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Comparative copper toxicity impact and enzymatic cascade effect on Biosorption Activated Media and woodchips for nutrient removal in stormwater treatment

Dan Wen, Ni-Bin Chang*, Martin P. Wanielista

Department of Civil, Environmental, and Construction Engineering Department, University of Central Florida, Orlando, FL, USA

HIGHLIGHTS

- Copper toxicity impact on microbial community for nutrient removal is not homogeneous.
- Copper can enhance the denitrification process through the enzymatic cascade reactions.
- Bioactivity of microbial community related to nitrogen cycle is decreased under copper impact.

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ABSTRACT

Copper, a commonly occurring heavy metal in stormwater runoff, was tested for its inhibitory effects on key nitrogen cycle bacteria in Biosorption Activated Media (BAM) and woodchip. The information in this paper is used to show that copper can enhance the denitrification process through enzyme cascade reactions since nitrous reductase is the enzyme responsible for the last step of denitrification and is largely dependent on copper as its cofactor. However, media characteristics are critical for assessing multi-enzymatic cascade reactions from the microbial ecology point of view. Moreover, both media showed significant copper removal through various mechanisms at 30 cm depth. The bioactivity evaluation indicates that other bacteria (fermentative bacteria, etc.) can be largely depressed with the presence of copper, hence the biofilm structure would be more vulnerable under shearing effects, which may result in holistic depression on the microbial community.

1. Introduction

Biosorption Activated Media (BAM) and woodchip have been studied, modified, and applied to different stormwater Best Management Practices (BMPs) for nutrient removal before the stormwater recharges to groundwater aquifers or surface water bodies (Chang et al., 2010; Greenan et al., 2009; Hood, 2012; Jones et al., 2015; Lens et al., 1994; O'Reilly et al., 2012; O'Reilly et al., 2014; Robertson, 2010; Xuan et al., 2009; Xuan et al. 2010; Xuan et al. 2013). Nutrient removal, particularly removal of nitrogen, relies primarily on a series of biological reactions, namely ammonification, nitrification, and denitrification, all of which are substantially affected by the water quality of stormwater runoff (Her and Huang, 1995; Matter, 2014; McElmurry et al., 2013). Stormwater runoff may introduce substantial impacts on microbial ecology in terms of bacteria population and structure changes or soil degradation when encountering toxic/hazardous materials (Cheng et al., 2018; Recanatesi et al., 2017). One of the major impact factors is a toxicant such as heavy metal copper (Holtan-Hartwig et al., 2002; Sobolev and Begonia, 2008) which commonly exists in stormwater runoff from urbanized areas (Booth et al., 2013; Michels et al., 2002; Prestes et al., 2006; TDC Environmental, 2004) with various concentrations normally around 15–30 μg/L (Borne et al., 2013), but sometimes even in the magnitude of hundreds of microgram per liter (Malmqvist, 1983). Copper is also found to be more active in association with dissolved organic matter in the dissolved phase when compared with common metals like lead and calcium (Prestes et al., 2006). Although copper is an essential element for life on earth, an excess dosage of copper may have an inhibitory impact on microbial communities and, as related to this research,

* Corresponding author.
E-mail address: nibinchang@gmail.com (N.-B. Chang).

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may cause a decrease in nutrient removal effectiveness (Thurman et al., 1989; Yoon et al., 2007). However, there are very limited studies regarding how copper may affect different evolvement of the Nitrogen cycle (N-cycle) microbial community in BAM and woodchip. This is related to the biological reactions for critical nitrogen removal as listed in Eqs. (1)–(7) (Felgate et al., 2012), in which four types of enzyme are needed in denitrification (Eqs. (1)–(4)) to sequentially reduce NO_3^- to N_2 with the help of a redox metal cofactor in each of those enzymes; Eqs. (6) and (7) represent the sequential steps of nitrification. Eq. (5) is another pathway of denitrification performed by anammox (AMX) that requires an anaerobic condition and the existence of ammonium and nitrite as food/energy source. Eqs. (1)–(4) represent the involvement of different enzymes, and they are nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (NosZ), respectively. The four types of enzyme might have a possible enzymatic cascade effect for the denitrifying process when one or multiple enzymes are affected by copper.

$$2NO_3^- + 4H^+ + 4e^- \xrightarrow{Fe\&Mo} 2NO_2^- + 2H_2O \quad (1)$$

$$2NO_2^- + 4H^+ + 2e^- \xrightarrow{Fe} 2NO + 2H_2O \quad (2)$$

$$2NO + 2H^+ + 2e^- \xrightarrow{Fe} 2N_2O + H_2O \quad (3)$$

$$N_2O + 2H^+ + 2e^- \xrightarrow{Cu} N_2 + H_2O \quad (4)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (5)$$

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (6)$$

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \quad (7)$$

The different characteristics of BAM and woodchip may result in entirely different outcomes on copper exposure. Some major differences between BAM and woodchip shall be noted, one of which is hydraulic difference due to very different porous structure. Another one relates to carbon source availability; woodchip itself can provide a carbon source through a slow decaying process, while BAM cannot provide much by itself. These major differences may lead to interesting metabolic pathways driven by enzymatic cascade effect, which produce different nutrient removal efficiencies. Enzymatic cascade effect through confined multiple enzymatic reactions has been studied for decades in different fields such as medical and chemical science (Hemker and Hemker, 1969; Macfarlane, 1964; van Dongen et al., 2009). One of its applications is for optimizing the over-all reaction process (velocity of product formation) through a series of reaction chains with multiple enzyme involvement in structural biochemistry. Even though many studies indicated that copper is toxic to microorganisms in general, including nitrifying and denitrifying bacteria (Bardgett et al., 1994; Ladomersky and Petris, 2015; Magalhães et al., 2011; Thurman et al., 1989; Yoon et al., 2007), Black et al. (2016) found that additional copper in farmlands is surprisingly helpful in the conversion process of N_2O to N_2, which is the last step of denitrification, as shown in Eq. (4). Black et al. (2016) may therefore have touched the base of enzymatic cascade catalysis for denitrification process initiated from the copper dosage. There is an acute need to investigate nutrient control and the potential of using BAM to treat stormwater runoff in order to deepen understanding of this unique phenomenon.

The objectives of this study were to evaluate the copper removal potential of BAM and woodchip and their toxic impact on changes of microbial ecology and enzymatic cascade effect for nitrogen removal from stormwater runoffs. Some science questions to be answered in this study include: 1) how the copper addition affects the nitrogen removal of BAM and woodchip holistically throughout the ammonification, nitrification, and denitrification processes, 2) how the copper addition influences the evolvement of the microbial community resulting in an enzymatic cascade effect on the denitrifying bacteria in BAM, and 3) how effective the two types of media are in copper removal. We hypothesized that 1) copper addition could decrease only some types of bacteria population in the N-cycle, 2) copper is an effective inhibitor for the whole microbial community in terms of the effectiveness of nutrient removal, 3) BAM and woodchip should have different reactions on nitrogen removal performance with different copper removal potentials.

2. Material and method

2.1. Experiment setup

BAM contains sand, tire crumb, and clay, which are all environmentally friendly or recyclable materials. In the laboratory, one column 15 cm in diameter and 1.2 m in height with 3 water sample ports of 30 cm intervals on the side (Fig. 1) was setup. BAM mixes applied by volume in this study contained sand (85%), tire crumb (10%) and clay (5%) by volume. In stage 1, stormwater was added to the column at 15 mL/min for two months with 5 mg/L spike of nitrate for cultivating the microbial communities. In stage 2, additional copper was added to the inlet with a concentration of 50 μg/L which ran for 1 day, since most stormwater runoff usually ends within less than one day. Concentration of 50 μg/L was chosen as the worst-case scenario when compared with 15–30 μg/L copper in normal stormwater runoff. All stormwater was collected from the stormwater retention pond on the campus of the University of Central Florida (UCF). Water samples were collected from inlet, outlet, and three ports at the end of each stage. Water quality parameters of dissolved oxygen (DO) and pH were measured in the laboratory right after water sample collection. Total nitrogen (TN), ammonia, nitrate and nitrite (NO_X), and alkalinity were determined by a certificated laboratory, namely Environmental Research & Design, and all methods for water sample analysis are listed in Table 1. The BAM media samples were collected at the top, port 1,

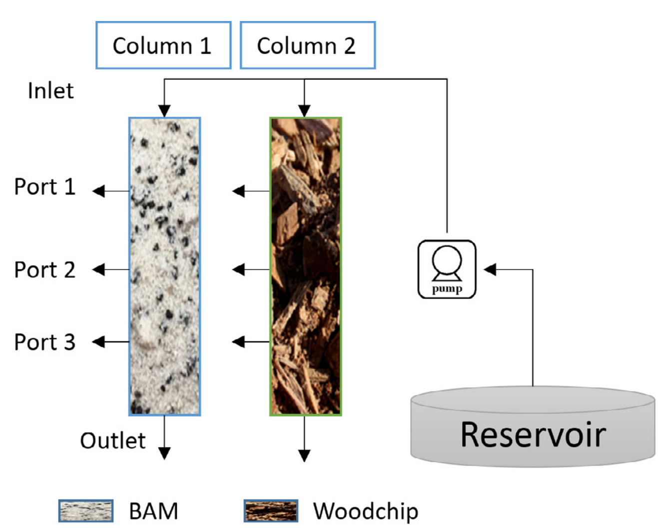

Fig. 1. Schematic diagram for column setup.