

# BACTERIAL ADHESION MECHANISM BY THERMODYNAMIC APPROACH

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**Abstract:** In this paper, the adhesion mechanism of microorganisms to various surfaces was explained by thermodynamic approach. A number of reports indicate that the surface energy of substrates affects bacterial adhesion. In this study the surface energies of four types of coatings were calculated based on the contact angle values of three test liquids. The adhesion energies of *Pseudomonas aeruginosa* to the surfaces were calculated. It is found that the bacterial adhesion is favourable when the adhesion energy is negative.

**Keywords:** Bacterial adhesion; Surface energy; Surface thermodynamics

## Introduction

Bacterial adhesion and biofilm formation on the surfaces of medical devices and implants, heat exchangers, food processing equipment, membrane filters, cooling water systems and ship hulls have been recognized as a widespread problem. Bacterial adhesion on implanted biomedical devices could lead to device-related infections, causing increased morbidity and mortality [1,2,3]. It is very important to understand bacterial adhesion mechanism in order to develop novel anti-bacterial coatings for medical devices and other industrial equipment.

Since microbial adhesion on the surfaces is a prerequisite for biofouling formation, prevention of microbial adhesion on the equipment surfaces will have a major impact in preventing biofouling. When microorganisms and substratum surfaces are in an aqueous environment, substratum surfaces will first become covered with a layer of adsorbed, organic molecules, generally called 'conditioning film' [4]. The second step in biofilm formation is the transportation of

microorganisms towards a substratum surface. Neu and Marshall [5] and Dufrière et al. [6] reported that microorganisms in suspension may transport towards each other and microbial co-aggregates can form. Subsequently, microbial adhesion may occur which is often initially reversible and becomes irreversible in time, amongst others through excretion of exopolymeric substances by the adhering microorganisms. Harris et al. [7] reported that the staphylococcus aureus adhered to coated surfaces as clumping, but sometimes the microorganisms attached to the surfaces directly. Initial microbial adhesive interactions are divided into adhesion to substratum surfaces, co-aggregation between microbial pairs and co-adhesion between sessile and planktonic microorganisms of different strains or species [8].

In this paper, the bacterial adhesion mechanism was explained with thermodynamics approach.

## Materials and Methods

*Thermodynamics approach* is based on surface energies of the interacting surfaces and does not include an explicit role for electrostatic interactions. It is expressed in terms of free energy of adhesion as:

$$\Delta G_{adh} = \gamma_{sb} - \gamma_{sl} - \gamma_{bl} \quad (1)$$

where  $\gamma_{sb}$ ,  $\gamma_{sl}$  and  $\gamma_{bl}$  are the solid-bacteria, solid-liquid, and bacteria-liquid interfacial free energies, respectively.  $\Delta G_{adh}$  can be calculated by Lifshitz-van der Waals acid/base (LW-AB) approach. The total

surface energy can be divided into Lifshitz van der Waals (LW) component and acid-base (AB) component[9]. In equation (1),  $\gamma_{sb}$ ,  $\gamma_{sl}$  and  $\gamma_{bl}$  can be described using following equations [10]:

$$\gamma_{sb} = \left( \sqrt{\gamma_s^{LW}} - \sqrt{\gamma_b^{LW}} \right)^2 + 2 \left( \sqrt{\gamma_s^+ \gamma_b^-} + \sqrt{\gamma_b^+ \gamma_s^-} - \sqrt{\gamma_s^- \gamma_b^+} - \sqrt{\gamma_s^+ \gamma_b^-} \right) \quad (2)$$

$$\gamma_{sl} = \left( \sqrt{\gamma_s^{LW}} - \sqrt{\gamma_l^{LW}} \right)^2 + 2 \left( \sqrt{\gamma_s^+ \gamma_l^-} + \sqrt{\gamma_l^+ \gamma_s^-} - \sqrt{\gamma_s^- \gamma_l^+} - \sqrt{\gamma_s^+ \gamma_l^-} \right) \quad (3)$$

$$\gamma_{bl} = \left( \sqrt{\gamma_b^{LW}} - \sqrt{\gamma_l^{LW}} \right)^2 + 2 \left( \sqrt{\gamma_b^+ \gamma_l^-} + \sqrt{\gamma_l^+ \gamma_b^-} - \sqrt{\gamma_b^- \gamma_l^+} - \sqrt{\gamma_b^+ \gamma_l^-} \right) \quad (4)$$

Substitute equations (2)-(4) into (1), the total free energy of adhesion  $\Delta G_{adh}$  is obtained:

$$\Delta G_{adh} = \left( -2\sqrt{\gamma_s^{LW} \gamma_b^{LW}} + 2\sqrt{\gamma_s^{LW} \gamma_l^{LW}} \right) + 2 \left( -\sqrt{\gamma_s^- \gamma_b^+} - \sqrt{\gamma_s^+ \gamma_b^-} - \sqrt{\gamma_l^+ \gamma_l^-} + \sqrt{\gamma_s^- \gamma_l^+} \right) + 2 \left( +\sqrt{\gamma_s^+ \gamma_l^-} - \sqrt{\gamma_l^+ \gamma_l^-} + \sqrt{\gamma_b^- \gamma_l^+} + \sqrt{\gamma_b^+ \gamma_l^-} \right) \quad (5)$$

From [8], we finally obtained:

$$\Delta G_{adh} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB} \quad (6)$$

The extended DLVO theory was proposed by van Oss [11], The principle interaction energies determining hetero-coagulation by the extended DLVO theory include a Lifshitz-van der Waals (LW) attractive interaction component, an electrostatic double-layer (EL) repulsive component, a Lewis acid-base (AB) component and a Brownian motion (Br) [10]. The total interaction energy  $\Delta E_{132}^{TOT}$  between a particle 1 and a solid surface 2 in liquid 3 can be written as the sum of these corresponding interaction terms:

$$\Delta E_{132}^{TOT} = \Delta E_{132}^{LW} + \Delta E_{132}^{AB} + \Delta E_{132}^{EL} + \Delta E_{132}^{Br} \quad (7)$$

The Lifshitz-van der Waals interaction energy between a particle 1 and a solid surface 2 in liquid 3 can be calculated using the following equations:

$$\Delta E_{132}^{LW} = -\frac{A_{132} \cdot R}{6H} \quad (8)$$

$$A_{132} = (\sqrt{A_{11}} - \sqrt{A_{33}})(\sqrt{A_{22}} - \sqrt{A_{33}}) \quad (9)$$

where  $H$  is the distance of separation and  $A$  is the Hamaker constant related to the properties of the interacting materials. van Oss [10] presented a very simple method for the calculation of Hamaker constant based on the surface energy of the interaction materials:

$$A_{ii} = 24\pi H_0^2 \cdot \gamma_i^{LW} \quad (10)$$

where  $\gamma_i^{LW}$  is the Lifshitz-van der Waals apolar component of the surface energy and  $H_0$  is the minimum equilibrium distance between the two interacting bodies, which has been found for a large range of materials to be equal to 0.157 nm. Combining equation (8)-(10), we obtained:

$$\Delta E_{132}^{LW} = \frac{2\pi H_o^2 \cdot R}{H} \cdot \left[ -2 \left( \sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}} \right) \left( \sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}} \right) \right] \quad (11)$$

The contribution of  $\Delta E_{132}^{AB}$  to the overall interaction energy  $\Delta E_{132}^{TOT}$  between the particle 1 and the surface 2 in medium 3 is given by:

$$\begin{aligned} \Delta E_{132}^{AB} &= 2\pi R \lambda \int_H^\infty \Delta E_{132(H_o)}^{AB} \exp\left(\frac{H_o - H}{\lambda}\right) dH \\ &= 2\pi R \lambda \exp\left(\frac{H_o - H}{\lambda}\right) \Delta E_{132(H_o)}^{AB} \end{aligned} \quad (12)$$

$$\Delta E_{132(H_o)}^{AB} = 2 \left( \begin{aligned} & -\sqrt{\gamma_1^- \gamma_2^+} - \sqrt{\gamma_1^+ \gamma_2^-} - \sqrt{\gamma_3^+ \gamma_3^-} \\ & + \sqrt{\gamma_1^- \gamma_3^+} + \sqrt{\gamma_1^+ \gamma_3^-} - \sqrt{\gamma_3^+ \gamma_3^-} \\ & + \sqrt{\gamma_2^- \gamma_3^+} + \sqrt{\gamma_2^+ \gamma_3^-} \end{aligned} \right) \quad (13)$$

In above four interaction components in equation (7), only Lifshitz-van der Waals (LW) interaction component and Lewis acid-base (AB) component involve surface energy components of the solid surface. When  $H = H_o$ , the sum of  $\Delta E_{132}^{LW} + \Delta E_{132}^{AB}$  is equal to

$$\Delta G_{adh} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB}. \text{ In this case the extended}$$

DLVO theory is simplified to the thermodynamics approach.

In this study four various surface coating, (PTFE (PolyTetraFluoroEthylene), PFA (perfluoroalkoxy-alkane), Nylon and Ni) were prepared for bacterial adhesion experiment.

*Contact angle measurements:* Prior to contact angle measurement, samples were ultrasonically cleaned in acetone, ethanol and deionized water in sequence. Contact angles were obtained using the sessile drop

method with a Dataphysics OCA-20 contact angle analyzer. This instrument consists of a CCD video camera with a resolution of 768×576 pixel and up to 50 images per second, multiple dosing/microsyringe units and a temperature controlled environmental chamber. The digital drop image was processed by an image analysis system, which calculated both the left and right contact angles from the shape of the drop with an accuracy of ±0.1°. Three test liquids were used as a probe for surface free energy calculations: distilled water, diiodomethane (Sigma) and ethylene glycol (Sigma). The data for surface tension components of the test liquids at 20 °C are given in Table 1.

Table 1: Test liquids and their surface tension components

Surface tension (mN/m)	$\gamma_L$	$\gamma_L^{LW}$	$\gamma_L^{AB}$	$\gamma_L^+$	$\gamma_L^-$
Water, (H <sub>2</sub> O)	72.	21.	51.	25.	25.
	8	8	0	5	5
Diiodomethane, (CH <sub>2</sub> I <sub>2</sub> )	50.	50.	0	0	0
	8	8			
Ethylene glycol, (C <sub>2</sub> H <sub>6</sub> O <sub>2</sub> )	48.	29.	19.	1.9	47.
	0	0	0	2	0

The surface energies of the coatings were calculated based on the contact angle values of three test liquids. The free energy of adhesion was calculated by thermodynamics approach, using equation (5) and (6). All the calculation values are shown in Table 2.

Table 2: Surface energies of the coatings

Coating	Surface energy (mN/m)				$\Delta G_{adh}$ (mN/m)
	$\gamma^{LW}$	$\gamma^{AB}$	$\gamma^+$	$\gamma^-$	
PTFE	21.35	1.89	0	6.21	6.0
PFA	16.29	0.89	0	4.99	5.5
Nylon	12.59	0	0	0	-11.3
Ni	37.28	4.48	0.68	7.38	-0.6

## Results and Discussions

*Pseudomonas aeruginosa* was used in bacterial adhesion experiments. Figure 1 shows the effect of the adhesion energy  $\Delta G_{adh}$  on the bacterial adhesion to the

four surfaces coatings. Clearly, the number of adhered bacteria decreased with  $\Delta G_{adh}$  value increasing.

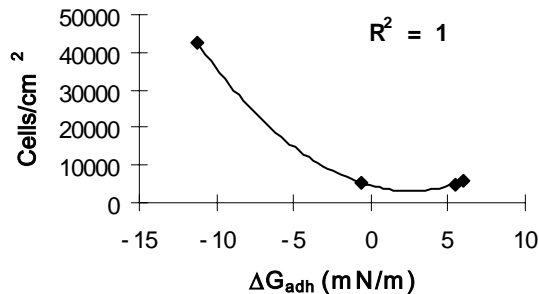


Figure 1: Effect of the adhesion energy on cell attachment.

This result is consistent with the results of Rolf Bos *et al.* [8] that bacterial adhesion is favourable to occur, when  $\Delta G_{adh}$  is negative ( $\Delta G_{adh} < 0$ ).

## Conclusions

The number of adhered bacteria decreased with  $\Delta G_{adh}$  value increasing. The surface energy of the substrates also has a significant influence on the bacterial adhesion. More detailed investigation will be performed to confirm the initial results.

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