Journal of Industrial Microbiology (1995) 15, 257–262

Interspecies bacterial interactions in biofilms

GA James[1,3], L Beaudette[2] and JW Costerton[3]

[1]*Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada;* [2]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada;* [3]*Center for Biofilm Engineering, Montana State University, Bozeman, Montana 59717, USA*

Interactions among bacterial populations can have a profound influence on the structure and physiology of microbial communities. Interspecies microbial interactions begin to influence a biofilm during the initial stages of formation, bacterial attachment and surface colonization, and continue to influence the structure and physiology of the biofilm as it develops. Although the majority of research on bacterial interactions has utilized planktonic communities, the characteristics of biofilm growth (cell positions that are relatively stable and local areas of hindered diffusion) suggest that interspecies interactions may be more significant in biofilms.

Keywords: bacteria; interaction; biofilm; mixed-species; community

Introduction

Biofilms in most natural and many engineered environments consist of a complex community of microorganisms rather than a single species (Figure 1). Microbial communities often have capabilities greater than those of the individual members alone and some processes, such as the mineralization of certain xenobiotic contaminants, can only be performed by a community [25,43,57]. For example, Wolfaardt *et al* demonstrated that a mixed culture of nine bacteria was capable of utilizing the herbicide diclofop methyl as a sole carbon and energy source although none of the bacteria was capable of this process in monoculture [57]. These enhanced faculties are due to interactions among populations, which influence the function, stability, and flexibility of microbial communities [6].

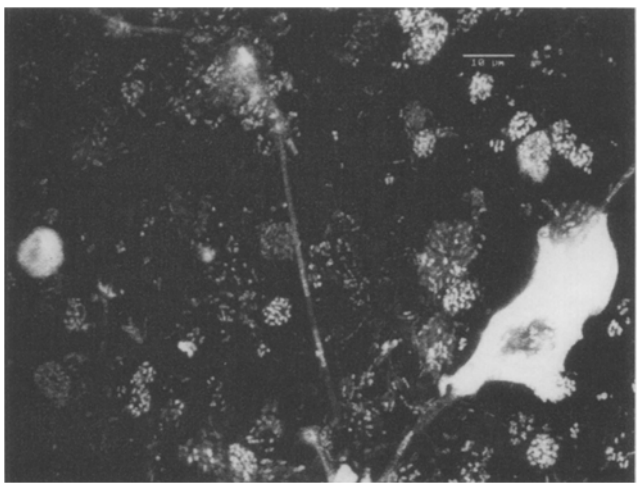

Figure 1 Scanning confocal laser micrograph of a biofilm that developed on a glass surface in a modified Robbins device irrigated with river water. The biofilm was prepared for microscopy by fixation with glutaraldehyde and treatment with the protein-binding stain, rhodamine isothiocyanate. This image is a projection of twenty optical sections through the biofilm, obtained at 1-μm intervals. The biofilms that developed in this system were complex communities consisting of various bacterial morphotypes, as well as algae (diatoms) and protozoa

Correspondence: JW Costerton, Center for Biofilm Engineering, Montana State University, Bozeman, Montana 59717, USA
Received 15 November 1994; accepted 24 March 1995

Microbial interactions are commonly classified based on the effect of the interaction on each population in a binary system [6,15,35]. *Neutralism* occurs when neither population is affected by the presence of the other. *Competition* refers to an interaction where two populations are competing for a growth-limiting nutrient and which is detrimental to both populations. When one population benefits from the presence or activity of the other while the benefactor is unaffected, the phenomenon is termed *commensalism*. An interaction where both populations benefit is *mutualism*, which includes obligatory interactions (*symbiosis*), facultative interactions (*protocooperation*), or interactions that result in the enhanced production (or consumption) of a certain product (*synergism*). Protocooperation involving the mutual exchange of a growth factor or energy source (cross-feeding) is termed *syntrophy*. This term is also used to describe the interaction between H_2-producing fermentative bacteria and H_2-consuming methanogens (syntrophic methanogenesis). *Ammensalism* refers to an interaction where one population has an indirect (not involving cell–cell contact) negative impact on another, such as the production of a bacteriocin by one species that inhibits the growth of another. Direct negative interactions include *predation*, where one organism is consumed by another, and *parisitism*, where one organism is invaded intracellularly by another. Although this binary system classification is useful for defining interactions, in natural communities interactions can be complex and include mixed interactions, where more than one type of interaction occurs between two species, as well as interactions involving more than one species [6].

This review focuses on the interactions between biofilm bacteria; however, interactions among other micro- and macroorganisms can also have a significant influence in biofilms. For example, interactions often occur between algae and bacteria [20] and many biofilm communities contain algal members. Predation within biofilms is also com-

mon and has been demonstrated to influence the physiology of nitrifying biofilms [28]. Recent studies have also begun to examine the effect of parasitism on biofilms (Doolittle and Cooney, this issue).

Physiological interactions among bacteria have been studied primarily in planktonic culture systems, but are more likely to occur in biofilms. Typically, biofilms have a heterogeneous structure with local areas of high cell density [12,26,48] and hindered diffusion [12,14,27]. These characteristics likely enhance physiological interactions involving the diffusion of nutrients and/or extracellular products. Furthermore, the positions of cells in biofilms are relatively stable, which may promote the development of interactions between adjacent populations. As discussed in this review, the processes of attachment, surface colonization, and biofilm development involve interactions that are specific to biofilms and other aggregated microbial communities, in addition to classical microbial interactions.

Biofilm-specific interactions

Interactions influencing adhesion and biofilm formation

Bacterial interactions begin to influence a biofilm during the initial stages of biofilm development. The formation of a biofilm begins with the adsorption of molecules to a surface (ie, conditioning film formation), followed by bacterial adhesion and colonization. The conditioning film can have a significant effect on bacterial adhesion, which is dependent on both the type of substratum and the source of the molecules adsorbing to form the conditioning film [45]. McEldowney and Fletcher demonstrated that the adhesion of one bacterial species to a surface can have a negative, positive, or neutral influence upon another species, depending on the species involved and the nature of the substratum [34]. Inhibitory adhesion interactions may be due to one cell blocking the attachment site of another [4] or the secretion of inhibitory macromolecules that adsorb to the substratum and modify the conditioning film [34]. Positive interactions during adhesion may be due to an indirect mechanism whereby bacterial products modify the conditioning film or by a direct mechanism involving cell–cell contact. Direct interactions during adhesion have been demonstrated in dental plaque where specific ligand receptor interactions cause interspecies coaggregation. This phenomenon has been reviewed recently by Kolenbrander [21]. Although the majority of coaggregation studies have involved planktonic bacteria, the attachment of one member of a coaggregating pair to hydroxyapatite can enhance the adhesion of the other member [10,46]. Nonetheless, other interactions may also be involved in adhesion of oral bacteria. Cells of the mutans streptococci group adhered to other oral bacteria immobilized on a nitrocellulose filter even though they did not form coaggregates in suspension [24]. Overall, these observations demonstrate that interspecies interactions can begin to influence a biofilm during the initial stages of biofilm formation.

The attachment of one species to a substratum is not always affected by the simultaneous or prior attachment of another [13,34]. Neutral adhesion interactions are likely due to separate binding sites on the substratum for each species. Multiple binding sites (high- and low-affinity) have even been reported within species [16,22]. For example, the attachment of a nonmotile mutant strain of *Pseudomonas fluorescens* to glass was not inhibited by the simultaneous attachment of the parent strain, suggesting that these strains occupied different binding sites [22]. The occurrence of an interaction during adhesion was dependent on the nature of the surface as well as the species involved [34]. This may be due to a variation in the mechanism of bacterial adhesion for different substrata. The adhesion of *Vibrio proteolytica* to a hydrophobic substratum was inhibited by proteases, whereas binding to a hydrophilic substratum was unaffected [38]. Thus, two species may have different binding sites on one substratum and not interact, while on a different substratum they may compete for the same binding site or produce an extracellular product which influences the attachment of another species.

The interaction of bacterial species during adhesion and surface colonization probably has a significant effect on the population structure (ie, which species are present) of biofilm communities. Initial colonizing species could promote the colonization of some species while inhibiting the adhesion of others, potentially recruiting species with which they are physiologically compatible. Further research is needed to elucidate the mechanisms of adhesion interactions as well as the role of these interactions in the structure and physiology of biofilm communities.

Effect of interactions on biofilm thickness and stability

Biofilms formed by microbial communities (ie, mixed-species biofilms) are often thicker and more stable than monospecies biofilms. For example, the mean thickness of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* monospecies biofilms in an annular reactor were 15 and 30 μm, respectively, while a biofilm consisting of both species was 40 μm thick [47]. This enhancement of biofilm thickness may be the result of one species enhancing the stability of another within a biofilm. The presence of *Pseudomonas fluorescens* enhanced biofilm formation by *Aeromonas hydrophila*, and surface coverage by mixed-species biofilms formed by *A. hydrophila*, *P. fluorescens*, and a coryneform was enhanced by the addition of *Xanthomonas maltophila* [13]. Similarly, a binary species biofilm consisting of *P. fluorescens* and *K. pneumoniae* detached from glass readily, but was more stable when a third species, *P. aeruginosa*, was added [49]. It is possible that one species copiously produces exopolymer that enhances the stability of other species within a biofilm and/or that stabilizing interactions occur between polymers of the different species [34,51]. Biofilm stabilization can be considered a commensal interaction, where one species benefits from the ability of another to form a stable film.

Classical bacterial interactions

Neutralism

Neutral interactions (ie, non-interactions) occur not only between populations that are too far apart spatially, but can also occur between closely-associated populations such as in yogurt starter cultures and activated sludge communities

[6]. For example, the population densities of a *Lactobacillus* sp and a *Streptococcus* sp grown in continuous culture, using whey as a substrate, were similar in mixed-cultures and individual cultures of each species [31]. In this case, it was proposed that neutralism was due to a different growth-limiting nutrient for each species. In a relatively thick biofilm it seems unlikely that adjacent species would have a neutral relationship. Nonetheless, the product (cellular and extracellular carbon) formation rate as well as the glucose to oxygen consumption ratio of *P. aeruginosa* and *K. pneumoniae* determined for monoculture biofilms in an annular reactor were unaffected when the organisms were grown as a mixed-species biofilm [47]. However, these bacteria formed a competitive relationship when cultivated in a packed-bed reactor system, with *K. pneumoniae* out-competing *P. aeruginosa* due to the faster growth rate of the former [50]. The differences in the nature of this interaction may be due to differences in the thickness of the biofilms and the diffusion of nutrients and metabolic wastes. The annular reactor biofilm was exposed to a higher shear and turbulent flow which probably resulted in more efficient nutrient and waste diffusion to both populations, eliminating competitive effects.

Competition

Competition for nutrients between bacteria in planktonic culture systems has received considerable study [35]. However, the results of these studies cannot necessarily be extrapolated to biofilm systems. In a recent study of ruminal fibrolytic bacteria by Odenyo *et al* [37], using 16S rRNA probes to quantitate population proportions, it was demonstrated that although *Ruminococcus flavefaciens* outcompeted *Fibrobacter succinogenes* when a soluble substrate (cellulose or cellobiose) was supplied, this competition was eliminated when a particulate substrate (wheat straw) was used. Since the digestion of particulate substrates by cellulolytic bacteria often requires attachment to the substrate [8], the enhanced competitiveness of *F. succinogenes* may be related to its ability to colonize the substratum effectively.

Competition in a biofilm can result in dominance by one population, but the other population often persists in the biofilm. Invasion of an established *Hyphomicrobium* sp biofilm by *Pseudomonas putida* resulted in dominance by *P. putida*, but the population of *Hyphomicrobium* sp cells remained constant [2]. Similarly, *P. aeruginosa* biofilms in a packed-bed reactor were outgrown by invading *K. pneumoniae* cells but the *P. aeruginosa* population (although probably inactive) remained at relatively high numbers [50]. In both of the previously mentioned studies when the roles of the established population and invader were reversed, the same organism dominated the biofilm. The outcome of competition in these cases was dependent upon the growth rate of the species involved. However, competition in natural microbial communities may have other mechanisms such as production of siderophores.

Ammensalism

Ammensalism occurs when one microorganism produces a compound that is inhibitory to another. Bacteriocins are antagonistic compounds produced by one species of bacteria that directly inhibit another. Ammensalism can also occur by an indirect mechanism, such as the production of organic acids by one species which lowers the medium pH and inhibits another species. A recent study by Odenyo *et al* [37] characterized an ammensal interaction between *Ruminococcus albus* and *R. flavefaciens* that was based on the production of a bacteriocin by *R. albus* [36]. This interaction occurred when cells were grown on both soluble (cellulose) and insoluble (wheat straw) substrates indicating that the interaction occurred during both planktonic and sessile growth. Further study of ammensalism in biofilms is required to understand the significance of this phenomenon in nature and perhaps lead to novel biofilm control strategies.

Commensalism

Commensal interactions occur when one population benefits and the other is unaffected. Such interactions are probably common in biofilm systems. One type of commensalism involves the consumption of oxygen by aerobic and/or facultative microorganisms, allowing the growth of obligate anerobes. This interaction may be particularly significant in biofilms, where oxygen gradients are often created [12,14,30]. This type of interaction can play an important role in microbially-induced corrosion, where the creation of anaerobic microniches within the biofilm permits the growth of sulfate-reducing bacteria [19,29]. Commensalism involving oxygen consumption has also been investigated in dental plaque development, where an initially high proportion of aerobic bacteria declined with a concurrent increase in the number of anaerobic bacteria [40]. Fluorescent antibody staining of dental plaque samples revealed that anaerobic *Veillonella* spp predominated in the deeper plaque layers, while aerobic species predominated in the upper layers [41]. Layering of aerobic and anaerobic bacterial species has also been observed in waste-water treatment biofilms [1]. Other commensal interactions such as provision of a substrate to one species by another are probably also common in biofilms, but have not received sufficient study.

Protocooperation

When each species benefits from the presence of the other, the interaction is termed protocooperation. This type of interaction has been demonstrated in many biofilm communities. In an alpine stream biofilm, a heterotrophic population utilized the excretion products of a phototrophic population [18]. This interaction likely enabled higher heterotrophic cell density and activity than the oligotrophic environment would allow, and may also have reduced photooxidative damage to the phototrophic population. Heterotrophic bacteria are often directly associated with cyanobacterial heterocysts in both planktonic and mat communities [33], and it is likely that such associations also exist in biofilm communities. Interactions between phototrophic and heterotrophic biofilm populations may also be important for contaminant degradation in the environment. For example, the degradation of a pesticide by a bacterial consortium was enhanced by the presence of an alga, presumably due to provision of alternative carbon sources to the bacteria by the alga [57].

Protocooperative interactions often involve synergism, a situation where more of a particular compound is produced or consumed by a microbial community than by a single population. For example, the rate and extent of cellulose degradation of rumen isolates *in vitro* are only a fraction of the natural rates observed in the rumen [7]. Observations of biofilms on cellulose particles from the rumen revealed cellulolytic as well as noncellulolytic bacteria enmeshed in the exopolysaccharide matrix of the biofilm [9]. Addition of a noncellulolytic species, *Treponema bryantii*, to cultures of a cellulolytic species, *Fibrobacter succinogenes* or *Ruminococcus albus*, resulted in an enhanced rate of cellulose degradation [23]. Presumably, *T. bryantii* utilized the hydrolytic products (eg, glucose or cellobiose) from the cellulolytic bacteria [23] which may repress and/or inhibit the cellulolytic enzymes [54]. However, this synergistic interaction is dependent on the growth-rate of the cellulolytic species. The rate of cellulose degradation by slowly-growing cultures of *R. flavefaciens* was enhanced by *T. bryantii*, but that of faster-growing cultures was not [3]. This difference may be due to differences in the nature of the interaction at the different growth rates. *R. flavefaciens* cells in the slowly-growing cultures benefited from cellulolytic product removal by *T. bryantii* forming a protocooperative (and synergistic) interaction. While in the fast-growing cultures, the *T. bryantii* population may not have been able to scavenge enough product to benefit the cellulolytic population and the interaction was commensal (and nonsynergistic). Thus, the activities of a biofilm community ultimately depend on the types of interactions occurring between the constituent populations.

Interspecies hydrogen or formate transfer is the most extensively studied protocooperative interaction and is important in many methanogenic microbial communities. Methane is one of the main gases produced in the rumen, but is not produced by cellulolytic bacteria, fungi, or protozoa. The main products of cellulolytic microorganisms are formate, H_2, and CO_2, which can be utilized by methanogenic bacteria resulting in the reduction of CO_2 or formate to CH_4 [55]. The production of hydrogen by fermentative bacteria is thermodynamically favorable only at a low hydrogen partial pressure, which is created by the utilization of hydrogen by the methanogens. Utilization of formate or H_2 by the methanogens shifts the products of the fermentative bacteria by preventing the accumulation of reduced nucleotides [58]. This interaction benefits both populations because the methanogens receive a substrate and the shift in fermentation products increases the molar ATP yield for the fermentative bacteria [41,58].

The relationship between interspecies hydrogen transfer (IHT) and biofilm architecture is unclear. The partial pressure of hydrogen in the fluid phase of floc-containing anaerobic digestors was too high to account for the rates of methanogenesis in these reactors, which suggest that IHT occurred within the floc fraction [11,52]. Thiele *et al* [52] concluded that microbial aggregates from an anaerobic digestor had a lattice structure, with a mixed species distribution rather than segregation of species or compartmentalized structure. However, other investigators described distinctly layered organization of both methanogenic microbial aggregates [5,32] and biofilms [42]. These differences in aggregate or biofilm structure may reflect differences in the communities developing in these systems, hydrolytic metabolism from the reactor producing the lattice-type granules was associated with the planktonic phase rather than the floc phase [53]. Whereas in the layered aggregates, hydrolytic bacteria were presumably located in the floc fraction [5,32]. The rumen bacteria, *Methanobrevibacter smithii* and *R. flavefaciens*, form a typical IHT interaction when cultured as biofilms on cellulose particles [3]. Examination of fluorescent antibody-stained preparations of these biofilms revealed that the methanogen, *M. smithii*, formed discrete microcolonies [3], rather than mixed microcolonies that are often assumed to form in interspecies hydrogen transfer interactions [39,44,52,58].

Biofilm architecture may also be important in other protocooperative interactions. A degradative community formed a specific biofilm architecture when grown on aromatic ring compounds that was not apparent when the biofilm was supplied with more labile substrates [56]. Microscopy of biofilms formed during protocooperative cellulose digestion by *R. flavefaciens* and *T. bryantii* revealed that cellulolytic *R. flavefaciens* cells were attached directly to cellulose particles, while the spirochete, *T. bryantii*, was located in the upper biofilm layers [3]. This spatial arrangement and the mobility of spirochetes in viscous environments [17] suggest that this organism may move through the biofilm, scavenging the products of the cellulolytic bacteria.

Conclusions

Interspecies bacterial interactions have a profound influence on the formation, structure, and physiology of biofilms. Initial interactions during bacterial adhesion determine the community structure (ie, which species are present) of the developing biofilm. As biofilm accumulation proceeds, stabilizing interactions between species lead to increased biofilm thickness and stability. Physiological interactions between microbial populations increase the metabolic flexibility of the community and may influence biofilm architecture. Many industrial and natural biofilm processes are more efficient with microbial communities than with single-species culture (or can only be accomplished by a community). Further study of microbial interactions in biofilms will lead to a better understanding of these processes and how they can be improved.

Acknowledgements

The Natural Sciences and Engineering Research Council of Canada and National Science Foundation (cooperative agreement ECD–8907039 with Montana State University) are acknowledged for financial support.

References

1 Alleman JE, JA Veil and JT Canaday. 1982. Scanning electron microscope evaluation of rotating biological contactor biofilm. Water Res 16: 543–550.
2 Banks MK and JD Bryers. 1991. Bacterial species dominance within a binary culture biofilm. Appl Environ Microbiol 57: 1974–1979.
3 Beaudette LA. 1994. The effect of methanogenesis on the consortial

degradation of cellulose. PhD thesis, The University of Calgary, Calgary, Alberta, Canada.
4 Belas MR and RR Colwell. 1982. Adsorption kinetics of laterally and polarly flagellated vibrio. J Bacteriol 151: 1568–1580.
5 Bochem HP, SM Schoberth, B Sprey and P Wengler. 1982. Thermophilic biomethanation of acetic acid: morphology and ultrastructure of a granular consortium. Can J Microbiol 28: 500–510.
6 Bull AT and JH Slater. 1982. Microbial interactions and community structure. In: Microbial Interactions and Communities (Bull AT and JH Slater, eds), pp 13–44, Academic Press, New York.
7 Cheng KC, CW Forsberg, H Minato and JW Costerton. 1990. Microbial ecology and physiology of feed degradation within the rumen. In: Physiological Aspects of Digestion and Metabolism in Ruminants (Tsuda T, Y Sasaki and R Kawashima, eds), pp 596–624, Academic Press, Orlando, Florida.
8 Cheng KJ, TA McAllister, H Kudo, CW Forsberg and JW Costerton. 1991. Microbial strategy in feed digestion. In: Recent Advances on the Nutrition of Herbivores (Ho YW, HK Wong, N Abdullah and Z Tajuddin, eds), pp 181–187, Malaysian Society of Animal Production, Serdang, Selangor Darul Ehsan, Malaysia.
9 Cheng KJ, CS Stewart, D Dinsdale and JW Costerton. 1983/84. Electron microscopy of bacteria involved in the digestion of plant cell walls. Anim Feed Sci Tech 10: 93–120.
10 Ciardi JE, GFA McCray, PE Kolenbrander and A Lau. 1987. Cell-to-cell interaction of *Streptococcus sanguis* and *Propionibacterium acnes* on saliva-coated hydroxyappetite. Infect Immun 55: 1441–1446.
11 Conrad R, TJ Phelps and JG Zeikus. 1985. Gas metabolism in support of juxtapositioning of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. Appl Environ Microbiol 50: 595–601.
12 Costerton JW, Z Lewandowski, D de Beer, DE Caldwell and GA James. 1994. Biofilms, the customized microniche. J Bacteriol 176: 2137–2142.
13 Cowan MM, TM Warren and M Fletcher. 1991. Mixed-species colonization of solid surfaces in laboratory biofilms. Biofouling 3: 23–34.
14 de Beer D, P Stoodley, F Roe and Z Lewandowski. 1993. Effects of biofilm structures on oxygen distribution and mass transport. Biotech Bioeng 43: 1131–1138.
15 Fredrickson AG. 1977. Behavior of mixed cultures of microorganisms. Ann Rev Microbiol 31: 63–87.
16 Gibbons RJ, EC Moreno and I Etherdan. 1983. Concentration-dependent multiple binding sites on saliva treated hydroxyapatite for *Streptococcus sanguis*. Infect Immun 39: 280–289.
17 Greenberg EP and E Canale-Parola. 1977. Motility of flagellated bacteria in viscous environments. J Bacteriol 132: 356–358.
18 Haack TK and GA Mcfeters. 1982. Nutritional relationships among microorganisms in an epilithic biofilm community. Microb Ecol 8: 115–126.
19 Hamilton WA. 1985. Sulphate-reducing bacteria and anaerobic corrosion. Ann Rev Microbiol 39: 195–217.
20 Jones AK. 1982. The interactions of algae and bacteria. In: Microbial Interactions and Communities (Bull AT and JH Slater, eds), pp 189–248, Academic Press, New York.
21 Kolenbrander PE. 1989. Surface recognition among oral bacteria: multigeneric coaggregations and their mediators. CRC Crit Rev Microbiol 17: 137–159.
22 Korber DK, JR Lawrence and DE Caldwell. 1994. Effect of motility on surface colonization and reproductive success of *Pseudomonas fluorescens* in dual-dilution continuous culture and batch culture systems. Appl Environ Microbiol 60: 1421–1429.
23 Kudo H, KJ Cheng and JW Costerton. 1987. Interactions between *Treponema bryantii* and cellulolytic bacteria in the *in vivo* degradation of straw cellulose. Can J Microbiol 33: 244–248.
24 Lamont RJ and B Rosan. 1990. Adherence of mutans streptococci to other oral bacteria. Infect Immun 58: 1738–1743.
25 Lappin HM, MP Greaves and JH Slater. 1985. Degradation of the herbicide mecoprop (2-(2-methyl-4-chlorophenoxy) propionic acid) by a synergistic microbial community. Appl Environ Microbiol 49: 429–433.
26 Lawrence JR, DR Korber, BD Hoyle, JW Costerton and DE Caldwell. 1991. Optical sectioning of microbial biofilms. J Bacteriol 173: 6558–6567.
27 Lawrence JR, GM Wolfaardt and DR Korber. 1994. Determination of diffusion coefficients in biofilms by confocal laser microscopy. Appl Environ Microbiol 60: 1166–1173.
28 Lee NM and T Welander. 1994. Influence of predators on nitrification in aerobic biofilm processes. Wat Sci Technol 29: 355–363.
29 Lee W, Z Lewandowski, M Morrison, WG Characklis, R Avci and PH Nielsen. 1993. Corrosion of mild steel underneath aerobic biofilms containing sulfate reducing bacteria. Biofouling 7: 197–239.
30 Lewandowski Z. 1994. Dissolved oxygen gradients near microbial colonized surfaces. In: Biofouling and Biocorrosion in Industrial Water Systems (Geesey G, Z Lewandowski and H Flemming, eds), pp 175–188, Lewis Publishers, Boco Raton, FL.
31 Lewis PM. 1967. A note on the continuous flow culture of mixed populations of lactobacilli and streptococci. J Appl Bacteriol 30: 406–409.
32 MacLeod FA, SR Guiot and JW Costerton. 1990. Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. Appl Environ Microbiol 56: 1598–1607.
33 Marshall KC. 1989. Cynobacterial-heterotrophic bacterial interaction. In: Microbial Mats: Physiological Ecology of Benthic Microbial Communities (Cohen, Y and E Rosenberg, eds), pp 239–245, ASM Press, Washington, DC.
34 McEldowney S and M Fletcher. 1987. Adhesion of bacteria from mixed cell suspension to solid surfaces. Arch Microbiol 148: 57–62.
35 Meers JL. 1973. Growth of bacteria in mixed cultures. CRC Crit Rev Microbiol 2: 139–184.
36 Odenyo AA, RI Mackie, DA Stahl and BA White. 1994. The use of 16S rRNA-targeted oligonucleotide probes to study competition between ruminal fibrolytic bacteria: development of probes for *Ruminococcus* species and evidence for bacteriocin production. Appl Environ Microbiol 60: 3688–3696.
37 Odenyo AA, RI Mackie, DA Stahl and BA White. 1994. The use of 16S rRNA-targeted oligonucleotide probes to study competition between ruminal fibrolytic bacteria: pure-culture studies with cellulose and alkaline peroxide-treated wheat straw. Appl Env Microbiol 60: 3697–3703.
38 Paul JH and WH Jeffrey. 1985. Evidence for separate adhesion mechanisms for hydrophilic and hydrophobic surfaces in *Vibrio proteolytica*. Appl Environ Microbiol 50: 431–437.
39 Pavlostathis SG, TL Miller and MJ Wolin. 1990. Cellulose fermentation by continuous cultures of *Ruminococcus albus* and *Methanobrevibacter smithii*. Appl Microbiol Biotechnol 33: 109–116.
40 Ritz HL. 1967. Microbiol population shifts in developing human dental plaque. Arch Oral Biol 12: 1561–1568.
41 Ritz HL. 1969. Fluorescent antibody staining of *Neisseria, Streptococcus* and *Veillonella* in frozen sections of human dental plaque. Arch Oral Biol 14: 1073–1083.
42 Robinson RW, DE Akin, RA Nordstedt and MV Thomas. 1984. Light and electron microscopic examinations of methane-producing biofilms from anaerobic fixed-bed reactors. Appl Environ Microbiol 48: 127–136.
43 Rozgaj R and M Glancer-Soljan. 1992. Total degradation of 6-aminonapthalene-2-sulfonic acid by a mixed culture consisting of different bacterial genera. FEMS Microb Ecol 86: 229–235.
44 Scheifinger CC, B Linehan and MJ Wolin. 1975. H_2 production by *Selenomonas ruminantium* in the absence and presence of methanogenic bacteria. Appl Microbiol 29: 480–483.
45 Schneider RP and KC Marshall. 1994. Retention of the gram-negative marine bacterium SW8 on surfaces: effects of microbial physiology, substratum nature and conditioning films. Colloids Surfaces B. Biointerfaces 2: 387–396.
46 Schwartz S, RP Ellen and DA Grove. 1987. *Bacteroides gingivalis–Actinomyces viscosus* cohesive interactions as measured by a quantitative binding assay. Infect Immun 55: 2391–2397.
47 Siebel MA and WG Characklis. 1991. Observations of binary population biofilms. Biotech Bioeng 37: 778–789.
48 Stewart PS, BM Peyton, WJ Drury and R Murga. 1993. Quantitative observations of heterogenieties in *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 59: 327–329.
49 Stoodley P, D de Beer and Z Lewandowsky. 1994. Liquid flow in biofilm systems. Appl Environ Microbiol 60: 2711–2716.
50 Sturman PJ, WL Jones and WG Characklis. 1994. Interspecies competition in colonized porous pellets. Wat Res 28: 831–839.
51 Sutherland IW. 1983. Microbial exopolysaccharides—their role in

microbial adhesion in aqueous systems. CRC Crit Rev Microbiol 10: 173–201.
52 Thiele JH, M Chartrain and JG Zeikus. 1988. Control of interspecies electron flow during anaerobic digestion: role of floc formation in syntrophic methanogenesis. Appl Environ Microbiol 54: 10–19.
53 Thiele JH and JG Zeikus. 1988. Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl Environ Microbiol 54: 20–29.
54 Weimer PJ. 1992. Cellulose degradation by ruminal microorganisms. Crit Rev Biotechnol 12: 189–223.
55 Whitman WB. 1985. Methanogenic bacteria. In: The Bacteria (Woese CR and RS Wolfe, eds), pp 3–84, Academic Press, New York.
56 Wolfaardt GM, JR Lawrence, RD Robarts, SJ Caldwell and DE Caldwell. 1994. Multicellular organization in a degradative biofilm community. Appl Environ Microbiol 60: 434–446.
57 Wolfaardt GM, JR Lawrence, RD Robarts and DE Caldwell. 1994. The role of interactions, sessile growth, and nutrient amendments on the degradative efficiency of a microbial consortium. Can J Microbiol 40: 331–340.
58 Wolin MJ and TL Miller. 1988. Microbe–microbe interactions. In: The Rumen Microbial Ecosystem (Hobson PN, ed), pp 343–385, Elsevier Science Publishers, New York.