Journal of Hazardous Materials 177 (2010) 633–638

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Aquatic worms eat sludge: Mass balances and processing of worm faeces

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ARTICLE INFO

Article history:
Received 12 August 2009
Received in revised form
23 November 2009
Accepted 16 December 2009
Available online 23 December 2009

Keywords:
Aquatic worm reactor
Lumbriculus variegatus
Biological sludge
Mass balance
Dewatering
Methanisation

ABSTRACT

Reduction of the amount of waste sludge from waste water treatment plants (WWTPs) can be achieved with the aquatic worm *Lumbriculus variegatus* in a new reactor concept. In addition to reducing the amount of waste sludge, further processing of produced worm faeces and released nutrients should also be considered. This study gives the mass balances for sludge consumed by *L. variegatus*, showing the fate of the consumed organic material, nutrients and heavy metals associated with the sludge. A distinction is made between conversion into worm biomass, release as dissolved metabolites and what remains 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, which has potential for reuse. Experiments showed that settling of the worm faeces 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. 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.

1. Introduction

1.1. Waste sludge production from waste water treatment

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 is subject to increasingly stringent legislation [1]. The associated high sludge processing costs of up to 50–60% of the operational costs at a WWTP have led to an increased interest in sludge reduction techniques [2]. Several mechanical, physical and chemical sludge disruption techniques are available and applied in practice [2]. However, these techniques may be costly and are mainly aimed at lowering the amount of waste sludge, without a focus on resource recovery. A biological approach for reducing the amount of waste sludge is the use of the aquatic worms [3]. Initially, research focused on extending the food web with aquatic worms that naturally occur in WWTPs. However, this process proved impossible to control and high worm densities could not be maintained [3,4]. Further research therefore focused on separate worm reactors in which conditions could be optimized for the worms [2]. A new reactor concept was recently introduced (Fig. 1) in which the aquatic worm *Lumbriculus variegatus* is immobilized in a carrier material, which also acts as a separator for waste sludge and worm faeces [5]. Initial batch experiments were very promising with 36–75% total suspended solids (TSS) reduction, growth of new worm biomass and collection of worm faeces with a higher settleability than the initial waste sludge [5,6]. It was also shown that this reactor concept can be applied in a continuous system, with a clear contribution of the worms towards sludge breakdown and with growth of worms in the reactor [7].

1.2. Aquatic worms for waste sludge processing

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, with potential for reuse.

Most aquatic worm reactors described in recent literature (e.g. [8,9]) have both sludge and worms in one aerated reactor volume, which essentially is an additional aeration tank with conditions optimized for the aquatic worms. Sludge is passed over the worm

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doi:10.1016/j.jhazmat.2009.12.079

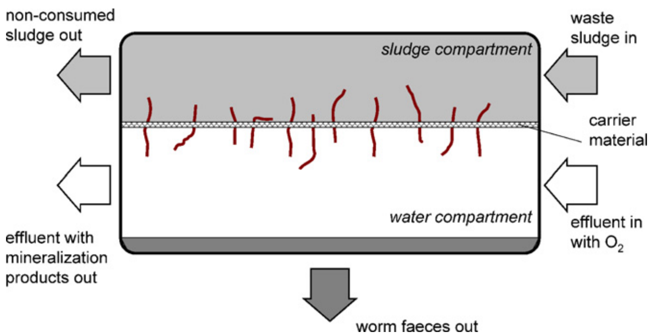

Fig. 1. Reactor concept for sludge reduction using the aquatic worm *Lumbriculus variegatus*.

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 by the sludge present in the worm reactor [10], e.g. the conversion of released ammonia into nitrate. Compared to such systems, our reactor concept (Fig. 1) allows for a separate collection and characterization of metabolites, worm faeces and worms.

Separate collection and processing of the worm faeces could have advantages. The faeces have a better settleability compared to waste sludge and can, therefore, be collected at a high TSS concentration [5]. 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 [11]. 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 [12]. 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.

1.3. Objective

This study 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 Fig. 1. The effect of the worms on the heavy metals present in the sludge was also 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, 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 are discussed.

2. Materials and methods

2.1. Aquatic worm experiments

The mass balances were established in sequencing batch experiments as described previously [6]. Each experiment consisted of 4 consecutive batches of 23 h each. Thus, results were obtained for a total of 92 h. Dissolved oxygen (DO) concentration was 8.6–9.7 mg O_2/L, pH 8.4–8.7 and temperature was 18–20 °C. Sludge was supplied in excess to the worms and ±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 μm polyamide mesh (SEFAR) was used as a carrier material. Effluent, sludge and supernatant of the sludge were analyzed for TSS, VSS, COD, total N, total P, ammonia 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.

2.2. Materials

Sludge and effluent from the Leeuwarden municipal WWTP were used, which applied 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 μm, Schleicher and Schuell), before being used in the experiments.

2.3. Analyses

Chemical Oxygen Demand (COD), total nitrogen (total N), total phosphorus (total P), and ammonia ($NH_4 + NH_3$) were determined according to Standard Methods [13] using Dr Lange® test kits. Phosphate (PO_4) was determined according to Standard Methods [13] using ion chromatography (Metrohm 761 Compact IC). Total, fixed and volatile suspended solids (TSS, FSS and VSS) concentrations were determined according to Standard Methods [13] 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 min 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 analyzed 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 [13] 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 [13] 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 was recorded as a function of time to allow calculation of the specific resistance to filtration (SRF) [14]. The SRF showed good reproducibility amongst measurements on the same sludge (relative standard deviation < 5%).

Centrifugation tests were performed with 50 mL of sludge or worm faeces in glass centrifuge tubes during 10 min 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 min 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 1 week, gas composition was measured using a Shimadzu GC-2010 Gas Chromatograph containing GS-Q (CO_2) and HP molsieve (O_2, N_2 and CH_4) columns. TSS, VSS and COD of the seed sludge, substrate and final sludge were determined.

3. Results and discussion

3.1. Mass balances

The mass balances determined for sludge that was consumed by the worms are summarized in Fig. 2. TSS and VSS reduction in these experiments were 21 and 26%, respectively, which was lower than the 35–75% TSS reduction in previous experiments [5,6]. 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 a 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 (<1% in all cases). For phosphorus however, a clear increase in soluble phosphate concentration in the water compartment was measured (from 0.36 to 1.6 mg PO_4-P/L). 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 CO_2. 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 NH_4-N/kg TSS consumed and 5.4 g PO_4-P/kg TSS consumed (58.0 g NH_4-N and 25.8 g PO_4-P per kg TSS digested). This ammonia release is higher than what was found in previous, less elaborate, experiments [6]. The effluent from the water compartment with these released nutrients will require treatment. As estimated previously [6], treatment of the released ammonia would increase the nitrogen load on a WWTP with less than 5%. The released phosphates were estimated to represent an additional phosphorus load of approximately 10%.

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.

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 [15].

3.2. 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%. The worm biomass contains a high fraction of protein and has several potential applications for reuse, which is currently under detailed investigation.

3.3. Metals

Sludge, worm faeces and worms were analyzed for metals. The results for Fe, Cu and Zn are shown in Table 1; concentrations of the

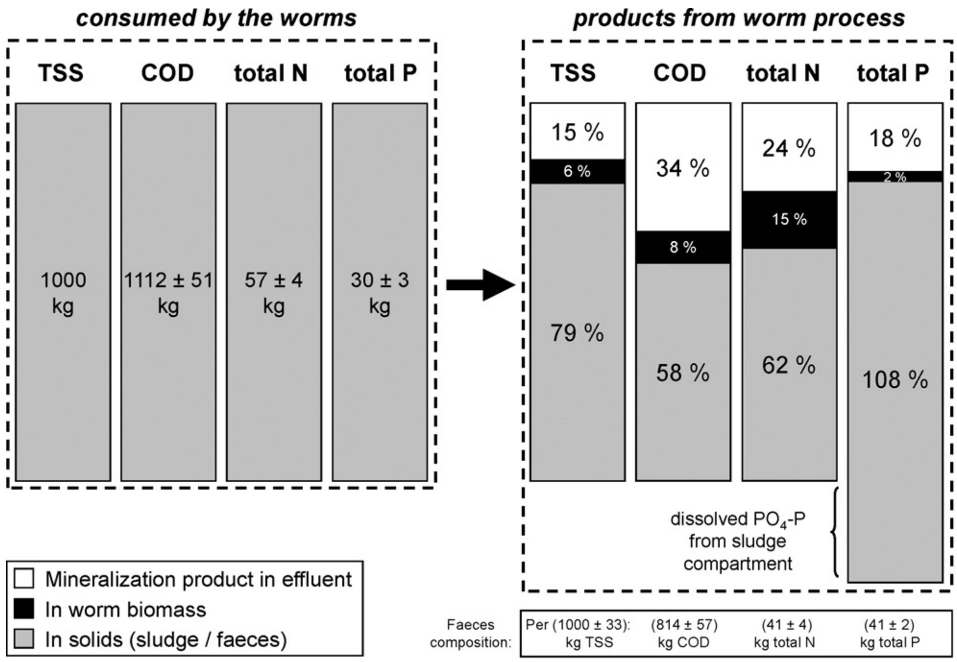

Fig. 2. Mass balances for COD, total N and total P over the sludge consumed by worms in sequencing batch experiments. Values show the average and standard deviation of three separate mass balancing experiments. Sludge from the Leeuwarden WWTP was used in these experiments.

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) [12,16]. 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 [6], it is possible that a fraction of the metals will be released from the sludge. However, this was not investigated further. Table 1 also shows that the metal concentrations in the worms are much lower than in the sludge. The amount of metals in newly formed worm biomass represents less than 0.8% of the amount of metals in the sludge consumed by the worms. This shows that the worms do not specifically bioaccumulate the metals from the sludge.

3.4. 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 [5].

Using the pre-settled solids, the effect on further dewatering was evaluated using specific resistance to filtration (SRF) and centrifugation tests. The SRF of $(2.5 \pm 0.7) \times 10^{12}$ m/kg for waste sludge was about 30% lower than the SRF for worm faeces, which was $(3.3 \pm 0.8) \times 10^{12}$ 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 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 [17].

Table 1
Metal concentrations in the waste sludge and the worm faeces. The values for worm biomass are in g metal/g dw.

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

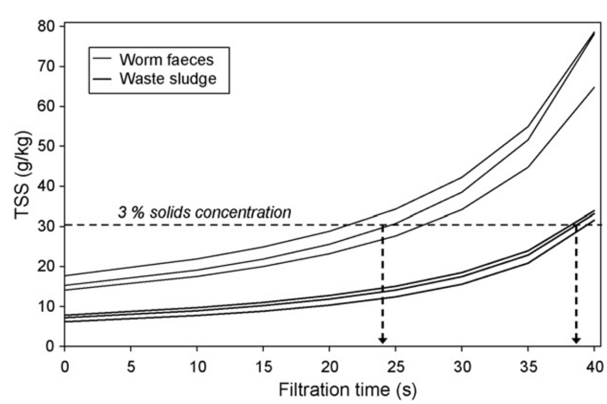

Fig. 3. Typical curves obtained in the filtration tests with worm faeces and waste sludge. A vacuum pressure of 50 kPa was applied and Whatman grade 2 filter paper was used as filter material. The dotted lines indicate the filtration times needed to reach a solids concentration of 3%.

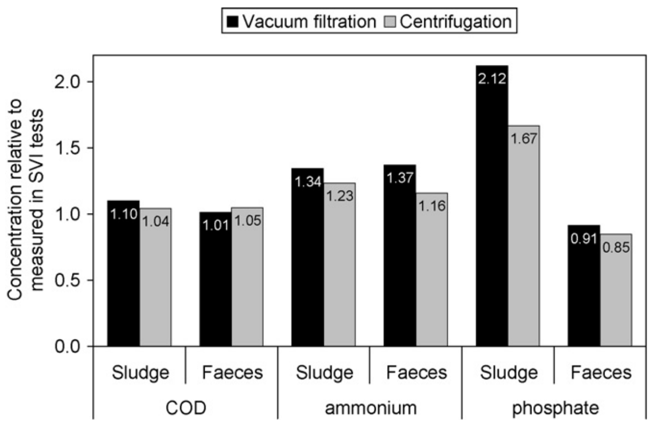

Fig. 4. Effect of dewatering/thickening method on COD and nutrient "release" from Leeuwarden WWTP sludge and faeces. Bars represent the concentration measured relative to that measured in the supernatant from the SVI tests. A value of 1 means the concentration in the reject water is equal to that in the supernatant after settling.

The dewaterability tests also showed (Fig. 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 concentration or that the same time can be used to achieve a higher TSS concentration.

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. The combined effect of TSS reduction and collection of the worm faeces at a much higher TSS 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 found in SVI measurements, a 67% reduction in the volume of waste biosolids (in that case worm faeces) can be achieved. This reduction in the volume of waste solids is of most benefit to smaller WWTPs, where the sludge processing costs are mainly dictated by transportation.

3.5. Reject water from settling and dewatering

Fig. 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 PO_4-P/L compared to the supernatant of the waste sludge (3.9 mg PO_4-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.

3.6. Anaerobic digestion of sludge and worm faeces

The results of the anaerobic digestion experiments with sludge, worm faeces and worms are summarized in Fig. 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 [6]. Nonetheless, the worms could digest the anaerobically

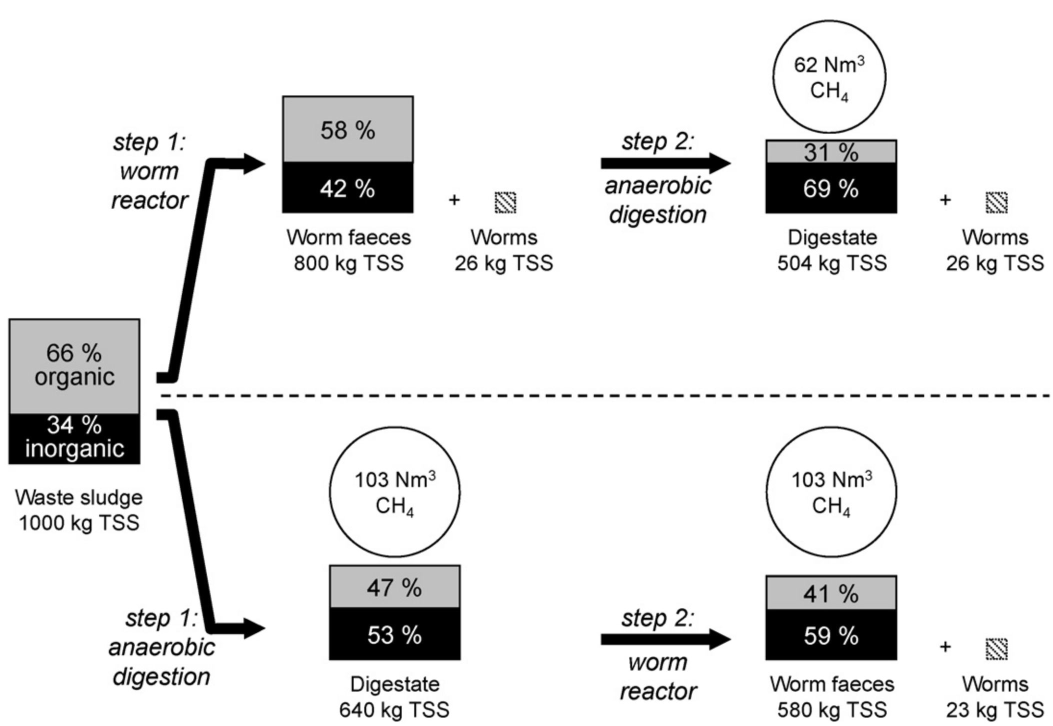

Fig. 5. Mass balances for two alternatives of combining sludge consumption by worms and biogas production through anaerobic digestion. Biogas volumes were calculated at 25 °C.

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 CH_4-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 one centralized location. 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.

4. Conclusions

The results presented in this paper showed that an aquatic worm reactor has 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.

The experiments also showed that:

- Treatment of the nutrients released by the worms is estimated to result in an additional internal load on the WWTP of 5% for nitrogen and 10% for phosphorus.
- 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.
- 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.

Acknowledgements

We thank Nadilsa Eloisa Rodrigues Tavares (University of Minho, Braga, Portugal) and Enna Klaversma for their assistance in the experimental work presented in this study. We also thank the operators from the Leeuwarden WWTP for their assistance in obtaining the effluent and (anaerobic) sludge used in the experiments. This work was performed in the TTIW-cooperation framework of Wetsus, Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs, the European Union Regional Development Fund, the Province of Fryslân, the City of Leeuwarden and the EZ/Kompas program of the "Samenwerkingsverband Noord-Nederland". We thank the participants of the research theme "Membrane Bioreactors" for their financial support.

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