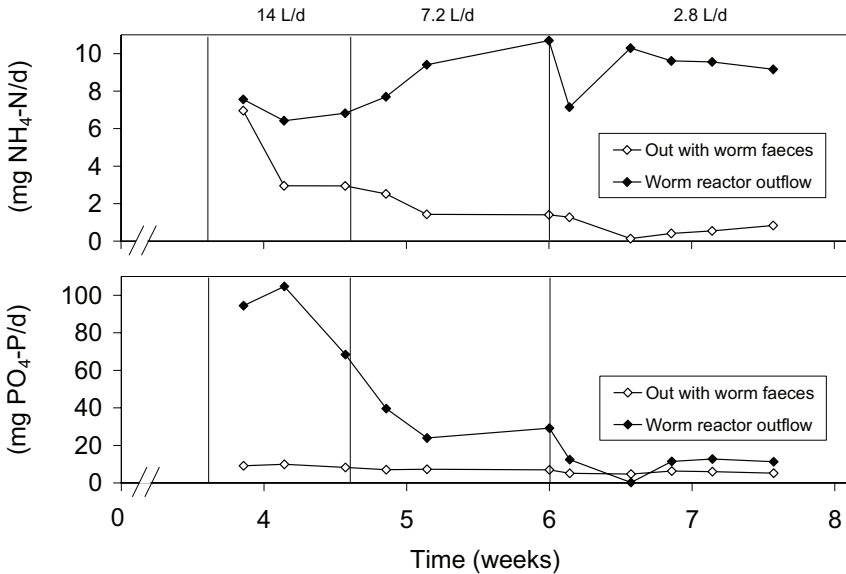


Figure 7.3 Ammonia and phosphate load removed with the outflow from the worm reactor and with the worm faeces.

Air input

Inside the worm reactor the effluent in the water compartment was aerated with a single bubble diffuser. Oxygen was also introduced to the reactor with the effluent, which had an average dissolved oxygen (DO) concentration of 5.8 mg/L. In the first weeks, the average DO concentration in the reactor was 7.7 mg/L, whilst during the last couple of weeks, this had dropped to 6.7 mg/L. This may have been caused by the increased oxygen consumption by the worms (as the amount of worm biomass increased over time), by the lower input of DO with the effluent that was pumped into the worm reactor (this flow rate was decreased stepwise over time), by oxygen consumption due to nitrification of the ammonia that was released by the worms and by the respiration of the sludge bed in the mesh cylinders. The temperature was constant at $20.6 \pm 0.9^\circ\text{C}$ and could therefore not have caused the decrease in DO concentration.

7.3.3 General discussion

The new reactor configuration with a vertically placed carrier material demonstrated a stable operation over a period of 8 weeks. Although only a low TSS reduction of 11% was found, worm faeces with a high settleability were continuously produced. The latter was shown to be the main benefit of a worm reactor (Chapter 5).

The new configuration has several advantages over the initial configuration with a horizontal carrier material. Most importantly, a higher net growth rate of 0.014 d^{-1} over 8 weeks was achieved, compared to 0.013 d^{-1} over 3 weeks for the horizontal carrier reactor described in Chapter 3. Despite their horizontal orientation, still some worms fell from the carrier material. But this was less than 0.5% per day of the amount of worms in the mesh cylinders, which was much lower than the 4% found in sequencing batch experiments with the same, but horizontally orientated, mesh material (Chapter 6). A second advantage is the efficient use of reactor volume. With the new configuration 22.5 m^2 of carrier material per m^3 of reactor volume can be achieved by spacing the mesh cylinders at a distance of 4 cm. In contrast, to achieve $22.5 \text{ m}^2/\text{m}^3$ in a reactor with a flat horizontal carrier, sections consisting of a water- and sludge compartment would have to be stacked. Each section could then only be 4 cm high; 2 cm for the sludge compartment and 2 cm for the water compartment. Such a small height would make sludge distribution and faeces collection very difficult. And finally, the new configuration has practical advantages with respect to faeces collection (one collection system for a large number of mesh cylinders) and aeration (air bubbles cannot get trapped under the carrier material). Additionally, the combined collection of non-consumed sludge and effluent with worm metabolites excludes the need to control two levels in the reactor (one in the water compartment and one in the sludge

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compartment). A separate collection is not required as both effluent and non-consumed sludge need to be returned to the WWTP.

A worm reactor such as the one described in this chapter is expected to be most interesting for smaller WWTPs with relatively high sludge handling costs. As an example, the results from the current experiments (110 mg TSS/(g ww·d) and 1.1 kg ww/m²) were used to estimate the size of a worm reactor for a 35,000 p.e. WWTP. For a waste sludge production of 1600 kg TSS/d, the footprint of the worm reactor would be 195 m² (assuming 22.5 m²/m³ and a height of 3 m). This is roughly one tenth of the surface area of a settler of such a WWTP. This additional surface space is expected to be available at a small WWTP, which is generally not located in densely populated areas.

7.4 Conclusions

A new configuration of our reactor concept was introduced with worms immobilised in a vertically orientated carrier material. This new configuration allows easy aeration of the water compartment, easy removal of worm faeces and a low footprint of the reactor and is therefore suitable for scaling-up of the process.

A continuous worm reactor treating the waste sludge from a lab-scale activated sludge reactor was successfully operated during a period of nearly 8 weeks. Net growth of worm biomass clearly took place in the worm reactor at a rate of 0.014 d⁻¹.

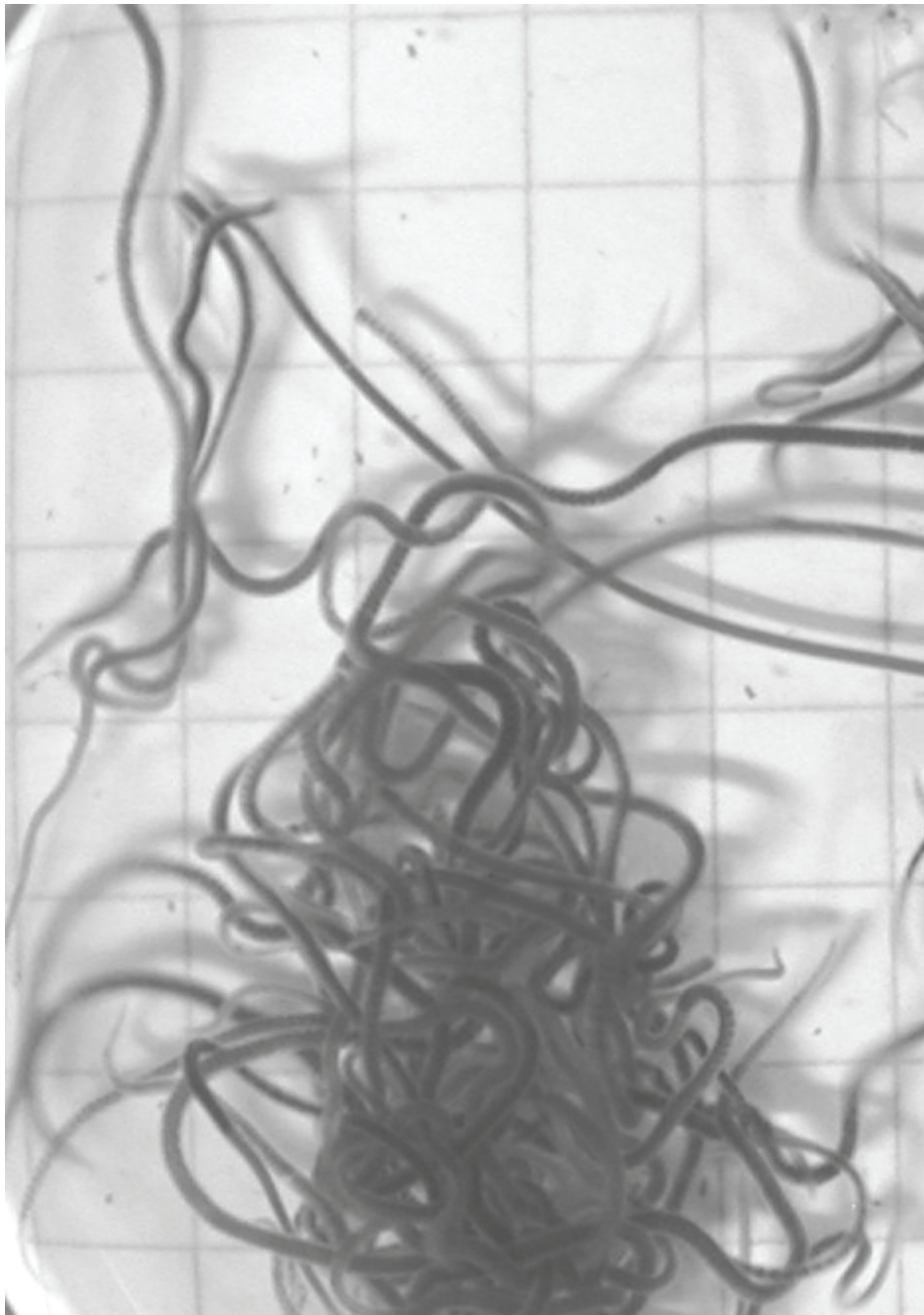
Release of nutrients by the worms was 20 mg PO₄-P/g TSS digested and 64 mg (NH₄-N + NO₃-N)/g TSS digested. The ammonia released by the worms as a product of their metabolism, was partially converted to nitrate in the worm reactor. The remaining ammonia load was removed from the worm reactor mainly with the worm faeces.

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Chapter 8

Aquatic worms grown on biosolids: biomass composition and potential applications

Abstract

The increasing production of biological waste sludge from waste water treatment plants is a problem, because stricter legislation inhibits the use of traditional disposal methods. The use of the aquatic worm *Lumbriculus variegatus* can minimise sludge production. Due to the fact that the worms can feed and grow on this waste sludge, valuable compounds that are present in the sludge can be recovered by the worms. This chapter describes a systematic approach for finding possible applications of the produced biomass. The worm biomass mainly consists of protein and smaller fractions of fat, sugar and ash. It also contains low concentrations of heavy metals. The potential produced amount is relatively small, compared to other waste streams, and is produced decentrally. Therefore, the most promising applications are specific components of the biomass, for example specific amino acids or fatty acids. However, until the process is optimized and there is a stable supply of worms, the focus should be on simple applications, later on followed by specific applications, depending on the market demand. Worm biomass grown on clean sludges has a broader application potential, for example as consumption fish feed.

Keywords: waste activated sludge, aquatic worms, *Lumbriculus variegatus*, biomass re-use and applications

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8.1 Introduction

Biological waste water treatment plants (WWTPs) produce biological waste sludge (biosolids), which is a complex mixture of water (up to more than 95 %), bacteria, dead organic and inorganic materials, containing phosphorus and nitrogen components and various pollutants (e.g. heavy metals, organic pollutants and pathogens) (Rulkens, 2004). In Europe alone, more than 40,000 WWTPs produce around 7 million tons of dry solids (ds) per year (Roman *et al.*, 2006) and this production is expected to increase, also on a global scale.

In Europe, most sludges are settled, stabilised, thickened, anaerobically digested and then disposed of (Roman *et al.*, 2006). Traditional disposal methods consist of application as agricultural fertilizer, disposal in landfills or the sea, or incineration. The costs of these treatment and disposal methods are high and estimated to be up to half of the operational costs of waste water treatment (Wei *et al.*, 2003). Also, stricter regulations for sludge, concerning for example maximum permissible heavy metal concentrations, to an increasing extent prohibit the first two disposal methods. These regulations will become even stricter due to the upcoming version of the European Urban Waste Water Treatment Directive (UWWTD). As a result, there is a strong need to develop technologies for minimizing sludge production and preventing disposal, such as recycling of valuable components in the sludge (e.g. Wei *et al.*, 2003).

A biological method that addresses both the minimization of sludge production and the recovery of valuable components is sludge reduction by terrestrial or aquatic worms. The consumption of sludge particles by worms not only leads to a decrease in the dry solids and volume of the sludge that has to be disposed of as worm faeces, but also to a conversion of part of the sludge into new worm biomass with potential for re-use because of, for example, its high protein content.

Sludge reduction by earthworms (vermicomposting) is a relatively common technology, especially in developing countries in small-scale settings (e.g. Ndegwa and Thompson, 2001). The main product of this technology is 'vermicompost' (earthworm faeces) that can be used as fertilizer due to its higher nitrogen content, higher microbial activity and lower heavy metal content (e.g. Ndegwa and Thompson, 2001). Also, new protein-rich worm biomass is produced that can be re-used for example as compost, enzyme source for the production of detergents or animal feed (e.g. Boer and Sova, 1998, Edwards and Neuhauser, 1988).

Sludge reduction with aquatic worms has been described less frequently and has not been applied in practice yet. Most research was conducted in China (e.g. Liang *et al.*, 2006), Japan (e.g. Luxmy *et al.*, 2001) and the Netherlands (e.g. Rensink and Rulkens, 1997). These investigations focused on sludge reduction but not on the re-use of worm biomass. In addition, most of the investigated worm species (e.g. Aeolosomatidae) are too small to be

separated from the sludge. Recently however, a reactor concept for sludge reduction with the larger aquatic species *Lumbriculus variegatus* was described in which the waste sludge, worm faeces and worms can be separated (Elissen *et al.*, 2006, Hendrickx *et al.*, 2009). This species is capable of stable sludge reduction and worm growth rates (Buys *et al.*, 2008) in contrast to other aquatic worm species (Ratsak and Verkuijlen, 2006). Reduction percentages show large variations (between 5 and 75 % of the dry matter), depending on the experimental conditions. Also, the doubling time of *L. variegatus* on sludge can be as low as 7 days, which is relatively short in comparison to those on other feeds like organic material in sediments (10-40 days) (e.g. Elissen, 2007, Mount *et al.*, 2006, Williams, 2005). In batch experiments, around 7 % of the total amount of sludge provided is converted into worm biomass, based on dry matter (Elissen, 2007). A 100,000 p.e. (person equivalent) WWTP with a typical yearly waste sludge production of almost 2000 tons dry solids (CBS, 2007) could thus produce 135 tons of worm dry weight (dw), which equals 1000 ton of wet weight (ww) per year. Application of *L. variegatus* for both minimizing sludge production and recovering valuable sludge components therefore has high potential. The basic composition of deperated (with empty guts) *L. variegatus* grown on other feeds than sludge, i.e. fish feed or sediments, has been determined (Hansen *et al.*, 2004) and is shown in Table 8.1.

Table 8.1 Biomass composition of deperated *Lumbriculus variegatus* (in % of dw) grown on fish feed or sediments (Hansen *et al.*, 2004). Worm dw was 15-16 % of ww.

Component	% of dw
Protein	62-66
Fat	11-12
Sugar	13-18
Ash	9-11
Fatty acids	7-12
Calcium	0.2-0.3
Phosphorus	1.4-2.1
Calories (kcal/g dw)	4.8-4.9

Based on its composition and other characteristics, Mount *et al.* (2006) concluded that *L. variegatus* is very suitable as a prey organism for conducting dietary exposure studies with fish. They found similar values for fat and fatty acid content when the worms were grown on fish feed, but different values for soluble protein and ash (47 and 4 % of the dw respectively). However, it seems that these worms were not deperated. They also determined the amino and fatty acid composition. In general, *L. variegatus* is an excellent feed for fish or other aquatic animals (Mount *et al.*, 2006, Drewes, 2005, Hansen *et al.*,

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2004) and is also a standard test organism world-wide used for bioaccumulation and toxicity assays.

The composition of *L. variegatus* grown on sewage sludge is unknown, but very important for determining re-use possibilities of worm biomass produced during sludge reduction. For this biomass, applications as feed for consumption animals are not an option, since it may contain (micro)pollutants which could end up in the human food chain.

This chapter therefore describes the composition of *L. variegatus* grown on sewage sludge in terms of biomass (protein, fat, sugar, ash, phosphorus, nitrogen and amino acids) and pollutants (heavy metals). Based on this information possible applications of the worm biomass are discussed. Also, suggestions for further research on interesting components are given.

8.2 Materials and methods

8.2.1 Organisms

L. variegatus cultures originated from commercially available ‘*Tubifex*’ mixtures (pet shops). They were maintained in an artificial ditch in our laboratory, which was constantly fed with effluent and sludge particles from a lab-scale activated sludge system treating waste water from the municipal WWTP of the village of Bennekom. For comparison, also *L. variegatus* fed with sludge from the municipal WWTP of the city of Leeuwarden were used for heavy metal analyses.

8.2.2 Composition of *L. variegatus* grown on sludge

8.2.2.1 Biomass

Dry weight

Dry weight (dw) of the worms was determined after drying overnight at 105°C and ash content after overnight ignition at 525°C. Dry solids (ds) of the sludge were determined according to Standard Methods (APHA, 1998) using black ribbon filters (12-25 µm, Schleicher and Schuell).

Protein analysis

Dried and milled worm material (20-50 mg protein) was put in a Kjeldahl tube to which 1 Kjeltab and 9 mL of concentrated sulphuric acid were added. Destruction was performed for 50 minutes at 420°C in a Gerhardt Kjeldatherm apparatus. After 10 minutes of cooling, 75 mL water was added. Subsequently steam distillation using Gerhardt Vapodist was performed for 4.5 minutes. Finally, the nitrogen content was determined using titration with 0.1 M HCl. Protein amount was calculated using a Kjeldahl factor of 6.25. Protein in sludge was measured by the Biuret method.

Protein molecular weight analysis

Molecular weight distribution of the protein fraction was determined by gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out with 15 % polyacrylamide gel. Samples (10 mg protein) were mixed with 600 μL sample buffer with B-mercaptoethanol, heated at 90°C for 5 minutes and centrifuged. The samples (10 μL) were applied on the gel. The gels were stained with Coomassie brilliant blue.

Fat analysis

Fat was determined by Soxhlet extraction with hexane. The samples were extracted with soxtec-extraction using hexane at boiling temperature for 30 minutes and then washed with hexane during 75 minutes at room temperature. The extracted samples were allowed to dry at 60°C during 16 hours. The weight of the samples was measured before and after extraction.

Sugar analysis

The milled samples were extracted with soxtec-extraction using ethanol:toluene 2:1, 96 % (v/v) ethanol and hot water (1 hour) at boiling temperature. The extracted samples were dried at 60°C for 16 hours. The content of neutral sugars of the ethanol-extracted material was determined after a two-step hydrolysis with sulfuric acid (12 M for 1 hour at 30°C; 1 M for 3 hours at 100°C) according to modified TAPPI methods. Neutral sugars were determined by HPAEC with pulsed amperometric detection on a CarboPac PA1 column (Dionex) with a water-sodium hydroxide gradient. The total sugar content of sludge was determined by the phenol sulphuric acid method with glucose as a standard.

Amino acid analysis

To dry worm samples (about 1 mg protein) 300-500 μL 6 M HCl was added and hydrolysis of the protein took place during 24 hours at 100°C. After centrifugation, about 500 μL 20 mM HCl was added in order to get a concentration of about 0.2 mg/mL. The amino acids were derivatised with AccQ.Flour reagens. 5 μL of the obtained solution was injected in a HPLC having a Nova-PakTM C18 column. The eluents was a 40/60 water/acetonitril mixture. The column temperature was 30 °C, the flow rate was 1 mL/min. Identification of the amino acids took place based on the retention times. Using this method tryptophan is being destroyed. Therefore, tryptophan was determined separately by Ansynth Service BV (Roosendaal, the Netherlands).

Nitrogen and phosphorus analysis

Total nitrogen and total phosphorus were determined according to Standard Methods (APHA, 1998), using Dr Lange[®] test kits.

8.2.2.2 Pollutants (heavy metals)

For determining the heavy metal concentrations in *L. variegatus*, two long-term experiments were performed. In the first experiment, *L. variegatus* cultures were grown on

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sludge from municipal WWTP Bennekom, the Netherlands, for six months. As control, a *L. variegatus* culture was grown on Tetra Min[®] fish feed (for tropical fish) during the same period. According to the label, the fish feed contained 49 % protein, 9 % fat, 2 % cellulose and 12 % ash (dry solids based) plus added vitamins A, D₃ and E. The cultures were fed weekly in excess. After six months, Cd, Cr, Cu, Ni, Pb and Zn were extracted from the worms, the control worms, the sludge and the fish feed by a microwave assisted aqua regia destruction step. Destruates were filled up to 100 mL with milliQ and filtered. 1 mL from each solution was dissolved in 9 mL milliQ and then analysed on an ICP-MS (0.14 M HNO₃ matrix) by a commercial laboratory (Soil Chemical and Biological Laboratory, Wageningen, the Netherlands).

In the second experiment, *L. variegatus* cultures were grown on sludges from municipal WWTPs Bennekom and Leeuwarden, the Netherlands, for five months. The cultures were fed weekly in excess. After five months, As, Cd, Cu, Cr, Pb, Hg, Ni and Zn in the two worm cultures and the two sludges were extracted and analyzed by the same laboratory as in the first experiment.

8.2.3 Applications of *L. variegatus* grown on sludge

To find suitable applications for *L. variegatus*, a systematic approach for finding new applications for by-products was used (Meeusen-van Onna *et al.*, 2008). The main principle is to generate as many options as possible and evaluating these in the least expensive way as early in the process as possible. The approach consists of a number of phases:

1. Information phase

Relevant information for characterizing the by-product is collected (e.g. chemical composition, regulations, production process, current applications and market expectations). Successful applications are defined.

2. Quick-scan and brainstorm sessions

Areas of interest from phase 1 are selected and brainstorm sessions with experts are held to make a potential list with valorisation options characterised by technical, market, economical, chain and legal aspects.

3. Analysis, prioritizing and selection

The new options are worked out in more detail. A selection is made of the most promising options which can be implemented or for which a market analysis or technical feasibility study is necessary.

8.3 Results and discussion

8.3.1 Organisms

Specimens of *L. variegatus* grown on sludge generally are larger (up to 45 mg) than those grown on other feeds like sediments or fish feed (typically 5-10 mg) (e.g. Mount *et al.*, 2006, Williams, 2005) (Figure 8.1). This indicates that sludge has a very high nutritional value, as Leppänen and Kukkonen (1998) found that individual wet weight increased in feeds with higher organic material content, while reproduction rates remained the same. Figure 8.1 also shows that the tissue colour of *L. variegatus* grown on sludge is darker (dark red) compared to that of worms fed with fish feed (pink). The reason for this is unknown.

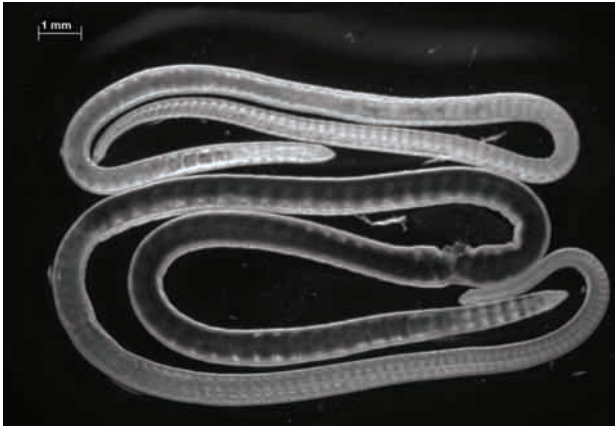


Figure 8.1 *L. variegatus* specimens grown on Tetra Min[®] fish feed (upper) and sludge from WWTP Leeuwarden (lower)

8.3.2 Composition of *L. variegatus* grown on sludge

8.3.2.1 Biomass

The main components of *L. variegatus* biomass grown on sludge are shown in Table 8.2. Results for amino acids and sugar are presented in separate figures.

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Table 8.2 Main components of depurated *L. variegatus* (in % of dw) and the sludge from WWTP Bennekom used to grow the worms (in % of ds). Worm dw was around 13 % of the ww.

Component	Worms % of dw	Sludge % of ds
Protein	63	34-43
Fat	25	n.d.*
Sugar	7**	23-26
Ash	6	14-22
Phosphorus	0.9-2.2	1.6-1.7
Nitrogen	11-13	6-10

* Fat was not determined in the sludge but constituted most likely the major part of the missing dw fraction (19-25 %), which also contained other components like humic acids, bacterial DNA and RNA.

** Sugar content was calculated

The high protein and phosphorus contents were similar to the data found by Hansen *et al.* (2004) for *L. variegatus* grown on fish feed (Table 8.1). Sugar and ash content were somewhat lower in *L. variegatus* grown on sludge compared to worms on fish feed, while the fat content was twice as high. A higher fat content can indicate a higher nutritional value of the feed, like was found for amphipods (shrimp-like invertebrates) (Perrone *et al.*, 2003). Fat content was found to vary between 7 and 18 % of the dry weight, with increasing fat content related to higher food availability. Based on their similar basic composition (Table 8.2 and Materials and Methods section), it is not clear why sludge would be more nutritious than fish feed. However, sludge contains living bacteria and this may explain the differences. Most results for *L. variegatus* grown on sludge, except for the fat content, were also similar to those for other aquatic Oligochaeta. Examples are *Tubifex tubifex* from riverbeds where organic waste is discharged (Yanar *et al.*, 2003) and several terrestrial Oligochaeta grown on undefined organic wastes (Edwards and Neuhauser, 1988).

Typical values for protein content in activated sludge are rather stable (32-41 %) and comparable to what we found, but those for ash and sugar content are variable, respectively 12-41 % and 10-45 % of the dry solids (e.g. Tchobanoglous *et al.*, 2003). In comparison to the feed sludge (Table 8.2), *L. variegatus* biomass is significantly enriched in protein and (naturally) nitrogen, but contained lower concentrations of ash and sugar. Fat and phosphorus concentrations were comparable.

The proteins isolated from *L. variegatus* have a broad molecular weight distribution varying from 10 kD to 300 kD. Some protein fractions were found with a very high molecular weight, which may be related to the presence of erythrocrucorin. This is the large

hemoglobin-like oxygen-binding blood protein found in *L. variegatus* and many other Annelida (e.g. Drewes, 2005, Frossard, 1982). However, the major part of the protein had a molecular weight between 14 and 20 kD under reduced conditions.

The amino acid composition of *L. variegatus* grown on sludge from WWTP Bennekom is shown in Figure 8.2.

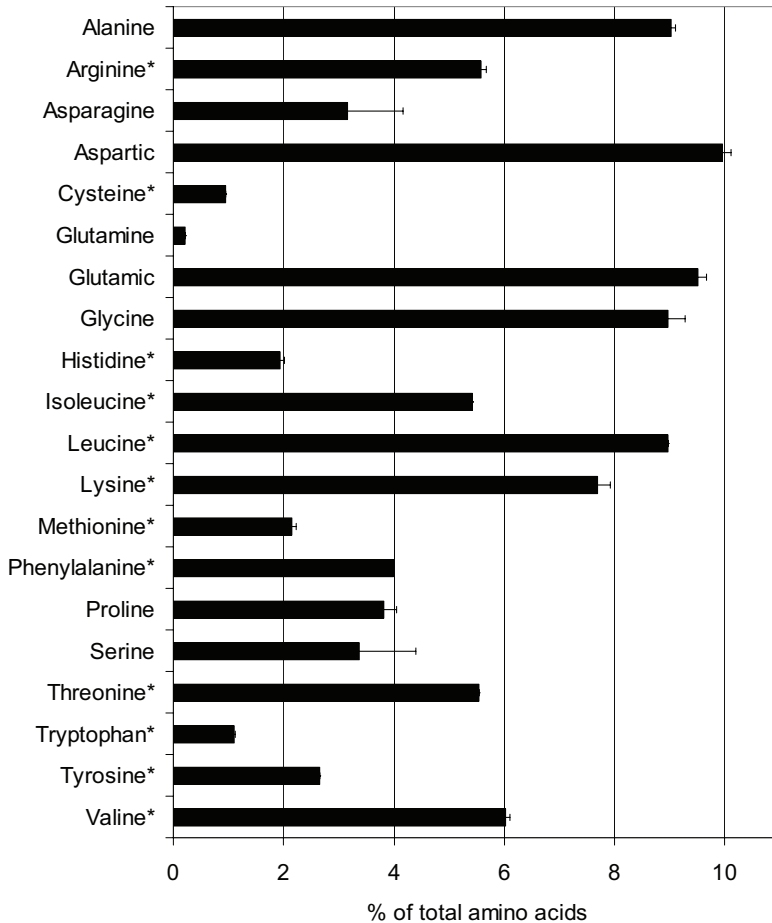


Figure 8.2 Amino acid composition of *L. variegatus* (in % of total amino acids) grown on sludge from WWTP Bennekom (n=2). (*='essential' amino acids, that can not be synthesized by mammalian cells)

8. Re-use of worm biomass

The amino acid composition was comparable to that described for *L. variegatus* grown on fish feed, with high percentages of alanine, aspartic acid, glutamic acid, glycine, leucine and lysine (Mount *et al.*, 2006). In contrast, the latter authors did not find asparagine, cysteine and glutamine, while in the current research no cystine was found. However, during the analysis process, these amino acids can be easily converted into aspartic acid, cystine and glutamic acid respectively, which may explain the different results. Again, the results were similar to those for other aquatic Oligochaeta, for example *T. tubifex* (Yanar *et al.*, 2003) and several terrestrial Oligochaeta (Edwards and Neuhauser, 1988).

The sugar (monosaccharide) composition of *L. variegatus* grown on sludge is shown in Figure 8.3.

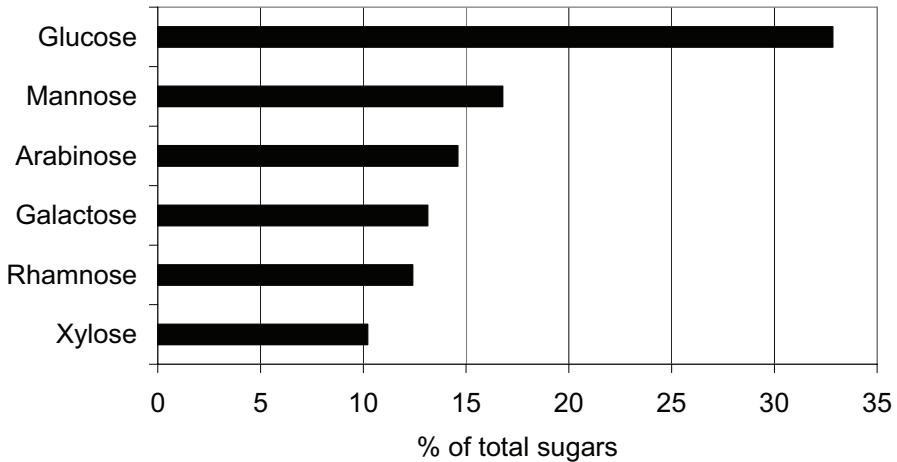


Figure 8.3 Sugar composition of *L. variegatus* (in % of total sugar) grown on sludge from WWTP Bennekom (n=1).

The sugar composition of *L. variegatus* was not determined before and also for other worm species data are scarce, except for glucose concentrations in some earthworm species (Holmstrup and Overgaard, 2007). These authors investigated the positive correlation between glucose concentration and freeze tolerance and found concentrations up to 4 % of the dry weight in reference worms, which is roughly in the same range as was found for *L. variegatus* (Figure 8.3), considering a total sugar content of 7 % of the dw (Table 8.2).

8.3.2.2 Pollutants (heavy metals)

The heavy metal concentrations in *L. variegatus* grown on different sludges and a control feed (Tetra Min[®] fish feed) from two long-term experiments are shown in Figure 8.4.

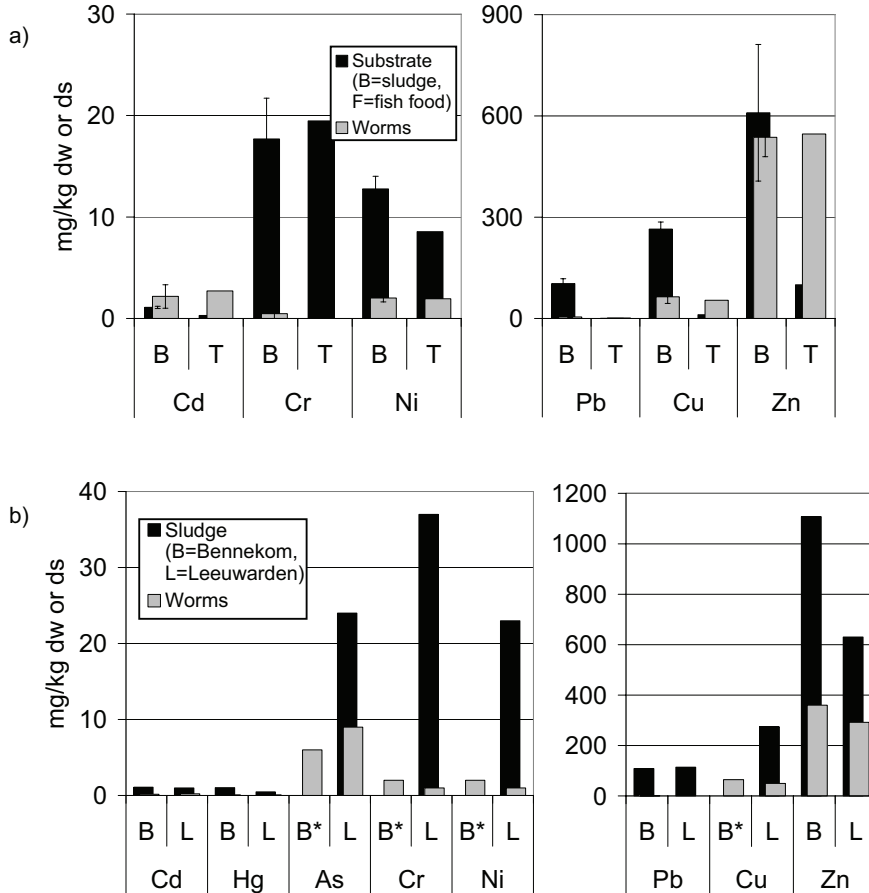


Figure 8.4 Heavy metal concentrations (in mg/ kg dw (worms) or ds (feed)) in

a) Experiment 1: Sludge from WWTP Bennekom (B), Tetra Min[®] fish feed (F) and *L. variegatus* grown on these two feeds. Hg and As were not analyzed.

b) Experiment 2: Sludges from WWTPs Bennekom (B) and Leeuwarden (L) and *L. variegatus* grown on these two sludges. (*= Sludge concentrations were not determined)

When invisible in the figures, concentrations of metals were very low.

8. Re-use of worm biomass

L. variegatus is capable of accumulating heavy metals in very high concentrations. Chapman *et al.* (1999) for example mentioned bioconcentration factors of 22x and 16x for Cu and Cd respectively in only 14 days from a sediment with low organic content. Clearly however, in both experiments in this chapter the concentrations of heavy metals in *L. variegatus* grown on sludge for long periods remained usually below those in sludge. Only Cd and Zn in Experiment 1 were found in similar concentrations in sludge and worms. Chapman *et al.* (1999) found that bioaccumulation of heavy metals by *L. variegatus* from contaminated sediments is negatively correlated to the organic matter content of these sediments. The low bioaccumulation may therefore result from binding of the metals to the organic fraction of the sludge (57-66 %, Table 8.2), which is much larger than that of sediments (typically a few percent). In analogy, Tubificidae are known to bioaccumulate heavy metals, dependent on environmental conditions like organic matter concentrations (e.g. Bervoets *et al.*, 1997). However, similar to Tubificidae, *L. variegatus* almost exclusively digests the organic fraction of the sludge (which contains most metals) and most likely regulates metal uptake. Tubificidae are known to possess detoxification mechanisms for metals like internal compartmentalization and binding to metallothionein-proteins (e.g. Mosleh *et al.*, 2006). These proteins possibly are also involved in excretion of the metals and a similar protein was detected in *L. variegatus* (Bauer-Hilty *et al.*, 1989). In support of this, the metal concentrations in the worms in both experiments were independent of the concentrations in the feeds (sludge or fish feed). This was especially obvious in Experiment 1 for Cu and Zn (Figure 8.4a). Gunn *et al.* (1989) also found that concentrations of Zn in Tubificidae were independent of the substrate concentration and they proposed this was due to uptake regulation. Hansen *et al.* (2004) measured heavy metal concentrations in *L. variegatus* grown on clean control substrate and polluted sediments for 43-65 days. The concentrations in the worms were in the same range as in Figure 8.4. They also concluded that heavy metal concentrations in the worms did not appear to reflect the concentrations in the substrate. Bioaccumulation can thus be limited, which is consistent with the results in Figure 8.4.

Metal sequestration in invertebrates is a complex mechanism and known to depend on several factors: metal type, life history and metal pre-exposure (Vijver *et al.*, 2004). Metals can be present in many chemical forms, for example bound to certain proteins (e.g. metallothioneins) or stored in extracellular granules. It is therefore not clear which basic fraction(s) of worm biomass (protein, fat, sugar and ash) contain(s) the heavy metals. This requires further investigation.

8.3.3 Applications of *L. variegatus* grown on sludge

8.3.3.1 Worm biomass production

Selection of useful applications for (components of) worm biomass is firstly determined by its composition, but also by the produced quantity, as it possibly has to compete with other waste streams of similar composition.

The current waste sludge production of the almost 400 WWTPs in the Netherlands is around 350,000 tons of dry solids per year (CBS, 2007). If all this sludge would be consumed by *L. variegatus*, around 7 % of this amount could be converted into new worm biomass, which equals almost 25,000 tons of dry weight (and 190,000 tons of wet weight). In comparison to other protein rich waste streams, for example 240,000 tons of dry weight per year of meat and poultry meal in the Netherlands, this is a small stream, which is produced decentrally. Local processing is therefore preferable for ecological and economical reasons. Also, applications based on rare components of the worm biomass are most promising, because the produced amount is too small to compete with waste streams of comparable composition.

8.3.3.2 Applications

Worm biomass grown on sludge contains heavy metals, which may limit applications, but most concentrations (except Cd and Zn in some cases) were much lower in worm biomass than in the sludge. Also, worms grown on commercial fish feed with much lower heavy metal concentrations, contained similar concentrations. Other pollutants originating from these undefined sludges may be present, such as organic micropollutants. Depending on their binding to the organic fraction of the sludge, their concentrations in worm biomass may be low, as was the case for heavy metals, but this needs to be analyzed. Another topic for further investigation is the possible presence of human pathogens (e.g. bacteria like *E. coli* and enterococci, viruses and protozoa) from the sludge in worm biomass. It is unknown if pathogens in the sludge are killed during gut passage in *L. variegatus*, similar to the 85-98 % decrease in coliforms in certain earthworm species (Monroy *et al.*, 2008). As mentioned before, applications in the human food chain (e.g. as cattle or consumption fish feed) are therefore not an option. However, for worm biomass grown on defined sludges originating from safe sources (e.g. sludges from food industries) such applications may be an option.

Applications based on the results for the biomass composition are presented in Table 8.3. They are subdivided in three categories: total biomass (live or dead) or specific components of the biomass. These specific components can be obtained for example by rendering (heating of the material, which results in separation of the fat fraction). The applications are discussed below in more detail.

8. Re-use of worm biomass

Table 8.3 Applications for *L. variegatus* grown on sludge.

Product	Application
1. <i>Live biomass</i>	Feed for non-food animals
2. <i>Dead biomass</i>	Feed for non-food animals Energy Fertilizer
3. <i>Specific components</i>	Various applications
Protein / amino acids	
Fat / fatty acids	
Sugar	
Other	

1. *Live biomass*

- Feed for non-food animals

Based on its protein and amino acid content, live *L. variegatus* is a very suitable feed for aquarium fish or other ornamental aquatic animals (flatworms, crayfish, leeches, shrimps, insect larvae, reptiles and fiddler crabs) (Drewes, 2005, Mount *et al.*, 2006).

2. *Dead biomass*

- Feed for non-food animals

Dead biomass can be used as (addition to) feed for non-food animals.

- Energy

Dead biomass can be used for biogas production in existing anaerobic digesters at WWTPs. Experiments have shown that the specific biogas production of worms is three times that of sludge (Chapter 5). However, during production of worms, sludge is broken down which in turn decreases biogas production from sludge. If we assume a sludge dry matter breakdown percentage of 20 % and a worm growth yield of 7 %, the net biogas gain will be the same for a system with or without worms. This could be further investigated. Another application may be biofuel. This is further discussed under the next category. Co-incineration, as is currently done with most waste sludge in the Netherlands, is an option, if the worm biomass can not be used for other purposes. In this case, it should be investigated whether reduced costs for sludge processing and transport, resulting from sludge reduction by worms, can compensate the loss in energy generation (less biomass for incineration).

- Fertilizer

Worm biomass has a high nitrogen content and therefore a high potential as fertilizer. However, for use as fertilizer, heavy metal concentrations in the biomass are important. As a result of this, the use of sewage sludge for this purpose is no longer allowed. The current limit values in the Netherlands according to the BOOM regulations for sludge are 1.25 mg Cd, 30 mg Ni, 75 mg Cr, 100 mg Pb, 75 mg Cu and 300 mg Zn per kg of dry solids. The concentrations measured in the worms (Figure 8.4) exceed these limits for Cd and Zn. Therefore, only a partial replacement of other fertilizer materials would be an option. In most other countries, the permitted concentrations are however higher (e.g. EU limits). As mentioned before, the presence of pathogens is another point of concern.

3. Specific components

- Protein or amino acids

Protein constitutes the largest fraction of *L. variegatus* and can for example be extracted under acidic or basic conditions followed by iso-electric precipitation. If this fraction is unpolluted, application as animal feed is an option. Other outlets for this protein could be technical applications like coatings, glues, emulsifiers, dispersing, foaming or wetting agents (Vaz *et al.*, 2003). For Tubificidae protein, the first two applications (coatings and adhesives) were tested (Winters, 2004). From the latter research it was concluded that the use of this protein as glue had potential. This is most likely also true for *L. variegatus* protein, because of its mainly low molecular weight (14-20 kD). Regarding the relationship between the molecular weight and possible technical applications, the desired molecular weight decreases in the order thermoplastic materials and coatings, adhesives and surfactants. The protein thus has the most perspectives in the field of adhesives and surfactants. Another option, which combines glueing and surfactant properties, would be use as adjuvants in agro-chemicals.

Amino acids have multiple applications. Separate amino acids are traditionally used as additions to animal feeds or as taste enhancer in human food. Arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine are essential amino acids that can not be synthesized de novo by mammalian cells. Methionine and lysine are produced in the largest quantities, followed by threonine and tryptophan. These amino acids constitute respectively 2, 7, 6 and 1 % of *L. variegatus* protein. More and more, amino acids are used for manufacturing industrial products (e.g. Scott *et al.*, 2007). They describe the production of a large range of valuable chemicals from amino acids, e.g. from plants. Lysine can for example be used for modification reactions.

8. Re-use of worm biomass

- Fat or fatty acids

Vegetable oils and animal fats are mainly applied in food (80 %). The remainder is used for industrial applications. Poly-unsaturated oils like linseed oil and soy oil are used for manufacturing resins in paint and ink industries. Recently, much attention has been given to the application of vegetable oils and animal fats as biodiesel. The fat fraction of *L. variegatus*, which can be obtained by rendering the worm biomass, could be used for this purpose. However, other organisms grown on waste water contain higher fat concentrations. Angerbauer *et al.* (2008) for example describe the conversion of sewage sludge into yeast biomass and finally biodiesel. The yeast biomass has a fat content of up to 60 % of the dry weight. Also, micro-algae grown on waste water and sunlight can be used for the production of biofuels and contain similar fat concentrations to *L. variegatus*.

Fatty acids are mainly applied in the cosmetic (e.g. soaps and other surfactants) and lubricant industry. Other important applications of fatty acid derivatives are cleaning products, plastics and fabric softeners. The fatty acid composition of *L. variegatus* has not been determined but when grown on fish feed (Figure 1 in Mount *et al.*, 2006), this species contains interesting fatty acids, such as the polyunsaturated (omega-3 and omega-6) EPA, DHA and AA. Recently, much attention has been paid to these PUFAs (polyunsaturated fatty acids). They are classified according to the fatty acids they are derived from: omega-6 PUFAs from linoleic acid and omega-3 PUFAs from alpha-linolenic acid. These essential fatty acids are very important for mammalian growth and development but can not be synthesized *de novo*. They must be obtained from the food. Related species, for example earthworms, are known to contain unusual fatty acids that are otherwise found in marine mammals, fish and herbivorous land animals (Hansen and Czochanska, 1975). Essential fatty acid concentrations measured in *T. tubifex* and earthworms are 22-37 and 5 % of total fatty acids respectively (e.g. Yanar *et al.*, 2003). Fatty acid composition and total content is known to vary in natural populations of aquatic and terrestrial Oligochaeta (Hansen and Czochanska, 1975, Sushchik *et al.*, 2006). This is related to the composition and availability of their feed. Apparently the feed dictates appearance, fat content, fatty acid content and composition and possibly other biomass characteristics as well. Analysis of fatty acids in *L. variegatus* grown on different sludges is therefore very interesting.

- Sugar

This fraction can be used as starting materials for the production of biobased chemicals.

- Other components

The presence of other components, like enzymes, hormones, aromas and flavourings has not been investigated thoroughly, but deserves more attention. Some of the enzymes that have been found in *L. variegatus* are proteases, catalase, peroxidase, glutathione S-

transferase, cholinesterase and δ -aminolevulinic acid dehydratase (e.g. Wiegand *et al.*, 2007). Also, carbohydrases (e.g. cellulase) and proteases have been detected in earthworms (e.g. Lattaud *et al.*, 1998). Cellulases, proteases and lipases can for example be used for sludge reduction and improved sludge settling (Roman *et al.*, 2006) or in detergents (Boer and Sova, 1998).

8.4 Conclusions

This chapter describes a systematic approach for finding new applications for *L. variegatus* biomass grown on biosolids. This biomass can be produced during waste sludge reduction at municipal waste water treatments plants and mainly consists of protein and smaller fractions of fat, sugar and ash respectively. The worms contain relatively low amounts of heavy metals, compared to the concentrations in the sludge, but the presence of these and possible other pollutants (i.e. micropollutants and pathogens) makes direct application in the human food chain impossible. Applications were divided into three categories:

1. live biomass (e.g. (addition to) non-consumption animal feed)
2. dead biomass (e.g. (addition to) non-consumption animal feed, fertilizer or energy)
3. specific components (e.g. amino acids, fatty acids and possibly enzymes).

Fatty acids content has not been determined but seems very interesting, based on literature data.

The biomass is produced decentrally in a relatively small amount, which makes competition with comparable waste streams (e.g. slaughter waste) difficult. Therefore, specific components (specific amino acids, fatty acids, etc) of worm biomass have more added value than bulk biomass or the protein or fat fraction. Worm biomass grown on defined sludges has a broader application potential, and is very suitable as, for example, consumption fish feed. Both points deserve further investigation. However, until the process is optimized and there is a stable supply of worms, the focus should be on simple applications, later on followed by specific applications, depending on the market demand.

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Chapter 9

Concluding discussion

Abstract

The research presented in this thesis has shown that sludge reduction with *Lumbriculus variegatus* is a feasible technology. A lab-scale batch technique was successfully transformed into a continuous reactor configuration that is suitable for a full-scale application. Continuation of this research can be along two promising paths of application. Firstly, focus can be on the application of sludge reduction and compaction at municipal waste water treatment plants (WWTPs). A pilot reactor should be operated to allow further optimisation of the process, including the further thickening of worm faeces and a suitable technique to harvest the worms. It will also provide information for a more accurate economic evaluation. Based on the current estimates, the economic perspective for a worm reactor at a small WWTP is good, with a payback period on the construction costs of 7-15 years. Secondly, focus on the production of clean worm biomass allows the use of excess worm biomass in aquaculture. This greatly increases the economic value of the worms and thereby the economic perspective of the process. Finally, a third, more fundamentally orientated line of research is to determine which fraction of the sludge is digested by the worms. This can provide valuable information on the applicability of the worm process.

9. Concluding discussion

9.1 Introduction

This thesis has demonstrated that the aquatic worm *Lumbriculus variegatus* can be used to reduce the amount of biological sludge produced by waste water treatment plants (WWTPs). A new concept for a worm reactor was introduced where the *L. variegatus* is immobilised in a carrier material (Chapter 2). The main advantages of this concept are the retention of the worm biomass in the reactor and separate collection of the compact worm faeces, which is favourable for further processing. An additional advantage is the production of protein-rich worm biomass, as this may have several applications for re-use (Chapter 8). The experimental work described in this thesis provides the design parameters for a worm reactor and information on the conditions under which it should be operated (Chapters 3, 4 and 6). As mentioned in the previous chapters, a worm reactor will be most interesting for smaller WWTPs, which do not have an anaerobic digester and sludge dewatering facilities. This chapter will therefore discuss the implications and economic perspective of a worm reactor at such a small scale WWTP (35,000 p.e.). This is followed by a discussion on the technological issues that need further investigation before a full-scale worm reactor can be applied. The work in this thesis focussed on reducing and compacting the amount of sludge from municipal WWTPs. The application of the worm process on other, “cleaner”, sludges will be discussed, as it may produce worm biomass that can be used as a live food source in aquaculture.

9.2 Worm reactor at a small WWTP

Figure 9.1 summarizes the implications of a worm reactor treating the waste sludge from a 35,000 p.e. WWTP, based on the results presented in this thesis and shown in Table 9.1.

Table 9.1 Design parameters for a worm reactor.

TSS reduction by worms	25	%
Worm biomass density	1	kg ww/m ²
TSS consumption rate by worms	50	g TSS/(kg ww · d)
dry weight / wet weight of worms	0.14	(-)
Worm biomass yield	0.2	kg dw/kg TSS digested
Carrier surface per reactor volume	22.5	m ² /m ³
Height of worm reactor	3	m

The worm reactor reduces the amount of waste sludge by 25 % (TSS reduction by the worms) and, most importantly, causes a 62 % reduction in the volume that needs to be transported to centralized sludge processing facilities. Additionally, 80 kg/d dry weight (dw) of potentially valuable worm biomass is produced. The worm reactor would have a footprint of 439 m². This compares to about 20 % of the surface area of the secondary

settler at such a WWTP and is therefore considered feasible. With the worm reactor, the additional internal nutrient loads on the WWTP are 5 and 17 % for total nitrogen and total phosphorus, respectively. The additional internal nitrogen load is small and the WWTP can be expected to have sufficient capacity to deal with this. For phosphorus, removal of the additional internal load will require expansion of the existing capacity, which can for example be achieved by dosing (more) ferric chloride. The additional internal hydraulic load is 11 % (assuming that the influent / sludge return ratio is 1) and its impact on the secondary clarifiers should be taken into consideration. However, experience with a lab-scale worm reactor demonstrated that the required effluent flow through the worm reactor (Chapter 7) may in practise be much lower.

Further treatment of the thickened worm faeces will have to take place at a centralised location. Here, the methane production by anaerobic digestion will be about 50 % lower, due to the lower organic fraction of the faeces and a lower methanisation yield of the remaining organic material (Chapter 5). However, the load on dewatering, transport of the dewatered sludge and incineration of the sludge will be reduced by about 25 %.

9. Concluding discussion

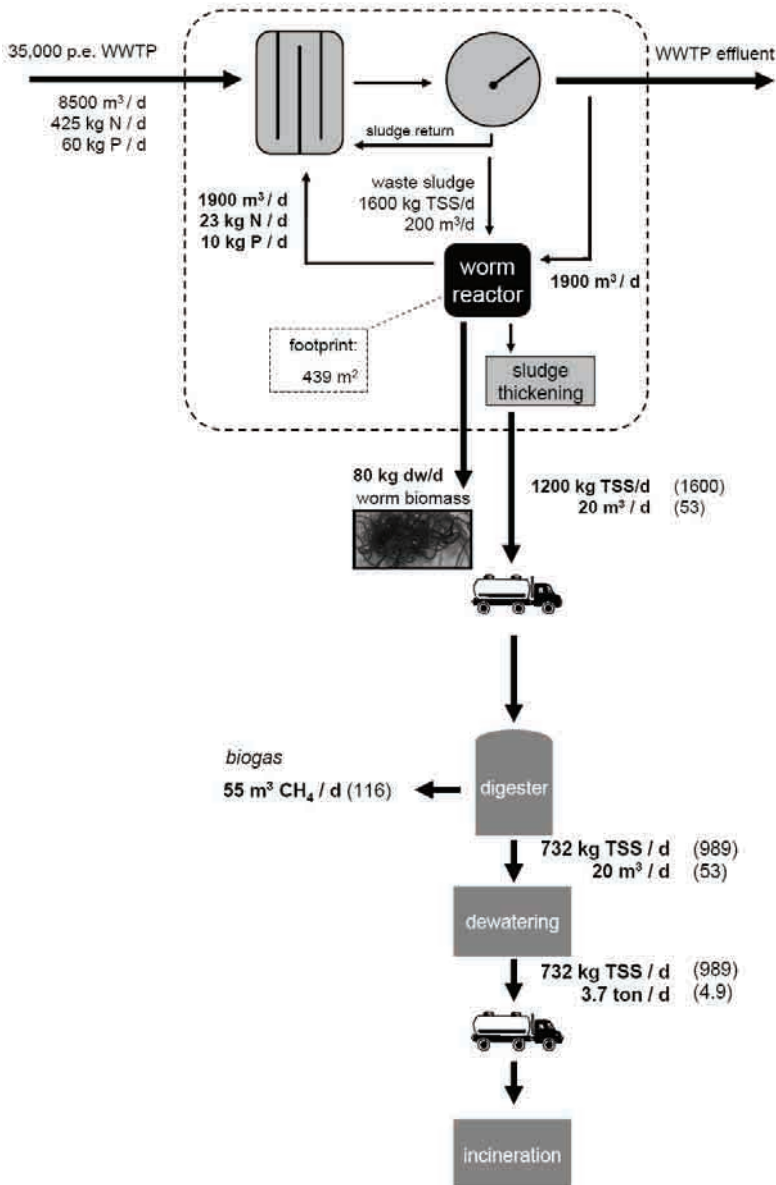


Figure 9.1 Overview of the effects of a worm reactor at a 35,000 p.e. WWTP and the further sludge processing steps. The numbers in brackets give the sludge load leaving the WWTP before installation of the worm reactor.

9.3 Economic perspective

The yearly costs and savings of a worm reactor treating the waste sludge at a 35,000 p.e. WWTP were estimated and are presented in Table 9.2. The estimation is based on the design data determined in this thesis (Table 9.1) and the information provided by STOWA (2005) and Elissen (2007), shown in Table 9.3. The thickened sludge from such a small WWTP is further treated at a centralised location, where it may represent only a small part of the total sludge load (Chapter 1). The potential impact on the required size of sludge processing equipment (and its maintenance costs) at this centralised location has, therefore, not been included in the evaluation, but should be included in a detailed economic feasibility study. The following economic perspective is mainly aimed at identifying where most cost savings can be made.

Table 9.2 Estimated costs and savings of the worm reactor at a 35,000 p.e. WWTP.

<i>x 1000 € / y</i>	WWTP	WWTP + worm reactor	Difference
Thickening	11.8	8.9	3.0
Transport thickened sludge	35.0	13.1	21.9
Sludge digestion, biogas	7.4	3.5	-/ 3.9
Nutrients in worm biomass		-/ 0.5	0.5
Dewatering	16.2	12.0	4.2
Transport dewatered sludge	8.1	6.0	2.1
Incineration	90.3	66.8	23.5
subtotal – cost savings			51.3
Aeration worm reactor		1.3	-/ 1.3
Operational costs worm reactor		23.7	-/ 23.7
subtotal – additional costs			-/ 25.0
Total			26.3
potential income worm biomass			29.2

9. Concluding discussion

Table 9.3 Assumptions for the calculations on the economic perspective (STOWA, 2005, Elissen, 2007). PE = poly-electrolyte, ds = dry solids.

Sludge incineration	250	€/ton ds
Cost of electricity	0.05	€/kWh
Electricity from biogas	3.5	kWh/m ³ methane
Transport thickened sludge	1.8	€/m ³
Transport dewatered sludge	4.5	€/m ³
Use of PE for thickening	4.5	g PE/kg TSS
Use of PE for dewatering digestate	10	g PE/kg TSS
Use of FeCl ₃ for P-removal	1.8	kg FeCl ₃ /kg P
Cost of PE	4.5	€/kg PE
Cost of FeCl ₃	173	€/ton
Aeration	0.5	kWh/kg O ₂
Construction costs	300	€/m ³
Operational costs	6	% of capital costs/y
Worm biomass	1	€/kg dw

At the WWTP itself, only a small cost reduction in thickening is achieved due to the 25 % reduction in the amount of solids accomplished by the worm reactor. By far the largest cost reduction is achieved on transportation of the thickened solids, because of the two times higher solids concentration that can be achieved by thickening of worm faeces compared to the original waste sludge. This was shown to be feasible for sludge from the Leeuwarden municipal WWTP and further improvements are expected, as thickening of the worm faeces was not yet optimised (Chapter 5). An improved settleability compared to the original waste sludge was achieved for several other types of sludge (Figure 9.2), indicating that this improved thickening is not exclusively related to the sludge we used in our experiments. Considering the nutrient removal at both the small WWTP (nutrient release in the worm reactor) and the centralised sludge processing location (nutrient release in anaerobic digestion), an overall lower load may be expected, as part of the nitrogen and phosphorus loads is removed with the worm biomass.

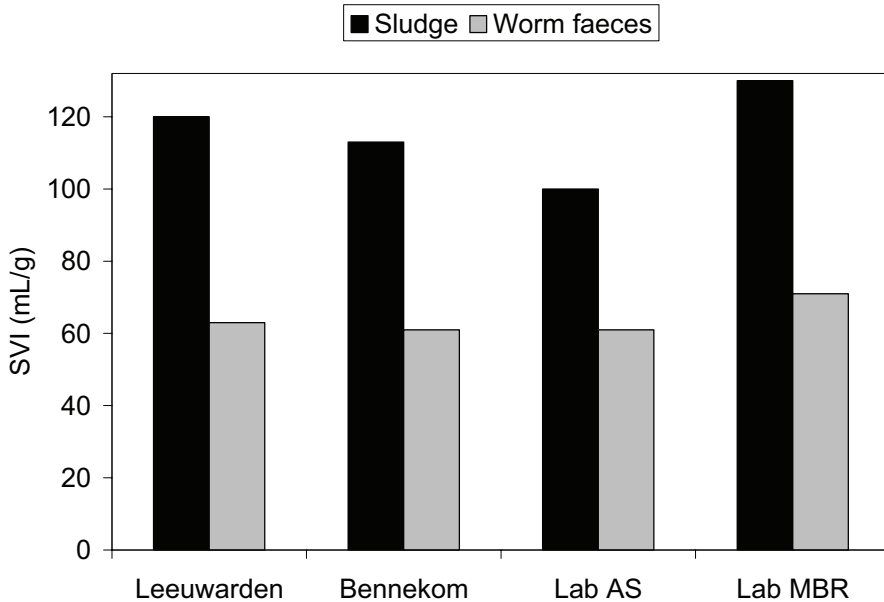


Figure 9.2 Comparison of the SVI of sludge and worm faeces for the four types of sludge (adapted from Hendrickx *et al.*, 2008). Leeuwarden and Bennekom are municipal WWTPs, the AS (conventional activated sludge) and MBR (membrane bioreactor) systems were operated in the lab.

After transportation of the sludge to centralized sludge processing facilities (Figure 9.1), some of the cost savings are lost because less biogas is produced (Chapter 5). However, this is more than compensated for by savings made on further dewatering of the digested sludge and transportation of the dewatered sludge to incineration facilities. Here, further cost savings can be made, due to the lower amount of solids than need to be incinerated compared to the original waste sludge. However, as the worms already digested mainly the organic fraction of sludge, the solids can be expected to have a lower heat value, which may result in higher associated incineration costs. From an energy point of view, the lower heat value of the worm faeces and the lower biogas production may be offset by the savings on energy consumption during solids transportation and dewatering. This could be included in a detailed feasibility study.

The extra costs that are introduced with a worm reactor are for aeration of the worm reactor and for its operational costs. However, Table 9.2 shows that the operational costs of the

9. Concluding discussion

worm reactor represent the majority of these additional costs. These operational costs include operation costs of the reactor, maintenance costs and depreciation costs.

The potential income from the excess worm biomass has a huge impact on the economic perspective of a worm reactor. Based on the estimates in Table 9.3, an income of 1 €/kg dw can offset the additional costs of the worm reactor. As a result, the payback period on the construction costs is only 7 years, compared to 15 years without income from worm biomass, provided that a suitable market for the worm biomass is found. When used as live feed for ornamental fish, 1 €/kg dw is a reasonable estimate. However, when the worms are applied as fish feed in aquaculture (fish for human consumption), the value of the worms can be as high as 40 €/kg dw (Australian blackworms, 2009). This will, however, be limited to worms grown on “cleaner” sludges, such as those produced in the food industry, which will be further discussed in paragraph 9.6. The worst case scenario is that no application for the worm biomass is found, which leaves methanisation of the worm biomass as the final option (Chapter 5). This would yield approximately 0.05 €/kg dw as electrical power, thereby only marginally improving the economic perspective.

Finally, the construction costs of a worm reactor were estimated at 300 €/m³, resulting in a total construction cost of € 395,000. With the information from Table 9.1, the payback period on the construction costs would be only 7 years, which makes it a very attractive process. Higher or lower construction costs will obviously have a huge impact on the payback period. In the range of 200 to 400 €/m³, this leads to 4 to 11 years of payback period, respectively (with selling the worms). Also the operational costs of a worm reactor (now 6% of the investment costs per year) have an important impact on the economic perspective. More accurate estimates of these costs can be obtained once a more detailed design and more experience with operating a larger worm reactor are available. Both can be achieved by constructing and operating a pilot reactor of e.g. 1-2 m³, which (partially) treats the waste sludge from a WWTP.

9

9.4 Pilot-scale worm reactor

A larger reactor was operated successfully for 8 weeks in the lab (Chapter 7), yet several technological questions remain to be answered. Most importantly, the reactor was not operated until the complete surface area of carrier material was populated by worms and all the sludge was consumed by the worms. Longer operation of a pilot reactor will show how much sludge passes unconsumed through the worm reactor and whether worms are washed out with this reactor outflow. The significance of operating a pilot reactor for a longer period is also to assess the response to daily and yearly process fluctuations, such as changes in temperature, ammonia concentration in the effluent, sludge composition and

amount of waste sludge. Particularly the effect of temperature is important, as it has a large impact on consumption rate by the worms (Chapter 4). Furthermore, sludge characteristics were shown to result in large differences in sludge consumption rates by the worms (Chapter 6). To a lesser extent this can also be expected to occur at a WWTP due to the variation in sludge composition over the year, caused by seasonal changes in waste water composition, process temperature and sludge retention time. Another aspect that is expected to be improved, is thickening of the worm faeces (Chapter 5). With a pilot reactor, larger amounts of worm faeces become available, which can be tested in larger scale and more realistic thickening equipment. This is expected to lead to an even higher compaction of the faeces than was found in the batch experiments.

An issue that has not been addressed in this thesis is how to harvest the worms. When there is no application for the worm biomass and they are not harvested from the reactor, a dynamic equilibrium will be reached between worm growth in the reactor and loss of worms with the worm faeces and potentially the reactor outflow. However, when the worm biomass is a valuable resource, it needs to be harvested from the reactor. The sequencing batch experiments showed that the worms remained in the carrier material despite lowering the DO concentration in the water compartment (Chapter 4) or not providing substrate in the sludge compartment (Chapter 6). A large salt gradient is known to remove worms from the carrier material (Elissen, 2007), but this does not seem like a feasible option in the current reactor configuration. An alternative method for harvesting the worms is therefore required.

9.5 What do worms digest?

Large differences were observed in the sludge consumption rates by the worms when different sludge types were used, ranging from 50 to 260 g TSS/(m²·d) (Chapter 6). The reason for these differences remained unclear. Sludge is a complex mixture of (dead) bacterial cells, extracellular polymeric substances, adsorbed organic and inorganic material and other components. Although the worms could consume all the sludge, a wide range of digestion percentages was found (5-75 %). It is not clear which components or fractions are preferentially taken up by the worms, apart from perhaps nitrogen which appeared to be specifically taken up by the worms (Chapter 5). Little is known about the digestive processes of *L. variegatus*, for example whether the worm produces its own digestive fluids (Moore, 1978) or possesses a (specific) bacterial community in its gut. A detailed investigation into the composition of the worm faeces, compared to the waste sludge, could provide information on which components or fractions of the sludge are used by the worms. The potential of a worm reactor for certain sludge types may then also be predicted, which can now only be assessed in time-consuming batch experiments. Sludge characteristics can

9. Concluding discussion

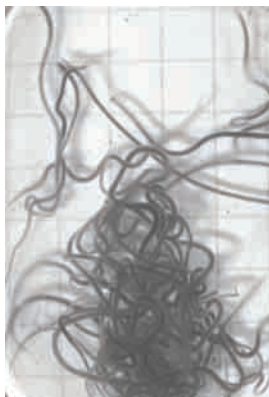
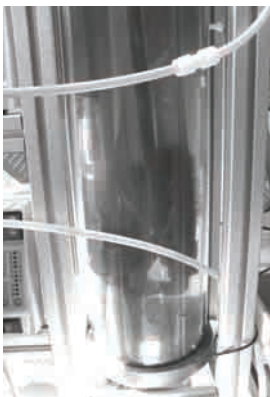
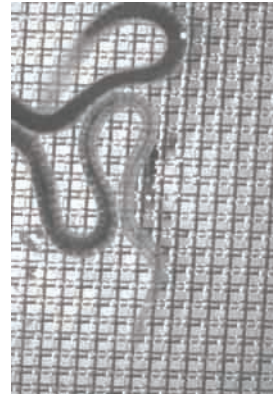
have a huge impact on the size of a worm reactor, which is best seen when comparing the estimated reactor sizes for the same amount of waste sludge but for two different sludge types. The results used in this chapter lead to a reactor volume of 1300 m³, whereas a volume of only 590 m³ was calculated in Chapter 7 where a different sludge type, with a higher specific sludge consumption rate, formed the basis for design.

9.6 Worm biomass production on clean sludge

The specific protein and amino acid content of *L. variegatus* make it a very suitable live fish feed, as was discussed in Chapter 8 and is already applied in practise (Australian blackworms, 2009). For worms grown on sludge from municipal WWTPs, this application is not an option, because of possible accumulation of pollutants in the worm biomass. But when “cleaner” and defined sludges are used, such as those from certain food industries, the worms have a much higher economic value and the main focus can then be on producing as much worm biomass as possible. Immobilising the worms in a carrier material is a disadvantage for this application, as it limits the growth rate of the worms (Chapter 6). Instead, depending on the characteristics of the sludge, a simple system with sieves could easily separate the (larger) worms from a mixture of sludge and worms. An additional benefit is that this would significantly reduce the investment costs in a worm reactor, thereby greatly improving the economic perspective of such a system. An important next step for this application is to determine the detailed composition of the worm biomass and how this is dependent on the food source of the worms. Also here, more detailed information on the digestive processes in the worms’ gut is required to make this link between food source and worm biomass composition.

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Summary

Biological waste water treatment plants (WWTPs) produce large amounts of waste sludge. This sludge contains valuable nutrients and organic carbon, but due to increasingly stringent legislation on the presence of heavy metals and pathogens, the application of this sludge in agriculture has become a limited disposal route. As a result, incineration is becoming the main disposal route for WWTP sludge. **Chapter 1** describes the whole sludge processing chain with incineration as the final treatment step. A beneficial use of sludge is currently limited to the (partial) recovery of energy by anaerobic digestion (as methane) and by (co)incineration of dewatered sludge. The cost of sludge processing may represent as much as 50 % of the operational costs at a WWTP. Consequently, sludge reduction techniques have gained a lot of attention. Several chemical, physical and mechanical techniques are available to disrupt the sludge, followed by mineralization of the (recycled) lysis products in the WWTP. However, these techniques require a significant input of energy and / or chemicals and are costly. A biological approach may be more attractive, such as the use of aquatic worms, which decreases net biomass production by extending the food chain. Initially, attempts were made to promote the growth of the aquatic worms that naturally occur at WWTPs. Unfortunately, this proved impossible to control and therefore focus shifted towards a separate worm reactor to treat the sludge after it has been wasted from the WWTP. This has, however, not yet led to a concept that is applicable at full-scale. Recently, sludge reduction with *Lumbriculus variegatus* was introduced, an aquatic worm which is not commonly observed at WWTPs. In a new reactor concept these worms are immobilised in a carrier material. The objective of this thesis is to investigate the feasibility of this new concept and to develop a continuous worm reactor suitable for sludge reduction by *L. variegatus* at small full-scale WWTPs.

In **chapter 2**, initial sequencing batch experiments show the potential of the new reactor concept for sludge reduction with *L. variegatus*. In this concept, the worms are immobilised in a carrier material made of a polyamide mesh. The carrier material also separates the sludge compartment from the water compartment, which contains WWTP effluent. As the worms use their tails to take up oxygen, they are positioned with their head in the sludge compartment and with their tails protruding through the carrier material into the aerated water compartment. As a result, worm faeces are collected in the water compartment, and are therefore separated from the sludge. A clear sludge reduction by the worms was observed compared to a blank experiment without worms. The collected worm faeces had a compact morphology, which resulted in a sludge volume index (SVI) that was only half that of the initial sludge, which is very beneficial for further processing. Because the worm

Summary

biomass has a high protein content, it has potential for re-use. The main conclusion is that the new reactor concept has a large potential for decreasing the sludge processing costs at WWTPs.

For a full-scale application of the proposed worm reactor, one of the requirements is continuous operation with a stable worm population in the worm reactor. In **chapter 3** experiments were performed with continuous worm reactors receiving sludge from a lab-scale activated sludge system. Two mesh sizes of carrier material were used in the experiments, 300 μm and 350 μm . Mass balances over the worm reactors and blank reactors operated in parallel, made a clear distinction between the contributions of sludge reduction by the worms, natural sludge breakdown and accumulation of sludge in the reactor. After an experimental run of 3 weeks, no worm growth was observed with the 300 μm mesh, while a net worm biomass growth rate of 0.013 d^{-1} was found when using a 350 μm mesh. Another experiment was performed in which the sludge that was not consumed by the worms, was returned to the activated sludge system. This did not affect the performance (COD removal and nitrification) of the lab-scale activated sludge system.

To assess under which conditions the worm reactor can and should be operated, sequencing batch experiments with the worm reactor were performed in **chapter 4**. Dissolved oxygen (DO) concentration is an important factor, as it affected both sludge consumption rate and sludge digestion rate by the worms. A high consumption rate was found at a DO concentration above 8.1 mg/L with a total suspended solids (TSS) reduction of 36 % of the sludge consumed by the worms. At DO concentrations below 2.5 mg/L the consumption rate was four times lower, but corresponded with a much higher TSS reduction of 77 %. Temperature had an effect on sludge consumption rate and on TSS reduction. Consumption rates had a maximum around 15°C, whilst TSS reduction gradually decreased with increasing temperature in the range of 10 to 25°C. Temperatures below 25°C were required to ensure survival of the worms. An increased ammonia concentration in the water compartment, and in particular the unionised ammonia concentration, had a negative effect on the sludge consumption rate. Ammonia release by the worms was estimated at 20 mg N/g TSS digested. To maintain a sufficiently low ammonia concentration in the worm reactor (below 0.1 mg N/L of unionised ammonia), the effluent in the water compartment needs to be replaced at a certain rate. After passing through the worm reactor, this effluent is returned to the WWTP, along with the ammonia released by the worms. This was estimated to result in an additional hydraulic load of 5-15 % and an additional nitrogen load of 5 % on the WWTP. Aeration of the worm reactor to supply the worms with oxygen, was estimated to increase the overall oxygen demand at the WWTP with 15-20 %. Supply of

oxygen to the worms and removal of ammonia from the worm reactor were identified as the two main challenges for full-scale application.

To quantify the release of metabolic products and uptake of nutrients by the worms, mass balances were constructed in sequencing batch experiments in **chapter 5**. Of the nutrients in the sludge digested by the worms, 39 % of total nitrogen and 12 % of total phosphorus were used to form new worm biomass. Ammonia and phosphate were released by the worms into the water compartment at a rate of 58.0 mg NH₄-N/gTSS digested and 25.8 mg PO₄-P/g TSS digested. Heavy metals were not released from the sludge by the worms, but remained in the worm faeces. Only a small fraction (less than 0.8 %) of the total heavy metal load on a worm reactor is incorporated into new worm biomass. Thickening of worm faeces resulted in a two times higher solids concentration compared to sludge, resulting in a 67 % decrease in the volume of sludge that needs to be transported to centralised sludge treatment facilities. This makes a worm reactor most interesting for smaller WWTPs without an on-site digester and dewatering equipment, and therefore high costs for transport of thickened sludge. In further processing of the thickened worm faeces, methanisation of worm faeces resulted in a 40 % lower methane production than methanisation of the initial waste sludge.

The most important design parameter for a worm reactor is the surface specific sludge consumption rate. This is the product of stable worm biomass density in the carrier material and the worm specific sludge consumption rate. **Chapter 6** describes the sequencing batch experiments that were performed to determine this stable worm density, and how this is dependent on the mesh size of the carrier material. Stable worm densities of 0.87 and 1.1 kg ww/m² were achieved in a 300 µm and 350 µm mesh size carrier material, respectively. The surface specific consumption rates at these densities were 45 and 58 g TSS/(m²·d), respectively, which would lead to a 29 % smaller worm reactor when a 350 µm mesh is used instead of a 300 µm mesh. To maintain a maximum sludge consumption rate by the worms, a sludge load of at least 100 mg TSS/(gww·d) was required. Other sludge types were assessed for their surface specific sludge consumption rate as well. This indicated that large differences can be expected, but the cause for this remained unclear. The biomass decay rate of 0.023 d⁻¹ for immobilised worms was much higher than the decay rate of 0.018 d⁻¹ for free worms. Consequently, the net biomass growth rate of 0.009-0.011 d⁻¹ for worms immobilised in a carrier material is lower than net biomass growth rate of 0.026 d⁻¹ for free worms. Finally, the specific oxygen uptake rate by the worms was 4.9 mg O₂/(g ww·d), which needs to be supplied to the worms by aeration of the worm compartment.

Summary

To overcome the problems of a limited worm growth, a new reactor configuration was designed in **chapter 7**. In this new configuration the carrier material is not orientated horizontally, but vertically and placed as cylinders in the water compartment. A larger worm reactor of this new configuration treated 8 g TSS/d of waste sludge from a lab-scale activated sludge system. The net worm biomass growth rate was 0.014 d^{-1} , which is higher than in experiments with a horizontal carrier, but still lower than for free worms. Mass balances for nutrients over the worm reactor showed a nitrogen release of 64 mg ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$)/g TSS digested by the worms and a phosphate release of 20 mg $\text{PO}_4\text{-P}$ /g TSS digested. The ammonia released by the worms was partially nitrified in the worm reactor. Consequently, a lower effluent flow rate through the worm reactor would be required to maintain a sufficiently low ammonia concentration in the worm reactor (below 0.1 mg N/L of unionised ammonia). Compared to a horizontal carrier material, the main advantages of the new reactor configuration are a higher net worm biomass growth rate, a smaller footprint, easier collection of the worm faeces and easier aeration of the water compartment to supply the worms with oxygen.

Potential applications of worm biomass grown on sludge from municipal WWTPs are discussed in **chapter 8**. An analysis of biomass composition showed that the worms mainly contain protein and smaller fractions of fat, sugar and ash. Although no bioaccumulation was observed, contamination of the worm biomass with heavy metals and potentially pathogens and organic micropollutants excludes the application in the human food chain. The remaining most promising applications are: 1) live worm biomass as an addition to non-consumption animal feed, 2) dead worm biomass as food additive to non-consumption animal feed, fertilizer or energy source and 3) use of specific compounds from the worm biomass. Particularly the latter deserves more attention, as specific amino acids and fatty acids may have a high added value. Also the production of worm biomass grown on more defined sludges, from e.g. the food industry, deserves more attention, as these may produce worm biomass that can be used in the human food chain and, therefore, has a higher added value. Additional aspects that should be considered in selection of a suitable application for the worm biomass are the decentralised production (at several WWTPs) and the competitiveness with existing resources of comparable composition.

Chapter 9 concludes with the economic perspective of a worm reactor at a small 35,000 p.e. WWTP without an on-site sludge digester and dewatering equipment. An overview of the estimated savings showed that the largest cost reductions were achieved on transport of the thickened worm faeces and on incineration of the solids at a centralised location. An additional income is the worm biomass that is produced, although a suitable market is yet to be found. Some costs savings are made in nutrient removal, as nitrogen and phosphorus are

partially removed with the worm biomass, but some income is lost due to a lower biogas production at a centralised location. Most costs are associated with the construction and operational costs of the worm reactor. The current perspective of a worm reactor at a small WWTP is good, with an estimated payback period of 7-15 years. Operation of a pilot reactor is recommended to further optimise the technological details and to obtain more operational experience with a large worm reactor. This will provide the information required for a more detailed economic evaluation. Such a pilot reactor can also be used to further optimise thickening of worm faeces and to develop a method to harvest the worms. Production of clean worm biomass is a second recommendation, as it may have a much higher added value than worm biomass grown on sludge from municipal WWTPs. This requires worm grown on clean sludges, such as those from the food industry. When growth of worm biomass is the main objective, immobilisation of the worms is not desirable, as it decreases the growth rate of the worms. Finally, more information is required on which fractions of the sludge are digested by the worms. This may also predict which type of sludge has most potential for applying a worm reactor.

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Samenvatting

Rioolwaterzuiveringinstallaties (RWZI's) produceren grote hoeveelheden biologisch afval-slib. Dit slib bevat kostbare nutriënten en organisch koolstof, maar de toepassing van dit slib in de landbouw is een beperkte afzetroute vanwege de strengere wetgeving op de aanwezigheid van zware metalen en pathogenen. Gevolg is dat verbranding de meest belangrijke afzetroute geworden is voor afvalslib. **Hoofdstuk 1** beschrijft de gehele slibverwerkingsketen met verbranding als de laatste behandelstap. De nuttige toepassing van slib is momenteel beperkt tot de (gedeeltelijke) terugwinning van energie door anaerobe vergisting (in de vorm van methaan) en door (co)verbranding van ontwaterd slib. De kosten van slibverwerking kunnen tot wel 50 % van de totale operationele kosten van een RWZI bedragen. Technieken om de hoeveelheid slib te verminderen hebben daardoor veel aandacht gekregen. Verscheidene chemische, fysische en biologische technieken zijn beschikbaar om het slib op te breken, gevolgd door de mineralisatie van de teruggevoerde afbraakproducten in de RWZI. Deze technieken vereisen echter een aanzienlijke inbreng van energie en chemicaliën en zijn kostbaar. Een biologische benadering zou aantrekkelijker kunnen zijn, zoals het gebruik van aquatische wormen die de netto biomassa-productie verminderen door het uitbreiden van de voedselketen. In eerste instantie werden pogingen gedaan om de groei te bevorderen van aquatische wormen die van nature voorkomen in RWZI's. Dit bleek echter een onbeheersbaar proces te zijn, waardoor de aandacht verschoof naar aparte wormenreactoren die het slib behandelen, afzonderlijk van de bestaande processen van een RWZI. Dit heeft echter nog niet tot een concept geleid dat toepasbaar is op volle schaal. Recentelijk werd de afbraak van slib door *Lumbriculus variegatus* geïntroduceerd, een aquatische worm die doorgaans niet bij RWZI's te vinden is. In een nieuw reactor concept zijn deze wormen geïmmobiliseerd in een dragermateriaal. Het doel van dit proefschrift is het onderzoeken van de haalbaarheid van dit nieuwe concept en het ontwikkelen van een continue wormenreactor die geschikt is voor slibreductie door *L. variegatus* bij kleine RWZI's.

In **hoofdstuk 2** laten batch experimenten het potentieel zien van het nieuwe reactor concept voor slibreductie met *L. variegatus*. In dit concept zijn de wormen geïmmobiliseerd in een dragermateriaal bestaande uit een polyamide gaas. Het dragermateriaal scheidt tevens het slibcompartiment van het watercompartiment, dat RWZI effluent bevat. Aangezien de wormen zuurstof opnemen met hun staart, positioneren ze zichzelf met de koppen in het slibcompartiment, terwijl de staarten door het dragermateriaal steken in het beluchte watercompartiment. Gevolg is dat de wormenkeutels in het watercompartiment worden opgevangen, gescheiden van het slib. Een duidelijke slibafbraak door de wormen werd

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waargenomen, vergeleken met een blanco experiment zonder wormen. Vergeleken met het oorspronkelijke slib, hadden de verzamelde wormenkeutels een compacte samenstelling, wat resulteerde in een twee keer zo lage slib volume index (SVI), hetgeen zeer gunstig is voor verdere slibverwerking. De eiwitrijke wormenbiomassa kan potentieel hergebruikt worden. De voornaamste conclusie was dat het nieuwe reactorconcept een grote potentie heeft voor het verminderen van de slibverwerkingskosten van een RWZI.

Eén van de voorwaarden voor toepassing op volle schaal van de wormenreactor is de continue bedrijving ervan met een stabiele wormenpopulatie. In **hoofdstuk 3** werden experimenten uitgevoerd met continue wormenreactoren die gevoed werden met slib uit een lab-schaal actief slib reactor. Twee dragermaterialen met verschillende maaswijdtes werden gebruikt, namelijk 300 en 350 μm . Massabalansen over de wormenreactoren en de parallel draaiende blanco reactoren, lieten een duidelijk onderscheid zien tussen de slibafbraak door wormen, natuurlijke slibafbraak en accumulatie van slib in de reactor. Na een experimentele periode van 3 weken, werd er geen wormengroei gemeten in de reactor met het 300 μm dragermateriaal, terwijl een netto groei van wormenbiomassa optrad bij gebruik van een 350 μm dragermateriaal. In een apart experiment werd slib dat niet door de wormen geconsumeerd werd, teruggevoerd naar het actief slib system. Dit had geen invloed op de zuiveringsprestaties (COD verwijdering en nitrificatie) van het lab-schaal actief slib systeem.

Het bepalen van de condities waaronder de wormenreactor kan en zou moeten worden bedreven zijn bepaald in **hoofdstuk 4**. De opgeloste zuurstof (DO) concentratie is een belangrijke factor, die zowel de slibconsumptiesnelheid als de slibverteringssnelheid door de wormen beïnvloedt. Boven een DO concentratie van 8.1 mg/L werd een hoge consumptiesnelheid gevonden, met een totaal gesuspendeerd stof (TSS) reductie van 36 % van het door de wormen geconsumeerde slib. Bij DO concentraties beneden 2.5 mg/L de consumptiesnelheid was een factor 4 lager, maar hier werd een hogere TSS reductie van 77 % bereikt. Temperatuur had een effect op zowel de slibconsumptiesnelheid als de TSS reductie. De consumptiesnelheid had een maximum rond 15°C, terwijl TSS reductie geleidelijk afnam bij oplopende temperaturen tussen 10 en 25°C. Een temperatuur beneden 25°C was vereist voor overleving van de wormen. Een toenemende ammonia concentratie in het watercompartiment, en in het bijzonder de concentratie niet-geioniseerd ammonia, had een negatief effect op de slibconsumptiesnelheid. Uitstoot van ammonia door de wormen werd geschat op 20 mg N/g TSS verteerd. Om een voldoende lage ammonia concentratie te behouden (lager dan 0.1 mg N/L niet-geioniseerd ammonia) moet het effluent in het watercompartiment vervangen worden met een bepaalde debiet. Na de wormenreactor gaat dit effluent met opgelost ammonia terug naar de RWZI. De extra

hydraulische belasting op de RWZI die dit oplevert, werd geschat op 5-15 % met een extra stikstofbelasting van 5 %. Beluchting van de wormenreactor om de wormen te voorzien van zuurstof resulteerde in een geschatte 15-20 % extra zuurstofbelasting voor de RWZI. Het voorzien van de wormen van zuurstof en het verwijderen van ammonia uit de wormenreactor werden aangemerkt als de twee belangrijkste uitdagingen voor toepassing op volle schaal.

Het kwantificeren van de uitstoot van metabolische producten en van de opname van nutriënten door de wormen, werd gedaan aan de hand van batch experimenten, beschreven in **hoofdstuk 5**. Van de nutriënten in het door de wormen verteerde slib werd 39 % van het totaal stikstof en 12 % van het totaal fosfaat gebruikt voor de groei van nieuwe wormenbiomassa. Ammonia en fosfaat werden uitgestoten in het watercompartiment door de wormen met een snelheid van 58.0 mg NH₄-N/g TSS verteerd en 25.8 mg PO₄-P/g TSS verteerd. Zware metalen werden niet door de wormen vrijgemaakt uit het slib, maar bleven in de wormenkeutels. Slechts een kleine fractie (< 0.8 %) van de totale lading zware metalen die door de wormen geconsumeerd werd, kwam terecht in de nieuw gevormde wormenbiomassa. Indikken van de wormenkeutels leidde tot een twee keer zo hoge droge stof concentratie vergeleken met indikken van slib, wat kan leiden tot een afname van 67 % in het slibvolume dat naar gecentraliseerde slibverwerkingsinstallaties moet worden getransporteerd. Een wormenreactor is hierdoor het aantrekkelijkst voor kleinere RWZI's zonder een eigen vergister en ontwateringsapparatuur en daardoor hoge kosten voor het transporteren van ingedikt slib. Tijdens het verder verwerken van ingedikt slib, leverde de methanisatie van wormenkeutels een 40 % lagere methaanproductie op vergeleken met methanisatie van slib.

De belangrijkste ontwerpparameter voor een wormenreactor is de oppervlakte specifieke consumptiesnelheid. Deze is het product van de stabiele wormendichtheid in het dragermateriaal en de worm specifieke consumptiesnelheid. **Hoofdstuk 6** beschrijft de batchexperimenten die uitgevoerd werden om deze stabiele wormendichtheid te bepalen, en de afhankelijkheid van de maaswijdte van het dragermateriaal. Stabiele wormendichtheden van 0.87 en 1.1 kg ww/m² werden gehaald met maaswijdtes van respectievelijk 300 en 350 µm. De oppervlakte specifieke consumptiesnelheden bij deze dichtheden waren respectievelijk 45 en 58 mg TSS/(m²·d), wat tot een 29 % kleinere wormenreactor zou leiden wanneer een 300 µm in plaats van een 350 µm maaswijdte gebruikt zou worden. Om een maximale consumptiesnelheid van de wormen te behouden, was een slibbelasting van minstens 100 mg TSS/(g ww·d) nodig. Andere soorten slib werden vergeleken op hun oppervlakte specifieke slibconsumptiesnelheid. Dit liet zien dat grote verschillen verwacht kunnen worden, de reden hiervoor bleef echter onduidelijk. De biomassa afbraaksnelheid

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voor wormen geïmmobiliseerd in het dragermateriaal was met 0.023 d^{-1} veel hoger dan de 0.018 d^{-1} voor vrije wormen. Het gevolg was dat de netto biomassa groeisnelheid voor geïmmobiliseerde wormen met $0.009\text{-}0.011 \text{ d}^{-1}$ lager was dan de groeisnelheid van 0.026 d^{-1} voor vrije wormen. Tot slot werd de specifieke zuurstofopname door de wormen bepaald op $4.9 \text{ mg O}_2/(\text{g ww}\cdot\text{d})$. Deze zuurstof moet aan de wormen toegediend worden door beluchting van het watercompartiment.

Het probleem van gelimiteerde wormengroei werd overkomen door het ontwerpen van een nieuwe configuratie voor de wormenreactor, beschreven in **hoofdstuk 7**. In deze nieuwe configuratie is het dragermateriaal niet horizontaal, maar verticaal geplaatst als cilindervormig in het watercompartiment. Een grotere wormenreactor van deze nieuwe configuratie behandelde 8 g TSS/d afvalslib van een lab-schaal actief slib systeem. De netto biomassa groeisnelheid was 0.014 d^{-1} , wat hoger is dan de groeisnelheid in experimenten met een horizontale drager, maar nog steeds lager dan voor vrije wormen. Uit massabalansen over de wormenreactor volgde een stikstofuitstoot van $64 \text{ mg (NH}_4 + \text{NO}_3\text{)-N/g TSS}$ verteerd door de wormen en een fosfaat uitstoot van $20 \text{ mg PO}_4\text{-P/g TSS}$ verteerd. Het door de wormen uitgestote ammoniak werd gedeeltelijk genitrificeerd in de wormenreactor. Dit heeft tot gevolg dat een lager effluent debiet nodig zou zijn om de ammoniak concentratie laag genoeg (lager dan 0.1 mg N/L niet-geïoniseerd ammoniak) te houden in het watercompartiment. Vergeleken met een horizontaal geplaatste drager, zijn de belangrijkste voordelen een hogere netto wormenbiomassa groeisnelheid, een kleinere footprint, eenvoudigere inzameling van wormenkeutels en eenvoudigere beluchting van het watercompartiment om de wormen van zuurstof te voorzien.

De potentiële toepassingen van wormenbiomassa gegroeid op afvalslib van gemeentelijke RWZI's worden besproken in **hoofdstuk 8**. Een analyse van de biomassa samenstelling liet zien dat de wormen voornamelijk eiwitten bevatten en kleinere fracties vetten, suikers en as. Ook al werd er geen bioaccumulatie waargenomen, vervuiling van de wormenbiomassa met zware metalen en mogelijk pathogenen en organische microverontreinigingen sluit toepassing van de wormen in de menselijke voedselketen uit. De overblijvende, meest veelbelovende toepassingen zijn: 1) levende wormenbiomassa als toevoeging aan voer voor niet-consumptie dieren, 2) dode wormenbiomassa als toevoeging aan voer voor niet-consumptie dieren, als meststof of als energiebron en 3) gebruik van specifieke componenten uit de wormenbiomassa. Vooral deze laatste toepassing verdient meer aandacht, aangezien specifieke aminozuren en vetzuren een hoge toegevoegde waarde kunnen hebben. Ook de productie van de wormenbiomassa gegroeid op meer gedefinieerde slibsoorten, zoals die uit bijvoorbeeld de voedselindustrie, verdient meer aandacht aangezien deze wormenbiomassa mogelijk in de menselijke voedselketen toegepast kan

worden en daardoor een hogere toegevoegde waarde heeft. Verdere aspecten die overwogen moeten worden bij de selectie van een geschikte toepassing van de wormenbiomassa zijn de decentrale productie (bij verschillende RWZI's) en de concurrentiepositie ten opzichte van bestaande stromen van vergelijkbare samenstelling.

Hoofdstuk 9 besluit met een economisch perspectief voor een wormenreactor bij een kleine 35,000 i.e. RWZI zonder een eigen vergister en ontwateringsapparatuur. Een overzicht van de geschatte kostenreducties liet zien dat de grootste besparingen behaald kunnen worden op het transport van ingedikt slib en de verbranding van ontwaterd slib op een centrale locatie. Daarnaast zorgt de overtollige wormenbiomassa voor extra inkomen, hoewel hier nog wel een geschikte afzetmarkt voor gevonden moet worden. Kleinere kostenbesparingen kunnen gemaakt worden op verwijdering van nutriënten, aangezien stikstof en fosfaat gedeeltelijk met de wormenbiomassa afgevoerd worden, maar er wordt wat minder inkomen gegenereerd door de lagere productie van biogas uit wormenkeutels op een centrale locatie. De meeste kosten zijn verbonden aan de bouw en de operationele kosten van de wormenreactor. Het huidige vooruitzicht voor een wormenreactor bij een kleine RWZI is goed, met een geschatte terugverdientijd van 7-15 jaar. Het opzetten van een pilot wormenreactor wordt aanbevolen om de technologische details verder te optimaliseren en om meer operationele ervaring op te doen met een grotere wormenreactor. Hierdoor wordt tevens de informatie verkregen die nodig is voor een meer gedetailleerde economische evaluatie. De pilot reactor kan ook gebruikt worden voor het verder optimaliseren van het indikken van wormenkeutels en voor het ontwikkelen van een methode om de wormen te oogsten. De productie van schonere wormenbiomassa wordt als tweede aanbeveling gegeven, aangezien dit een veel hogere toegevoegde waarde kan hebben vergeleken met wormenbiomassa gegroeid op slib van RWZI's. Dit vereist het groeien van wormen op schonere slibsoorten, zoals die uit de voedingsindustrie. Indien hierbij de groei van de wormen het primaire doel is, zal immobilisatie in een dragermateriaal niet gewenst zijn, aangezien dit de groeisnelheid van de wormen limiteert. Tot slot is er meer informatie vereist over welke fracties uit het slib door de wormen verteerd kunnen worden. Dit zou tevens een voorspellende functie kunnen hebben voor toepasbaarheid van een wormenreactor voor een bepaald type slib.

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Tim Hendrickx
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About the author ...

Tim Hendrickx was born on the 15th July 1975. After finishing secondary education at the Rhedens Lyceum in Rozendaal, he studied Chemical Engineering at the University of Twente. His MSc project was on treatment of waste water from a car wash installation under supervision of Henk van den Beld. After this he spent two years at the University of Oulu (Finland). There he obtained his Licentiate degree on the control of a fungal growth in the biofilm process at the municipal waste water treatment plant, supervised by prof. Riitta Keiski and Eero Meskus. After a brief break from research, he started his PhD project in November 2004 as a PhD student at Wageningen University, but stationed at Wetsus in Leeuwarden. Since the beginning of 2009 he is working as a postdoc at Wageningen University on a project dealing with anaerobic waste water treatment followed by ammonia removal with the anammox bacteria.





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- Writing a grant proposal

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Presentations:

- Oral Presentation: IWA specialized conference on sustainable sludge management, 29 May 2006, Moscow, Russia
- Oral Presentation: Sensible water technology (SENSE meeting), 12 April 2007, Leeuwarden, The Netherlands
- Poster Presentation: Novel Cost Effective Technologies for Wastewater Treatment and Bio-energy production (SENSE meeting), 5 September 2008, Wageningen, The Netherlands
- Oral Presentation: 13th European Biosolids and Organic Resources Conference, 11 October 2008, Manchester, UK

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