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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