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Antioxidant and behavior responses of earthworms after introduction to a simulated vermifilter environment

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ABSTRACT

Vermifiltration is a new technology for treating wastewater with a high organic loading, but there is almost no published information on the biochemical and behavioral response of earthworms in a vermifilter environment. This study measured antioxidase enzyme (AOE) activities and reactive oxygen species (ROS) and malondialdehyde (MDA) levels in tissues of earthworms for a period of 56 d after transfer into simulated vermifilters. Three vermifilter media were tested. In general AOE activities, ROS and MDA were elevated for the first 3–5 d after transfer, variable for approximately the next 7 d, and statistically indistinguishable from controls after 15 d. Similarly, earthworm activity as measured by daily burrowing length was initially enhanced but converged with that of controls after 6 d. Removal of chemical oxygen demand (COD) from artificial wastewater was significantly correlated with AOE and ROS in earthworm tissues, and daily burrowing length of earthworms, but none of these parameters was correlated to NH_4^+-N removal rate, suggesting earthworms do not play a direct role in nitrogen removal in vermifilters. The data provide useful information for vermifilter operators about the biochemical adaptive process earthworms undergo when introduced to a vermifilter environment and the lag time between set up and reliable operation of a vermifilter. The results could potentially lead to development of preconditioning treatments to shorten the settling-down time for newly set up vermifilters. Maximum burrow depth was reduced in simulated vermifilters, suggesting an optimal depth for vermifilter substrates. The three vermifilter media tested differed in performance and possible reasons are briefly discussed.

1. Introduction

Earthworms are an important group of soil fauna whose activities contribute strongly to development of soil structure, nutrient cycling and soil fertility (Zhang et al., 2007). Vermifiltration is a new technology using earthworms to process organically polluted water (Li et al., 2008; Xing et al., 2012). It has been developed to treat sewage or effluent from household sources or indoor rearing of animals with a mixture of solid and liquid phases and high organic matter load, as well as municipal sewage with relatively low organic matter loads (Bouché and Soto, 2004; Li et al., 2008; Sinha et al., 2008). Vermifiltration effectively reduces carbon (C), nitrogen (N), and phosphorus (P) concentration in wastewater resulting in sludge reduction and stabilization and on-site sludge sanitization (Li et al., 2009; Xu et al., 2013; Zhao et al., 2010). The efficacy of vermifiltration is indicated by the results from a commercial scale pilot plant tested in Shanghai in 2009 and 2010 (Li et al., 2009; Zhao et al., 2010). Over the first 18 months of operation 60 m^3 of village wastewater was treated daily with an average removal rate for chemical oxygen demand (COD), biochemical oxygen demand (BOD), NH_4^+-N, and total suspended solids of 67.6%, 78.0%, 92.1% and 89.8%, respectively. The treated water was compliant with current minimum standards for irrigation water in China (Zhao et al., 2010).

In recent years, many studies on the physical process of vermifiltration have been published and these are helpful for understanding and managing vermifilters (VFs) (Xing et al., 2012; Yang et al., 2013b). Additionally the range of microorganisms which form surface films on the VF substrate, and together with the earthworms contribute to the VF pollutant removal process has now been characterized (Yang et al., 2013a).

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However, few publications have examined the physiological and behavioral responses of earthworms after they are placed in a VF. Transfer to a VF is a substantive change in environment for earthworms. There is much higher occupancy of pore spaces by water and a greater contaminant (C, N, P etc.) concentration in VF than in soil. Therefore, earthworms may suffer a stress response while their physiological processes adapt to the new environment. Furthermore, it is possible that changes in earthworm physiology in response to the VF environment may affect the earthworm/microbial community relationship with consequential effects on the operation and treatment efficiency of the VF (Zhao et al., 2010). It can also be assumed that the health of the earthworms in the vermifilter (VF) provides a self-bioindication of VF functionality. For these reasons, it is important to understand the stress responses of earthworms when transferred into VF environments.

One major stress factor for earthworms in VF is oxidative stress generated by an imbalance between reactive oxygen species (ROS) and antioxidase activity. The formation of ROS is a normal consequence of a variety of essential biochemical reactions including mitochondrial and microsomal electron transport systems, and phagocytosis among others (Saint-Denis et al., 1998). The role of the antioxidase system is as a protective mechanism for earthworms to reduce or avoid oxidative injury to tissues and cells (Halliwell and Gutteridge, 1999; Honsi et al., 1999; Saint-Denis et al., 2001). Antioxidases make an important contribution to the prevention of oxidative stresses by scavenging ROS. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-px) form a defensive complex against endogenously-produced and exogenous ROS (Sanchez-Hernandez, 2006). Excess ROS may cause lipid peroxidation in the cells of an organism with malondialdehyde (MDA), the product of the reaction between free radicals and unsaturated fatty acids in cellular membranes, considered to be an indication of lipid peroxidation (Halliwell and Gutteridge, 1999).

Behavioral responses may also be an indication of stress. In recent years, the burrowing activity of earthworms has been used as a biomarker of soil quality and of the presence of certain chemical pollutants (Dittbrenner et al., 2010). 2D terraria provide a relatively simple method to observe earthworm burrowing in the laboratory and have been used by a number of authors to study the effect of the nature and placement of food on burrowing behavior (Cook and Linden, 1996). Previous studies using 2 dimensional (2D) terraria have investigated ecologically endogeic and anecic species (Bouché, 1977; Capowiez, 2000). There is very little data on species that are epigeic in their natural environment, such as *Eisenia fetida* and *Eisenia andrei*, which are the most commonly used species for soil pollution and vermifiltration research (Bouché and Soto, 2004; Hong and James, 2009). Hence, information on any changes in burrowing activity or related behavioral changes would be a useful indicator of earthworm metabolic stress levels as a component part of research into VF operation.

No information about the antioxidant response and behavior changes of earthworms in a VF environment is currently available. In this study we investigated oxidative stress and burrowing behavior changes in earthworms introduced into a VF environment. The objective was to determine the extent and time course of adaptive responses in the earthworm body tissues and to estimate the period of time needed to adapt. Such information will provide insight into the adaption of earthworms to adverse environments, and should lead to changes in practice that improve VF performance.

2. Materials and methods

2.1. Vermifilter and terraria design

Small scale VFs of cylindrical cross section were constructed for determination of earthworm physiological responses, while 2D terraria were built for determination of burrowing responses (Fig. 1). A VF typically comprises 3 layers: an organic layer to support earthworms and to facilitate active decomposition of organic matter, a middle layer of fine stones to intercept particulate matter and partly decompose contaminants, and a bottom layer of larger stones to support the upper filter materials and improve efficacy of contaminant reduction (Li et al., 2009; Sinha et al., 2008). In these experiments, only the active layer was used as the main objective was to investigate the stress response of the earthworms. We did not include the other two layers because the earthworms do not go into them and thus their absence does not affect the responses of earthworms. The simulated VFs for determination of physiological responses were plastic cylinders with a diameter of 20 cm (cross-sectional area 0.0314 m^2) and height of 30 cm (Fig. 1a). Metal mesh was placed at the bottom of the cylinder to allow the drainage of wastewater through the filter material. Three combinations of filter materials sourced from commercial suppliers were used: VF1 – sawdust, peat, zeolite 1:2:2 (v/v/v); VF2 – sawdust, peat, crushed autoclaved aerated concrete blocks1:2:2 (v/v/v); VF3 – sawdust, peat, crushed volcanic scoria 1:2:2 (v/v/v). The diameter of zeolite, concrete block and volcanic scoria fragments was 2–10 mm. Three replicates were set up for each VF type.

Earthworm burrowing behavior was examined using terraria adapted from a design of Capowiez (Capowiez, 2000). Terraria were made from two glass sheets (each 33 cm × 48 cm) separated by 3-mm-thick strips of PVC on the two long sides (Fig. 1b). Two pieces of latex tape were used to bind the glass sheets together and cotton gauze used at the bottom edge of the glass sheets to retain filter materials after wastewater application. The upper edge remained open. Terraria were filled with the same filter materials as the VFs described above (denoted VF1, VF2, and VF3 in the following text), except that volcanic scoria, zeolite and concrete block media were crushed and sieved to obtain a fraction with particle size <1.5 mm diameter for each of the 3 materials, so that the particles could be filled into the 3 mm wide terraria. The prepared filter materials were spread on one of the glass sheets with the PVC strips attached, and then the other glass sheet was placed on top. Three replicate terraria were prepared with each filter material while 3 terraria containing a normal earthworm culture medium of mixture of 50% sphagnum and 50% cow manure in place of filter material were used as controls. The terraria were then arranged on a shelf and fitted with a wastewater distribution system comprising an aluminum channel at the top of each VF terrarium with 20 slots of 0.5 cm length to deliver the wastewater across the soil surface of each terrarium.

2.2. Artificial wastewater preparation

Stock artificial wastewater was prepared using a method adapted from a procedure for preparing standard artificial domestic wastewater (Wu et al., 2012). The ingredients of the artificial wastewater were (in mg l^{-1}): glucose (1406.25), NH_4Cl (143), KH_2PO_4 (66.25), $NaHCO_3$ (375), peptone (125), Yeast extract (50), $MgSO_4.7H_2O$ (82.5), $MnSO_4.7H_2O$ (7.5), $Fe_2(SO_4)_3$ (0.5). All these chemicals were put in a cylindrical plastic barrel with diameter of 1.04 m and height of 1.31 m, then tap water was slowly added while stirring with a bar to make 1000 l artificial wastewater. Up to 6000 l of artificial wastewater were prepared at a time, and prepared wastewater was stored in the barrels for 1–4 weeks prior to use. As the water was not sterile, COD and NH_4^+-N concentrations did change a little during storage as a result of bacterial growth. Hence, these concentrations were measured just prior to waste water application. This artificial wastewater was used in the experimental VF and terraria, but the control terraria did not receive any water application.