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Research paper

Effects of microbial community variation on bio-clogging in drip irrigation emitters using reclaimed water

Bo Zhou[a,b,1], Tianzhi Wang[a,1], Yunkai Li[a,*], Vincent Bralts[c]

[a] College of Water Resources and Civil Engineering, China Agricultural University, Beijing 100083, China
[b] College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA
[c] Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907, USA

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ABSTRACT

Drip irrigation emitter clogging is one of the key barriers to the development and application of reclaimed water drip irrigation technology. Reclaimed water typically contains large amounts of bacteria, and their excretions, the sticky extracellular polymeric substances (EPS). Indeed, different amounts and types of microorganisms affect drip irrigation emitter clogging, especially bio-clogging process, via excretive EPS. Therefore, it is important to study the dynamic microbial community structure and its effect on the emitter bio-clogging process. In this paper, a drip irrigation experiment using reclaimed water was carried out. Phospholipid fatty acids (PLFAs) in biofilm (bio-clogging substance) were taken as the biomarker of microbial community inside emitters, and the dynamic variation of microbial community in drip irrigation emitter and its effect on the clogging process were studied. The results showed that the microbial growth of biofilms inside 9 types of drip irrigation emitters could enhance emitter clogging, and the PLFAs showed S-shaped pattern with emitter clogging degrees ($R^2 > 0.95$, $p < 0.01$), which was closely associated with the variation of microbial community. There were 3–7 types of PLFAs commonly in biofilms within drip irrigation emitters, among which gram positive bacteria (i15:0, 16:0 and 18:0) were dominant in amounts, with the close contents of 24.4%–34.2%, 24.8%–37.2% and 24.2%–39.0%, respectively, and their total proportion exceeding 76.3%. The *Pseudomonas* (16:0) was found to be the most critical bacteria to affect emitter clogging as it performed better to decompose and utilize organic matters, and showed the best relation with clogging degrees, along with significant relations with other types of bacteria. Therefore, controlling gram positive bacteria, especially *Pseudomonas* was the most effective way to relieve emitter clogging. Their variation also changed the microbial community structure, and the diversity index (H), evenness index (J) and dominance index (D) of microbial community in biofilms varied within 1.08–1.53, 0.75–1.11 and 0.65–0.75. The diversity index and dominance index both decreased as the amounts and types of microorganisms increased. The total amount of the gram positive bacteria increased after their decrement, and resulted in the similar variation of the microbial community evenness index. The results of this study will establish a theoretical basis for exploring the effects of microbial community variation on emitter bio-clogging, and provide insight into the emitter clogging mechanisms and possible mitigation strategies.

1. Introduction

Drip irrigation emitter clogging directly affects irrigation uniformity, service life and operational performance of the whole system (Li et al., 2009; Pei et al., 2014). This issue becomes more serious when using reclaimed water in agriculture with drip irrigation technology. Although the reclaimed water quality meets the basic standards of agricultural irrigation, it contains large amounts of microorganisms, solid particles, organic matters, etc. (Li et al., 2009; Liu and Huang, 2009). Microorganisms are especially important, as microorganisms are not only the critical components of biofilms, but also secret sticky extracellular polymeric substances (EPS). EPS are able to maintain the biofilm structure stable, meanwhile adsorbing multiple matters in water source to promote biofilm growth (Zhou et al., 2016). So microorganisms and sticky EPS are

* Corresponding author.
E-mail address: liyunkai@126.com (Y. Li).
[1] Bo Zhou and Tianzhi Wang equally contributed to this paper.

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the basis of biofilm growth, and they are influenced by the amounts and types of other microorganisms (Yan et al., 2009). Therefore, studying the dynamic variation of microbial community inside drip irrigation emitters using reclaimed water and its effects on emitter bio-clogging are the premise of controlling microbial activity and the sticky EPS, and sequentially reducing biofilm growth, in order to control emitter clogging effectively.

The role of microorganisms in emitter clogging was studied from the 1980's. Picologlou et al. (1980) found microbial growth inside drip lines would finally form membrane structure, due to the interactions among microorganisms and the polysaccharide layer they secreted. Ravina et al. (1992, 1997), Taylor et al. (1995) also conducted related researches and acquired some valuable qualitative results. With the development of molecular microbiology and testing technologies, the quantitative testing of biofilm components became possible in recent years. Among these technologies, phospholipid fatty acids (PLFAs) analysis is a culture-independent technique commonly used to characterize the structure of the microbial community in natural ecosystems (Vestal and White, 1989; Gómez-Brandón et al., 2010). It is based on the analysis of the profile of the ester-linked fatty acids of the phospholipids which constitute essential components of microbial membranes. The variation of phospholipids along with their rapid degradation after cell death makes PLFAs useful indicators of the living microbial community (Findlay, 2008). PLFAs have been used for estimating the total soil microbial biomass (Bååth and Anderson, 2003), assessing the impact of agronomic practices (Bossio et al., 1998), pesticides (Spyrou et al., 2009), and heavy metal pollution (Frostegård et al., 1993; Hinojosa et al., 2005) on the structure of the soil microbial community. Based on these, Yan et al. (2009) investigated the microbial characteristics of mature biofilms presented in the emitters and the effect of flow path structures on the biofilm microbial communities. The PLFAs were used as the indicator to describe the difference among microbial communities inside biofilms attached in different types of emitters. Zhou et al. (2013) studied the differences of biofilm components among eight types of emitters, and quantified the linear relations between biofilm PLFAs and emitter clogging parameters with four batches of biofilm samples. Besides, the emitter clogging degree in this study was within 75% and biofilm samples were limited due to experimental restrictions. So the study could not eliminate the randomness of biofilm growth so well. In order to control microorganism growth and bio-clogging progress in an environmentally friendly way, Boari et al. (2008) carried out the experiment using drip lines, and demonstrated that conidial suspensions (10^6 conidia ml^{-1}) could pass through the emitters without causing clogging, regardless of their size, and remained viable. found that *Bacillus* spp ERZ, OSU-142 and *Burkholdria* spp OSU-7 were the antagonistic bacterial strains which could be used as anti-clogging agents for treatment of drip irrigation emitters. Their research group continued the research and additionally demonstrated that OSU and A1 application significantly increased the outflows of $CaCO_3$-clogged emitters (Eroglu et al., 2012). Overall, the studies analyzed neither dynamic distribution of microbial communities in biofilms inside emitters, nor the effects of microbial community on the bio-clogging process until now. Almost no results were reported about the critical bacteria and their relations with biofilm growth and emitter clogging, and it was hard to reveal the mechanism of emitter bio-clogging. Therefore, it's hard to establish the appropriate environmental friendly controlling mode.

The dynamic variations of microbial communities in the biofilms attached inside drip irrigation emitters using reclaimed water were studied in this paper, to acquire knowledge about the critical bacteria and their impact on emitter bio-clogging. The results then provide insight into the emitter clogging mechanisms and possible controlling strategies.

2. Materials and methods

2.1. Reclaimed water drip irrigation experiment

The experiment was carried out in the Beiqijia wastewater treatment plant in Changping District, Beijing, China. In this plant, the reclaimed water, which was treated with cyclic activated sludge system (CASS), was the water source of the drip irrigation system. Before the inlet of the drip irrigation laterals, there was a backflow unit consisting of two horizontal pipes and three vertical pipes, and one valve was placed before the backflow unit while the other ones were placed between the backflow unit and the drip irrigation laterals. Thus the working pressure and the flow rates were controlled mainly according to the shunt principle. The drip irrigation system operated 8 h every day and 1224 h in total. The detailed experiment settlements and operation mode were introduced by Zhou et al. (2016).

During the experiment, reclaimed water quality characteristics were tested and recorded by the online monitoring system in the wastewater treatment plant. The monitoring system contains water quality automatic measuring instruments and automatic samplers. The monitoring included chemical oxygen demand (COD_{cr}), biological oxygen demand (BOD_5), total suspended solid (TSS), total phosphorus (TP), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), pH and water temperature at a fixed time every day. The testing results are shown in Fig. 1.

In the experiment, four types of non-pressure-compensating flat emitters (marked as FE1-FE4) and five types of non-pressure-compensating cylindrical emitters (marked as CE1-CE5), all of which were commonly used, were selected. There were ten laterals for each type of emitter to collect biofilms samples during the clogging process. Each drip irrigation lateral was 12 m in length, with the emitter spacing of 0.3 m. So the emitters in each lateral were numbered from No.1 to No.40 from the inlet to the outlet of the lateral. The detailed structural parameters of emitters used in the experiment are shown in Table 1.

2.2. Biofilm sampling and microbial community testing methods

During the experiment, the emitter samples were taken ten times in total to test microbial community, when the system operated for 240 h, 408 h, 600 h, 736 h, 832 h, 920 h, 1016 h, 1088 h, 1160 h and 1224 h, and those were also the times when emitter clogging degrees reached approximately 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%, respectively. At each sampling time, one lateral of each emitter type treatment was selected. Then the emitters at the head part (No.1-8), middle part (No.17-24) and end part (No.33-40) of each lateral selected were tested for 3 min, and outflows were weighted and then used to calculate the clogging parameters. In order to eliminate the errors caused by water temperature, the emitter outflows were modified according to the method used by Pei et al. (2014). The clogging degree of the single emitter was determined by the tested outflow/initial outflow. Out of the total eight emitters, five emitters, which were closer to the expected clogging degree, were selected at the head, middle and end part, respectively. The selected emitter samples were cut off from the drip irrigation laterals and the emitters were stripped off with pinchers to test PLFAs, according to the detailed testing method used by Zhou et al. (2013). After collecting samples, the remaining parts of each lateral were removed from the drip irrigation system.