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REVIEW ARTICLE

Impact of bacterial biofilms: the importance of quantitative biofilm studies

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Abstract The impact of various parameters, such as nutrient, temperature, surface materials and condition and hydrodynamics, on biofilm formation is well studied. Extensive research has focused on the relationship between these parameters and bacterial biofilms, with the aim of gaining an understanding of biofilm behaviour under different growth conditions so that relevant control strategies can be implemented. In such studies, model simulations have been used to qualitative study the behaviour of the biofilms respond to change in parameters. However, little is known about the quantitative study of biofilm behaviour in response to change in these parameters. In previous studies, it was indicated that nutrient concentrations influence biofilm morphology (biomass, structures and thickness) but the concentration levels at which biofilm change in structure and thickness is not mentioned. These observations were based on determining biofilms structure without considering the biomass. Findings that are based on qualitative studies only may be insufficient and not in supportive due to the fact that may be pose many speculations and debates. The biomass, structures and thickness form biofilm morphology, therefore if one part is affected, the other parts may also be affected. It is important to conduct research that will focus on both qualitative and qualitative analysis on the impact of parameters on biofilm formation and growth. The aim of this review is to highlight the importance of conducting parallel research on quantitative and qualitative study on microbial biofilms with respect to biomass, structure and thickness.

Keywords Biofilms · Biomass · Structures · Parameters

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Introduction

The study of microbial biofilm formation is of paramount importance in a wide range of industries, including those associated with health products, food, water, paper mills, medical health and pharmaceuticals. Research on the impact of various parameters on biofilm formation has been conducted within the framework of these industries, with varying results depending on the specific methods used in the industry. The information reported in this review is not restricted to any one industry.

Bacteria can transition from the planktonic to sessile stage, and when they are in the sessile stage, they form biofilms. Biofilms can be described as a group of microorganisms that attach and colonise on any surface that is immersed in an aqueous medium (Costerton 1995; Prakash et al. 2003; Smith 2005; Coleman et al. 2010). Figure 1 illustrates the processes taking place during biofilm formation. These biofilms are protected by extracellular polymeric substances (EPS) produced by the microorganisms themselves (Fig. 2; Jiao et al. 2010). Biofilms are highly structured communities, and biofilm cells are able to intercommunicate to some degree via biochemical signalling. Several studies have investigated the growth conditions of biofilms and found that a number of parameters, such as nutrients, temperature, surface material and conditions, and hydrodynamics, have major impact on the growth of the biofilms whereby its structural integrity is determined by the growth conditions (Florjanic and Kristl 2011).

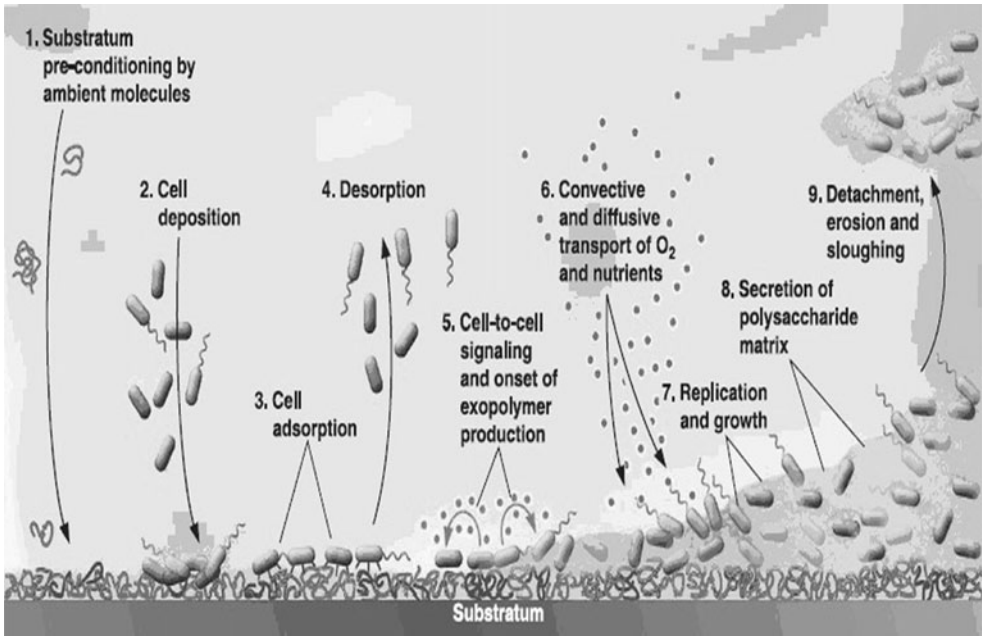

Fig. 1 Processes (stages) of biofilm formation (Breyers and Ratner 2004). *1* Reversible attachment of bacterial cells to pre-conditioned surface (biotic or abiotic) immersed in aqueous medium (Prakash et al. 2003; Ghannoum and O'Toole 2004). *2–4* Cells attach irreversibly, forming microcolonies, a step mediated mainly by the production of extracellular polymeric substances (EPS), and the cells lose their flagella-driven motility (O'Toole et al. 2000; Kumar and Prasad 2006). *5–8* Maturation of biofilms, the process whereby biofilm cells communicate through the exchange of genetic materials and other processes taking place during biofilm growth and development (An et al. 2000; Rachid et al. 2000). *9* Dispersion or detachment stage during which single motile cells disperse from the microcolonies; the detachment process can be through erosion or sloughing (Dunne 2002; Li et al. 2007)

The existence of these parameters in a wide range of environmental conditions indicate that microbial biofilms are able to respond positively to the growth environment and to change their structures and adhesion abilities (Liu et al. 2010) depending on the condition of the surface to which they attach (Vu et al. 2009). Comparison studies based on EPS structures of biofilms in response to nutrient concentrations and hydrodynamics have revealed that biofilms grown under high concentrations of nutrients and high shear will be more abundant, filamentous, and densely packed and have thicker EPS, while those growing under low nutrient concentrations and low shear will be less packed, with a single layer of microcolonies, and have thinner EPS (Purevdorj et al. 2002; Prakash et al. 2003).

Bonaventura et al. (2008) conducted a study on the effect of temperature on *Listeria monocytogenes* biofilm formation. These biofilms were exposed to different temperatures of 4, 12, 22, 27 and 37°C. The outcomes showed that the structure of *L. monocytogenes* biofilms based on visual studies (microscopic analysis) differed depending on the temperature. More specifically, with increasing temperature, the biofilm structure became more complex. However, the thickness of the biofilm biomass and structure was not quantified in terms of changing temperature. Another study by Rinaudi et al. (2006) showed that *Sinorhizobium meliloti*

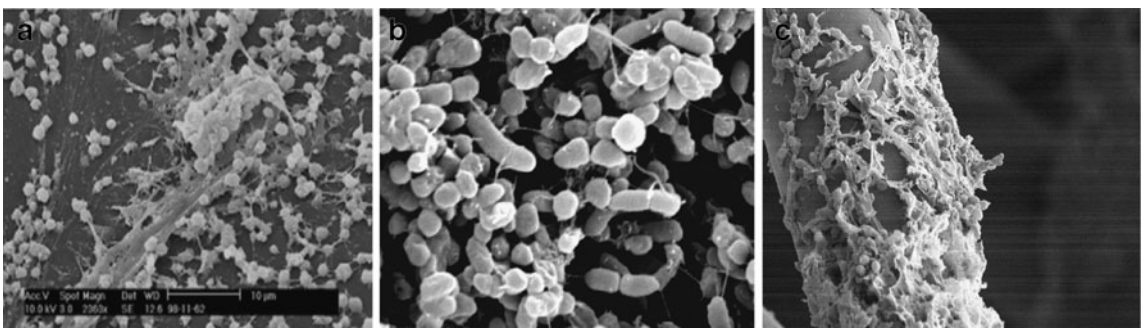

Fig. 2 Micrographs of *Staphylococcus* spp. (**a**), *Pseudomonas aeruginosa* (Donlan 2002) (**b**) and *Pseudomonas fluorescens* biofilm (**c**) (Molobela et al. 2010)

were able to grow and form biofilms in a wide range of temperatures, but no difference in terms of growth were observed at 28° and 37°C.

In these studies, the results were generated from qualitative studies whereby the aim was to study the structures of the biofilms. No mention was made of biofilm morphology in response to changes in growth conditions in terms of various parameters. A parallel quantitative and qualitative analysis will provide information on how biofilms respond in terms of biomass, structure and thickness to changes in growth conditions.

Extracellular polymeric substances

The extracellular polymeric substances provide the structural integrity of the biofilm (Yongqin et al. 2010). Biofilms produce different EPS, and the structural composition of these EPS determine the conditions in which a biofilm will grow (Dignac et al. 1998; Flemming et al. 2000). EPS are composed of high-molecular-weight compounds, including polysaccharides, proteins, nucleic acids, lipids and DNA, with proteins and carbohydrates representing the major components (Donlan 2002; Liu et al. 2004; Cheng et al. 2007; Vu et al. 2009). Humic substances are also found in some EPS (Hoyle 1992). The ionisable functional groups, such as the carboxylic, phosphoric amino and hydroxyl groups, play a key role in the formation of biofilm EPS (Pan et al. 2010). Figure 3 illustrates different components of extracellular polymeric substances (EPS).

Published studies on the composition of biofilm EPS in terms of the ratio of carbohydrates to proteins have shown that biofilms produce unequal amounts of EPS depending on the growth conditions. Some researchers have found that certain biofilm EPS have a higher concentration of proteins than polysaccharides, while others have found that in some instances polysaccharides were the dominant component of the biofilm EPS (Sutherland 1994; Liu et al. 2004, Girbal-Neuhauser 2011). In the study of Ras et al. (2008), carbohydrates and proteins were the two major constituents relative to other components (lipid, amino acids, DNA) of the biofilm EPS. Similar observations were reported by Liu and Fang (2002) regarding the protein and carbohydrate ratio except that DNA components were also found in the wastewater.

Flemming et al. (2007) found protein components to be more dominant than carbohydrate components, while Jiao et al. (2010) found the reverse. These results imply that the EPS structural composition is variable amongst bacterial strains. Nonetheless, the quantity and quality and the biofilm EPS is dependent on a number of factors, including microbial species, nutrients, the type of limiting substrate (carbon, nitrogen and phosphorus), oxygen limitation, temperature and shear force (Zhang and Fang 2001; Fang et al. 2002; Liu and Fang 2002; Liu et al. 2004; Bhaskar and Bhosle 2005). Due to structural complexity and other influential factors, including parameters that contribute to the formation of biofilm EPS, the study and analysis of biofilm structures, biomass and thickness may be quite difficult and demanding.

Growth parameters of biofilm

Nutrients

In an open reticulating system, abundant nutrients can be derived from water, particularly in cooling towers that

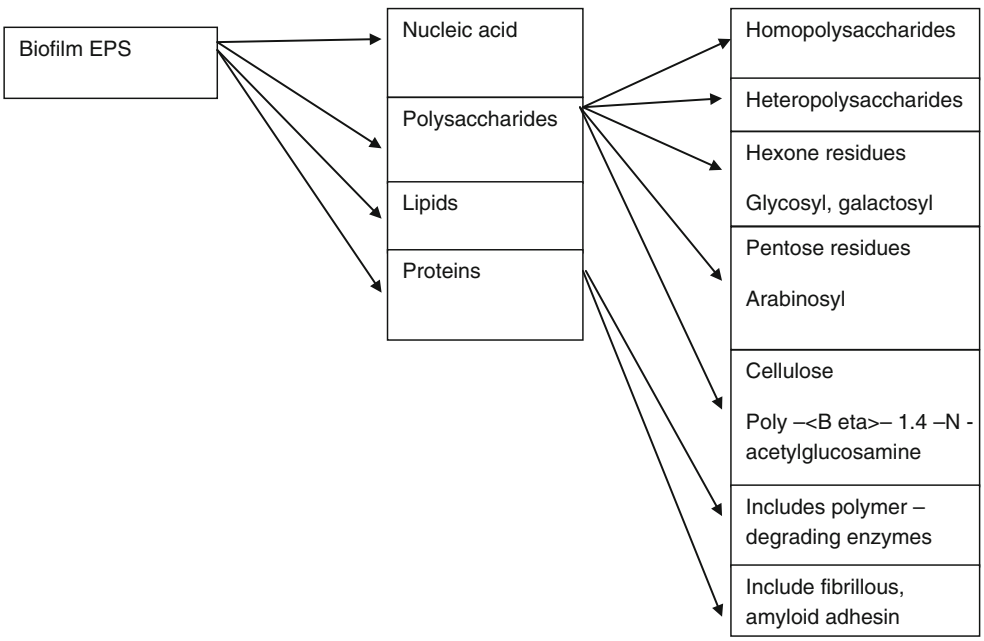

Fig. 3 Schematic overview of the structural components of EPS involved in biofilm formation (Kristensen et al. 2008)

favour the growth of biofilms (Camper et al. 1996; Mains 2008; Klahre and Flemming 2000). The structures of biofilms growing under low and high nutrient concentrations, respectively, differ (Purevdorj et al. 2002). It has been reported that biofilms growing under high nutrient concentrations have a high cell density and that the biofilm structure (EPS) appears to be thick and complex, whereas lower nutrient concentrations tend to lead to a more compact structure with a lower cell density. This variability demonstrates that the structural morphology of the biofilm is dependent on the availability of nutrients. In addition, an open structure of the biofilm will facilitate the diffusion of nutrients more easier that the compact structure (Allison 2003).

Studies performed by Sauer and Camper (2001) and Rochex and Lebeault (2007) demonstrated that *Pseudomonas aeruginosa* and *P. putida* biofilms detached at higher nutrient concentrations relative to low nutrient concentrations. It is possible that as more nutrients accumulate, more biofilm cells are produced and the conditions become unfavourable for some cells due to oxygen limitation and other activities within the biofilm, eventually resulting in these cells disintegrating from the biofilm. This process would explain the detachment of the *P. aeruginosa* and *P. putida* biofilms at high nutrient levels.

Oh and Jo (2007) showed that *Escherichia coli* O157:H7 biofilms formed faster, with more cells attaching to a glass surface, under low nutrient conditions than under high nutrient conditions. Rice et al. (2005) indicated that *Serratia marcescens* formed thicker and more filamentous biofilms at high nutrient concentrations and thinner biofilms at low nutrient concentrations. These results show that biofilm's attachment and detachment is dependent on bacterial strength.

In these studies, the influence of nutrients on biofilm formation was discussed based on the thickness of the biofilm EPS (quality), but the level of the thickness (quantity) in response to a certain nutrient level was not mentioned. It would be much more informative if both a qualitative and quantitative study on the effect of nutrients on biofilm growth and formation had been done.

Temperature

Biofilm formation is significantly influenced by temperature, often associated with warmer temperatures, and can be seasonal in nature (Chmielewski and Frank 2003; Bonaventura et al. 2008; Rao 2010; Simoes et al. 2010). The results of a study conducted by Bonaventura et al. (2008) (whereby *Listeria monocytogenes* isolated from industrial food processes tested for biofilm formation at low and high temperatures) indicated that biofilm structures become more complex with increasing temperature. These results are contradictory to those of Sauer and Camper (2001) and Rochex and Lebeault (2007). In their study, Bonaventura et al. (2008) only examined the response of structures of the *L. monocytogenes* biofilms temperature changes; no information on biomass was provided.

A study conducted by Rinaudi et al. (2006) showed that *Sinorhizobium meliloti* were able to grow and form biofilms at a wide range of temperatures and that there was no difference in terms of growth between 28 and 37^{O}C. This result possibly indicates that biofilm biomass had reached the stationary phase, but this cannot be based on prediction. If the biofilm biomass and thickness had been quantified, the information would have provided more support to the findings on the biofilm structures.

Surface materials

Microbial biofilms can attach to a wide range of surfaces, including rubber, glass, plastic etc. and the surface may be living or dead. A material surface exposed in an aqueous medium will become conditioned or coated by polymers from that medium, and the resulting chemical modification will affect the rate and extent of microbial attachment (Apilanez et al. 1998). The surface may also have several characteristics that are important in the attachment process of the biofilms, such as roughness, hydrophobicity among others (Zacheus et al. 2000; Carlen et al. 2001; Dunne 2002; Faille et al. 2002).

Materials used in industrial distribution systems are highly susceptible to biofilm attachment and development (Kalmokoff et al. 2001; Mains 2008). Biofilms develop more rapidly and are more dense on iron pipes, especially older pipes, than on PVC pipes. Other components that can support biofilm growth include materials used in valves, gaskets, washers, pump lubricants, and pipe coatings (Mains 2008).

Kalmokoff et al. (2001) studied the impact of surface materials on biofilms and found that the levels of adsorption or adhesion of the *Listeria monocytogenes* strain tested were comparable to those of the control, which was the same species but isolated from a different source. These findings show that the degree of adhesion or adsorption is dependent on the type of microorganism, its response to a specific surface material, and the production of specific protein molecules. One possible explanation for the similarity of the results in terms of the degree of adhesion among the biofilms could be that both of the strains tested belonged to the same species (*Listeria monocytogenes*), with the chance that both biofilms may use the same protein molecules for attachment. If different species had been used as controls, different results may have been found. The degree of adsorption may also be dependent on the structural integrity of the biofilms: the stronger the EPS, the harder it attaches. In this regard, methods to test the

structural integrity and thickness of the biofilm should have been applied. If mathematical statistical models need to be applied, how would it be possible to do this without the results of a quantitative analysis? These findings show that there is lack of information on quantitative studies of microbial biofilms and parameters. If both qualitative and quantitative studies were to be conducted, strategies would be improved and better information would become available on biofilms, leading to improved modelling.

Cloete et al. (2003) also found no significant difference in the colonisation rates of bacteria isolated from potable water distribution systems on the surface materials tested (asbestos–cement, cast iron in a red epoxy coating, galvanized steel and PVC). These studies show that the degree of adhesion/adsorption is dependent on the type of microorganism and the protein molecules responsible for attachment. Hydrophobicity and surface topography also promote the attachment of biofilms.

Hydrodynamics

Biofilm development, behaviour and population characteristics are strongly influenced by hydrodynamic conditions during growth (Sauer and Camper 2001; Whiteley et al. 2001; Dunsmore et al. 2002; Purevdorj et al. 2002; Stoodley et al. 2002; Simoes et al. 2007). One of the most important factors affecting biofilm structures, biomass and behaviour is the velocity field of the fluid in contact with the microbial layer (Vieira et al. 1993; Purevdorj et al. 2002; Horswill et al. 2007).

Studies have shown that biofilms growing in low shear environments tend to form isolated microcolonies, often of irregular shape with little or no indication of direction of flow. On the other hand, those growing in high shear environments tend to form long filaments or streamers leading to the downstream direction. These results were based on the visual investigation of the structures of the biofilms but not on biomass and thickness. However, if the biomass were to be quantified, it would indicate the thickness of the structure over flow velocity (Stoodley et al. 1999).

Pei-shi et al. (2008) studied the effect of shear stress on the structures of wastewater biofilms and found that carbohydrate components were more abundant than protein components at a velocity of 0.032 m/s. However, when the velocity was increased to 0.056 and 0.089 m/s, the proportion of both proteins and carbohydrate decreased. Based on these findings, it is possible to conclude that biofilms respond differently to changes in growth conditions with a subsequent effect on structures. However, these findings do not provide any information on the biomass, and some researchers may find these results insufficient. Quantitative analysis is recommended for verification of the results.

The importance of research model simulation to study biofilm processes

Despite the large range of morphologies observed for biofilms, there is strong experimental and theoretical evidence that the complex nature of biofilm structure dynamics is primarily a consequence of the effect of environmental conditions on biofilm development. Biofilm models have mostly been used as simulation tools and as research tools to study and identify the complexity of bacterial biofilm processes. However, biofilm models can also be used to evaluate experimental observations (qualitative and quantitative analysis) when studying a diversity of biofilm-related phenomena, such as the impact of environmental parameters on biofilm growth and development (Noguera et al. 1999).

A sound knowledge of the fundamentals of biofilm models, the development of mathematical models for the real time control of biofilm processes and the ability to engineer biofilm structure and function have been identified as the most important objectives for the practical application of biofilm models. As mathematical research tools, biofilm models can be used to gain a better understanding of biofilm structure, function and population dynamics and the transition (structure and behaviour) of biofilms growing under different environmental conditions (Noguera et al. 1999). Specific topics identified as priorities using model simulation are the study of (1) the behaviour of microorganisms within a biofilm, (2) the elucidation of attachment and detachment mechanisms of the biofilms, (3) the determination of the mechanical properties of EPS, (4) ecological interaction(s) among different microorganisms within the habitat and (5) the impact of environmental parameters on biofilm growth and development (Noguera et al. 1999).

The combination of mathematical models and adequate sensitivity analyses (qualitative and quantitative) will provide insights into the degree of accuracy needed in the experimental evaluation of parameters. For example, the behaviour of biofilms growing under conditions of low nutrient concentrations, high shear stress, rough surface and low temperature might be significantly different from those growing under conditions of high nutrient concentrations, low shear, smooth surface and high temperature.

Discussion and conclusion

Parameters, such as nutrients, temperature, surface material and hydrodynamics, have been well studied in terms of their impact on microbial biofilm formation. However, to date, research has largely focused on examining the structural behaviour of the biofilm with respect to changes in growth conditions. Little is known on how the biofilms

respond to these parameters in terms of biomass and thickness. Investigations of biofilm behaviour may not provide in-depth information on how the biofilms respond to changes in growth conditions with respect to various parameters.

If a model is proposed or is intended to be implemented in an industry to study the impact of parameters on biofilms and if the results found are based only on the visualization of the response of structures (EPS) of the biofilms to such parameters, then the results may be insufficient and unreliable. Further studies in terms of quantitative analysis need to be conducted to investigate the correlation between biofilm biomass, structure and thickness. In addition, it is advisable to quantify the EPS structures to determine the dominant components (proteins, carbohydrates, lipids).

Sauer and Camper (2001) and Rochex and Lebeault (2007) reported that *Pseudomonas aeruginosa* and *P. putida* biofilms detached at high nutrient concentrations in comparison to low nutrient concentrations. The findings of these studies only focused on the behavioural change of the biofilms in terms of structures responsive to change in nutritional concentrations; the thickness of the EPS structures and biofilm biomass was not quantified. Therefore, these results may not be sufficient due to the fact that information on biofilm biomass and thickness was not provided. Correlation studies on the behaviour of biofilm biomass and structure with regards to quantitative and qualitative studies are strongly advised.

The removal of microbial biofilms may be difficult. Removal strategies such as use of antimicrobials do not seem to be effective in the control and removal of biofilms, possibly due to multiprocesses during biofilm formation, including the various parameters discussed. Villa et al. (2010) indicated that new approaches to defeat deleterious biofilms are of vital importance and that the best strategy is to anticipate biofilm formation. Many researchers have focused on qualitative analysis to conduct research on bacterial biofilms; however, this analysis does not reveal in-depth information on the relationship between biofilm structure, biomass and thickness. Hence, parallel research on quantitative and qualitative analysis would significantly improve the results. In conclusion, the need to evaluate parameter sensitivity to the growth and development of bacterial biofilms is an essential component of modelling research. The current use of biofilm models as research tools has broader objectives, most of which relate to gaining a better understanding of biofilm structure and behaviour, population dynamics, structural heterogeneities and the environmental habitat in which a specific biofilm grows. The design of the experiment (DoE) through research model simulation may be of paramount importance as a proper design would facilitate the study of specific and identified topics of biofilm processes.

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References

Allison DG (2003) The biofilm matrix. Biofoul 19:139–150

An YH, Dickison R, Doyle RJ (2000) Mechanisms of bacterial adhesion and pathogenesis of implant and tissue infections. In: An YH, Friedman RJ (eds) Handbook of bacterial adhesion: Principles, methods and applications. Humana Press, Totowa, pp 1–27

Apilanez I, Gutierrez A, Diaz M (1998) Effect of surface material on initial biofilm development. Biores Technol 66:225–230

Bhaskar PV, Bhosle NB (2005) Microbial extracellular polymeric substances in marine biogeochemical processes. Curr Sci 88:47–53

Breyers JD, Ratner JP (2004) Bioinspired implant materials befuddle bacteria. ASM News 70:232–237

Bonaventura GD, Piccolomini R, Paludi D, D'Orio V, Vergara A, Conter M, Ianieri A (2008) Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: relationship with motility and cell surface hydrophobicity. J Appl Microbiol 104:1552–1561

Camper AK, Warren LJ, Jason TH (1996) Effect of growth conditions and substratum composition on the persistence of coliforms in mixed-population biofilms. Appl Environ Microbiol 62:4014–4018

Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J (2001) Surface characteristics and in vitro biofilm formation on glass ionomer and composite resin. Biomaterials 22:481–487

Cheng G, Zhang Z, Chen S, Bryers J, Jiang S (2007) Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces. Biomaterials 29:4192–4199

Chmielewski RAN, Frank JF (2003) Biofilm Formation and control in food processing facilities. Comprehensive review in food science and food safety. Inst Food Technol 2:22–32

Cloete TE, Westaard D, van Vuuren SJ (2003) Dynamic response of biofilm to pipe surface and fluid velocity. Water Sc iTechnol 47 (5):57–59

Coleman DC, O'Donnell MJ, Shore AC, Swan J, Russell RJ (2010) The role of manufacturers in reducing biofilms in dental chair waterlines. J Dent 35:701–711

Costerton JW (1995) Overview of microbial biofilms. J Ind Microbiol 15:137–140

Dignac MF, Urbain V, Rybacki D, Bruchet A, Snidaro D, Scribe P (1998) Chemical description of extracellular polymers: implication on activated sludge floc structure. Water Sci Technol 38:45–53

Donlan RM (2002) Biofilms and device-associated infections. Emerg Infect Dis 7(2):277–281

Dunne WM (2002) Bacterial adhesion: Seen any good biofilms lately? J Clin Microbiol 15:155–166

Dunsmore BC, Jacobsen A, Hall-Stoodley L, Bass CJ, Lappin-Scott HM, Stoodley P (2002) The influence of fluid shear on the structure and material properties of sulphate-reducing bacterial biofilms. J Ind Microbiol Biotechnol 29:347–353

Faille C, Jullien C, Fontaine F, Bellon-Fontaine MN, Slomianny C, Bénézech T (2002) Adhesion of *Bacillus* spores and *Escherichia coli* cells to inert surfaces: role of surface hydrophobicity. Can J Microbiol 48:728–738

Fang HHP, Liu H, Zhang T (2002) Characterization of hydrogen producing granular sludge. Biotechnol Bioeng 78:44–52

Flemming HC, Wingender J, Mayer C, Kostgens V, Borchard W (2000) Cohesiveness in biofilm matrix polymers. In: Community structure and cooperation in biofilms. Press Syndicate, Cambridge, p 91

Flemming HC, Neu TR, Wozniak D (2007) The EPS matrix: The "house of biofilms cells". J Bacteriol 189(22):1–6

Florjanic M, Kristl J (2011) The control of biofilm formation by hydrodynamics of purified water in industrial distribution system. Int J Pharm 405:16–22

Ghannoum M, O'Toole GA (2004) Microbial biofilms. American Soc Microbiol Press, Washington D.C., pp 250–268

Girbal-Neuhauser E (2011) Extracellular polymeric substances diversity of biofilms grown under contrasted environmental conditions. Water Res 45:1529–1538

Horswill AR, Stoodley P, Stewart PS, Parsek MR (2007) The effect of the chemical, biological, and physical environment on quorum sensing in structured microbial communities. Anal Bioanal Chem 387:371–380

Hoyle B (1992) Pseudomonas aeruginosa biofilm as a diffusion barrier to piperacillin. J Ant Agents Chem 36:2054–2056

Jiao Y, Cody GD, Harding AK, Wilmes P, Schrenk M, Wheeler KE, Banfield JF, Thelen MP (2010) Characterization of extracellular polymeric substances from acidophilic microbial biofilms. Appl Environ Microbiol 76(9):2916–2922

Kalmokoff ML, Austin JW, Wan XD, Sanders G, Banerjee S, Farber JM (2001) Adsorption, attachment and biofilm formation among isolates of *Listeria monocytogenes* using model conditions. J Appl Microbiol 91:725–734

Klahre J, Flemming HC (2000) Monitoring of biofouling in papermill process. Water Res 34(14):3657–3665

Kristensen JB, Meyer RL, Lauren BS, Shipovskov S, Besenbacher F, Poulsen CH (2008) Antifouling enzymes and the biochemistry of marine settlement. J Biotechnol 26:471–481

Kumar A, Prasad R (2006) Biofilms. JK Sci 8:15–17

Li Y, Hao G, Galvani CD, Meng Y, de la Fuente L, Hoch HC, Burr TJ (2007) Type I and type IV pili of *Xylella fastidiosa* affect twitching motility, biofilm formation and cell- cell aggregation. J Microbiol 153:719–726

Liu H, Fang HP (2002) Extraction of extracellular polymeric substances (EPS) of sludge. J Biotechnol 95:249–256

Liu L, Chu L, Liu Q, Wang C, Xia Y, Peng X (2010) A comparative study on biofilm formation of nontypeable *Haemophilus influenzae* and *Pseudomonas aeruginosa* under single culture or co-culture. Afr J Microbiol Res 4(3):180–184

Liu Y, Yang SF, Li Y, Xu H, Qin L, Tay JH (2004) The influence of cell substratum surface hydrophobicities on microbial attachment. J Biotechnol 110:251–256

Mains C (2008) Biofilm control in distribution systems. Natl Environ Serv Center (NESC) 8(2):1–4

Molobela IP, Cloete TE, Beukes M (2010) Protease and amylase enzymes for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by *Pseudomonas fluorescens* bacteria. Afr J Microbiol Res 4(14):1515–1524

Noguera DR, Okabe S, Picioreanu C (1999) Biofilm modelling: present status and future dicetions. Water Sci Technol 39(7):273–278

O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79

Oh YJ, Jo W (2007) Biofilm formation and local electrostatic force characteristics of Escherichia coli 0157:H7 observed by electrostatic microscopy. Appl Phys Letters 90:143901

Pan X, Liu J, Zhang D, Chen X, Li L, Song W, Yang J (2010) A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy. Water SA 36(1):111–116

Pei-shi QI, Wen-bin W, Zheng, QI (2008) Effect of shear stress on biofilm morphological characteristics and the secretion of extracellular polymeric substances. School of Municipal & Environmental Engineering. Harbin Institute of Technology. Harbin, pp 3438–3441

Prakash B, Veeregowda BM, Krishnappa G (2003) Biofilms: A survival strategy of bacteria. J Curr Sci 85:9–10

Purevdorj B, Costerton JW, Stoodley P (2002) A influence of hydrodynamics and cell signaling on the structure and behavior of *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 68(9):4457–4464

Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W (2000) Effect of sub inhibitory antibiotic concentrations on polysaccharide intercellular adhesion expression in biofilm forming *Staphylococcus epidermidis*. J Ant Agents Chem 44:3357–3363

Rao TS (2010) Comparative effect of temperature on biofilm formation in natural and modified marine environment. Aquat Ecol 44:463–478

Ras M, Girbal-Neuhauser E, Paul EM, Sperandio M, Lefebvre D (2008) Protein extraction from activated sludge: An analytical approach. Water Res 42:1867–1878

Rice SA, Koh KS, Queck SY, Labbate M, Lam KW, Kjelleberg S (2005) Biofilm formation and sloughing in *Serratia marcescens* are controlled by quorum sensing and nutrient cues. J Bacteriol 187(10):3477–85

Rinaudi L, Fujishinge NA, Hirsch AM, Banchio E, Zorreguieta A, Giordano W (2006) Effects of nutritional and environmental conditions on *Sinorhizobium meliloti* biofilm formation. Res Microbiol 157:867–875

Rochex A, Lebeault JM (2007) Effects of nutrients on biofilm formation and detachment of a *Pseudomonas putida* strain isolated from a paper machine. Water Res 41:2885–2892

Sauer K, Camper AK (2001) Characterization of phenotypic changes in *Pseudomonas putida* in response to surface associated growth. J Bacteriol 183:6579–6589

Simoes M, Simoes LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. Food Sci Technol 43:573–583

Simoes M, Pereira MO, Sillankorva S, Azeredo J, Viera MJ (2007) The effect of hydrodynamic conditions on the phenotype of *Pseudomonas fluorescens* biofilms. Biofoul 23(3/4):249–258

Smith AW (2005) Biofilms and antibiotic therapy: Is there a role for combating bacterial resistance by the use of novel drug delivery system? J Adv Drug Delivery Review 57:1539–1550

Stoodley P, Dodds I, Boyle JD, Lappin-Scott HM (1999) Influence of hydrodynamics and nutrients on biofilm structure. J Appl Microbiol 85:19–28

Stoodley P, Cargo R, Rupp CJ, Wilson S, Klapper I (2002) Biofilm material properties as related to shear-induced deformation and detachment phenomena. J Ind Microbiol Biotechnol 29:361–367

Sutherland IW (1994) Structure- function relationship in microbial exopolysaccharides. J Biotechnol Adv 12:393–448

Vieira MJ, Melo LF, Pinheiro MM (1993) Biofilm formation: Hydrodynamic effects on internal diffusion and structure. J Bioad Biofilm Res 7(1):67–80

Villa F, Albanese D, Giussani B, Stewart P, Daffonchio D, Cappitelli F (2010) Hindering biofilm formation with zosteric acid. Biofoul 26:739–752

Vu B, Chen M, Russell JC, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535–2554

Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. Nature 413:860–864

Yongqin JY, Cody GD, Harding AK, Wilmes P, Schrenk M, Wheeler KE, Banfield JF, Thelen MP (2010) Characterization of Extracellular Polymeric Substances from acidophilic microbial biofilms. Appl Environ Microbiol 76(9):2916–2922

Zacheus OM, Livanainen EK, Nissinen TK, Lehtola MJ, Martikainen PJ (2000) Bacterial biofilm formation on polyvinyl chloride, polyethylene and stainless steel exposed to ozonated water. Water Res 1:63–70

Zhang T, Fang HP (2001) Quantification of extracellular polymeric substances in biofilms by confocal laser scanning microscopy. J Biotechnol 23:405–409