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Can aquatic worms enhance methane production from waste activated sludge?

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HIGHLIGHTS

- *Lumbriculus variegatus* has no synergistic effect on digestion of high-loaded sludge.
- High-loaded sludge provides an excellent feed source for aquatic worms.
- Worms give the highest methane yield, followed by waste sludge and worm feces.

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ABSTRACT

Although literature suggests that aquatic worms can help to enhance the methane production from excess activated sludge, clear evidence for this is missing. Therefore, anaerobic digestion tests were performed at 20 and at 30 °C with sludge from a high-loaded membrane bioreactor, the aquatic worm *Lumbriculus variegatus*, feces from these worms and with mixtures of these substrates. A significant synergistic effect of the worms or their feces on methane production from the high-loaded sludge or on its digestion rate was not observed. However, a positive effect on low-loaded activated sludge, which generally has a lower anaerobic biodegradability, cannot be excluded. The results furthermore showed that the high-loaded sludge provides an excellent feed for *L. variegatus*, which is promising for concepts where worm biomass is considered a resource for technical grade products such as coatings and glues.

1. Introduction

The activated sludge process is the most commonly used biological treatment technology for municipal and industrial wastewater. It is highly efficient in the removal of organic matter and nutrients but also produces large amounts of excess sludge. This sludge contains heavy metals, organic micropollutants and pathogens, which has led to stringent legislation for sludge applications (Leschber et al., 2002). Not only from an environmental, but also from an economical point of view, a reduction of the amount of sludge solids is important since treatment of these solids in small wastewater treatment plants constitutes up to 50–60% of the total operational costs (Wei et al., 2001).

Different technologies can be applied for reduction of the amount of sludge solids. Of these, mesophilic anaerobic digestion (typically at a temperature around 35 °C) is the most widely applied process because it produces methane which can be used as an energy source. If allowed by legislation, the digestate can be used as a stabilized fertilizer (Koroneos and Nanaki, 2012). However, the biodegradable fraction of activated sludge solids generally is low and therefore solids reduction (13–27% of the volatile solids) and biogas production (0.07–0.18 Nm^3/kg volatile solids) during digestion are limited (Bolzonella et al., 2005).

Different types of worm reactors have been developed over the years, mainly with the objective to reduce the amount and volume of waste activated sludge (e.g. Elissen et al., 2006; Hendrickx et al., 2009; Lou et al., 2011; Tamis et al., 2011; Wei et al., 2009). These reactors consist of a second or adjusted aeration tank that is inoculated with aquatic worms. In this manner the food chain is

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extended, giving a larger overall reduction of complex organic matter and a reduced amount of waste solids (Elissen et al., 2006). The products of such a worm reactor are worm biomass, worm feces and, if not fully consumed by the worms, remaining waste sludge. Hendrickx et al. (2010) found that treating waste activated sludge by the aquatic worm *Lumbriculus variegatus*, followed by mesophilic anaerobic digestion of the remaining products, resulted in a 76% overall reduction of volatile solids (VS). This was 22% more compared to anaerobic digestion of the waste sludge alone. Tamis et al. (2011) operated a full scale worm reactor using the aquatic worm *Aulophorus furcatus*, combined with subsequent anaerobic digestion of the products. They concluded that an overall 65% reduction of total solids (TS) could be achieved, which is much better than a typical reduction of 20–30% TS when only anaerobic digestion is applied. Anaerobic digestion of the sludge, worms and worm feces took place under ambient (psychrophilic) temperatures of 4–20 °C. Based on the occurrence of anaerobic digestion at such low temperatures they assumed that the worms or their feces must have contributed to an improved digestibility of the waste sludge. A similar synergistic phenomenon was observed by Feng et al. (2012): addition of 3% earthworm manure improved biogas production from food waste by approximately 8%. Presumably this was caused by external enzymes and/or bacteria producing enzymes in the worm manure that promoted degradation of complex and otherwise poorly biodegradable organic matter.

In particular for an aquatic worm such as the sediment dwelling *L. variegatus* a similar phenomenon can be expected, although specific information on the hydrolytic enzymes of this species is scarce (e.g. Kuz'mina and Ushakova, 2007; Tweeten and Reiner, 2012). In its natural environment this worm depends for its nutrition on low concentrations of highly complex organic matter and it is selectively attracted to colonies of bacteria (Milbrink, 1993). As only a few researchers have investigated anaerobic digestibility of the products from a worm reactor, anaerobic digestion of *L. variegatus* and of its feces was studied in more detail. To test the hypothesis that this worm can stimulate anaerobic degradation of excess sludge, anaerobic digestion tests were carried out with and without the addition of *L. variegatus* and/or its feces. The results are of interest for aquatic worm technologies to reduce the amount and volume of excess sludge, for production of worm biomass as a starting material for coatings and glues or for production of worm biomass from by-products from the food industry to serve as a fish feed for the aquaculture industry (Elissen et al., 2010, 2015).

2. Material and methods

2.1. Substrates

Anaerobic digestion tests were carried out with the following substrates (Fig. 1): waste activated sludge, the aquatic worm *L. variegatus* cultivated on this waste activated sludge (adapted worms), worms cultivated on the commercial fish feed Tetramin® (non-adapted worms) and the feces from adapted worms.

Waste activated sludge was collected from a bench scale, aerobic high-loaded membrane bioreactor (HL-MBR) treating municipal wastewater from the city of Leeuwarden, The Netherlands. This HL-MBR was operated at a very short solids retention time (SRT) of 0.5 d and a very short hydraulic retention time (HRT) of 0.7 h. The total suspended solids (TSS) and volatile suspended solids (VSS) concentration of this sludge approximately were 3 and 2.5 g/L, respectively. More details about this HL-MBR and its operation can be found in Faust et al. (2014a) and Faust et al. (2014b).

Worm feces produced by worms growing on waste activated sludge were harvested from batch experiments for which 4 plastic trays (90% covered with lids and aerated by means of aquarium air pumps) were used. The feed sludge was centrifuged in a Beckman Coulter centrifuge at 8000 rpm (JLA-8.1000 rotor) for 20 min and the supernatant was discarded to remove most of the ammonia because this compound can inhibit worm growth. In every tray, a 3 g sludge pellet was re-suspended in 3 l of tap water plus 3 l of effluent from the HL-MBR system that the sludge originated from. Per tray 12 g of live worms were added, which originated from a breeding system fed with fish feed (Tetramin®). The worm to sludge ratio on dry matter basis at the start of the experiment was around 0.6. The dry weight to live weight ratio of worms was around 0.15. After approximately one week it was concluded by visual inspection that the worms had consumed all the sludge pellets and converted them into compact feces. The worms were subsequently separated from the feces by sieving at 250 μm (Retsch). The collected feces were left to settle in a large bucket for 3 h after which the supernatant was discarded. All collected feces were stored at 4 °C until use in the digestion tests. The worms were subsequently put back into the trays to receive new sludge, effluent and water. This procedure was repeated weekly for four weeks in a row.

2.2. Anaerobic digestion tests

Anaerobic digestion tests were carried out with the individual substrates, i.e. waste activated sludge (S), adapted and non-adapted worms (W) and worm feces (F) (Table 1). In addition, mixtures of waste sludge and worms and of sludge and worm feces were used, both at a COD ratio of 7:3. At such a ratio a sufficient amount of worm or worm feces should be present to be able to test whether they stimulate anaerobic sludge digestion. With adapted worms an additional digestion test was carried out with a mixture of sludge, adapted worms and feces at a COD ratio of 2:1:1. All substrates were homogeneously blended prior to preparing the mixtures and were kept at 4 °C to avoid fermentation.

The digestion tests were carried out at 20 and 30 °C for a period of 30 days, in duplicate (tests 1–5 in Table 1 with non-adapted worms) or triplicate (tests 6–11 in Table 1 with adapted worms) in glass serum bottles with a volume of 117 ± 1 mL. The bottles were continuously mixed at 300 rpm by orbital stirrers. The inoculum was crushed granular sludge from a paper mill wastewater treatment plant situated in Eerbeek, The Netherlands. The concentrations of (total) substrate and inoculum were 1 g COD/L and 2 g VSS/L, respectively. A pH buffer and trace element solution as described by Fannin (1987) and Field et al. (1988) were added as well as macro elements to establish an adequate nutrient balance close to 300:5:1 (C:N:P) as described by Aiyuk et al. (2006). The liquid volume was increased to 50 mL with distilled water, and a gentle flow of nitrogen gas was used to exclude oxygen from the headspace, ensuring anaerobic conditions in the bottles. Biogas production was followed over time by measuring pressure in the headspace at time intervals of 48 h. Liquid and gas samples were taken and analyzed at the start of each test and at the end of the tests to determine biogas composition and to confirm that no accumulation of volatile fatty acids (VFA) had taken place. The pressure data were corrected for the (average) pressure measured in blank tests only containing the 2 g VSS/L of inoculum.

2.3. Analyses

The following parameters were determined to characterize the substrates for the digestion tests and the contents of the serum bottles at the end of these tests: pH, total COD and soluble COD, NH_4-N, PO_4-P and volatile fatty acids (VFA). At the end of each test the biogas composition was determined with a gas chromatograph (Shimadzu), equipped with serially connected columns

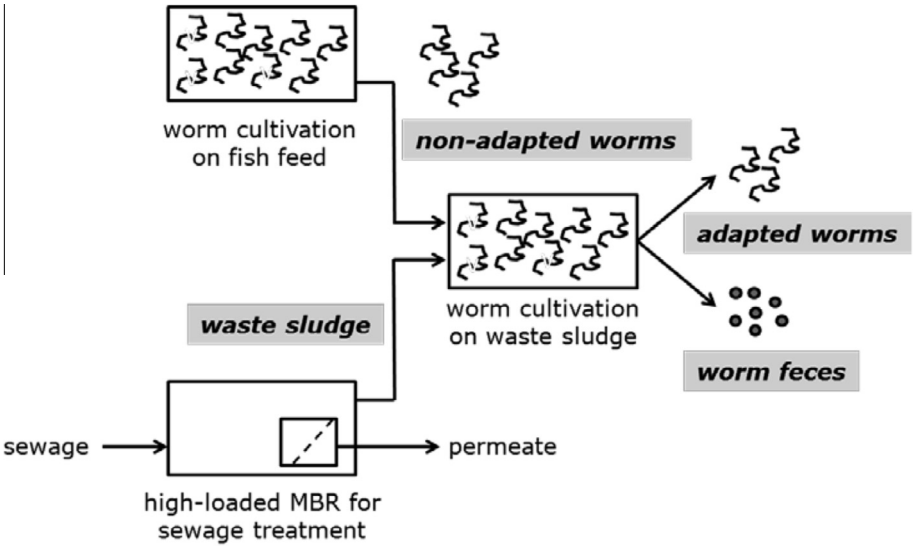

Fig. 1. Experimental set-up to obtain substrates (in gray boxes) for the anaerobic digestion tests.

Table 1
Substrates used in the anaerobic digestion tests, and the methane yield and degree of solubilization in these test (W = worms, F = worm feces, S = sludge).

		Substrates g COD/L			Methane yield $mgCH_4$-COD/g COD		Solubilization %	
Test		W	F	S	20 °C	30 °C	20 °C	30 °C
Non-adapted worms								
1	W	–	–	1	630	579	84	71
2	F	–	1	–	176	429	51	74
3	S	1	–	–	494	704	59	72
4	W/S	0.3	–	0.7	402	556	48	69
5	F/S	–	0.3	0.7	323	682	44	73
Adapted worms								
6	W	–	–	1	800	896	93	105
7	F	–	1	–	99	275	32	53
8	S	1	–	–	491	659	66	82
9	W/S	0.3	–	0.7	579	689	70	83
10	F/S	–	0.3	0.7	416	539	58	69
11	W/F/S	0.25	0.25	0.5	513	453	62	62

(CP-Moliseve 5A and CPPorabond Q) and a thermal conductivity detector. Detector and inlet temperatures were 150 and 120 °C, respectively. Hach Lange kits were used to determine COD, NH_4-N and PO_4-P. VFA was determined by a gas chromatograph according to a procedure described by Weijma et al. (2000).

2.4. Data elaboration

Methane yields were expressed in mg CH_4-COD produced per gram of substrate COD and were calculated from the pressure data (corrected for the pressure in the blank tests) and from the fraction of methane in the biogas. The degree of solubilization was calculated as follows:

$$\text{solubilization}\,(\%) = 100 \times \frac{\text{COD of produced } CH_4 + \text{final soluble COD}}{\text{initial total COD}} \quad (1)$$

Using a least-squares method, methane production in time, corrected for methane production by the inoculum, was fitted with a pseudo-first order kinetic model as proposed by Borja et al. (1995):

$$Y_{CH4}(t) = Y_{CH4,max}(1 - e^{-kt}) \quad (2)$$

with $Y_{CH4}(t)$ and $Y_{CH4,max}$ the methane production after t days and the maximum methane yield in mg CH_4-COD/g COD, respectively and with k the first-order rate constant in d^{-1}.

3. Results and discussion

3.1. General observations

Table 1 gives the methane production achieved after 30 days digestion time of the different substrates and the degree of solubilization of these substrates. Differences between duplicates of tests 1–5 and between triplicates of tests 6–11 varied, but all within a range of 1–10%. In Table 1 only average results are reported. Fig. 2, as an example, shows the development of methane production in time during tests 6–11 with adapted worms, worm feces and waste sludge as substrates.

Maximum methane production always was achieved within 30 days, except for the test with worm feces at 20 °C (test 8 in Table 1). The biogas contained 77–79% of methane. As expected, digestion tests at 30 °C generally gave more methane than the tests at 20 °C, although the extent of this temperature effect exhibited considerable variation between the tests. Only for tests 1 (non-adapted worms) and 11 (mixture of worms, feces and sludge) a negative effect of temperature was observed for which no obvious explanation is available ($p < 0.05$ and $p < 0.005$ in t-test, respectively). The most pronounced temperature effect occurred for digestion of worm feces with a 2.5 to 3 times higher methane production at 30 °C compared to 20 °C.

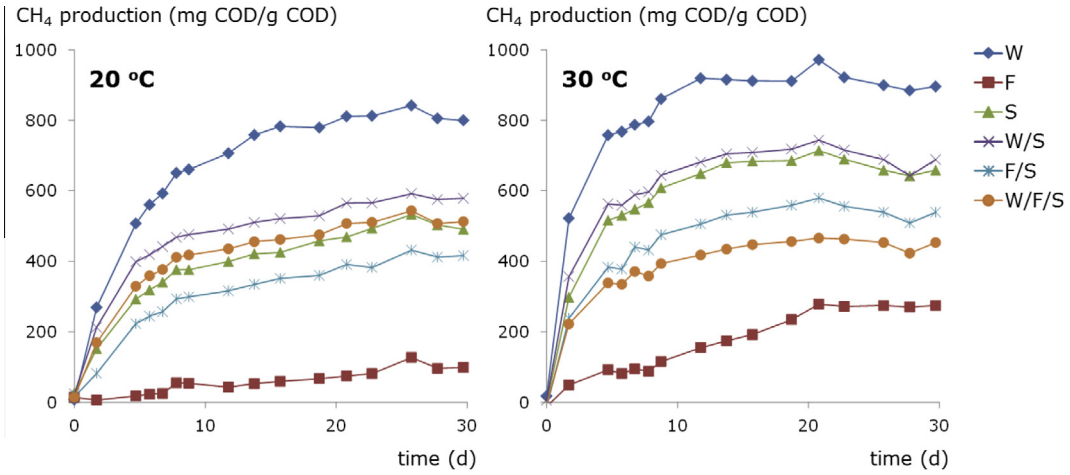

Fig. 2. Methane production during digestion of different substrates and substrate mixtures at 20 °C (left) and 30 °C (right). W = (adapted) worms, F = worm feces and S = waste sludge.

At the end of the digestion period in the test with waste sludge and non-adapted worms at 20 °C (test 4 in Table 1) the highest VFA concentration was detected, but this concentration only was 30 mg VFA-COD/L. VFA accumulation and inhibition of methane production by VFA (typically taking place at VFA concentrations above several grams per liter) therefore can be safely neglected. It also implies that all solubilized biodegradable COD was subsequently converted into methane. The average degree of solubilization was 12–13% higher than anaerobic biodegradability calculated from methane production (Table 1). This indicates that after 30 days of digestion time 12–13% of the substrate COD had ended up as soluble inert COD. Only in the tests with worm feces this percentage was considerably higher, i.e. 22–33%.

3.2. Digestion of single substrates

With the exception of test 1, carried out at 30 °C with non-adapted worms, the worms gave the highest methane production per gram of substrate COD, followed by waste sludge and worm feces (Table 1). The methane yields from adapted worms of 800 and 896 mg CH_4-COD/g COD at 20 °C and 30 °C, respectively, are even higher than a yield of 720 mg CH_4-COD/g COD at 35 °C that was reported by Hendrickx et al. (2010) for anaerobic digestion of *L. variegatus* worms feeding on activated sludge. According to Elissen et al. (2010) the organic fraction of *L. variegatus* not only is high (around 90–95%) but apparently also consists of biopolymers that can be easily degraded under anaerobic conditions.

The (adapted) worms cultivated on waste sludge resulted in a 1.3 (at 20 °C) to 1.5 times (at 30 °C) higher methane production per gram of COD than the worms that were fed with fish feed (tests 1 and 6, respectively). This suggests a difference in biomass composition between adapted and non-adapted worms, which is supported by Elissen et al. (2010) who compared the biopolymer composition of these worm types. The protein levels of these worms were similar (62–66%), but worms growing on waste sludge contained more fat (25% compared to 11–12% when grown on fish feed) and less sugar (7% compared to 11–12% when grown on fish feed).

The waste sludge of the HL-MBR gave a methane production of 491–494 mg CH_4-COD/g COD at 20 °C and of 659–704 mg CH_4-COD/g COD at 30 °C. Interestingly, even at 20 °C methane production was much higher than what is typically found for mesophilic digestion of waste activated sludge, i.e. 140–370 mg CH_4-COD/g COD as estimated from data provided by Bolzonella et al. (2005). The waste sludge in the present study was collected from a HL-MBR, which was operated at an extremely short SRT of 0.5 d. Under such conditions less than 10% of the biodegradable sewage COD is (aerobically) mineralized and at least 75% of this COD is distributed to the waste sludge (Faust et al., 2014b; Khiewwijit et al., 2015a). Thus, most of the biodegradable sewage COD was still present in the high-loaded waste sludge and available for methane production. A similar high anaerobic digestibility of HL-MBR waste sludge was reported by Akanyeti et al. (2010) and by Khiewwijit et al. (2015b). Most activated sludge plants operate at much longer SRTs (typically 15–20 days), which results in extensive aerobic mineralization of the biodegradable organic matter, a higher fraction of inert organic matter in the waste sludge and herewith a lower methane potential per gram of COD.

Worm feces gave the lowest methane yield of 99–429 mg CH_4-COD/g COD. This was expected as the feces are a waste product after the worms have already digested most of the biodegradable organic substrate, and is in agreement with a low methane yield from worm feces reported by Hendrickx et al. (2010). The worm feces that were used in test 2 gave approximately a two times higher methane yield compared to the feces that were used in test 7. We cannot explain this unless in the case of test 2 the contact time between worms and waste sludge perhaps had not been sufficiently long to fully convert all the waste sludge into fecal pellets. In general, because of the low methane production, anaerobic digestion of the worm feces is not recommended and their incineration is a more logical disposal route. An application as a (stabilized) organic fertilizer cannot be excluded, although this would require more information regarding the level of pathogens, heavy metals and organic micropollutants in the feces.

With the adapted worms the difference between the methane yield from the substrate of these worms (the waste sludge, test 8) and their feces (test 7) was very large. Assuming this difference in anaerobic biodegradability is a good measure for the fraction of substrate that was digested by the worms, it was calculated that no less than 80% and 58% of the biodegradable fraction of the waste sludge was digested by the worms at 20 and 30 °C, respectively. This is in agreement with observations that specific growth rates of the worms on the same type of high-loaded waste sludge (0.08 d^{-1}, unpublished result) were even higher than growth rates on Tetramin® of 0.02–0.05 d^{-1} (Williams, 2005), while the latter is considered to be a very efficient substrate for aquatic worms such as *L. variegatus* (Ducrot et al., 2007; Elissen et al., 2015). In contrast, (Hendrickx et al., 2010) reported that methane production from

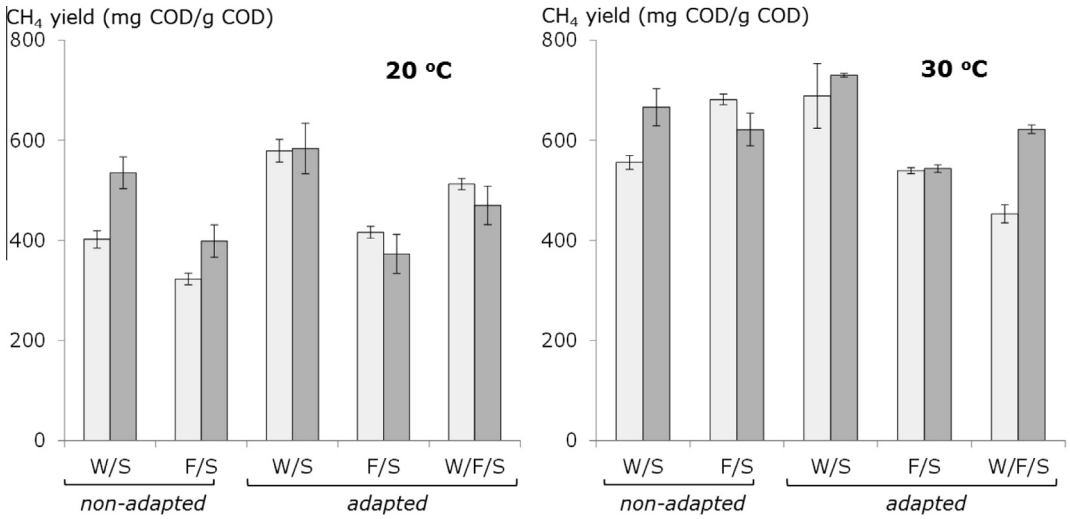

Fig. 3. Measured (light bars) and calculated (dark bars) methane yields for digestion tests with mixtures of non-adapted and adapted worms (W), worm feces (F) and waste sludge (S) at 20 °C (left graph) and 30 °C (right graph). Error bars represent standard deviations (in measured methane yields) and calculated, propagated standard deviations (in calculated methane yields).

Table 2
Estimated pseudo first-order rate constants *k* for the digestion tests 6–11 with adapted worms (W = worms, F = worm feces, S = waste sludge).

		Substrates g COD/L			Rate constant k d^{-1}	
Test		W	F	S	20 °C	30 °C
6	W	–	–	1	0.202	0.391
7	F	–	1	–	0.022	0.041
8	S	1	–	–	0.173	0.284
9	W/S	0.3	–	0.7	0.238	0.328
10	F/S	–	0.3	0.7	0.146	0.243
11	W/F/S	0.25	0.25	0.5	0.207	0.277

worm feces (0.13 Nm^3 CH_4/kg VS or 262 mg COD/g COD assuming 1.42 g COD/g VSS) only was 23% lower than from the low-loaded waste activated sludge that was used to produce these worm feces (0.16 Nm^3 CH_4/kg VS or 322 mg COD/g COD). Also the specific growth rate of *L. variegatus* on this type of sludge of 0.02 d^{-1} was much lower (Elissen, 2007). That high-loaded activated sludge provides a better feed source for the worms than low loaded sludge can be of great interest, for instance to produce technical grade coatings and glues from the worm biomass as proposed by Elissen et al. (2010).

3.3. Synergistic effect of worms or worm feces on sludge digestion

For all the substrate mixtures (tests 4–5 and 9–11 in Table 1), a methane yield was calculated from the measured yields of the individual substrates and from the COD contributions of these individual substrates. For example, in test 9 carried out at 20 °C with a mixture of 0.3 g COD/L of worms and 0.7 g COD/L of waste sludge, the (theoretical) methane yield was calculated as $(0.3 \times 800) + (0.7 \times 491) = 584$ mg CH_4-COD/gCOD. A measured methane yield of a substrate mixture which exceeds this calculated yield points to a synergistic effect of the worms or worm feces on digestion of the waste sludge. Fig. 3 compares measured and calculated yields for all the digestion tests in which substrate mixtures were used. For most mixtures the measured methane yield was similar to, or even lower than the calculated yield. A slight positive effect was calculated at 20 °C for the feces/sludge mixture (+10%, test 10) and worms/feces/sludge mixture (+8%, test 11) and at 30 °C for the feces/sludge mixture (+9%, test 5). However, from the standard deviations in the duplicate and triplicate tests (varying between 1% and 10%) it can be shown that none of these effects was statistically significant.

To analyze the effect of the presence of worms or their feces on the rate of anaerobic sludge digestion, the methane production data of Fig. 2 were fitted against the pseudo first-order model of Eq. (2). This was only done for tests 6–11 with adapted worms. Table 2 gives estimated values for the rate constant *k* (Eq. (2)). For all the tests the correlation coefficient was 0.98 or higher. As expected, a temperature increase from 20 to 30 °C had a positive effect on the rate of methane production, with a 1.3 to 1.9 times higher first-order rate constant.

Table 2 shows that worms gave the highest methane production rate, followed by the waste sludge and the feces, which is in line with the methane yield data of Table 1. Using the estimated parameter values for the HL-MBR sludge, it was calculated that at 30 °C only 10 days digestion time is needed to achieve 95% of the maximum methane production from the HL-MBR sludge. Remark that this is extremely short compared to typical digestion times for (conventional) waste activated sludge of 20–30 days and implies that for this type of sludge much smaller and therefore cheaper digesters can be designed.

Using estimated values for Y_{CH4max} and *k* for single substrates, the development of the methane yield in time for tests 9 (mixture of waste sludge and worms) and 10 (mixture of waste sludge and worm feces) in time was calculated and compared to measured methane production data. Fig. 4 shows that irrespective of the temperature measured methane production in these tests (markers) was similar to, or lower than calculated methane production

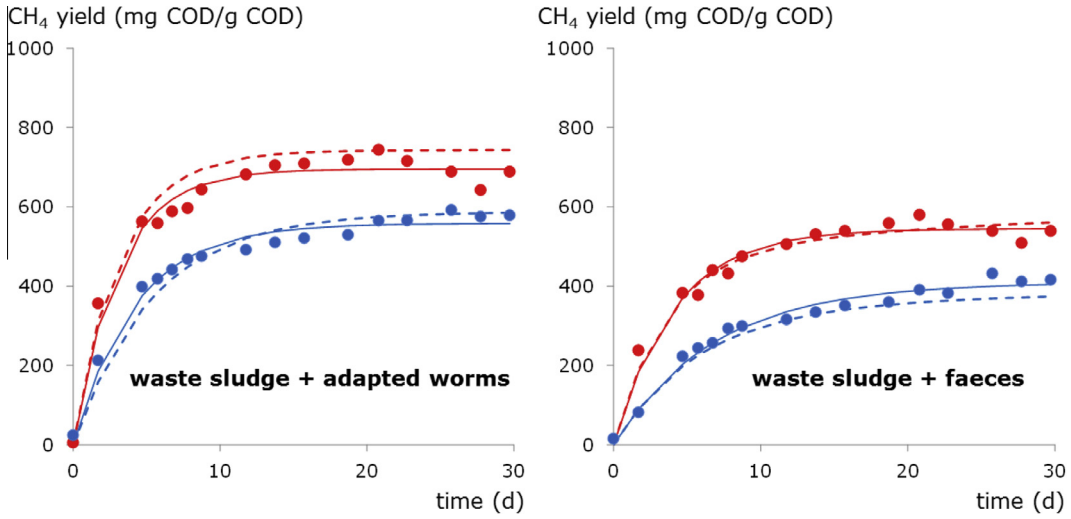

Fig. 4. Measured (markers), fitted (solid lines) and calculated (dotted lines) methane production in time during anaerobic digestion of a mixture of waste sludge and adapted worms (left) and of a mixture of waste sludge and worm feces (right), at 20 °C (blue) and 30 °C (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(dotted lines). Thus, a positive effect of (adapted) worms or of their feces on anaerobic digestion rate of the HL-MBR sludge can be excluded.

Experiments with the aquatic worm *A. furcatus* by Tamis et al. (2011) and with manure from earthworms by Feng et al. (2012) suggested that such organisms can improve the digestibility of waste activated sludge, presumably by enzymes and/or bacteria producing enzymes in the worm manure that promote degradation of complex organic matter. However, even though these were added at appreciable quantities (0.3 g COD/0.7 g COD), a clear and consistent positive effect of the aquatic worm *L. variegatus* or of its feces on methane production was not observed in this study. One explanation for the absence of such a synergistic effect may be that *L. variegates* worms or their gut microorganisms do not possess the proper enzymes to improve the anaerobic digestibility of waste sludge. A second explanation could be that such enzymes are present, but become inactive under methane producing redox conditions or other (unknown) changes in environmental conditions. Thirdly, in the experiments described by Tamis et al. (2011), a sludge-worm mixture was continuously supplied from a heated worm reactor (25 °C) to a sludge buffer where anaerobic conversion took place. In our study the substrates for the digestion tests were temporarily stored at a low temperature (4 °C), which may have led to inactivation of the relevant enzymes. A fourth and perhaps most probable reason for the absence of a synergistic effect is the very high (anaerobic) biodegradability of the HL-MBR sludge that was used. This could have masked a positive effect of worms or worm feces on the small fraction of poorly biodegradable organic matter of this sludge. A synergistic effect of worms or worm feces on anaerobic digestion of other types of waste activated sludge that containing a higher fraction of recalcitrant or less easily biodegradable organic matter therefore cannot be entirely excluded. It is therefore recommended to perform similar experiments as in the present study, but using waste sludges grown at different SRTs, i.e. different digestibilities. This can be combined with enzyme activity tests, further building on earlier research by Kuz'mina and Ushakova (2007) and Tweeten and Reiner (2012) who measured protease activity in *L. variegatus*.

4. Conclusions

L. variegatus or the feces of these worms did not have an effect on the amount or rate of methane production from high-loaded waste sludge. Worms gave the highest methane yield, followed by the sludge and the worm feces. Worms grown on fish feed gave a lower methane yield than worms grown on the waste sludge, which suggests that the feed source of the worms has a strong effect on their biomass composition. The high-loaded sludge proved to be an excellent feed source for the worms, which is promising for technologies and concepts that aim to produce worm biomass.

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