

## Hydrophobicities and Electrostatic Behavior of Different Micro-organisms

Abbas YOUSEFI RAD,

Bayındır Hospital, Medical Center, Söğütözü, Ankara–TURKEY

Hakan AYHAN, Erhan PIŞKİN\*

Hacettepe University, Chemical Engineering Department, Bioengineering Division, and  
TÜBİTAK–Centre of Excellence: Polymeric Biomaterials, Beytepe, Ankara–TURKEY

Özgül KISA

Gülhane Medicine Academy of Military, GATA, Microbiology and Clinic Microbiology Division,  
Ankara–TURKEY

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**Abstract:** The surface properties of 7 different micro-organisms with their 14 different strains were examined. The contact angles of the micro-organism films were measured by a captive-bubble technique. Surface free energies were calculated from the contact angles. Hydrophobicities were also evaluated by adhesion to *p*-xylene. The zeta potentials and surface charges of the micro-organisms were measured using a zeta potential analyser system. The contact angles of the gram positive micro-organisms, gram negative micro-organisms and *Candida albicans* were in the ranges 48–70°, 43.5–55°, and 69–75° respectively, while the corresponding surface free energies were in the ranges 44.9–60.4 erg.cm<sup>-2</sup>, 55.3–61.8 erg.cm<sup>-2</sup> and 46.9–50.6 erg.cm<sup>-2</sup> respectively. The *p*-xylene adhesions were in parallel to the hydrophobicities defined by the contact angles: 35.4–80.3%, 2.3–36.6%, and 67.3–86.2 for the gram positive bacteria, gram negative bacteria and *Candida albicans* yeasts respectively. The zeta potentials for these bacteria were in the ranges 14.5–650.2 mV, 6.0–159.6 mV, and 20.7–21.7 mV respectively. Most of the bacteria were negatively charged, except for the CNS-2 and CPS-1 strains.

**Key Words:** *Micro-organisms, hydrophobicity, contact angle, surface free energy, p-xylene adhesion, zeta potential, surface charge.*

### Farklı Mikroorganizmaların Hidrofobisitesi ve Elektrostatik Davranışları

**Özet:** Bu çalışmada 7 farklı mikroorganizmanın 14 değişik suşlarının yüzey özellikleri araştırılmıştır. Mikroorganizma filmlerinin temas açıları kabarcık-yakalama tekniği ile ölçülmüştür. Yüzey serbest enerjileri, temas açılarından yararlanılarak hesaplanmıştır. Hidrofobisiteyi *p*-ksilen'e yapışma miktarı ile değerlendirilmiştir. Gram pozitif mikroorganizmaların, gram negatif mikroorganizmaların ve *Candida albicans*'in temas açıları sırası ile 48–70°, 43.5–55° ve 69–75° aralığındadır. Aynı zamanda bu açılara karşılık gelen yüzey serbest enerjileri de sırasıyla 44.9–60.4 erg.cm<sup>-2</sup>, 55.3–61.8 erg.cm<sup>-2</sup> ve 46.9–50.6 erg.cm<sup>-2</sup> dir. *p*-Klisen'e yapışma miktarları temas açıları olarak tanımlanan hidrofobisitelere paralel olarak Gram pozitif, gram negatif bakterilere ve *Candida albicans*'a sırası ile % 35.4–80.3, % 2.3–36.6 ve % 67.3–86.2 olarak bulunmuştur. Bu bakteriler için zeta potansiyel değerleri de sırası ile 14.5–650.2 mV, 6.0–159.6 mV ve 20.7–21.7 mV aralığındadır. CNS-2 ve CPS-1 suşlarının dışındaki bakteriler negatif yüklüdür.

**Anahtar Sözcükler:** *Mikroorganizmalar, hidrofobisite, Temas açısı, Yüzey serbest enerjisi, p-ksilen'e yapışma, zeta potansiyel, yüzey yükü.*

\* Author for further correspondence.

## Introduction

Microbial adhesion to a surface is known to play an important role in a wide variety of situations, e.g., infection of various tissues (1), dental decay (2), ship fouling (3), fermentation (4), and waste water and sewage treatment (5). However, the fundamental mechanisms governing microbial adhesion are poorly understood and have not been well defined. Most work to date on microbial adhesion has dealt with the influence of substrata surface properties on the extent of the relative adhesion (6), growth of the adhering microbes, and their subsequent behavior (7).

In medical applications, the adhesion of micro-organisms on to inserted or implanted biomaterials is thought to be an essential step in the pathogenesis of material- and medical device-associated infections (8–10). In recent years, an increasing number of attempts have been made to understand the mechanisms of microbial adhesion to biomaterials, prevention of infection at the time of surgery, and, more interestingly, the development of antimicrobial surfaces (11–17). The data reported in the related literature indicate the complexity of the phenomenon of biomaterial-associated infection and the increasing likelihood that there is no single simple explanation. A series of events occurs at the time of implantation. Microbial adherence to biomaterials is the initial event, followed by colonization and the formation of an adherent biofilm which embeds bacteria on the material surface, rendering antibiotic treatment and the host defense mechanism ineffective, and which is also assumed to be a continuous focus of infection (18, 19). The surface properties of both the micro-organisms and biomaterial are considered essential factors in the adhesion and proliferation of micro-organisms on the biomaterial surface (20, 21). Hydrophobicity and electrostatic charges of both surfaces seem to be the most important factors in the nonspecific adhesion of bacteria to interfaces (22–26). Moreover, several recent studies with pathogenic bacteria indicate that specific surface receptors may influence the interaction (27). Recently, strategies that change (or improve) the surface properties of the biomaterials used to decrease microbial adhesion, in other words to develop antimicrobial surfaces, have received increased attention (28, 29). In our recent related studies we attempted to prepare antimicrobial surfaces by a novel technique, i.e., low-temperature plasma technology (28). In this paper, we report the surface properties of the micro-organisms that were utilized in these studies, which provide the essential data for the discussion of microbial adhesion in the later part of the study.

## Material and Methods

### Micro-organisms: Storage and Growth

The micro-organisms investigated in this study were isolated from different sources at Bayındır Hospital (Ankara, Turkey). They are described in Table 1 and their respective abbreviations are given. A total of 7 different micro-organisms with their 14 different strains were evaluated. Note that none of the micro-organisms had a capsule, determined using India ink (30).

Table 1. Sources of micro-organisms used in this study.

Micro-organisms	Clinical Source
Gram Positive Micro-organisms	
<i>Coagulas positive staphylococcus</i> Strain-1 (CPS-1)	From wound due to suture infection
<i>Coagulas positive staphylococcus</i> Strain-2 (CPS-2)	Aspiration catheter
<i>Coagulas negative staphylococcus</i> Strain-1 (CNS-1)	Blood culture
<i>Coagulas negative staphylococcus</i> Strain-2 (CNS-2)	Blood cluture
<i>Corynebacterium spp.</i> Strain-1 (CB-1)	Vaginitis
<i>Corynebacterium spp.</i> Strain-2 (CB-2)	Skin wound
<i>Streptococcus pyogene</i> Strain-1 ( <i>S.pyog</i> -1)	Blood culture
<i>Streptococcus pyogene</i> Strain-2 ( <i>S.pyog</i> -2)	Skin wound
Gram Negative ve Micro-organisms	
<i>Escherichia coli</i> Strain-1 ( <i>E. coli</i> -1)	Catheter from infected urinary tract
<i>Escherichia coli</i> Strain-2 ( <i>E. coli</i> -2)	Blood culture
<i>Pseudomonas aeruginosae</i> Strain-1 ( <i>P. aeru</i> -1)	Skin wound
<i>Pseudomonas aeruginosae</i> Strain-2 ( <i>P. aeru</i> -2)	Blood culture
Candida albicans	
<i>Candida albicans</i> Strain-1 ( <i>C. albicans</i> -1)	Catheter from infected uninary tract
<i>Candida albicans</i> Strain-2 ( <i>C. albicans</i> -2)	From infected urinary tract

After primary isolation and classical identification, all of the micro-organisms except the *Streptococcus pyogene* and *Corynebacterium spp* strains were first grown in brain-heart infusion borth (BHI) (Difco Laboratories, Detroit, Mich., USA) overnight at 37°C. Glycerol (10%) was added to these media, and they were divided into 2 ml batches in several small tubes (5 ml), and then stored frozen at -40°C for long-term storage (3-6 months). The same procedure was applied for the *Streptococcus pyogene* and *Corynebacterium spp* strains, but Todd-Hewith broth (TOH) (Difco Laboratories, Detroit, Mich., USA) containing glycerol 10% was used instead of BHI.

For short-term (one month or less) storage, the frozen samples were first brought to room temperature, and were then cultured on sheep blood agar plates (Oxoid Ltd., London, England) overnight at 37°C. All of the micro-organisms, except for the *Streptococcus pyogene* and *Corynebacterium spp* strains, were recultured in tubes (held in the horizontal position) containing nutrient agar overnight at 37°C. Samples in which growth was successful were maintained at 4°C for about 1 month. The same procedure was applied for the *Streptococcus pyogene* and *Corynebacterium spp* strains, using sheep blood agar in the horizontal tubes instead of nutrient agar.

The following procedure was employed to obtain suspensions of the micro-organisms for daily use: the micro-organisms were removed from the short-term storage conditions, placed

in 100 ml of liquid growth media (i.e., BHI or TOH), and then shaken in a rotary shaker (New Brunswick Scientific Co., USA) at 120 rpm at 37°C for 18 h. Note that TOH was used for the *Streptococcus pyogenes* and *Corynebacterium spp* strains, while BHI was the growth media for the other micro-organisms. The micro-organisms were harvested by centrifugation at 5000 rpm for 10 min and subsequently washed three times with PBS buffer, pH: 7.4 ( $8.1 \times 10^{-3}$  M  $\text{Na}_2\text{HPO}_4$ ,  $1.46 \times 10^{-3}$  M  $\text{KH}_2\text{PO}_4$  with 0.14 M NaCl,  $2.7 \times 10^{-3}$  M KCl,  $9.2 \times 10^{-4}$  M  $\text{CaCl}_2$  and  $1.07 \times 10^{-3}$  M  $\text{MgCl}_2$ ). The washed micro-organisms were resuspended in PBS. In order to remove the clusters of micro-organisms, the suspensions were filtered through a membrane filter (Type SC; Milipore Corp., USA) with a pore size of 0.8  $\mu\text{m}$ . The concentration of the micro-organisms in the suspensions was adjusted to  $2 \times 10^9$  CFU/ml. Concentrations were measured by a spectrophotometer (Model 259, Ciba Corning Diagnostics Ltd., Halstead, Essex, UK) at 660 nm for *Candida albicans* and 540 nm for the other micro-organisms. The final concentration was also confirmed by means of the Colony Forming Unit (cfu) Method.

#### Contact Angle Measurements

In order to define the relative hydrophobicities of the micro-organisms used in this study, the contact angles of the microbial-films were measured using a captive-bubble technique (31). In short, the suspension of the micro-organisms carrying  $1 \times 10^9$  cells/ml was filtered through a cellulose acetate filter (Type SC; Milipore Corp., USA) with a nominal pore size of 0.45  $\mu\text{m}$  under vacuum to remove the aqueous phase, which formed a homogeneous multilayer microbial film containing approximately  $10^8$  cells/ $\text{mm}^2$ . The filter with the micro-organism-film was placed upside down in a distilled water chamber. An air bubble of about 10  $\mu\text{L}$  volume was injected into the chamber onto the surface of the microbial film. A micrograph of the captured bubble was taken about 30 sec after air bubble-surface contact, using an optical microscope and camera system (PM-6 Camera, serial no: 225060, VS-IV optical system, Olympus, Tokyo, Japan). The contact angle ( $\theta_{\text{air}}$ ) was evaluated from the height (h) and the width (d) of the air bubble at the specimen surface, and calculated by the equations below (32). Five measurements were taken and the average values and standard deviations were calculated for each surface.

$$\text{for } \theta_{\text{air}} < 90 \quad \theta_{\text{air}} = \cos^{-1} (2h/d - 1) \quad (1)$$

$$\text{for } \theta_{\text{air}} > 90 \quad \theta_{\text{air}} = 180 - 2 \tan^{-1} (2h/d) \quad (2)$$

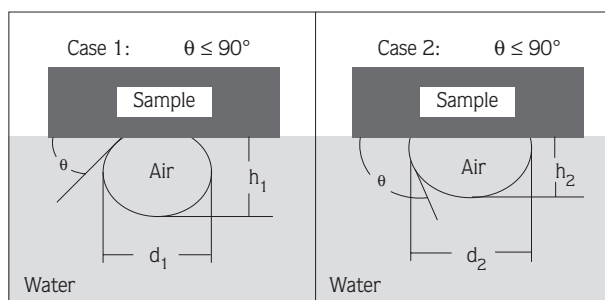


Figure 1. Contact angle measurements.

### Surface Free Energies

The surface free energies of the micro-organism films were calculated from the contact angle values using the equation below, which was originally applied to obtain the surface free energies of solid surfaces (33), and was then adapted to micro-organisms films by Minagi et al. (31).

$$\cos \theta = \frac{(0.015\gamma^{MV} - 2) (\gamma^{MV} \cdot \gamma^{LV})^{1/2} + \gamma^{LV}}{[0.015 (\gamma^{MV} \cdot \gamma^{LV})^{1/2} - 1]} \quad (3)$$

Here,  $\gamma^{MV}$  and  $\gamma^{LV}$  are the surface free energies of the micro-organism film and water respectively ( $\text{erg/cm}^2$ ).  $\gamma^{LV}$  was  $64 \text{ erg/cm}^2$  (34).

### Adhesion to p-Xylene

The relative hydrophobicities of the micro-organisms were also measured using the *p*-xylene method described in the literature (35). In short, 3 ml of the suspensions of the micro-organism were placed in a series of round-bottom glass test tubes (5 ml), and different amounts of *p*-xylene, ranging from 0 to 1 ml, were added to each tube. The mixtures were vigorously agitated for 60 sec using a vortex-mixer (Stuart Scientific Co. Ltd., UK). Then, two phases were allowed to fully separate in 20 min. The optical densities of the samples taken from the aqueous phase were measured: 660 nm for *Candida albicans* and 540 nm for the other micro-organisms. The relative optical densities and percentage of micro-organisms in the hydrocarbon phase (i.e., *p*-xylene) were calculated using the equations stated below. Note that these tests were repeated at least three times and the mean values  $\pm$  standard deviations were evaluated. The optical densities (ODs) of both the original and *p*-xylene treated micro-organisms were measured, and the relative optical density and percentage of micro organisms in the *p*-xylene phase were calculated from the following equations:

$$\text{Relative optical density (ROD)} = \frac{\text{Optical density of the } p\text{-xylene treated micro-organisms}}{\text{Optical density of micro-organisms}} \quad (4)$$

$$\text{Percentage of micro-organisms in } p\text{-xylene phase (\%)} = 100 - 100 \times \text{ROD} \quad (5)$$

### Zeta Potentials

The zeta potentials and surface charges of the micro-organisms were measured by a zeta potential analyser system (Freeport Sulphur Com., New York, USA). This system contains two chambers. Both chambers were filled with the same micro-organism suspension. The smaller chamber was weight ed precisely, and then placed in the larger one. The window between the chambers was removed to bring the suspensions into direct contact. The current level and the time were 2 amp and 4 min respectively. The polarity applied to the small chamber was positive in all cases. After this procedure, the window was closed, and the small chamber was removed

and weighted. Note that a decrease in the weight of the small chamber shows that the micro-organisms were positively charged. Conversely, an increase in weight is obtained if the micro-organisms are negatively charged.

The densities of the micro-organism suspensions and PBS (which was used for the preparation of the suspensions) were measured using appropriate picnometers. The electrical resistance of the micro-organism suspensions was measured using the same zeta potential analyser system, following the method given in the manufacturer's manual (36). Using the weights of the small chambers before and after the test, the densities, electrical resistances, the viscosity of the water, the dielectric constant of the water (relative to the air), and the conductivity of the cell constant of the small chamber, the zeta potentials were calculated from the formula given below (36):

$$\Phi = \frac{w_1/\rho_1}{(w_1/\rho_1) + (w_2/\rho_2)} \quad (6)$$

$$V_E = \pm \frac{K \cdot \Delta W}{R \cdot t \cdot I \cdot \Phi (1-\Phi) (\rho_1 - \rho_2)} \quad (7)$$

$$\xi = \frac{36 \times 10^4 \cdot \pi \cdot V_E \cdot \eta}{D} \quad (8)$$

Here,  $\Phi$ : volume fraction of micro-organisms;  $V_E$ : electrophoretic mobility ( $\text{cm} \cdot \text{sec}^{-1} \cdot \text{mV}^{-1} \cdot \text{cm}^{-1}$ );  $\xi$ : zeta potential (mV);  $\rho_1$  and  $\rho_2$ : densities of the micro-organisms and liquid medium (i.e., PBS) respectively ( $\text{g} \cdot \text{cm}^{-3}$ );  $w_1$  and  $w_2$ : weight fractions of the dried micro-organisms and PBS respectively;  $\Delta W$ : weight change of the small chamber before and after each test (g);  $K$ : cell constant (which was  $0.803 \text{ cm}^{-1}$ );  $R$ : resistance of the micro-organisms suspension (ohm);  $t$ : time (sec);  $I$ : current (amp);  $\eta$ : viscosity of the water (which was  $0.008937$  poise); and  $D$ : dielectric constant of the water (which was  $78.54$ ).

## Results and Discussion

In this study, the surface properties of seven different micro-organism with their 14 different strains (see Table 1) were examined. In order to define the relative hydrophobicities of the micro-organisms used, the contact angles of the micro-organism films were measured by a captive-bubble technique. The surface free energies were calculated from the contact angles. In parallel, hydrophobicities were evaluated by the *p*-xylene method. The zeta potentials and surface charges of the micro-organisms were also determined. The hydrophobicities and zeta potentials of the micro-organisms are presented and discussed separately in the subsections below.

### Hydrophobicities

The hydrophobicity of micro-organisms is considered one of the most important parameters, especially in terms of adhesion to solid surfaces (37, 38). Several methods have been used to determine the hydrophobicities of various micro-organisms, including measuring the contact angles of a water-droplet on a microbial lawn (21–23), adhesion of micro-organisms from an aqueous suspension to a liquid hydrocarbon phase (35), and using hydrophobic chromatography techniques (40). A comparison of the different approaches shows that very hydrophobic and very hydrophilic micro-organisms behave similarly in these tests but micro-organisms with intermediate hydrophobicity behave differently in different tests (39, 40). In the present study, the two most widely employed techniques, i.e., contact angle measurements and adhesion to *p*-xylene, were applied to measure the hydrophobicities of the selected microbial strains. The hydrophobicities of the micro-organisms are compared below by categorization into three basic subgroups, i.e., gram positive bacteria, gram negative bacteria and *Candida albicans*.

#### Gram Positive Bacteria

The contact angles of the gram positive bacteria used in this study are given in Figure 2, in the form of bar graphs. Note that the average values and standard deviations are also presented in Table 2. The contact angle values were in the range 48–70°, which may be considered

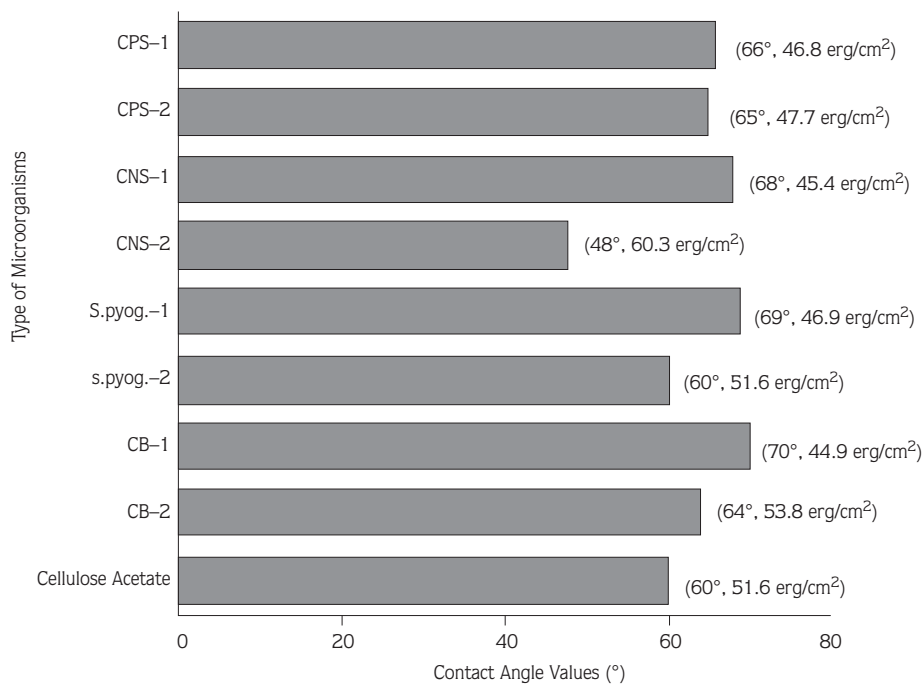


Figure 2. Contact angles of gram positive bacteria.

moderately hydrophobic. Notice that the contact angle value for the reference surface (i.e., the cellulose acetate membrane) was 60. Only the CNS-2 strain was relatively hydrophilic (relative to the reference surface), while the contact angles of the other gram positive bacteria were similar to each other, but were greater (meaning more hydrophobic) than those for the reference surface. In parallel to the contact angle values, the surface free energies of the micro-organisms were similar to each other and were in the range 44.9–60.4 erg.cm<sup>2</sup>. The surface free energies for several gram positive bacteria, including *S. aureus*, *S. epidermidis* and *L. monocytogenes*, were around 66–69 erg.cm<sup>2</sup>, as reported by Absolom et al. (37).

Figure 3 gives the relative optical densities (ROD) and percentages of the bacteria in the hydrocarbon phase (i.e., *p*-xylene), which were calculated using Eqs. 4 and 5. The average values and standard deviations are also shown in Table 2. Note that lower values of the ROD, and, in contrast, higher values of the percentages of the micro-organisms in the *p*-xylene phase correspond to more hydrophobic bacteria. According to the *p*-xylene method, the CNS-1, CB-1 and CB-2 strains were the most hydrophobic bacteria, while the CNS-2 strain was the most hydrophilic micro-organisms, which was parallel to the results obtained by the contact angle measurements. However, the correlation between these two alternative methods was not that great in the case of some other bacteria, such as in the case of the *S. pyog.*-2 and CPS-2 strains.

Hogt et al. investigated the adherence of a series of coagulase-negative *Staphylococci* (CNS) to xylene-water emulsions and FEB films, and reported adherence values over a wide range (40). Pascual et al. studied hydrophobicities in CNS strains, again using the *p*-xylene method, and showed that some strains are hydrophobic while the others are quite hydrophilic (41). Hogt et al. measured the hydrophobicities of two *S. epidermidis* strains and one *S. saprophyticus* strain by the *p*-xylene method, and reported that *S. epidermidis* strains behaved in a hydrophobic manner, while the *S. saprophyticus* strain was very hydrophilic (42). In conclusion, our results showed that gram positive bacteria are usually moderately hydrophobic with only a few exceptions, as determined by both contact angle measurements and the *p*-xylene method. In contrast, in some of the related studies in the literature, in which both hydrophobic and

Table 2. Properties of gram positive bacteria used.

Bacteria	Contact angle (°)	Adherence to <i>p</i> -xylene (%)	Surface free energy (erg/cm <sup>2</sup> )
<i>CPS-1</i>	66±5	69,7±1.5	46.8
<i>CPS-2</i>	65±4	68.8±1	47.7
<i>CNS-1</i>	68±5	80±2	45.4
<i>CNS-2</i>	48±5	35,4±0.9	60.3
<i>S. pyog-1</i>	69±5	82,1±2.1	46.9
<i>S. pyog-2</i>	60±4	58,6±1.9	51.6
<i>CB-1</i>	70±5	70±0.95	44.9
<i>CB-2</i>	64±4	64±1.4	53.8

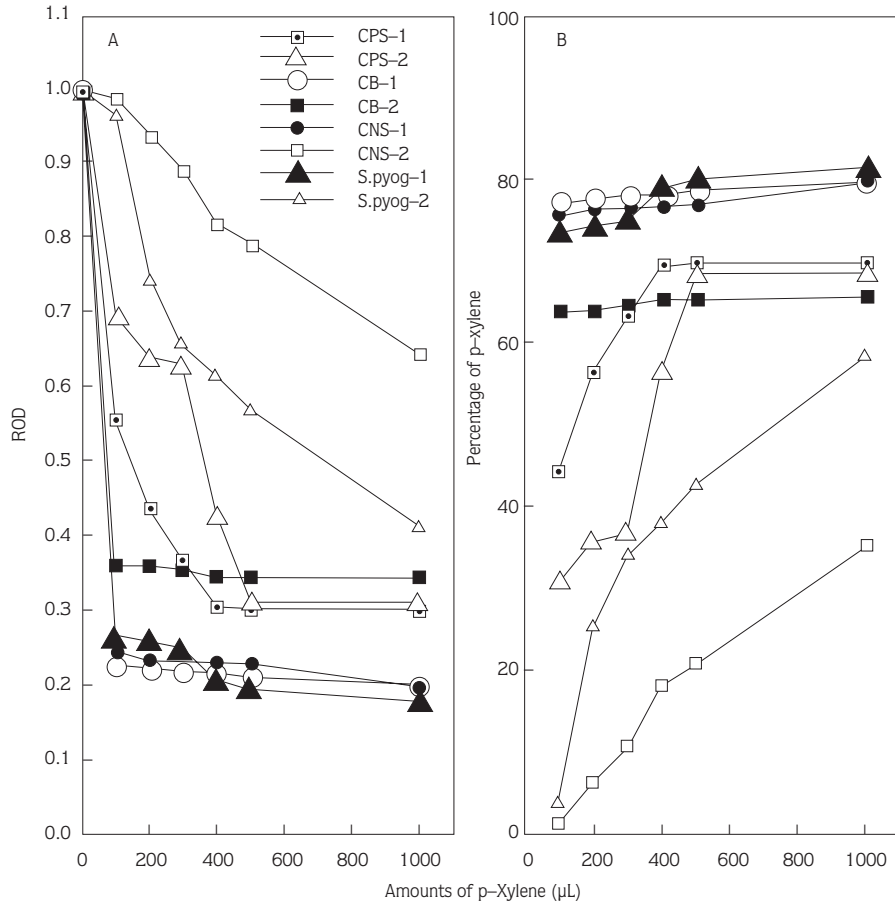


Figure 3. Adherence of gram positive bacteria to p-xylene: (A) relative optical densities (ROD); and (B) percentages of the micro-organisms in the hydrocarbon phase.

hydrophilic gram positive bacteria strains were reported, no correlations were found between these two methods. It should be noted that, in most of the previous studies, contact angle measurements were taken usually by placing a water droplet (or other test liquid droplets) on "dry" bacterial lawns. It should be understood that the surface characteristics of bacteria may be influenced significantly by their state of hydration and also the composition of the fluids in which the bacteria are suspended. In the present study, we measured the contact angles of the bacterial film under water, in other words, in the wetted state, and perhaps because of this, we observed a correlation in the results obtained with these two methods in most cases. Table 2 shows that all of the bacteria were negatively charged (except CPS-1 and CNS-2), as reported in the related literature (40, 44, 48). The zeta potentials varied over a wide range, 14.5–650.2 mV. When we compared the hydrophobicity (as contact angles and adherences of p-xylene) and

electrostatic behaviour of the micro-organisms, we did not observe any clear correlation in most of the cases.

### Gram Negative Bacteria

Two *Pseudomonas aeruginosa* and two *Escherichia coli* strains were studied as examples of gram negative bacteria. The contact angles of these strains and also the reference material (i.e., cellulose acetate) are given in Figure 4. Note that the average values and standard deviations are also given in Table 3. Notice that all the strains were hydrophilic (relative to the reference

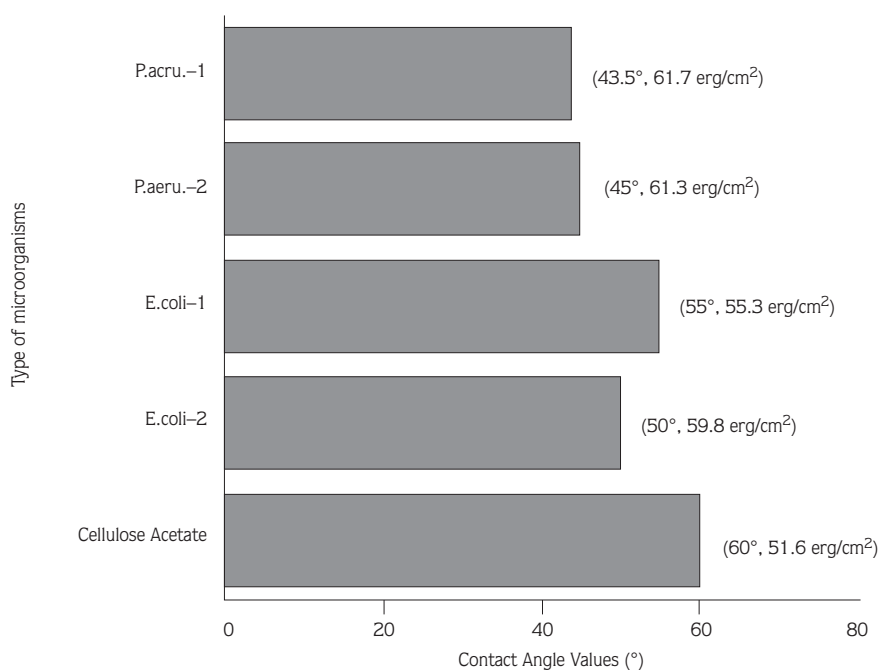


Figure 4. Contact angles of gram negative bacteria.

Table 3. Properties of gram negative bacteria used.

Bacteria	Contact angle (°)	Adherence to p-xylene (%)	Surface free energy (erg/cm <sup>2</sup> )
<i>E. coli-1</i>	55±3	36,6±2.3	55.3
<i>E.coli-2</i>	50±2	29,6±0.6	59.8
<i>P.aeru-1</i>	43.5±3	2,3±0.1	61.7
<i>P.aeru-2</i>	45±2	33,7±0.3	61.3

material surface). Both of the *P.aeru.* strains exhibited smaller contact angle values (meaning more hydrophilic) than those observed in the *E. coli* strains. The surface free energies of the gram negative bacteria strains used in this study were similar, in the range 55.3–61.8 erg.cm<sup>-2</sup>. Similar values were also reported by Absolom et al. (37) for several *E. coli* strains (around 70 erg.cm<sup>-2</sup>).

Figure 5 shows the relative optical densities (ROD) and percentages of the micro-organisms adhered to the hydrocarbon phase (i.e., *p*-xylene). The average values and standard deviations are also given in Table 3. All of the gram negative bacteria studied were hydrophilic, as determined with the *p*-xylene method. The percentage of the gram negative bacteria adhered to the *p*-xylene phase was significantly lower than was observed for the gram positive bacteria

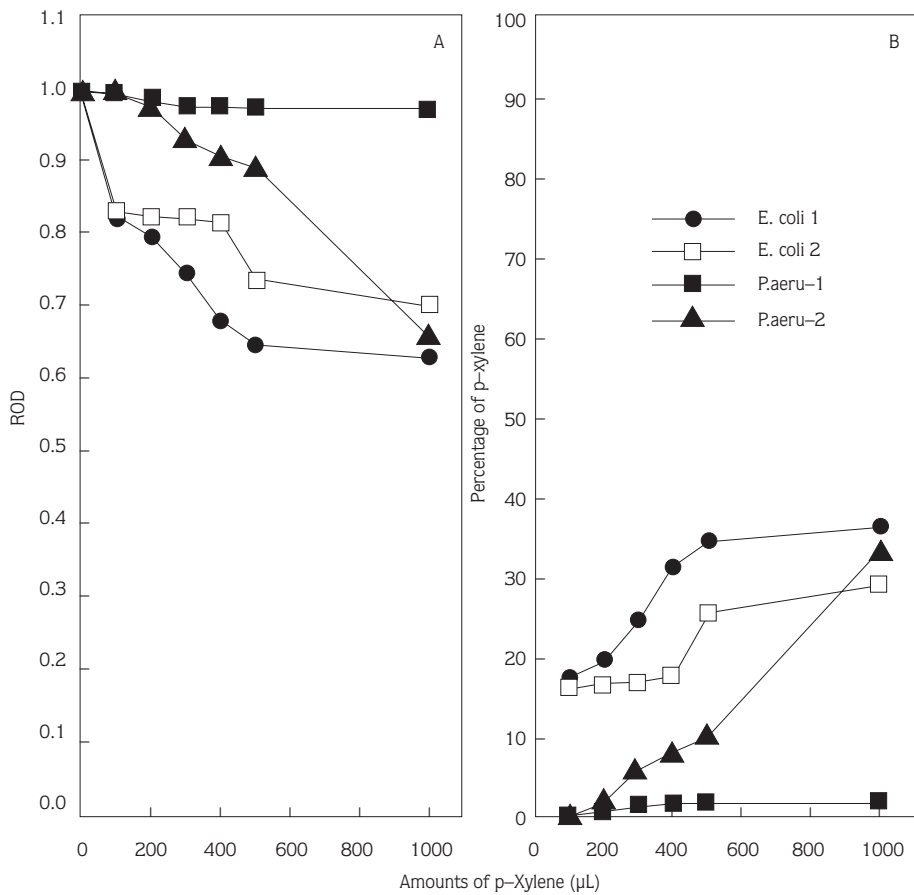


Figure 5. Adherence of gram negative bacteria to *p*-xylene: (A) relative optical densities (ROD); and (B) percentages of the micro-organisms in the hydrocarbon phase.

(see Figure 3). The *p*-Xylene data were parallel to the results of the contact angle measurements in the case of the *E. coli* strains. However, the *p*-xylene data for the *P. aeru*-1 strain showed that this strain had almost no affinity for the xylene/water interface. The data obtained by these two methods did not correlate for the *P. aeru*-2 strain, especially when lower amounts of *p*-xylene were used. Eight *E. coli* strains were characterized by Harkes et al. In their study, both adhesion to xylene and water contact angles were measured.

They found no correlation between the results of these two methods (43). The lack of agreement between water contact angles and adhesion on the hydrocarbon phase has also been reported by other authors (22, 24). One reason may be the state of the bacteria (which was in the dry state) in the contact angle tests performed in these studies, as mentioned above (i.e., in the wet state). Table 3 shows that all of the bacteria were negatively charged, as reported in the related literature (40, 44, 48). The zeta potentials varied over a wide range, 6.0–159.6 mV. When we compared the hydrophobicity (contact angles and adherences of *p*-xylene) and electrostatic behaviour of the micro-organisms, we did not observe any clear correlation in most of the cases.

#### **Candida albicans**

Two strains of *Candida albicans* yeast were examined. Figures 6 and 7 show the contact angles and *p*-Xylene adhesion data of these strains. Note that the average values and standard deviations are also given in Table 4. The hydrophobicity of the *Candida albicans*-1 strain was significantly higher than second strain. The contact angles, surface free energies and ROD values of Strain-1 and Strain-2 were 75, 50.57, and 0.138; and 69, 46.95, and 0.328 respectively. Notice that there were similar data for the gram positive and negative bacteria, and a correlation was observable in the hydrophobicities determined using the two methods. Table 4 shows that these micro-organisms were negatively charged, as reported in the related literature (42, 46, 48). Minagi et al. described *Candida albicans* as relatively hydrophilic and *Candida tropicalis* as more hydrophobic, while surface free energies for both types were in the range 20–40 erg.cm<sup>-2</sup> (31).

#### **Zeta Potentials**

Microbial attachment on material surfaces has generally been interpreted in terms of hydrophobicity (37, 43, 44). In addition, some researchers have indicated the influence of electrical charges of bacteria and material surfaces on adhesion (42, 38, 45). Most bacteria are negatively charged (46). In aqueous media, these surface charges are counter-balanced by

Table 4. Properties of *Candida albicans* used.

Micro-organisms	Contact angle (°)	Adherence to <i>p</i> -xylene (%)	Surface free energy (erg/cm <sup>2</sup> )
<i>Candida albicans</i> -1	75±3	75±1.9	50.6
<i>Candida albicans</i> -2	69±4	64±2.2	46.9

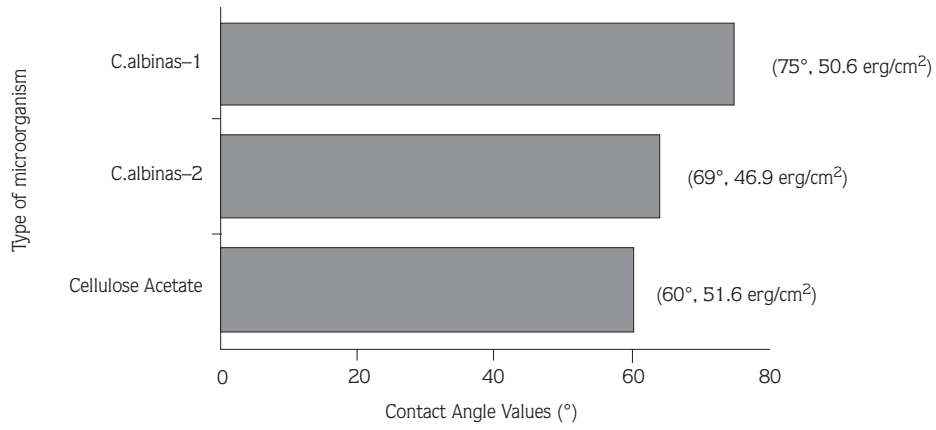


Figure 6. Contact angles of *Candida albicans*.

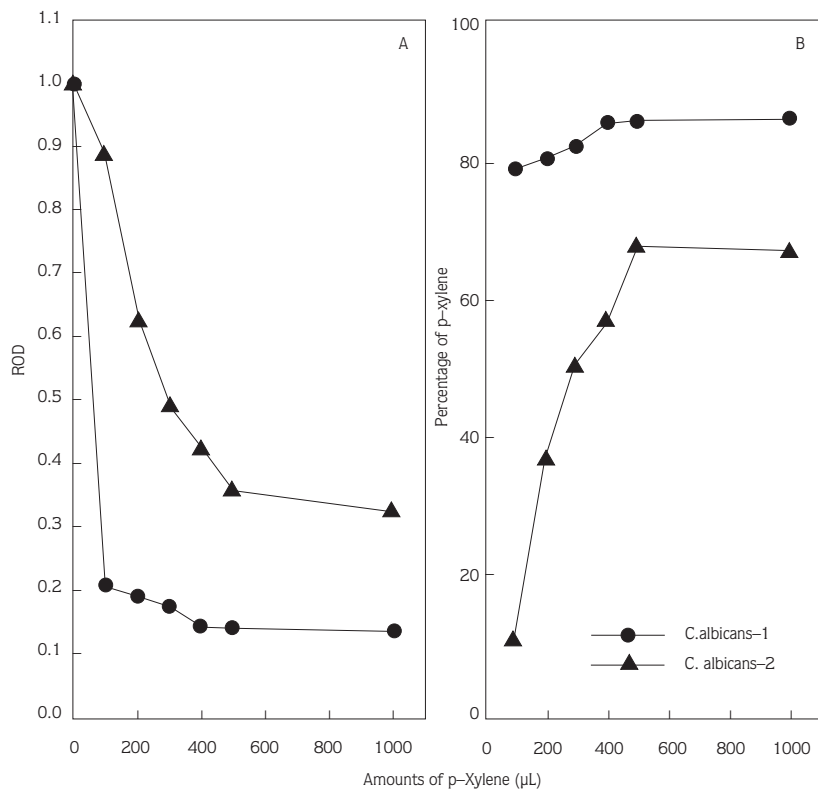


Figure 7. Adherence of *Candida albicans* to p-xylene: (A) relative optical densities (ROD); and (B) percentages of the micro-organisms in the hydrocarbon phase.

oppositely charged ions, some of which are bound to the surface whereas the rest are distributed in a diffusive layer (47). The thickness of this diffusive layer depends on pH, the ionic strength of the solution and the valencies of the counterions. The electrical interaction between the particles (including bacteria) in solution are governed by the extension of the diffusive layer.

There are different ways to obtain information about electrostatic interactions (44, 46, 47). In this study, zeta potential was used to describe the extent of micro-organism electrostatic behavior. Zeta potentials were measured in PBS with an ionic strength of  $\mu=0.264$  and at the physiological pH of 7.4, using a zeta potential analyser system, as described in the section "Materials and Methods".

Table 5 gives the zeta potentials and surface charges of the micro-organisms examined in this study. All of the micro-organisms were negatively charged, as reported in the related literature (38, 44, 48), except for CPS-1 and CNS-2. Zeta potentials varying over a wide range, from  $-650.20$  to  $+17.5$  mV, were determined and these were in the ranges  $-650.20$  to  $+17.5$  mV,  $-159.6$  to  $-6.0$  mV, and  $-21.0$  mV to  $-22.0$  mV for the gram positive bacteria, gram negative bacteria, and *Candida albicans* respectively.

Micro-organisms	Zeta potential (mV)	Surface charge
<i>CPS-1</i>	17.5	+
<i>CPS-2</i>	-78.1	-
<i>CNS-1</i>	-86.2	-
<i>CNS-2</i>	18.57	+
<i>S.pyog-1</i>	-650.2	-
<i>S.pyog-2</i>	-14.5	-
<i>CB-1</i>	-480	-
<i>CB-2</i>	-345	-
<i>E.coli-1</i>	-17.9	-
<i>E.coli-2</i>	-35.1	-
<i>P.aeru-1</i>	-159.6	-
<i>P.aeru-2</i>	-6.0	-
<i>Candida albicans-1</i>	-21.0	-
<i>Candida albicans-2</i>	-22.0	-

Table 5. Zeta potentials and surface charges of the micro-organisms.

## Conclusions

In this study, the surface properties of seven different micro-organisms with their 14 different strains were examined. The hydrophobicities of the micro-organisms were determined by means of two alternative methods, namely, contact angles (also known as surface free energies) and *p*-xylene adhesion. There was quite a good correlation between the hydrophobicities of the micro-organisms with these two methods in most cases. This was attributed to the contact angle test method (i.e., a captive-bubble technique), in which the

micro-organism film was in the wet state. The electrostatic behaviour of the micro-organism surfaces was analysed in terms of the type of surface charge and zeta potential. Most of the micro-organisms utilized in this study were negatively charged, except for two strains. The zeta potentials for the gram positive, gram negative and *Candida Albicans* were in the ranges 14.5–650.2 mV, 6.0–159.6 mV, and 20.7–21.7 mV respectively. There were no clear correlations in most cases between the hydrophobicity (contact angles and adherences of p-xylene) and electrostatic behaviour of the micro-organisms.

### References

1. Woods, D.E., Straus, D.C., Johanson, W.G., Berry, V.K., and Bass, J.A., Role of pili of *Paeruginosa* to mammalian buccal epithelial cells, *Infect. Immun.* 29: 1146–1151, 1980.
2. Rosan, B., Appelbaum, B., and Holt, S.C., Isolation and identification of the surface receptor *S.sanguis* responsible for adherence to hydroxyapatite, p: 537–540. In R.C. Berkeley, J.M. Lynch, J. Melling, P.R. Rutter, and B. Vincent eds., *Microbial adhesion to surfaces*, Halstead Press, New York, 1981.
3. Marshall, K.C., Stout, R., Mitchell, R., Selective sorption of bacteria from sea water, *Can. J. Microbio.*, 17: 1413–1416, 1971.
4. Atkinson, B., and Fowler, H.W., The significance of microbial film in fermenters, *Adv. Biochem. Eng.*, 3: 221–227, 1974.
5. Characklis, W.G., Attached microbial growth. I. Attachment and growth, *Water Res.* 70: 1113–1127, 1973.
6. Dexter, S.C., Influence of substratum critical surface tension on bacterial adhesion: in situ studies, *J. Colloid Interface Sci.*, 70: 346–354, 1979.
7. Fletcher, M., The effect of culture concentration and age, time and temperature and bacterial attachment to polystyrene, *Can. J. Microbiol.*, 23: 1–6, 1977.
8. Rozalska, B., Infection associated with the use of biomaterials in medicine. *Postepy. Hig. Med. Dosw.* 48: 143–60, 1994.
9. Cristina, A.G., Giridhar, G., Gabriel, B.L., Naylar, P.T., & Myrvik, Q.N., Cell biology and molecular mechanisms in artificial device infections. *Int. J. Artif. Organs.* 16: 755–63, 1993.
10. Tang, L., & J.W. Eaton. Inflammatory responses to biomaterials. *Am. J. Clin. Pathol.* 103: 466–71, 1995.
11. Johnson, J.R., Robert, P.L., Olsen, J., Moyer, K.A., & Stamm, W.E., Prevention of Catheter-associated Urinary Tract Infection with a Silver-Oxide Urinary Catheter. *Clinical and Microbiologic Correlats. J. Infect. Dis.* 162: 1145–1150, 1990.
12. Merritt, K. Infection at Site of Implants with Various Materials and Organisms. *Biomater. Clin. App.* 705–710, 1987.
13. Gemmell, C.G., Gorham, S.D., Monsour, M.J., Mcmillan, F., & Scott, R., The in-vitro Assessment of a Collagen/Vicryl (Polyglactin) Composite Film Together with Candidate Suture Materials for Potential Use in Urinary Tract Surgery. *Urol. Res.* 16: 381–384, 1988.
14. Singhal, J.P., Singh, J., Ray, A. R., & Singh, H.T., Antibacterial multifilament nylon sutures. *Biomater. Art. Cells and Immob. Biotech.* 19 (3): 631–648, 1991.

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15. Samuel, R., Farrah & Gregory W. Erdos., The production of antibacterial tubing, sutures, and bandages by in situ precipitation of metallic salts. *Can. J. Microbiol.* 37: 445–449, 1990.
16. Jansen, B., Schierholz, J., Schmacher–Perdreau F., Peters, G., & Pulverer, G., Modification of Polymers for Prevention of Foreign Body Infection. *Clinical Implant Materials.* 9: 117–122, 1990.
17. Golomb, G.P., & Spigelman, A., Prevention of bacterial colonization on polyurethane in–vitro by incorporated antibacterial agent. *J. Biomed. Mat. Res.* 25 (8): 937–952, 1991.
18. Cristina, A.G., Barth, E., & Webb, X.L., Microbial Adhesion an Molecular Mechanisms in Biomaterial and Compromised Tissue Centered Infection. *Biomat. Clin. Appl.*, p. 461–474, 1987.
19. Wadström, T., Molecular Aspects on Pathogenesis of Wound and Foreign body Infections due to Staphylococci. *zbl. Bakt. Hyg. A.* 266: 191–211, 1987.
20. Peters, G., Locci, R. & Pulverer, G., Adherence and Growth of Coagulase–Negative Staphylococci on Surface of Intravenous Catheters. *J. Infec. Dise.* 146 (4): 423–426, 1982.
21. Chang Chu, C., & Williams, D.F., Effects of Physical Configuration and Chemical Structure of Suture Materials on Bacterial Adhesion. *The American J. of Surgery.* 147: 197–204, 1984.
22. Minagi, S., Miyake, Y., Fujika, Y., Tsuru, H., & Suginaka, H., Cell Surface Hydrophobicity of *Candida* species as Determined by the Contact–angle and Hydrocarbon–adherence Methods. *J. Gen. Microbio.* 132: 1111–1115, 1986.
23. Stenström, T.A. Bacterial Hydrophobicity, an overall Parameter for the Measurement of Adhesion Potential to Soil Particles. *Appl. Env. Microbiol.* 55 (1): 142–147, 1989.
24. Fattom, A., & Shylo, M., Hydrophobicity as an Adhesion Mechanism of Bentic Cyanobacteria. *Appl. Env. Microbiol.* 47 (1): 135–143, 1984.
25. Van Lloosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, & Zehnder, A.J.B., Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacteria adhesion. *Appl. Env. Microbiol.* 53 (8): 1898–1901, 1987.
26. Hogt, A.H., Dankert, J., Feijen, J., Adhesion of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* to a Hydrophobic. *Biomaterial. J. Gen. Microbiol.* 131: 2485–2491, 1985.
27. Veenstra, G.J.C., Cremer, F.F.M., Diyk, H.V., Fleer, A., Ultra Structural Organization and Regulation of a Biomaterial Adhesion of *Staphylococcus epidermidis*. *J. Bacteriol.*, 178 (2): 537–541, 1996.
28. Jansen, B., Schierholz, J., Schareina, S., Schumacher–Perdreau, F., Peters, G., & Pulverer, G., Modifaction of Polymers for The Prevention of Foreign Body Infection. *Cli. Imp. Mater.* 9: 117–122, 1990.
29. Yousefi rad, A. Ph.D. thesis. Interaction of Bacteria with Polymeric Surfaces, University of Hacettepe, Ankara, 1997.
30. Bailey & Scott's *Ellen, J. Baron & Sydney M. Finegold.*, *Diagnostic Microbiology*, 8 th Edition. Mosby Company. Copyright, p. A–37, 1990.
31. Minagi, S., Miyake, Y., Inagaki, K., Tsuru, H., & Suginaka, H., Hydrophobic interaction in *Candida albicans* and *Candida tropicalis* adherence to various denture base resin materials. *Infect. Immun.* 47: 11–14, 1985.

32. Andrade, J.D., King, R., Gregonis, D.E., Surface Characterization of Poly (HEMA) and Related Polymers. 1. Contact Angle Method in Water. *J. Polym. Sci. Symp. C.*, 66: 313–336, 1979.
33. Neumann, A.Q., Good, R.J., Hope, C.J., & Sejpal, M., An Equation of State approach to Determine Surface Tensions of Low–Energy Solids from Contact Angles. *J. Colloid Interface Sci.* 49: 291–304, 1974.
34. Perry, R.H., Green, D., (2nd printing) *Perry's Chemical Engineerings Handbook*, Sixth Ed., Section 3, p. 288–289, 1985.
35. Rosenberg, M., Gutnik, D., & Rosenberg, E., Adherence of bacteria to hydrocarbons: a simple method for measuring cell–surface hydrophobicity. *FEMS Microbiol. Letters.* 9: 29–33, 1980.
36. *Manual of Zeta Potential Analyser System*, U.S. patent no: 3, 208, 919, Freeport Sulphur Com., New York, USA, 1965.
37. Absolom, D.R., Lamberti, F.V., Policova, Z., Zingg, W.J., Van Oss, C.J., & Neumann, A.W., Surface thermodynamics of bacteria adherence. *Appl. Microbiol.* 46(1): 90–97, 1983.
38. Van Der Mei, H.C., Weerkamp, A.H., & Busscher, H.J., A comparison of various methods to determine hydrophobic properties of streptococcal cell surface. *J. Microbiol. Methods.* 6: 277–287, 1987.
39. Hogt, A.H., Dankert, J., & Feijen, J., Adhesion of coagulase–negative staphylococci to methacrylate polymers and copolymers. *J. of Biomed. Mater. Res.* 20: 533–545, 1986.
40. Hogt, H., Dankert, J., Hulstaert, C.E., & Feijen J., Cell Surface Characteristics Coagulase–Negative Staphylococci and Their Adherence to Fluorinated Poly (Ethylene–propylene). *Infection and Immunity.* 294–301, 1986.
41. Pascual, A., Fler, A., Westerdaal, N.A.C., & Verhoef, J., Modulation of adherence of Coagulase–Negative Staphylococci to Teflon Catheters in Vitro. *Eur. J. Clin. Microbiol.* 5 (5): 518–522, 1986.
42. Hogt, A.H., Dankert, J., Hulstaert, C.E., & Feijen, J., Adhesion of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* to a Hydrophobic Biomaterial. *J. General Microbiol.* 131: 2485–2491, 1985.
43. Harkes, G., Feijen, J. & Dankert, J., Adhesion of *Escherichia coli* on to a Series of poly (methacrylates) differing in charge and hydrophobicity. *Biomaterials.* 2: 853–860, 1991.
44. Van Loosdericht, M.C.M., Lyklema, J., Norde, W., Schraa, G., & Zehnder, A.J.B., Electrophoretic Mobility and Hydrophobicity as a Measure to Predict the Initial Steps of Bacterial Adhesion. *Appl. Env. Microbiol.* 55 (8): 1898–1901, 1987.
45. Gordon, A.S., & Millero, F.J., Electrolyte effect on attachment of an estuarine bacterium. *Applied and Environmental Microbiology.* 47: 495–499, 1984.
46. James, A.M., Electrophoresis of particles in suspension, in: *Surface and Colloide Sci.*, 11, 174–177, (Good, R.J., and Stromberg, R.R. eds.) Plenum, New York, 1979.
47. Van Der Mei H.C., Leonard, J.A., Weekamp, H.A., Rouxhet, G.P., & Busscher J.H., Surface properties of *Strep. salivarius* HB and nonfibrillar mutants: measurement of Zeta potential and elemental composition with X–Ray photoelectron spectroscopy. *J. Bacteriol.*, 170 (6): 246–258, 1988.
48. Weekamp, A.H., Uyen, H.M., & Busscher, J., Effect of zeta potential and surface energy on bacterial adhesion to uncoated and Saliva–coated Human Enamel and dentin. *J. Dental Res.* 67: 12, 1483–1487, 1988.