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Biofilm research within irrigation water distribution systems: trends, knowledge gaps, and future perspectives

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Highlights: (max 85 characters with 3-5 points)

- Significant research gaps concerning IWDS biofilms have been identified.
- IWDS conditions affecting biofilm formation, pathogen spreading, and food safety are reviewed
- Distinct various multidisciplinary aspects in performing IWDS biofilm research are discussed
- Future perspectives and advanced technology that can be applied are provided

Abstract:

Biofilms in irrigation water distribution systems (IWDSs) play an essential role in spreading pathogens, chemical pollutants, and environmental pollutants into downstream irrigated crops and thus should be considered a potential threat to food safety. Although the role of biofilms in drinking water distribution systems has been extensively studied in the last decade, the research on IWDS biofilms in this period has been limited. This review identifies research gaps in the field of IWDS biofilms, provides perspectives on experimental designs for investigating IWDS biofilms, and suggests potential strategies worth pursuing in IWDS management. The current state of the art of IWDS biofilms is discussed, and an analysis of the challenges in IWDS biofilm research is presented. Furthermore, this review proposes useful advanced technologies that allow a practical, in-depth fundamental understanding of IWDS biofilms. In a nutshell, this article provides future directions and insights into detailed experimental designs on a relatively under-reported research topic: “IWDS biofilms.”

Keywords: Irrigation water distribution system, biofilm, food safety, risk assessment

1. Introduction

Irrigation systems consist of a complex combination of freshwater source, canals, channels, and pipes. While there are a multitude of different irrigation methods (i.e. surface irrigation, micro-irrigation, sprinkler irrigation and subirrigation), they all share common basic features that include a water source and a piping system for distributing that water. Agricultural productivity has always relied on the careful management and distribution of water, and as such, water is considered as the primary ingredient for ensuring proper produce growth and processing in the pre- and post-harvest stages of production, respectively. As for all raw ingredients, good water quality is essential to prevent produce contamination, and ensuring water safety in agricultural processes is crucial. Regrettably, the use of contaminated irrigation water continues to affect the safety of agricultural produce. An estimated one-third of the world’s food supply originates from 17% of the world’s irrigated croplands, 70% of which are located in developing countries where the use of sewage or sewage-contaminated water is common and widely unregulated (Shanan, 1998; WHO, 2006). In developed countries, the adoption of municipal clean water for crop irrigation is considered unrealistic for large-scale crop production, and only surface water, which makes up only 0.01% of the Earth's 2.5% freshwater, is a viable irrigation source. However, the surface water quality is deteriorating rapidly as the world population continues to increase (Bogardi et al., 2012; Vorosmarty et al., 2010), leading to the current problem of water scarcity in addition to the decrease in available safe water (Shanan, 1998). To meet the irrigation demand for agricultural production, reservoirs in the form of dams, wells, or catchments are created by diverting rivers, pumping groundwater, or collecting rainwater runoffs, respectively. These constructed reservoirs are then used to channel water into extensive canal systems and piping systems, which can be susceptible to microbial contamination and pollution exposure, especially when the contaminated water originates from the polluted surface water. Pathogens such as *E. coli* ,*Salmonella enterica*, and HAV (Hepatitis A virus) originating from irrigation water sources can be internalized by growing fruits and vegetables, for example, lettuce, cantaloupe and green onions, respectively, which makes it difficult to remove these pathogens by washing, physical scrubbing, or routine surface inactivation using antimicrobial agents/disinfectants such as alkyl sodium sulfonate and sodium fatty alcohol ether sulfate (Solomon, Pang, & Matthews, 2003; Stine, Song, Choi, & Gerba, 2005). Similarly, the presence of metal ions including lead, copper, manganese, magnesium, zinc, cadmium, iron and nickel and chemical pollutants ranging from petroleum, explosives, pesticides in contaminated water poses a risk to fresh produce as they can be internalized in ready to eat foods (Fang, Xu, & Chan, 2002; Mitra & Mukhopadhyay, 2016). As such metal ions and other chemical pollutants can also be absorbed and concentrated within the extracellular polymeric substances (EPSs) of biofilms, their potential release into irrigated crops may result to downstream food poisoning in consumers. Nevertheless, there is insufficient knowledge about the survival, ecology, and spread of pathogens within irrigation water distribution systems (IWDSs) or about the potential role they play in the downstream release of chemical or physical pollutants.

To ensure IWDS safety, water source quality is a primary factor; however, most parameters used for assessing microbial irrigation water quality are vaguely defined, e.g., guideline limits for fecal coliform bacteria range from <100 to <1000 fecal coliforms per 100 ml of irrigation source water (US-EPA, 1992; WHO, 1989). These inconsistent parameters suggest a lack of understanding about the effect of coliforms on water safety, which is a flaw in the current risk assessment techniques for irrigation water safety and its link to foodborne diseases. Moreover, bacterial microorganisms have a natural propensity to adhere to and form biofilms on abiotic surfaces, and therefore, the likelihood of biofilm formation within IWDSs cannot be ignored. In the IWDS safety context, poor management, potentially uncontrolled biofilm formation, and the subsequent harboring of unwanted biological, chemical, and physical pollutants within biofilms may cause safety concerns in downstream applications such as crop irrigation.

Although biofilms in drinking water distribution systems (DWDSs) have been extensively studied and characterized, little is known about the presence of biofilms in IWDSs and their potential role in harboring, releasing, and spreading pathogens in the environment, from which they affect downstream food produce (Mandrell, 2011; Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011). Even though DWDS and IWDS may at times be considered similar based on the piping infrastructure used for transporting water, the main difference lies in the source and quality of water being distributed. While drinking water meant for domestic use would have gone through strict water treatment for ensuring safe consumption by end-users, water meant for irrigation can have various sources having least risky to most risky water attributes originating from sources such as treated water, collected rain water, surface water and waste water**.** Moreover, water safety standards for irrigation purposes vary from different regions of the world (Jeong, Kim, & Jang, 2016). As presented in Figure 1, there is a large discrepancy of the number of peer-reviewed reports on the topics of biofilms in DWDSs and IWDSs in recent decades. Both water distribution systems are associated to the sustainable development notion of food-water-energy nexus, in which sectors involving areas of water security, energy security and food security are inevitably linked and impact each other (FAO, 2019). Interestingly, while the number of research papers on topics related to the food-energy-water nexus has exponentially increased in the past 10 years, research pertaining to biofilm and IWDS also increased, however, when dealing with endpoint of the distribution system. In the last decade literature pertaining to “biofilm and drip irrigation” and “biofilm and emitter irrigation” accumulated to around 30 publications, while “biofilm and sprinkler irrigation” amounted to only three publications. Interestingly, concerning “biofilm and irrigation distribution network” only three articles were published in the last decade, in contrast “biofilm and drinking distribution network” which generated more than 130 articles in the same period. A comprehensive list of recent research on the biofilm research within various irrigation strategies is presented in Table 1.

Similar to most water distribution systems, irrigation piping infrastructure is susceptible to colonization by microorganisms present in the microbial ecosystem of the source from which the water is drawn in the irrigation system. In DWDS, the most recognized pathogenic organisms include legionellae, Nontuberculous Mycobacteria (NTM) and *Pseudomonas aeruginosa* (Ashbolt, 2015). Additionally coliform bacteria have also been isolated in DWDS, which is an indicator of other disease-causing organisms (pathogens) (LeChevallier, Welch, & Smith, 1996). Although the biofilms in DWDSs are critical potential reservoirs of pathogenic organisms, little is known about the relationship between biofilms formed in IWDSs and their specific role in harboring and releasing pathogens (Mandrell, 2011; Pachepsky et al., 2011). These uncertainties have mostly been associated with the challenging task of adequately characterizing biofilms formed under IWDS conditions, especially in the context of deteriorating water quality. This knowledge gap is primarily attributed to the lack of implementation of the latest molecular tools, which can provide a better understanding of the diversity and functional roles of IWDS biofilms. Implementing the latest molecular tools such as next generation sequencing or quantitative PCR for studying biofilms formed in IWDS is feasible. However, barriers may be experienced in terms of linking generated results with the age of the biofilm, or specific historical pollution events. Other problems may still be experienced while estimating and characterizing the biomass of such biofilms without having to remove parts or sections of the IWDS line. Hence, biofilm research concerning IWDS is further challenged with respect to dynamic changes to source freshwater from either sporadic environmental pollution events, or changes associated with seasonal drifts, and the lack of proper models for studying such biofilm in a fundamental manner within a laboratory setting.

Although bacteria in water distribution systems are most likely found in the form of established biofilms within the interior pipe wall (95%) and bulk water (5%) (LeChevallier, Babcock, & Lee, 1987; Lehtola et al., 2004; Simões, Simões, & Vieira, 2010; Wingender & Flemming, 2004), irrigation water quality assessments are, for the most part, primarily based on water sampling methods to quantify enteric indicators. To the best of our knowledge, few reports have been published on the microbial ecology of IWDSs based on biofilm sample collections (Lapidot

& Yaron, 2009; Pachepsky et al., 2012; Sanchez et al., 2014; Yan et al., 2009). The few published studies have investigated bacterial community composition in IWDSs in terms of changes in freshwater quality caused by human activities or environmental changes.

Biofilms in IWDSs cannot be ignored as they are a potential source of contamination to downstream irrigated produce, and several ready-to-eat produce outbreaks have been showed to directly linked with irrigation (J. Gelting, A. Baloch, Zarate-Bermudez, & Selman, 2011; Nygard et al., 2008). In 2004, a *Salmonella* Thompson outbreak in Norway resulted in the contamination of Rucola Lettuce following irrigation using non-potable water (Nygard et al., 2008). Although most produce outbreak investigations link produce contamination with irrigation using questionable water quality, the specific role of IWDS biofilms in the harbor and proliferation of pathogenic agents should therefore be further elucidated, especially considering that the presence of biofilms are known as threats in other environments such as in the food processing industry. In 2006, a multistate *E. coli* O157:H7 outbreak associated with the consumption of contaminated spinach in the USA, was traced to faulty irrigation using biologically compromised groundwater from its interactions with surface water (J. Gelting et al., 2011). Therefore, there is an urgent need to improve our understanding of the microbial ecology within IWDSs, especially in terms of the presence, structure, and function of established biofilms within these systems. As part of a management framework, the implementation of the acquired knowledge about IWDS biofilms would serve as a basis for developing and optimizing risk assessment techniques, which would help improve intervention strategies for mitigating the risks to ensure the safety of downstream agricultural produce.

This review focuses on biofilms formed in IWDSs, identifies research areas that would help to better manage IWDSs, and provides perspectives on experimental designs for studying IWDS biofilms.

2. Biofilm structure and response to extrinsic factors

2.1. Biofilm formation and detachment

From the first surface attachment of a bacterial cell to its development into a mature biofilm, surface colonization by bacteria can be regarded as a dynamic process. This process is typically characterized by the irreversible attachment phase of planktonic cells onto an abiotic surface and is followed by further development into microcolonies when exposed to ideal growing conditions. The final stage in the biofilm development process consists of cell accumulation leading to the formation of macrocolonies, which are defined as a mature biofilm (Li, Xia, Tao, & Wang, 2017). Biofilm detachment can occur at any stage of the biofilm formation process and may lead to the release of pathogenic bacterial cells and other embedded chemical and physical pollutants further downstream. The detachment can either be triggered by intrinsic factors such as natural biofilm cycle for colonizing new surfaces, or initiated by environmental forces, such as hydrodynamic shear conditions, physical contact and disinfectants (Petrova & Sauer, 2016). The simplified biofilm cycle dynamics is presented in Figure 2. Extracellular polymeric substances (EPSs), called the "house of biofilm cells," are secreted by biofilm microorganisms function as both a protective diffusion barrier and a nutrient trap to protect and facilitate bacterial growth (Flemming, Neu, & Wozniak, 2007). It is composed of polysaccharides and a wide variety of proteins, glycoproteins, glycolipids, and in some situations, substantial amounts of extracellular DNA (Flemming et al., 2007). The living condition of biofilms is largely determined by EPSs as they modulate several parameters of the biofilms, such as porosity, density, water content, charge, hydrophobicity, and mechanical stability. EPSs provide a protective diffusion barrier especially when exposed to environmental stress, where the biofilm will be reacting differently compared to their planktonic counterpart, which will be further elucidated in the following section.

2.2. Biofilm response to extrinsic environmental factors

Bacteria cells within biofilm generally present very different metabolic properties compared to their planktonic counterparts (Fletcher, 1992). One physiological reason is that the cell's surrounding extracellular polymeric substances within biofilms acts directly as a nutrient source or indirectly through the concentration of nutrients from the environment. Moreover, cell density and distance between cells within biofilms can also promote ecological interactions in the form of synergism, mutualism, competition, and antagonism.

Biofilms are naturally better equipped to survive environmental stresses characterized by extreme temperature, pH, shear, or pollution conditions, such as chemical and metallic contamination (Fechner, Gourlay-France, & Tusseau-Vuillemin, 2014) than their planktonic counterparts. Although few studies have evaluated the role of IWDS biofilms, the biofilm's reactivity to environmental stresses have been well studied in DWDSs. For example, in one study, the effects of flushing on biofilm detachment were evaluated and compared between three types of pipe with different materials (Liu et al., 2017). The results revealed that pipe materials play an important role in determining whether biofilms are resistant or sensitive to flushing (Liu et al., 2017). Another study by Shen *et al.* investigated the biofilm response to long-term exposure to disinfectants within DWDS environment and found that 3-month exposure to disinfectant treatments did not affect the biofilm thickness, suggesting that that long-term disinfection may not be able to remove the complete biofilm biomass (Shen et al., 2016). Furthermore, water stagnation and temperature changes affect both chemical and microbial quality in domestic drinking water systems and the changes in biofilm community composition (Zlatanovic, van der Hoek, & Vreeburg, 2017).

The piping material has also been shown to induce metabolic responses in the established biofilms. One study on the difference in the metabolism between the biofilms of the multi-metal-resistant bacterium *Pseudomonas fluorescens* and its planktonic counterpart under metal stress showed that the planktonic cells undergo several reactive changes when exposed to copper, including alterations in the TCA cycle, glycolysis, and pyruvate, nicotinate, and nicotinamide metabolism (Booth et al., 2011). However, such changes were not observed in biofilms, which instead showed changes in the exopolysaccharide-related metabolism, including galactose and sucrose metabolism, and in the phosphotransferase system, indicating that metal stress induces a protective response rather than reactive changes. The increase in the exopolysaccharide-related metabolism could possibly result in more newly synthesized EPSs or induce metabolic changes in the EPSs for protecting the biofilm against copper stress.

IWDSs and DWDSs have several similar properties. Both systems include a centralized treatment plant/well and piping systems to carry water to a final user end-point (the agricultural produce or the consumer's tap). Hence, research on biofilm reactivity to multiple stresses in

IWDSs is warranted. The following chapters illustrate biofilm dynamics in the context of irrigation and address unevaluated research issues in this field.

3. IWDS conditions that affect biofilm formation, detachment, pathogen spread, and food safety

3.1. Water source

The irrigation water source plays a vital role in determining irrigation water quality and safety as well as the extent of biofilm formation in the pipe. Although different countries around the world may have existing standards for controlling irrigation water quality (Jeong et al., 2016), compounded by the current water stress issues observed worldwide, a large fraction of unregulated fresh water from groundwater to retreated water and surface water such that found in lakes, rivers, and reservoirs may be redirected for use in irrigation (Figure 3). However, these fresh water sources may not be safe for direct irrigation use. More particularly, using treated wastewater for irrigation may lead to the accumulation of chemical and biological contaminants in soil, ended up in posing risks to both human and environmental health (Becerra-Castro et al., 2015). Besides, there is an increase of active pharmaceutical ingredients entering into sewage networks that's potentially used for irrigation (Lees, Fitzsimons, Snape, Tappin, & Comber, 2016). Reversely, it has been found long-term wastewater irrigation also has an impact on groundwater pollution (Jampani et al., 2018). As a consequence, more than one billion people lack access to clean, safe water (Tiyo et al., 2015) and are forced to use potentially contaminated water. A study conducted in Brazil investigated the bacteriological status of 20 wells for irrigation, including 10 artesian and 10 conventional wells. From artisanal wells, 42.5% of water samples tested positive for total coliforms, and 5.0% showed abnormal coloration. In contrast, 87.5% of conventional wells were contaminated with total coliforms, 82.5% of which showed thermotolerant coliforms contamination and 12.5% showed abnormal coloration. Measurement of fecal indicator bacteria (FIB), such as *Escherichia coli* and *Enterobacteriaceae* endemic to warm-blooded animals, is considered as the basis of microbiological water analysis (Cabral, 2010). While most FIB do not pose a threat in most healthy adults, they may nevertheless cause harm in infants, the elderly and people with compromised immune systems. Furthermore, the presence of FIB may be considered a proxy for the presence of harmful pathogens that cause gastrointestinal diseases, such as salmonellosis and shigellosis. Moreover, agriculture produce with internalized fecal pathogens transmitted through irrigation may lead to foodborne infections in humans (Tiyo et al., 2015). Another investigation on the quality of water from surface water sources used for drinking and irrigation in Zaria, Nigeria (Chigor et al., 2012) showed that 228 water samples collected from 10 sites had high fecal coliform counts ranging from 2.0 to 1.6×10^6 MPN/100 ml, which exceeded the WHO-specified limits for drinking and irrigation water. A recently published paper reported evidence that the microbial quality of water in reservoirs is affected by the hydraulic concrete structure (HCS) (Cai et al., 2017). Biofilms attached to different types of HCS surfaces showed notable variations in microbial diversity. These results provided implications for better source water safety management in terms of HCS.

In developing countries, the use of sewage or sewage-contaminated water for irrigation is frequent and widely unregulated because of water scarcity (Jaramillo & Restrepo, 2017), whereas in developed countries, the use of municipal clean water for irrigation is considered to be unrealistic for large-scale crop irrigation, as 1 ha of crop land would require 6 m^3 of irrigation water. Hence, to meet the requirement of water for large-scale irrigation, reservoirs in the forms of dams, wells, or catchment are created by diverting rivers, pumping groundwater, or collecting rainwater, respectively. These developed reservoirs then channel water into extensive canal systems, where pathogenic contamination and pollution could readily occur. For example, surface runoff from active grazelands may transfer pathogens into freshwater systems. The systematic emphasis on water availability rather than safety/quality has led to crop contamination through irrigation.

Regardless of the geographical location, the water source used for irrigation will mostly contain some types of pollutants. Because of the ubiquitous presence of biofilms within the piping infrastructure, the relationship between pollutants from the water source and established biofilms within the piping structure of IWDSs is still misunderstood from an irrigation management standpoint. This knowledge gap limits the existing irrigation risk assessments by overlooking the presence of piping biofilms and their functional attributes. The following section provides examples of biofilm function in terms of antibiotic resistance and its consequences if left untreated in IWDSs.

3.2. IWDS environment

Freshwater pollution is usually characterized when a freshwater source is contaminated by inland water containing substances causing the freshwater source to be no longer fit for its natural or intended use, such as for irrigation and faming purposes. One example of inland water pollution is sewage discharges that contain not only fecal matter, but also pharmaceutical agents, which also include antibiotics and other antimicrobials. Reportedly, antibiotic-resistant bacteria can spread through the systems of reclaimed wastewater, sewage effluent, drinking water, surface water system, and especially hospital discharge water (Armstrong, Shigeno, Calomiris, & Seidler, 1981; Czekalski, Berthold, Caucci, Egli, & Burgmann, 2012; Gomez-Alvarez, Revetta, & Santo Domingo, 2012; Lehmann et al., 2016; Tejedor Junco, Gonzalez Martin, Pita Toledo, Lupiola Gomez, & Martin Barrasa, 2001; Yomoda, Okubo, Takahashi, Murakami, & Iyobe, 2003). The study by Blaustein *et al.* was perhaps the first to explore the proliferation of antibiotic-resistant bacteria in irrigation systems. In this study, irrigation events were conducted at a perennial stream on a weekly basis, where ampicillin- and tetracycline- resistant microorganisms were monitored at the intake water (Blaustein et al., 2016). Biofilms were isolated from the inner piping wall and were screened for resistance to seven antibiotics. The results showed that sampled microbial cells, mostly fecal coliforms, were multi-drug resistant (Blaustein et al., 2016). Further, high-level resistance to β-lactams; limited resistance to chloramphenicol, tetracycline, and aminoglycosides; and little to no resistance to ciprofloxacin was observed in the general biofilm-associated bacteria (Blaustein et al., 2016). These findings revealed that similar to most water distribution systems, IWDSs are likely to spread antibiotic-resistant bacteria, thereby increasing potential health risks through downstream dissemination into the food supply chain (Blaustein et al., 2016).

Maal-Bared *et al.* explored the antimicrobial resistance properties of bacterial samples isolated from an agricultural watershed in British Columbia and revealed the difference in prevalence and resistance between *E. coli* isolates, including the pathogenic *E. coli* O157 (Maal-Bared, Bartlett, Bowie, & Hall, 2013). Compared with the bacterial cells suspended in water, those associated with benthic biofilms or sediments were found to be more resistant to a range of antibiotics and found to proliferate against tetracycline, ampicillin, and streptomycin (Maal-Bared et al., 2013). Interestingly, the authors performed multivariate logistic regressions, revealing that water depth, nutrient concentrations, temperature, dissolved oxygen and salinity had significant associations with frequency of *E. coli* resistance to nalidixic acid, streptomycin, ampicillin and tetracycline. It was therefore suggested that *E. coli* be used as proxies to predict the frequency of antimicrobial-resistant organisms in a given water distribution system. In their study it was shown that water depth is positively correlated with *E. coli* resistance towards ciprofloxacin and nalidixic acid, and negatively correlated with its resistance towards tetracycline. In one other example it was shown that temperature was positively correlated with *E. coli* resistance towards streptomycin, and negatively correlated with its resistance towards nalidixic acid. However, more research in this area would help improve water quality risk assessments. In the context of IWDSs, the proposed proxy approach would still require an in-depth understanding of the interaction between the biofilm and the transported bulk water, especially in terms of the mechanisms of bacterial proliferation within the biofilms because biofilms are considered as dynamic biological systems harboring antimicrobial-resistant pathogens. The high density of cells, which is a characteristic of biofilms, may also favor physical contact between cells, thereby allowing the transfer and exchange of genetic material between cells, including antimicrobial resistance genes, in a process called horizontal gene transfer.

Studies have already demonstrated that horizontal gene transfer plays an important role in the spread of antibiotic resistance genes through biofilms in agriculture systems, stock breeding, and clinical environments (Haug, Tanner, Lacroix, Meile, & Stevens, 2010). Conjugative mediators, such as transposons and plasmids, are primary carriers in horizontal gene transfer. Other types of horizontal gene transfer include mobilization and retromobilization. In conjugation, genetic material is transferred between bacterial cells by direct cell-to-cell contact or by a bridge structure formed between two bacterial cells; it is the most common method of horizontal gene transfer (Holmes & Jobling, 1996). In mobilization, the transfer of plasmids that cannot cause their own transfer is mediated by a co-resident conjugative plasmid (Timmery, Modrie, Minet, & Mahillon, 2009). In retromobilization, some conjugative plasmids mobilize genes in the opposite direction, i.e., from the recipient to donor, which provides a way for the donor bacteria to acquire new genes from other species.

Reportedly, *E. coli* cells are involved in the horizontal gene transfer of antimicrobial resistance genes and thus pose risks to human health. Interactions between aquatic systems, such as by runoffs or leaching, could result in the transfer of antibiotic resistance genes between the bacteria of different microbial ecosystems. Moreover, the antimicrobial resistance pattern of *E. coli* has been observed in not only aquatic systems but also in broiler chicken and farmed animals in watershed, indicating that the possible communication of antimicrobial resistance genes between the animal population and aquatic systems (Diarra et al., 2007; Maal-Bared et al., 2013). This has been reported to occur mainly in the case of *E. coli* O157 isolates (Maal-Bared et al., 2013). Some studies have revealed that plasmid-mediated horizontal transfer of antibiotic resistance genes also occurs within the biofilm complex (Anjum & Krakat, 2016; Timmery et al., 2009). The high density of cells in biofilms may facilitate the interaction and genetic material transfer between cells.

The following section provides examples of current research on biofilms in the irrigation systems, with a discussion of the advantages and challenges in conducting such investigations.

4. Examples of current research on biofilms in IWDSs (cf. Table 2)

Biofilm structure in drip irrigation emitters was first evaluated by Yan *et al.* by quantifying phospholipid-derived fatty acids (PFLAs) and analyzing micrographs following scanning electron microscopy (Yan et al., 2009). Their study involved six types of emitters with different flow path structures, thus allowing the investigation of the effect of path structure on the microbial community and emitter clogging. Total PLFA levels were used to estimate the total microbial biomass based on the assumptions that differences in the fatty acid levels indicate the community-level microbial profiles and that the phospholipid content is directly proportional to cell biomass. Among the six emitter groups, high total PLFA levels, especially bacteria PLFA levels, were found to be significantly associated with reductions in discharge and distribution uniformity. This finding suggests that the total microbial biomass, especially bacterial biomass, is strongly correlated with emitter clogging. Moreover, total EPS and total polysaccharide contents were correlated with discharge reduction. Interestingly, among the six emitter groups, the highest reductions in discharge and distribution uniformity were observed in two types of emitters with biofilms containing fungal and algal PLFAs. Future research is warranted to evaluate the complete microbial community diversity in different types of emitters and to establish optimal emitter designs with anti-clogging properties. Further, efficiency of the application of disinfectants/antimicrobial agents should be investigated and compared among different types of emitters.

Salmonella enterica is one of the most common pathogens causing foodborne diseases and has the endophytic ability to invade the internal part of plants (Lapidot & Yaron, 2009). To investigate whether two biofilm components, curli and cellulose, play a role in facilitating the invasion of *S. enteric* in the internal plants, Lapidot and Yaron used wild-type *S. enterica* and mutants, namely MAE52 (biofilm sufficient, curli sufficient, and cellulose sufficient), MAE190 (curly deficient and cellulose deficient), MAE97 (curli deficient), and MAE150 (cellulose deficient) (Lapidot & Yaron, 2009). Parsley was selected as the target plant. GFP plasmids were transformed to obtain GFP-labeled cells. The confocal laser scanning micrographs revealed the presence of wild-type bacteria on the leaves as aggregates or single cells after irrigation with water containing wild-type *S. enterica*, indicating that irrigation water contaminated with *S. enterica* caused contamination of the edible part of parsley. Moreover, the MAE52 (biofilm sufficient, curli sufficient, and cellulose sufficient) group presented a contamination profile similar to that of wild-type bacteria as well as a significantly higher colony count than all other tested mutants, indicating that curli and cellulose play an essential role in transferring *S. enterica* into the plant. This is one of the few studies to reveal the role of biofilm in transferring pathogens into plants, highlighting the importance of disinfection when using wastewater for irrigation.

The effect of pipe-based biofilms on the microbial quality of irrigation water was first investigated by Pachepsky *et al.* using *E. coli* as the microbial water quality indicator (Pachepsky et al., 2012). The water source was demonstrated to be an essential factor for microbial water quality. They revealed a positive correlation between *E. coli* density and total heterotrophic bacterial count and concluded that the biofilm-associated *E. coli* can affect the microbial quality of irrigation water and should therefore not be neglected when estimating microbial mass balances for irrigation systems. Nevertheless, it should be noted that one of the drawbacks of using *E. coli* or any other fecal indicator bacteria from sampled water is amplified by the absence of coliform counts, which does not necessarily reflect the absolute contamination level of the studied sample. However, the sampling of biofilms may help determine the risk towards an irrigation system, based on the fact that biofilms can release planktonic cells. Given that biofilms in the freshwater environments or IWDS are composed of a plethora of different organisms, quantifying the level of pathogens embedded within them will therefore be an important measure for assessing the level of risk of pathogenic contamination. Moreover, such an approach would also help acquire fundamental knowledge of the ecological drivers allowing for the persistence and survival of these pathogens within biofilm ecosystems.

Sanchez *et al.* investigated the microbial communities in irrigation water (both treated wastewater and reclaimed water), pipe biofilm, and dripper biofilm in the recently developed greenhouse-enclosed drip irrigation system using denaturing gradient gel electrophoresis and 16s rRNA sequencing (Sanchez et al., 2014). They found that almost all obtained sequences had some similarity to thermophilic microorganisms and were mainly related to potentially spore-forming organisms, suggesting that microbial communities with the ability to proliferate at high temperatures were sourced from the microorganisms present in the incoming water. Interestingly, biofilm samples showed a lower bacterial diversity than water samples, possibly due to the selection of certain bacterial populations capable of attaching to the surfaces of irrigation drippers and pipes. This study provided useful detailed information for predicting the risk of potential crop contamination and offered suggestions to develop efficient biofilm management strategies within irrigation systems. For example, one should also take into account of sample temperature and the level of thermophilic microorganisms in designing biofilm management strategies. This is because endospores of spore-forming thermophiles could become a problem when attempting to control biofilms with biocides because endospores are highly resistant to disinfectants.

The examples mentioned above were the only studies addressing the issue of structure and functionality of biofilms within IWDSs. Considering the critical role of IWDS biofilms with regards to food safety, more fundamental research is warranted to better understand the biofilm structure and function, especially in terms of horizontal gene transfer, as well as biofilm reactivity in terms of incoming pathogens, pollutants, and microbicides. Technologies that can be used in IWDS biofilm research and the challenges in designing IWDS studies are addressed in the following chapter (chapter 5).

5. Challenges in IWDS biofilm research and the application of advanced technologies

5.1. Challenges and limitations in biofilm research.

In the past few years, only a few studies have investigated IWDS biofilms; these include field-based and laboratory-based studies. The reason for the limited investigations may be the complexity and challenges in IWDS biofilm research. Investigations relating to IWDS biofilm research are met with challenges caused by the limited or inexistent standardized protocols and experimental systems for studying biofilms (Parsek & Fuqua, 2004). Moreover, an integrated multidisciplinary approach, involving fields such as microbial ecology, molecular biology analytical chemistry, and bioinformatics, is needed to acquire a comprehensive understanding of IWDS biofilm community structure and functional attributes.

IWDS biofilm research can take the form of a field-based study or/and a laboratory-based study. As for laboratory-based studies, several types of biofilm reactors are available for conducting laboratory-based studies. However, reactor selection must take into consideration the scope and objectives of the biofilm investigation, and this also applies to IWDS biofilm research. Compared with field-based studies, biofilm formation conditions in a laboratory setting can be efficiently controlled, thus allowing fundamental research. However, laboratory-based biofilm studies require proper infrastructure and equipment. With regards to future IWDS biofilm research direction, the fundamental research question concerning the reactivity of simulated irrigation piping biofilms towards different class of pollutants can be achievable through the means of a relevant water source and biofilm reactor/piping systems.

Field-based IWDS biofilm research can be challenging considering the difficulty in collecting relevant biofilm samples and in filtering out impurities because IWDSs are closed-end systems.

Sampling of IWDS biofilms usually involves performing an autopsy of the IWDS infrastructure, a task that would requiring cutting out sections that would otherwise compromise the purpose of distributing irrigation water. This is especially relevant when the IWDS in question is still actively in use. A potential solution to this technical problem would require researchers to focus on investigating easily accessible dripper-type irrigation systems from which biofilms can be characterized based on the original water source and its identified pollutants. Such findings would then serve as a basis for formulating hypothesis-led IWDS biofilm investigations in laboratories. The other potential challenge in biomass collection from real IWDSs is the contamination with impurities that can potentially influence the outcomes to the following experiments, especially those based on omics tools. Omics studies are mostly based on sequencing of nucleic acids, such as DNA and RNA, and impurities could degrade nucleic acids, particularly during RNA extraction, because RNA are fragile molecules that degrade easily. In addition, samples to be used in omics studies must be handled using proper techniques to minimize DNA/RNA degradation. For example, raw samples aimed for RNA extraction may need to be immediately frozen and stored at −80°C after sample collection and initial processing because slow freezing may activate endogenous RNase, causing RNA degradation. Moreover, products such as RNAlater® can also be added to the raw sample to prevent subsequent degradation. In addition, the period between sample collection and storage also should be short to maximize the RNA/DNA integrity because integrity is considered to be the most important factor for further sequencing because the use of low integrity RNA can lead to significant issues during bioinformatic analysis, in the form of increased mapping inaccuracy and decreased number of mapped/detected genes/species.

Despite the challenges in conducting biofilm studies, there is potential to conduct fundamental research on biofilm communities present in IWDS environments. Such research can be successfully conducted using a combination of technologies, which are discussed in the following section.

5.2. Omics technology

Although the application of omics technologies has allowed a better understanding of the genomic composition, gene expression profiles, and metabolic activities within biofilms, its application in IWDS biofilm research has hardly been considered in previous studies. In one rare exception, Sanchez *et al.* investigated the microbial communities and prevalence of thermophilic microorganisms in irrigation water, pipe biofilm, and dripper biofilm from a greenhouse-enclosed dripper irrigation system using 16S amplicon-based metagenomics (Sanchez et al., 2014). Future IWDS biofilm research should consider using a combination of omics tools, including genomic, transcriptomic, and metabolomics approaches, to obtain a comprehensive understanding of biofilm at a fundamental level as the biofilms are affected by environmental factors.

5.3. Fluorescence in situ hybridization (Nygard et al.)

For decades, FISH has been a method of choice for the in-situ detection and localization of specific organisms within complex microbial consortia. Specifically, in biofilm-related studies, FISH can be used to investigate the biodiversity of organisms inhabiting a biofilm by enabling a rapid and precise visualization of groups of microorganisms in their natural habitat, which may otherwise be difficult to quantify using traditional culturing methods (Wolf, 2017). In combination with omics tools, FISH could be applied in parallel studies as an essential in-situ validation step following bioinformatics analyses. To the best of our knowledge, FISH is yet to be applied in IWDS biofilm research. This technique would provide additional invaluable fundamental information on the spatial localization of targeted organisms within a biofilm to help elucidate the survival or persistence of these localized organisms when faced with environmental stresses.

6. Future perspectives

6.1. IWDS biofilm structure, composition, and functionality

Several types of irrigation systems are available for different irrigation applications, such as surface irrigation, localized irrigation, drip irrigation, sprinkler irrigation, center pivot irrigation, lateral move irrigation, sub-irrigation, and manual irrigation (Figure 4). Of these, most types involve either pipes, drippers, or sprinklers that provide the necessary surface for allowing biofilm formation. Although Sanchez *et al.* (Sanchez et al., 2014) investigated the microbial communities of biofilms developing in irrigation water, pipe biofilm, and dripper biofilm in a greenhouse-enclosed drip irrigation system, no such study has been performed on other types of IWDSs. Future research should investigate such unexplored areas of IWDS biofilm structure, function, and reactivity. Advanced microscopy techniques could be used to characterize the structural properties of biofilms at different biofilm formation stages. Omics technology in combination with bioinformatics tools and FISH techniques could be applied to determine the microbial composition and spatial localization of specific microbial groups or individuals within an IWDS biofilm. In terms of biofilm function, researchers should focus on exploring how intraspecific and interspecific transfer of specific genes, such as antibiotic resistance genes, occurs through biofilms, such as by horizontal gene transfer.

6.2. Effect and impacts of pollutants and other environmental factors on IWDS biofilms

To investigate the effects of pollutants or other environmental factors on the formation of IWDS biofilms, the development of a laboratory-based simulated IWDS is necessary. Once such a system is available, harvested IWDS biofilms can be exposed to relevant pollutants to assess biofilm–pollutant interactions at a fundamental level. Of particular interest, environmental pollutants, such as micro-plastic, which provide a surface for microorganisms/biofilm to attach and serve as a media for the spread of pathogens, are worth investigating for their influence on biofilm reactivity and water quality (Harrison et al., 2018). Also, of interest, chemical pollutants, such as DEET, antibiotics, and biological pollutants, and microorganisms, of food safety relevance should be investigated.

In addition, the role of environmental factors, such as temperature, sunlight intensity (Schultz-Fademrecht, Wichern, & Horn, 2008), and nutrient levels, on biofilm stability and water quality are also worth investigating. All such studies should be designed to involve a parallel control group under standard conditions to assess the effect of the changed parameter on test groups.

6.3. IWDS biofilm management

Specific biofilm management strategies are required in different water distribution systems. Many studies have extensively investigated biofilm management strategies in DWDSs and food processing systems, resulting in the development of various biofilm management methods for such well-explored systems. However, the research on biofilm management in irrigation systems has been limited. Facilitating a functional IWDS biofilm management structure by ensuring a quality-controlled irrigation water source is not sufficient, as biofilms develop within the piping surface. Therefore, establishing biofilm management strategies covering the entire IWDS is crucial. According to water distribution management concepts, the choice of materials could be a potential target for managing biofilms within irrigation systems through the use of antimicrobial materials, such as antifouling coating material. Moreover, ultrasound, hydrodynamic cavitation, and electron-beam radiation can serve as potential environmental management strategies for the mechanical disruption of biofilms within irrigation systems. With the emergence of biofilms with multi-antibiotic resistance, further fundamental and applied research are needed to promote safer produce. In one fundamental study, biofilm treatment using quorum sensing inhibitors or quorum quenchers followed by antibiotic treatment was shown to be able to kill or at least inhibit bacterial growth (Bhardwaj, Vinothkumar, & Rajpara, 2013); the question now remains whether similar approaches or strategies would be efficient enough to manage IWDS biofilms.

7. Conclusion

Although DWDS biofilms have been extensively investigated, research on IWDS biofilms has been limited. It is well known that biofilms are capable of spreading pathogens that can be internalized by fresh produce; therefore, it is crucial to have a deeper understanding of IWDS biofilms for establishing and designing biofilm-managing strategies. Although a few studies have characterized the biofilm structure in various irrigation emitters (Yan et al., 2009) or the propensity of foodborne pathogens to be internalized by fresh produce following irrigation (Lapidot & Yaron, 2009), further research is warranted for unravelling the IWDS biofilm structure, composition, and function. Furthermore, understanding of IWDS biofilm reactivity toward pollutants or other environmental factors will enable the development of optimal IWDS biofilm management strategies. Considering the current challenges in IWDS biofilm research, a proper and relevant experimental design for hypothesis-based fundamental and applied research needs to be carefully selected. Omics tools in combination with advanced in-situ hybridization techniques should be considered to obtain invaluable fundamental information regarding IWDS biofilms. The issue of IWDS biofilms has downstream effects in the large food–water–energy nexus, wherein biofilm management could help obtain safer fresh produce, diminish produce wastage, and sustain the livelihood of millions working in the agricultural sectors.

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CAPTIONS TO TABLES AND FIGURES.

Table 1: Studies related to biofilm research within various irrigation systems.

Table 2: Summary of significant findings related to biofilm research within IWDSs.

Figure 1: “Web of Science” search results of nine keyword combinations related to biofilm and agricultural irrigation research in recent decades.

Figure 2: Schematized biofilm development cycle.

Figure 3: Examples of water sources with naturally occurring biofilms that may potentially spread pathogens to downstream irrigations systems.

Figure 4: Examples of different forms of irrigation systems involving either pipes, drippers or sprinklers, known for providing surfaces for biofilm formation.

Table 1: Studies related to biofilm research within various irrigation systems.

keywords used for searching on Web of Science	Number of publications based on searching result	Number of qualified publications after screening	reference
Biofilm + “drip irrigation”	30	30	(Ait-Mouheb et al., 2019; Batista, Costa, Lopes, Coelho, & Paiva, 2011; Batista, de Matos, da Cunha, Lo Monaco, & dos Santos, 2012; Batista, dos Santos, Neto, Santos, & Barreto, 2012; Batista et al., 2009; Batista, Soares, Moreira, Feitosa, & Bezerra, 2011; Brigmon, Martin, & Aldrich, 1997; Gamri, Soric, Tomas, Molle, & Roche, 2014; Green, Katz, Tarchitzky, & Chen, 2018; Hao, Li, Wang, & Li, 2017; Katz, Dosoretz, Chen, & Tarchitzky, 2014; Katz, Wagner, Horn, Tarchitzky, & Chen, 2018; Li et al., 2012; Li, Song, Pei, & Feng, 2015; Li et al., 2013; Oliver, Hewa, & Pezzaniti, 2014; Qian et al., 2017; Raudales, Parke, Guy, & Fisher, 2014; Ribeiro et al., 2018; Tajrishy, Hills, & Tchobanoglous, 1994; Vale, Costa, Batista, Coelho, & Feitosa, 2018; D. Yan, Yang, Rowan, Ren, & Pitts, 2010; D. Z. Yan et al., 2009; B. Zhou et al., 2014; B. Zhou et al., 2015; B. Zhou et al., 2013; B. Zhou, Li, Song, Wang, et al., 2016; B. Zhou, Li, Song, Xu, & Bralts, 2016; B. Zhou, Wang, Li, & Bralts, 2017; H. X. Zhou, Li, Wang, Zhou, & Bhattarai, 2019)
Biofilm + “sub-irrigation”	0	0	Not applicable
Biofilm + “surface irrigation”	1	0	Not applicable (the article is related to wastewater treatment but not actually irrigation)
Biofilm + “manual irrigation”	1	0	Not applicable (the article is about middle ear irrigation)
Biofilm + “lateral move irrigation”	0	0	Not applicable
Biofilm + “center-pivot irrigation”	0	0	Not applicable

Biofilm + "sprinkler irrigation"	3	2	(Laury-Shaw, Gragg, Echeverry, & Brashears, 2019; Pachepsky et al., 2012)

Reference:

Ait-Mouheb, N., Schillings, J., Al-Muhammad, J., Bendoula, R., Tomas, S., Amielh, M., & Anselmet, F. (2019). Impact of hydrodynamics on clay particle deposition and biofilm development in a labyrinth-channel dripper. *Irrigation Science, 37*(1), 1-10. doi:10.1007/s00271-018-0595-7

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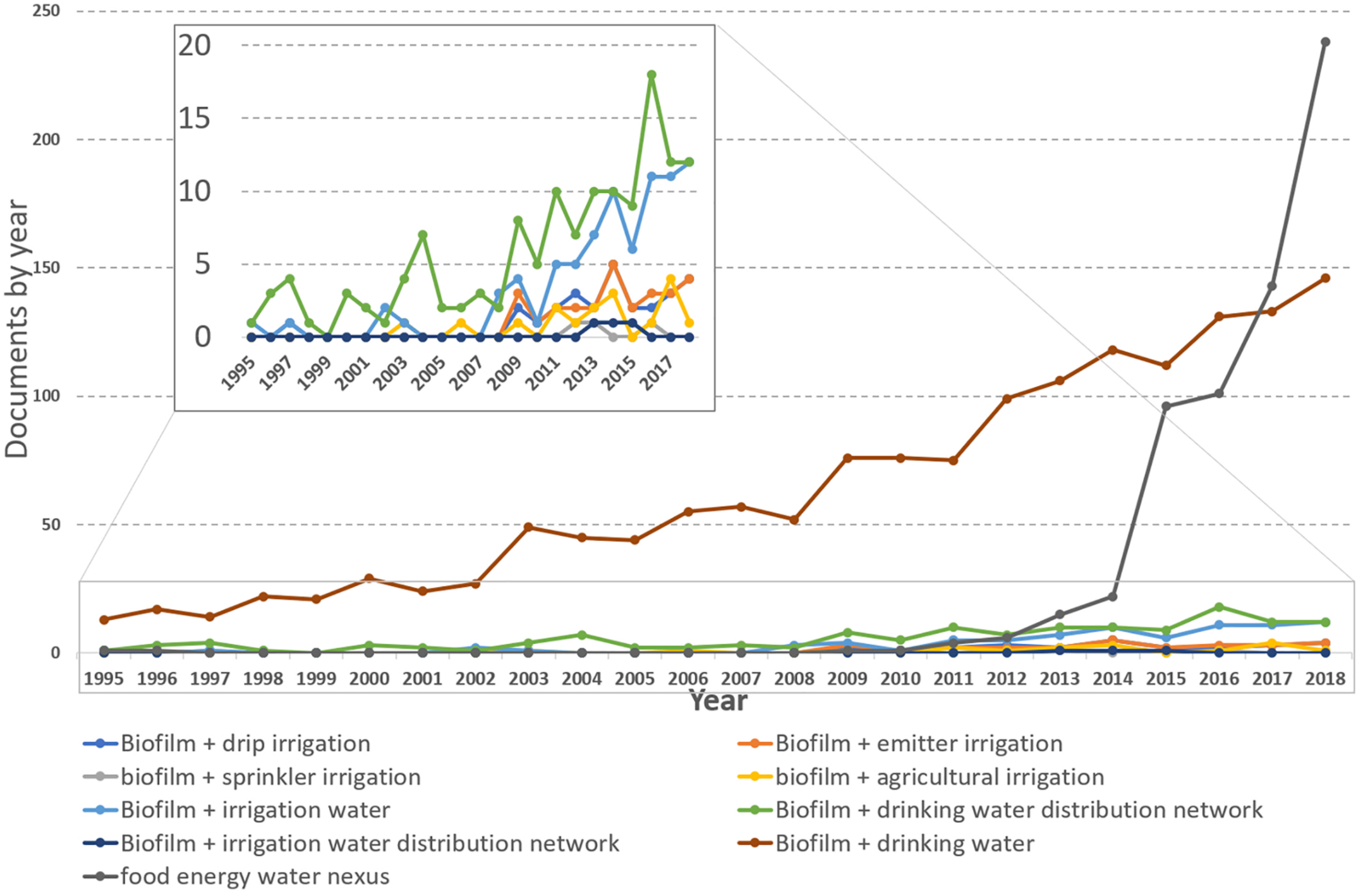
Table 2

Country & Reference	Aim	Methods/Technologies	Study findings
China (Yan et al., 2009)	Investigate the effect of six types of emitters with different path structures on the microbial community and emitter clogging	Quantifying phospholipid-derived fatty acids (PFLAs); Scanning electron microscopy (SEM)	(1) Total microbial biomass, especially bacterial biomass, is strongly correlated with emitter clogging. (2) Total EPS and total polysaccharide contents were correlated with discharge reduction. (3) The highest reductions in discharge and distribution uniformity were observed in two types of emitters with biofilms containing fungal and algal PLFAs.
Israel (Lapidot & Yaron, 2009)	Investigate the role of biofilm curli and cellulose in facilitating the internalization of *S. enteric* into internal plants	Use of wild type of *S.* enteric and mutants (Curli and cellulose sufficient; curli and cellulose deficient; curli deficient; cellulose deficient) for comparison; Plasmid transformation; Confocal laser scanning microscope	Irrigation water contaminated with wild type S. enteric as well as curli and cellulose sufficient group caused contamination of the edible part of parsley, indicating that curli and cellulose play an essential role in transferring *S. enterica* into the plant.
USA (Pachepsky et al., 2012)	Investigate the effect of pipe-based biofilms on the microbial quality of irrigation water	Assembled an irrigation system	(1) Water source was demonstrated to be an essential factor for microbial water quality. (2) A positive correlation between *E. coli* density and total heterotrophic bacterial count were observed, indicating that the biofilm-associated *E. coli* can affect the microbial quality of irrigation water
Spain (Sanchez et al., 2014)	Investigate the microbial communities in irrigation water (both treated wastewater and reclaimed water), pipe biofilm, and dripper biofilm in the recently developed greenhouse-enclosed drip irrigation system	Denaturing gradient gel electrophoresis and 16s rRNA sequencing	(1) Almost all obtained sequences had some similarity to thermophilic microorganisms and were mainly related to potentially spore-forming organisms, suggesting that microbial communities with the ability to proliferate at high temperatures were sourced from the microorganisms present in the incoming water.

(2) Biofilm samples showed a lower bacterial diversity than water samples, possibly due to the selection of certain bacterial populations capable of attaching to the surfaces of irrigation drippers and pipes.

Highlights

- Significant research gaps concerning IWDS biofilms have been identified.
- IWDS conditions affecting biofilm formation, pathogen spreading, and food safety are reviewed.
- Distinct various multidisciplinary aspects in performing IWDS biofilm research are discussed.
- Future perspectives and advanced technology that can be applied are provided.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Biofilm + drip irrigation
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biofilm + sprinkler irrigation
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Biofilm + irrigation water distribution network
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food energy water nexus

Figure 1

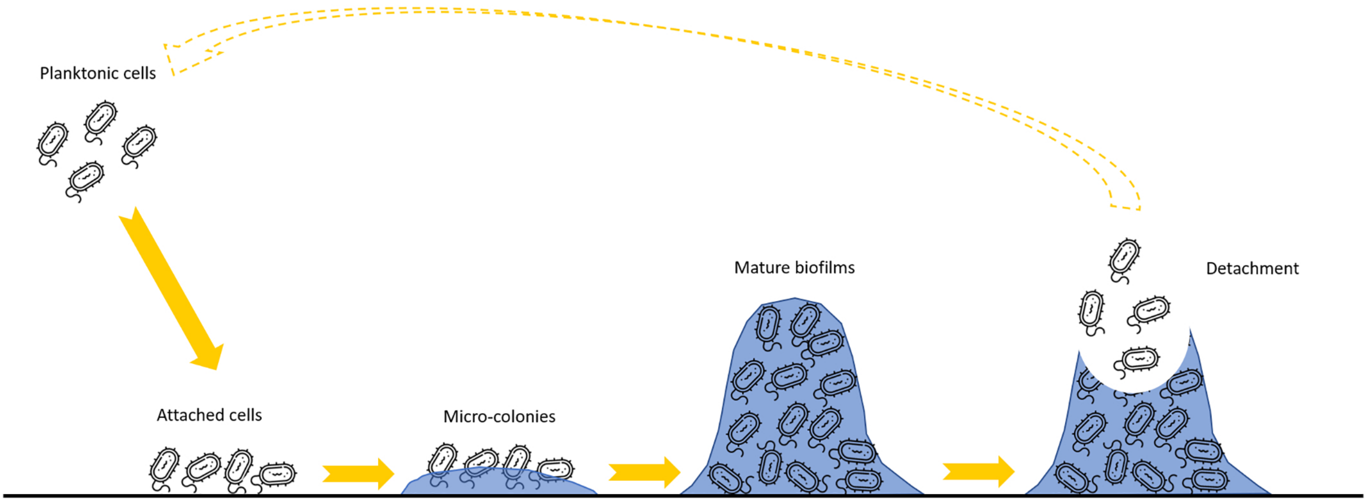
Planktonic cells
Attached cells
Micro-colonies
Mature biofilms
Detachment

Figure 2

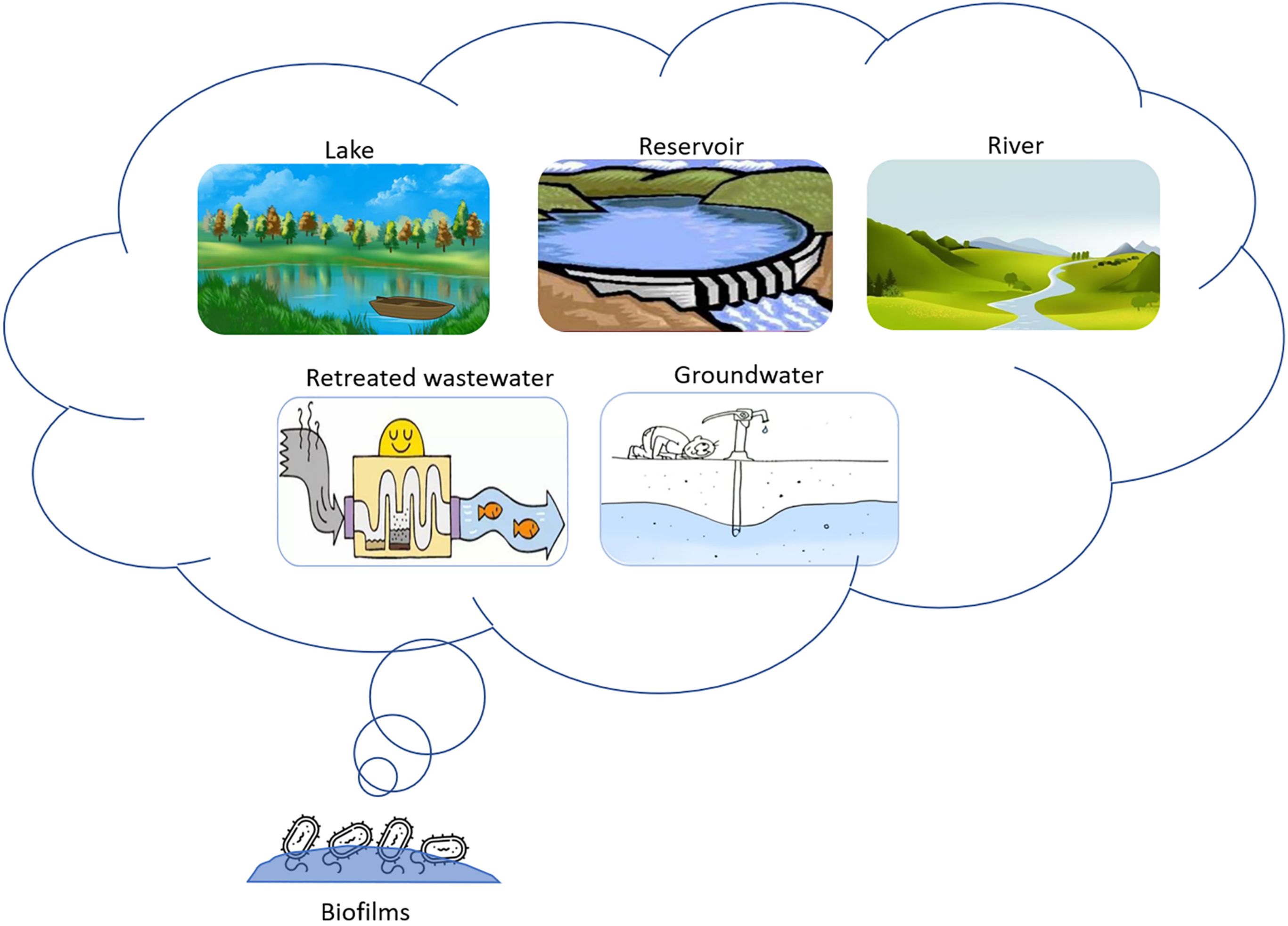
Lake
Reservoir
River
Retreated wastewater
Groundwater
Biofilms

Figure 3

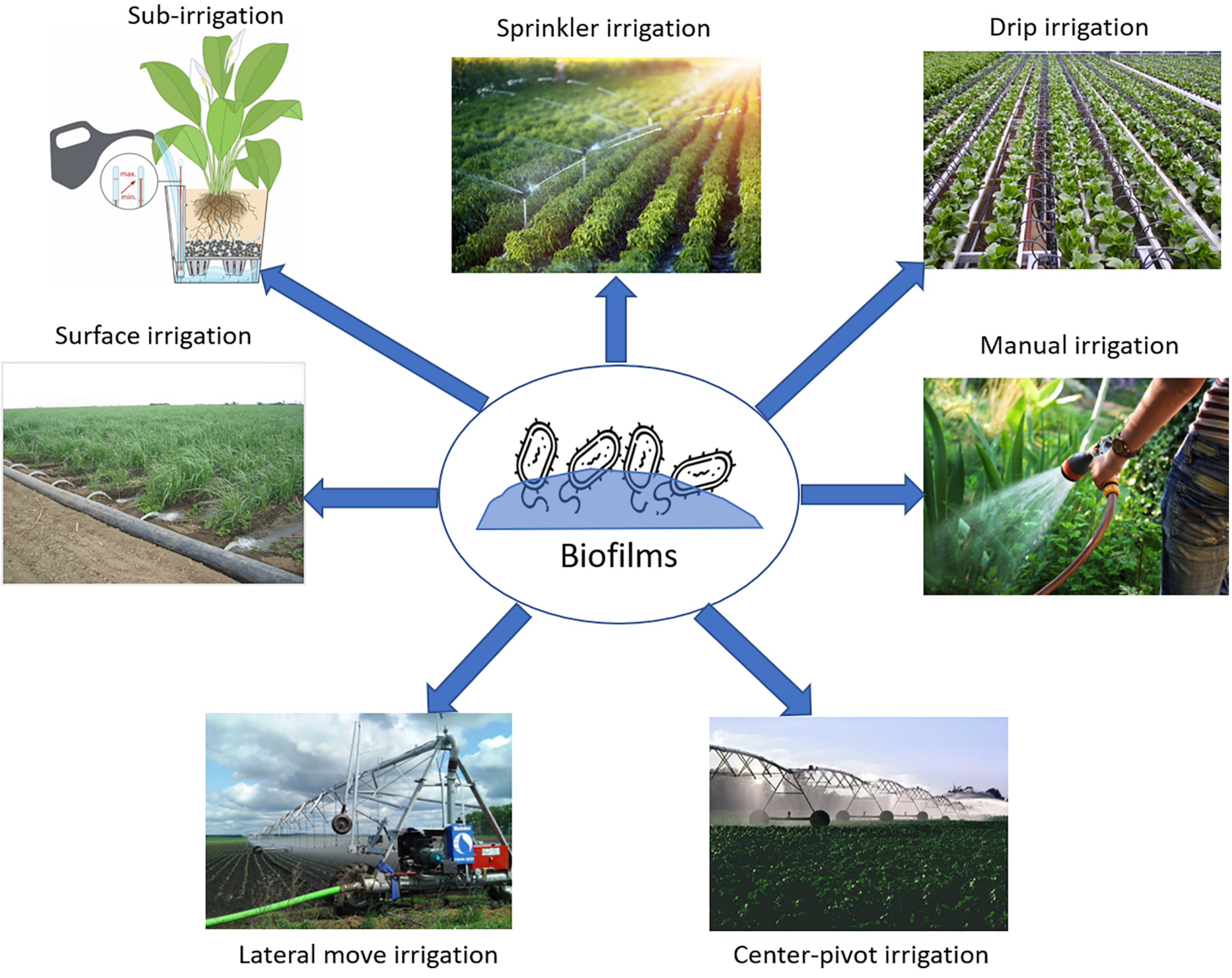
Sub-irrigation
max.
min.
Sprinkler irrigation
Drip irrigation
Surface irrigation
Manual irrigation
Biofilms
Lateral move irrigation
Center-pivot irrigation

Figure 4