Physicochemical properties modification of stainless steels 304l by using sodium hypochlorite in disinfection procedures and consequences on *Escherichia coli* adhesion potential

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Abstract— The aim of this study was to assess the effect of using sodium hypochlorite in disinfection protocols on surface properties (hydrophobicity, electron donor–electron acceptor (acid–base) properties and roughness) of stainless steel 304L known as the main materials of catering equipment and food processing industries. Also, the impact of surfaces modifications on the adhesive potential of four *Escherichia coli* isolated from surfaces in food environment was investigated. The physiochemical properties of stainless steels before and after treatment with concentrations (6°, 8°,10°, 12°, 14° 16°, 18° Chl) of sodium hypochlorite was evaluated using the contact angle method. The topographic properties were examined by the atomic force microscopy (AFM). Also, surface characterization of *Escherichia coli* strains was performed with contact angle technique. The observed results revealed that the initial characteristics of stainless steel 304L was hydrophilic (θw = 27.7°±4.9°; ΔGiwi = 35.6 mJ/m^2) with high electron donor and low acceptor properties (γ^- = 53.8 mJ/m^2; γ^+ = 0.6 mJ/m^2). After 15 min of treatments, the hydrophobicity and donor/acceptor characters was highly instable by changing from a hydrophilic character to a hydrophobic character especially with the concentrations 8°, 10°, 12° Chl while the electron donor component decreased considerably (γ^- = 6.0 mJ/m^2 , 25.2 mJ/m^2,19.4 mJ/m^2) with these concentrations. Moreover, The surface topography and the arithmetic average roughness (Ra) before and after each disinfection experiment didn't change significantly. The prediction adhesion demonstrated that the adhesion potential of *Escherichia coli* strains before treatment was unfavorable from thermodynamically point of view, however, their adhesion ability was significantly enhanced after treatments with concentrations 8°, 10°, 12°Chl. Furthermore, stainless supports seem to be more resistant to *E. coli* adhesions outside this interval. This study contributes to demonstrate the significant impact of sodium hypochlorite on the physicochemical stability of stainless steel and subsequently, its hygienic status against microbial adhesion.

Index Terms— stainless steel, sodium hypochlorite, physicochemical properties, roughness, Escherichia coli, adhesion, Food processing surfaces.

1 Introduction

SURFACES in contact with food, especially material of catering equipment and food industries are daily disinfected to preserve and maintain a high hygienic quality of food. One of the decisive arguments when choosing materials to be in contact with food, long with their mechanical and anticorrosive properties, has become hygienic status but also the stability against various chemical products for cleaning and disinfection procedure is also highly recommended. Of these materials, stainless steel, which is widely used for constructing food process equipment, has previously been demonstrated to be highly hygienic [1]. Austenitic stainless steels containing chromium and nickel, such as AISI 304L are widely used in the food industry and catering equipment due to their high resistance to corrosion by food products and detergents.

Disinfection treatments are used in industrial, catering and in the domestic environments to control the biocontamination of surfaces. Although these biocide treatments eliminate most surface contamination, some microorganisms may survive and give rise to serious problems in terms of public health. Indeed, numerous reports have highlighted the survival of microorganisms after cleaning and disinfection in food [2], [3], [4], and domestic environments [5]. The resistance of microorganisms to disinfection is frequently associated with the presence of biofilms on surfaces [6], [7].

Moreover, the disinfecting products are generally effective in the treatment and elimination of microorganisms in planktonic cell form but not as well as in sessile cell form in biofilms. For that, many researches are currently being conducted to determine the mechanisms involved to enable certain products resistance. The major part of these researches is the study of biofilms, which play an important role in protecting bacteria against exposure to disinfectants, however, limited data concerning the effect of using chemical products on physicochemical surfaces properties and consequently, the

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probability of increasing microbial adhesion and enhancing the risk of bio-contamination in food industrial environment are available.

Therefore, the aim of the present work was to study the effect of using sodium hypochlorite in disinfecting protocols on the hygienic status of stainless steel 304L by evaluating its surface stability and subsequently the impact of these treatments on the adhesion potential of four *Escherichia coli* stains isolated from food environment.

2 Materiel and Methods

2.1 *Escherichia coli* strains

Sampling and collection of strains was performed in the laboratory of microbiology, food hygiene and environment of the "Institut Pasteur – Maroc". One hundred samples received for a month from different food sources (Catering, Food industries, hotels, and supermarkets) was analyzed. From twelve samples contaminated by *E. coli* strains, we choose four *E. coli* strains based on their high adhesion potential. The strains have been previously identified using the API 20E system. Frozen cells have been transferred to LB broth and incubated at 37˚C for 24h and sub cultured on solid LB medium [8].

The Four strains of *Escherichia coli were* codified *(13, EM3, 19, EM4)* and each strain was identified by serotyping according to Qrskov and Orskov [9] : (*E. coli* 13 (O_{124}, H_8, K_{25}); *E. coli* EM3 (O_{114}, H_8, K_{11}); *E. coli* 19 (O_{126}); *E. coli* EM4 (O_{126}, H_{40}, K_{11}).

2.2 Disinfecting product: Sodium Hypochlorite

The present study was performed with concentered sodium hypochlorite 48°Chl (containing 12.9% of active chlorine, pH=12,5) frequently used in industrial environment. We diluted the concentered solution of sodium hypochlorite in sterile distilled water just before use. The test concentration range was: 6°, 8°, 10°, 12°, 14°, 16°, 18° Chl.

2.3 Preparation and treatment of solid surface

The substrate used for adhesion experiments was stainless steel (304L). Before each experiment, the substrate was soaked for 15 min in ethanol and rinsed for 5 min under softened water. Finally, the substrate was autoclaved for 15 min at 120◦C [10]. The coupons were subjected to the following disinfecting procedure: they were treated with sodium hypochlorite with different concentration (6°, 8°, 10°, 12°, 14°, 16°, 18° Chl) by immersion for 15min (One cycle of treatment), and then rinsed six times with distilled water. Each coupon was used only once in order to avoid any surface change due to stainless steel ageing.

2.4 Contact angle measurements (CAM)

Stainless steel surface free energies were determined from contact angle measurements of water, diiodomethane and formamide using the sessile drop technique described by van Oss, Chaudury, and Good [11], with a goniometer (Digidrop, GBX Scientific Instruments, France) through a Digidrop camera. Image analysis was performed with Win drop++. The surface energy components of a surface (γ_S^+, γ_S^- and γ_S^{LW}) were determined by performing contact angle measurements using two polar liquids (water and formamide) and one apolar liquid (diiodomethane) with known energy characteristics (Table 1) (γ_L^+, γ_L^- and γ_L^{LW}) and employing Young's Eq:

$$\mathbf{Cos\ \theta = -1 + 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2}/\gamma_L + 2(\gamma_S^{+}\gamma_L^{-})^{1/2}/\gamma_L + 2(\gamma_S^{-}\gamma_L^{+})^{1/2}/\gamma_L} \quad (1)$$

Where θ is the measured contact angle, γ^{LW} is the van der Waals free energy component, γ+ is the electron acceptor component, γ− is the electron donor component and the subscripts (S) and (L) denote solid surface and liquid phases respectively. The surface free energy is expressed as: $\gamma_S=\gamma_S^{LW}+\gamma_S^{AB}$ where $\gamma_S^{AB}=2(\gamma_S^+\gamma_S^-)^{1/2}$ (**2**) is the acid–base free energy component.

The cell surface hydrophobicity was evaluated through contact angle measurements and by the approach of Van Oss [12]. 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 (ΔGiwi < 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:

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

For each concentration, three to six measurements were performed.

2.5 The total free energy of interaction (ΔG_{TOT})

The total free energy of interaction between microbial cell (M) and substratum (S) through Water (W) is calculated as the sum of the van Der Waals (LW), Acid-base (AB) interactions and the electrical interactions. Since the suspending liquid (KNO_3 solution) employed in this work has high ionic strength (0.1M). we neglected electrical interactions free energy versus the sum of ΔG^{LW} and ΔG^{AB} as done by Absolom [13] et Busscher [14]:

Where $$\mathbf{\Delta G_{MLS}^{Tot} = \Delta G_{MLS}^{Lw} + \Delta G_{MLS}^{AB}} \quad \mathbf{(3)}$$

$$\mathbf{\Delta G^{LW} = ((\gamma_M^{LW})^{1/2} - (\gamma_S^{LW})^{1/2})^2 - ((\gamma_M^{LW})^{1/2} - (\gamma_L^{LW})^{1/2})^2 - ((\gamma_S^{LW})^{1/2} -}$$

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$(\gamma_L^{LW})^{1/2})^2$ **(4)**

And $\Delta G^{AB} = 2\,((\gamma_L^{+})^{1/2}((\gamma_C^{-})^{1/2}+(\gamma_S^{-})^{1/2}-(\gamma_L^{-})^{1/2})) + (\gamma_L)^{1/2}((\gamma_C^{+})^{1/2}+(\gamma_S^{+})^{1/2}-(\gamma_L^{+})^{1/2}) - (\gamma_L^{-}\gamma_S^{+})^{1/2}-(\gamma_L^{+}\gamma_S^{-})^{1/2}))$ **(5)**

The ΔG_{TOT} values allow for evaluation of the thermodynamics of the adhesion process: if $\Delta G_{TOT} < 0$, the process is favorable; if $\Delta G_{TOT} > 0$, the process is unfavorable.

Table 1: Surface tension parameters (mJ/m²) of the liquids commonly used in contact angle measurements and thin-layer wicking

Liquids	γ^{TOT}	γ^{LW}	γ^{+}	γ^{-}
Water	72.8	21.8	25.5	25.5
Formamide	58.0	39.0	2.3	39.6
Diiodomethane	50.8	50.8	0.0	0.0

2.6 Atomic Force Microscope (AFM)

The surface topography and the arithmetic average roughness (Ra) of stainless steel were evaluated before and after each disinfection experiment using the Atomic Force Microscope (AFM).

2.7 Statistical Analysis

All Angle contact measurements were conducted in completely randomized design (CRD) with three repetitions and duplicate. Roughness measurements were conducted in CRD with three repetitions and duplicate. A significance level of 5% was used for Duncan's test using SPSS 20.0 software.

3 RESULTS AND DISCUSSIONS

3.1. Impact of Sodium hypochlorite's treatments on the physicochemical surface properties of stainless steel

The surface free energy characteristics of each substrate of stainless steel under various treatment by sodium hypochlorite, determined from the measurements of contact angle and calculation using the Young–van Oss equations are presented in Table 2.

According to Vogler [15], hydrophobic surface exhibit a water contact angle values higher than 65°, whereas hydrophilic one exhibit water contact angle values lower than 65°. Considering the contact angle with water (Ow) results in table 2, the supports 304L were hydrophilic (27.7°). The same stainless steel surfaces have remarkably changed after treatment with 8°, 10°, 12° and 16° Chl of sodium hypochlorite by becoming hydrophobic (64.0°; 87.9°; 83.4°; 74.0°; 75.2°), however, surfaces treated with 14° and 18°Chl remained hydrophilic (15.5°; 21.7°).

With this approach it is only possible to assess hydrophobicity qualitatively while using the thermodynamic approach of Van Oss and co-workers [11], [16], the surface free energy ΔGiwi (3) gives a quantitative indication of the surface substrate hydrophobicity.

Thus, as it can be seen in table 2, the value of the surface free energy obtained for the untreated stainless steel 304L was hydrophilic (35.62mJm^{-2}). By increasing the concentration of sodium hypochlorite, especially with 8°, 10° and 12°, the results show that the value of hydrophobicity (ΔGiwi) decreases significantly and changed from a hydrophilic to a hydrophobic character (-52.9 mJm^{-2}; -2.7 mJm^{-2}; -15.5 mJm^{-2}). However, the support has kept its hydrophilicity nature for treatment by 6 °, 14 ° 16 ° and 18 °Chl of sodium hypochlorite (12.6 mJm^{-2}; 31.9 mJm^{-2}; 10.2 mJm^{-2}; 29.2 mJm^{-2}).

Table 2: Contact angle measurements relative to water (θw) , formamide (θf) and diidométhane (θd). The electron donor-acceptor (γ-, γ +), components (acid, basic) (γab), Lifshitz-van Der Waals (γlw) and the free energy of interactions (ΔGiwi) of stainless steel 304L treated by sodium hypochlorite.

	Contact angles (°)			Surface tension: components and parameters (mJm^{-2})			ΔGiwi (mJm^{-2})
Concentration (°Chl)	O_w	O_f	O_d	γ^{lw}	γ^{+}	γ^{-}	ΔGiwi
0°	*27.7 (4.9)*	*34.0 (2.7)*	*47.5 (2.4)*	*35.5*	*0.6*	*53.8*	*35.6*
6°	*64.0 (2.7)*	*74.4 (2.3)*	*38.8 (0.1)*	*40.1*	*3.8*	*42.6*	*12.6*
8°	*87.9 (1.7)*	*72.6 (0)*	*56.4 (0.5)*	*30.5*	*0.0*	*6.0*	*-52.9*
10°	*83.4 (1.5)*	*89.9 (0.3)*	*51.4 (1.7)*	*33.4*	*5.2*	*25.2*	*-2.7*
12°	*74.0 (0.7)*	*68.7 (3.3)*	*40.7 (1.9)*	*39.1*	*0.9*	*19.4*	*-15.5*
14°	*15.5 (3.6)*	*15.7 (2.3)*	*27.5 (3.1)*	*45.1*	*0.4*	*53.9*	*31.9*
16°	*75.2 (4.0)*	*89.4 (4.9)*	*51.9 (5.3)*	*33.1*	*6.4*	*39.7*	*10.2*
18°	*21.7 (2.1)*	*20.5 (1.0)*	*33.5 (4.6)*	*42.6*	*0.5*	*51.2*	*29.2*

Note: Standard deviation was given in parentheses.

This clear variation of hydrophobicity as a function of treatment could be the result of alteration of apolar chemical groups. Mozes [17], have also found that hydrophobicities of glass surface and polymer surface change with solution used in cleaning procedure. Furthermore, other works [18], [19], [20], [21], suggest that great variations in the surface free energies could be induced by various surface treatments including food contact and cleaning and disinfection procedures.

The results of the electron-donor, the electron acceptor and the Lifshitz van der Waals components are also reported in Table 2.

Before disinfection, stainless steel was predominantly electron donor and weakly electron acceptor with values which are respectively γ^{-}= 53.8 mJ/m^2 and γ^{+}= 0.6 mJ/m^2. These findings correlate with those of Rubio [22], who reported that the measurement of the contact angle of the stainless surface 304L has a low electron acceptor character. Similar statements were found by Hamadi [10], who showed that stainless steel 304 was predominantly electron donors and weakly electron acceptor.

After Treatment, it can be seen that concentration 6°, 10° and 16°Chl affects considerably the electron acceptor property by increasing from low value (0.63 mJ/m^2) to relatively high value (3.89 mJ/m^2; 5.28 mJ/m^2; 6.44 mJ/m^2). In addition, the electron donor property has significantly changed after treatment by 8°, 10° and 12°Chl by decreasing from (53.82 mJ/m^2) to respectively (6.08 mJ/m^2, 25.29 mJ/m^2, 19.44 mJ/m^2). Moreover, the Lifshitz van der Waals component didn't change significantly by different treatments.

The changing of the electron donor and acceptor properties of strainless steel due to treatment could be the result of the modification of the characteristics of surface functional groups. The protonation of the Lewis acid–base sites could be the origin of the variation of electron donor/electron acceptor properties as proposed by van Oss [23].

Boulange Petermann [24], demonstrates that any contact between stainless steels and detergents would alter the energy characteristics of the materials and consequently their hygienic properties. However, cleanability experiments performed on various materials with very different surface properties (stainless steel, enameled steel, polycarbonate and mineral resins) failed in demonstrating any influence on the ease to be cleaned [1], at least when unused materials were tested.

3.2. Impact of Sodium hypochlorite's treatments on surface roughness of stainless steel

The surface topography and the arithmetic average roughness (Ra) of stainless steel were evaluated before and after disinfection experiment using the Atomic Force Microscope (AFM). The results are presented in Fig.1 and Fig.2.

For all stainless steel samples, the images produced by the Atomic Force Microscopy (AFM) indicates that the topography of the supports has not been changed by sodium hypochlorite treatment (10°, 12°, 14°Chl) (Fig.1). Moreover, the variation of surface roughness (Ra) after treatments seems to be not significant (Fig. 2).

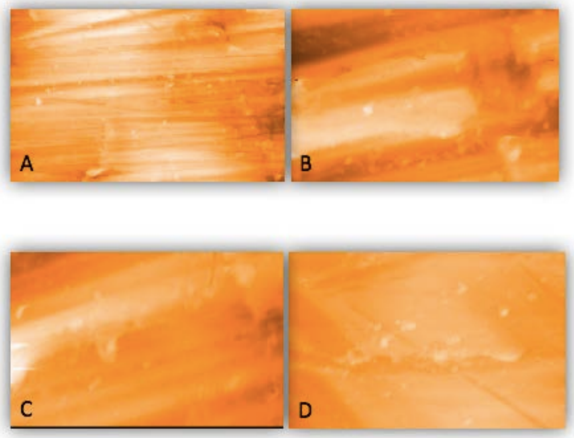

Fig. 1: Observations by atomic force microscopy of stainless steel (304L) after treatments with sodium hypochlorite (8°, 10 °, 12 ° Chl): A: untreated; B: 8°Chl; C: 12°Chl; D: 14°Chl

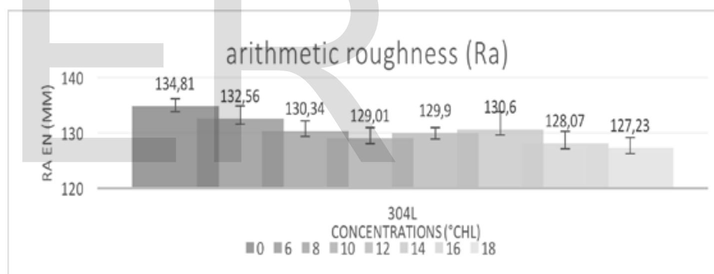

Fig. 2: variation of the roughness of stainless steel 304L after treatment with different concentrations of sodium hypochlorite

According to Kerherve [25], all corrosive solutions contained ions chlorides (Cl^-), hydroxyl (OH^-) and sodium (Na+). Moreover, the OH- ions and Na+ are not corrosion agents to stainless steels, the risk of corrosion are entirely due to Cl^- ions in sodium hypochlorite. In addition, the simultaneous presence of aggressive ions (chloride: Cl^-) and strong oxidizing ions (hypochlorite: ClO^-) in bleach solutions can cause the appearance and development of crevice corrosion and pitting on stainless steels and consequently, modify surface roughness. These modifications affect highly surface properties and can either accentuate or inhibit microbial adhesion [26].

Substratum topography surface is recognized as a parameter that affects the bio adhesion of bacterial cells and biofilm formation [26]. However, in this study, all treated supports did not experience important changes in their surfaces roughness (Fig.2). This can be explained by the contact time which wasn't sufficient enough to modify their initial roughness (Ra) even if we've increased the concentration of hypochlorite sodium from low value (6°Chl) to high value (18°Chl).

3.3 Prediction of Adhesion ability of *Escherichia coli* strains on treated surfaces

The effect of the changes in the energy characteristics of stainless steel substrate on bacterial adhesion of four pathogenic *E. coli* strains collected from food environment was evaluated through the use of theoretical prediction of adhesion. The physicochemical surface properties of *E. coli* strains are measured by contact angle (Table 3).

Bacterial characterization indicates that *E. coli* 19 and EM3 are qualitatively and quantitatively hydrophobic (negative value of ΔGiwi, Ow>65°) while *E. coli* EM4 and 13 are hydrophilic (positive value of ΔGiwi. Ow>65°). In addition, the electron donor (γ-) did not vary greatly among bacteria with the same hydrophobicity (*E. coli* 19 and *E. coli* EM3; *E. coli* EM4 and *E. coli* 13). However, results indicate that E. coli strains are weakly electron acceptor (γ+) except *E. coli* EM4 (High value of γ^{+}=7 mJm^{-2}). Moreover, the Lifshitz van der Waals component didn't change significantly by different treatments.

Table 3: Contact angle measurements relative to water (θw), formamide (θf) and diidométhane (θd). The electron donor-acceptor (γ-, γ +), components (acid. basic) (γab), Lifshitz-van DerWaals (γlw) and quantitative hydrophobicity (ΔGiwi) values of *Escherichia coli* strains isolated from food origins.

Strains	Contact angles (°)			Surface tension: components and parameters (mJm−2)			**ΔGiwi** (mJm−2)
E.Coli	O_w	O_f	O_d	y^{lw}	y^{+}	y^{-}	**ΔGiwi**
***E.coli* 19**	***74.0 (3.2)***	***63.3 (2.8)***	***46.8 (2.0)***	***35.9***	***0.1***	***15.2***	***-25.0***
***E.coli* EM3**	***74.2 (3.4)***	***63.8 (4.6)***	***64.6 (1.4)***	***25.8***	***0.6***	***14.9***	***-20.7***
***E.coli* EM4**	***69.4 (2.3)***	***84.2 (2.0)***	***42.2 (3.6)***	***38.3***	***7.0***	***45.8***	***11.8***
***E.Coli* 13**	***54.1 (1.4)***	***53.6 (1.2)***	***45.4 (0.9)***	***36.7***	***0.0***	***35.3***	***13.4***

The prediction of adhesion behavior of *E. coli* strains on stainless steel support before and after treatements with hypochlorite is calculated with ΔG_{Tot} equations (Formula 3, 4, 5). Results are presented in table 4. it is widely known that when a lower interaction free energy ΔG_{Tot} is obtained, a higher adhesion is expected. In a more precise way, only negative values of ΔG_{Tot} predict favorable adhesion.

Strains	0°	6°	8°	10°	12°	14°	16°	18 °
***E.coli* 19**	*8.2*	*2.4*	***-9.9***	***-37.8***	***-19.5***	*6.0*	*3.1*	*5.0*
***E.coli* EM3**	*6.9*	*1.9*	***-10.0***	***-38.4***	***-19.8***	*5.1*	*2.5*	*4.0*
***E.coli* EM4**	*8.3*	*3.9*	***-7.7***	***-34.6***	***-16.5***	*7.4*	*3.8*	*6.3*
***E.coli* 13**	*26.2*	*15.2*	***-0.9***	***-18.5***	***-3.3***	*13.6*	*22.9*	*24. 4*

Table 4: Total interaction free energy ΔG_{Tot} (mJ.m−2) for the adhesion between *E. coli* strains and stainless steel supports (304L) after treatment with sodium hypochlorite.

Before treatment, the positive values of the ΔG_{tot} indicate unfavorable adhesion of all *Escherichia coli* strains from a thermodynamical point of view. After treatments, the bacterial adhesion behavior changed remarquadly: ΔG_{tot} values increased significantly at 6°Chl until becaming negative on the treated support with 8°, 10°, 12°Chl. These results indicate a favorable adhesion potential between (8° to 12°Chl) and especially on surfaces treated by 10°Chl. However, outside this range, the adhesion behavior remains thermodynamically unfavorable.

It should be expected that concentrations (10° and 12°Chl) commonly used in disinfecting protocols and almost all food industries, make surfaces more hygienic. However, these concentrations seem to not be theoretically efficient on sessile cell form.

By analyzing the results of surface properties (Table 2), we observed that the electron donor character (y-) seems to be related to the hygienic status of the stainless steel surfaces: low y- values were associated to high theoretical adhesion (low hygienic status) on stainless steel with all *E. coli* strains. Furthermore, quantitative hydrophobicity (ΔGiwi) of surfaces after treatment seems to affect the potential adhesion of *E. coli* strains especially when it is hydrophobic (table 2): adhesion behavior become more prenounced when the surface treated become more quantitatively hydrophobic. This finding is similar to previous works that indicated an increased adhesion of bacterial vegetative cells [27] and Bacillus spores [28], [29] on hydrophobic materials.

As a consequence, adhesion ability of *Escherichia coli* strains on stainless steel surfaces may greatly increase when the treated surfaces become hydrophobic and the electron donor character (y-) decreases significantly.

4 CONCLUSION

Stainless steel surfaces demonstrated to be highly instable after treatment with different concentrations of sodium hypochlorite. The surface properties (hydrophobicity, electron donor/acceptor) were shown to be significantly affected with treatments while the stainless steel roughness was shown to be poorly changed with one cycle of treatment. All these variations in the physicochemical surface properties affected the hygienic status of the supports and consequently leaded to reinforce (or weaken) bacterial attachment depending on concentrations used in treatment.

Under the conditions of this study, the obtained results showed that adhesion ability of *Escherichia coli* strains on stainless steel was favorable when the treated surfaces become hydrophobic and electron donor character (y-) decreases significantly. However, it was difficult to link the adhesion ability to other surface properties.

Roughness parameter Ra has been chosen to define the minimum requirements for hygienic design of product contact areas [30], [31]. But, we suggest by this work to consider stability of physicochemical properties against disinfecting treatments as important parameters to define hygienic supports to be used especially in food industries.

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