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Adhesion of *Candida albicans* to polythene in Sodium hypochlorite disinfected aquatic microcosm and potential impact of cell surface properties

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ABSTRACT

The adhesion of C. albicans cells harvested from different growth stages to polythene in Sodium hypochlorite (NaOCl) treated water, under static and dynamic conditions was carried out. With cells harvested from the lag, exponential, stationary and decline growth phases, the highest abundance of cells adhered was respectively 0.75, 1.18, 0.85 and 0.95 units (Log (CFU/cm^2)), when considering the static and dynamic experimental condition. The hydrophobicity values ranged respectively from 17 to 99, 45 to 93, 8 to 93, and from 10 to 83 %. The increase in incubation duration was significantly correlated (P<0.01) to the decrease in the abundance of adhered C. albicans when considering the NaOCl concentrations used. The increase in the NaOCl concentration in water was significantly correlated (P<0.01) to the increase in the abundance C. albicans adhered to polythene when considering the incubation durations. An increase in NaOCl concentration in the water seems significantly correlated with the decrease of the abundance of C. albicans adhered to the substrate (P<0.01) when considering each cell growth phase. Using the H (Kruskal-Wallis) test to compare the registered mean abundances of cells adhered to polythene after living in water treated with various concentrations of NaOCl with respect to the growth phase, a significant difference (P<0.05) was noted with cells coming from the exponential, stationary and decline growth stages. The adhesion of C. albicans to polythene seems to be impacted by various factors with some depending on the cells status.

Keywords: *Candida albicans*, adhesion, NaOCl, Cell growth phases, aquatic microcosm, hydrophobicity.

INTRODUCTION

In order to respect the standards of potable water, and preserve public health, one of the major concerns of companies in charge of the treatment of drinking water is to effectively meet the demand and maintain a distribution of water of good quality [1]. Many interactions between disinfectants, pipe walls, free and fixed biomasses always take place and often resulted in many physicochemical and biological reactions. These reactions may be the cause of the degradation of the organoleptic properties of the water supply [2]. The control analyses of drinking water are based not only on physicochemical parameters, but also on microbiology [3].

The purpose of drinking water treatment is not to produce sterile water, but water that does not pose a risk to public health. These analyses relate mostly to faecal and/or pathogenic bacteria like *Escherichia coli* and *Streptococcus faecalis* [4]. The presence of these bacteria species indicates the relatively high probability of the presence of germs that cause waterborne diseases.

Candida albicans is one of the specific germs species on which research has increasingly being of interest to the scientific community. It has been indicated that *C. albicans* is a cosmopolitan yeast. Its isolation frequency varies with respect to the sampling sites [5]. Its main reservoir is the gut where the frequency of carriage varies with respect to individuals. This yeast is the major cause of opportunistic nosocomial infections of fungal origin. A large majority of deep infections is of endogenous origin and develop from saprophytic yeasts present in the digestive tract of the host and the mucosa. A small proportion of candidiasis may also be of exogenous origin from skin colonization due to equipment care and insertion of catheter into the human body [5].

Previous works allowed the understanding of the mechanisms of emergence of *C. albicans* in the drinking water distribution system [5]. They also showed that *C. albicans* which is a facultative anaerobic microorganism can exceptionally be isolated from soil and water, but this usually resulted from faecal contamination [5, 6].

The adhesion of *C. albicans* to a surface is a key step in the process of *Candida* pathogenesis and resistance against current treatments [7]. Many studies have also been focused on measuring the hydrophobicity of *C. albicans* at different growth phases [8], monitoring water supply, treatment plants, and health risks associated with the dysfunctional network of drinking water distribution in Cameroon [9-11]. They showed on one hand that the cells coming from exponential and stationary growth phases appear as some of the important factors impacting the *C. albicans* adhesion on solid surface [8]. On the other hand they also showed that despite treatment done upstream in factories, in addition to disinfectant residuals in pipes and the oligotrophic nature of the medium, some microorganisms adapt and proliferate in the water distribution network [1]. The latter are sometimes the cause of nests and microbial biofilm formation, among others. In addition, the variation in the abundance of microorganisms in response to disinfectants can be linked to changes in their cell wall which may be due to a change in their growth stage [12] as well as their cell surface properties [13, 14].

Although many studies have been focused on the mechanisms of emergence of *C. albicans* in drinking water distribution systems [5], there is little information on the importance of growth stages or metabolic processes and cell surface properties in the presence of disinfectants on the cell adhesion process to surface. This study aimed at evaluating in microcosm condition, the effect of cell surface hydrophobicity variation of *C. albicans* cells on their adhesion at different growth stages to polythene immersed in water treated with different concentrations of Sodium hypochlorite (NaOCl), under stationary and dynamic conditions over time.

MATERIALS AND METHODS

Collection and identification of *C. albicans* strains, and cell storage

Candida albicans strains was provided by the Microbial Laboratory of the Yaounde Central Hospital (YCH). Its identification was firstly based on its culture characteristics on a supplemented chloramphenicol Sabouraud culture medium after 48 hours incubation at 37°C, then on cell morphological and growth's characters under microscope (filamentation test, chlamydosporulation, growth at 45°C) and biochemical properties using ELIchrom fungi gallery (BioMérieux, France). Biochemical tests underlined that *C. albicans* strains used is able in aerobic conditions to assimilate (auxanogram) or in anaerobic conditions to ferment (zymogram) an organic carbon for its growth. In both conditions, these cells use glucose, maltose, sucrose and galactose. They do not synthesize urease and they develop resistance to acitidione. It was then stored in glycerol at -70°C before use to avoid excessive subcultures.

Assessment of *C. albicans* cell growth phase

Three replicates of 15 test tubes each containing 10 mL sterile tryptone (Biokar) solution were used. Tubes of each set were labeled t_0, t_2, t_4, t_6, t_8, t_{10}, t_{12}, t_{14}, t_{16}, t_{18}, t_{20}, t_{22}, t_{24}, t_{26} and t_{28}. Prior to the experiment, a frozen vial containing *C. albicans* was defrosted at room temperature. The culture (300 µL) was then transferred into 10 mL of nutrient broth (Oxford) and incubated at 37°C for 24 hours. Cells were then collected by centrifugation (8000 rpm for 10 min at 10°C) and washed twice with sterile NaCl (8.5 g/L) solution. The sediment was then diluted in 10 mL of sterile NaCl solution. After dilution, 100 µL of the suspension was added to each of the 15 tubes containing 10 mL of sterilized peptone solution.

The cell suspensions in 3 tubes labeled t_0 were immediately analyzed. Those in the tubes labeled t_2, t_4, t_6 … t_{28} were incubated for 2, 4, 6… 28 hours at 37°C. After each incubation duration, analyses were carried out using plate count method on a supplemented Chloramphenicol Sabouraud culture medium, incubated for 48 hours at 37°C. The colony forming units (CFUs) were then counted. Mean CFUs were calculated from the results of the triplicates and Log(CFU) also calculated. The straight Log(number of CFUs) curve against storage period was plotted and compare to the cell growth curve. The cell growth phases of *C. albicans* were then determined.

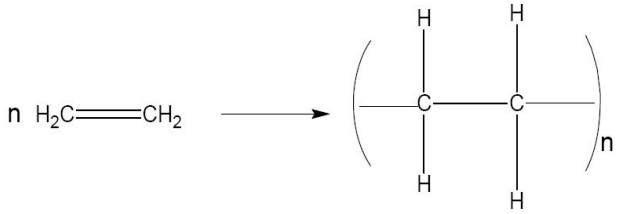
Disinfectant used

Sodium hypochlorite (NaOCl) which belongs to the group of halogen derivatives was used. NaOCl used was from of the Colgate-Palmolive (USA) brand. The ease with which this disinfectant is generally used for drinking water treatment justified its choice for this study. Concentrations of NaOCl used ranged from 0.25 to 0.75‰. These concentrations were evaluated by simple method of dilution of crude solution obtained directly from the manufacturer. To count the surviving

microorganisms after disinfection, the sterile NaCl solution was used as a diluent.

Adsorbing substrate used

The adsorbing substrate used was high dense polythene. It differed from low radical dense polythene and low linear dense polythene by sparsely branched chains of its molecular structure, and its relatively high resistance to shocks, high temperatures and ultraviolet rays [15, 16]. It is a plastic piping material obtained directly from the manufacturer and used in drinking water distribution networks. High dense Polythene resulted from polymerization of macromolecules of polyolefin family. This polymerization is obtained from gaseous ethylene according to the following equation [17, 18]:



The polythene used in this study is commercialized by Goodfellow SARL (France).

Adhesion test of *C. albicans* on polythene in the solution containing NaOCl

On the basis of previous studies, parallelepipedic shaped fragments of polythene with 13.28 cm^2 of total surface area suspended to a wire of 0.1 mm diameter were immersed in triplicate in two sets named A and B. Set A contained four subsets each having three Duran's 250 mL flasks labeled as follows: A1, A1', A1'', A2, A2', A2'', A3, A3', A3'', and A4, A4', A4''. Same for set B with labeling as follows: B1, B1', B1''; B2, B2', B2''; B3, B3', B3'' and B4, B4', B4''. Each flask contained 99 mL of NaCl solution. Meanwhile, controls were made and coded $A_0$1, $A_0$2, $A_0$3, $A_0$4 and $B_0$1, $B_0$2, $B_0$3, $B_0$4 [19]. The whole was then autoclaved. Prior to the experiment, a frozen vial containing *C. albicans* strains was defrosted at room temperature. The culture (300 µL) was then transferred into 10 mL of nutrient broth (Oxford) and incubated at 37°C for 24 hours and the cells latter collected by centrifugation at 8000 rpm for 10 min at 10°C, then washed twice with sterile NaCl solution. The sediment was then diluted in 10 mL NaCl solution.

After serial dilutions, the initial concentration of yeast cells (concentration at t=0) in each mother solution was adjusted to 6x10^8 CFU/mL. This was performed by reading the optical density (OD) at 600 nm using a spectrophotometer (DR 2800) followed by culture on Sabouraud agar medium [19]. Afterwards, 1 mL of the suspension was added to 99 mL of sterilized NaCl solution contained in Erlenmeyer flasks. For adhesion tests, the final concentrations of NaOCl 0.25, 0.5 and 0.75‰ were respectively prepared in the following three sets of 8 Erlenmeyer flasks: A1, A2, A3, A4, B1, B2, B3, B4, A1', A2', A3', A4', B1', B2', B3', B4' and A1'', A2'', A3'', A4'', B1'', B2'', B3'', B4''.

A set of these erlenmeyer flasks were incubated in triplicates under dynamic condition by stirring at a speed of 60 rpm, using a stirrer (Rotatest brand) and another set under static condition for 180, 360, 540 and 720 min. All these incubations were done at laboratory temperature (25 ± 1°C).

Unhooking cells from fragment

After each incubation period, fragments of polythene were dripped for 10 seconds in a sterile environment created by the Bunsen burner flame and then introduced into 10 mL of sterilized NaCl solution. The unhooking of adherent cells was performed by vortex agitation at increasing speeds for 30 seconds in three consecutive series of 10 mL sterilized NaCl solution. This technique allows the unhooking of maximum adhered cells [20, 21]. The total volume of the suspension containing unhooked *C. albicans* was 30 mL. Isolation and numbering of unhooked cells were performed using the spread plate method on a supplemented chloramphenicol Sabouraud medium, followed by the incubation of Petri dishes at 37°C for 24 to 48 hours. The disinfectant was not evaluated after incubation.

Assessment of the hydrophobicity cell surface of *C. albicans*

C. albicans surface hydrophobicity was measured by the adhesion test on polythene using the MATH (Microbial Adhesion To Hydrocarbons) method [13], as recently described by Jain *et al.* [14]. After culture on supplemented chloramphenicol Sabouraud medium, cells were centrifuged at 8000 rpm for 10 min at 10°C, washed and resuspended at a concentration of 10^8 CFU/mL in distilled water (pH 4.9) by reading the spectrophotometer DR 2800. The optical density (OD) of the solution was measured at 400 nm (Ao). Then 1 mL of this solution is introduced into two sets (A and B) of 5 Erlenmeyer flasks each in triplicates A0, A0', A0'' and B0 , B0', B0'', A1, A1', A1'' and B1, B1', B1'', A2, A2', A2'' and B2, B2', B2'', A3, A3', A3'', and B3, B3', B3'', and A4, A4', A4'' and B4, B4', B4'' containing 99 mL sterilized NaCl solution and parallelepipedic shaped fragments of polythene of 13.28 cm^2 total surface area suspended to a wire of 0.1 mm diameter. The mixture was incubated at room temperature (25±1°C) in stationary and dynamic regimes for 180, 360, 540, and 720 min. After each incubation period, the OD of the aqueous phase was measured (A1). Concentrations of *C. albicans* inocula were assessed by turbidity and expressed by measuring the OD at 600 nm on a spectrophotometer DR 2800. A density of 0.12 to 0.15 corresponded to 1-5 x 10^5 CFU/mL [22].

The percentage of cells adhered to the polythene was calculated using the following formula [14]:

$$\text{Cell adhered (\%)} = \frac{A0 - A1}{A0} \times 100$$

In this formula, A0 is the OD of the solution measured at 400 nm before the substrate immersion, and A1 is

the OD of the aqueous phase measured after cell adhesion process. According to Rosenberg [13] and Jain *et al.* [14], the percentage of cells adhered to the polythene in this condition is closely proportional to the cell surface hydrophobicity. According to those authors, the more the value obtained is closer to 100% the more cell surface is hydrophobic. The percentages of cells adhered were then compared to the hydrophobicity percentages [14, 23].

Data analysis

Variations in abundance of adhered *C. albicans* in each experimental condition were illustrated by semi-Logarithmic curves. Standard deviations were not considered because the curves were too close. Spearman "*r*" correlation test was used to assess the degree of relation between the abundance of adhered cells and other parameters considered. To compare the mean abundance of adhered *C. albicans* from one experimental condition to another; Kruskal-Wallis H-test and Mann-Whitney U-test were used using the statistical software package SPSS 17.0. A *p*-value of 0.05 was assumed to be statistically significant.

RESULTS AND DISCUSSION

Growth curve

The *C. albicans* growth in sterile tryptone (Biokar) solution exhibited a hyperbolic curve of 4 phases: a lag growth phase of 5 hours duration, an exponential growth from the 5th to the 13th hour of incubation, a stationary growth phase of 9 hours duration, and a decline growth phase which begin from the 22nd hour of incubation (Figure 1).

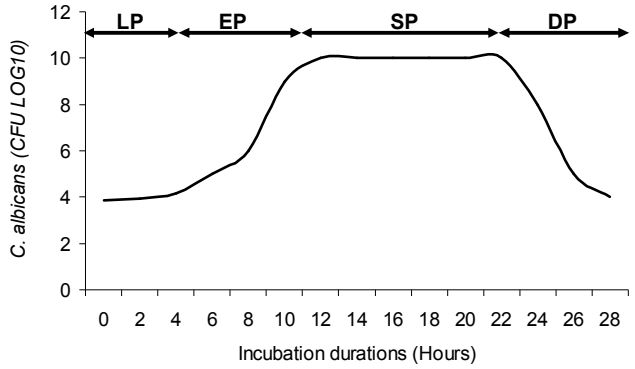


Figure 1. Growth curve of *C. albicans* (**LP**: Lag growth phase, **EP**: Exponential growth phase, **SP**: Stationary growth phase, **DP**: Decline growth phase).

Adhesion Kinetics in stationary and dynamic regimes

The hourly *C. albicans* cells adhesion rate on to the considered substrate was assessed in static and dynamic conditions. It has been noted that it varied from one cell growth phase to another (Table 1). Under static condition, adhesion speeds varied from 1.744 to 17.389 cells adhered/cm²/h whereas under dynamic condition, it varied from 2.169 to 8.989 cells adhered/cm²/h. Under these experimental conditions, the lowest adhesion speed (1.744 cells adhered/cm²/h) was recorded under static condition with the cells harvested from the lag growth phase. The highest adhesion speed (17.389 cells adhered/cm²/h) was obtained in the same experimental condition but with cells harvested from the stationary growth phase (Table 1).

Table 1. Hourly adhesion speeds (and regression coefficient) of *C. albicans* with respect to cell growth phases under static and dynamic conditions.

Cell growth phases	Adhesion speed (Cell adhered/cm² /h)	
	Static	Dynamic
Lag	1,7436 (0,8313)	2,1687 (0,9863)
Exponential	11,9666 (0,9144)	8,989 (0,8327)
Stationary	17,389 (0,9675)	8,811 (0,9117)
Decline	2,6444 (0,8342)	2,9777 (0,9672)

Abundance of adhered *C. albicans* to polythene after a stay period in the NaOCl solution

Abundance of adhered *C. albicans* in the solution treated with NaOCl sometimes reached 1.18 units (Log (CFU/cm²)). The maximum abundance of cells adhered was recorded during the exponential growth phase under static condition after 180 min in the solution containing 0.25‰ NaOCl. *C. albicans* was sometimes completely decimated by NaOCl. This result was recorded under static condition during the lag phase in the solutions containing different concentrations of NaOCl and under dynamic condition in the solution disinfected with 0.75‰ NaOCl. The same observation was made in the stationary and decline growth phases under dynamic condition in the 0.75‰ NaOCl solution.

With cells harvested from the lag phase, abundance of adhered *C. albicans* to the control substrate ranged from 1.27 to 1.95 units (Log (CFU/cm²)) and was always higher than those adhered to the substrate in the NaOCl treated solution. Furthermore, they increased with incubation periods. The maximum abundance of cells adhered was recorded after an adhesion test of 720 min under dynamic condition. In the solution disinfected with NaOCl, abundance of adhered *C. albicans* sometimes reached 0.75 units (Log (CFU/cm²)). The densities of adhered cells in the solution treated with NaOCl decreased with incubation periods. The maximum density was observed under dynamic condition after an adhesion test of 180 min in the solution containing 0.25‰ NaOCl (Figure 2).

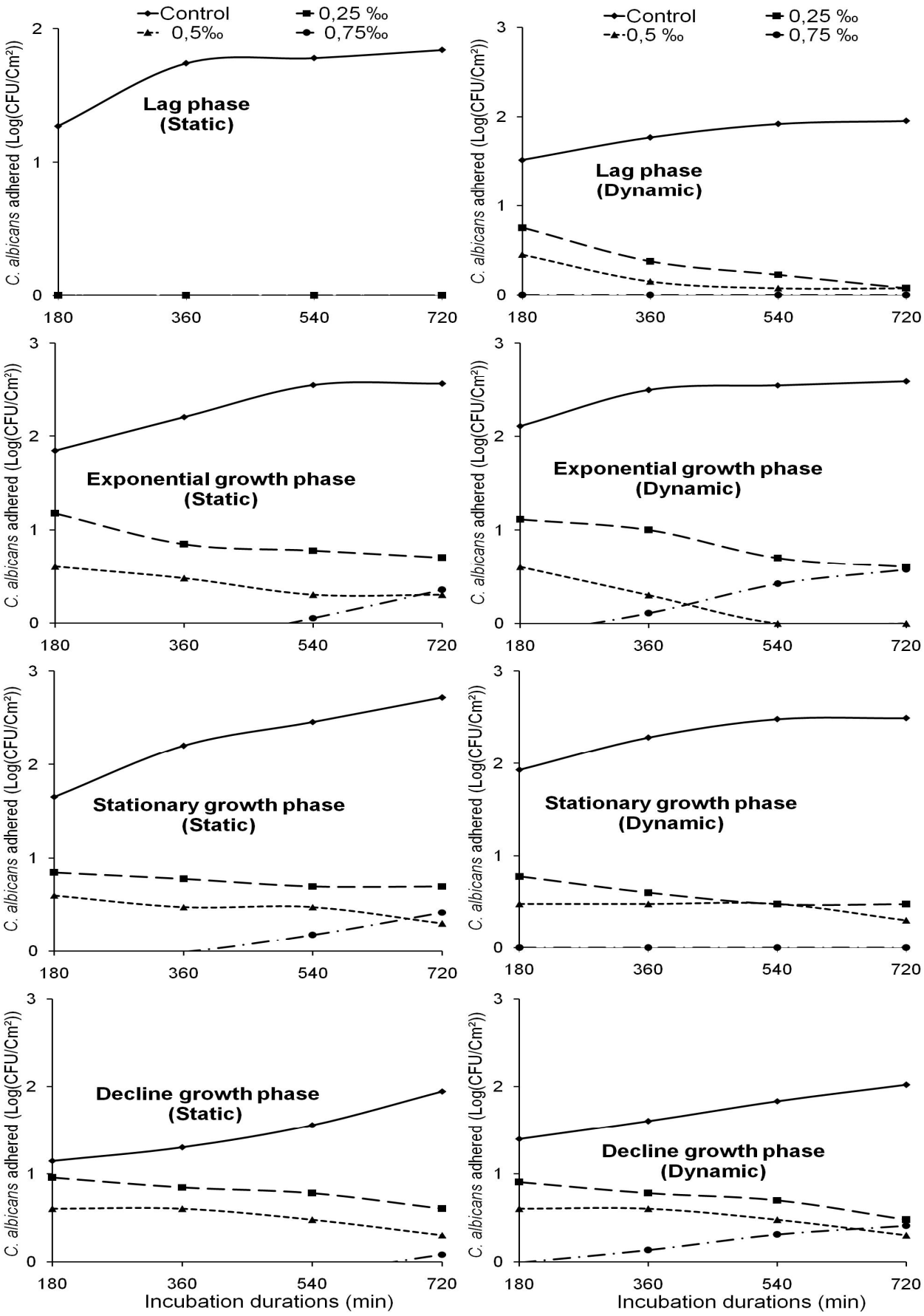


Figure 2. Temporal variation of the abundance of cells adhered under static and dynamic conditions in the solution containing NaOCl at the various concentrations.

With cells reaching the exponentially growth phase, abundance of adhered *C. albicans* to the control substrate ranged from 1.85 to 2.59 units (Log (CFU/cm^2)) and was always higher than those adhered to the substrate in the NaOCl treated solution and also they increased with incubation periods. The maximum abundance was recorded after an adhesion test of 720 min under dynamic condition. In the solution disinfected with NaOCl, abundance of *C. albicans* sometimes reached 1.18 units (Log (CFU/cm^2)). The densities of adhered cells in the solution treated with NaOCl decreased with incubation periods. The maximum density was observed under static condition after an adhesion test of 180 min in the 0.25‰ NaOCl solution (Figure 2).

The stationary growth phase exhibited abundance of *C. albicans* adhered to control substrate ranging from 1.65 to 2.72 units (Log (CFU/cm^2)) and was always higher than those adhered to the substrate in the NaOCl treated solution and increased with incubation periods. The minimum and maximum abundance were respectively recorded after an adhesion test of 180 and 720 min under static condition. In the solution disinfected with NaOCl, abundance of adhered *C. albicans* often reached 0.85 units (Log (CFU/cm^2)). The densities of adhered cells in the solution treated with NaOCl decreased with incubation periods. The maximum density was observed under static condition after an adhesion test of 180 min in the 0.25‰ NaOCl solution (Figure 2).

With cells harvested from the decline growth phase, the abundance of *C. albicans* adhered to control substrate ranged from 1.15 to 2.02 units (Log (CFU/cm^2)) and was always higher than those adhered to the substrate in the NaOCl treated solution. In addition, they increase with incubation periods. The maximum abundance of adhered cells was recorded after 720 min incubation period under dynamic condition. In the solution disinfected with NaOCl, abundance of adhered *C. albicans* sometimes reached 0.95 units (Log (CFU/cm^2)). The densities of adhered cells in the solution treated with NaOCl decreased with incubation periods. The maximum density was observed under static condition after an adhesion test of 180 min in the 0.25‰ NaOCl solution (Figure 2).

Surface hydrophobicity of adhered *C. albicans* cells after a stay period in the NaOCl solution

The percentages of cells adhered were calculated and then compared to the hydrophobicity percentages. The percentage of cells adhered to the polythene in experimental conditions is closely proportional to the cell surface hydrophobicity. Without any treatment with NaOCl, the hydrophobicity ranged from 17 to 95%. It increased with incubation periods. The minimum value was recorded after 180 min under static condition with cells coming from the decline growth phase. During the same growth phase, the maximum value of hydrophobicity percentage was observed after 720 min under dynamic condition. Without any treatment, *C. albicans* surface seems more hydrophobic in the decline growth phase under dynamic condition after 720 min. In addition, it was observed that the *C. albicans* cell surface hydrophobicity varied with the NaOCl concentrations used.

With cells harvested from the lag phase, the hydrophobicity ranged from 17 to 99% after living in the NaOCl treated solution. It relatively decreased with incubation periods. The lowest value was recorded under dynamic condition after 720 min in the solution disinfected with 0.75‰ NaOCl. The highest value was observed under static condition after 180 min in the solution treated with 0.25‰ NaOCl. Thus in the 0.25‰ NaOCl solution, *C. albicans* seems more hydrophobic under static condition after 180 min (Figure 3).

Cell surface hydrophobicity of *C. albicans* coming from the exponential growth phase ranged from 45 to 93% after living in the solution containing NaOCl. It also relatively decreased with incubation periods. The lowest value was recorded under dynamic condition after 720 min in the solution disinfected with 0.75‰ NaOCl. The highest value was noted under static condition after 180 min in the solution containing 0.25‰ NaOCl. In the NaOCl 0.25‰ treated solution, *C. albicans* seems is more hydrophobic under static condition after 180 min (Figure 3).

With cell harvested from the stationary growth phase, the hydrophobicity ranged from 8 to 93% after living in the solution treated with NaOCl. With different NaOCl concentrations, it decreased with incubation periods. The minimum value was observed under static condition after 720 min with NaOCl concentration 0.75‰. The maximum value was recorded under dynamic condition after 180, 360, and 540 min in the solution disinfected with 0.25‰ and 0.50‰ NaOCl. In the 0.25‰ and 0.50‰ NaOCl solutions, *C. albicans* cells seem more hydrophobic under dynamic condition after 180, 360, and 540 min (Figure 3).

The hydrophobicity ranged from 10 to 83% with the cells coming from decline growth phase. It relatively decreased with incubation periods. The maximum and minimum values were recorded under static condition, respectively after 180 and 720 min in the NaOCl 0.25‰ and 0.75‰ treated solutions. With 0.25‰ NaOCl solution, *C. albicans* seems more hydrophobic under static condition after 180 min (Figure 3).

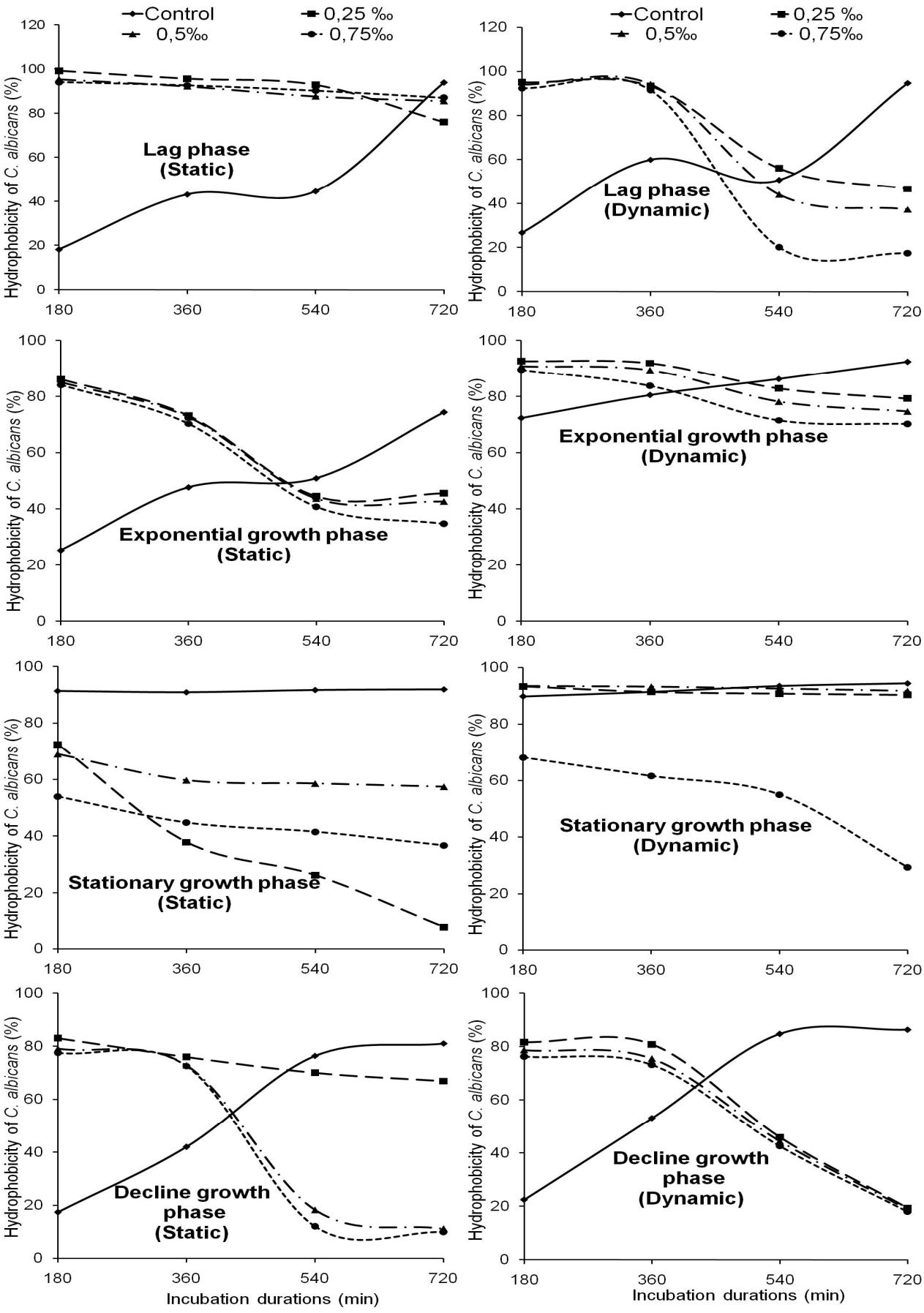


Figure 3. Variation of the hydrophobicity of *C. albicans* cells surface under static and dynamic conditions in the solution containing NaOCl at various concentrations, with respect to the incubation durations.

Correlation between the abundance of *C. albicans* cells adhered and incubation duration or concentrations of NaOCl

Spearman *"r"* correlation coefficients between the abundance of adhered *C. albicans* and incubation durations for each concentration of NaOCl and under each experimental condition were assessed and presented in Table 2. It was noted that the increase in incubation duration is significantly correlated (P<0.01) to the decrease in the abundance of adhered *C. albicans* in the solution disinfected with NaOCl.

Spearman *"r"* correlation coefficients between abundance of adhered *C. albicans* and NaOCl concentrations for each incubation period and under each experimental condition were also assessed. Under static as well as dynamic condition, it was noted that the increase in the NaOCl concentration in water is significantly correlated (P<0.01) to the increase in the abundance *C. albicans* adhered to polythene (Table 3).

The degrees of relationship between NaOCl concentrations and abundance of adhered *C. albicans* harvested from each growth stage were assessed and are presented in Table 4. It resulted that an increase in NaOCl concentration in the water seems significantly correlated with the decrease of the abundance of *C. albicans* adhered to the substrate (P<0.01).

Table 2. Spearman "*r*" correlation coefficients between the abundance of adhered *C. albicans* and incubation periods for each concentration of NaOCl and each experimental condition.

Experimental condition	NaOCl concentrations		
	0.25‰	0.5‰	0.75‰
Static	-0.775*	-0.833**	-0.500**
Dynamic	-0.949*	-0.400*	-0.500**

**: P <0.01; *: P <0.05 ddl=15

Table 3. Spearman "*r*" correlation coefficients between the abundance of adhered *C. albicans* and concentrations of NaOCl for each incubation period and under each experimental condition.

Experimental condition	Incubation periods			
	180 min	360 min	540 min	720 min
Static	0.992**	0.976**	0.986**	0.994**
Dynamic	0.954**	0.940**	0.873**	0.947**

**: P <0.01 ; ddl=15

Table 4. Spearman "*r*" correlation coefficients between NaOCl concentrations and abundance of adhered *C. albicans* harvested from each growth stage.

Experimental condition	Cell growth phases			
	Lag	Exponential	Stationary	Decline
Static	-0.993**	-0.982**	-0.991**	-0.979**
Dynamic	0.499	-0.957**	-0.944**	-0.967**

**: P <0.01 ; ddl=11

Comparison of mean abundance of adhered *C. albicans* amongst the different NaOCl concentrations used at each growth stage

For each NaOCl concentration used, the mean abundance of adhered *C. albicans* from the three incubation periods was calculated for each cell growth stage. The mean abundances noted from the three NaOCl concentrations used were compared using the H test of Kruskal-Wallis. It was noted that with cells harvested from the lag growth phase, no significant difference was registered amongst the mean abundance of *C. albicans* adhered to polythene (P>0.05). On the contrary, using cells coming from the exponential, stationary and decline growth stages, there was a significant difference (P<0.05) amongst the mean abundance of adhered *C. albicans* after living in the solutions treated with the various concentrations of NaOCl.

DISCUSSION

This study carried in aquatic microcosm conditions, showed that *C. albicans* adhered to polythene with varying abundance. Adhesion of microorganisms to surfaces is the first step in biofilm formation which is a form of microbial life in the aquatic environment [24]. It is the source of biocontamination problems in various fields such as health, environment, food industry, water purification [25-27].

It has been indicated that the development of *C. albicans* biofilm starts with the adhesion of blastospores to a surface substrate. This adhesion is ensured by non-specific hydrophobic and electrostatic interactions and specific adhesins on the surface of fungal cells [28-31]. The blastospores layer is in close contact with the anchor surface and the final three-dimensional structure on the substrate colonized. The initial adhesion is followed after about 180 to 240 minutes by the formation of micro-colonies to the colonized surface. After 660 minutes, a thick layer of *C. albicans* can be observed. The intermediate phase (720-1440 minutes) is mainly characterized by the synthesis of the extracellular matrix, which covers the premature *C. albicans* biofilm [32]. After 1440 to 2880 minutes, a complex network of yeast cells, pseudohyphae and true hyphae is established.

The adhesion is governed by physicochemical interactions of Van Der Waals and acid-Lewis base types. The greatest adhesion speed (17.389 cells adhered/cm^2/h) was recorded under static condition with *C. albicans* harvested from stationary growth phase. Fluctuating velocities of adhesion of cells observed during different growth phases in stationary and dynamic regimes could be due on one hand by changes in physiology of microorganism at each growth stage and on the other hand by changes in the cell surface hydrophobicity of *C. albicans* after living in NaOCl treated solution [8, 33, 34]. The fight against biofilm formation involves the following steps: (i) the disinfection time before the biofilm develops, (ii) the disinfection of biofilms using aggressive disinfectants, (iii) the inhibition of fixing microbes choosing surface materials that do not promote adhesion [35]. Adhesion to abiotic surfaces is provided primarily by hydrophobic interactions [36].

The hydrophobicity percentages without treatment by NaOCl increased with incubation durations. The

minimum value was recorded under static condition with *C. albicans* cells harvested from decline growth phase after 180 minutes. The more the incubation period increased, the more *C. albicans* became hydrophobic and adhered more to substrate. The hydrophobicity percentages of *C. albicans* after living in the_NaOCl disinfected solutions decreased both with incubation periods and concentrations of this disinfectant. It was proven that whatever the cell growth phases or experimental condition considered, a decrease of hydrophobicity percentages was inversely proportional to the NaOCl concentrations and incubation periods. This can be explained by the fact that the increase of cell surface hydrophobicity improves the adhesion of *C. albicans* to polythene [37].

The abundance of *C. albicans* adhered to the polythene in the solutions containing NaOCl reached 1.18 units (Log (CFU/cm^2)) although sometimes rare. Irrespective of the growth phases or experimental conditions considered, the abundance of *C. albicans* adhered to the polythene also decreased with incubation periods and different concentrations of NaOCl contained in solution. The considerable differences in antimicrobial susceptibility between planktonic *C. albicans* and adhered cells were noted. Several mechanisms explain the increased resistance of adhered *C. albicans* to antimicrobial agents. The first being that environmental gradients within the structure of adhered cells can lead to changes in NaOCl concentrations reaching individual cells. In fact, chemical gradients such as pH can affect the antimicrobial activity [38, 39]. The increased resistance of adhered cells was also explained by a delay of penetration of the antimicrobial agents through the extracellular matrix. Antimicrobial agents must diffuse through the extracellular matrix by means of water channels to reach the cells. The matrix may act as a barrier to antimicrobial compounds because the target cells are in the adhered biomass [40]. The matrix components can also bind directly to antimicrobial agents [41-43]. Another hypothesis is that the adhered cells are in a metabolic rest, grow more slowly than their planktonic counterparts, which makes them refractory to antimicrobial therapy. It is also known that nutrients limitation and the production of toxic metabolites promote biofilm formation [44].

By considering separately each experimental condition, it was noted that increasing incubation durations lead to a significant decrease of the effectiveness of NaOCl ($P<0.01$). This resulted in a decrease of the abundance of adhered *C. albicans*. It is indicated that a biofilm can develop within a few hours and allow the microorganisms therein become resistant to external agents causing any contamination [45, 46]. Under static as well as dynamic condition, increased efficiency and concentrations of NaOCl on *C. albicans* adhered to the polythene was noted. This resulted in a significant decrease of the abundance of adhered *C. albicans* after living in the NaOCl disinfected solution. According to Ji-Hyoung *et al*. [47], treatment of biofilms with antimicrobial agents entails removing adhered cells. Furthermore, the variation of *C albicans* cells behavior against the action of NaOCl may be related to changes in their surface hydrophobicity due to a change in their growth stage [12]. Some previous studies have shown the effect of pH values on the *C. albicans* adhesion [8]. Other indicated the effects of disinfectants on the adhesion of some eubacteria [48].

It was also noted that for each incubation period and each growth phase, an increase of the concentration of the disinfectant contained in the solution significantly decreased the abundance of *C. albicans* adhered to the substrate ($P<0.01$). *C. albicans* develop hyphae and form biofilms as a survival strategy to face antimicrobial agent [8, 49, 50]. Several mechanisms are involved in antimicrobial resistance of *C. albicans* adhered to the substrate namely (i) the slow penetration of the antimicrobial agent into the biofilm, (ii) chemical changes in the microenvironment formed by the adhered cells, leading to areas of slow or zero growth (iii) the adaptation of responses to stress, and (iv) the presence of a small population of highly resistant cells [39, 51, 52].

A significant difference ($P<0.05$) was observed between the mean densities of adhered *C. albicans* after living in the solutions treated with different concentrations of NaOCl in the exponential, stationary, and decline growth phases. The effectiveness of any method of disinfection depends on biotic factors such as the physiological state and the intrinsic microbial resistance to lethal agents [53]. It is important to remember that microorganisms contained in a biofilm have very different characteristics from their planktonic counterparts including the production of exopolymers [54], a significant increase in antimicrobial resistance and environmental stress [52, 55]. The exopolymer matrix that acts as a mechanical barrier, reducing the rate of penetration of the compounds through the biofilm environment, thus protecting the cells embedded in the biofilm. This explains the fact that the increase of the concentration of NaOCl in solutions for each growth stage lead to a significant decrease ($P<0.01$) of the abundance of *C. albicans* adhered to substrates. In addition, the adhesion of *C. albicans* to substrate is influenced by the cell growth phase. *C. albicans* harvested from stationary growth phase adhered in greater numbers than the cells harvested from exponentially growth phase [8].

CONCLUSION

This study showed that *C. albicans* harvested from stationary growth phase and under static condition has a high adhesion speed. The adhesion of *C. albicans* to polythene was influenced by the cell growth phase. In the solutions containing different concentrations of NaOCl, a significant difference was observed amongst the mean densities of *C. albicans* adhered in exponential, stationary, and decline growth phases. The hydrophobicity percentages of *C. albicans* in the absence of NaOCl increased with incubation periods. In

the solution treated with NaOCl, the hydrophobicity percentages decreased both with incubation periods and concentrations of the NaOCl. *C. albicans* became hydrophobic in the presence of these NaOCl when their concentrations and incubation periods decreased. The increase of cell surface hydrophobicity thus improved the adhesion of *C. albicans* to polythene. Although *C. albicans* adhered to polythene was hydrophobic in the NaOCl disinfected solution, it was noted that cell surface hydrophobicity was more important under static condition and short incubation periods.

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