Overview

The importance of sewer biofilms

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In spite of being under ground and out of sight, sewers are important parts of the urban infrastructure for transporting used, contaminated water for safe treatment. Within sewers, during the transport of waste water, processes take place, transforming the chemical components of the waste water. These processes are largely carried out by bacteria, a significant part of which live in biofilms. These microbial processes impact the sewers by causing odor and corrosion of the sewer pipes, leading to the need costly repair and control strategies. The biofilms may also impact the environment by contributing to greenhouse gasses in the atmosphere and pollution in natural aquatic environments. However, improved understanding of the function of biofilms and the novel techniques and approaches for manipulating biofilms may provide us with strategies for controlling these problems. Moreover, such advances may allow us to design in-sewer biofilms for beneficial purposes such as in-pipe treatment of waste water, potentially leading to decreased environmental impact. © 2016 The Authors. *WIREs Water* published by Wiley Periodicals, Inc.

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INTRODUCTION

Sewers are extremely important components of urban infrastructure that helps keep the urban environment safe from flooding and prevents the spread of water-borne diseases by safely transporting waste water to the waste water treatment works and through the transport of rain water from urban surfaces.[1] Naturally, the focus on sewer design and construction has been on the function of the sewer networks with respect to safe transport of water to fulfill this vital role within urban infrastructure. However, over the years, the understanding of sewer function has been expanded, so that expectations of the urban drainage systems is now also to help minimize the impact of urban activities on the natural environment[2,3] and to prevent odor from waste water in the urban environment.[4] In light of increasing expectations, it is necessary now, more than ever, to design robust sewer networks to fulfil the expected design life of 50–100 years.

Existing sewer networks are under increasing strain mainly due to population growth, increasing urbanization, and climate change. The increasing strain on the sewers manifests itself as operational failures leading to flooding events, increased loads on treatment facilities leading to poor quality of water discharge, and leaks linked to enhanced structural deterioration due to concrete corrosion. Early on in the history of the modern sewer networks, it was discovered that the degradation of the materials that the sewers are built from, i.e., mostly concrete, is a consequence of the environment within the sewers.[5] This has led to an increased focus on the processes taking place inside the sewers during the transport of the waste water.[6] It is now generally recognized that the chemical transformations of the waste water and the environmental conditions in the sewers are largely dictated by the microorganisms present in the sewer. The microorganism-facilitated loss of structural integrity of sewers is a major challenge facing existing urban drainage systems.[7] However, the complete eradication of all microorganisms from sewers is impossible; if anything, part of the purpose of sewers

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is the safe handling of water with bacteria. Moreover, in sewers, certain microbial processes, especially those that break down complex organic matter, are potentially desirable as they help improve the quality of the waste water reaching the treatment plants.[8] Therefore, it has emerged that the design of effective sewer systems should also take into account the positive and negative effects of microbial processes that occur in the sewer.[9]

In order to develop the understanding of the processes and process interactions taking place in the sewer, the sewer can be approached as a reactor where chemical and biological processes occur.[10] When using this approach, it is still important to consider the vast underground pipe networks and the waste water transport but at the same time, look at the individual pipes and the different 'phases' or ecosystems that can be identified here.[10] Figure 1 illustrates a cross section of a sewer pipe with these different ecosystems marked. When looking at the sewer in this way, in each pipe, there will of course be the flowing bulk of waste water but also a sediment phase, a sewer atmosphere, a submerged sewer biofilm, and a moist unsubmerged surface of the sewer wall. Microorganisms, usually in complex communities called 'biofilms' are present in most of these ecosystems within the sewer.[11–14] The importance of processes carried out by microorganisms have been recognized from very early on, and hence, investigations of the sewer biofilms have been a part of the early research field.[15,16] Additionally, the biofilms have been investigated as they are a potential source of pathogenic bacteria that can be released into the environment under particular conditions.[2] In light of their ubiquity and impact on sewers, this paper will focus on providing an overview of the importance of the biofilms in the sewer networks and potential new advances that can be made with a systematic approach to the understanding of biofilms and exploitation of the new possibilities for biofilm control that such an increased understanding brings, for example, through emerging techniques such as synthetic biology, synthetic ecology, and bioaugmentation.

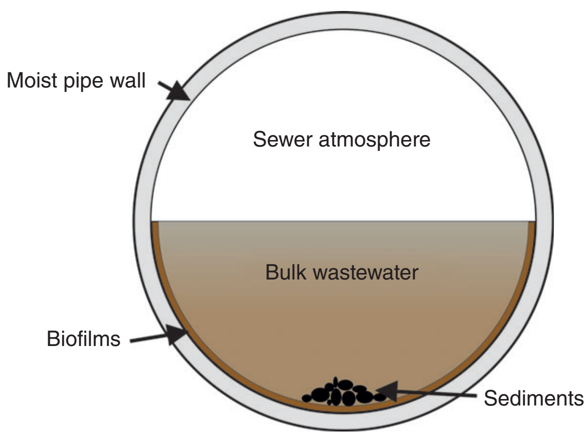

FIGURE 1 | Cross section of a sewer pipe highlighting the different environments for microbial growth, i.e., bulk of waste water, submerged biofilms, sediments, and moist, unsubmerged surfaces.

WHAT ARE BIOFILMS?

In the simplest of terms, biofilms can be described as a community of microorganisms living attached to a surface.[17] Biofilms have been described as a 'city of microorganisms' where the structural components are afforded by a self-produced matrix of sticky, complex polymers like proteins, sugars, and DNA. These complex polymers are called extracellular polymeric substances (EPS).[18] Observation of microorganisms has revealed that their predominant mode of survival in their natural habitat is within a biofilm and that they rarely are found as single dispersed units functioning independently. Within a biofilm, different types of microorganisms interact with each other and their environment and coordinate their activities, usually in a mutually beneficial way, to ensure their survival.[19] It has emerged that the behavior of microorganisms in a biofilm is markedly different from their dispersed counterparts, and they respond to environment onslaughts in a unique fashion.[19] This illustrates that when assessing microbial communities, not just in sewers, the understanding of biofilms is very important. Therefore, in order to be relevant, the study of microbial processes in sewers must focus on biofilms.

The formation of a biofilm is a developmental process with mature biofilms often displaying specialized structures such as microcolonies, aerial hyphae, and fruiting bodies and channels to allow the supply of water, oxygen, and nutrients to the microorganisms within a biofilm.[20,21] The thickness of a biofilm can range from a few micrometers to a few centimetres. Biofilms provide the organisms with a microenvironment where conditions are relatively stable compared to the bulk environment. Microorganisms within a biofilm display enhanced resistance to environmental stressors, including antimicrobial compounds and cleaning agents.[21] The persistence of biofilms can be attributed either to the unique behavior of the cells within a biofilm[22,23] or to the presence of the EPS matrix.[24] Although many laboratory-based studies in the current literature are focused toward studying biofilms of single species, in nature as well as in sewers, biofilms typically contain many different species displaying marked metabolic cooperativity[19] as well as intercellular communication via quorum-sensing molecules.[25] Apart from biological drivers, biofilm formation is also affected by physical and chemical drivers, such as roughness of the surface on which they form,[26] the composition of the waste water in the sewers, and the temperature of the sewer as well as the nature of the fluid flow across the surface.[27] Therefore, not all biofilms within a sewer are the same. The microbial composition of the biofilm dictates the consequences of biofilm formation in sewers. Admittedly, the formation of biofilms in sewers affords both challenges as well as opportunities for maintaining robust sewer networks. These challenges and opportunities are discussed in the following sections.

UNDERSTANDING AND SOLVING THE PROBLEM OF HYDROGEN SULPHIDE

In sewer networks, the initial driver for studying biological transformation in general, and biofilms in particular, has been the need to control the formation of hydrogen sulphide.[28] Hydrogen sulphide is a gas and is odorous, toxic, and causes degradation of concrete surfaces within the sewer.[29–31] These are all problems that cause genuine concerns and costs for sewer network operators. The risk to sewer workers due to hydrogen sulfide toxicity is part of the risk associated with human entry into the sewer; however, in spite of safety equipment and safety procedures, fatalities still occur due to this exposure.[30,32] Odor causes nuisances and hence gives cause for complaints that need to be addressed by the sewer operators.[33] Additionally, the degradation of the concrete pipes can cause collapse of the concrete pipes, which result in costly repair and service disruption.[34]

The understanding of the biofilms in relation to hydrogen sulphide is important as hydrogen sulphide is generally accepted to be formed by a special type of bacteria, called sulphate-reducing bacteria (SRB), living in biofilms formed in the portion of the sewer submerged under waste water.[28] Hence, the majority of the studies of biofilms in sewers have, in some way or another, been related to the problem of hydrogen sulphide. The biofilm is important in this context because it provides an environment where the residence time is higher than is the case for the waste water flowing in the sewer network. Residence time for waste water in the sewer network is generally less than 2 days.[35] The bacteria that produce hydrogen sulphide, however, are slow growing and can therefore not generally establish a large population in the flowing waste water. The biofilms, however, can provide a niche environment that provides just the right growth conditions for these bacteria, also providing a favorable environment in places where the conditions within the flowing waste water would not permit activity of these bacteria.[12]

The knowledge of the function of the hydrogen sulphide-producing bacteria has also been the key to many of the mitigation strategies used for limiting the production of hydrogen sulphide in sewers. The SRB are only active in the absence of oxygen and nitrate; hence, the addition of these compounds to the waste water is widely used for hydrogen sulphide control.[7,36,37] However, in spite of the ability of these compounds to inhibit the activity of the hydrogen sulphide-producing SRB, the protection provided by the biofilm environment have meant that even with the addition of oxygen, the activity of the bacteria continued within the biofilm. This is because the oxygen could not penetrate through as the transport is limited by diffusion through the biofilm and the simultaneous use of oxygen by other bacteria within the outer layers of the biofilm.[38] Hence, the main effect of the oxygen injection was found to be direct oxidation of the hydrogen sulphide in the waste water and outer layers of biofilms rather than removing the cause of the problem by removing the hydrogen sulphide-producing bacteria.[36] Similar results were found for the addition of nitrate to achieve a similar removal of hydrogen sulphide.[9] The protection of the bacteria within the biofilms also means that it is difficult to achieve a long-term change within the biofilm using these methods.[9]

CORROSION

It is not just within the submerged part of the sewer that biofilms cause problems. As mentioned previously, one of the main concerns regarding the hydrogen sulphide formation is the corrosion of concrete pipes, which takes place on the moist surfaces in the sewer above the water level[31] (Figure 1). It was found relatively early that the corrosion caused by hydrogen sulphide cannot take place without a biofilm on the corroding surfaces.[15] When installed initially, however, unsubmerged concrete sewer surfaces are a hostile place for biofilms to form as the pH of the surface is high (11–12).[39–41] As the surface pH is lowered due to chemical processes on the surfaces, it reaches a pH around 9 where it becomes possible for bacteria to colonize the surface.[34,39,42] These bacteria are utilizing hydrogen sulphide and oxygen for energy and producing sulphuric acid as the waste product of this reaction. The sulphuric acid reacts with the alkaline components of the concrete, thus accelerating the degradation of the concrete. Due to the generation of sulphuric acid, the pH on the surfaces will continue to drop, requiring a more and more specialized microbial community on the surfaces to withstand the hostile environment generated by the acid.[39] It has long been assumed that this community would be very limited in species richness and that one particular type of bacteria was particularly indicative of the corrosion.[43] However, recent investigations of the biofilms on these surfaces using new microbial sequencing techniques have revealed that the communities in these biofilms are complex, and the role in the corrosion process of most of the bacteria found here are yet to be determined.[13,44–46]

CONTRIBUTION TO ENVIRONMENTAL POLLUTION

One of the ways that sewer biofilms have also been considered is as a direct source of pollution for the aquatic environment. Many sewer networks across the world are so-called combined sewers, where rain water and sewage from houses and industry are transported in the same pipe network.[1] In such systems, structures known as combined sewer overflows (CSOs) are installed. These structures allow the sewer to spill into the natural environment during rain events that exceed the capacity of the sewers. Allowing the sewers to spill in a controlled way prevents uncontrolled flooding of the urban environment.[1] The intermittent flow of rain water in the sewers dramatically increases the flow rate, velocity and, hence, the shear stresses on the sewer biofilms, leading to detachment of the biofilms. The spillage from the CSOs will thus transfer pollutants from the sewer into the natural environment.[47] In addition to the direct pollution with organic matter and bacteria from the biofilms, the biofilms may also act as a sink for micropollutants in the waste water, leading to the discharge of higher concentrations of micropollutants during biofilm detachment.[48] The impact of this is hard to quantify experimentally. Rocher et al. concluded that the biofilms detached from the sewer walls were not directly a significant source of pollutants, whereas a layer of organic matter on the sewer sediments was the source of the majority of the pollutants during wet weather discharges from CSOs.[2] It would be possible to argue that parts of the organic layer on sewer sediments or sewer deposits do constitute a biofilm, making parts of the pollution from the discharge of sediments related to biofilms[49], and the microbial activity has even been related to the sediment stability. Hence, biofilms may be important in controlling how much sediment is discharged from sewers, making this an important type of biofilm to gain further understanding of.[50]

CONTRIBUTION TO CLIMATE CHANGE

A more recent concern than the corrosion and other hydrogen sulphide-related problems is the contribution from sewers to climate change. Greenhouse gases such as nitrous oxide (laughing gas) and methane have been known to form in sewers.[51–54] Methane has a direct global warming potential 25× that of carbon dioxide, and for nitrous oxide, this is 298× that of carbon dioxide.[55] These gases are generally produced as a waste product of microbial activity within the biofilms in the sewers.[56,57] However, differing conclusions have been arrived at regarding the significance of greenhouse gases produced in the sewers. It is likely that the variations in these studies are a consequence of the differences in the microbial composition within biofilms in different regions of the world as a result of differences in the waste water composition and sewer conditions. A study from Germany has suggested that the contribution of nitrous oxide from sewers was insignificant,[53] whereas a newer evaluation of the evidence suggests that the contributions of nitrous acid from sewers are significant.[58] A study from Australia suggested that a significant contribution of methane can also be expected from sewers,[56] produced both in sewer biofilms on the submerged pipe walls and in biofilms associated with the sewer sediment.[51] Methane-producing bacteria have also been identified in communities from other sewer systems; however, how much methane production this relates to is not certain.[9,46] More data from multiple sewer networks across the world are, however, needed to truly assess the contribution of processes in sewer biofilms to climate change.

An interesting angle on the significance of nitrous oxide in sewers is that it has been suggested as a warning system that can be used to detect potential biocides that have been discharged into the sewer network, which may cause problems in the waste water treatment plant. Such potentially harmful compounds are suggested to cause a rise in the nitrous oxide concentration in the sewer atmosphere as the sewer biofilm is influenced by the compounds.[57] This is an interesting approach for using naturally occurring biofilm and biofilm processes as sensors or indicators.

FUTURE PROSPECTS

More systematic investigations of the sewer biofilms with respect to identification of microorganisms are starting to emerge, providing an unprecedented knowledge of the composition of the microbial communities in the sewer networks.[13,44,59–61] One of the next challenges, however, is to link this information about species to biofilm function, in terms of contribution to processes, in a comprehensive and effective way. Doing so will further enable the inclusion of microbial processes and waste water transformations in considerations for the design of robust and sustainable sewer networks. However, more information is also needed regarding the changes over time within the sewer biofilms as these are dynamic systems.[44] As previously discussed, the microbial generation of hydrogen sulfide is being incorporated into the design of new sewer systems.[61]

The knowledge of microbial processes also opens up the exciting possibility of targeted remedial measures for some of the challenges facing the existing sewer networks. For instance, the presence of certain microorganisms, such as SRBs, within sewer biofilms is the cause of hydrogen sulfide generation and subsequent concrete corrosion in sewers. Preventing the activity of SRBs or selectively targeting the SRBs for removal will break the concrete corrosion cycle. Although conventional technologies for remediation do not afford this level of precision, emerging technologies such as synthetic biology, synthetic ecology, and bioaugmentation may provide innovative solutions to current challenges. The solutions afforded by these emerging technologies for microbial challenges in the sewers are already being demonstrated in the academic literature. De Gusseme et al. have demonstrated that the introduction of a nitrate-reducing, sulfide-oxidizing bacterial consortium actively converts the hydrogen sulfide produced in sewage to elemental sulfur and polysulfide ions.[62] Production of elemental sulfur is desirable in sewers because it enables the increased dissolution of hydrogen sulfide in waste water, which prevents hydrogen sulfide from entering the sewer atmosphere and subsequently causing concrete corrosion. Incorporation of these bacteria within sewer biofilms, deliberately altering the existing communities, is an interesting approach toward the prevention of concrete corrosion in sewers. This approach could be more cost-effective and efficient compared to continual dosing of sewage with nitrates.[7] A similar bioaugmentation approach may be possible for the reduction in greenhouse gas emissions, for example, by enhancing colonization of methane-oxidizing bacteria on the moist sewer surfaces (Figure 1) to degrade methane before it is released from the sewer pipes. Such an approach would require an in-depth understanding of the biofilm-forming properties of the methane-oxidizing bacteria; however, this kind of work is already ongoing for many different bacteria.[63]

Moreover, the investigation of biofilm-based solutions for sewer management has yielded further innovative avenues. Soleimani et al. have investigated the feasibility of coating sewer surfaces with a biofilm of fast-growing bacteria that are capable of surviving in the oxygen-deficient environment in the sewers.[64,65] They hypothesize that the presence of such a biofilm layer will preclude the establishment of SRBs as they will be out-competed for nutrients by the fast-growing bacteria. This approach could also prevent methane formation and reduce the potential contribution to climate change. With the smaller microbial diversity in the biofilms on the moist sewer surfaces,[45] this might be an easier environment in which to achieve an augmentation of the biofilm than in the submerged biofilms.

The new understanding of biofilm communities with sewers is likely the next step needed toward utilizing the potential for in-sewer treatment of waste water and, hence, ascribing a positive effect to the in-sewer biofilms rather than just focusing on the problems associated with biofilm processes. Treatment of waste water involves the removal of organic carbon, nitrogen, phosphorus, and pathogens from the waste water before discharge into water bodies. When looking at the capacity to remove organic matter from the waste water, the processes within the sewers, including the biofilm processes, can be said to perform as a treatment.[66–68] Moreover, studies have demonstrated that the biofilms exhibit far superior organic removal rates compared to suspended bacterial cells.[69] Incorporation of in situ treatment of waste water in sewer biofilms could be desirable in the right circumstances where it reduces the processing burden in the waste water treatment plants and improves the quality of water being discharged into the water bodies. In Hong Kong, opportunities for in-pipe denitrification are being explored, utilizing the particular local possibilities.[70] As this technology relies on urine-separating toilets it is not easily implemented everywhere; however, this kind of tailored in-pipe treatment opens interesting future possibilities.

With all these new opportunities, it is important to remember that sewers are not completely closed systems. Many sewer networks across the world are combined systems, where rain water and waste water are transported in the same pipes during rain events.[1] This means that during very heavy rain, these systems will become overloaded, and a mixture of waste water and rainwater will be released into the environment. All solutions applied to such sewers therefore need to be assessed against any potential adverse effects they may have within the natural environment.[2]

CONCLUSION

In conclusion, biofilms in sewers are important in a range of ways, from causing dangerous conditions for sewer workers to potentially offering opportunities for some level of in-sewer treatment of waste water, providing a broad-ranging impact, touching a lot of different fields of research and work in general. Biofilms offer complex niche environments for bacteria, making the detailed understanding of these systems challenging, but recent advances in the field show a lot of promise in future opportunities for prediction, control, and use of biofilms.

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