Water Research xxx (2015) 1–9

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Ecotoxicological assessment of flocculant modified soil for lake restoration using an integrated biotic toxicity index

Zhibin Wang, Honggang Zhang, Gang Pan*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form
22 July 2015
Accepted 18 August 2015
Available online xxx

Keywords:
Chitosan
Cationic starch
Modified soil
Ecotoxicity
Aquatic organisms

ABSTRACT

Flocculant modified soils/clays are being increasingly studied as geo-engineering materials for lake restoration and harmful algal bloom control. However, the potential impacts of adding these materials in aquatic ecological systems remain unclear. This study investigated the potential effects of chitosan, cationic starch, chitosan modified soils (MS-C) and cationic starch modified soils (MS-S) on the aquatic organisms by using a bioassay battery. The toxicity potential of these four flocculants was quantitatively assessed using an integrated biotic toxicity index (BTI). The test system includes four aquatic species, namely *Chlorella vulgaris*, *Daphnia magna*, *Cyprinus carpio* and *Limnodrilus hoffmeisteri*, which represent four trophic levels in the freshwater ecosystem. Results showed that median effect concentrations (EC_{50}) of the MS-C and MS-S were 31–124 times higher than chitosan and cationic starch, respectively. *D. magna* was the most sensitive species to the four flocculants. Histological examination of *C. carpio* showed that significant pathological changes were found in gills. Different from chitosan and cationic starch, MS-C and MS-S significantly alleviated the acute toxicities of chitosan and cationic starch. The toxicity order of the four flocculants based on BTI were cationic starch > chitosan > MS-S > MS-C. The results suggested that BTI can be used as a quantitative and comparable indicator to assess biotic toxicity for aquatic geo-engineering materials. Chitosan or cationic starch modified soil/clay materials can be used at their optimal dosage without causing substantial adverse effects to the bioassay battery in aquatic ecosystem.

1. Introduction

Over the past several decades, harmful algae blooms (HABs) have frequently occurred worldwide, causing serious ecological and economic impacts to aquatic ecosystems and human health (Akyuz et al., 2014; Paerl and Huisman, 2008). Several chemical (Burson et al., 2014; Fan et al., 2013), mechanical (Li et al., 2014) and biological techniques (Kim et al., 2007; Nan et al., 2008) have been developed to reduce these impacts. Recently, lake geo-engineering techniques are discussed in solving this problem. The term “geo-engineering”, defined as achieving a desired chemical or ecological response by adding materials such as a modified clay or metal compound to a lake (Mackay et al., 2014). The range of materials used is growing and includes engineered materials, commercially available salts, flocculants, clay/soils and industrial by-products (Spears et al., 2014).

Although these materials may be useful in controlling nutrient level, there is a need to evaluate the impacts of adding exogenous materials to the aquatic ecosystem. Reports indicate that some chemical materials exhibit toxicity to aquatic biota. The lanthanum-modified clay (Phoslock®) is promising in holding phosphorus in the sediment (Meis et al., 2013), but the population growth rates of daphnia are 6% and 20% lower than the control at 100 and 1000 μg La/L, respectively (Lürling and Tolman, 2010). Clearwater et al. (2014) demonstrate that fingernail clam survival is adversely affected by high dosage (344 g alum/m^2) of alum application and some aluminum accumulation occurred in the crayfish and mussels (Clearwater et al., 2014). The aqueous Al can increase the risk of infection in the crayfish by impairing the ability of haemocytes to recognise and/or remove bacteria from the circulation (Ward et al., 2006). Recent studies indicate that toxic Al^{3+} could be released after alum application at low pH (<6.0), and sediment-capping with alum could inhibit microbial nitrification and denitrification under aerobic conditions (Gibbs and Oezkundakci, 2011).

Recently, natural flocculant materials, such as chitosan and

* Corresponding author.
E-mail address: gpan@rcees.ac.cn (G. Pan).

http://dx.doi.org/10.1016/j.watres.2015.08.033

cationic starch, were developed as environmental friendly materials to control harmful algal blooms because of their high flocculation efficiency (Anthony and Sims, 2013; Hansel et al., 2014; Letelier-Gordo et al., 2014; Xu et al., 2013). To improve the HABs removal using clays, chitosan is used to modify the local soils and applied to small natural waters to control both cyanobacteria blooms and sediment nutrient release, leading to recovery in submerged macrophytes (Li and Pan, 2015, 2013; Pan et al., 2012). Anthony and Sims (2013) find that cationic starch can effectively flocculate algae cells and remove total phosphorus in wastewater with an upward trend of TP removal with increasing dosage. Cationic starches serve as substrates in anaerobic digestion or fermentation processes using the harvested biomass as feedstock and such biomass can be safely used as animal feed or fertilizer (Anthony and Sims, 2013). Cationic starch modified soil has been reported by Shi et al. (2015) as the effective algae flocculant with the loading of 0.11 g/L for a removal efficiency of 86%. Although chitosan and cationic starch have been used in wastewater treatment and the removal of HABs in aquatic system, there are little studies on their toxicity effects on aquatic ecological system when they are applied in field (Li and Pan, 2013). It is necessary to evaluate the biotic toxicity of chitosan and cationic starch by using appropriate test methods.

Conventional methods of assessing toxicity effect of flocculants are to expose a single species to the flocculent solutions over a range of concentrations for a certain period of time, but the results may be not sufficient because a single organism cannot represent an aquatic ecosystem. Therefore, the application of a battery of bioassay tests with organisms belonging to different trophic levels is recommended and developed (Hartwell, 1997; Nowell et al., 2014; Wei et al., 2011). Antunes et al. (2007) use a battery of bioassays (algae, crustaceans and dipterans) to screen the acute toxicity of water column and sediment from an abandoned uranium mine, and find that *Daphnia longispina* is the most sensitive organisms (Antunes et al., 2007). In order to evaluate the effects of human activities on the biosafety of water quality, Wei et al. (2008) develop an evaluation method using algae, daphnia and larval medaka (Wei et al., 2008). Tigini et al. (2011) study the toxicity of simulated textile and tannery wastewaters by using a battery of seven organism bioassays and find that the algae *Pseudokirchneriella subcapitata* is the most sensitive organism (Tigini et al., 2011). While bioassay battery tests can provide more information than single species test to assess the toxicity of chemicals, it is still hard to quantitatively evaluate the biotic toxicity of biodegradable and/or non-degradable chemicals to the aquatic ecosystem and to the food chain.

Several integrated assessment toxicity models have been developed to evaluate the biotic toxicity in the field of pesticide and wastewater treatment. Potential ecotoxic effects probe (PEEP) index was developed to assess and compare the toxic potential of industrial effluents (Costan et al., 1993). Nowell et al. (2014) used Pesticide Toxic Index (PTI) to evaluate relationships between pesticide exposure and biological condition (Nowell et al., 2014). However, the information about the biotic toxicity of flocculants to the aquatic organisms is very limited. There is an urgent need to develop an integrated biotic toxicity index to assess toxicological effects of chemicals on the aquatic organisms.

This paper aims to investigate the biotic toxicity of chitosan, cationic starch, chitosan modified soil (MS-C) and cationic starch modified soil (MS-S) to the aquatic organisms and elucidate the mechanism of the toxic effect by means of a battery of four bioassays that belong to different trophic levels. An integrated biotic toxicity index (BTI) was developed to make a comprehensive and comparable assessment on the biotic toxicity of the added flocculants on the aquatic organisms.

2. Materials and methods

2.1. Soil and flocculants

The soils and chitosan used in this study were described in a previous study (Li and Pan, 2013). Cationic starch was obtained from Minsheng Environmental Technology Co. Ltd, Dalian, China. The cationic starch was dissolved by adding 250 mg cationic starch to 100 mL deionized water. The molecular weights (MW) of chitosan and cationic starch are 5×10^5 g/mol and 1×10^8 g/mol, respectively. The chitosan modified soils (MS-C) and cationic starch modified soils (MS-S) were obtained by adding 100 mL chitosan solution (5 mg/mL) or 100 mL cationic starch solution (2.5 mg/mL) to 100 mL soil suspension (50 mg/mL), respectively. The mixture was well stirred and then ready for use in the toxicity experiment.

2.2. Test solution

BG11 medium was used for algae growth inhibition test only. The solution was adjust to pH 8.2 by adding either 0.5 mol/L NaOH or 0.5 mol/L HCl solutions after autoclaving (Li and Pan, 2013). The artificial water with a pH of 7.8, a total hardness of 250 mg $CaCO_3$/L was used for the other tests. The dissolved oxygen values were maintained at 8.0 mg/L.

2.3. Aquatic organisms

2.3.1. Chlorella vulgaris

The green algae *C. vulgaris* (FACHB-1227) were obtained from the FACHB, Institute of Hydrobiology, Chinese Academy of Sciences, and cultured in BG11 medium, at 25 ± 1°C and with a 12 L: 12 D h photoperiod in an illuminating incubator. At the start of new cultures, algae were harvested during the exponential growth phase and inoculated in fresh medium.

2.3.2. Daphnia magna and Limnodrilus hoffmeisteri

The *D. magna* and *L. hoffmeisteri* were isolated from Lake Taihu, China and were maintained in artificial water at 25 ± 1 °C, on a 16 h light and 8 h darkness regimen. The average weight of the *L. hoffmeisteri* was 40 ± 10 mg, and the average body length was 10 ± 2 mm. *D. magna* were fed with *Scenedesmus obliquus* (10^6 cells/mL) and *L. hoffmeisteri* were fed with approximately 100 mg powder fish food every day.

2.3.3. Cyprinus carpio

C. carpio, were obtained from a fish farm and acclimated for a month to lab conditions in 100 L tank filled with artificial water prior to the tests. The average mass/size of *C. carpio* used in the test was 0.5 ± 0.1 g/3.0 ± 0.2 cm. The fish were fed with commercial carp food at a rate of 1.5% of body weight. The tank water was changed weekly. Ammonia, nitrate and nitrite levels were kept below toxic concentrations (<0.1 mg/L) (Eyckmans et al., 2012).

2.4. Experiment design

2.4.1. Soil leachate and toxicity tests

Soil materials may potentially release heavy metals into water phase under a variety of conditions. The toxicity characteristic leaching procedure (TCLP) was carried out to determine the mobility of metal elements in soil (USEPA, 1992). The metal elements leached from the soil by three different extraction fluids were analyzed using Inductively Coupled Plasma Emission Spectrometry (ICP-OES, Optima 8300, PerkinElmer, USA). As a complementary test, the effects of soil on four species were determined. Following a static design, the organisms were exposed to five