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Research article

Bacterial toxicity assessment of drinking water treatment residue (DWTR) and lake sediment amended with DWTR

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ABSTRACT

Drinking water treatment residue (DWTR) seems to be very promising for controlling lake sediment pollution. Logically, acquisition of the potential toxicity of DWTR will be beneficial for its applications. In this study, the toxicity of DWTR and sediments amended with DWTR to *Aliivibrio fischeri* was evaluated based on the Microtox® solid and leachate phase assays, in combination with flow cytometry analyses and the kinetic luminescent bacteria test. The results showed that both solid particles and aqueous/organic extracts of DWTR exhibited no toxicity to the bacterial luminescence and growth. The solid particles of DWTR even promoted bacterial luminescence, possibly because DWTR particles could act as a microbial carrier and provide nutrients for bacteria growth. Bacterial toxicity (either luminescence or growth) was observed from the solid phase and aqueous/organic extracts of sediments with or without DWTR addition. Further analysis showed that the solid phase toxicity was determined to be related mainly to the fixation of bacteria to fine particles and/or organic matter, and all of the observed inhibition resulting from aqueous/organic extracts was identified as non-significant. Moreover, DWTR addition not only had no adverse effect on the aqueous/organic extract toxicity of the sediment but also reduced the solid phase toxicity of the sediment. Overall, in practical application, the solid particles, the water-soluble substances transferred to surface water or the organic substances in DWTR had no toxicity or any delayed effect on bacteria in lakes, and DWTR can therefore be considered as a non-hazardous material.

1. Introduction

Drinking water treatment residue (DWTR) is an inevitable by-product generated during tap water production (Babatunde and Zhao, 2007). Commonly, DWTR can be classified into coagulant, groundwater or softening, natural and manganese residue. The coagulant residue constitutes the majority of DWTR and is also studied mostly (Babatunde and Zhao, 2007). In conventional coagulation and filtration treatment process, aluminum (Al) and iron (Fe) coagulants are used to remove suspended solids from raw water, which results in the produced DWTR being highly porous and containing high concentrations of amorphous Al and Fe (Wang et al., 2015). DWTR has been demonstrated to have high adsorption capability for many contaminants such as antibiotics (Punamiya et al., 2015), metals (metalloids) (i.e., Castaldi et al., 2015), organic pesticides (Zhao et al., 2013, 2015), perchloric acid (Makris et al., 2006), phosphorus (P) (Oliver et al., 2011), and sulfide (Sun et al., 2015). Therefore, scientists have been attempting to reuse DWTR for environmental pollution control. DWTR can be used to reduce the loss of P from P-rich soils for non-point pollution source control (Agyin-Birikorang et al., 2009), to remove excessive P from wastewater as the main substrate for a constructed wetland (Zhao et al., 2011), and to remediate metal- and arsenic (As)-contaminated soils (e.g., Wang et al., 2012b). Recently, DWTR has shown a high potential for use as a stabilizer to reduce lake internal P loading (Wang et al., 2013a; Wang and Jiang, 2016) and control the release of As and metals from lake sediment (i.e., Chiang et al., 2012) for lake restoration. As a by-product with a high contaminant adsorption capability, the successful reuse of DWTR will create a win-win situation for both the environment and the economy (Wang et al., 2014a).

Therefore, comprehensive understanding of the potential pollution risk of DWTR is essential prior to application for environmental remediation. Our laboratory has assessed the metal pollution risk of DWTR using chemical processes and found that DWTR could be considered non-hazardous according to the toxicity

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characteristic leaching procedure recommended (TCLP) by the US Environmental Protection Agency (Wang et al., 2014a, 2014c, 2014d). DWTR contained varying quantities of As, barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), strontium (Sr), vanadium (V) and zinc (Zn), while the concentrations of most metals tended to be low (Wang et al., 2014a; Elliott et al., 1990). Most of these meals also tended to have high bioaccessibility for humans but were largely non-extractable by the sequential extraction protocol of the European Community Bureau of Reference (BCR, now superseded by the Standards, Measurement and Testing Programme) (Wang et al., 2014a). Furthermore, most of the metals in DWTR were stable at pH levels of 6–9 (Wang et al., 2014d) and anaerobic conditions (Wang et al., 2014c).

However, the chemical analysis only determines the components and concentrations of the contaminants that may be released into the environment (Li et al., 2013b). Comparatively, biological analysis could intuitively reflect the toxic effects of contaminants on organisms and profoundly reveal the mechanisms (Li et al., 2013a). Typically, the luminescent bacteria toxicity assay, which is fast (the exposure time of conventional luminescent bacteria toxicity assay is 5–30 min), convenient and cost-effective, has attracted the attention of researchers (Ma et al., 2014). Recently, the Microtox® solid phase assay (MSPA) combining leachate phase assay (MLPA) and flow cytometry analyses was further developed to assess the direct ecotoxicity of the solid phase of soils, sludges and sediments (Burga Pérez et al., 2012), and the kinetic luminescent bacteria test (kinetic LBT) was improved to evaluate the long-term (12–24 h) inhibition of luminescence and growth (Menz et al., 2013). Therefore, the combination analysis of MSPA and the kinetic LBT could be conducive to a thorough understanding of the ecotoxicity of solid samples to luminescent bacteria.

In this study, we evaluated the toxicity of solid phase and aqueous/organic extracts of DWTR and lake sediments with and without DWTR addition using the luminescent bacteria *Aliivibrio fischeri* (*A. fischeri,* previously named *Vibrio fischeri*) based on the MSPA, MLPA, flow cytometry analyses and the kinetic LBT. The results reported herein will facilitate the productive reuse of DWTR in environmental remediation.

2. Materials and methods

2.1. Samples and bacteria

The dewatered DWTR was collected from the dewatering workshop of the Beijing City No. 9 Waterworks in China. The DWTR was air-dried, ground and sieved to a diameter of less than 1 mm. The physic-chemical characteristics of DWTR are detailed in our previous study (Wang et al., 2014c). The lake sediment was obtained from the Jiaozhuang Village in Lake Baiyangdian (38°53′ N, 115°59′ E) in China. The upper 10 cm of the sediment was collected and filtered through a 1.8-mm screen to remove impurities and then was mechanically homogenized. The freeze-dried *A. fischeri* was purchased from Hamamatsu Photonics, Beijing, China, and stored until use at −20 °C.

The DWTR was mixed with sediment at doses accounting for 0, 10 and 50% of sediment in dry weight. After incubation for 10 days (Wang et al., 2012a), the mixtures were freeze-dried, ground and sieved to a diameter of less than 1 mm. The raw sediment was represented as RS, and the sediment with the addition of 10% and 50% DWTR was represented as WAS-10 p and WAS-50 p, respectively. DWTR can immobilize lake internal P within 10 d at a dose ratio to sediment of 10% in dry weight in both aerobic and anaerobic/anoxic conditions (Wang et al., 2013a; Wang and Jiang, 2016). Considering that doses of P-inactivating agents for lake restoration in practice would likely be greater than the calculated theoretical doses due to various factors affecting natural environments (Meis et al., 2013), doses of 10% and 50% were selected to determine the effect of DWTR addition dosage-wise.

2.2. The solid phase assessment tests

2.2.1. Microtox® solid and leachate phase assays

The moisture content of freshly dewatered DWTR and sediment is approximately 80% and 50%, so the solid/liquid ratio of sample and diluent [3% (w/v) aqua sodium chloride (NaCl)] was 1:10 (g/mL) rather than 1:5 (g/mL) (Burga Pérez et al., 2012). The solution was mixed on a magnetic stirrer for 10 min, and 1:2 dilutions were then prepared from it. These dilutions are isotonic to *A. fischeri*.

Regenerated luminescent bacteria were exposed to control (3% (w/v) aqua NaCl) and each dilution for 15 min. All solutions were filtered using 15-μm Millipore filter paper at the end of exposure, and filtrate containing exposed bacteria was transferred to a new test tube. Finally, light emission was measured after 5 min.

The MLPA was conducted under the same conditions as the MSPA with the only difference being that *A. fischeri* was exposed after filtration of sample dilutions rather than before. The dilutions and control were filtered first, and bacteria were subsequently exposed to filtrate and then light emission was measured (Burga Pérez et al., 2012).

2.2.2. Flow cytometry analysis

Bacterial DNA was stained with Syto 13 (Invitrogen S7575) dye. According to Burga Pérez et al. (2012), in addition to sampling with bacteria, sampling without bacteria was also conducted to consider the endogenous bacterial DNA quantity. Aqua NaCl (3% (w/v)) was used as a control to exclude background noise and determine the total bacteria count. Control without filtration was also carried out to analyze the bacteria retained by the filter. Accordingly, the percentage of bacteria retained by the DWTR and sediments was quantified by subtracting the endogenous bacteria from the bacterial count in the filtrate of samples with *A. fischeri* addition and then subtracted from the bacteria count in the control. Syto 13 was added to the 1-mL dilution and control to a final concentration of 5 μM and then incubated at room temperature for 15 min in the dark.

A Facs Calibur flow cytometer (Becton Dickinson) was used to acquire green fluorescence (FL1) emission measured at 530 ± 30 nm and angle light scatter (SSC, related to cell size).

2.3. The kinetic luminescent bacteria test

2.3.1. Aqueous extracts and organic extraction preparation

Aqueous and organic solvent extracts of samples were prepared according to Ocampo-Duque et al. (2008) with some modification. Aqueous extracts were obtained by mixing 3 g of sample with 30 mL of 3% (w/v) aqua NaCl solution, shaking in a constant temperature shaker (20 °C) for 12 h at 160 rpm, and then filtering with 0.45-μm pore diameter membrane filters.

Organic extraction was performed by mixing 1 g of sample and 30 mL of acetone:hexane (1:1) and treating for 20 min in a microwave closed digester (Ethos S, Milestone, USA) at 115 °C. Extracts were then filtered using 0.45-μm pore diameter membrane filters (glass fibre) and evaporated by pressured gas blowing concentrators (ND200, Shanghai Joyn Electronic Co., Ltd.). Finally, the remaining residue was dissolved in 4 mL of dimethyl sulfoxide (DMSO).

2.3.2. Kinetic luminescent bacteria test procedure

Organic extracts were previously diluted in 3% (w/v) aqua NaCl