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RESEARCH ARTICLE

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Bacillus amyloliquefaciens application to prevent biofilms in reclaimed water microirrigation systems*

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Abstract

Reclaimed water (RW) applied in drip irrigation systems could be a solution to cope with the challenges of limited freshwater resources. Biofilm growth in irrigation systems is an unavoidable issue when using RW. To date, strong chemical bactericides mostly dominate the commercial market in controlling biofilms. However, recently scientists have been concerned about their real efficacy and environmental risks. This study assesses a low-concentration microbial antagonist (i.e., *Bacillus amyloliquefaciens* inoculums, BAI) intermittently injected into the systems to mitigate biofilm formation in three types of drip emitters using two kinds of treated RW. The results indicated that the application of BAI significantly ($p < .05$) mitigated the microbial biomass within biofilms when compared with the control groups (no-BAI). In addition, BAI reduced the contents of extracellular polysaccharides and proteins of biofilms, which decreased the total biomass in BAI treatments by 44.9%–73.8%. Consequently, BAI effectively improved the emitter performances and increased the discharge variation rate by 31.9%–44.3%. These findings might provide a new perspective to control biofouling when applying RW in irrigation systems, with potential implications for sustainable water management in agricultural production.

KEYWORDS

Bacillus amyloliquefaciens, biofilm, drip irrigation, emitter clogging, microbial antagonism

Abstract

L'eau recyclée (RW) appliquée dans les systèmes d'irrigation goutte à goutte pourrait être une solution pour faire face aux défis de la limitation des ressources en eau douce. La croissance du biofilm dans les systèmes d'irrigation est un problème inévitable lors de l'utilisation de RW. À ce jour, un bactéricide chimique puissant domine principalement le marché commercial du contrôle des biofilms. Cependant, récemment, les scientifiques se sont inquiétés de leur efficacité réelle et des risques environnementaux. Cette étude a évalué un antagoniste microbien de faible concentration (c.-à-d. *Bacillus amyloliquefaciens*

* Application de Bacillus amyloliquefaciens pour empêcher les biofilms dans les systèmes de microirrigation avec de l'eau recyclée

inoculums, BAI) injecté par intermittence dans les systèmes pour atténuer la formation de biofilm dans trois types d'émetteurs goutte à goutte en utilisant deux types de RW traité. Les résultats ont indiqué que l'application de BAI de manière significative ($p < 0,05$) atténuait la biomasse microbienne dans les biofilms par rapport aux groupes témoins (sans BAI). En outre, BAI a réduit les teneurs en polysaccharides extracellulaires et en protéines des biofilms, ce qui a diminué la biomasse totale des traitements BAI de 44,9 à 73,8%. Par conséquent, BAI a effectivement amélioré les performances de l'émetteur et augmenté le taux de variation de décharge (Dra) de 31,9 à 44,3%. Ces résultats pourraient fournir une nouvelle perspective pour contrôler l'encrassement biologique lors de l'application de RW dans les systèmes d'irrigation, avec des implications potentielles pour la gestion durable de l'eau dans la production agricole.

MOTS CLÉS

antagonisme microbien, *Bacillus amyloliquefaciens*, irrigation goutte à goutte, colmatage des émetteurs, biofilm

1 | INTRODUCTION

With the rapid development of the global economy and population, the contradiction between supply and global demand for freshwater is increasingly prominent—four billion people are suffering from severe water scarcity (Mekonnen & Hoekstra, 2016). Agricultural irrigation accounts for 69% of global freshwater consumption (Food and Agriculture Organization of the United Nations [FAO], 2016). Thus, reusing the reclaimed water (RW) for agricultural irrigation is an alternative method to cope with shortages of global freshwater resources (Jaramillo & Restrepo, 2017; Angelakis *et al*., 2018). From the perspective of public health and environmental concern, drip irrigation is an appropriate irrigation technique for the application of RW (World Health Organization [WHO], 2006). Drip irrigation could effectively refrain RW from contact with human beings or animals, thus preventing health risks (Capra & Scicolone, 2007), as well as reduce the content of contaminants in RW leaching to soil and water (Fonseca *et al*., 2011). However, since plenty of microorganisms, nitrogen, phosphorous, and inorganic salt are present in RW, biofilms inevitably appear in the narrow flow path (0.5–1.2 mm) (Li *et al*., 2006) of drip emitters, thus causing emitter clogging (Xiao *et al*., 2020a). Emitter clogging can reduce irrigation uniformity and even leads to the scrapping of irrigation systems (Dosoretz *et al*., 2010). So far, biofilm mitigation was mainly achieved by the periodical application of chemicals acids (e.g., chlorination) (Katz *et al*., 2014; Song *et al*., 2017). However, the application of chemical fungicides has caused severe negative impacts on soil and crop growth (Li & Li, 2010) and has led to water pollution issues (Carrow *et al*., 2008; Agnelo *et al*., 2020). Therefore, more effective and greener anti-clogging methods for RW drip irrigation systems are urgently needed.

Biofilms are caused by the accumulation of multiple microorganisms (Wong & O'Toole, 2011). The biofilm community contains complex interactions among microorganisms, such as mutualism, symbiosis, parasitism, antagonism, and predation. The interactions among microbes could directly change the formation of biofilms (Faust & Raes, 2012; Xiao *et al*., 2020b). Souza *et al*. (2020) reported that the synergetic cooperation between *Candida albicans* and *Streptococcus* promoted the growth of a *Streptococcus* biofilm. Conversely, Kreth *et al*. (2008) observed that the antagonistic effect of *Streptococcus sanguis* and *Streptococcus gordonii* on *Streptococcus mutans* inhibited biofilm growth. Therefore, the antagonistic effect of microorganisms could be used to prevent or even kill the existing microorganisms, so as to interfere with the formation of biofilms (Simões *et al*., 2010), which constitutes a new and cleaner proposal for minimizing biofilm growth in RW drip irrigation systems. Sahin *et al*. (2005) applied *Bacillus* spp (ERZ, OSU-142) and *Burkholdria* spp (OSU-7) with a high concentration of agricultural antagonists to recover the completely clogged emitters in a drip irrigation system for 14 days. However, the application of high-intensity and high-concentration antagonistic bacteria for emitter flow recovery would inevitably destroy the original microbial community of the irrigated soil (Yuan *et al*., 2017) and consequently affect crop growth and yield (Dozet *et al*., 2014). Recently, intermittent- and low-concentration *B. amyloliquefaciens* has received increasing attention for biofilm mitigation. As a species of *Bacillus*, *B. amyloliquefaciens* has a broad-spectrum bacteriostatic activity and has an antagonistic effect on many bacteria and fungi (Gautam *et al*., 2019; Saravanakumar et al., 2019). For example, Wu *et al*. (2014) showed that the bacteriocin produced by *B. amyloliquefaciens* FZB42 could inhibit the activity of *Cyanobacteria*. Moreover, *B. amyloliquefaciens* has been proved to be a plant growth-promoting rhizobacterium that produces antibacterial substances to reduce crop disease risks (Wang *et al*., 2002; Kim & Chung, 2004) and increases crop yield (Cui *et al*., 2019; Zafar-ul-Hye *et al*., 2019). Thus, the application of *B. amyloliquefaciens* in RW drip irrigation systems is expected to be a potential means of synergistically alleviating emitter clogging and promoting crop growth. Despite of these research advances, the effects of *B. amyloliquefaciens* on biofilm inhibition in RW drip irrigation systems have not yet been reported.

In this study, wastewater treated with two different technologies was selected, and *B. amyloliquefaciens* was applied as antagonist to inhibit the formation of attached biofilm in emitters of a drip irrigation system. The main objectives of the present study were to (a) clarify the effect of *B. amyloliquefaciens* application on controlling emitters' bio-clogging in drip irrigation systems using RW and (b) reveal the dynamic variations of key biofilm components during the inhibition process of *B. amyloliquefaciens*.

2 | MATERIALS AND METHODS

2.1 | Experimental design and operating process

The experiment was carried out in the Beijing Tongzhou Experimental Station of China Agricultural University (116°41′ E, 39°42′ N). A home-designed testing system was used (Figure 1). The testing system consisted of RW storage tanks, an equalizing tank, booster pumps, disk filters of 106-μm filtration level, pressure gauges, and drip irrigation laterals. Three layers of 2-m-long drip irrigation laterals, with nine laterals arranged at equal intervals on each layer, each consisted of six emitters equally distributed. Three types of non-pressure-compensated drip emitters with different flow rates were subjected to the experiment. The used emitters' flow path geometrical parameters are listed in Table 1. The applied water was stored in the RW storage tanks. During the experiment, the system operating pressure was maintained at 0.1 MPa. The filters were cleaned manually during the experiment after every 3 days. Finally, the system was operated for 12 hr (7:00–19:00) every day, with 720 hr (60 days) in total.

The two types of RW treated by the cyclic activated sludge system (CASS; from the Yongledian Sewage Treatment Plant, 116°82′ E, 39°70′ N) and sequencing batch aeration wastewater recycling (SBWL; from the Bishui Sewage Treatment Plant, 116°70′ E, 39°87′ N) were used in this experiment. The basic water quality characteristics tested during the experiment are listed in Table 2, with the RW being replaced every 6 days.

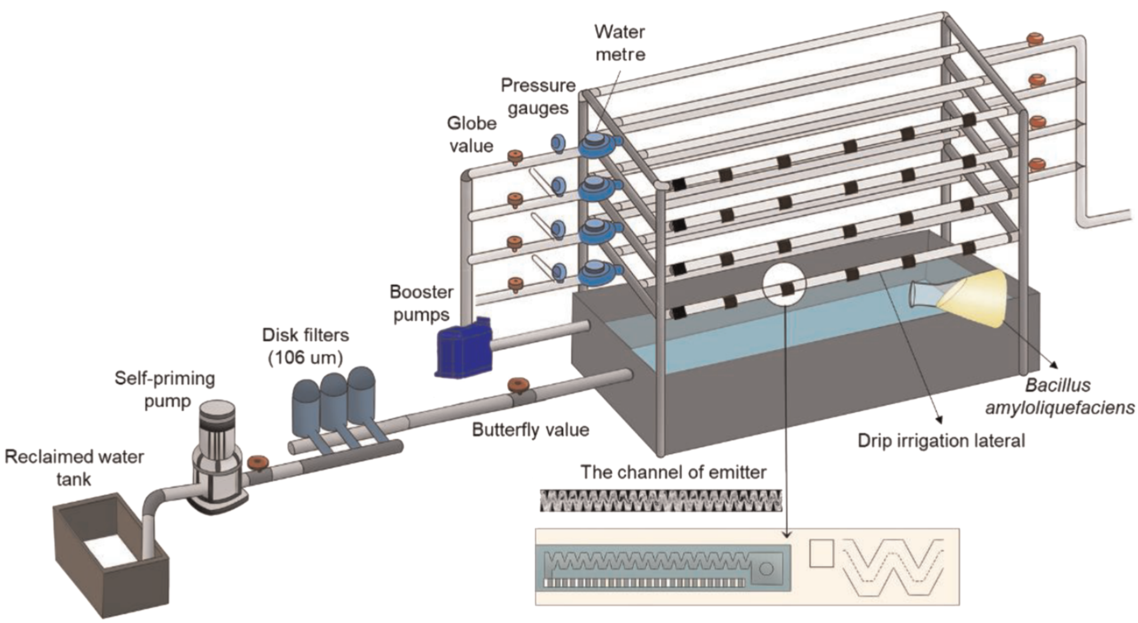

FIGURE 1 Schematic layout of testing apparatus [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Structural parameters of drip irrigation emitters

No.	Emitter discharge q (L/hr)	Emitter discharge exponent (x)	Discharge coefficient (K_d)	Manufacturing coefficient (%)	Geometry parameters Length (mm)	Width (mm)	Depth (mm)	Emitter channel structure
E_1	1.51	0.510	4.90	2.6	24.73	0.77	0.41	
E_2	1.60	0.507	5.13	3.8	37.99	1.28	0.42	
E_3	2.59	0.504	8.27	4.2	40.20	1.31	0.45	

The treatment without antagonistic agent was designed as control group, marked as CK ; *B. amyloliquefaciens* isolated from the corn soil root system of the experimental station was cultured into a bacterial sole marked as BAI (*B. amyloliquefaciens* inoculums). The concentration of antagonistic bacteria was kept at 10^8 CFU/ml, and the application frequency was set as 120 hr (10 days), which lasted for 24 hr each time. After operating 96 h in total, the system started to add *B. amyloliquefaciens* directly into the water tank for a duration of 24 h. The system operated normally after adjusting the concentration of *B. amyloliquefaciens* to the experimental design value.

2.2 | Cultivation of antagonists

The BAI were prepared as follows: A frozen stock of *B. amyloliquefaciens* was grown on a Luria-Bertani plate for 24 hr, and the *B. amyloliquefaciens* colonies were then transferred into a Luria-Bertani liquid culture using a sterile inoculating loop. The cultures were grown in conical flasks shaking at 150 rpm and 25°C for 48 hr. The growth of the culture was monitored until the spore density reached 10^8 CFU/mL. The detailed methods of testing can be found in the supplementary material. The flat-plate antagonism test was used to evaluate the antagonistic effect of *B. amyloliquefaciens* on the flora of the emitter-attached biofilm. The detailed methods of testing are referred to in Bai *et al*. (2008).

2.3 | Sampling and testing content

2.3.1 | Emitter outflow

During the experiment, the emitter outflow in each treatment was tested every 60 hr of system operation. The emitter outflow was tested using the weighing method. To eliminate the effects of water temperature, pressure, and emitter clogging, the emitter outflow was corrected as described by Pei *et al*. (2014). The evaluation indexes of emitter clogging including discharge ratio variation (Dra) and coefficient of uniformity (CU) were calculated referring to the calculation method used by Li *et al*. (2015) (see supplementary material).

2.3.2 | Biofilm dry weight

Biofilm biomass was characterized by analysing biofilm dry weight (DW). Biofilm samples were taken from each treatment and emitter type, after the system had been

TABLE 2 Averages ± standard deviations of water quality parameters

Parameter	CASS	SBWL	Irrigation standards[a]
Total amount of bacteria (CFU/ml)	(8.2 ± 1.3) $*10^4$	(9.3 ± 1.7) $*10^4$	-
Cl^- concentration (mg/L)	57.5 ± 10.1	112.4 ± 11.8	≤350
pH	7.2 ± 0.2	7.5 ± 0.5	5.5 ∼ 8.5
Chemical oxygen demand (mg/L)	41.2 ± 5.2	56.4 ± 3.6	≤200
Biochemical oxygen demand (mg/L)	15.1 ± 4.3	19.6 ± 4.6	≤100
Total suspended solids (mg/L)	16.1 ± 3.6	20.3 ± 4.0	≤100
Total phosphorus (mg/L)	3.9 ± 0.3	2.7 ± 1.0	≤5.0

Abbreviations: CASS, cyclic activated sludge system; SBWL, sequencing batch aeration wastewater recycling.
[a]Stand for the China irrigation water standard (GB5084-2005). The two types of reclaimed water were tested every 6 days a total of 10 times.

operated for 240 hr, 480 hr, and 720 hr, respectively. One complete drip lateral (six emitters involved) was collected during each sampling event. DW samples were randomly taken from identical emitters with three replicates and were cut with a knife. A high-precision electronic balance (accuracy: 10^{-4} g, ME104E, Mettler Toledo, Switzerland) was used to weigh emitters (with biofilm attached). Later, the attached biofilms in the emitter flow path were removed physically with a brush and other related materials, and the biofilm inside the emitters was thoroughly rinsed off with 15 ml of deionized water. The cleaned emitters (without biofilm attached) were dried in an oven and weighed again. The differences between these two results obtained were recognized as the biofilm DW in the emitters. In addition, the biofilms inside emitters contain few solid particles.

2.3.3 | Extracellular polymers analysis

The sampling times of extracellular polymer substance (EPS) were similar to those of DW; the biofilms of the three types of emitters were mixed. The EPS of attached biofilms inside the emitter flow path mainly included the contents of extracellular polysaccharide (EPO) and extracellular protein (EPR); EPO and EPR were analysed by the phenol-sulfuric acid method and the Lowry method (Raunkjær *et al.*, 1994), respectively. The detailed testing methods are presented in the supplementary material.

2.3.4 | Total microorganisms

A quantitative polymerase chain reaction (qPCR) method was adopted to determine the total amount of microorganisms at the same sampling points as for DW and EPS. Total biofilm DNA was extracted from each emitter (the internal surface area of each type of emitter was fixed) using the FastDNA® SPIN Kit (MP-Biomedicals) according to the manufacturer's protocols. DNA was finally resuspended in 50 μl diethylstilbestrol (deoxyribonuclease/pyrogen-free water) and measured by Qubit® 3.0 Fluorometer (Thermofisher). Each PCR reaction mixture (20 μL) contained 10 ng of template DNA, 10 μL ChamQ SYBR Colour qPCR Master Mix (Vazyme), and 0.5 μL of each primer (5 μm; Forward: 5'-ACTCCTACGGGAGGCAGCAG-3′; Reverse: 5'-GGAC TACHVGGGTWTCTAAT-3′) (Chu *et al.*, 2015). The qPCR reactions were performed in triplicate under thermal cycler conditions of 5 min at 95°C, and 40 cycles of 30 s at 95°C, 30 s at 55°C, and 40 s at 72°C in LineGene9600plus Real-Time PCR Detection System (BioRad) (Guo *et al.*, 2018). All results were normalized and calculated using the ΔCt method (Livak & Schmittgen, 2001).

2.4 | Statistical analysis

One-way analysis of variance was conducted to compare the means of Dra, biofilm biomasses, EPS content, and total microbial biomasses. The least significant difference and Duncan methods were applied to separate treatment means exhibiting significant differences at $\alpha = .05$ level. The regression and statistical analyses were carried out using SPSS (ver. 20.0, IBM Analytics).

3 | RESULTS

3.1 | Effect of B. amyloliquefaciens on total microorganisms

The distribution of the total amount of microorganisms in the emitters' clogging substances after the application of *B. amyloliquefaciens* are shown in Figure 2. The total amounts of microorganisms in both CK and BAI treatments increased gradually with the accumulated system

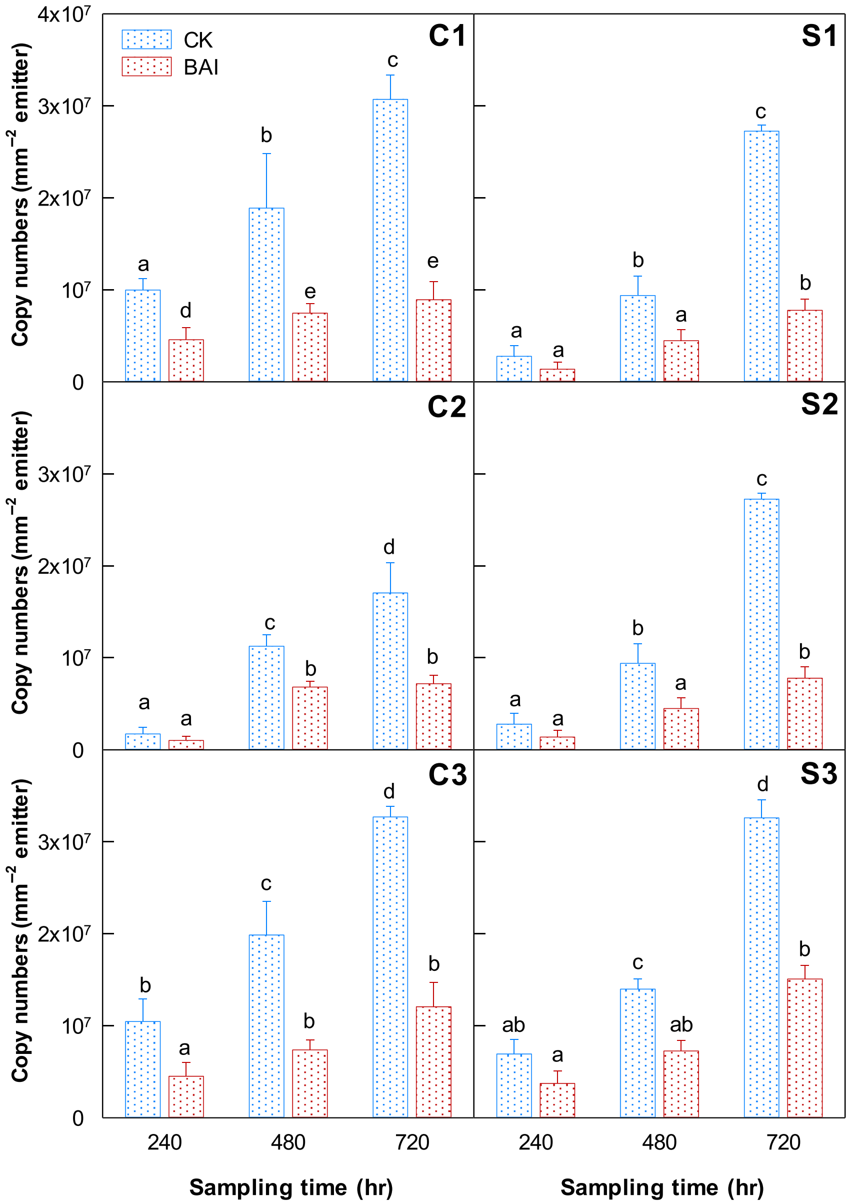

FIGURE 2 Effect of *Bacillus amyloliquefaciens* on the number of microorganisms in drip irrigation emitters. C and S indicate CASS- and SBWL-treated RW; 1, 2, 3 indicate the three emitter types; a–e indicate the difference of each treatment, the presence of the same letters between the two groups indicates not significant at the a = 0.05 level, and the absence of the same letter indicates a significant difference between the two groups. BAI, *Bacillus amyloliquefaciens* inoculum; CASS, cyclic activated sludge system; CK, control group; RW, reclaimed water; SBWL, sequencing batch aeration wastewater recycling [Colour figure can be viewed at wileyonlinelibrary.com]

operating. Compared with the CK, the total amount of biofilm microorganisms in BAI treatments decreased by 37.5%–77.4%, being significantly different at 480 h and 720 h ($p < 0.05$, Figure 2). The reduction effect of BAI on the total amount of microorganisms was affected by water quality, emitter type, and operating time. Under CASS water quality treatment, the total amount of microorganisms decreased by 37.5%–70.9%, while those in the SBWL treatments decreased by 46.1%–77.4%. This demonstrated that the antagonist antibacterial agent played an effective role in SBWL water quality. The total quantity of microorganisms in emitter E1 decreased by 50.5–71.5% and in E3 by 56.9–77.4%. No significant difference was observed between E1 and E3. However, in emitter E2 the total quantity of microorganisms was decreased by 37.5–58.0%. With longer system operation, the antagonistic effect of BAI on microorganisms showed an upward trend, indicating that the antagonistic antibacterial agents also had an inhibition effect on biofilm accumulation with the growth of biofilms in the emitters.

3.2 | Effect of *B. amyloliquefaciens* on EPS

The dynamic variations of EPS content in emitters are shown in Figure 3. Similarly, the total content of EPS in each treatment increased gradually. After the application of BAI, the total amount of EPS in biofilm decreased significantly ($p < 0.05$, Table S1), reaching 37.2–89.4%. The inhibition rates of *B. amyloliquefaciens* on EPS were 44.2–89.4%, 37.2–82.6%, and 59.5–77.7% at 240, 480, and 720 hr, respectively. BAI presented a different controlling effect on EPS and EPR, with a decrease of 27.5–88.3%

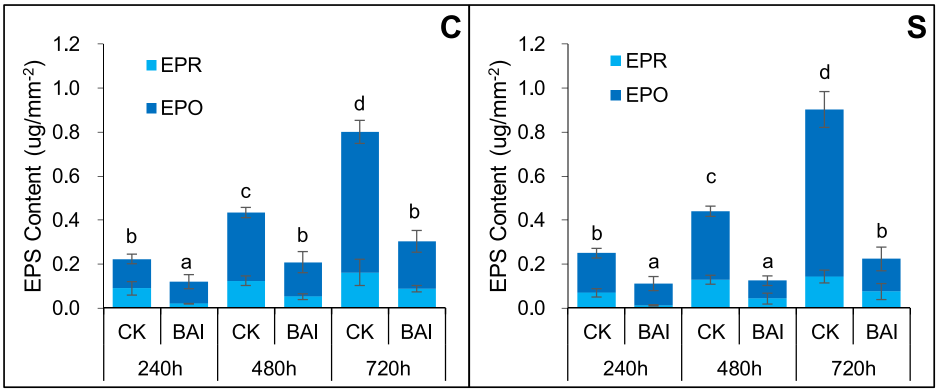

FIGURE 3 Variation of extracellular polymer content in emitters. C and S are the EPS content in the three types of emitters under the CASS and SBWL water quality treatments, respectively. a–d indicate the difference of each treatment, the same letter between the two groups indicates not significant at the a = 0.05 level, and a different letter indicates a significant difference between the two groups. BAI, *Bacillus amyloliquefaciens* inoculum; CASS, cyclic activated sludge system; CK, control group; EPO, extracellular polysaccharide; EPR, extracellular protein; EPS, extracellular polymer substance; SBWL, sequencing batch aeration wastewater recycling [Colour figure can be viewed at wileyonlinelibrary.com]

and 18.4–91.7%, respectively. Significant differences were observed in EPR content between BAI and CK ($p < 0.05$, Table S1). Particularly, the contents of EPR increased rapidly after 480 h in the CK, while no significant differences were observed between 480 and 720 h in BAI. In addition, the application of BAI significantly ($p < 0.05$, Table S1) controlled EPO contents during the whole experimental periods in both types of RW. Particularly, no significant ($p > 0.05$) differences were found in EPO contents at 240 h, 480 h, and 720 h for CASS RW.

3.3 | Effect of *B. amyloliquefaciens* on total DW

The clogging substances were cultured after 720 h, and both the culturable bacteria and *B. amyloliquefaciens* showed antagonistic effects (Figure 4) and resulted in decreased content of biofilm (clogging substances). The biofilm biomasses in CK and BAI treatments at 240–720 h ranged between 8 and 16 × 10^{-2} and 2–5 × 10^{-2} g cm^{-2}, respectively. After the application of *B. amyloliquefaciens*, the content of attached biofilm in emitters decreased significantly ($p < 0.05$), reaching 44.9%–73.8%. Along with system operation time, the efficiency of reducing the biofilm DW in the CK and other treatment increased from 44.9–68.4% (240 h) to 60.3–73.8% (720 h), suggesting that the controlling effect of BAI on biofilm biomasses was comparatively better afterwards. In addition, the difference in DW between the two water qualities and the three emitter types was found non-significant ($p > 0.05$, Figure 4), indicated that the controlling effect of BAI on biofilm in emitters had not been affected by either water quality or emitter type.

3.4 | Effect of *B. amyloliquefaciens* on Dra and CU

The dynamic variations in Dra and CU of RW drip irrigation systems in BAI and CK treatments are shown in Figure 5. In the CK, the Dra showed a gradual declining trend during the early stage (0–360 h), which, however, initiated a sharp decline during the later stage (480–720 h). Similarly, the CU in the CK decreased rapidly after 480 h, while BAI treatment significantly maintained the emitters' Dra > 80% and the CU > 90% during the whole experimental period ($p < 0.05$, Figure 5). The differences for Dra and CU between BAI and CK treatments became larger over system operation time, indicating that the effect of BAI application was comparatively more effective in the later periods. At the end of system operation, BAI treatments increased Dra and CU by 31.9–44.3% and 58.1–67.1%, compared with the CK, respectively. The differences between the CK and BAI treatments for Dra and CU in the two types of RW were non-significant ($p > 0.05$). There were also no significant differences in Dra and CU among the three emitter types ($p > 0.05$). This indicates that BAI has broad-spectrum characteristics that are not affected by water quality and emitter type.

4 | DISCUSSION

4.1 | Effect of *B. amyloliquefaciens* on emitter clogging inhibition

The results of the present study demonstrate that *B. amyloliquefaciens* could effectively control the total

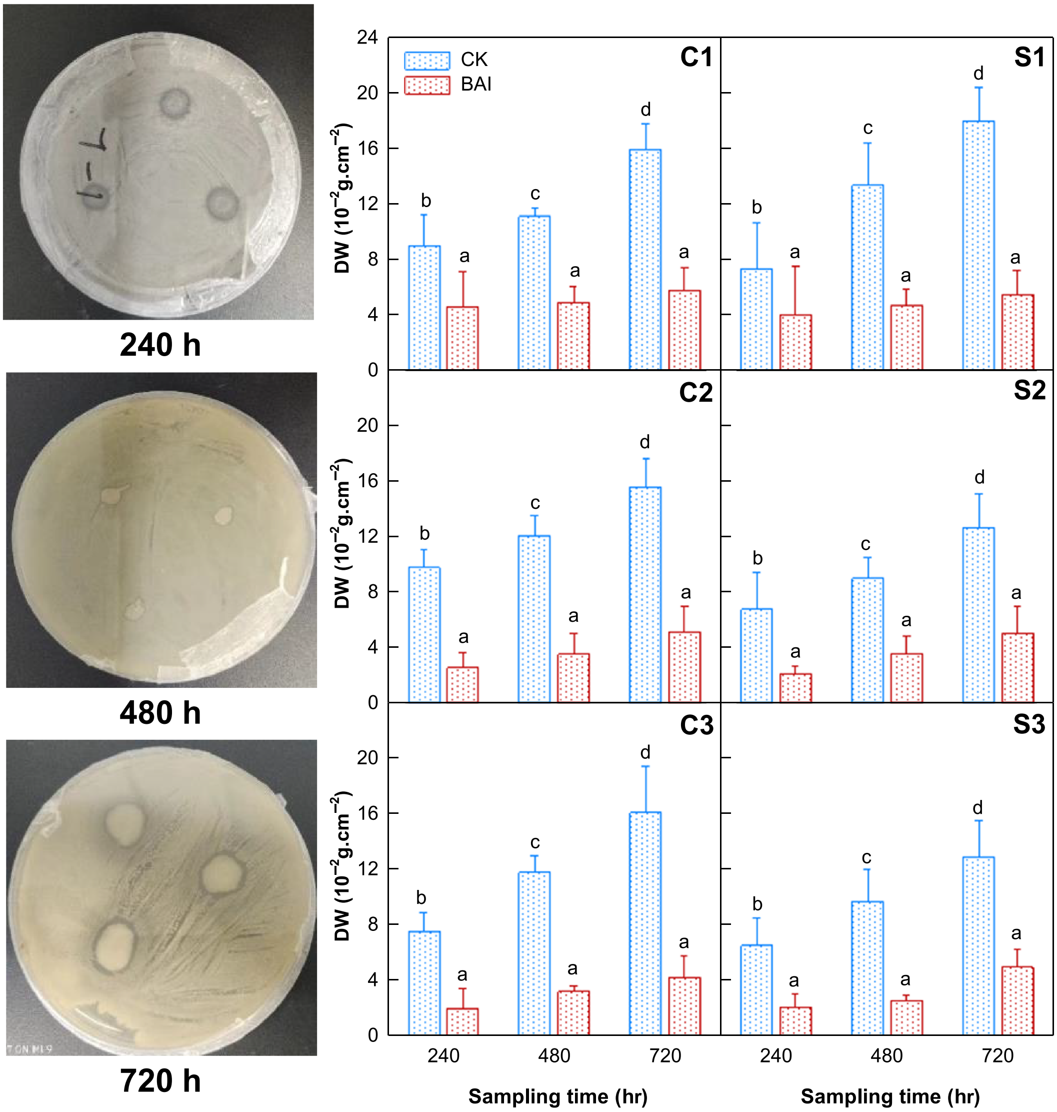

FIGURE 4 Effect of *Bacillus amyloliquefaciens* on total dry weight in drip irrigation system emitters. C and S indicate CASS- and SBWL-treated RW; 1, 2, 3 indicate emitter types; a–d indicate the difference of each treatment, the same letter between the two groups indicates not significant at the a = 0.05 level, and a different letter indicates a significant difference between the two groups. BAI, *Bacillus amyloliquefaciens* inoculum; CASS, cyclic activated sludge system; CK, control group; DW, dry weight; RW, reclaimed water; SBWL, sequencing batch aeration wastewater recycling [Colour figure can be viewed at wileyonlinelibrary.com]

content of microorganisms in biofilm clogging substance in drip irrigation emitters, decreasing it by 40.3–98.2%. Similarly, Zhang *et al*. (2008) reported that the application of *B. amyloliquefaciens* had an inhibitory effect on soybean root rot, with a bacteriostatic rate of 80.2–96.7%. The inhibitory effect of BAI on microorganisms was different depending on the types of microorganisms and fungus concentration. A good antibacterial effect of *B. amyloliquefaciens* mainly depended on the antagonistic correction with the dominant microorganisms in the biofilms. BAI could produce a wide variety of antibacterial substances, such as lipopeptide, polyketone, and protein-active antibacterial substance. The amylocyclicin isolated from *B. amyloliquefaciens* FZB42 had a strong inhibitory effect on the gram-positive bacteria. The bacteriocin (PPB) isolated from SP-1-13LM has a broad-spectrum bacteriostatic activity and can inhibit *Listeria*, *Salmonella,* and *Shigella* (Kim & Chung, 2004). It has been reported that *Bacteroides* (Zhou *et al*., 2016), sequences belonging to the *Firmicutes* (Sanchez *et al*., 2014), and *Cyanobacteria* (Lequette *et al*., 2019) were the dominant microorganisms in biofilms attached in RW drip irrigation. The antagonistic relationship of *B. amyloliquefaciens* with these flora, by which it reduces the total amount of microorganisms in biofilms, has been shown by Wu *et al*. (2014).

The antagonism between BAI and other microorganisms in biofilm will definitely affect the EPS and biofilm

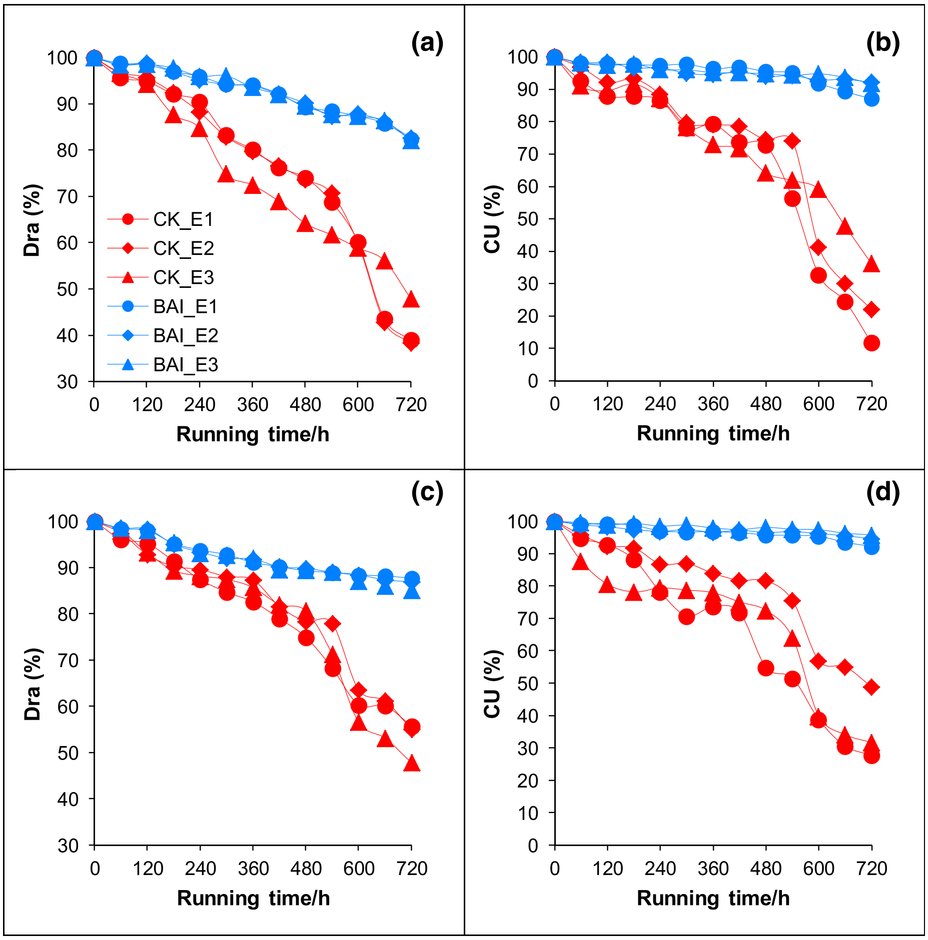

FIGURE 5 Clogging effect of *Bacillus amyloliquefaciens* on emitters in drip irrigation systems. a, c represent the changes of Dra of emitters under the CASS and SBWL water quality with time, respectively; b, d represent the variation of CU with time under CASS and SBWL water quality, respectively. BAI, *Bacillus amyloliquefaciens* inoculum; CASS, cyclic activated sludge system; CK, control group; CU, coefficient of uniformity; Dra, discharge variation rate; E1–E3, emitter types; SBWL, sequencing batch aeration wastewater recycling [Colour figure can be viewed at wileyonlinelibrary.com]

biomasses. De la Fuente *et al.* (2013) found that the antagonist strain of *Flavobacterium psychrophilum* (i.e., *Pseudomonas fluorescens* FF48) effectively prevented biofilm formation and reduced the biofilm total biomass. In addition, EPS, as an important component in biofilm, provided mechanical stability of biofilms (Flemming & Wingender, 2010)and the reduction of EPS content directly affects biofilm growth and formation (Xiao *et al.*, 2020c). This could be attributed to the obvious differences in water quality characteristics in CASS and SBWL, the difference between the dominant bacteria in biofilm formed, and the interaction between BAI and the dominant bacteria in both water qualities. With the accumulated operation, the biofilm removal efficiency of BAI also gradually increased, caused by the rapid growth of biofilm in the CK during the operating hours 480–720. Similarly, Pei *et al.* (2014) reported that the drip irrigation emitter flow rate showed a fluctuating trend during the early stage but rapidly declined later on. BAI had a good inhibitory effect on microorganisms during different operating periods so that the total content of biofilm was always comparatively low and the removal rate of biofilm in the later period was higher. The content of each component in biofilm directly affected the performance of the drip irrigation system. A significant ($p < 0.05$) linear negative correlation was found between Dra-CU and the contents of total microorganisms, EPS, and clogging substances (Figure S1). Thus, the application of BAI effectively maintained the performance of the drip irrigation system.

4.2 | Engineering implications

Due to the productive inhibitory effects of BAI on biofilm growth, it became necessary to evaluate the engineering implications of these results for further productive application in RW distribution systems. Many methods were studied to control the growth of biofilms for RW drip irrigation systems, including chemical chlorination, emitter material modification, electrochemical treatment, etc. (Li *et al.*, 2018). Among them, chemical chlorination is the most traditional and widely used method to control biofilm in engineering practice (Li *et al.*, 2010; Katz *et al.*, 2014), which could be compared with BAI application in terms of anti-biofouling capacity, safety, and environmental risks. Compared with chlorination, the control effect of microbial antagonistic methods was more obvious. In this study, with application of BAI, after the system had been operated for about 700 h, the Dra of the system could be maintained larger than 85%, while the conventional chemical chlorination application was comparatively low at 70% (Song *et al*., 2017). Moreover, the long duration of chemical chlorination would cause the occurrence of chlorine-resistant bacteria in biofilm, which significantly promotes microbial activity and total biomass of biofilms (Song *et al*., 2019a). Secondly, compared to the application of BAI, chemical chlorination being a strong oxidant could be much more inconvenient to store and poses potential risks to human health. Environmental risks need to be considered as well: Chlorination increases the risk of soil hazards (Song *et al*., 2019b), and damages crop roots and drip irrigation pipeline systems (Rubens & Ronaldo, 2001). The antagonist is a kind of microbial agent that obviously had both biocontrol and rhizosphere promoting function. *B. amyloliquefaciens,* found in this study to be frequently obtained from soils, is considered safe for use in the environment and with mammals (Stabb *et al*., 1994; Zhao *et al*., 2015). At the same time, *B. amyloliquefaciens* is also the most studied rhizosphere growth-promoting bacterium, which could advance cutting rooting and fine root development (Díaz *et al*., 2009), and can also dissolve phosphorus and prevent soil-borne diseases (Madhaiyan *et al*., 2010). The antagonist as a liquid could mix well with irrigation water and enter into the drip irrigation system. Periodically added bacteria are able to enter the soil to exert their biological control and rhizosphere promotion on the basis of solving the attached biofilm accumulation in emitters, thus avoiding the environmental risks caused by traditional chlorination methods.

In summary, this paper discussed the inhibition effect of *B. amyloliquefaciens* on bio-clogging and biofilm components in RW drip irrigation systems and obtained some preliminary conclusions. However, microbial inoculants increased the number of exogenous microorganisms. Reasonable concentration and frequency of microbial inoculants was necessary to effectively control the bio-clogging of emitters. Therefore, the suitable application mode of antagonistic agents has become an urgent problem to solve.

5 | CONCLUSIONS

The following conclusions have been drawn:

- *B. amyloliquefaciens* effectively reduced the microorganisms' total quantity by 37.5–77.4%. The controlling effect of microorganisms using SBWL-treated RW was higher than with CASS treatments.
- The contents of EPS in biofilms decreased significantly ($p < 0.05$) after the application of antagonists by 37.2–89.4%. Among these, EPR decreased by 27.5–88.3%, while the content of EPO decreased by 18.4–91.7%.
- Application of *B. amyloliquefaciens* effectively reduced the content of emitter clogging substances by 44.9–73.8% in RW drip irrigation, resulting in a 31.9–44.3% increase in emitters' Dra and a 45.8–75.5% increase in CU.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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