EFFICACY OF SODIUM HYPOCHLORITE AND QUATERNARY AMMONIUM COMPOUNDS ON YEASTS ISOLATED FROM APPLE JUICE

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ABSTRACT

In this work, we evaluated the effectiveness of sodium hypochlorite and a commercial quaternary ammonium compound against four wild yeast strains in their different growth forms: planktonic, sessile and forming microcolonies. These yeasts had been isolated from apple juice processing plant, and appropriate environmental conditions were employed throughout the work. For planktonic cells, 4 log reduction was obtained using the lowest concentration of sodium hypochlorite (200 ppm) on all tested strains. However, quaternary ammonium chloride was effective only on Zygosaccharomyces *sp. in all in-use concentrations tested, and only the stronger in-use concentration (1:30) was effective on* Rhodotorula mucilaginosa*. Because of the above results, the disinfection on sessile yeast cells was performed only with sodium hypochlorite solutions. In cells attached in batch conditions, the highest concentration of sodium hypochlorite (500 ppm) was necessary to get 4 log reduction. In order to obtain a 3 log reduction on* Zygosaccharomyces *sp. microcolonies formed in laminar flow conditions, the combined action of 0.5% w/v NaOH and 500 ppm sodium hypochlorite was required. We concluded, thus, that cells forming microcolonies under laminar flow conditions were less susceptible to disinfectants than those suspended or attached in batch conditions. These results also indicated that attachment and colonization of autochthonous yeasts must be properly identified to evaluate the effectiveness of disinfectants used in apple juice processing plants.*

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PRACTICAL APPLICATIONS

Biofilm formation is a serious hygienic problem in food industry. In fruit processing plants, the microbial attachment causes serious engineering problems such as decreases in permeate flow in membrane processes, greatly reducing operating efficiency of ultrafiltration process. Moreover, the presence of microorganisms attached to food contact surfaces could increase the fluid frictional resistance of surfaces, increases the corrosion rate of surfaces leading to energy and production losses and compromises the hygienic quality of the final product causing significant economic losses. Our results show that the evaluation of the effectiveness of a disinfectant on biofilms should be performed using a method where the adhesion and colonization of cells was carried out in flow conditions similar to those of the environment where the disinfectant is applied. We observed that even when using new surfaces, and performing both cleaning and sanitizing under optimal conditions, the risk that the number of viable cells remaining on the surface can generate a new biofilm subsists.

INTRODUCTION

The persistence of microorganisms in biofilms is a serious hygienic problem in food industry. For example, in fruit processing plants, biofilm formation greatly reduces the permeability of filtration membranes (Flemming *et al.* 1992). Moreover, communities of microorganisms, which adhere to surfaces forming biofilms, have advantages in both growth and survival as compared with their planktonic forms (Vatanyoopaisarn *et al.* 2000).

Biofilm formation in industrial systems reflects a disturbance in the process. Biofilms are less likely to accumulate in well-designed systems, which are effectively cleaned. The European Hygienic Engineering and Design Group (http://www.ehedg.org) promotes safe food by improving hygienic engineering and design in all aspects of food manufacture, but problems with the accumulation of particulates and cells occur whenever cleaning is inappropriate for any reason.

Contamination control in food industry generally involves a first step of cleaning followed by disinfection of food contact surfaces. In practice, a biofilm on improperly cleaned surfaces is a barrier between microbes and disinfectants, antibiotics or biocides.

The current disinfectant testing position for the food industry is that disinfectant manufacturers rely on suspension tests, performed on planktonic cells, to provide data for recommended in-use concentrations. However, it is generally accepted that sessile organisms are more resistant to biocides than

the same species in their planktonic form. The biofilm will act as continuous source of food spoilage microorganisms and pathogens that will contaminate food if this increased resistance is not taken into account during disinfection (Meyer 2003; Cabo *et al.* 2009; Shi and Zhu 2009).

Few papers on yeast adhesion on solid surfaces (and not porous ones) could be seen in the literature. Guillemot *et al.* (2007) evaluated the adhesion force between *Saccharomyces cerevisiae* yeast cells and polystyrene using laboratory and industrial yeast strains, and in a previous work (Brugnoni *et al.* in press), we concluded that the yeasts strains more frequently isolated from concentrated apple juice processing plants in Argentine present a rapid capacity of adhesion to the stainless steel (SS), the more used surface in the food processing plants, still in the shortest times of assay, both under static or laminar flow conditions. Once the microbial attachment has occurred, the formation of a complex and dynamic adherent microbial community begins. For fruit processing industries, yeasts represent a major problem. Spoilage yeasts of foods and drinks are of increasing importance in food technology, being responsible for significant economic losses.

Argentine is the largest apple juice producing country in the Southern Hemisphere and exports large amounts of concentrated apple juice. The juice processing industries have recently made important capital investments in new machinery and now, there is an increasing effort to improve the juice quality (Blanco *et al.* 2005). Although yeast biofilm development may cause adverse effects on processing equipment (Bott 1992; Lehmann *et al.* 1992), there is little information about the effectiveness of cleaning-in-place system in eliminating surface adherent yeasts in the apple juice industry.

Because yeasts are usually contaminants that affect the quality and the shelf life of fruit juices and the attachment and biofilm formation of yeasts on surfaces as well as the presence of organic material impair the efficacy of disinfectants, the purposes of the current work was to study the consequences of the initial steps of yeast biofilm formation on sanitation process in the apple juice industry under batch and flow conditions. To carry out this objective, we evaluated disinfectants and cleaners commonly used in food industry against four wild yeast strains in their different growth forms: planktonic, sessile and forming microcolonies.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions

Kluyveromyces marxianus, *Candida krusei*, *Rhodotorula mucilaginosa* and *Zygosaccharomyces* sp. which isolated and identified in a previous study

from an apple juice processing industry in Argentine were used (Brugnoni *et al.* 2007). They were selected because of their high prevalence in different stages of concentrated apple juice production.

Stock cultures of each strain were suspended in 20% (v/v) glycerol in yeast-extract glucose chloramphenicol broth: 0.5% w/v yeast extract (Merck KGaA, Darmstadt, Germany), 2% w/v glucose (Merck KGaA) and 0.01% w/v chloramphenicol (Fluka Chemie AG, Buchs, Zwitzerland) and stored at −70C. For experiments, a loop of frozen yeast cells was subcultured twice in YGC broth at 25C and 100 rpm on an orbital shaker (Vicking M23, Vicking s.r.l., Argentina) until it reached the stationary phase (48 h).

Food Soiling System

The 12°Brix clarified apple juice (mean composition: fructose: 70 g/L, glucose: 35 g/L, sucrose: 16 g/L, malic acid: 0.4–3.4 g/L, citric acid: <1 g/L, ascorbic acid: <40 mg/L, potassium: 1 g/L, calcium: 0.05–0.4 g/L, phosphorus: 70–100 mg/L, sodium: 20 mg/L, free amino acids: 1–5 g/L, pH: 3.2 ± 0.2, ionic strength: 0.023 mol/L [Lozano 2006]) was prepared from 72°Brix concentrated juice and sterilized by microfiltration (pore size 0.45 μm) (Metricel®Grid, GelmanSciences, Ann Arbor, MI).

Growth Conditions and Preparation of Yeast Suspensions

Each yeast strain cultured as previously described was harvested by centrifugation at 3000 × g for 5 min (Labofuge 200, Kendro, Germany) and subsequently washed and resuspended in sterile 12°Brix clarified apple juice prepared as explained in food soiling system. The optical density was adjusted at 550 nm to 0.125 (approximately 10^6 cells/mL) using a visible light spectrophotometer (Thermo Spectronic Genesys 20, Thermo Electron Corporation, Waltham, MA).

Disinfectants and Cleaning Agents

For all experiences, the sanitizing solutions were prepared in sterile distilled water at room temperature. Sodium hypochlorite (55 g/L active chlorine, Clorox Argentine, S.A., Buenos Aires, Argentine) was used at 200 (pH 6.5), 350 (pH 8.0) and 500 ppm (pH 9.0) of sodium hypochlorite. The concentrations of the disinfectant were chosen based on the recommendations for the industry (Qin *et al.* 2002; Sharma and Anand 2002).

In the case of quaternary ammonium compound (BacterAction, Casa Thames, Buenos Aires, Argentine), three dilutions were made according to the concentration recommended by manufacturer (1:30, 1:60 and 1:90). The com-

position declared by the manufacturer was the following: quaternary ammonium chloride, nonionic tensoactive, EDTA and water (pH 6.0–7.0).

Sodium hydroxide (Merck KGaA) was used at 0.50% p/v (pH 12.0).

Suspension Tests

Suspensions test were carried out to investigate the effectiveness of the disinfectants against planktonic yeast cells and to obtain basic information to perform the tests on sessile yeast cells.

Two milliliters of yeast suspension in apple juice, prepared as indicated in growth conditions and preparation of yeast suspensions, were added to 18 mL of each disinfectant solution in sterile 150 mL Erlenmeyer flask under low stirring (50 rpm) on an orbital shaker at 23 ± 1°C. Final yeast concentration resulted approximately in 10^5 cells/mL. Samples were taken at 5 min. Each treatment was performed in triplicate per disinfectant and yeast. Each sample was diluted immediately in 9 mL of 0.1% w/v peptone water (Merck KGaA) to reduce residual disinfectant effects and to produce serial dilutions necessary for plate count method (Winniczuk and Parish 1997). Colonies were enumerated on yeast glucose agar: 0.5% w/v yeast extract (Merck KGaA), 2% w/v glucose (Merck KGaA) and 1.2% w/v agar (Merck KGaA). The plates were incubated at 25C for 5 days. The results expressed as cfu/mL were transformed to $\log_{10}$ and the log reduction was calculated.

Substrate

For adhesion experiments, the surface used was AISI 304 polished 0.5 mm SS. For the assays in batch conditions, SS was cut into rectangular chips (15×25 mm), and cut into 15×75 mm for assays in flow systems. Before the experiments, the chips were soaked for 15 min with 2% w/v of a detergent solution (Extran MA 02 neutral, Merck KGaA) at 50C and rinsed five times for 5 min each with hot tap water, followed by five rinses with distilled water. Finally, the chips were autoclaved for 15 min at 120C.

Effectiveness of Sodium Hypochlorite on Attached Yeast Cells under Batch Conditions

The experiments were carried out in sterile glass Petri dishes divided in six sections by glass pieces (Brugnoni *et al.* 2007). The divisions were made to avoid overlapping of the chips during the experiment. A chip was put into each Petri dish section.

The yeasts were prepared as indicated in growth conditions and preparation of yeast suspensions. Six milliliters of the yeast suspension in apple juice were poured into each Petri dish section. This volume was enough to cover up

the surfaces. The coupons were placed flat into Petri dishes allowing the cell adhesion and colonization only on one side. The plates were incubated at 23 ± 1C under low stirring (70 rpm). After 2 h incubation, the coupons were taken out of the experiment and rinsed in sterile distilled water at 100 rpm for 1 min to remove reversely attached cells. Then, the coupons with adhered cells were transferred aseptically to sterile Petri dishes (one Petri dish was used for each time of colonization) and covered by 6 mL of sterile 12°Brix clarified apple juice to allow colonization. After 5, 16 and 24 h of incubation, the coupons (six for each Petri dish and time of colonization) were rinsed with sterile distilled water (3 × 1 min). These coupons with adherent cells, obtained as described above, were immersed in sodium hypochlorite solutions (200, 350 500 ppm) at 23 ± 1C for 5 min. Control coupons were treated with distilled water (pH 6.5). After disinfection or control treatment, the coupons were rinsed with sterile distilled water. Each treatment was performed in triplicate per adhesion time and yeast. The number of viable yeast cells, on treated and control coupons, was evaluated by epifluorescence microscopy as is indicated in count of viable cells.

The log reduction after exposure to a disinfectant concentration was calculated by subtracting the average $\log_{10}$ number of cells per square centimeter for disinfectant-exposed biofilms from the average $\log_{10}$ number of cells per square centimeter for control biofilms. The obtained results indicate by how many log units the disinfectant reduced the microbial population during the exposure. This measurement combines the effects of killing and removal: the number of viable microorganisms on the surface can be reduced by either the physical removal of cells from the surface or by the killing of a cell that remains attached (Chen and Stewart 2000).

Effectiveness of Cleaning and Disinfection on Attached Yeast Cells Under Laminar Flow Conditions

The experiments were carried out in a flow model system (flow rate = 3.60 L/h, Reynolds = 66). The shear stress flow chamber, the flow conditions and the fluid properties were described in Brugnoni *et al.* (in press). The yeast suspensions were prepared as indicated in growth conditions and preparation of yeast suspensions and circulated for 2 h through the system at 23 ± 1C. Then, the SS coupons were rinsed by circulation of distilled sterile water for 2 min at 23 ± 1C to remove poorly adhering cells. Thereafter, sterile 12°Brix clarified apple juice was continuously supplied at the same flow rate for 16 h. Then, the coupons were rinsed by circulation of distilled sterile water for 2 min at 23 ± 1C.

To determine the effectiveness of different sanitizing sequences on young biofilm removal, a 16-h old biofilm developed on SS was exposed to the

following cleaning and disinfection sequences. Contact time and solution temperature were detailed between brackets:

- Sequence 1:
  (1) 500 ppm sodium hypochlorite (5 min, 20C),
  (2) Rinse with sterile distilled water (2 min, 20C).
- Sequence 2:
  (1) BacterAction (dilution 1:90, 5 min, 20C),
  (2) Rinse with sterile distilled water (2 min, 20C),
  (3) 500 ppm sodium hypochlorite (5 min, 20C),
  (4) Rinse with sterile distilled water (2 min, 20C).
- Sequence 3:
  (1) 0.50% NaOH pH 12.0 (10 min, 50C),
  (2) Rinse with sterile distilled water (2 min, 20C),
  (3) 500 ppm sodium hypochlorite (5 min, 20C),
  (4) Rinse with sterile distilled water (2 min, 20C).
- Control:
  Rinse with sterile distilled water (2 min, 20C).

Sequence 1 was designed to evaluate the characteristics of disinfection and removal attributed to sodium hypochlorite.

In Sequence 2, a surface active-disinfectant agent (BacterAction) was added. We wanted to observe if the adding of this agent increased the proportion of cells removed during the cleaning step prior to disinfection.

Sequence 3 simulates the traditional system of cleaning and disinfection in fruits and vegetables processing plants. In those SS accessories that are not subject to process at high temperatures, the recommended doses range from 0.1 to 0.5% with a weekly frequency of washing.

The number of viable yeast cells on the SS was evaluated by epifluorescence microscopy as is indicated in count of viable cells. Each sequence was performed in triplicate by yeast.

Count of Viable Cells

A standard stock solution of 2 mg/mL (0.2% w/v) fluorescein diacetate (FDA) ($C_{24}H_{16}0_7$, Sigma-Aldrich Chemical Co., St. Louis, MO) was prepared in acetone (Dorwil, Industria Argentina) and stored at −18C. To evaluate the number of viable yeast cells (Ki-Bong and Hideaki 2002) on SS after control and disinfection treatment, the coupons from each time were stained with sterile FDA acetonic solution in 0.1 M phosphate buffer (0.04% w/v), pH 7.5. After 90 min shaking at 23 ± 1C in the darkness, the coupons were rinsed twice with sterile distilled water. The stained surfaces were examined under an Olympus BX 51 epifluorescence microscope (Olympus, Buenos Aires, Argen-

tina) with a suitable filter combination, using a 100× oil-immersion objective. At least 20 fields (area: 0.038 mm^2) were examined per coupon.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to perform multiple analyses of the interactions between all factors. The ANOVAs were done on log data, and zero values were not encountered. Probabilities less than 0.05 were considered significant.

RESULTS

Susceptibility of Planktonic Yeast Cells to Disinfectants

The effect of disinfectants on the studied yeasts in suspension tests is shown in Table 1. In order to be considered effective, disinfectants must reduce the amount of yeasts by at least 4 log units in the case of planktonic cells in 5 min (Anon 1997, 2000). The obtained results indicated that sodium hypochlorite was more effective than quaternary ammonium chloride against planktonic yeasts. We found that all in-use concentrations of sodium hypochlorite were efficient on all tested strains producing at least 4 log reduction (Table 1). Quaternary ammonium chloride was effective only on *Zygosaccharomyces* sp. in all in-use concentrations tested, and only the stronger in-use

TABLE 1.
LOG REDUCTION OF VARIOUS IN-USE CONCENTRATIONS OF SODIUM HYPOCHLORITE AND QUATERNARY AMMONIUM CHLORIDE OBTAINED ON *KLUYVEROMYCES MARXIANUS, CANDIDA KRUSEI, ZYGOSACCHAROMYCES* SP. AND *RHODOTORULA MUCILAGINOSA*. THE DISINFECTANT EFFICACY WAS EVALUATED IN SUSPENSIONS FOR 5 min AT 23 ± 1C

Yeast	Disinfectant					
	BacterAction Use dilution			Sodium hypochlorite Use concentration (ppm)		
	1:90	1:60	1:30	200	350	500
	Log reduction					
K. marxianus	2.32	3.31	3.65	>4.00	>4.00	>4.00
C. krusei	1.25	1.39	1.68	>4.00	>4.00	>4.00
Zygosaccharomyces sp.	>4.00	>4.00	>4.00	>4.00	>4.00	>4.00
R. mucilaginosa	1.42	2.29	>4.00	>4.00	>4.00	>4.00

concentration (1:30) was effective on *R. mucilaginosa*. This test was carried out to obtain preliminary information for guidance for conducting trials with sessile cells. Because of the obtained results with quaternary ammonium chloride in suspension tests, the disinfection on sessile yeast cells were performed only with sodium hypochlorite solutions.

Effectiveness of Disinfection against Cells Adhered under Batch Conditions

In order to be considered effective, disinfectants must reduce the amount of yeasts by at least 3 log units in the case of cells which adhered to the surface (Mosteller and Bishop 1993). Figure 1 shows the susceptibility of yeast cells, grown on SS coupons at 23 ± 1C, to sodium hypochlorite at three in-use concentrations (200, 350 and 500 0ppm) after exposure for 5 min. With colonization time of 5 h, *K. marxianus*, *C. krusei* and *R. mucilaginosa* showed no significant differences ($P > 0.05$) in reduction percentage of viable cell counts to concentrations of 200 ppm (38, 38 and 39%, respectively) and 350 ppm (48, 44 and 50%, respectively), while *Zygosaccharomyces* sp. showed the greatest resistance to these concentrations (9 and 12% of reduction, respectively) (Fig. 1A). With concentrations of 500 ppm, all strains showed no significant differences ($P > 0.05$) in reduction percentage of viable cell counts (approximately 95%), except *R. mucilaginosa*, the most resistant to this concentration of sodium hypochlorite (70% of reduction) (Fig. 1A). With colonization times of 16 and 24 h, the four yeast strains showed similar reduction patterns (approximately 20% of reduction) in viable cells counts to concentrations of 200 ppm (Fig. 1B,C). *K. marxianus*, *C. krusei* and *Zygosaccharomyces* sp. showed similar reduction patterns in viable cells counts to concentrations of 350 and 500 ppm, but for *R. mucilaginosa*, the inactivation was lower compared with the three remaining yeast at these concentrations (49 and 62% of reduction, respectively, Fig. 1B) and 32 and 54% of reduction, respectively (Fig. 1C).

For *C. krusei* and *Zygosaccharomyces* sp. in all colonization times, concentrations of 200 and 350 ppm showed no significant differences ($P > 0.05$) in reduction percentage of viable cells (Fig. 1).

In summary, the disinfection with 200 and 350 ppm for 5 min was not effective in reducing the number of viable cells by at least 3 log units, with respect to the control in all time of experiences. Only with 500 ppm was a reduction of at least 3 log units obtained.

Following the results obtained in this assay, the work continued using two yeasts: *Zygosaccharomyces* sp., the most dangerous yeast for wine, concentrates, fruit juices and soft drinks industries, and *C. krusei*. In previous work

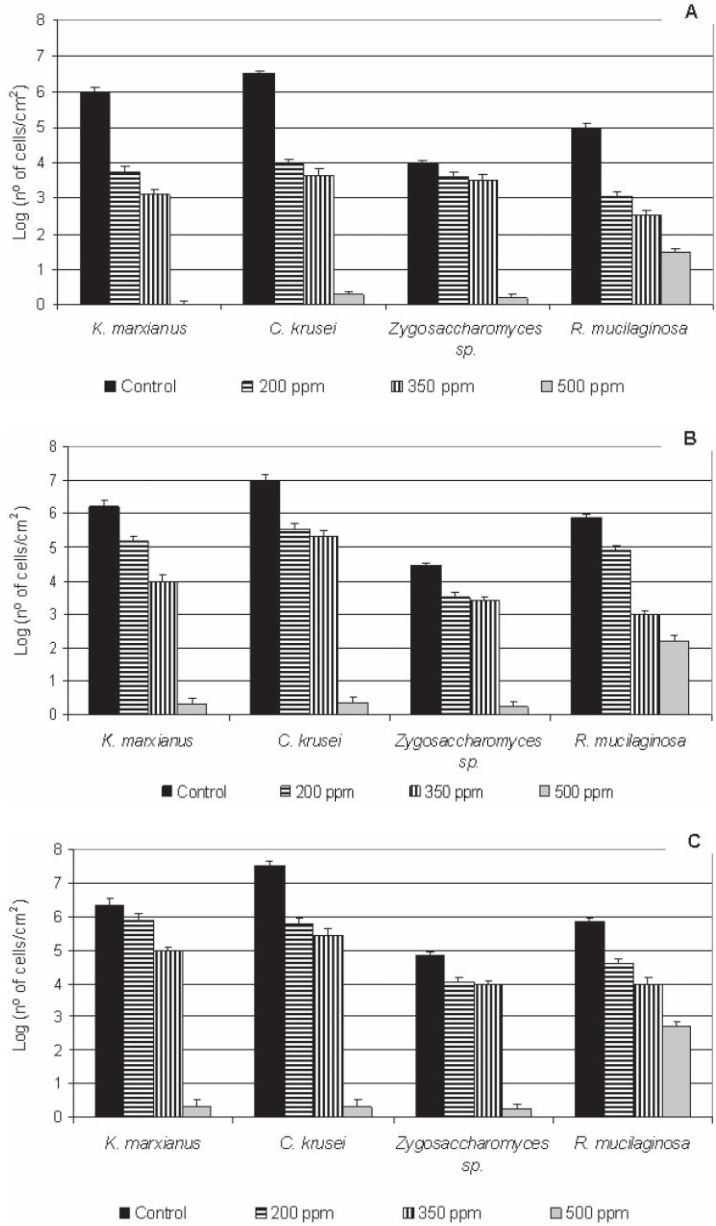

FIG. 1. LOG REDUCTION OF SODIUM HYPOCHLORITE AGAINST *KLUYVEROMYCES MARXIANUS*, *CANDIDA KRUSEI*, *ZYGOSACCHAROMYCES* SP. AND *RHODOTORULA MUCILAGINOSA*

(A) colonization time: 5 h, (B) colonization time: 16 h and (C) colonization time: 24 h. The disinfectant efficacy was evaluated for 5 min at 23 ± 1C. The log reduction in viable cell numbers measures loss of viable bacteria by the combined effects of killing and removal.

(Brugnoni *et al.* 2007, in press), we determined that *C. krusei* presents the fastest colonization and the highest covered surface on SS compared with the other yeasts.

Effectiveness of Cleaning and Disinfection against Cells Attached on Laminar Flow Conditions

In previous work (Brugnoni *et al.* in press), we observed that under laminar flow conditions, *C. krusei* reduced by at least 1 log unit the attachment and colonization with respect to the tests in batch conditions. In contrast, *Zygosaccharomyces* sp. increased the number of cells by cm^2 by approximately 1 log unit compared with batch conditions.

Figure 2 shows the results obtained for sequences 1, 2 and 3. Sequences 1 and 2 reduce by more than 50% the number of viable cells of *C. krusei* on the surfaces (3.45 log reduction). Despite Sequence 2 including a step further as the application of cleaning sequence with quaternary ammonium chloride preceding disinfection with sodium hypochlorite, no significant differences were detected between these treatments ($P > 0.05$). For *Zygosaccharomyces* sp., neither Sequence 1 nor 2 were efficient against the adhered yeast cells (more than 3 log reduction). Only Sequence 3 produced a reduction of viable

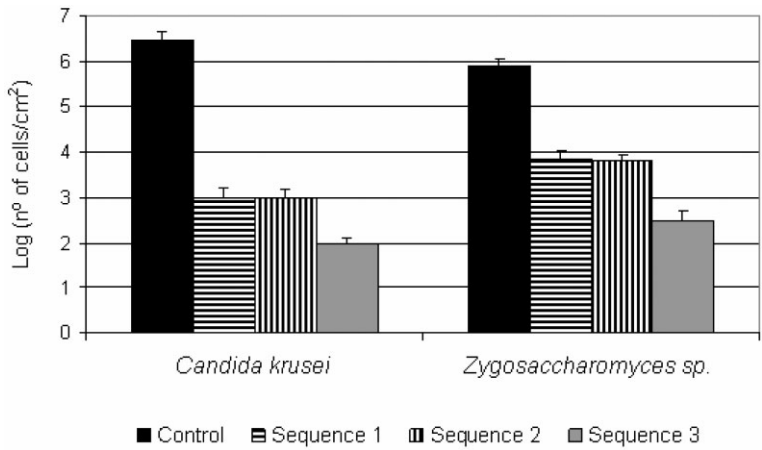

FIG. 2. LOG REDUCTION OF THREE CLEANING AND DISINFECTION SEQUENCES[a] AGAINST *CANDIDA KRUSEI* AND *ZYGOSACCHAROMYCES* SP. 16-HOUR-OLD BIOFILM DEVELOPED ON STAINLESS STEEL UNDER LAMINAR FLOW CONDITIONS

The log reduction in viable cell numbers measures loss of viable bacteria by the combined effects of killing and removal. [a]Sequence 1: sodium hypochlorite 500 ppm, Sequence 2: quaternary ammonium compound (1:90) + sodium hypochlorite 500 ppm and Sequence 3: NaOH 0.5% p/v + sodium hypochlorite 500 ppm

cells greater than 70% (4 log reduction) for *C. krusei* and greater than 55% in the case of *Zygosaccharomyces* sp. (3.4 log reduction).

DISCUSSION

The microbial biofilm formation in food processing equipment is one of the most significant sources of microorganisms and generates contamination pulses that are difficult to predict and control. Research in biofilms has shown that the physiology of cells involved in this mode of growth (sessile) differs significantly from planktonic ones. Especially, it has been exposed that the cells in a biofilm are more resistant to antimicrobial agents (Carpentier and Cerf 1993), enabling them to persist in industrial areas despite the implementation of an adequate sanitation program. Numerous studies of biofilm development have been largely devoted to bacterial species. The capacity of yeasts to form biofilm and the strategies of control have been studied particularly for species of medical importance (Crump and Collignon 2000; Singleton *et al.* 2001; Gallardo-Moreno *et al.* 2004; Polaquini *et al.* 2006). However, there is little information about yeast adhesion capacity and biofilm formation in fruit juice processing and its consequences on sanitization process.

The main goal of this work was to study the consequences of the initial steps of yeast biofilm formation on the processes of sanitization in the apple juice industry. We evaluated some disinfectants and cleaners commonly used in food industry against wild yeast strains attached to SS in batch and flow conditions. Studies conducted under batch conditions, where there is no flow of liquid relative to the surface, successfully contributed to an understanding of the phenomenon of attachment. However, in a typical liquid food processing plant, equipment inlet surfaces are either continuously or periodically in contact with flowing liquids. The experiences described in this work are an attempt to compare disinfectant efficacy on adhered cells under shear forces (laminar flow) generated by the liquid flow and no fluid forces (batch).

Under laminar flow conditions, we observed the greatest resistance to the action of sodium hypochlorite in the case of *Zygosaccharomyces* sp., and it was necessary to combine the action of an alkaline detergent with the highest concentration of sodium hypochlorite tested to obtain at least 3 log reductions (Sequence 3). In previous studies (Brugnoni *et al.* in press), we observed significant changes in *Zygosaccharomyces* sp. cell morphology under different hydrodynamic conditions. These observations seem to indicate that adhesion and colonization of this yeast under laminar flow conditions differed from that observed under batch conditions (Fig. 3). Delissalde and Amábile-Cuevas (2004) stated that during colonization and biofilm growth, cells are affected by phenotypic modifications affecting the cell–surface interactions. According to

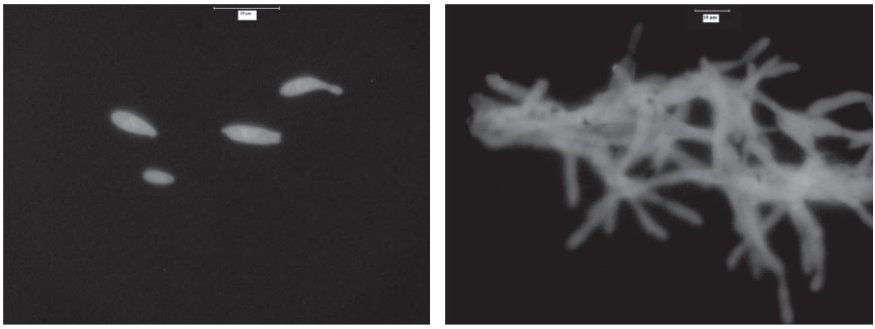

FIG. 3. EPIFLUORESCENCE MICROSCOPIC CHARACTERISTICS OF *ZYGOSACCHAROMYCES* SP. CELLS AFTER 16 H COLONIZATION: UNDER LAMINAR FLOW CONDITIONS (LEFT PANEL) AND UNDER BATCH CONDITIONS (RIGHT PANEL)

Sauer *et al.* (2002), a switch from planktonic and sessile growth involves phenotypic changes. In the present study, this phenomenon was evident, and cell morphology (size and form) was affected by the stress imposed by flux. This behavior could have serious consequences on sanitation process.

These results clearly showed that the evaluation of the effectiveness of disinfectants on yeasts isolated from an apple juice processing plant should be conducted using a method where attachment and colonization of cells are taken into account. The tests should be carried out under similar flow conditions to the environment where the disinfectant is applied. Suspension tests may be used for the food industry to screen disinfectants that must then be evaluated using surface tests. So the in-use concentrations derived from suspension tests can only be used as a guide and should be confirmed by the user in practical field trials. There is a need to evaluate the disinfectants against the strains most commonly isolated from the processing plants to establish optimal in-use conditions (concentration, time, temperature, etc.). These results should serve as a warning about the extrapolation of data. In addition, the use of appropriate disinfectant concentration is critical because, if applied at low concentrations, it not only increases the risk of contamination of the food but also the risk of resistance to disinfectant by microorganisms. At concentrations above the effective, industrial costs and environmental consequences are increased. Moreover in food industry, the possibility to increase the hypochlorite concentrations contrasts with its corrosive effect on the SS.

CONCLUSIONS

We confirmed that yeast cells forming microcolonies under laminar flow conditions are less susceptible to disinfectants than those attached in batch

conditions. The sequence that reduced the number of viable cells to a level considered acceptable by the literature is one that combines the effects of 0.5% NaOH and sodium hypochlorite at 500 ppm. It is noteworthy that the cleaning and disinfection sequences were evaluated on yeast cells forming microcolonies and it is likely that, on a biofilm already established, it becomes necessary to further increase the concentration of the agents use to achieve the expected log reductions. In the case of the food industry, the possibility of increasing concentrations of hypochlorite is limited by its corrosive effect on SS.

Despite the fact that the recommended log reduction was reached (3 log reduction), no treatment was successful in reducing the viable attached cells to non-detectable levels. The number of cells remained on surface could restart a biofilm. In addition to an adequate sanitation program, the industry should know and implement the strategies needed to prevent the establishment of biofilms, or fix the problem if it happens. At present, the implementation of good manufacturing practice and standard operating procedures, as part of an appropriate design Hazard Analysis Critical Control Point, should ensure the microbiological quality of the final product.

The experimental technique used in this study has been shown to be reproducible and to have the potential to provide valuable insights into the effectiveness and limitations of sanitization processes. Based on obtained results, the authors expect to extend the studies on mature biofilms and sanitization process under turbulent flow conditions.

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