Success and Failure of Colloidal Approaches in Adhesion of Microorganisms to Surfaces

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Abstract

Biofilms are communities of cells attached to surfaces, their contributions to biological process may be either a benefit or a threat depending on the microorganism involved and on the type of substrate and environment. Biofilms formation is a complex series of steps; due to the size of microorganisms, the initial phase of biofilm formation, the bacterial adhesion to the surface, has been studied and modeled using theories developed in colloidal science. In this review the application of approaches such as: Derjaguin, Landau, Verwey, Overbeek (DLVO) theory and its extended version (xDLVO), to bacterial adhesion is described along with the suitability and applicability of such approaches to the investigation of the interface phenomena regulating cells adhesion. A further refinement of the xDLVO theory encompassing the brush model is also discussed. Finally, the evidences of phenomena neglected in colloidal approaches, such as: surface heterogeneity and fluid flow, likely to be the source of failure are defined.

Keywords: Bacterial adhesion, Biofilm, DLVO theory, xDLVO theory, steric model

Content

1 Biofilms and biofilms formation

In nature, bacteria can be present as either planktonic cells, which are freely flowing in a bulk solution, or as biofilm which exist as a unit attached to a surface [1]. Microbial biofilms have been defined as complex, three-dimensional functional consortia of adherent microorganisms, bound to, and growing at, an interface and encased by a extracellular polymeric matrix [2]-[4].

Claude ZoBell in 1943, a marine biologist, first introduced the concept of the bottle effect, whereby the number of free living microorganisms in fresh sea water gradually declines when kept in a glass bottle, whilst the number of attached micro-organisms increases. Yet, it took time, about 30 years, before it was accepted that for microorganisms, both bacteria and fungi, the biofilm mode of life is the rule rather than the exception [5]-[7]. Biofilms develop as result of cells adhesion to a surface that can be either abiotic (like a medical device) or biotic (like another biological tissue, i.e. intestine walls) and then may, depending on the bacterial strain, produce the extracellular polymers that provides a matrix for further adhesion. From a medical perspective, for example, it is well accepted that *Staphylococcus epidermidis* infections are dependent on this species ability to adhere to artificial surfaces and to assemble large biofilm consortia [6]. Commonly, biofilms will form on indwelling medical devices that act as a substrate for growth; this is a major problem in medical healthcare and accounts for serious complications and expensive care [8],[9]. Infections caused by biofilms are difficult to eradicate as a result of the higher survivability of cells in this physiological state [10]-[12], therefore suggesting that biofilm contributes to the survival of bacteria by providing better growth conditions when cells are placed in hostile environments or when exposed to antimicrobial compounds. Biofilms may be also detrimental when found on food, slaughter house equipments as well as on ship hulls and in oral cavities [13]-[15].

Despite this apparent negative description of biofilms, they are not always unwelcomed as some of them play important roles in many environmental and industrial aspects, for example: by degrading environmental hazardous substances in soil (bioremediation). Moreover, biofilms are also employed in many engineering processes such as: bioreactors or bioflocculants in the separation of coal particles from mineral matter [14],[16]-[18] and waste water treatment systems [19].

The development of biofilms is generally considered a multi-steps process [20],[21] as depicted in Figure 1. When organic matter is present, such as: milk, tear fluid, urine, blood, saliva [13],[22], during the initial stage of attachment the substratum surface become covered by a layer of adsorbed organic material, known as the "conditioning film" [23],[24]. This conditioning film (Figure 1b) determines the outcome of the biofilm formation process as bacterial cells do not immediately contact naked surfaces. Any surface that may be exposed to an aqueous medium will be coated with molecules that are adsorbed from the medium, thereby forming the conditioning film. For example, in dentistry, a tooth may become coated in a proteinaceous layer made of albumin, lysozome, glycoproteins, lipids, and gingival crevice fluid [3],[25].

Bacterial adhesion is the critical step in biofilm formation [26]; once the microorganisms have attached themselves to the surface, the chances of further transport of other free-floating organisms increase thus resulting in coaggregation and formation of multiple layers (Figure 1c); this process is generally considered stochastic [27]. It is possible for coaggregation to occur in a variety of mechanisms depending on the system under consideration, for example by Brownian motion, gravitation, diffusion, convection and intrinsic motility of a microorganism.

Closely followed by the coaggregation process, is the "reversible stage" that involves the adhesion of single organisms and of microbial coaggregates; immediately after attachment to a substrate cells may deattach and return in the fluid bulk, however this adhesion becomes irreversible with time through the excretion of exopolymeric substances from the adhering microorganisms. These excreted exopolymeric substances adsorb to the substratum forming a microbially derived conditioning film as opposed to host or environmentally derived conditioning film. Figure 1c illustrates the initial biofilm formation due to the coaggregation and exopolymeric excretions of the adhering microorganisms; and it is represented by the blue film wrapping around the microorganisms.

As the process matures, further co-adhesion occurs between microbial pairs and the adhering microorganisms that are in contact with the substratum. Because of the conditioning film, the strength of the biofilm is dependent on the cohesiveness of the conditioning film [13],[28],[29] rather than on the direct interactions with the actual substrate. A firm irreversible adhesion through the exopolymer production and anchoring will occur with some sessile microorganisms which can stimulate the

adhesion of other, still suspended, planktonic microorganisms [1],[13],[30],[31]. Evidence suggests that some sessile microorganisms slow down on the approach to the surfaces, therefore, increasing the chances of adhering to the substratum [13][32]. Irreversible adhesion is represented in Figure 1d. Once the microorganisms have adhered, they start to grow (growth phase); this step is a major contributing factor to the accumulation of a high number of cells on a substratum surface. The growth phase is demonstrated in Figure 1e along with the accumulation of cells; this may also cause morphologic changes to the appearance of the structure [2],[33]-[35]. The final phase of formation (Figure 1f) is when the bacteria is released into the environment through the detachment of cells from the biofilm leading to a repeat of the cycle from the first stage in another location [2].

The problems or solutions resulting from the formation of biofilms, normally dependent on the microorganism nature (pathogenic or with some relevant industrial application), have stimulated the development of predictive models to forecast the possibility of cells adhesion or to further govern it. The control of microbial adhesion and biofilm formation can have two opposite goals: its prevention and inhibition or its enhancement and promotions [14]. As biofilms comprise of two distinct entities: the cell and the substrate, the focus can be directed on the properties either of the cell or of the substrate surface. Based on this classification, two types of studies can be identified; in one the properties of the substrate are modified and the resulting impact on the biofilm formation predicted [36]-[40]. In the other group of investigations, the focus is on the cell surface and how the genome or environmental conditions impact the microorganism cell surface properties and, consequently, its ability to form biofilms. These later works try to elucidate the role of specific compounds expressed by the cell such as: LPS [41],[42], DNA [43], surface proteins [44],[45] and other cell appendages [36] on the adhesion forces and overall biofim formation. More recently, colloidal approaches have been employed to describe the adhesion of viruses [46] and phages [47] to surfaces.

In this review, the fundamental theory in colloid science (Derjaguin, Landau, Verwey and Overbeek - DLVO) will be described and its application to bacterial adhesion will be presented along with subsequent improvements (xDLVO). Later, evidences of possible discrepancies between theory assumptions and experimental data will be introduced highlighting the pitfalls and cautions in employing a colloidal approach to biofilm formation.

2 Thermodynamic approach

The simplest method to predict whether a cell will adhere to a surface or not is the thermodynamic approach. Bacterial cell and liquid or solid surface thermodynamics are described by the surface tension [48]. When a cell adheres to a surface with a resulting contact area *A*, the system energy transition is from $A\gamma_{BL} + A\gamma_{SL}$ to $A\gamma_{BL}$ [48],[49] as the two interfaces liquid-solid and bacteria-liquid are replaced by one bacteria-solid (Figure 2a). The thermodynamic potential, or the free energy, variation as consequence of a bacteria adhering to a surface is, therefore:

$$\Delta G = A\gamma_{BS} - (A\gamma_{BL} + A\gamma_{SL}) \quad (1)$$

Where:

ΔG is the variation of free energy,

γ_{BS} is the bacterium-substratum interfacial energy

γ_{BL} is the bacterium-liquid interfacial energy

γ_{SL} is the substratum-liquid interfacial energy

A contact area

In order for this transition to be thermodynamically favored, the ΔG associate must be negative [50],[51], hence, the condition for a bacteria cell to adhere to a surface can be described as:

$$\gamma_{BS} < \gamma_{BL} + \gamma_{SL} \quad (2)$$

Similarly, coadhesion can be predicted. In this case two liquid-bacteria interfaces are replaced by one bacteria-bacteria (Figure 2b); the condition for co-adhesion to occur is:

$$\gamma_{BB} < 2\gamma_{BL} \quad (3)$$

This approach has been proven accurate in predicting cell adhesion only in few cases [38],[52],[53]; however, it is generally regarded too simplistic and inaccurate [54]-[56].

3 Surface energy components

The total surface free energy γ^{TOT} consists of two components:

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \tag{4}$$

γ^{LW} is the apolar component of the surface free energy associated with Lifshitz-Van der Waals interactions and γ^{AB} is the acid-base component of surface free energy. γ^{AB} results from the electron-donor (γ^-) and electron-acceptor (γ^+) molecular interactions (i.e. Lewis acid-base interactions). The acid-base term is expressed as the product of the electron donor and electron acceptor parameters:

$$\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-} \tag{5}$$

The interfacial energy between two phases *x* and *y* (γ_{xy}) is defined as van Oss et al. (1988) [57]:

$$\gamma_{xy} = \left(\sqrt{\gamma_x^{LW}} - \sqrt{\gamma_y^{LW}}\right)^2 + 2\left(\sqrt{\gamma_x^+\gamma_x^-} + \sqrt{\gamma_y^+\gamma_y^-} - \sqrt{\gamma_x^+\gamma_y^-} - \sqrt{\gamma_x^-\gamma_y^+}\right) \tag{6}$$

where the subscripts *x* and *y* refer to the two phases, respectively.

The surface energy parameters of a surface and contact angle of a liquid over it are linked by the Young-Dupre equation:

$$\gamma_L(1+\cos\theta) = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^+\gamma_L^-} + \sqrt{\gamma_S^-\gamma_L^+}\right) \tag{7}$$

This approach is commonly used to determine the surface energy components of a material [58]-[60] or of a bacteria [61]-[63] once the surface energy components of the chosen liquids are known. In case the Lewis acid-base interactions are neglected, the simplified van Oss-Chaudhury-Good equation [64]:

$$\cos\theta = \frac{2(\sqrt{\gamma_S^{LW}\gamma_L^{LW}}}{\gamma_L} - 1 \quad (8)$$

Can be used directly to estimate γ_S^{LW} for a bacteria or a surface once γ_L^{LW} is known.

4 Derjaguin, Landau, Verwey, Overbeek (DLVO) theory

4.1 Introduction

The DLVO theory forms the basis of modern colloid and interface science [65],[66]. The principal concept regarding the stability of lyophobic colloids was developed over 50 years ago by Derjaguin, Landau, Verwey and Overbeek. With added research, the theory was refined for the calculation of electrostatic and dispersion forces and their direct measurement [65]. Famously, the Derjaguin school has dealt with the physical aspects of the theory and colloid stability, whereas the Rehbinder school concerned with the colloid chemistry and physico-chemical mechanics. This resulted in both schools accounting for the same problems concerning the main factor of the theory, colloid stability. From Derjaguin, this main effect was thought to be secured by electrostatic repulsion forces; however, Rehbinder considered an absorption-solvation barrier as the main factor. Further developments came about when Derjaguin introduced the disjoining pressure as a measure of forces acting between two plane interfaces. This resulted in the DLVO theory been used to measure the interaction energy of flat surfaces directly using spherical bodies or crossed cylinders [65]. Derjaguin and Landau used the complete Debye-Huckel equation for electrical potential distributions between two similarly charged plates when applied to strong electrolytes.

In the DLVO theory, the energy of the system (ΔG^{TOT}) comprising of two particles immersed in a medium is the sum of the electrostatic interactions (ΔG^{EL}) and of the Lifshitz-van der Waals forces (ΔG^{LW}), with both interactions depending on the separation between particles (d).

$$\Delta G^{TOT}(d) = \Delta G^{LW}(d) + \Delta G^{EL}(d) \quad (9)$$

The Lifshitz-van der Waals forces have electromagnetic nature and originate from second-order perturbation theory to dipoles as first shown by London in 1930 [67]. The theory of dispersion interactions between macroscopic bodies separated by an interlayer has been developed since then using a microscopic or a macroscopic approach. In the former the resulting interaction is the sum of the individual contribution each pair of molecules, instead the macroscopic approach considers the two bodies as continuous separated by a thin interlayer (considered greater than the molecules forming the two bodies) and interacting through fluctuating electromagnetic field [68].

The electrostatic element originates from the Coulomb interaction between charged cells and substrate, it is usually described through the zeta potentials ϕ of the cell and substrate; its strength and range are strongly affected by the presence of surrounding ions [55],[69].

For identical particles the Lifshitz-van der Waals are attractive, whilst for non-identical particles these can be either attractive or repulsive [70],[71]; electrostatic forces can be attractive e or repulsive, furthermore the sign of such forces can also change with the separation distance [72].

However, there are cases where other interfacial phenomena (non considered in the DLVO theory) play a significant role in colloids interaction; these forces such as: hydration, hydrophobic and capillary, are grouped under the term "non DLVL forces" [73]-[75].

4.2 Application of DLVO to biofilms

The DLVO is extensively employed to predict colloids stability; however, in consequence of the microorganisms size being about 0.5-2 μm, it has been employed, since Marshal et al. [76], to study the initial phase of biofilm formation [13],[14] ,[77] and to predict the biofilm capability of microorganisms on different substrates [39],[40],[78]. Such investigations have been carried out using the equations developed to describe a model system made of one spherical particle and a flat surface:

The van der Waals interaction (ΔG^{LW}) can be estimated as:

$$\Delta G^{LW}(d) = -\frac{Ar}{6d} \tag{10}$$

Where :

A is the Hamaker constant

d is the separation distance between the cell and the substratum

r is the radius of the cell (as the cells are assumed to be spherical).

The Hamaker constant can be calculated from:

$$\Delta G_{slb}^{LW} = \frac{A}{12\pi l_o^2} \tag{11}$$

Where :

l_o is the minimum separation distance and assumed to be 0.157 nm [64]

$\Delta G_{slb}{}^{LW}$ is the Lifshitz-van der Waals component of the free energy of adhesion and it estimated as:

$$\Delta G_{slb}^{LW} = -2\left(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}}\right)\left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}}\right) \tag{12}$$

The electrostatic interaction (ΔG^{EL}) can be estimates through the Hogg, Healy and Fuerstenau (HHF) equation [79],[80][81]:

$$\Delta G^{EL}(d) = \pi\varepsilon\varepsilon_o r\left(\phi_b^2 + \phi_s^2\right)\left[\frac{2\phi_b\phi_s}{\phi_b^2 + \phi_s^2}\ln\left(\frac{1+e^{-kd}}{1-e^{-kd}}\right) + \ln\left(1-e^{-2kd}\right)\right] \tag{13}$$

This equation is valid for ϕ_b and ϕ_s < 25 mV and κ r > 10 [79] and:

ϕ_b and ϕ_s are the zeta potential of the bacteria and substrate

$\varepsilon\varepsilon_0$ is the dielectric permeability of the medium

κ is the reciprocal Debye length and it is calculated as:

$$\kappa = \sqrt{\frac{e^2\sum n_{i,\infty} z_i^2}{\varepsilon\varepsilon_o K_b T}} \tag{14}$$

Where:

$n_{i,\infty}$ is the bulk density of ions in solution for i-th species

K_b is Boltzmann's constant

e is the charge of the electron

z_i is the valence of i-th ion species

T is the Temperature

Each system will develop towards the energy minimum; assuming attractive van der Waals interactions, because of the various relations of the energy components upon the reciprocal distance, different scenarios can occur. In case the electrostatic forces are attractive or negligible as result of high ionic strength, the cells will adhere to the surface as the energy minimum is at a separation distance equal to 0 (Figure 3a). When the electrostatic forces are repulsive two cases can arise; the repulsive forces do not overcome the adhesive van der Waals forces at any separation distance, therefore, the energy minimum is at a separation distance equal to 0 (Figure 3b) and cell adhesion occurs. In the other case, the van der Waals forces prevail at long separation distances, whilst the repulsive electrostatic forces prevail at short separation distances; in this situation the energy presents a local minimum at a separation distance equal d. The cells will not adhere to the surface and remains separated (Figure 3c) Finally, the van der Waals forces can be predominant at long and short distances; in this case the energy profiles presents two minima and a local maximum (Figure 3d). The minimum at $d = 0$ is call primary minimum, whilst the other is called secondary minimum and the corresponding separation distance is denoted as d_{sm}. When this situation occurs, the cell is stable at a separation distance d_{sm} from the surface (reversible adhesion), however if the cells can overcome the energy barrier constituted by the local maximum, another stable situation can be reached (irreversible adhesion). Energy barriers up to 800 k_bT have been shown surmountable for bacteria to reach primary minimum position; alsoadhesion counts in the primary minimum increased with lowering energy barriers [83]-[85]. The switch from one situation to another can be the results of Brownian motion or the formation of cell surface features (fimbrie, curli and other adhesion exopolymer) that act like a bridge between cell and substrate [54],[83]. In absence of an energy barrier, the adhesion is proportional to the extent of absolute value of the total interaction energy [86]. When shear forces are

present because of fluid flow, bacteria captured in the energy minimum can slide along the fluid direction until desorption or irreversible adsorption occur [87].

4.3 xDLVO in bacterial cells adhesion

Numerous discrepancies between DLVO predictions and experimental evidences have been described highlighting the limitation of the DLVO theory in studying biofilm formation [42],[45],[83],[88]-[91]. In order to take into consideration other phenomena involved in colloidal adhesion and neglected by the DLVO approach, extended DLVO (xDLVO) theories have been developed. One of the most common form of xDLVO used in biofilm formation studies considers the total free energy of interactions (ΔG^{TOT}) between two surfaces immersed in an aqueous environment as the sum of the Lifshitz -van der Waals (ΔG^{LW}) forces, polar interactions (ΔG^{AB}) and electrical double layer (ΔG^{EL}) interactions [55],[83],[92]-[94], The mathematical formulation of xDLVO is:

$$\Delta G^{TOT} = \Delta G^{LW} + \Delta G^{AB} + \Delta G^{EL} \tag{15}$$

In both the classic DLVO and extended DLVO theories, the main components of colloid-size particles interaction are: apolar or Lifshitz-van der Waals components [48],[95] and polar components. The only difference is that, in the polar element the interaction in xDLVO, also the Lewis acid-base component is considered. The Lewis acid-base component is governed by the potential formation of coordinate covalent bonds by Lewis acids, i.e. electron pair acceptors and Lewis bases, i.e. electron donors. Acid–base interactions often play the most important role in bacterial attachment to surfaces [18],[52],[63],[86],[96], consequently the xDLVO theory provides better predictions than DLVO [97]. The Lewis acid-base component of the total energy can be calculated as Van Oss (1994) [64]:

$$\Delta G^{AB}(d) = 2\pi r \lambda \Delta G_{slb}^{AB} e^{\frac{l_0 - d}{\lambda}} \tag{16}$$

Where:

l_o is the minimum separation distance and assumed to be 0.157 nm [64]

λ is the correlation length of molecules in the liquid medium

ΔG_{slb}^{AB} is the acid Lewis component of the free energy of adhesion and is estimated as:

$$\Delta G_{slb}^{AB} = -2\left[\left(\sqrt{\gamma_B^+} - \sqrt{\gamma_S^+}\right)\left(\sqrt{\gamma_B^-} - \sqrt{\gamma_S^-}\right) - \left(\sqrt{\gamma_B^+} - \sqrt{\gamma_L^+}\right)\left(\sqrt{\gamma_B^-} - \sqrt{\gamma_L^-}\right) - \left(\sqrt{\gamma_S^+} - \sqrt{\gamma_L^+}\right)\left(\sqrt{\gamma_S^-} - \sqrt{\gamma_L^-}\right)\right] \quad (17)$$

λ is 0.6 nm for hydrophilic bacteria and 13 nm for hydrophobic bacteria [64]

4.4 Role of the secondary minimum in cell attachment to surfaces

The secondary minimum seems to play a critical role in explaining reversible attachment of cells [41],[84],[98],[99]; they can be loosely attach in the secondary minimum or "well" in virtue of the high energy barrier required to reach the primary minimum. As consequence of such weak attachment, these cells can be released from the surface when the electrostatic repulsion is enhanced though a reduction of the ionic strength or thorough Brownian motion. It has been demonstrated that adhesion increases with increasing energy associated to the secondary minimum [41],[100],[101]. A dimensionless parameter N_{DLVO} was also introduced by Elimelech (1992) [102] and defined as:

$$N_{DLVO} = \frac{kA}{\varepsilon_o \phi_b \phi_s} \quad (18)$$

Where the parameters have the same meaning as in Eq. 10 and 13.

N_{DLVO} incorporates the factors controlling the height of the DLVO energy barrier as well as the depth of the secondary energy minimum; its increase reflects a decrease in electrostatic repulsive forces and a corresponding increase in the depth of the secondary minimum [41]. Deposition rates of cells have been positively correlated to N_{DLVO} proving further evidence of the role of the secondary minimum in microbial adhesion [41].

Secondary minima with corresponding energy in the range -3 to -5 k_bT are considered to be necessary to support adhesion; this range originates from the assumption that the Brownian movement of a bacterium, as the principal detachment process, possesses energy of about 1.5 k_bT [103]-[105].

Additional evidences to this were presented by Jacobs et al. (2007) [106], who showed that bacteria-substrate curves exhibiting a secondary minima of less than 1 k_bT returned a very poor bacterial adhesion as consequence of the cell detachment. Similar results were also presented by Jucker et al. (1998)[107],[108]. These values are based on studies that employ xDLVO theory and neglect the Browning component in Eq. 16. If this is considered, such guidelines values need offsetting of 1 k_bT. The Brownian component can be estimated considering that particles adhering to a surface have two degrees of freedom instead of three as the perpendicular direction to the surface has been blocked by bonding. Brownian motion energy comprises of 1/2 k_bT per degree of freedom, the corresponding free energy term equal to 1 k_bT = 0.414 10^{-20} J (at 300 K); therefore [37],[64],[86]:

$$\Delta G^{BR} = 0.414 \ \ 10^{-20} \text{ J} \tag{19}$$

4.5 Estimation of the interaction forces

Both DLVO and xDLVO theories have been employed to estimate the forces involved in the adhesion of cells to substrates. In physic, it is well known that the force acting in direction k (F_k) is the first derivate of the energy (ϕ) in the direction k [110],[111]:

$$F_k = -\frac{\partial \phi}{\partial k} \tag{20}$$

The total force acting on a cell (F_d) can be estimated substituting in Eq 21 the formulation of the ΔG^{TOT} according to the DLVO or xDLVO model (equation Eq. 9 and 16 respectively). Assuming a monodimensional problem where only the separation distance (d) between cell and substrate is considered, the force acting on a cell is [81],[109],[111]:

$$F_d = -\frac{\partial \Delta G^{LW}}{\partial d} - \frac{\partial \Delta G^{EL}}{\partial d} - \frac{\partial \Delta G^{AB}}{\partial d} \tag{21}$$

This force is equal to zero when the separation distance corresponds to a point of minimum or maximum of the energy as the attractive and negative forces balance each other out; however,

interaction forces can be estimated determining the maximum attractive force at the minimum of the DLVO and xDLVO models [81],[109]. Despite this approach success in predicting interaction forces between membranes and colloids [112], it failed in biofilms studies [113],[90]

It has been reported that high adhesion forces enhance microbial adhesion, but are detrimental to the survival of bacteria after attachment as these forces can induce stress on the cells and impinging on their ability to duplicate [53]. Furthermore, the forces required to detach an adhering cell can increase with time after the initial attachment [114],[115], the rate of such change is dependent on the genetic profile of the cell and it is being shown to be strain dependent not only species specific [115],[116]. Adhesion forces between AFM tips and cells have been successfully modeled using DLVO [117],[118] or its extension xDLVO [119]. In order to apply the DLVO theory to this situation, the equations for the van der Waals and electrostatic forces between two dissimilar spheres are used. Such investigations have allowed the elucidation of the role of ionic strength [85] and growth temperature [118],[120] on *Listeria monocytogenes* adhesion properties.

5 Reasons for failure

Despite the numerous successes of xDLVO in predicting bacterial adhesion [18],[23],[39],[40], [83],[86],[100],[107],[108],[121], as described earlier, there also many evidences of its shortcomings [27],[41],[56],[78],[89],[97],[106],[122],[123]. These discrepancies have been described and attributed to the complexity of bacterial cells as living organisms, which is far from the ideal colloidal particles that these theories are based on. The main assumptions of the xDLVO theory are that substrate and cell surfaces are perfectly smooth and homogenous and the shear forces (lateral) caused by media flow are not considered. In the next section the evidences of the limitations of this theory are presented and discussed. Further refinements of the xDLVO theory to take into consideration such phenomena are also introduced.

5.1 Interactions between polymers covering surfaces

Some bacteria species excreted extracellular polymeric substances that can offer a steric interference when interactions between a bacterial cell and a substrate are considered. Such steric effects result in additional repulsive forces that can alter the DLVO prediction. A way to consider the steric interaction is thorough the inclusion of another term (ΔG^{ST}) in the overall estimation of ΔG^{TOT}; with this additional contribution, Eq. 16 becomes:

$$\Delta G^{TOT} = \Delta G^{LW} + \Delta G^{AB} + \Delta G^{EL} + \Delta G^{ST} \tag{22}$$

The Alexander-de Gennes equation [124],[125] is used to estimate the extent of repulsive interactions between two surfaces covered by polymers in a solvent [78],[122],[123]. According to this model, the repulsive force per unit area F/A_s (repulsive pressure) for two flat plates (at a separation distance d) covered with a neutral polymer of thickness L is [126]:

$$\frac{F(d)}{A_s} = \frac{k_b T}{s^3}\left[\left(\frac{2L}{d}\right)^{9/4} - \left(\frac{d}{2L}\right)^{3/4}\right] \quad \text{for } d < 2\text{L} \tag{23}$$

where:

s is the distance between polymer chains on the surface of the plates

d is the separation distance between the plates.

K_b is the Boltzmann constant

T is the Temperature

To consider the interaction between two spheres of radii R_1 and R_2, the Derjaguin approximation is used:

$$F(d) = 2\pi \frac{(R_1 R_2)}{R_1 + R_2} \int_d^{\infty} \frac{F(u)}{A_s} du \tag{24}$$

setting the upper limit of integration to $2L$ instead of ∞, Eq. 25 becomes [116]:

$$F(d) = 16\pi L \frac{(R_1 R_2)}{R_1 + R_2}\left(\frac{k_b T}{35 s^3}\right)\left[7\left(\frac{2L}{d}\right)^{5/4} + 5\left(\frac{d}{2L}\right)^{7/4} - 12\right] \tag{25}$$

For the case of asymmetric brush (brush layer against a solid substrate) the integration limit is *L* and Eq. 25 becomes [123]:

$$F(d) = 8\pi L \frac{(R_1 R_2)}{R_1 + R_2}\left(\frac{k_b T}{35 s^3}\right)\left[7\left(\frac{L}{d}\right)^{5/4} + 5\left(\frac{d}{L}\right)^{7/4} - 12\right] \tag{26}$$

The steric interaction energy $\Delta G^{ST}(h)$ is obtained integrating Eq. 26 or 27 [78]:

$$\Delta G^{ST}(d) = -\int_{\infty}^{d} F(u)du \tag{27}$$

Consequently, for the symmetric case, setting the upper limit of integration to *2L* instead of ∞, because of the range of validity of Eq. 24, results in:

$$\Delta G^{ST}(d) = -16\pi L \frac{(R_1 R_2)}{R_1 + R_2}\left(\frac{k_b T}{35 s^3}\right)\int_{2L}^{d} 7\left[\left(\frac{2L}{u}\right)^{5/4} + 5\left(\frac{u}{2L}\right)^{7/4} - 12\right]du \tag{28}$$

That is [123]:

(29)

Analogously, for the asymmetric case, when Eq. 27 is integrated (upper limit of integration *L*), the result is [123]:

$$\Delta G^{ST}(d) = 8\pi L^2 \frac{(R_1 R_2)}{R_1 + R_2}\left(\frac{k_b T}{35 s^3}\right)\left[28\left(\frac{L}{d}\right)^{1/4} - \frac{20}{11}\left(\frac{d}{L}\right)^{11/4} + 12\frac{d}{L} - \frac{420}{11}\right] \tag{30}$$

These equations can be applied to a system of a bacteria and a solid surface (a sphere-plate model) letting $R_2 \to \infty$, therefore:

$$\frac{(R_1 R_2)}{R_1 + R_2} = R_1 \tag{31}$$

Polymer-mediated interactions are not always repulsive (repulsive steric interactions) but can also be attractive (polymer bridging) depending on the properties of the polymers and the solid surface [123]; these attractive polymer-mediated interactions can occur in case the affinity of the polymers for another surface exceeds a certain critical value. For two spherical particles of radius R_1 and R_2, the bridging force can be approximated as [127]:

$$F(d) = -4\pi \frac{(R_1 R_2)}{R_1 + R_2} \varepsilon \Gamma \left(\frac{L_c - d}{l_s} \right) \tag{32}$$

Where:

Γ is the tailing density

d is separation distance

l_s is a segment length

L_c is the contour length

ε is the binding energy per segment

Furthermore, if:

$$\Gamma = \frac{1}{s^2} \tag{33}$$

Where: s is the distance between polymer chains on the surface of the plates; Eq. 33, becomes:

$$F(d) = -\frac{4\pi}{s^2} \frac{(R_1 R_2)}{R_1 + R_2} \varepsilon \left(\frac{L_c - d}{l_s} \right) \tag{34}$$

And the corresponding interaction energy term to be added in the xDLVO model (Eq. 23) is [123]:

$$\Delta G^{ST}(d) = \frac{4\pi}{s^2} \frac{(R_1 R_2)}{R_1 + R_2} \varepsilon \left(\frac{L_c d - d^2/2 - L_c^2/2}{l_s} \right) \tag{35}$$

Bridging forces are also involved in DNA condensation and are usually mediated by charged molecules in the media [128], it is therefore foreseeable that also bacterial polymers interaction forces are subjected to similar phenomena and that electrostatic could play a role in such interactions.

Predictions of *Pseudomonas* cells, a bacterium well known for producing exopolymers, adhesion to surfaces are, generally, not satisfactory when the xDLVO model is employed [41], but they greatly improve when the steric model is added to the other interaction terms of the theory [78],[122],[123][149], shedding lightly on the relevant importance of the steric interactions of the polymeric chains present on the surface.

5.2 Heterogeneity of surface properties

Colloidal theories rely on surfaces being homogeneous. A growing body of evidence indicates also that the degree of surface charge heterogeneity plays a critical factor in cell adhesion determining zones of favorable and other of unfavorable adhesion [98],[99],[129][130]-[133].

Not only material surfaces present levels of heterogeneity, but alsothe increasing application of AFM to biological investigations has demonstrated that bacterial cell surface is strongly heterogeneous. Some studies reported the cell properties such as: adhesion forces between AFM tip and cell as a single value (with some sort of experimental error, i.e. standard deviation from cell to cell). However, some authors have measured the cell surface properties on many points on the same cell, compiling a surface mapping of the cell surface (Figure 4). These studies have, therefore, given unconfutable evidences of the heterogeneity of cells surfaces [134]-[141].

5.3 Fluid flow

Fluid flow is an important factor in cells deposition and adhesion [142]; increasing fluid flow velocity increases microbial transport towards the substratum, i.e. convective diffusion; simultaneously increasing the detachment forces [41],[81],[116],[143]. Katsikogianni and coworkers [38][56][89] gave evidences of the success of the thermodynamic model in predicting adhesion under static condition but its failure when flow was present.

The dominant effect of fluid flow is the shear stress [81] that causes a microbial cell immersed in a moving fluid to be subjected to an additional force. In the general condition of liquid flow over a substrate, the shear force is tangential to the surface, therefore, perpendicular to the adhesive force considered by colloidal theories such as DLVO and xDLVO.

There are two critical values for shear stress: the first refers to the critical shear rate preventing adhesion; the second critical shear rate refers to the detachment of already adhering organisms. These two values of shear stress are not equivalent as the shear forces that are needed to stimulate the detachment are generally higher than those that can prevent attachment [48],[55],[81]. This is because, after the initial attachment, cells develop other adhering mechanisms (Extracellular Polymeric Substances (EPS), adhering proteins etc.) that result in the force required to detach a cell being greater than the force required to prevent a cell from sticking to a surface. The time required by a microorganism to develop the highest resistance against detachment by shear forces has been found to be linked to the hydrodynamic conditions of the location were the strain has been isolated [116]. The energy associated with the secondary minimum has also been correlated with the critical shear forces required for cells to adhere to a surface [109]; it can be assumed that cells will travel on the surface before attaching and a proportionality has been found between the energy associated with the secondary minimum and the highest shear rate that a cell can withstand before being unable to adhere, in other words, the higher the energy of secondary minimum the higher the shear force needed to prevent adhesion. In case the cell movement on the surface is predominantly "sliding" and not active swarming, the friction coefficient links the critical shear rate and the energy of the secondary minimum [109] and only when the friction force is greater than the shear force, adhesion is possible. Colloidal approaches can be successful in static conditions or low fluid velocity; nevertheless as shear forces increase with increasing flow rates., the impact of hydrodynamic conditions on biofilm formation is greater at high fluid velocity; hence the difference between colloidal predictions and experimental evidences is progressively marked with increasing flow.

5.4 Surface roughness

Biofilm formation can be influenced by surface roughness [143]-[145], however assessment of the impact of surface roughness and bacterial adhesion is still inconclusive as numerous reports also state that the number of adhering cells is unaffected by this [146]-[148]. The most likely sources of such discrepancies are the different nature of microorganisms investigated, the range of roughness analysed and the description of surface topographic features with a single parameter.

Colloidal approaches generally assume that the surfaces are perfectly smooth, therefore a direct investigation of surface roughness on cells adhesion is not possible through the classical formulation of DLVO or xDLVO. Recently, computational simulations using surface discretisation schemes have been employed to take into consideration the surface topography heterogeneity in DLVO predictions [150],[153]. In these works, that do not involve biofilms yet, the interacting surfaces have been divided into small areas, each with its own properties (charge and spatial coordinates) and the individual contribution estimated and added.

6 Conclusions

Microorganisms can colonise surfaces developing a layers of cells with a 3 dimensional structure denoted “biofilm”; such biofilms have properties distinguished from their floating counter parts and play significant role in many environmental, medical and industrial processes. In virtue of their resistance to potentially toxic compounds, they can be either a health concern or a biotechnological tool.

The initial stage of biofilm formation is the adhesion of cell to the surface; this process is governed by the physical chemical nature of both cell and substrate and can be interpreted using colloidal theories because of the microorganisms dimensions. DLVO is the classical approach in colloidal science, it is based on the overlapping effect of electrostatic and van der Waals forces and has been extensively employed in studying bacterial adhesion. Nevertheless, DLVO fails to completely predict the adhesion behavior of bacteria because it neglects other phenomena such as: acid-base and steric interactions. Extended forms of the DLVO theory, often called xDLVO, have made significant steps in improving predictions. However, cell adhesion is controlled also by fluid flow that is not considered in any of xDLVO theory proposed, therefore, when shear forces are the predominant factor influencing cell adhesion, DLVO can not be relied upon. Furthermore, growing evidences of surface heterogeneity gained through AFM surface mapping of cells highlight the limitation of such approach. In this review, DLVO and the corresponding extensions have been described and the reason

for failure presented proving an alerts for when xDLVO is likely to be unreliable, for example when cells exhibit polymers on the surface (like *Pseudomonas* spp) the steric interactions need considering. We have also introduced the recent approaches proposed to improve DLVO predictions, through computer simulation after surface discretisation, that allow considering heterogeneity.

7 References

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8 Figures caption

Figure 1 Schematic representation of the steps of the biofilm formation process.

Figure 2 Representation of the thermodynamic considerations in (a) cell adhesion and (b) co-adhesion.

Figure 3 Possible profiles of the system energy components according to the DLVO theory.

———— van der Waals interactions

- - - - - - - - - electrostatic interactions

———— total energy

Figure 4 Mechanical properties of alive or dead bacteria: (a) AFM deflection image of single living E. coli bacterium. A foot print can be seen on the right of the cell. (d) AFM deflection image of the inset in panel a; (g) AFM deflection image of the same single bacteria killed by thermal treatment (20 min, 45 °C). The foot print has disappeared. (b,e,h) Elasticity maps (z-range = 10 MPa) corresponding, respectively, to images a, d, and g inset. (c,f,i) Elasticity distribution with a typical force curve corresponding to b, e, and h Reprinted with permission from Cerf et al. Langmuir.2009;25(10):5731-5736. Copyright 2009 American Chemical Society

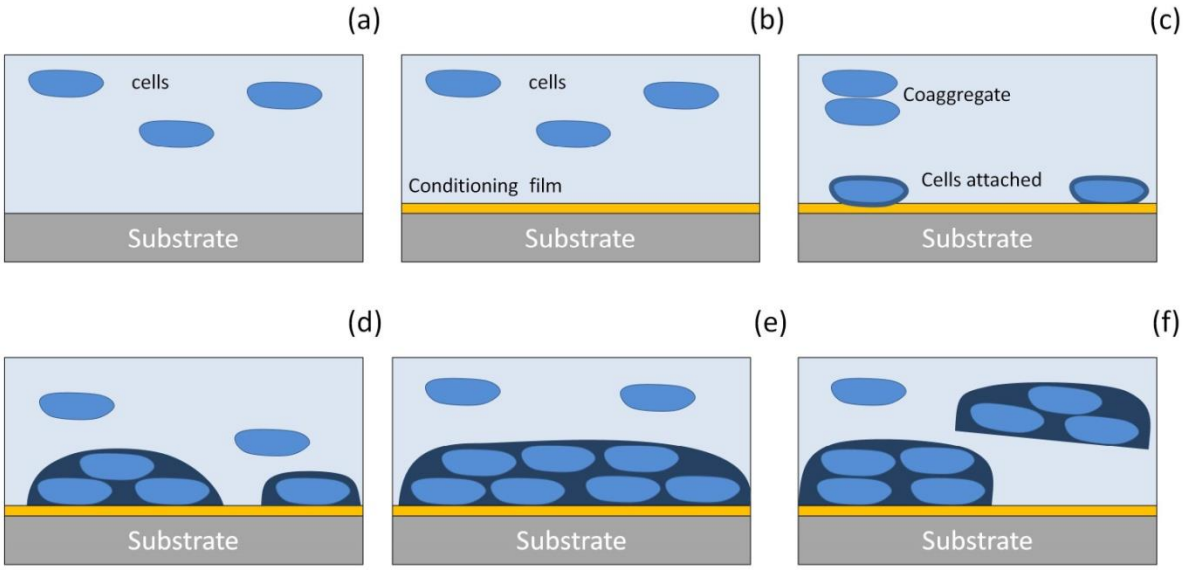
(a)
cells
Substrate
(b)
cells
Conditioning film
Substrate
(c)
Coaggregate
Cells attached
Substrate
(d)
Substrate
(e)
Substrate
(f)
Substrate

Figure 1

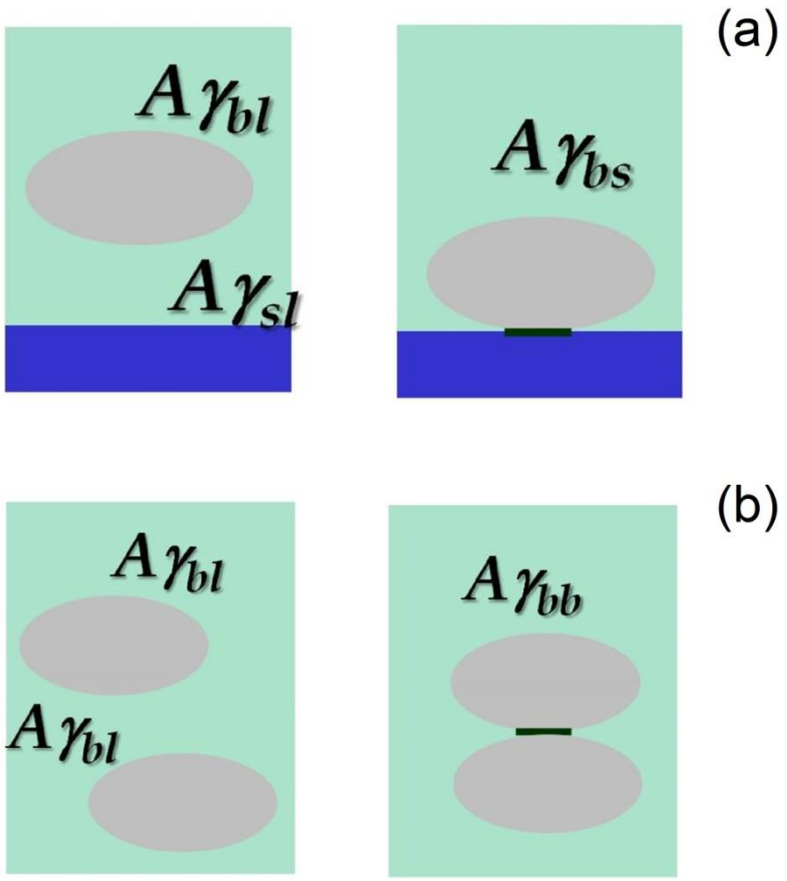
(a)
$A\gamma_{bl}$
$A\gamma_{sl}$
$A\gamma_{bs}$
(b)
$A\gamma_{bl}$
$A\gamma_{bl}$
$A\gamma_{bb}$

Figure 2

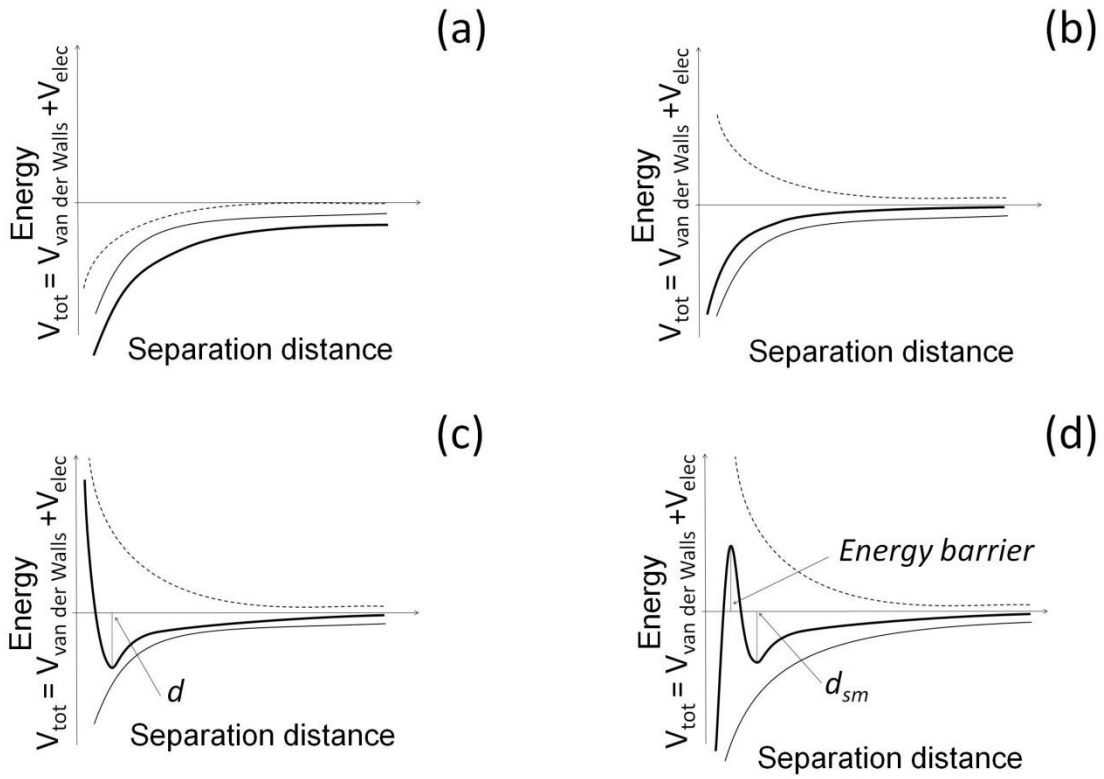
(a)
Energy
$V_{tot} = V_{van\ der\ Walls} + V_{elec}$
Separation distance
(b)
Energy
$V_{tot} = V_{van\ der\ Walls} + V_{elec}$
Separation distance
(c)
Energy
$V_{tot} = V_{van\ der\ Walls} + V_{elec}$
d
Separation distance
(d)
Energy
$V_{tot} = V_{van\ der\ Walls} + V_{elec}$
Energy barrier
d_{sm}
Separation distance

Figure 3

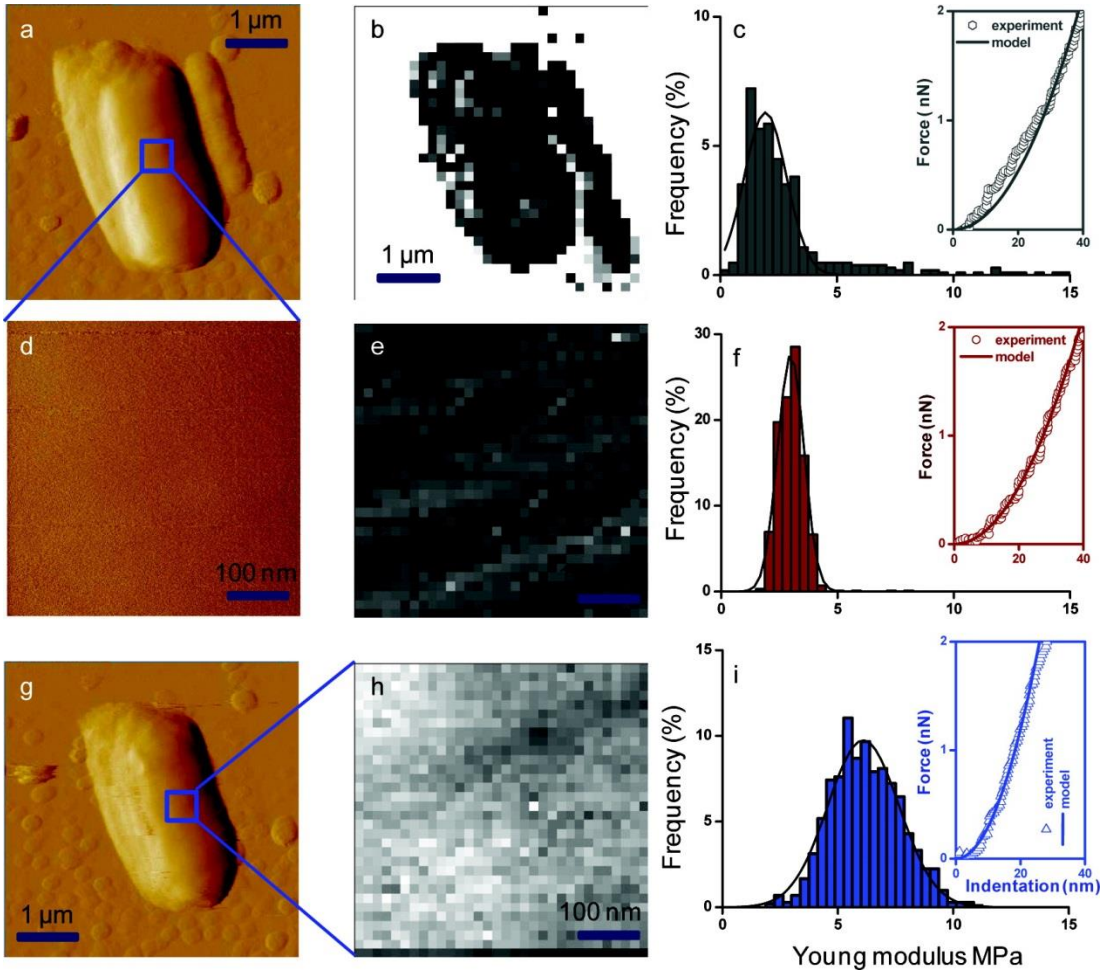
a
1 µm
b
1 µm
c
Frequency (%)
Force (nN)
experiment
model
d
100 nm
e
f
Frequency (%)
Force (nN)
experiment
model
g
1 µm
h
100 nm
i
Frequency (%)
Force (nN)
experiment
model
Indentation (nm)
Young modulus MPa

Figure 4