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Vermicomposting of sludge from recirculating aquaculture system using *Eisenia andrei*: Technological feasibility and quality assessment of end-products

Antonín Kouba*, Roman Lunda, David Hlaváč, Iryna Kuklina, Jitka Hamáčková, Tomáš Randák, Pavel Kozák, Anna Koubová, Miloš Buřič

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, CZ-389 25 Vodňany, Czech Republic

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ABSTRACT

Intensive aquaculture is an important and fast-growing food production industry generating significant amounts of nutrient-rich sludge, which represents a potential environmental threat. Vermicomposting aquacultural sludge has been suggested, but remained poorly understood – only survival and growth of initial earthworm stocks have been assessed so far. The present study provides a comprehensive evaluation of the production system, examining vermicomposting of three types of sludge each at four inclusion levels and the possibility of further utilising end-products (vermicomposts and earthworms). Through an 18-week experiment, high survival of initial earthworm stocks, exceeding 90% among treatments up to week 6, was documented. Higher inclusion levels and sludge types richer in nutrients positively influenced individual weight of initial stocks and their reproduction indices (cocoon and juvenile production). The most progressive treatments sustained >300 juveniles in experimental incubators containing 200 g dw of initial substrates. Original sludge and final vermicomposts were found suitable for use in agriculture, complying with limits for heavy metals given in the most usually applied regulations. In relation to the heavy metals, earthworms were found to be a generally safe feed for fish. Only arsenic concentrations may occasionally exceed given limits. Still, observed concentrations are considered safe, presuming arsenic presence primarily in organic forms having largely reduced toxicity. Vermicomposting is recommended as a clean and sustainable technology transforming aquaculture sludge into highly valuable vermicompost and earthworm biomass.

1. Introduction

Fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. Since 2014, more than half of all fish for human consumption came from aquaculture. Its extent, diversification and intensification make aquaculture one of the fastest growing food-producing sectors globally. As a result of the magnitude and intensity of aquaculture production, issues related to its long-term sustainability and environmental impacts have become more pronounced (FAO, 2016).

Many flow-through and cage aquaculture systems have minimally effective, or a complete lack of, systems for treating effluent waters (van Rijn, 1996). This leads to the unacceptable rate of eutrophication of adjacent recipients. Increasingly strict regulations on discharged waters, combined with a limited number of suitable sites for conventional aquaculture systems, has led to the development of recirculating aquaculture systems (RAS). RAS have distinct advantages compared with conventional technologies, since the amount of effluent water is much lower, while the concentration of solid wastes is substantially higher. This makes treatment of effluent waters more effective, easier and cheaper (Blancheton et al., 2007). Despite improvements in digestibility of commercial feeds provided to the cultured fish, some 15% of consumed feeds is converted to faeces (Reid et al., 2009) and some 5% not consumed (Bureau et al., 2003). For RAS, an additional biomass of microorganisms is released mainly from biofilters (van Rijn, 1996). All of these resources are particularly rich in organic

* Corresponding author.
E-mail address: akouba@frov.jcu.cz (A. Kouba).

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matter and nutrients.

The resulting aquacultural sludge is extremely susceptible to putrefaction and may contain various pathogens. This makes its direct utilisation as a fertiliser applied on the agricultural lands problematic. Its dewatering and stabilisation prior to such application is recommended (Bergheim et al., 1998). Further ways of RAS sludge utilisation are rarely applied and include biogas production (del Campo et al., 2010), composting or vermicomposting (Marsh et al., 2005).

Vermicomposting is a complex biological and ecological process of accelerated bio-oxidation and stabilisation of organic material. In contrast to traditional composting, it involves the joint action of earthworms and microorganisms without a thermophilic phase (Edwards, 2004), exhibiting reduced emission of greenhouse gases (Nigussie et al., 2016). The applicability of this biotechnology has been shown for a wide range of organic matrices. Vermicomposting allows transformation of potentially problematic organic solid waste into highly valuable end-products – vermicompost and biomass of earthworms (Lim et al., 2016).

Marsh et al. (2005) proposed the possibility of vermicomposting RAS sludge mixed with shredded cardboard for use as a feedstocks for the earthworm *Eisenia fetida* (Savigny, 1826). However, this study evaluated survival and growth of initial stocks only, which is insufficient for complete evaluation of the applicability of this technology. The qualitative parameters of RAS sludge vary between farms and the same is expected for different technological sections of a given RAS. Low sludge inclusion levels do not promote a suitable vermicomposting process while overdosing may led to the mortality of initial stocks. In the present study vermicomposting of three kinds of sludge obtained from a commercial RAS mixed with shredded wheat straw at four inclusion levels each was tested. Expanding on previously evaluated parameters, cocoon and juvenile production of *E. andrei* Bouché, 1972 were assessed during an 18-week experiment. Final vermicomposts were characterised and, together with earthworm biomass, contents of selected heavy metals were measured. Two of the most commonly used commercial fish diets and a dominant market-sized fish conventionally reared on the farm from which RAS sludge originated were also analysed for heavy metals. This allows qualitative evaluation of resultant earthworms as an alternative diet for feeding fish.

2. Material and methods

2.1. Substrates and earthworms

Three kinds of aquaculture sludge were obtained from a Trout farm (Mlýny, Žár, Czech Republic). Two kinds of sludge were acquired directly from the RAS. The sludge sampling sites were located either in the outlet channel from the culturing units (derived vermicomposting treatments thereafter indicated as O) or in the immersed biofilter (B). The third sludge was sourced from an adjacent pond which is used for sedimentation of effluent water (P). For a detailed description of the RAS and location of sampling sites see Supplementary Information (Fig. S1) and Buřič et al. (2016).

The O sludge was taken manually with a fine hand-held mesh screen from sedimentation zones in the RAS. The B sludge was pumped from immersed biofilters during the desludging process. For P sludge, a top layer of fine sediment was scraped manually from close to the inflow of effluent water in the sedimentation pond. Sludge of B and P origin were further sieved through a stainless steel sieve with a mesh size of 0.65×0.65 cm in order to eliminate large particles, mainly plastic elements (RK Plast A/S, Skive, Denmark) used for biofiltration in the RAS. Resulting sludge samples were left on polyamide meshes (mesh size of 109 μm) for 2 h for gravitational dewatering. Composition of the sampled sludge is shown in Table 1. Unless further specified, analyses were done in the accredited laboratory of the AGRO-LA, spol. s.r.o., Jindřichův Hradec, Czech Republic. Organic carbon was determined at the Institute of Soil Biology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic. The analyses followed standardised methods of Zbíral and Honsa (2010) for dry matter, pH, calcium, magnesium, phosphorus, potassium and sodium, and Zbíral et al. (2011) for organic matter, total organic carbon and total nitrogen. Heavy metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc) were determined at the accredited laboratory of the State Veterinary Institute in Prague, Czech Republic according to Zbíral (2011). The mercury concentration was determined by the AAS (AMA254, Altec, Czech Republic), chromium (Cr) by GF-AAS (SpectrAA 220Z, Varian, Australia) and the other metals by ICP-MS, Varian, Australia.

Suitability of biological material for vermicomposting is often determined by its humidity and C:N ratio. Despite species-specific differences in requirements among earthworm species typically involved in vermicomposting, these parameters are usually around 80% humidity and 25:1 C:N (Ndegwa and Thompson, 2000). The raw RAS sludge had low dry matter content and was rich in nitrogen (Table 1). In order to mitigate the impacts of both high humidity and nitrogen content, dry carbonaceous material (shredded wheat straw) was included. Wheat is one of the most widely grown cereal crops globally, being cheap and widely available. For composition of the straw see Table 1.

The stock of earthworm species, originally purchased as *E. fetida*, was obtained from a commercial supplier (Tomsovy žížaly, 2008). This species and *E. andrei* are closely related epigeic earthworms often utilised in vermicomposting (Edwards, 2004), and species may sometimes have been incorrectly assigned, particularly in older literature. Applying molecular methods (Dvořák et al., 2013) to our earthworm stock revealed the correct species assignment to be *E. andrei* (Dvořák, personal communication, July 2012).

2.2. Experimental set-up

2.2.1. Tested treatments and incubators

Experimental substrate mixtures consisting of 5, 10, 20 and 30% dry weight (dw) of respective sludges and shredded wheat straw were manually mixed. The abbreviated codes thereafter refer to the sludge content e.g. B20 for a treatment containing 20% RAS sludge sourced from the biofilter (B). Shredded wheat straw itself served as the control. Each treatment was tested in triplicate, each 200 g, with an initial humidity of 75% (i.e. 800 g wet weight, ww). The initial humidity was adjusted using distilled water.

The substrates were placed in the experimental incubators, which were made from a polypropylene pipe (inner diameter 12.5 cm, height 22.5 cm) with a fine (mesh size of 109 μm) polyamide mesh fixed on its lower part. This mesh prevented escapes of earthworms and allowed drainage of any excessive water. The upper part was closed with a tightly fitting lid with a hole in the centre (2.2 cm in diameter), which was overlain with a mesh glued on its inner side to allow ventilation. Completed incubators were placed on polypropylene plates (17 cm in diameter) which collected excess water. For more details on the construction of experimental incubators see Supplementary Information (Fig. S2).

2.2.2. Stocking of earthworms, temperature and humidity maintenance

In order to reduce the possibility of earthworm mortality in treatments with higher concentrations of RAS sludge (presumably caused by the toxicity of ammonia), all incubators, once filled, were left in a temperature-controlled room for a one-week pre-