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MINIREVIEWS

Bacterial Signaling Ecology and Potential Applications During Aquatic Biofilm Construction

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Abstract In their natural environment, bacteria and other microorganisms typically grow as surface-adherent biofilm communities. Cell signal processes, including quorum signaling, are now recognized as being intimately involved in the development and function of biofilms. In contrast to their planktonic (unattached) counterparts, bacteria within biofilms are notoriously resistant to many traditional antimicrobial agents and so represent a major challenge in industry and medicine. Although biofilms impact many human activities, they actually represent an ancient mode of bacterial growth as shown in the fossil record. Consequently, many aquatic organisms have evolved strategies involving signal manipulation to control or co-exist with biofilms. Here, we review the chemical ecology of biofilms and propose mechanisms whereby signal manipulation can be used to promote or control biofilms.

Biofilm Introduction

Surface-adherent microbial communities have been described by a number of early microbiologists including van Leeuwenhoek [1] and Zobell [2]. During the 1970s and 1980s, several investigators including Busscher [3], Caldwell [4], Costerton [5], Fletcher [6], Høiby [7], Marshall [8], White [9], and their colleagues conducted a number of pivotal studies on bacterial adhesion to surfaces and the formation of adherent bacterial communities. During this period, the term biofilm came into prominent use [10]. While a detailed description of these earlier accomplishments is beyond the scope of this review, we shall address several key concepts that provided the groundwork for our present understanding of biofilms and their now recognized association with quorum sensing (QS).

Early studies of biofilms were driven largely by microscopy and related culture-based approaches. Unlike traditional pure culture techniques, individual bacteria were not separated from their natural substrata or from other organisms. Rather, they were examined as closely as possible to their natural situation. Light microscopy and electron microscopy, with its higher resolution, were adapted to these studies. One notable issue found during this time was that biofilms are ubiquitous in most environments that were examined [11]. This included aquatic and marine environments [8, 12], soils [13], digestive processes in higher animals [14], industry (biofouling and trickling filter applications in wastewater treatment) [15, 16], and infectious disease [17, 18]. Based on their appearance with transmission electron microscopy, biofilms appeared to have complex structures with microcolonies of similar organisms interspersed with an organic matrix (formerly referred to as a glycocalyx) [19]. Detailed examination of bacterial colonies on agar plates [20] showed complex microbial community development and cellular organization, which provides an interesting correlation of biofilms in natural settings to conventional lab culturing techniques. Indeed, colony biofilms are now a commonly used approach during genetic studies of biofilms [21].

The advent of confocal microscopy revealed biofilms to have an even more elaborate structure than was seen with electron microscopy [22]. Although lacking the resolution of electron microscopy, confocal microscopy allowed the observation of fully hydrated structures and biofilm architecture (Fig. 1). Instead of a homogeneous distribution of cells as suggested by electron microscopy, confocal microscopy showed bacteria to be frequently clumped together into

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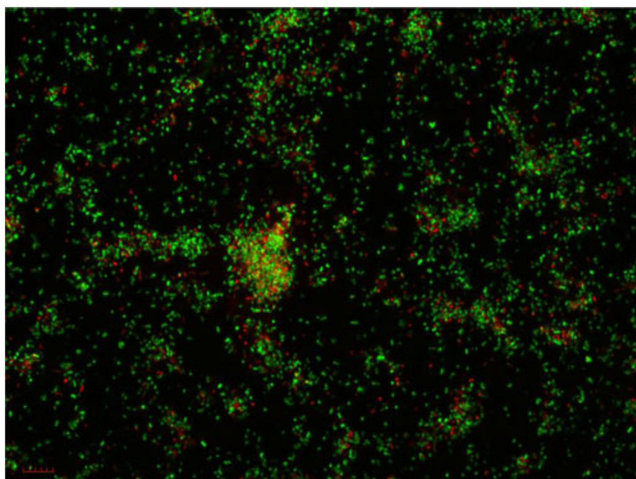

Fig. 1 Confocal microscopy image of a mixed culture biofilm consisting of *P. aeruginosa* and *E. coli*. Individual cells aggregate into clusters (microcolonies) that are surrounded by low cell density regions (water channels). *Bar* represents 10 μm

microcolonies that were surrounded by regions of low cell density referred to as water channels [22].

General Biofilm Characteristics

The prominence of biofilms is easily explained in flowing systems such as rivers [23]. Adhesion enables individual organisms to persist in spite of shear forces. As well in oligotrophic environments such as alpine streams, nutrients adsorb onto surfaces and microorganisms would therefore be attracted to those sources of nutrition [24]. Metabolic and genetic interactions are facilitated when organisms grow in close proximity within biofilms. Wolfaardt et al. [25] investigated the ability of soil bacteria to grow on a commercial herbicide, diclophop methyl (the methyl ester of 2-[4-(2,4-dichlorophenoxy)phenoxy] methyl propanoic acid), and found that some bacteria could survive on this compound as a sole carbon source only if present as a biofilm consortium. Pure cultures of the soil isolates were unable to grow on this herbicide regardless of whether they were grown as planktonic or biofilm cultures. Similarly, mixed planktonic cultures were unable to grow on diclophop methyl [25]. Syntrophic metabolism within microbial aggregates has also been reported in interspecies hydrogen transfer during anaerobic digestion of cellulose [26, 27]. Biofilm growth has also been shown to promote genetic exchange through transformation [28] and conjugation [29, 30] due to the close proximity of the donor and recipient organisms.

During the 1980s, robust biofilms were widely described in a number of industrial and medically important environments wherein unattached (planktonic) bacteria would be exposed to potentially lethal concentrations of antimicrobial chemicals and, in the case of medical environments, the host immune system [10]. In 1985, Nickel and co-workers [5] investigated the relative susceptibility of biofilm and planktonic populations of *Pseudomonas aeruginosa* to tobramycin. Under their experimental conditions, the minimal inhibitory concentration of tobramycin against planktonic *P. aeruginosa* populations was 1 μg/ml, whereas biofilm populations persisted at 1,000-fold greater concentrations (1 mg/ml). Other investigators have found similar differences in susceptibility of planktonic and biofilm populations to various antimicrobial agents, with the specific values depending upon culture conditions and the antimicrobial agent used [18, 31]. In aquatic and terrestrial environments, biofilm growth has also been shown to protect against predation from protozoa, snails and other invertebrates [32, 33], and viruses [34]. Biofilm growth also allows microbial persistence against other environmental stresses such as pH and oxidative stress [35] and facilitates genetic exchange [30] and enhanced mutation [36] to respond to evolutionary pressures.

In the 1990s, a number of theories were developed by Gilbert, Stewart, Costerton, and others to explain the resistance of biofilms against various stresses [37, 38]. These included penetration difficulties of antimicrobials through biofilm matrices, slow growth of biofilm organisms due to nutrient limitations, and differential gene expression and physiology of bacteria due to surface adhesion or biofilm growth. As a tribute to these predictions, subsequent studies have shown that slow growth [39, 40] and biofilm specific gene expression [41] are important mechanisms in antimicrobial resistance. Other contributing factors to biofilm drug resistance include the formation of slow growing subpopulations of persister cells [42] and metabolic interactions within mixed population biofilms [43]. Aside from the aforementioned issues of slow growth and biofilm-specific gene expression, the high population density within biofilms was predicted to be very important [37, 44].

Cell Signaling and Its Application to Biofilm Development

As reviewed in [45], time course observations of biofilm formation, coupled with genetic studies, showed that biofilm formation is a coordinated, developmental process wherein planktonic bacteria attach to a surface and then migrate together into clusters (microcolonies) using twitching motility [46] in which an organism's type IV pili attach to a surface and then retract in an analogous manner to a grappling hook, thereby pulling the organism along the substratum [47]. Matrix formation occurs during microcolony formation as well as during the maturation process. The final stage in biofilm life is a detachment process whereby some cells would detach from the sessile community and reenter the planktonic phase [48]. The highly coordinated growth of bacterial populations during biofilm development [45] is indicative of signal processes.

Since they were first observed [1], bacteria have been considered to be single celled life forms. In this context, the description of luciferase activity in *Vibrio fischeri* as a function of population density [49] was highly significant. Since a critical threshold of bacterial population is necessary for this group behavior [50], the term quorum sensing is now applied [51]. There are a number of small metabolites that are associated with QS. The first class of quorum signals described were *N*-acylated homoserine lactones (AHLs), which are commonly associated with signaling in a number of gram negative bacteria [51]. Here, the AHL signal, originally called an autoinducer, is synthesized by an AHL synthase (LuxI homolog) and, at concentrations above a threshold level, affects a transcriptional activator (LuxR homolog) [51]. While the threshold concentration for quorum signaling often exceeds 10^8 CFU/ml in planktonic cultures, Connell et al. [50] showed that under diffusion restricted lab conditions, which likely mimic biofilm conditions, as few as 10^2–10^3 cells could trigger a quorum response. There are now a number of additional signals identified including AI-2 (autoinducer 2) [52], quinolones [53], small peptides in gram positive organisms [54], and a genus specific signal in *Vibrio* sp. [55]. New signal molecules such as 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS) are being described in the literature [56]. Although initially considered to control individual characteristics such as light production in *V. fischeri* and elastase production in *P. aeruginosa*, QS is now recognized as a global regulatory mechanism in most bacteria [57]. A full description of QS is beyond the scope of this mini-review. Readers are referred to some excellent articles for further information [55, 58, 59].

The first experimental evidence of quorum signals in naturally occurring biofilms was demonstrated in freshwater stream biofilms [60] (Fig. 2) using an *Agrobacterium tumefaciens* AHL bioassay [61]. This same bioassay was used to document AHL production in clinical biofilm infections of urinary catheters [62]. AHL production was also confirmed in *P. aeruginosa* pulmonary infections associated with cystic fibrosis [63]. Using a biofilm flow cell and confocal microscopy, Davies et al. [64] showed that *P. aeruginosa lasI* (one of two *luxI* homolog genes in P. aeruginosa) mutants lost the characteristic microcolony and water channel structures seen in wt organisms, but that this structure could be restored by exogenous addition of 3-oxo-dodecanoyl-homoserine lactone (3-oxo-C12 HSL, the AHL that is synthesized by LasI). Although AHLs were later shown to be not necessary for biofilm structure formation under some nutritional conditions [65], these findings did suggest a notable role of cell signaling and QS during biofilm development. At least two research groups showed that QS was a major contributing factor to the antibiotic and immune resistance of *P. aeruginosa* biofilms [66, 67]. Extracellular polysaccharide production and water channel formation, two key morphological features of biofilms have also been linked to quorum regulation [68, 69]. Cell signaling has also been linked to biofilm dispersion, wherein bacteria disperse from biofilms and return to a planktonic lifestyle [45]. There have been a number of recent reports linking small fatty acids [70], D-amino acids [71], and polyamines [72] as signals for detachment. In this context, the dispersion signal-induced return to a planktonic lifestyle would reduce or potentially eliminate the biofilm-mediated antibiotic resistance and allow many antibiotics to be used. Signal manipulation offers an attractive strategy for controlling biofilms as many traditional antibiotics and disinfectants are ineffective [10].

Fig. 2 Bioassay using *A. tumefaciens* A136 bioassay [61] showing AHL production from biofilms associated with duckweed (*Lemna minor*). The same bioassay was used to show AHL production in aquatic biofilms on rocks [60]. Interestingly, bacterial association with *L. minor* was first reported by van Leeuwenhoek [1]

Cell Signaling and Biofilm Manipulation

Although biofilms are now recognized as a major factor in medicine and industry [10], they are an ancient mode of bacterial growth as recognized in the fossil record [73, 74]. As a result, many organisms have evolved strategies to control or in many cases coexist with biofilms. A key finding was the discovery of biofilm inhibition by quorum-inhibiting furanone compounds produced by the Australian macroalga, *Delissea pulchra* by Givskov, Steinberg, Kjelleberg, and their colleagues [75, 76]. Although *D. pulchra* lives in marine regions prone to biofouling, young thalli on this macroalga do not get colonized by bacteria [75]. The brominated furanone compounds produced by *D. pulchra* are not lethal to bacteria at naturally occurring concentrations, but block AHL-mediated gene activation by interfering with LuxR-DNA binding [77]. Early field tests showed that materials soaked in the natural furanone from *D. pulchra* were quite resistant to biofouling [76]. Quorum inhibitors including synthetic furanones [78] and garlic extract [79] have also proven effective in clearing biofilm-mediated *P. aeruginosa* pulmonary infections in animal models. In *P. aeruginosa*, several other key cellular functions are regulated by QS, notably virulence [80] as well as biofilm-mediated resistance to antibiotics and the innate

immune response [66]. QS regulation of virulence has also been described in a wide variety of bacteria (reviewed in [55, 59]). Traditional biofilm control strategies including the use of biocides, strong disinfectants, and conventional antibiotic therapy readily target planktonic bacteria but have a relatively poor track record in biofilm control [10]. In stark contrast, biofilm control strategies involving quorum signal disruption have considerable promise even in natural settings [78, 81] and often mimic successful biofilm controlling strategies of higher organisms.

Identification of QS Molecules and Quorum Sensing Inhibitors

The discovery of furanones [75] has sparked considerable interest in the biofilm community, particularly in the context of identifying additional quorum disrupting compounds. Of great utility to these investigations is the association of QS with pigmentation in some organisms, including *Chromobacterium violaceum* [82] and *Serratia marcescens* [83]. Both organisms grow readily on standard lab media such as Luria Bertani agar (LB agar). Pigmentation loss can be used to identify quorum inhibition (Fig. 3) due to signal disrupting chemicals, signal degrading (quorum quenching) enzymes [84], or competition with other AHLs [85]. In *C. violaceum*, the cognate AHL is decanoyl homoserine lactone (C10-HSL) [86] and the presence of other AHLs can also lead to a loss of pigmentation through competitive inhibition of C10-HSL binding to CviR (LuxR homolog) [85]. The *C. violaceum* bioassay can be supplemented with another AHL bioassay, such as *A. tumefaciens* A136 [85] in order to determine whether pigmentation inhibition in *C. violaceum* arises from competition with other AHLs or from an actual quorum inhibitor. *A. tumefaciens* A136, which contains a plasmid with a *traR::lacZ* fusion, recognizes a wide variety of AHLs

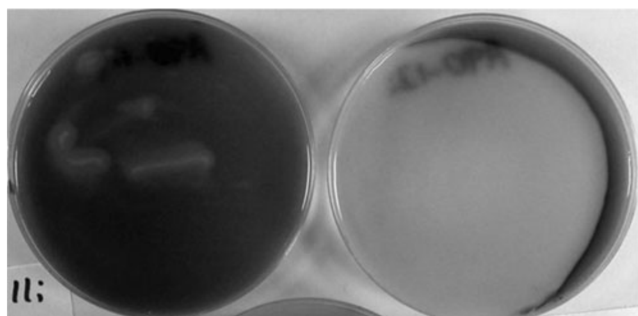

Fig. 3 Quorum signal inhibition is a very promising strategy for controlling biofilms [81]. As violacein pigmentation is quorum-regulated in *C. violaceum* [82], a pigmentation inhibition assay [82, 85], using this organism, can be used to screen other organisms or chemicals for potential quorum inhibiting activity. Using a *C. violaceum* overlay assay [85] quorum inhibition is evident in one aquatic bacterial isolate (*right*) but absent in another (*left*)

[87] and can be used as a second screen for competing AHLs [85].

Rasmussen et al. [88] developed an intriguing alternative strategy for identifying potential quorum sensing inhibitor (QSIs), using a positive selection approach. In this study, they engineered three different bacterial strains, referred to as QSI selectors (QSIS), so that they contained plasmid-borne genes conferring toxicity or susceptibility to kanamycin which were regulated by AHLs. Two of the strains, QSIS-1 and QSIS-3, were constructed in *Escherichia coli*, which is naturally unable to produce AHLs (lacks *luxI* homolog), whereas QSIS-2 was constructed in a *P. aeruginosa lasI rhlI* strain. QSIS-1 contained a toxic gene, *phlA*, originally from *Serratia liquefaciens* under the control of *luxR*, which responds to a variety of AHLs [89]. This organism was unable to grow in the presence of AHLs unless a QSI such as a furanone was present. In strain QSIS-3, kanamycin resistance mediated by *npt* was placed under regulation of the phage lambda cI repressor gene, which in turn was placed under LuxR regulation. Strain QSIS-3 would be unable to grow in the presence of AHLs and kanamycin unless QSI molecules are present. Strain QSIS-2, constructed in *P. aeruginosa*, contained a *sacB* gene under the control of the *lasB* promoter. Unlike the LuxR promoter, the *las* promoter responds exclusively to 3-oxo-C12-HSL. QSIS-2 is therefore unable to grow in the presence of 3-oxo-C12 HSL and sucrose unless QSI molecules are present. In their study, the authors found the greatest success using strains QSIS-1 and QSIS-2, although elevated sugar concentrations (particularly glucose) that are present in some fruit extracts, caused false-positive readings due to interference with the *sacB*-mediated sucrase killing in QSIS-2 [88]. The QSIS strategy has been used in a number of investigations, and candidate QSI molecules have been identified from a variety of plants including garlic extract and 4-nitro-pyridine-*N*-oxide from the original study [88] and an isothiocyanate compound from horseradish [90].

While QS and QSI reporter strains have been useful in qualitative assays including screens for quorum activity, quorum inhibition, and localization studies in microscopy (e.g., [50]), they have also been successfully adapted to quantitative assays. Shaw et al. [91] adapted an *A. tumefaciens traR::lacZ* reporter strain to detect AHL molecules in thin layer chromatography. In this case, the AHLs are separated using reverse phase chromatography. The reporter strain is mixed with agar containing X-gal (if a *lacZ* reporter is used) and then used to cover the plate. From our experience, the mixing of the organisms into the agar and the overlay must be completed quickly as many reporter strains including *A. tumefaciens* are heat sensitive. Following overnight incubation at an appropriate temperature for the reporter strain, the AHL molecules appear as colored spots on the plate (Fig. 4). Rasmussen et al. [88] used a similar strategy to detect QSI molecules in thin layer chromatography, in which QSIS strains containing lacZ

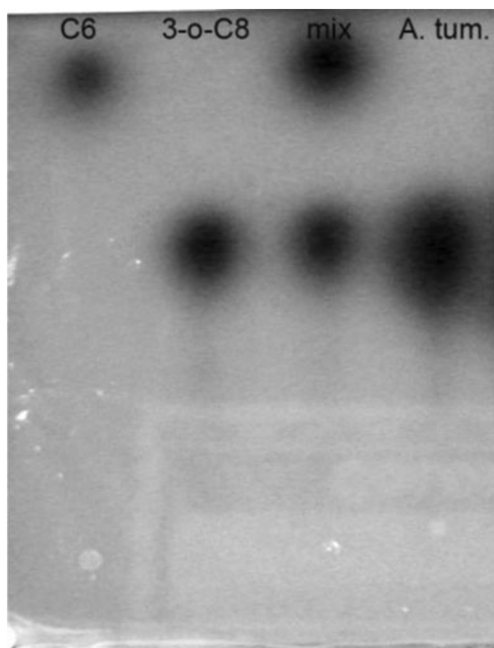

Fig. 4 AHL reporter strains such as *A. tumefaciens* A136 [61] can be used to detect AHLs at low concentrations during thin layer chromatography [91]. Shown in this figure are C6-HSL (C6) at 5 μmol, 3-o-C8 HSL (3-o-C8) at 5 pmol, a mixture of C6 and 3-o-C8 HSLs (mix), and an ethyl acetate extract of 3-o-HSL from an overnight culture of *A. tumefaciens* KYC6 [129]. The C6- and 3-o-C8-HSLs were obtained from a commercial source (Sigma/Aldrich)

reporter genes were incorporated into the agar overlay. Moré et al. [92] used an *A. tumefaciens* reporter strain during QS detection by HPLC. In this study, AHLs were separated by reversed phase HPLC and then the output was collected in a fraction collector and AHLs detected by incubating with a suitable reporter strain. A number of quorum molecules, notably many AHLs, are commercially available, and so detection limits and reporter strain responses can be correlated with known AHL concentrations. In our experience, the TLC protocol using *A. tumefaciens* in LB agar with X-gal could detect 3-oxo-octanoyl homoserine lactone (3-o-C8 HSL, cognate AHL of *A. tumefaciens*) [87] to the low picomolar range and some other AHLs to the micromolar or even nanomolar range. Other investigators using the same reporter with defined media have detected this 3-o-C8 HSL to sub-picomolar concentration [91]. Conceivably, this strategy of using reporter strains could be adapted to other non-AHL QS and QSI systems.

While there are a wide variety of bioassays capable of identifying quorum signals and quorum inhibition (reviewed in [93]), the pigmentation-based assays such as the *C. violaceum* assay [82] require no additional antibiotics or specialized detection equipment (such as bioluminescenc or fluorescence detectors for *lux*- and *gfp*-based reporters). As such, they are particularly useful for investigating quorum inhibition in regions of high biodiversity such as the tropics.

Bioassays have been used to identify a number of candidate quorum inhibiting materials in natural environments. Examples include a variety of plants [94], food extracts [79, 95], bacteria [96, 97], fungi [98], and some antibiotics [99]. Quorum inhibition has also been shown to play a role in bacterial competition [100]. Synthetic analogs of quorum signals have also shown promise in quorum inhibition [101], and there is considerable effort to refine rapid screening technology to identify other inhibitors.

Potential Quorum Signaling Applications for Biofilm Manipulation

Overall several strategies are used for quorum signal interruption (examples in parentheses refer to AHL-based quorum signaling):

1. Block production of quorum signals (LuxI target) [102, 103].
2. Enzymatic inactivation or degradation of quorum signals. This approach is often called quorum quenching [104, 105].
3. Use autoinducer analogues to block receptors (LuxR target) [101, 103].
4. Disrupt or inactivate the autoinducer receptor (LuxR target) [102].
5. Inhibit downstream effects of QS [77, 106, 107].

In some of these studies, the mechanism of inhibition has been well described [79, 96, 97]. There have been promising investigations of some quorum inhibitors against industrial biofouling and biofilm infections under natural conditions [78, 79]. Given the association of bacterial nutritional status with QS and biofilm formation [65], it would be prudent to evaluate quorum inhibiting compounds under a range of chemical and physical conditions. As well in animal testing and clinical testing, potential host toxicity and bacterial quorum inhibitor resistance would also need to be addressed.

Aside from the prevention of biofilm formation through quorum inhibitors, there are also considerable potential medical and industrial applications to removing an established biofilm by the use of dispersion signals [70]. The underlying assumption is that organisms returning to a planktonic growth state would lose biofilm-associated antibiotic and disinfectant resistance [5]. Dispersion agents could be used in combination with antibiotics or disinfectants. Aside from potential host toxicity and bacterial resistance concerns mentioned previously, one potential issue would be the mechanism by which biofilms would return to the planktonic stage. An ideal situation would involve the biofilm dispersing into individual planktonic cells. However, if large aggregates of cells were to become released from biofilms associated with a venous catheter or other medical device, then potentially serious

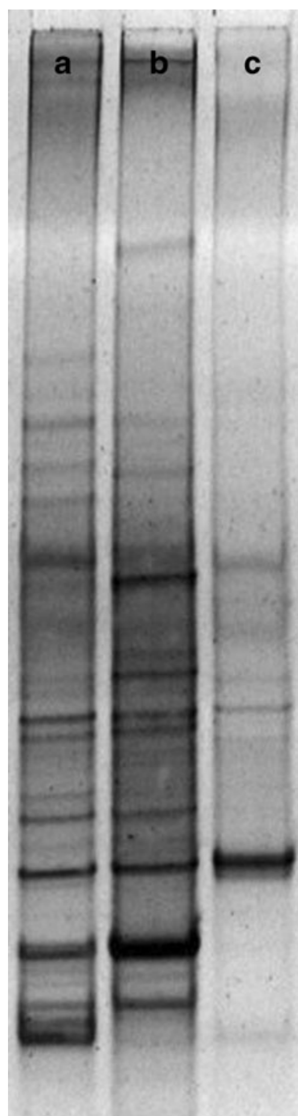

Fig. 5 DGGE profile of microbial community colonizing dialysis tubing suspended in a spring-fed lake [111]. As evidenced by the number of bands, the diversity of organisms colonizing dialysis tubing containing lake water (**a**) or media (**b**) is significantly higher than tubing containing autoclaved spent media (**c**), suggesting that organisms have the potential to control microbial succession in a biofilm

complications, such as a stroke or embolism, would arise if these aggregates blocked an important capillary.

Often in nature, a higher organism may promote the formation of a desired microbial community. Examples of this include protective bacterial populations associated with coral [97] and bioluminescent *V. fischeri* associated with the Hawaiian bobtail squid, *Euprymna scolopes* [108]. In both these situations, the surrounding seawater microbial environment is complex, yet the number of bacterial species that ultimately colonize the coral and squid is restricted. In the case of the *E. scolopes* light organ, colonization is restricted to one organism *V. fischeri* [108]. There are a number of factors involved in the specificity of the *E. scolopes*–*V. fischeri* association including an organic matrix component of the light organ and the innate immune system of the squid host, and cell surface molecules on the bacterium [109]. Although quorum-regulated proteins in *V. fischeri* are essential for light production, some proteins regulated by quorum signaling are involved in the maturation of the light organ [109]. While factors in some microbial community development populations are known in higher organisms (reviewed in [110]), little is known about microbial succession processes that occur on nutrient-lacking abiotic surfaces. Recently, we conducted a field study to investigate whether bacterial AHLs influenced bacterial colonization onto dialysis tubing that was suspended into a spring-fed lake [111]. In that study, culture supernatants from AHL-producing and non-AHL-producing strains of *A. tumefaciens* and *C. violaceum*, as well as several abiotic controls, were placed in dialysis tubing, suspended into a lake for 4–5 h, and colonized by ambient aquatic microorganisms. As shown by denaturing gradient gel electrophoresis (DGGE) (Fig. 5), the diversity of organisms that colonized tubing containing spent culture media was considerably lower than those colonizing the abiotic controls. Minor differences were observed in the presence and absence of AHL-containing supernatant. The major conclusion from this study was that a preexisting microbial community on a surface can control subsequent colonization and microbial succession [111]. Huang et al. [112] investigated microbial community development in intertidal marine biofilms. These investigators

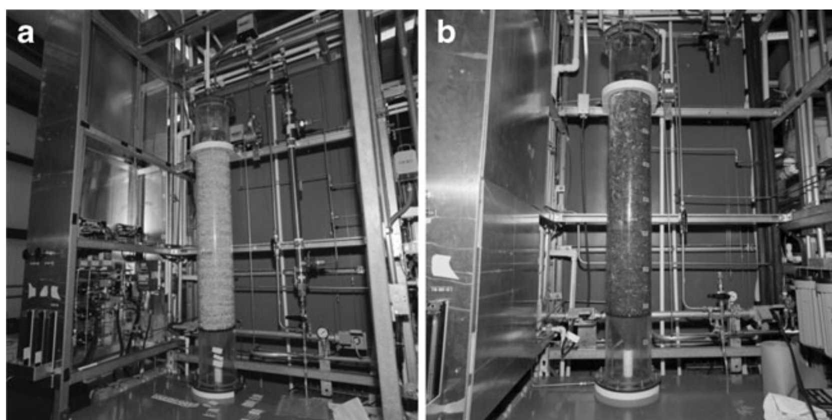

Fig. 6 Photograph of a packed bed reactor for treating wastewater before (**a**) and after (**b**) biofilm growth. Although biofilms grow quickly on some reactors (such as the denitrification reactor shown), in biofilm-based, nitrification reactors, a functioning biofilm may take as long as a month to become established. QS-based technology may provide a mechanism to accelerate this process

found that primary colonizing bacteria may influence microbial succession, possibly in part through AHL production. In some instances, there are situations wherein biofilm formation may be desired. In that context, quorum signal technology may be very relevant. We now address one situation involving the use of biofilms for wastewater treatment including its potential applications to the space program.

Wastewater is typically treated by microbial degradation of pollutants. In wastewater associated with agriculture or aquiculture, the water may contain elevated quantities of nitrogenous compounds such as protein or urea. Here, considerable levels of ammonia will arise through microbial deamination or urease activity [113]. Elevated levels of dissolved ammonia (NH_4^+) and nitrate (NO_3^-) can be toxic (reviewed in [114, 115]), but can be removed biologically. The first stage in biological nitrogen removal is the oxidation of NH_4^+ to NO_3^- by nitrifying bacteria [116], followed by a second anaerobic denitrification step involving the reduction of NO_3^- to N_2 [117]. There are a number of biofilm-based bioreactors that employ nitrification [116–119]. However, the lag time involved in establishing a fully functional nitrifying biofilm can be significant (approximately 3–4 weeks in one pilot scale investigation (LM Vega and K Pickering, unpublished observations). While an inconvenience for conventional wastewater processing on Earth, an extended lag time, or recovery of nitrifying organisms from stress [120] in any life support system providing potable water in space flight, would represent a major concern [121] and potential risk to astronaut health.

As shown in Fig. 6, one potential use of QS technology would be to accelerate biofilm formation. Based on two studies involving freshwater and marine biofilm formation, there is evidence that QS can influence microbial succession in biofilm development [111, 112]. Gonzales et al. [122] showed that long chain AHLs could promote surface colonization by *Acidithiobacillus ferrooxidans* on pyrite. As well, AHLs have been shown to promote recovery of ammonia oxidizing bacteria from starvation stress [123] and also promote the anoxic ammonia oxidizing activity in a mixed microbial community [124]. Several key issues would need to be addressed during the development of new QS technology. Based on prior work, one would certainly anticipate an interaction of QS and nutrients present [65]. Pertinent issues that would need to be addressed would include the identification of the relevant quorum signals, testing their effectiveness under ambient wastewater chemical conditions, optimizing their use, and finally evaluating whether any toxicity issues [81] may arise.

Concluding Thoughts and Future Perspectives

Biofilms, containing high concentrations of surface-adherent bacteria, have been widely described in a number of environments. While a successful mode of bacterial growth, they have proven to be remarkably resistant to control by the conventional use of disinfectants and antibiotics [10, 17]. A number of lab investigations have shown bacterial signaling, including QS, to be prominently associated with the development and eventual dispersion of biofilms [48, 64, 125, 126]. Ecological studies have shown that many higher organisms are capable of controlling biofilms by interfering with QS [75, 97]. Many QS-disrupting compounds including brominated furanones have been identified and are actively being exploited for their ability to prevent biofilms [127]. Biofilm dispersion signals [70–72] represent another very promising line of investigation. While biofilm growth is associated with antibiotic resistance, these sessile bacteria regain antibiotic susceptibility upon return to the planktonic growth [5]. Combination therapy involving dispersion signals and traditional antimicrobial agents may be promising in combating biofilm-associated infections and industrial biofouling. Finally, QS may provide a strategy whereby biofilm growth or desired microbial community composition and succession within biofilms may be promoted.

There is increasing evidence that higher organisms manipulate QS to control microbial populations including biofilms [97, 127, 128]. Although biofilms were once seen as an intractable microbial problem [10], we are now finding mechanisms whereby they can be controlled via manipulation of the cell signal ecology.

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