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Utilization of Sludge from African Catfish (*Clarias gariepinus*) Recirculating Aquaculture Systems for Vermifiltration

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Abstract: Vermifiltration is a low-energy and low-cost option to reduce the environmental impact of aquaculture. A comparative study was performed for two different stocking densities of the epigeic worm *Dendrobaena veneta* (Michaelsen, 1890, Annelida: Oligochaeta), which were fed with sediment sludge from African catfish, *Clarias gariepinus* (Burchell, 1822), recirculation aquaculture systems (RAS). The intensive (I) and extensive (E) systems were stocked with 15 and 10 g of worm/L filter substrate, respectively, and were compared with a control (C) for four weeks. The total weight gain was 9.4–13.5% for (I) and 13.8–19.5% for (E), with low mortality rates of 3.46–5.84% (I) and 3.57–5.19% (E). The temperature inside the vermifilters was slightly higher than that in the control, indicating a favorable milieu for microbial activity. The worms supported the pH buffering capacity in the systems, with the effluent reaching 7.10 ± 0.02 (I) and 7.26 ± 0.04 (E) at the end of the experiment while the pH in the (C) was significantly higher (7.51 ± 0.05). The removal rates were 68.02–98.84% (I), 71.85–98.67% (E), and 72.80–98.68% (C) for the total nitrogen bound (TNb); 82.77–96.64% (I), 81.65–94.84% (E), and 77.79–94.74% (C) for the total organic carbon (TOC); and 50.43–97.51% (I), 50.89–96.84% (E), and 48.23–96.34% (C) for the chemical oxygen demand (COD). By utilizing the sludge as feed, the worms and associated microbiota significantly altered the African catfish sediments, removing organic loads, upgrading the composition, and reducing the possible environmental impacts.

Keywords: organic reduction; worms; compost; waste treatment; aquaculture; aquaponics; effluent sludge; solids; recycling of fecal residues; pH neutralization; circular economy

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1. Introduction

Aquaculture is the fastest-growing sector in the agriculture industry. The global production of aquatic animals peaked at 87.5 million t in 2020 [1], and global aquaculture feed production was 41.6 million t in 2019 [2]. The resulting release of total suspended solids (TSS), the major pollutant in aquaculture sludge, generally ranges from 10% to 30% of the feed intake on a dry matter basis [3]. This translates to 4.16–12.48 million t of aquaculture-based TSS per year. For example, land-based Norwegian salmon smolt production alone produced approximately 54,000 t of sludge with 15% dry matter content in 2017 [4].

The African catfish is a silurid fish that is uniquely suitable for aquaculture, since it can withstand unfavorable water conditions such as high nutrient loads and salinity [5,6], can be produced under high stocking densities [7], and can breathe atmospheric oxygen and thus does not require an additional oxygen supply [8]. Its production reached 1,245,300 t 2018, mainly in open-water ponds and raceway systems [1].

Since 1985, recirculation aquaculture systems (RAS) have been developed in Europe as standalone or farmland-based production systems [9]. The African catfish land-based RAS operators usually collect and store accumulated sludge in settlement ponds or lagoons, following which they might spread it on agricultural land for watering and fertilization. The operators, therefore, contribute to greenhouse gas emissions through denitrification processes and critical nutrient loads in the soil and groundwater [10]. In this light, efforts are being made to treat effluent streams on site and to recycle the process water more effectively. However, conventional filtration procedures often require costly, sophisticated technology and may have high energy consumption [11]. With highly species-specific feed formulations and very efficient feed conversion, only 7.1–9.9% of the feed accumulates as solids in the African catfish RAS [12]. One alternative to conventional filtration procedures is aquaponics (sensu [13]), in which fish-based excretions serve as a nutrient source for plant cultivation. With the current state-of-the-art, only the dissolved nutrients from the process water are being used for plant cultivation; however, some nutrients are often lacking in this solution [14]. Further, if directly transferred into the hydroponics system, the significant amount of organic carbon inside the solids may lead to biofouling, clogging, and undesired oxygen depletion in the system [15].

Vermifiltration (VF) is a purification method that has been little explored in the context of aquaculture. Compost worms such as *Eisenia fetida* (Savigny, 1826) or *Dendrobena veneta* can digest and degrade effluents from fish production, support the elimination of aquaculture waste, and improve the overall RAS performance, especially for aquaponics farming [13]. Studies have already shown the high potential of this technique for physical and biological filtration [16–18]. Some authors have claimed the effective mineralization of nonsolvent plant nutrients. However, most studies thus far have investigated VF only as a potential treatment for municipal sewage sludge and land-based livestock manure [19–21].

For implementing VF or vermicomposting (VC) techniques in aquaculture or aquaponics, the design features and interlinkages of this biological approach must be investigated. This study aims to analyze the suitability of aquaculture sludge from African catfish RAS as worm feed. The degradation of organic matter is investigated, which originates from the metabolic activity of the worms and associated microorganisms. A possible re-use of the vermicompost nutrient-rich effluent, and worm biomass for future farming applications is discussed. Additionally, the potential for larger-scale implementation of this proof-of-concept in the future aquaculture industry is being considered.

2. Materials and Methods

2.1. System Design

Nine individual devices consisting of plastic boxes (dimensions: 36 × 36 × 12 cm) were arranged in a drawer setup (Figure 1). Each system had four drawers and a liquid catch tank (volume: 5 L) at the bottom. Three drawers per device were filled with 12 L (thickness: approximately 10 cm) of beech wood chips as a worm bedding substrate and additional carbon source. The remaining drawer was inaccessible to the worms, and it was filled with 12 L of "Kaldnes K1 Micro" biocarrier, a low-weight plastic filter medium with a particularly large surface area of approximately 500 m^2/m^3. It was used to elongate the retention time of the effluent trickling down under gravity and as an additional substrate for microbial colonization.

The drawer setup enabled the maximal use of the potential reactor volume. However, this setup is quite different from those used most frequently in VF. A conventional vermifilter usually consists of a thin worm-active layer on top (thickness: 5–10 cm) and supporting layers of sand, gravel, etc., underneath as described in [16]. The drawer setup used in this study enabled a very deep worm-active layer, thereby reducing the weight pressure of lower sections and making them accessible to the worms.

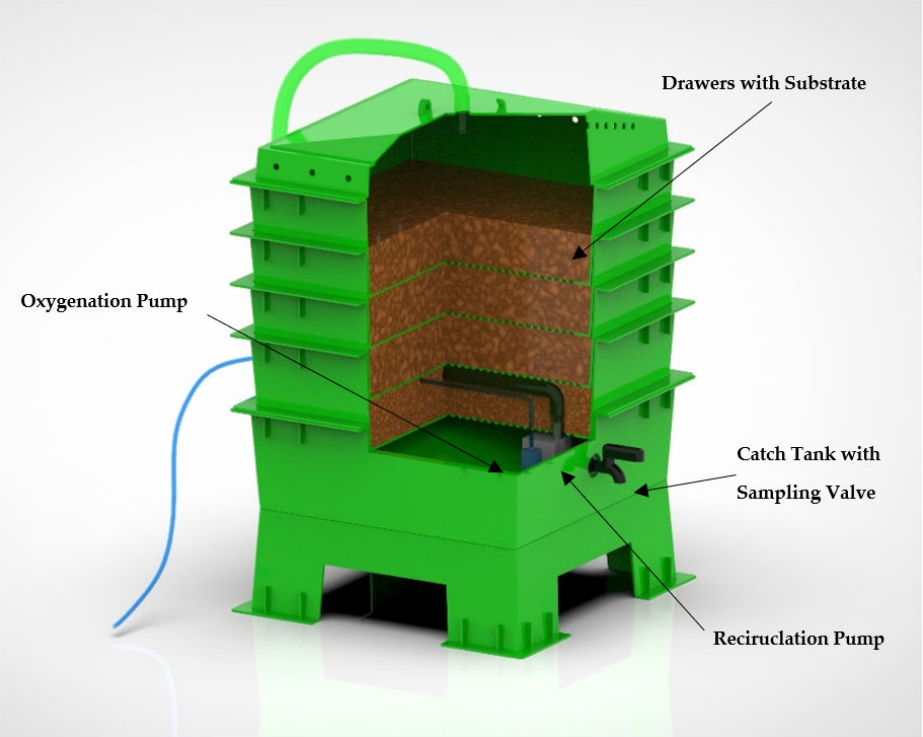


Figure 1. Schematic design of laboratory-scale bio-/vermifilter units.

VC is another biotechnology that uses worms to decompose organic matter [17]. The transition between VF and VC is fluid. In general, the focus of operation is either on the filtration of liquid influents or on the degradation of biosolids and the production of compost materials from organic matter. The present study could be seen as a hybrid approach between both.

The devices were divided into three groups in triplicates. Two groups were stocked with the oligochaete *Dendrobena veneta* (Michaelsen, 1890), also known as the European nightcrawler. An intensive group (I) and an extensive group (E) were stocked with 15 and 10 g of worm biomass per liter of substrate volume, respectively. The control group (C) was not inoculated with worms. Each filtration device was fed with sludge from the RAS-producing African catfish, *Clarias gariepinus* (Burchell, 1822). Sludge was collected from the semi-intensive aquaculture unit in the FishGlassHouse at the Faculty of Agriculture and Environmental Sciences at the University of Rostock, with a maximum fish stocking density of 100 kg/m^3. Fish were fed with *Coppens Meerval Special PRO EF* feed. The sludge was collected from mechanical filtration devices in the RAS system (sedimentation units) as described in [13]. The average dry matter content of the sludge was 3.13 $\pm$ 0.26%.

In keeping with the weekly routine management in the aquaculture facility, sludge was taken every 7 days after cleaning the sedimentation units. These were decoupled from recirculation, and the solids were left to settle for 15 min before the supernatant was pumped out from the surface, leaving only the settled solids. This sludge suspension was then thoroughly mixed, pumped out, and used as influent for the filtration units and sampling.

To prevent the sedimentation and unequal distribution of the sludge's dry matter to the filtration devices, it was constantly stirred manually and homogenized before and during feeding. The sludge was applied from the top of the filters and trickled through under gravity. The catch tank was equipped with a pump (*Tunze* 106 s 5 W) for the recirculation of effluent water. The solids remained trapped inside the bedding substrate. For ensuring the uniform distribution of the effluent, a plastic box that fit snugly into the top drawer was drilled with evenly distributed holes. The liquid was recirculated two times a day and spread again on the top drawer. The room temperature in the laboratory was kept

constant at 21 °C. After one week of recirculation, each catch tank was emptied, the effluent was kept for sampling, and the sludge for the next week was introduced into the systems. This procedure was continued for four weeks. The average C/N ratio of the sludge (serial measurements for six weeks before the experiment) was 9.57 ± 0.48. The optimum value for both worm activity and microbial decomposition is known to be approximately 25 [22]. To bridge this gap, carbonaceous matter (specifically, wood chips) was added as a substrate. A rather conservative feeding approach was chosen, because this material is organic and compostable, and how much and how fast the wooden bedding substrate and on-growing biofilm would be accessible for the worm's nutrition could not be predicted.

2.2. Feed Ration Calculation

The organic loading rate (*OLR*) in relation to the worm stocking density was calculated to gauge the appropriate amount of input sludge as feed for the worms. The average *OLR* over four weeks was 0.155 kg COD (chemical oxygen demand)/m^3/d, as calculated using the following equation:

$$OLR = \frac{(COD_L \times Q)}{V}$$

where COD_L is the chemical oxygen demand concentration of input sludge (kg/L); Q, the flow of influent in the filter (L/day); and V, the reactor volume (m^3).

The initial stocking densities were 360 g/device (E) and 540 g (I). This translated to 8.556 worms/m^3 and 12.833, respectively.

Each filtration device was fed with 3 L of sludge per week. Given the average dm content in the fed sludge, the fed sludge per filter was 11.72 g dm/d, 14.23 g dm/d, 13.20 g dm/d, and 14.74 g dm/d in the first, second, third, and fourth weeks (13.47 ± 1.15 g dm/d throughout the experiment), respectively. This corresponded to 2.49% and 3.74% of the initial worm body weight per day for groups (E) and (I), respectively. The sludge was directly applied with the maximum amount of available dry matter without any further concentration other than as described before.

2.3. Initial Worm Stocking Weigh-In

The worms came packed in plastic bags together with some fully matured vermicompost of unknown composition as the bedding. To eliminate bias, the input stock was carefully washed with tap water to remove any residues. Subsequently, the worm mass was put on a plastic sieve to remove excess water and carefully pat-dried on paper towels. Although the worms were widely homogenous in size, they were preselected, and small individuals and cocoons were removed. Thirty-three individual worms were separated three times and weighed to calculate the average weight per worm. Thereafter, the filter groups were stocked with 540 g of worms for group (I), 360 g of worms for group (E), and no worms for the control (C). The mean initial bodyweight of the worms at the beginning of the experiment was 1.17 ± 0.04 g/worm.

2.4. Weigh-Out

At the end of the experiment, the total worm biomass was collected from each filter, washed, placed on a plastic sieve, and pat-dried. The total biomass per filter and 3 × 33 individuals for obtaining the average weight per worm were weighed on a laboratory scale. The specific growth rate (*SGR*) and absolute daily growth rate per worm were calculated. The number of individual worms and mortality were calculated on the basis of the total and average single worm weight for 99 individuals at the beginning and end of the experiment.

The *SGR* was calculated as follows:

$$SGR\left(\% \, d^{-1}\right) = \frac{(lnBW_f - lnBW_i)}{t} \times 100$$

where BW_f is the body weight in grams at the end of the experiment; *Bwi*, the body weight in grams at the beginning of the experiment; t, the period, expressed in number of days; and *Ln*, the natural logarithm.

The absolute daily growth rate (*DGR*) per worm was calculated as follows:

$$DGR = \frac{W_f - W_i}{t}$$

where W_f is the final individual worm weight in mg; W_i, the initial individual worm weight in mg; and t, the duration of the experiment in days.

The mortality (*M*) was calculated as follows:

$$M = \frac{NW_i - NW_f}{NW_i} \times 100$$

where N_{wi} is the number of worms at the beginning of the experiment and N_{wf}, the number of worms at the end of the experiment.

2.5. Analysis and Sampling

The chemo-physical water parameters, pH and temperature, were measured weekly with a multimeter (HACH LANGE HQ40d) in the influent (sludge) and output effluent.

The total nitrogen bound (TNb), total organic carbon (TOC), and COD were measured weekly in the influent and effluent. For measuring the TOC and TNb, *compEAct N* CN Analyzer (Analytik Jena, Jena, Germany) was used. For measuring the COD, *LCK Cuvette Test System* and *DR3900* Spectrophotometer (Hach Lange, Ames, IA, USA) were used. For sampling and feeding, meticulous care was taken to use dedicated equipment for every single filter to avoid any bias arising from microbial exchange between stocked and unstocked filters.

One additional intensively stocked filter was continuously fed with 3 L of sludge per week for 8 months at the end of the experiment to assess the quality of the mature compost. One sample was sent to an external laboratory to test the compost quality as specified in the Bundesgütegemeinschaft Kompost BGK (German Association for Quality Assurance of Compost and Digestate) guidelines [23].

2.6. Statistical Analysis

IBM SPSS Statistics 27 was used for analyzing all data. The temperature and pH, growth, COD, TOC, and TNb were tested for normal distribution using the Shapiro–Wilk test. If data were normally distributed, one-way analysis of variance was used to test for significant differences in mean values between groups. If the homogeneity of variance showed nonsignificant results, the Tukey HSD post hoc test was used for multiple comparisons; otherwise, the nonparametric Dunnett-T3 test was used. The statistical significance level was set to $p < 0.05$. The graphs were plotted using GraphPad *Prism* version 8.0.0 for Windows 10.

3. Results

3.1. Total Worm Biomass and Growth Rate

The total worm biomass at the end of the experiment for groups (I) and (E) was 609 ± 11.52 g and 432 ± 13.72 g, respectively. This corresponded to a total weight gain of 56–84 g and 54–87 g, respectively. The SGR for groups (I) and (E) was 0.35–0.52% and 0.50–0.77%, respectively. The average weight per worm after four weeks for groups (I) and (E) was 1.36 ± 0.044–1.41 ± 0.025 g and 1.42 ± 0.015–1.52 ± 0.036 g, respectively (Figure 2). The worms from both groups (E) and (I) weighed significantly more than the initially stocked worms, and the individual worms from group (E) weighed significantly more than those from group (I) ($p < 0.05$). The DGR for groups (I) and (E) was 6.79–8.57 mg/d and

8.93–12.50 mg/d, respectively. The M for groups (I) and (E) was 3.46–5.84% and 3.57–5.19%, respectively(E).

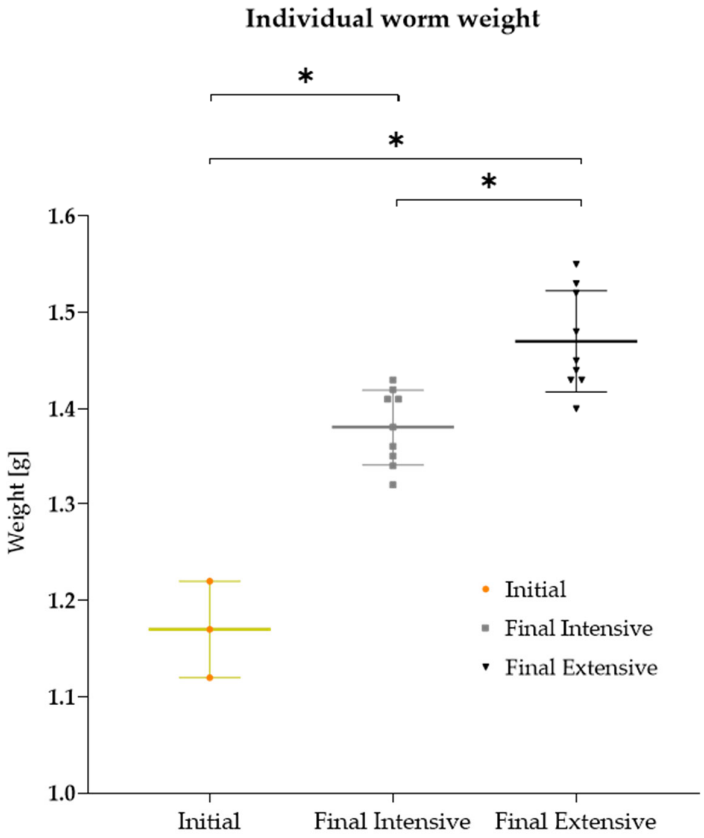
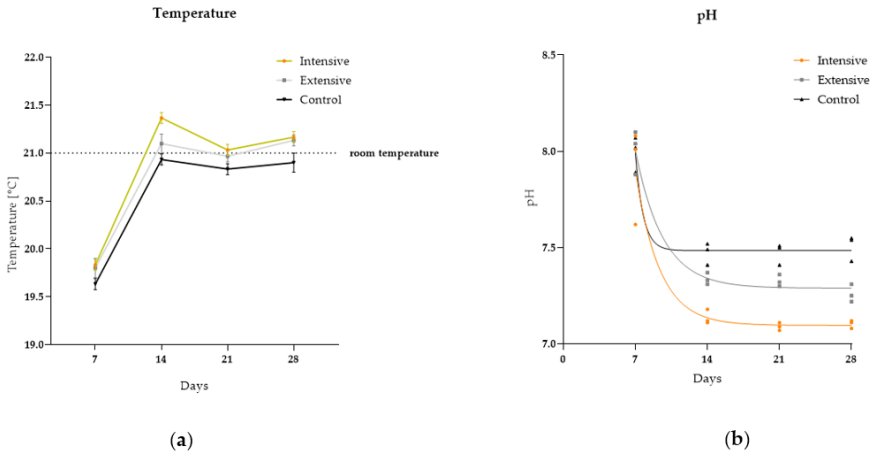


Figure 2. Individual worm weight at the beginning and end of experimental trial (* represents statistically significant difference).

3.2. Physicochemical Parameters (Influent and Effluent)

The pH and temperature plateaued in week 2, following which they did not change significantly for all groups. Only the pH of group (E) showed a small, albeit significant, difference between weeks 3 and 4; it decreased further to 7.26 ± 0.458 ($p < 0.05$) (Table 1, Figure 3).



(**a**) (**b**)

Figure 3. (**a**) Temperature and (**b**) pH in effluent over experimental period.

Table 1. Comparison between weeks (lowercase a–d represents statistically significant difference).

		rmANOVA, *p*-Value	Week 1	Week 2	Week 3	Week 4
pH	intensive	0.028	7.90 ^{a} ± 0.248	7.14 ^{b} ± 0.038	7.09 ^{b} ± 0.200	7.10 ^{b} ± 0.208
	extensive	0.005	8.01 ^{a} ± 0.114	7.34 ^{b} ± 0.031	7.33 ^{b} ± 0.031	7.26 ^{c} ± 0.458
	control	0.024	7.99 ^{a} ± 0.093	7.47 ^{b} ± 0.057	7.47 ^{b} ± 0.032	7.51 ^{b} ± 0.067
Temp °C	intensive	0	19.83 ^{a} ± 0.058	21.37 ^{b} ± 0.058	21.03 ^{b} ± 0.058	21.17 ^{b} ± 0.058
	extensive	0.005	19.80 ^{a} ± 0.100	21.10 ^{b} ± 0.100	20.97 ^{b} ± 0.058	21.13 ^{b} ± 0.058
	control	0.001	19.63 ^{a} ± 0.058	20.93 ^{b} ± 0.058	20.83 ^{b} ± 0.058	20.90 ^{b} ± 0.100
TnB mg/L	intensive	<0.01	100.07 ^{a} ± 17.156	23.66 ^{b} ± 0.795	11.47 ^{c} ± 0.927	8.91 ^{d} ± 0.632
	extensive	<0.01	88.11 ^{a} ± 7.215	23.14 ^{b} ± 3.454	11.67 ^{c} ± 1.194	10.21 ^{c} ± 0.436
	control	<0.01	85.15 ^{a} ± 2.562	27.25 ^{b} ± 5.065	13.84 ^{c} ±1.531	10.14 ^{d} ± 0.160
TOC mg/L	intensive	<0.01	1586.42 ^{a} ± 45.302	558.53 ^{b} ± 12.453	202.72 ^{c} ± 34.149	129.18 ^{a} ± 25.881
	extensive	<0.01	1601.15 ^{a} ± 54.970	594.95 ^{b} ± 199.529	207.22 ^{c} ± 36.221	198.47 ^{c} ± 18.349
	control	<0.01	1915.61 ^{a} ± 191.985	719.96 ^{b} ± 199.142	259.28 ^{c} ± 38.414	202.39 ^{d} ± 7.497
COD mg/L	intensive	<0.01	6157.0 ^{a} ± 28.627	2510.33 ^{b} ± 127.125	499.67 ^{c} ± 50.712	349.67 ^{d} ± 46.444
	extensive	<0.01	6100.33 ^{a} ± 27.382	2785.33 ^{b} ± 835.812	514.0 ^{c} ± 105.860	444.33 ^{c} ± 27.249
	control	<0.01	6430.67 ^{a} ± 88.026	3141.67 ^{b} ± 815.943	585.0 ^{c} ± 82.321	513.67 ^{c} ± 18.974

The TNb for all groups decreased significantly each week. Only group (E) showed no significant decrease between week 3 (11.67 ± 1.194 mg/L) and week 4 (10.21 ± 0.4636 mg/L) ($p < 0.05$).

The TOC for groups (I) and (E) decreased significantly each week. Only group (E) showed no significant decrease between week 3 (207.22 ± 36.221 mg/L) and week 4 (198.47 ± 18.349 mg/L) ($p < 0.05$).

The COD for all groups decreased significantly each week, except for groups (E) and (C) between weeks 3 and 4 (Table 2) ($p < 0.05$).

Table 2. Growth data for the worms.

	Final Total Biomass g	Total Weight Gain g	Final Weight/Worm g	DGR mg	Mortality %
Int. Group (I)	609 ± 11.52	56–84	(1.36 ± 0.044)–(1.41 ± 0.025)	6.79–8.57	3.46–5.84
Ext. Group (E)	432 ± 13.72	54–87	(1.42 ± 0.015)–(−1.52 ± 0.036)	8.93–(−12.50)	3.57–5.19

In week 1, no significant pH differences were seen between the groups. In week 2, significant differences were seen between all groups. The strongest pH neutralization was seen in group (I), with a pH of 7.14 ± 0.038 at the end of week 2. The pH of groups (E) and (C) was 7.34 ± 0.031 and 7.47 ± 0.057, respectively. In week 3, again, significant differences were seen for each group ((I): 7.09 ± 0.200, (E): 7.33 ± 0.031, (C): 7.47 ± 0.032) ($p < 0.05$). In week 4, significant variations were seen between the groups. At the end of week 4, groups (I), (E), and (C) had a pH of 7.10 ± 0.208, 7.26 ± 0.456, and 7.51 ± 0.067, respectively ($p < 0.05$).

Both groups (I) and (E) showed significant differences in temperature after week 1 compared to group (C) (19.63 ± 0.058 °C) ($p < 0.05$). However, no significant differences were seen between group (I) (19.83 ± 0.0508 °C) and group (E) (19.80 ± 0.100 °C). At the end of week 2, a significant difference was seen between (I) and groups (E) and (C) ((I): 21.37 ± 0.058 °C, (E): 21.10 ± 0.100 °C, (C): 20.93 ± 0.058 °C). At the end of week 3, significant differences were seen between groups (I) and (C) and between groups (E) and (C) but not between groups (I) and (E) ((I): 21.03 ± 0.058 °C, (E): 20.97 ± 0.058 °C, (C): 20.83 ± 0.058 °C). At the end of week 4, significant differences were seen between groups (I) and (C) and between groups (E) and (C) but not between groups (I) and (E) ((I): 21.17 ± 0.058 °C, (E): 21.13 ± 0.058 °C, (C): 20.90 ± 0.100 °C) ($p < 0.05$).

3.3. Organochemical Parameters (TNb, TOC, and COD)

After week 1, the TNb levels were significantly different between group (I) and the other two groups. No significant differences were seen between groups (E) and (C). After week 2, no differences were seen. After week 3, significant differences were seen between groups (I) and (E) on the one hand and group (C) on the other hand, but not between groups (I) and (E). At the end of week 4, group (I) showed the lowest TNb of 8.91 ± 0.632 mg/L; this was significantly lower than those of groups (E) (10.21 ± 0.436 mg/L) and (C) (10.14 ± 0.160 mg/L). No differences were seen between groups (E) and (C) (Figure 4a) ($p < 0.05$).

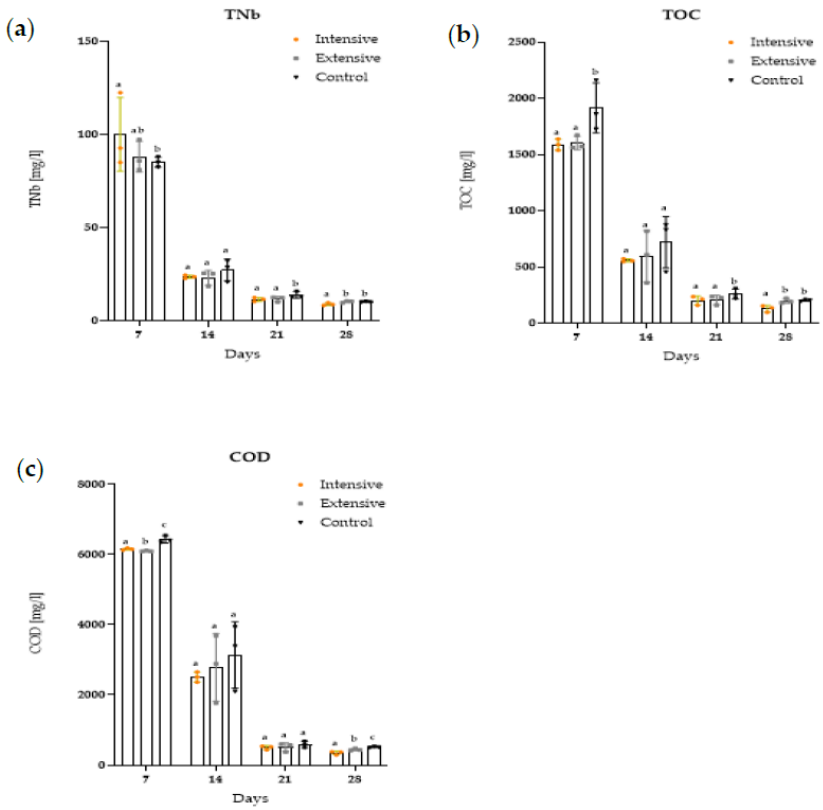


Figure 4. (**a**) TNb, (**b**) TOC, and (**c**) COD in effluent over experimental period (lowercase a–c represents statistically significant difference).

After week 1, the TOC was significantly higher in group (C) than in groups (I) and (E); however, no significant differences were seen between groups (I) and (E). After week 2, no significant differences were seen between the three groups ((I): 558.53 ± 12.453 mg/L, (E): 594.95 ± 199.529 mg/L, (C): 719.96 ± 199.142 mg/L). After week 3, groups (I) and (E) again showed significantly lower TOCs compared to group (C). After week 4, significant differences were seen between group (I) on the one hand and groups (E) and (C) on the other hand, but not between groups (E) and (C) (Figure 4b) ($p < 0.05$).

After week 1, the COD levels were significantly different between all groups ((I): 6157 ± 28.627, (E): 6100.33 ± 27.382, (C): 6430.67 ± 88.026). No significant differences were seen after week 2 ((I): 2510.33 ± 127.125, (E): 2785.33 ± 835.812, (C): 3141.67 ± 815.943) and week 3 ((I): 499.67 ± 50.712, (E): 514.0 ± 36.221, (C): 585.0 ± 82.321). After week 4, all groups showed significantly different COD levels ((I): 349.67 ± 46.444, (E): 444.33 ± 27.249, (C): 513.67 ± 18.974) (Figure 4c) ($p < 0.05$).

The additional filter (intensively stocked, 15 g worms/L) that was continuously fed with 3 L of sludge per week for 8 months after the end of the experiment produced mature compost. The compost quality parameters, as specified in the Bundesgütegemeinschaft Kompost BGK guidelines [23], are given in Table 1.

4. Discussion

4.1. Growth Rate

The average weight gain per worm after four weeks was 0.19–0.24 g and 0.25–0.35 g for the high- and low-density groups, respectively, with an individual growth rate of 6.79–8.57 mg/d and 8.93–12.50 mg/d for groups (I) and (E), respectively. There was a gap between the highest growth rate in group (E) and that reported in the literature. Viljoen [24] reported a mean growth rate of 11.9 mg/day/worm for *Dendrobena veneta* fed for 200 days from the cocoon onwards in cattle manure at 25 °C. The mean biomass achieved per worm was 2.350 mg at the end of the trial. For a growth period of 200 days, the fastest worm growth was seen at age 30–100 days after hatching, with a mean growth rate of 19.4 mg/d/w [25]. Subsequently, the growth rate decreased to 9 mg/w/d for a worm age of 100–140 days. In the present experiment with an average individual worm input weight of 1.17 ± 0.04 g and the preselection of same-sized worms, the initial stock was already mature and most likely older than its exponential growth phase. This could explain the observed growth rate in the present study.

The data shows that the worms from group (E) performed better than those from group (I). This might be attributable to an undersupply of food, as the worms in group (E) had more sludge per worm supplied to the filtration units. Compared with the other compost worms, *Dendobena veneta* grows relatively slowly [26]. However, it has the largest size and highest biomass yield among commonly used species. The compost worms obtain their nutrition from both decomposing organic matter and on-growing microorganisms (bacteria, fungi, etc.) [27]. Under favorable conditions, they can consume 35–50% of their body weight on a wet weight basis per day [28], at an optimal moisture level of 60–70% [29]. Before setting up the experiment, it was estimated that a significant part of the worms' nutrition would be derived from the wooden bedding substrate and on-growing biofilm. Therefore, the OLR (0.155 kg COD/m^3/d) was set lower than in the literature [30,31]. However, at the launch of the experiment, the coarse wood chips were not accessible to the worms. Moreover, there was no pre-rotting phase in terms of adding sludge to the devices prior to inoculation with the worms, giving time for microbial colonization. Therefore, it could be concluded that the worms were under suboptimal conditions and possibly underfed, especially at the initial stage. Other studies emphasized that worms needed an acclimatization phase to feed on new substrates effectively, thus implying that the worms did not reach their full growth potential during the experiment [32,33].

4.2. Physicochemical Parameters: Temperature and pH

The optimum temperatures for the activity of the different composting worm species ranged from 15.7 °C to 35.0 °C [34]. Very limited information was available on the optimal production temperatures for the less commonly used *Dendrobena veneta*. However, the known temperature range being lower than that of *E. fetida* had some implications [35]. For the commonly used *E. fetida*, most authors reported an optimal temperature of approximately 25 °C [24,36]. Loehr et al. [27] concluded that the optimal temperature range for *D. veneta* was 20–25 °C. Fayolle et al. [32] analyzed the influence of temperature on the life cycle of *D. veneta*. These authors reared the worms at different temperatures and found that the initial growth rate was the highest at 25 °C. Further, the worms reached sexual maturity faster at this temperature. For this experiment, the room temperature in the laboratories was set at 21 °C. The influent sludge was allowed to acclimatize to 21 °C before being supplied to the filtration devices. Consequently, the temperature settings were likely not optimal for the growth and activity of *D. veneta*, and slightly higher temperatures would have been better for the overall performance of the VF systems, especially considering the short duration of this experiment. However, the temperatures within the vermisystems were slightly higher than that in the control. The effluent from (I) was on average 0.27 ± 0.08 °C warmer than that from (C). This indicated a different microbial activity in the worm-stocked filters. Worm digestion affects the microfauna, in turn influencing the number of bacteria and fungi degrading and decomposing in the system. Furthermore,

worm burrowing supports aeration of the filter packing and aids the activity of aerobic mesophilic microorganisms.

Quicklime was used to adjust the pH of the aquaculture process water as a daily routine in the RAS. The settlement of nondissolved lime residues caused the sediment sludge to be highly alkaline. The pH of the influent sludge was 8.04, 8.10, 7.45, and 7.63 for the four consecutive weeks of the experiment. The average pH of the influent was 7.83 ± 0.25. The VF systems showed strong regulating effects on the effluent, as the pH evened out to an almost neutral level of approximately 7, whereas it was considerably higher in the non-worm-stocked filters. The strongest buffering effect was observed in group (I), with an average pH of 7.10 ± 0.02 at the end of the trial. Except for the first week, in which no statistical differences were seen between the groups, all subsequent sample points proved that groups (I) and (E) reduced the influent pH significantly more than did group (C). Different authors substantiated these findings [37–39]. Further, the acidic pH influents might be regulated by the worm and microbial activity [40]. This capability could be especially interesting for aquaponic producers, as they often face problems in adjusting extreme pH values within the process water, which is a time-consuming and therefore costly process [41]. The optimal pH for aquaponics is 6.5–7 [42]. Therefore, a coupled VF system to treat fish effluents upstream to the plant production might also be effective to reduce the use of expensive buffering agents. In general, the neutralization of both acidic and alkaline process waters from aquaculture might be of interest. Depending on the aquatic species used and the general management, the actual pH in RAS operations varies widely [43].

4.3. Organochemical Parameters (TNb, TOC, and COD)

After initial feeding and one week of recirculation, the TNb values were significantly higher in group (I). This might be attributable to the fact that the input worm stock was not starved before being stocked to the filters, and their gut contents were still rich in the nitrogenous compounds that were released into the systems. The second week with no significant differences between the groups was considered a turning point during which time the worms incorporated more feed-derived nitrogenous compounds from the sludge, thereby reducing the total nitrogen compared to the control. However, the residues from the gut contents from the beginning still had some influence.

After week 2, the TNb levels in the effluent were heavily reduced by 75.75 ± 3.90% for (I), 73.33 ± 5.44% for (E), and 67.88 ± 6.11% for (C), even though the input TNb of the sludge was 88.29% (589.40 mg/L) higher than in the first week (313.02 mg/L). This suggests a run-in period of two weeks for microbial colonization and adaptation. The conversion and removal of the nitrogenous compounds is dependent on various pathways such as adsorption, filtration, precipitation, volatilization, and biomass assimilation [44]. With regard to VF, especially ammonification, nitrification, and denitrification plus adsorption into the bedding material, utilization by the worms plays a key role [45]. The anammox process must also be considered as it contributes to the overall nitrogen removal in many wastewater treatment systems [46]. The main reason for the significant differences in the TNb reduction between the vermi and the control filters might be the burrowing and digesting activity of the worms, direct uptake into the biomass, and a reduction in the particle size leading to an enlargement of the active surface area for microbial colonization. Earthworms excrete the consumed soil and support the decomposition of organics and nitrogen. This conversion is aided by the metabolic activities of the indigenous intestinal bacteria in the worms [47]. Nitrogen removal is also highly pH-dependent. Lu et al. [48] suggested that a slight alkaline environment with a pH of 7.4 and 7.6 is ideal for the growth of ammonia-oxidizing (AOB) and anaerobic ammonium-oxidizing (Anammox) bacteria. Egli et al. [49] reported that an even more alkaline environment with a pH of 7.5–8 resulted in the best anammox activity. In addition, for the majority of denitrifying bacteria, the optimum pH is 7.5–9.5 [50]. In this regard, the control had some advantages in N_2 production and therefore in the overall removal of nitrogen. Toward the

end of the experiment, differences between the vermi and nonstocked filters decreased but remained significant.

Another important fact to be considered in this regard was the progressive clogging of the control filters. The worm burrowing activities in the vermisystems kept the substrate loose, and worm passages supported aerobic conditions. In the control, the substrate was dense, compact and clogged, as indicated by its drained weight after 24 h at the end of the experiment. With an average weight of 19.72 kg, the bedding substrate for group (C) exceeded the worm-stocked groups (E) and (I) by 1.21 kg (+6.14%) and 1.19 kg (+6.03%), respectively. This demonstrated that the drainage capacity of the nonstocked filters was heavily reduced at the end of this rather short experimental trial. The amount of water-filled pores directly affects nitrogen conversion as it promotes denitrification [51]. Worm burrowing and digestion activities help to prevent this condition. The incipient clogging of the control filters might be beneficial to overall nitrogen removal through the denitrification and anammox processes, as it promoted the anaerobic conditions. Further, clogging might cause a total malfunction of the filter system and even if only proportional, would lead to a severe reduction in the filters' lifespan. Aeration through worm activity can reduce anaerobic degradation and hinders the excess output of CH_4 and N_20 that have specifically high global warming potentials.

The average removal rates of the organic chemical parameters were 87.42 ± 6.52% (I), 86.32 ± 6.03% (E), and 83.54 ± 7.92% (C) for TOC and 81.10 ± 18.93% (I), 80.49 ± 18.62% (E), and 78.86 ± 19.55% (C) for COD. With regard to previous studies, these reduction rates were quite high. Different authors reported rates of 58–85% for TOC [52–55] and 70–98% for COD [52,56,57]. However, the TOC levels in all groups increased in relation to the levels of the sludge fed after the first week, but to a lesser extent in the vermifilters ((I): + 38.78%, (E): +40.07% and (C): +67.58%). The increase was probably due to the sawdust particles from the wood substrate that are fine enough to coalesce with the recirculating liquid and pass through the filter pores to reach the catch tanks where the samples were taken. However, the worms quickly started to incorporate these fine particles into their nutrition. As for the TNb, the differences in TOC and COD between the groups began to decrease toward the end of the experiment; however, after week 4, the TOC of (I) and COD of (I) and (E) were still significantly lower than those of the (C).

Comparing the results with those from previous studies seemed rather difficult because of the differences in the setup used. Kumar et al. [55] used a very thin worm-active layer of 5 cm of fully matured vermicompost in comparison to the 30 cm layer of beech wood chips used in the present study. Thus, the worms in the previous study were restricted to obtain nutrition mostly from the pure wastewater influent and to a much lesser extent from the on-growing biofilm. Depending on the bed media and general setup of the filters' lower sections, a vermifilter might be too dense and devoid of oxygen and was thus not preferred by the compost worms [58]. However, owing to the drawer-style setup in the present study, the media bed density was not affected by the filter depth, and the worms reached every layer. Most earlier studies used continuously fed filters, whereas in the present study, a batch fed approach was chosen in adaptation to the weekly cleaning interval of the aquaculture system, where the sludge originated. Therefore, the sludge liquid that remained trickling into the filter catch tanks could be recirculated, supporting the very high removal rates, although the inceptive organic loading of the fed sludge was much higher than those from previous authors.

Further, the effects of the initial worm stocking density on TNb, TOC, and COD removal were obvious. At the end of the experiment, the removal rate for all parameters was significantly higher in group (I). The unique inherent worm gut-associated microbiota contributed to a miscellaneous community of microbes in VF [59]. The worms excrete the ingested substrate, which is rich in bacteria and enzymes; support the decomposition of organic compounds; and directly and indirectly modify the microbial community [60]. A possible bias to consider was the exchange of those beneficial microorganisms between the stocked and nonstocked filters. For sampling and feeding, meticulous care was taken to use

dedicated equipment for every single filter to theoretically avoid this possibility. However, after one week of operation, a noticeable number of small flying insects, namely, trickling filter moth flies (*Psychoda alternata*, Say 1824), was established within the laboratory. They are a common occurrence in RAS. Unfortunately, with the used setup, it was not possible to stop the hatched flies from moving from the vermifilters to the control. The potential effect of these flies could not be quantified.

To assess whether liquid VF effluents were suitable for reintroduction to the RAS water loop, six representative samples were taken prior to the experiment over a one-year operation period at a local commercial African catfish farm producing around 300 t per year. The outflow water from the mechanical filtration units on this farm showed an average COD of 503 ± 32.42 mg/L. At the end of the experiment, the COD concentrations were 349.67 ± 41.61 mg/L for (I), 444.33 ± 23.46 for (E), and 513.67 ± 12.71 for (C). Thus, the effluent re-supply to the RAS water circulation from the vermifilters would have led to an improvement in the water quality, whereas that from the nonstocked filters would have resulted in a slight deterioration.

The nitrogen and COD removal rate for all vermifilters met the EU standards for the discharge of urban wastewater according to Directive 91/271/EEC [61], which demands a minimum removal of 70% nitrogen and 75% of the COD. For aquaculture practitioners, it is of special interest to reduce effluent pollution. The regulatory bodies in the United States have restrictions on individual farms and hatcheries in terms of nutrient levels in the effluents dumped into public surface waters [62]. In Denmark, efforts are being made to base the maximum production of aquacultures on transferable nitrogen output quotas [63]. Alongside other technical solutions to reduce the nutrient and organic loads to meet the increasingly stringent requirements for aquaculture effluents, VF is a beneficial contribution, especially in the context of the more sustainable food production and organic farming promoted by the EU Green-Deal policy [60].

4.4. Compost Quality

The compost quality was analyzed in compliance with the BGK guidelines (Table 3). The achieved quality met all of the requirements for fresh and finished compost, except for the water content. In this case, the water content as a quality criterion could be neglected as it could be easily adapted. The quality characteristic "hygiene," in terms of a desired thermophile phase in standard composting, was not applicable because of the low temperatures prevalent in vermitreatment. However, no *Salmonella* spp. was found in 50 g of the original matter. The sample was neither tested for germinable seeds nor for foreign matter, because the used raw materials were pure. As the macronutrient content varies substantially in different composts, a general comparison was difficult. However, it was interesting to note that the P content of 26 g/kg dm in the vermicompost was relatively high compared to that of other manure-based composts. The authors of [64] found an average P content of 15.5 g/kg dm in pig manure plus sawdust, and Richards [62] reported multisided mean values of 9–20 g/kg dm from livestock-manure-based conventional compost products. Referring to the values from the BGK Book of Methods [25] determined as a 10–90% percentile range for all performed quality-assured compost analyses from 2008, the P content there varied between 4–11 g/kg dm and the pH was 7.8–8.2 compared with the VC of 7.3. The total N in the VC was within the BGK´s range of 0.9–1.9% dm; however, at the high end of the range with 1.86%. The K in the VC was relatively low with 0.26% dm and not in the reported range of 0.6–1.8% dm. For heavy metal loads, some noteworthy differences were seen. The BGK values were 21–59 mg/kg dm for Pb (VC = 4.0), 14–36 for Cr (VC = 4.8), and 6.8–25 for Ni (VC = 4.0). The only heavy metal in the VC exceeding these values was Zn 120–249 (VC = 339); however, it still matched the BGK´s criteria and the European and American critical limit loads [63].

Table 3. Vermicompost quality parameters after 8 months of continuous feeding.

Chemical Parameters	Unit	Value in OS	Value in DM	Quality Requirements BGK	Method
Salinity	gKCI/L	0.73			EN 13038 /DIN EN 13038:2012-01
pH-value		7.3			DIN EN 13037: 2012-01
Physical Parameters					
Bulk density	g/L	1030			Methods of BGK:2006-09
Water content	%	83.5			DIN EN 13040: 2008-01
Soil improvement					
C/N-Ratio at 450 °C			20.7		Calculation from single parameters
Organic matter	%	11	66.4	>30	DIN EN 13039:2000-02
Alkaline constituents CaO	%	0.92	5.55		Methods of BGK: 2006-09
Plant nutrients					
Nitrogen total (N)	%	0.31	1.86		Methods of BGK: 2013-05
Phosphorus total (P_2O_5)	%	0.431	2.61		DIN EN ISO 11885: 2009-09
Potassium total (K_2O)	%	0.0429	0.26		
Magnesium total (MgO)	%	0.08	0.51		DIN EN ISO 11885: 2009-09
Nitrogen $CaCl_2$-soluable	mg/L	24.5			Methods of BGK: 2006-09
Ammonium (NH_4-N)	mg/L	0.722			Methods of BGK: 2006-09
Nitrate (NO_3-N)	mg/L	23.8			Methods of BGK: 2006-09
Biological Parameters					
Rotting degree		V		II	Methods of BGK: 2006-09(PT)
Hygiene					
Salmonella	in 50 g	not found		n.f	Methods of BGK, chapter IV C: 2006-09
Potential pollutants				Critical limits (mg/kg DM)	
Lead (Pb)	mg/kg	0.66	4	150	DIN EN ISO 17294-2: 2005-02
Cadmium (Cd)	mg/kg	0.076	0.461	1.5	DIN EN ISO 17294-2: 2017-01
Chromium (Cr)	mg/kg	0.797	4.83	100	DIN EN ISO 17294-2: 2017-01
Copper (Cu)	mg/kg	8.4	51.4	100	DIN EN ISO 17294-2: 2017-01
Nickel (Ni)	mg/kg	0.67	4.06	50	DIN EN ISO 17294-2: 2017-01
Mercury (Hg)	mg/kg	0.021	0.13	1	DIN EN 1483: 2007-07
Zinc (Zn)	mg/kg	55.9	339	400	DIN EN ISO 17294-2: 2017-01

5. Conclusions

This study shows that aquacultural sludge is a suitable source of nutriment for VF with *Dendrobena veneta.* The biomass gain was substantial for the applied mature worms. The vermifilters reduced the alkalinity of the influent to almost neutral levels, thus demonstrating the pH buffering capacity of VF.

The worms effectively reduced COD, TOC, and TNb loads in African catfish (*Clarias gariepinus*) sludge.

The nitrogen and COD removal rates met the EU standards for the discharge of urban wastewater, and the effluents from the vermifilters were below the COD levels at the mechanical filtration units from a local commercial African catfish production. The worm burrowing activity kept the filter packing loose and aerated, thus preventing clogging observed in the control filters.

Dendrobena veneta and associated microorganisms together were able to transform aquacultural sludge and beech wood chip substrate into a high-quality biological fertilizer that was particularly rich in nitrogen and phosphorus and low in contaminants. The low K and higher Zn contents reflected the peculiarities of African catfish RAS effluents that demonstrate a species-specific nutrient degradation that was also reflected in the compost. Compost worms have been reported to have a high protein content, including vast amounts

of lysine, thus being a suitable protein source for substituting fishmeal in future diets [61]. However, it has to be proven that the nutritional composition of worm-fed aquacultural sludges can compete with traditionally fed conspecifics and do not incorporate pollutants such as heavy metals to a critical degree. Further research should be encouraged to fully understand the benefits of VF systems for the more sustainable operation of aquaculture and aquaponics productions, thus encouraging actual implementations into existing operations.

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