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EXPERIMENTAL ARTICLES

Bacillus cereus Adhesion: Real Time Investigation of the Effect on the Chemistry of Industrial Stainless Steel[1]

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Abstract—The initial microorganism adhesion on substrate is an important step for the biofilm formation. The surface properties of the stainless steel and *B. cereus* were characterized by the sessile drop technique. Moreover, the physicochemical properties of surface adhesion and the impact of bio adhesion to the stainless steel were determined at different time of contact (2, 4, 7, 9 and 24 h). The results showed that the strain was hydrophilic (Giwi = 3.37 mJ/m^2), whereas the substratum has hydrophobic character (Giwi = −57.6 mJ/m^2). Stainless steel surface presents a weak electron–donor character ($\gamma^- = 4.1$ mJ/m^2) conversely to *B. cereus* that presents an important parameter ($\gamma^- = 31.6$ mJ/m^2). The bio adhesion was investigated at different time of contact. The data analysis after 2 h, confirmed the adhesion of *B. cereus* with an amount of 10 cfu/cm^2 which increased to 1.210^4 cfu/cm^2 after 24 h. Interestingly, despite the difference of hydropohbicity, the interaction between *B. cereus* and substratum was favored by the thermodynamic aspect of adhesion ($\Delta G_{adhesion} < 0$). Interestingly, the study of the effect of *B. cereus* adhesion on the stainless steel has revealed that, the substratum becomes hydrophilic ($\theta° = 41.3$, ΔGiwi = 39.6 mJ/m^2) and highly electron donor ($\gamma^- = 52.9$ mJ/m^2) after 2 h of bio adhesion.

Keywords: physicochemical proprieties, microbial adhesion, *Bacillus cereus* and stainless steel surface

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As all inert surfaces, the materials are potential site for biofilm formation after initial attachment of microorganisms. Once established, the biofilm can be responsible for the spoilage of engineering materials and can lead to product contamination, and surface destruction [1, 2]. The capacity of microorganisms to adhere rapidly to surfaces such as plastics, polypropylenes, rubbers, stainless steel and glass is now well established. Especially, adhesion of microorganisms to food processing equipment surfaces and the problems it causes are a matter of concern to food industry. Biofilms have the potential to act as a chronic source of microbial contamination, which may compromise food quality and represent a significant health hazard. To control these problems, it has been recognized that a greater understanding of the interactions between microorganisms and food-processing surface is required [3, 4]. In this context, several works [5, 6] have reported that Stainless steel, although susceptible to bacterial attachment, is the most frequently used material for construction of vessels, piping, valves and various types of equipment used in the food processing industry. Indeed, bacterial adhesion to surface is affected by the physicochemical properties of bacteria and substratum surface involved [7, 8] which can include hydrophobicity, charge, Lewis acid-base properties and surface topography. In addition, limited works have studied the physico-chemical proprieties changes after bacterial adhesion [9]. Furthermore, it is clear that the modification of each parameter involved in this process could be the origin of the increase or the decrease of surface adhesion potential. So, the better understanding of these features is of extreme importance for the development of effective adhesion control mechanisms that will ultimately prevent biofilm formation. Thus—in the present study—the characteristics of interfacial tension of *B. cereus* strain, a stainless steel surface and a stainless steel surface adhered by *B. cereus* at different time were examined by measurements of contact angles between the surfaces.

MATERIAL AND METHODS

***Bacillus cereus* strain, media and culture conditions.** A strain of *B. cereus* was originally isolated from biofilm taken from dairy processing line of the collect centre located in Nabeul, Tunisia. The strain has been previously identified using the API 50 CHB system

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Table 1. Surface tension properties of contact angle liquids*

Liquid	γ^{LW}, mJ/m^2	γ^+, mJ/m^2	γ^-, mJ/m^2
Water	21.8	25.5	25.5
Formamide	39	2.3	39.6
Diiodomethane	50.5	0	0

Note: * This result was obtained by van Oss [13].

and characterized by PCR-sequencing targeting 16S rDNA genes. Following BLAST analysis, strains was affiliated to *Bacillus cereus* R13 (97%) (NCBI Blast software). Frozen *B. cereus* had been transferred in Nutrient broth (Difco, United States) and subcultured in 10 mL BN broth at 30°C for 11–18 h before the cells were used. The culture was harvested by centrifugation for 15 min at 8400 *g*, and washed twice and resuspended in 0.1 M KNO_3 solution.

Stainless steel surface characterization. The substrata chosen for this study was stainless steel SMS 316 (25 by 25 by 1 mm). Squares were cut and rinsed six times with distilled water. After that, the substrate was autoclaved for 15 min at 120°C. The solid substrate was allowed to air dry and the contact angle measurements were carried out.

***B. cereus* surface characterization.** A suspension of cells in KNO_3 solution was deposited into a 0.45 um cellulose acetate filter (Sartorius) by first washing the filter with 10 mL of distilled water for wetting, and then 10 mL of the cell suspension was added obtaining a thick lawn of cells after filtration by means of negative pressure. The wet filters were placed carefully on a glass support with double-sided sticky tape and allowed to air dry until so-called stable "plateau contact angles" could be measured [10].

Adhesion assay of *B. cereus*. Stainless steel chips were first cleaned by washing them with neutral liquid detergent and water, followed by rinsing with distilled water, air-dried, and sterilized at 121°C for 15 min [11]. The cleaned and sanitized coupons were inserted in separate 55-mm-diameter petri dishes that contained 20 mL of nutrient broth. An overnight culture of *B. cereus* in BN at 37°C was used to inoculate Nutrient broth in petri dishes, which were incubated at 37°C. The medium was removed after 2 h, and then fresh BN (20 mL) was added. The initial number of cells was approximately 10^6 cfu/mL. Cell adhesion and biofilm development were evaluated after 2, 7 and 24 h at 37°C in a shaker rotating at 120 rpm. Following incubation, the medium was removed and 10 mL of saline solution (0.9% NaCl) was gently poured onto the chips to remove loosely adhering bacteria. Negative control was obtained by placing the coupon in a saline solution without bacterial cells. All experiments were done in duplicate and repeated in 2 independent assays.

Assessment of bacterial adhesion. The number of bacterial cells of *B. cereus* adhered to the stainless steel coupons was determined after 2, 7 and 24 hours of cultivation. Initially, coupons were immersed three times in 5 mL of sterile saline water (0.9% NaCl), to remove the planktonic cells, followed by the removal of the adhered cells using previously sterilized standardized swabs. The swabs were transferred to test tubes containing 10 mL of saline solution and stirred vigorously in vortex for one minute. For each measuring period, two randomly collected coupons were used as replicates. Serial dilutions of up to 10^{-6} were made in test tubes containing 9 mL of saline solution. Aliquots of 100 μL of each dilution were inoculated in petri dishes containing Nutrient agar, using the spread plate technique.

Contact angle measurements and surface tension components. Hydrophobicity and surface free energy characteristics were inferred from measured contact angles using a goniometer (GBX Instruments, France) by the sessile drop method [11].

Calculation of hydrophobicity. The hydrophobicity of each substratum was evaluated by water contact angle measurement and by the approach of Van Oss et al. [12]. In brief, one drop of a liquid was deposited onto a dry stainless steel. Three to six contact angle measurements were made on each substratum surface for water. Measurements of the contact angle of one 2.0 μL drop were taken each second for 30 s for liquid and surfaces. Hydrophobicity is determined by measuring the angle between the tangent to the surface of a distilled water droplet made with the surface of the solid sample, with well-known surface tension components (Table 1). Larger contact angles with water liquids indicate that the surface is more hydrophobic. In contrast, lower contact angles with water liquids indicate that the substrate is hydrophilic (less hydrophobic). In this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two entities of that material when immersed in water (*w*): ΔGiwi. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic (Giwi < 0); conversely, for a hydrophilic material, ΔGiwi > 0. ΔGiwi is calculated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta Giwi = -2\gamma_{iw} = -2[(\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2})^2$$
$$+ 2((\gamma_i^+\gamma_i^-)^{1/2} + (\gamma_w^+\gamma_w^-)^{1/2} - (\gamma_i^+\gamma_w^-)^{1/2} - (\gamma_w^+\gamma_i^-)^{1/2}].$$

Table 2. Contact angles values and free energy of interaction (Giwi) of *B. cereus* and stainless steel surface in the presence or absence of *B. cereus* adherence at different time of contact

	Liquid contact angles			
	θ_w, (°)	θ_F, (°)	θ_D, (°)	Giwi, mJ/m^2
Stainless steel	125.9 (2.95)	86.8 (6.15)	64.9 (1.65)	–57.6
Bacillus cereus	52.6 (1.9)	51.4 (1.25)	31.7 (2.48)	3.37
Biofilm 2 h	41.3 (0)	56 (1.5)	63.3 (0)	39.6
Biofilm 4 h	39.8 (0)	64.8 (4.55)	73.3 (3.07)	67.4
Biofilm 7 h	20.1 (1.7)	64.3 (1.55)	41.1 (1.75)	69.7
Biofilm 9 h	16.9 (2.5)	71.8 (0)	75.9 (0.95)	90.8
Biofilm 24 h	20.9 (5.88)	18.9 (0)	48.1 (2.78)	26.5

Where γ^{LW} accounts for the Lifshitz–van der Waals component of the surface free energy and γ^+ and γ^- are the electron acceptor and electron donor parameters, respectively, of the Lewis acid-base component (γ^{AB}), with $\gamma_S^{AB} = 2(\gamma_S^-\gamma_S^+)^{1/2}$.

Calculation of surface tension components. Three to six contact angle measurements were made on each substratum surface for all probe using two pure liquids (polar and apolar) with well-known surface tension components (Table 1): formamide (>99%) and diiodomethane (>99%). The Lifshitz–van der Waals (γ^{LW}), electron donor (γ_S^-) (or Lewis-base), electron acceptor (or Lewis acid) (γ_L^+) components of the surface tension of bacteria were estimated from the approach proposed by Van Oss et al. [13]. In this approach the pure liquid (L) contact angles (θ) can be expressed as:

$$\gamma_L(\cos\theta + 1) = 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2}/\gamma_L + 2(\gamma_S^+\gamma_L^-)^{1/2}/\gamma_L + 2(\gamma_S^-\gamma_L^+)^{1/2}.$$

Calculation of total free energy of interaction. From the values of the components of the interfacial tensions, it is possible to determine the Total Free Energy of Adhesion (ΔG adhesion) between two surfaces (microbial cells (b) and food processing surfaces (s)) at different times of contact:

$$\gamma_{bs} = \gamma_{bs}^{LW} + \gamma_{bs}^{AB}.$$

Where:

$$\gamma_{bs}^{LW} = \gamma_b^{LW} + \gamma_s^{LW} - 2(\gamma_b^{LW}\gamma_s^{LW})^{1/2},$$

and

$$\gamma_{bs}^{AB} = 2((\gamma_b^+\gamma_b^-)^{1/2} + (\gamma_s^+\gamma_s^-)^{1/2} - (\gamma_b^+\gamma_s^-)^{1/2} - (\gamma_b^+\gamma_s^-)^{1/2}).$$

When free energy is related to the interfacial tension, then ΔG adhesion can be represented by the following:

$$\Delta G_{adhesion} = \Delta G_{bls}^{LW} + \Delta G_{bls}^{AB}.$$

Where:

$$\Delta G_{bls}^{LW} = \gamma_{bs}^{LW} - \gamma_{bl}^{LW} - \gamma_{sl}^{LW},$$

and

$$\Delta G_{bls}^{AB} = (\gamma_{bs}^{AB} - \gamma_{bl}^{AB} - \gamma_{sl}^{AB}),$$

where γ_{bs} is the interfacial tension between the bacterial surfaces and the adhesion surface, γ_{bl} is the interfacial tension between the bacterial surfaces and the liquid, and γ_{sl} is the interfacial tension between the adhesion surfaces and the liquid. The $\Delta G_{adhesion}$ values allow for evaluation of the thermodynamics of the adhesion process: if $\Delta G_{adhesion} < 0$, the process is favorable, but if $\Delta G_{adhesion} > 0$, the process is unfavorable [9].

RESULTS

Physicochemical characterization of stainless steel and *B. cereus* surfaces. The surface free energy characteristics of both substrates (stainless steel and *B. cereus*), determined from the measurements of contact angle and calculation using the Young–van Oss equations are presented in Tables 2 and 3.

Water contact angle can be used as a qualitative indication of the surface material hydrophobicity, with higher values indicating a more hydrophobic surface ($\theta_w > 65°$) [12]. As it can be seen, stainless steel surface indicate of hydrophobic character ($\theta_w = 125.9°$) (Table 2). Using the approach of Van Oss and coworkers [13], it is possible to determine the absolute degree of hydrophobicity of any substance (i) vis-a-vis water (w), which can be precisely expressed in applicable System International (Formula 1). The values of free energy Giwi also showed that substratum is hydrophobic (ΔGiwi = –57.6 mJ/m^2) (Table 2). In opposite, *Bacillus cereus* isolate can be considered hydrophilic ($\theta_w = 52.6°$ and ΔGiwi = 3.37 mJ/m^2) (Table 2). On the other hand, the Lifshitz–van der Waals (γ^{LW}) component as well as electron donor (γ^-) and electron acceptor (γ^+) are also presented (Table 3). The result show that the stainless steel surface was weak electron donor ($\gamma^- = 4.1$ mJ/m^2)/acceptor ($\gamma^+ = 0.1$ mJ/m^2).

Table 3. Surface energy and their tension components of *B. cereus* and stainless steel surface in the presence or absence of *B. cereus* adherence at different time of contact

	γ^{LW}	γ^{+}	γ^{-}	γ^{AB}	γ^{total}
Stainless steel	25.8	0.1	4.1	1.28	27.08
Bacillus cereus	13.1	8.9	31.6	33.54	46.64
Biofilm 2 h	33.3	0.4	52.9	9.2	42.5
Biofilm 4 h	34.8	0.9	72.9	16.2	51
Biofilm 7 h	39.1	2.2	105.2	30.42	69.5
Biofilm 9 h	39.7	2	120.8	31.08	70.78
Biofilm 24 h	35.3	1.8	50.1	18.99	54.7

B. cereus strain predominantly affects more electron donor property ($\gamma^{-} = 31.6$ mJ/m^2) than acceptor property ($\gamma^{+} = 8.9$ mJ/m^2).

Adhesion of *B. cereus*. The assessment of adhesion of *B. cereus* cells to stainless steel (Fig. 1) at different contact time of 2, 7, and 24 h has confirmed the adhesion of *B. cereus* after 2 h with an amount of 10 cfu/cm^2 of adhered cells, which increased exponentially over 1.210^4 cfu/cm^2 after 24 h, with a significant spreading of biofilm.

Effect of adhesion the *Bacillus cereus* strain on surface free energy components of stainless steel surface. In the purpose of studied the effect of adhesion of *B. cereus* on the physicochemical characteristics of stainless steel, the hydrophobicity and surface tension parameters were evaluated at 2, 4, 7, 9 and 24 h of time contact.

The results show stainless steel surface adhered by *B. cereus* was hydrophilic at different time studied. The adhesion of *B. cereus* was accompanied with a decrease of hydrophobicity degree of the stainless steel surface after 2 h of contact, which becomes hydrophilic (Fig. 2). The level of this hydrophobicity ranged from ΔGiwi = –57.6 mJ/m^2 (not adhered) to ΔGiwi = 26.5 mJ/m^2 (adhered by *B. cereus*). Interestingly, after 24 h, stainless steel was become more hydrophilic ($\theta_w^\circ = 20.9$, ΔGiwi = 26.5 mJ/m^2) (Fig. 3).

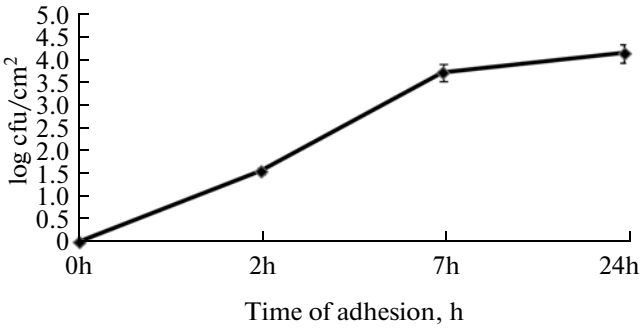

Fig. 1. Assessment of bacterial adhesion on stainless steel surface at 2, 7, and 24 hours.

In other hand, the determination of tension components of stainless steel surface has shown an increase of the electron donor character of substrate (γ^{-} has increased from 4.1 to 52.9 mJ/m^2) after 7 h of adhesion, which attempt 120.8 mJ/m^2 at 9 h of contact (Fig. 4). In addition, an increase of the dispersive compound (γ^{LW} = 39.1 mJ/m^2) and the acid-base components (γ^{AB} = 30.42 mJ/m^2) has been showed that can represents the hydration degree of substrata (Table 3).

Global free energy of adhesion ($\Delta G_{adhesion}$) of *B. cereus* and stainless steel surface. It is widely known that when a lower interaction free energy is obtained, a higher adhesion is expected. In a more precise way and referring to inert-colloidal particles, only negative values of $\Delta G_{adhesion}$ predict favorable adhesion. The results of free energy of interaction calculated are presented in Table 4. According to the thermodynamic theory of adhesion, the free energy of adhesion between stainless steel and *B. cereus* is negative ($\Delta G_{adhesion} = -7.95$ mJ/m^2), being thermodynamically favorable. Moreover, the Lifshitz van der Waals energy ΔG_{bls}^{LW} (–0.76 mJ/m^2) and acid-base energy

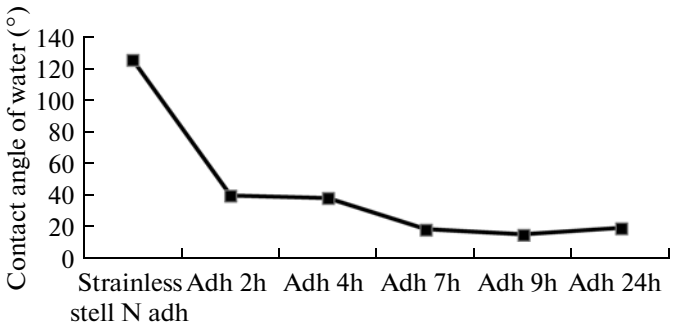

Fig. 2. Qualitative hydrophobicity (θ°) of stainless steel surface in the presence or absence of *B. cereus* adherence at different time of contact: stainless steel non adhered (N adh), adhesion 2 h (Adh 2 h), adhesion 4 h (Adh 4 h), adhesion 7 h (Adh 7 h), adhesion 9 h (Adh 9 h) and adhesion 24 h (Adh 24 h).

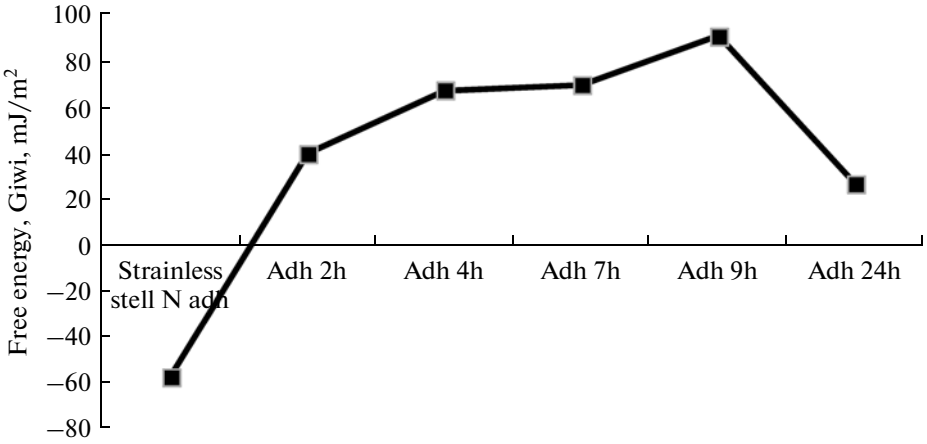

Fig. 3. Quantitative hydrophobicity (Giwi) of stainless steel surface in the presence or absence of *B. cereus* adherence at different time of contact: stainless steel non adhered (N adh), adhesion 2 h (Adh 2 h), adhesion 4 h (Adh 4 h), adhesion 7 h (Adh 7 h), adhesion 9 h (Adh 9 h) and adhesion 24 h (Adh 24 h).

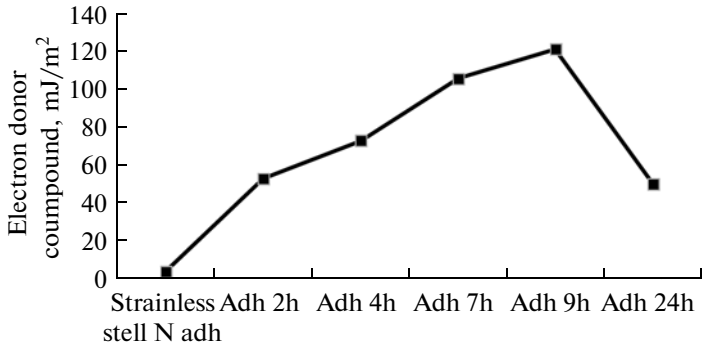

Fig. 4. Electro donor compound of stainless steel surface in the presence or absence of *B. cereus* adherence at different time of contact: γ^- (■): stainless steel non adhered (N adh), adhesion 2 h (Adh 2 h), adhesion 4 h (Adh 4 h), adhesion 7 h (Adh 7 h), adhesion 9 h (Adh 9 h) and adhesion 24 h (Adh 24 h).

ΔG^{AB}_{bls} (−7.18 mJ/m^2) values indicated attraction for stainless steel surface evaluated with adhered cells (Table 4).

DISCUSSION

The physicochemical characterization of the surface properties of microorganism and substrata has also received extensive interest. The characteristics of stainless steel and *B. cereus* have been determined to attempt to correlate the adhesion capability with the hydrophobicity and tension surface parameters of cells and substrata.

In what concerns cell surface hydrophobicity parameters determined, *B. cereus* is hydrophilic. These results are in accordance with previously obtained by Chaves et al. [14] that clearly demonstrate the hydrophilicity of *B. cereus* evaluated at different temperature of incubation. While, taking into consideration water contact angle value, the stainless steel is highly hydrophobic. This fact is corroborated by Flint et al. [15] and Bernardes et al. [9] that demonstrated a higher surface hydrophobicity of stainless steel. Inter-

Table 4. Global free energy of adhesion values ($\Delta G_{adhesion}$) between *B. cereus* and the stainless steel surface in aqueous liquid media and their apolar (ΔG^{LW}) and polar (ΔG^{AB}) components

	Global free energy of adhesion, mJ/m^2		
	ΔG^{LW}	ΔG^{AB}	$\Delta G_{adhesion}$
Bacillus cereus	−0.76	−7.18	−7.95

estingly, in spite of difference of hydrophobic character, the bio adhesion has been demonstrated on the stainless steel, showed by a significant level of adhered cells. Thus, no relationship was found between cell surface hydrophilicity of *B. cereus* and its ability to adhere to hydrophobic surface. These conflicting results are highlighted by several works of Bernardes et al. [9], El Abed et al. [17] that have observed the adhesion capability of *B. cereus* to stainless steel and wood substrata respectively, despite the difference of hydrophobicity. However, according to physicochemical approach, the hydrophobic cells tend to attach to a hydrophobic substrate and the hydrophilic cells tend to attach to hydrophilic substrata. Consequently, it seems that this approach is insufficient to explain our result since *B. cereus* which is hydrophilic, adhere better to hydrophobic stainless steel substrata.

In fact, previous studies of El Abed et al. [17] and Hamadi et al. [10] have suggested that other surface factors can as well contribute to the initial attachment to biomaterials surfaces, such as the free energy of surface [9, 18], the electron donor/electron acceptor (acid-base) properties [10], the substratum topography [19], forces and chemistry of surface and roughness level [16, 20, 21]. In this study, adhesion of *B. cereus* showed the ability of stainless steel to promote biofilm formation, mediated by changes in contact angle and surface tension parameters. In fact, non adhered stainless steel surface appeared lowly electron donors/acceptor but after *B. cereus* adhesion, the electron–donor/acceptor property increases. These results indicate that the adhesion of *B. cereus* increases as the electron–donor property of the stainless steel surface increases. The results of this work suggest that the electron donor character can be involved in adhesion of *B. cereus* to stainless steel. These finding are consistent with Van Oss [27], Henriques et al. [28], Hamadi et al. [14]. They have reported that the electron donor–electron acceptor plays a crucial role in microbial adhesion in bacterial interactions with heavy metals and mineral surfaces. In addition, Mahdavi et al. [26] have reported that all polymeric surfaces are predominantly electron donors because of the presence of oxygen in the atmosphere especially in the interface of substrata and the hydration of microbial cells. So, the involvement of electron donor/electron acceptor properties could also be important in explaining this bio adhesion highlighted by Van Oss et al. [12]. Moreover, the role of acid-base interactions can be considered in bio adhesion on stainless steel [30]. The increases of γ^{AB} values founded in these experiments may be explaining this cells attachment to substrata. Indeed, previous study of Bernardes et al. [12] have reported that a high γ^{AB} components value means more water of hydration on the surface and increased hydrophilicity and consequently favours the interaction between *B. cereus* and substrata despite hydrophobicity differences. Nevertheless, it must be noted that the global free energy of adhesion is thermodynamically favorable to stainless steel ($\Delta G_{adhesion} < 0$) according to the thermodynamic theory of adhesion. In fact, it should be expected that others parameters of surface such as van der Waals and acid-base energy, can play an important role in the interaction with substrata.

In other hand, the impact of *B. cereus* adhesion on stainless steel has been assessed at different time of contact. Interestingly, the variation of physicochemical characteristics of substratum has been highlighted due to the bio adhesion. In fact, the decreasing of hydrophobic character of surface could be correlated with metabolic activity for biofilm formed on stainless steel which decreased the hydrophobicity and favours the hydrophilic character. These results were recently reported [9] showed the decrease of hydrophobicity of stainless steel, that favours the adhesion of *B. cereus*, but after 10 days at 10°C. In addition, Chaves et al. [14] have observed that the surface of stainless steel, predominately hydrophobic and electron donor became hydrophilic after adhesion of *B. cereus*. Furthermore, results showed an increase of electon donor character after bio adhesion on stainless steel. So, a relationship between the increase of this parameter and bio adhesion can be highlighted [9]. In addition, adhesion of *B. cereus* on stainless steel surface has increased the van der Waals and the acid-base components and consequently favours the variation of substrata surface characteristics. Indeed, the highest γ^{LW} and γ^{AB} values founded in these experiments may be explaining this cells attachment to substrata. Indeed, a high component value means more water of hydration on the surface and increased hydrophilicity and consequently favours the interaction between *B. cereus* and substrata despite hydrophobicity differences [9]. This result was corroborated by Strevett and Chen [28] observed the variation of thermodynamic parameters of surface after adhesion of *E. coli, B. subtilis*, which favours the attraction of bacterium. Therefore, despite that the strain studied in this experiment is considered hydrophilic; the bio adhesion was established on hydrophobic substrata. Indeed, from a divergence of results, it is now clearly established that microbial adhesion, the first stage in the formation of biofilms, depends principally on the physicochemical surface characteristics of microorganisms and substrata [31]. Consequently, the effect of bio adhesion on the variation of surface properties of either of these compounds may result in a change to their bioadhesive behaviour.

This study demonstrates the effect of *B. cereus* adhesion on physicochemical properties (hydrophobicity; electron donor/electron acceptor) of stainless steel surface. While, the results of this work suggest that the hydrophobicity cannot be involved in the adhesion onto stainless steel, but the components of interfacial tension of surface especially the electron donor, acid-base and van der Waals property are strongly involved in *B. cereus* adhesion to substrata. Nevertheless, physicochemical characterization of the

adhesion process on inert material surface appears as interesting area of research. Current investigations in our laboratory are now focused in the development of biocontrol process of biofilm using preservative strain of lactic acid bacteria.

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