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Clean in place (CIP) of different stainless steel geometries contaminated with *Pseudomonas fluorescens*

Limpeza *clean in place* (CIP) de diferentes geometrias de aço inoxidável contaminadas com *Pseudomonas fluorescens*

Limpieza *clean in place* (CIP) de diferentes geometrías de acero inoxidable contaminadas con *Pseudomonas fluorescens*

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Abstract

The presence of biofilms on food processing surfaces is a constant concern and can cause economic damage and impacts on public health. The aim of this work was to evaluate the development of *P. fluorescens* on the stainless steel surface, to analyze the CIP (clean in place) hygiene considering different geometries, to investigate the flow fluid dynamics and to determine the consumption of the inputs in this process. A circulation line with the characteristics of a dairy was used. The surface sampling was done using the swab technique and the performance of the process was evaluated based on decimal reductions considering the initial population adhered. The fluid dynamics study was carried out with FLUENT software and the consumption was determined by means of flow and electric current sensors. The results showed that *P. fluorescens* adhered to the surface reaching an average of 4.31 ± 0.26 log CFU·cm^{-2}, with the production of exopolysaccharides during usual time of industry operation. The decimal reduction was not significantly different among the pipe geometries in straight section, elbow, expansion and reduction. The stretch with branching in tee was statistically different from the others due to a zone of stagnation and fluid recirculation. The rinses were the stages that consumed the most water and the alkaline cleaning demanded more energy to execute the CIP. The geometries showed microbiological safety after CIP process, except tee. In addition, the expressive demand for water and energy for the execution of the process was evident.

Keywords: Hygiene; Psychrotrophic; Fluidodynamics; Food security.

Resumo

A presença de biofilmes nas superfícies de processamento de alimentos é uma preocupação constante e pode causar prejuízos econômicos e impactos na saúde pública. O objetivo deste trabalho foi avaliar o desenvolvimento de *P. fluorescens* na superfície do aço inoxidável, analisar a higienização CIP (*clean in place*) considerando diferentes geometrias, investigar a fluidodinâmica do escoamento e determinar o consumo dos insumos neste processo. Uma linha de circulação com as características de um laticínio foi empregada. A amostragem da superfície foi feita por meio da técnica *swab* e o desempenho do processo foi avaliado com

base nas reduções decimais considerando a população inicial aderida. O estudo fluidodinâmico foi realizado com *software* FLUENT e o consumo foi determinado por meio de sensores de vazão e de corrente elétrica. Os resultados mostraram que a *P. fluorescens* aderiu a superfície alcançando em média 4,31 ± 0,26 log UFC·cm^{-2}, com a produção de exopolissacarídeos durante o tempo usual de operação da indústria. A redução decimal não foi significativamente diferente entre as geometrias da tubulação em trecho reto, cotovelo, expansão e redução. O trecho com ramificação em tê foi estatisticamente diferente das demais devido a uma zona de estagnação e recirculação de fluido. Os enxágues foram as etapas que mais consumiram água e a limpeza alcalina demandou mais energia para execução do CIP. As geometrias apresentaram segurança microbiológica após o processo CIP, exceto o tê. Além disso, ficou evidente a expressiva demanda de água e de energia para execução do processo.

Palavras-chave: Higiene; Psicrotrófica; Fluidodinâmica; Segurança alimentar.

Resumen

La presencia de biopelículas en superficies de procesamiento de alimentos es una preocupación constante y puede causar daños económicos e impactos en la salud pública. El objetivo de este trabajo fue evaluar el desarrollo de *P. fluorescens* en la superficie de acero inoxidable, analizar la higiene CIP (*clean in place*) considerando diferentes geometrías, investigar la dinámica de fluidos y determinar el consumo de los insumos en este proceso. Se utilizó una línea de circulación con las características de una lechería. El muestreo de superficie se realizó mediante la técnica *swab* y se evaluó el desempeño del proceso con base en reducciones decimales considerando la población inicial adherida. El estudio de dinámica de fluidos se realizó con el *software* FLUENT y el consumo se determinó mediante sensores de flujo y corriente eléctrica. Los resultados mostraron que *P. fluorescens* se adhirió a la superficie alcanzando un promedio de 4,31 ± 0,26 log UFC·cm^{-2}, con producción de exopolisacáridos durante el tiempo habitual de operación de la industria. La reducción decimal no fue significativamente diferente entre las geometrías de tubería en sección recta, codo, expansión y reducción. El tramo con ramificación T fue estadísticamente diferente a los demás debido a una zona de estancamiento y recirculación de fluidos. Los enjuagues fueron las etapas que consumieron más agua y la limpieza alcalina demandó más energía para ejecutar el CIP. Las geometrías mostraron seguridad microbiológica después del proceso CIP, excepto el T. Además, se evidenció la expresiva demanda de agua y energía para ejecución del proceso.

Palabras clave: Higiene; Psicrotrófica; Fluidodinámica; Seguridad alimentaria.

1. Introduction

Biofilms are a community of microorganisms, adhered to the surface and embedded by a protective slime. This system consists of cells, exopolymers and residual food. This arrangement is highly efficient and makes bacteria more protected against the action of antibiotics, sanitizers and the weather. (Wang et al., 2018). The presence of biofilms in the industrial environment can cause corrosion of equipment and pipes, harbor and disseminate deteriorating and pathogenic microorganisms and reduce the rates of energy transfer in the form of heat (Bremer et al., 2006).

In the dairy industry, *Pseudomonas fluorescens* stands out with the potential for deterioration and loss of food produced (Ge et al., 2017). This species is able to grow at low temperatures, produce exopolysaccharides and cause changes in the structure and color of foods due to the production of lecithinase (phospholipase C), proteolytic enzymes and pigmented molecules (Rossi et al., 2016).

In this context, it is essential to promote an efficient hygiene process and observe the frequency of execution of the procedure. Furthermore, it is the responsibility of the food producer and equipment manufacturer to know the hygienic design of the industrial plant, in order to consider the points of difficult hygiene, due to the geometry of the system and to avoid problems of contamination and losses (Faille et al., 2017).

The CIP hygiene process is an usual practice in food plants considering the cleaning and sanitization of equipment and pipes, without dismantling the equipment and with little or no manual involvement of the operators (Memisi et al., 2015). This process is carried out in stages: pre-rinse to remove coarse residues, alkaline cleaning for solubilization and removal of protein and fat deposits, rinse for removal of residual detergent and sanitization for destruction and reduction of microorganisms to levels considered safe for processing foods (Yang et al., 2018).

Several factors participate of hygiene procedure and its effectiveness is expressed by the combination of thermal energy, which is a function of the temperature of application of fluids, chemical energy, through chemical agents and their respective concentrations, and mechanical energy, expressed through fluid flow velocity. These factors are connected and acting together with the contact time for the effectiveness of the process (Tetra Pak, 2015). Among these variables, the flow fluid dynamics has a substantial influence on the effectiveness of the CIP process (Bode et al., 2007).

The computational fluid dynamics (CFD) technique is useful to investigate the

behavior of the fluid inside the pipes. This analysis allows to predict areas of difficult hygiene and consequently critical points considering the different geometries. The quality of this prediction is based on the selection of turbulence models to represent the flow's particularities. The models most used in this approach are k-ε e k-ω due to their robustness and precision for most industrial applications (Ansys Fluent, 2014).

In addition, the CIP hygiene procedure requires numerous resources such as water, chemical agents, energy and time. According to Li et al. (2019), the dairy industry consumes approximately 28% of the total water in hygiene practices. Furthermore, from an energy point of view, this practice consumes about 13% of energy expenditure in relation to the entire manufacturing process of the industry. Yang et al. (2018) showed that alkaline cleaning and sanitization are the steps that demand the most time in the CIP process and, consequently, the biggest industrial production stops.

In this perspective, the objective of this work was to investigate the contamination caused by *P. fluorescens* on the surface of stainless steel pipes in contact with milk, to evaluate the CIP hygiene procedure considering five different geometries commonly found in industrial processing units (straight cylindrical section, tee section, elbow, expansion and reduction of pipe diameter). In addition, to analyze the behavior of fluids in each geometry using the CFD technique, evaluating turbulence models and estimating the operational consumption of inputs to perform the CIP process.

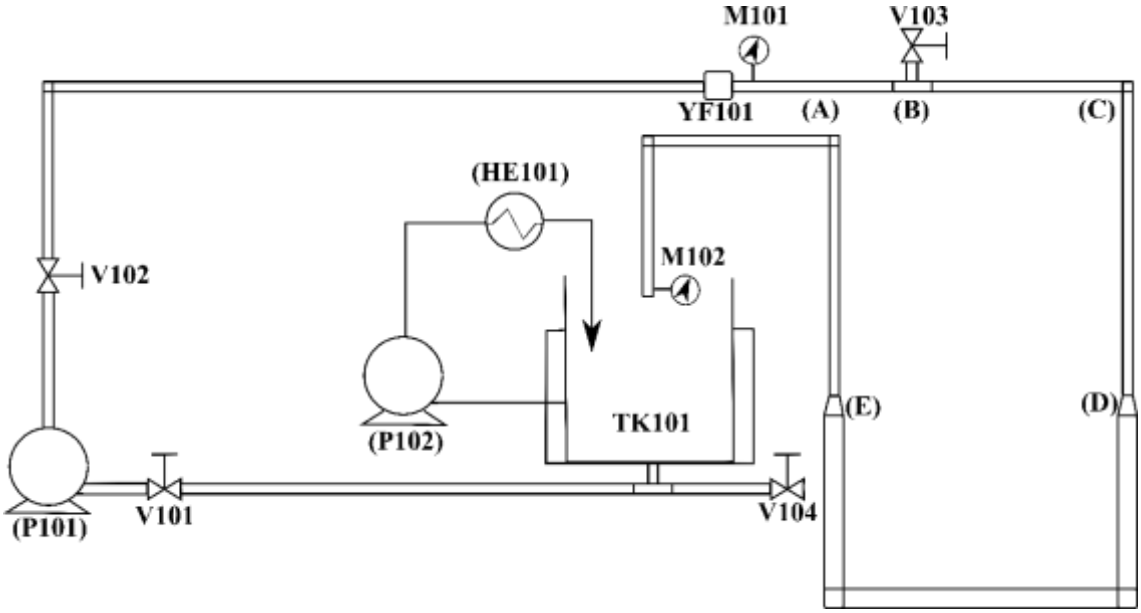
2. Materials and Methods

2.1 Experimental unit

A milk circulation system with structural characteristics similar to those used in dairy products was used, in stainless steel AISI 304, degree of polishing n° 4. The prototype of the circulation line model is shown in Fig. 1.

The connections of each section of pipe were threadable, which allowed the disassembly and sampling of the internal surface of each geometry at the end of the CIP process. The following test sections geometries were used: straight cylindrical section (A), tee (B), elbow (C), expansion (D) and reduction (E) in order to represent items commonly present in the dairy circulation lines and the different intensities of shear forces applied to them. The flow control system for the circulation line was described by Silva et al. (2019).

Figure 1 - Schematic representation of the milk circulation line model.



Legend: TK – milk storage tank and cleaning solutions (25 L capacity); V – ball type locking valves ½'' thread connection; P – centrifugal pump to promote circulation of fluids and cleaning agents in the system; YF – turbine flow sensor ½'', M - U-tube pressure gauge, HE – heat exchanger, different geometries: straight cylindrical section (A), tee (B), elbow (C), expansion (D) and reduction (E).
Source: Authors.

2.2 Microbial growth and surface contamination

All geometries were previously cleaned with potable water and neutral liquid detergent and subsequently autoclaved at 121 °C for 30 minutes. The geometries were filled with whole UHT milk, obtained from local market, sterile and inoculated at 1% (v/v) bacterial suspension of *Pseudomonas fluorescens* (ATCC 13525), previously activated in BHI broth (Merck, Germany).

The geometries were incubated at 24 °C for 15 hours for biofilm formation, according to the adhesion kinetics previously determined. For adhesion and biofilm formation, the static condition was chosen, since it standardizes the homogeneity on the geometry surface and isolates the hydrodynamic effects (Lelièvre et al., 2002). After incubation, the milk was drained and the geometries were installed in the circulation line to be submitted to the CIP process.

A replica of the geometry used in hygiene procedure was selected for the initial cell count on the surface. This geometry was filled with peptone water (Acumedia, Lansing, United States) 0.1 % (wt.), which remained inside the surface for 1 minute to remove planktonic cells. Then, the sessile cells were removed using a swab.

After the CIP process, the cells that remained adhered to the geometries were removed with a swab and transferred to a solution of peptone water 0.1 % (wt.) where they remained for 2 minutes in vortex agitation (Velp, Wizard Advanced IR) to release the cells into the solution. After this step, serial dilutions were prepared and plated using the spread plate method, in plate count agar (PCA – Kasvi, Italy). The plates were incubated for 36 h at 24 °C. The result was expressed in CFU.cm^{-2} using Eq. 1:

$$Count\left[\frac{CFU}{cm^2}\right]=\frac{C\cdot V_R}{V_A\cdot A} \quad (1)$$

where, C: average number of colonies after incubation [CFU]; V_R: Volume used in rinse [mL]; V_A: Volume used in plating [mL]; A: geometry area [cm²].

2.3 Exopolymerics (EPS) compounds of biofilm

The determination of the composition of the exopolysaccharides present in the biofilm was determined by FTIR as described by Wang et al. (2018). A stainless steel coupon (10 mm x 10 mm) after 24 hours of incubation with *P. fluorescens* was rinsed aseptically three times with 0.85% NaCl solution (wt.) to remove planktonic cells. The coupon with biofilm was air-dried at room temperature. The spectra were collected in the transmission mode from 2,000 to 800 cm^{-1} with a Shimadzu spectrometer with resolution 2 cm^{-1} e 128 scans.

The spectrum of the stainless steel plate without biofilm was used to remove the spectral background. The peaks corresponding to the functional groups were researched and identified according to references available in the literature.

2.4 CIP procedure

All geometries, after being subjected to the contamination process, were inserted in the circulation line to perform CIP, traditionally performed in a daily basis in the dairy industry and comprising the following steps: pre-rinse, alkaline detergent, rinse, sanitization and final rinse.

First, the storage tank was filled (TK 101) with potable water at room temperature for the pre-rinse step. The water circulation started in an open circuit for 5 minutes to remove milk residues. All steps of the procedure were performed at a velocity of 1.5 m·s^{-1} as

recommended by the literature (Tamime, 2008 & Andrade, 2008) for cleaning pipes, thus producing a Reynolds number equal to 23700.

After the pre-rinse step, cleaning started with the circulation of alkaline detergent NaOH 1 % (wt.) in a closed circuit for 15 minutes as suggested by Andrade (2008) and the temperature of 70 ºC as indicated by Tetra Park (2015) for pipes CIP procedure. The alkaline detergent was rinsed for approximately 5 minutes with potable water in open circuit to remove residual NaOH. The rinse was completed when the conductivity of the pipe effluent was equivalent to the conductivity of the potable water that was equal to 200 ± 12 $\mu S \cdot cm^{-1}$.

The sanitization step with peracetic acid was carried out at room temperature for 15 minutes at 100 ppm (Andrade, 2008 & Costa et al., 2017) in a closed circuit of circulation. Finally, the system was rinsed with potable water in open circuit for 5 minutes to remove the residual sanitizer.

2.5 Monitoring CIP procedure

After CIP procedure, the 5 geometries were removed and the swab technique was performed inside the tubes and accessories to remove the remaining cells. An aliquot of each sample was plated in PCA agar, after the cells were released in the vortex, and incubated by 36 h at 24 °C. The result was expressed in $CFU.cm^{-2}$ using Eq. 1.

The decimal reduction (DR) in number of cells observed in each geometry was determined by Eq. 2, according to Kumari & Sarkar (2014).

$$DR = \log N - \log n \quad (2)$$

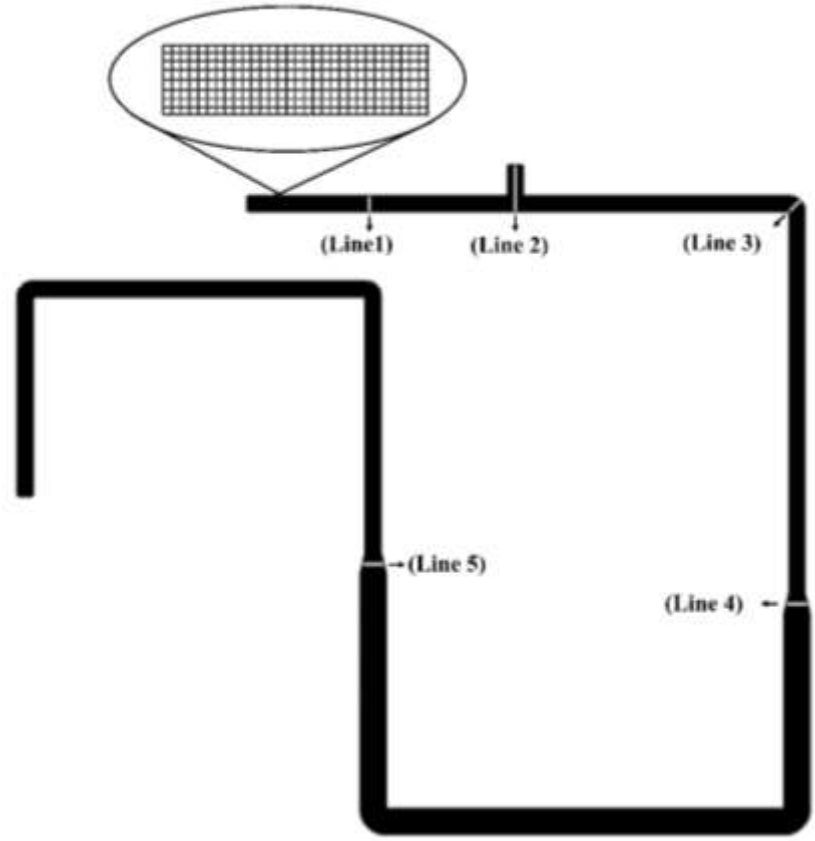
where, *N*: colony-forming units count $CFU \cdot cm^{-2}$ from sessile cells before CIP; *n*: colony-forming units count $CFU \cdot cm^{-2}$ after CIP.

2.6 Computational fluid dynamics (CFD)

For the study of the flow particularities, the CFD technique was used. The computational mesh created to represent the pipe system was developed using the software GAMBIT 2.4.6 and structured in two dimensions, mostly with quadrilateral cells, as shown in Fig. 2 and later exported to the numerical solver FLUENT 20.1 (Student).

For the simulation, the boundary condition was adopted on the left lateral end of the tube disposed in the horizontal position was defined as velocity inlet type. In this boundary condition, the value of 1.5 $m \cdot s^{-1}$ was informed and at the output, at the end of the vertical straight section, was specified pressure outlet. Thus, in relation to the relative pressure, (pressure gauge) he null value was adopted for the simulations. The fluids used in the CIP process were water and aqueous solutions of chemical cleaning agents such as NaOH and peracetic acid. Thus, the cleaning agent solution properties were assumed to be the same as water, as suggested by Yang et al. (2018).

Figure 2 - Representation of the two-dimensional mesh of the pipe system and indication of the lines to investigate the velocity and shear stress profiles.



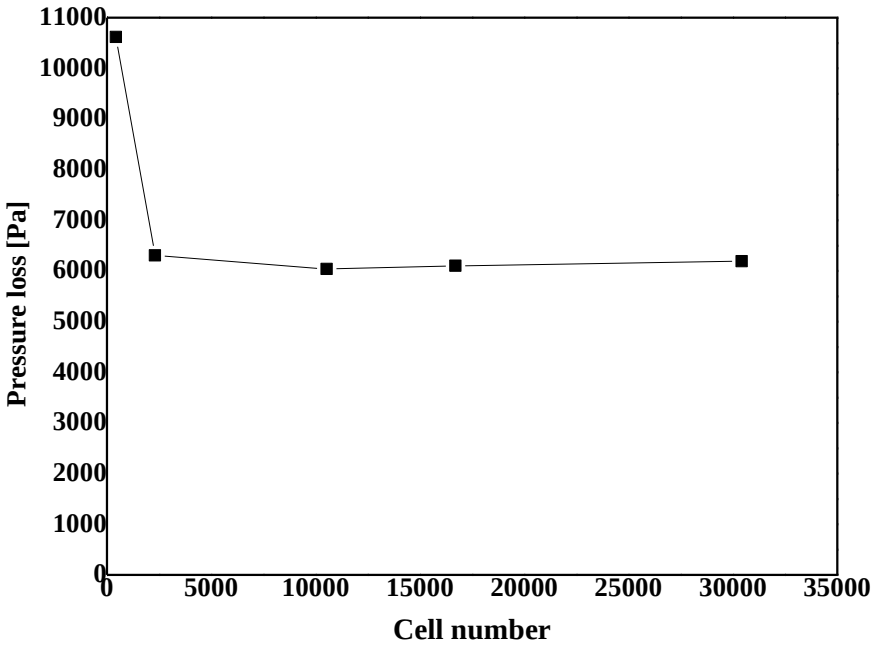
Source: Authors.

The investigation of the velocity and shear stress profiles was done in the average position of each geometry as shown in Fig. 2, with line 1 in the part of the straight cylindrical pipe, line 2 in the region of the tee, line 3 in the elbow, line 4 on the pipe expansion and line 5 on the reduction.

Mesh independence was assessed to minimize errors associated with the discretization

of sections of pipe and fittings. The mesh used in this study was refined in the radial direction, to explore the characteristics of the fluid near to the wall. The mesh density was increased until the pressure drop became constant. The results obtained are presented in Fig. 3. The mesh of 16701 cells was chosen, as it presented similarly accurate results with a more refined mesh. The use of a more refined mesh did not significantly affect the improvement of the results and would consume even more simulation time.

Figure 3 - Pressure drop as a function of the number of cells for analysis of mesh independence.



Source: Authors.

Some models are used to represent flows in turbulent conditions (Bhutta et al., 2012) and the models k–ε and k-ω are the more relevant. These two models were tested and compared to the system pressure drop values.

2.7 Operational consumption

The operational consumption of the CIP process was determined considering the electrical energy ($\delta_{eletricity}$) consumed by the pump for fluid circulation, the energy consumed for heating ($\delta_{heating}$) the alkaline solution and the volume of water used (δ_{water}).

The consumption of potable water for rinsing was determined based on the flow, as

shown in Eq. 3 and proposed by Silva & Gedraite (2018). The energy consumption for pumping was determined based on Eq. 4, as shown by Silva et al. (2020), with the electrical current measured with a clamp ammeter model ET-3200 Minipa. The energy spent on heating was calculated by Eq. 5 as suggested by Yang et al. (2019).

$$\delta_{water} = \int_{t=0}^{t} \dot{Q} \cdot dt \quad (3)$$

$$\delta_{eletricity} = \sqrt{3} \cdot V_L \cdot \cos\theta \cdot \int_{0}^{t} I_L(t) dt \quad (4)$$

$$\delta_{heating} = V \cdot \rho \cdot c_P \cdot (T - T_0) \quad (5)$$

where: V_L: line tension [V]; I_L: line current [A]; phase angle; V: solution volume [m³]; ρ: specific mass of water [kg·m^{-3}]; c_p: specific heat of water [J·(kg·ºC)$^{-1}$)] T: temperature [ºC]; T_0: room temperature [ºC] e Q volumetric flow [L·s^{-1}]

2.8 Statistical analysis

The experiments were carried out in triplicate. The results were analyzed using analysis of variance (ANOVA). The Tukey test was used to assess comparisons between means. The statistical treatments were performed considering the 5% probability level and using the program Statistica 7.

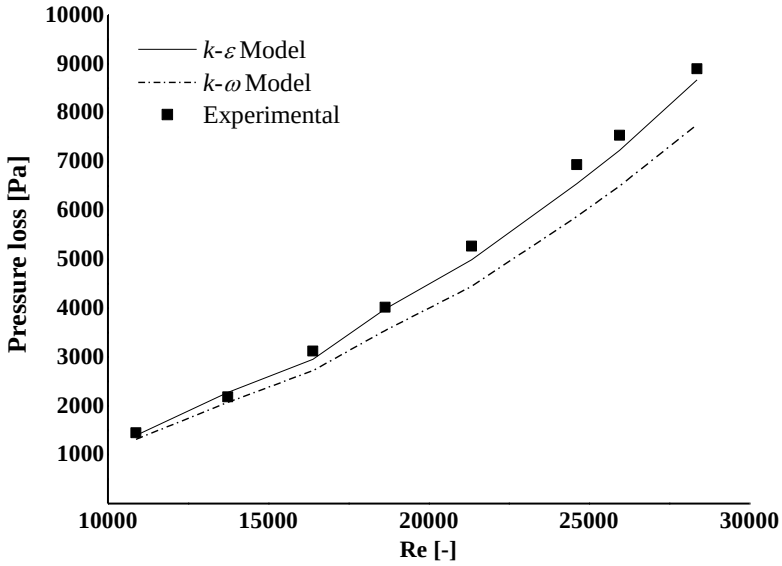
3. Results and discussion

3.1 Turbulence model

The results obtained with the turbulence model k-ω and k-ε differ from each other more significantly in flows with a higher number of Reynolds, so that the k-ε model presented better adjustment to the experimental results, as shown in Fig. 4. Thus, k-ε model was chosen to study the flow in the pipeline. Bouvier et al. (2014) also showed in their research related with flow in heat exchangers that the k-ε model exhibited a better adjustment to the experimental results. The turbulence model k-ε has numerical robustness, precision and good convergence capacity and computational efficiency (Pan et al., 2021; Bouvier et al., 2014).

Cunault et al. (2015) reported that the *k-ε* model is valid for totally turbulent and wall-confined flows, whereas the *k-ω* model generally provides better transport results close to the wall.

Figure 4 - Validation of the turbulence model through the experimental and simulated pressure drop in the processing line.



Source: Authors.

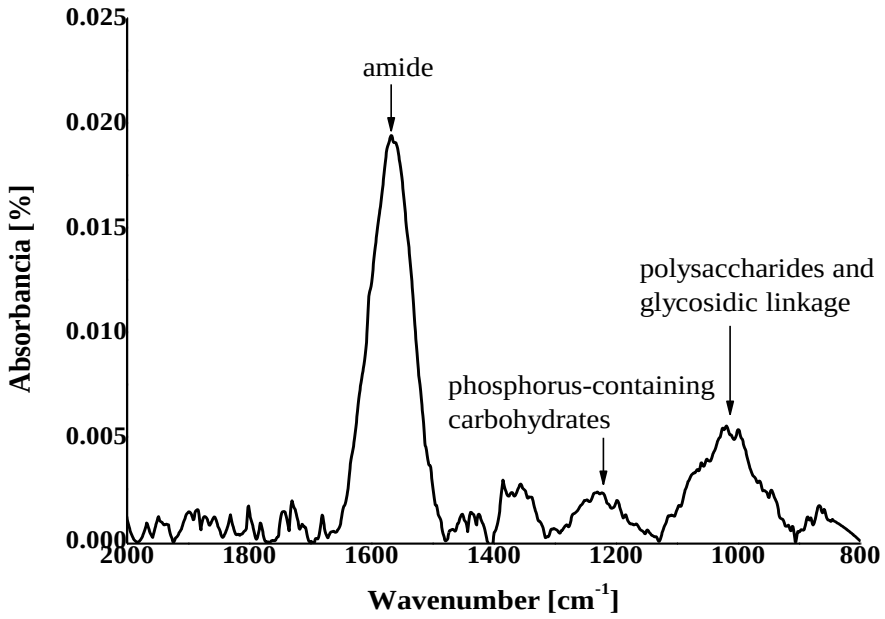
3.2 Surface contamination

After the *P. fluorescens* incubation period in the studied geometries, the contamination produced on the stainless steel surface was 4.31 ± 0.26 log CFU·cm^{-2}. Over the 24 hours period of operation, usually employed in the milk processing industries (Wang et al., 2018), the *P. fluorescens* was able to multiply at room temperature and cause contamination in the pipes and accessories. In addition, the cell count on the surface has shown that inadequate hygiene procedure can develop a high number of cells in this environment.

The analysis of the ATR-FTIR spectra of the biofilm *P. fluorescens* on the contaminated surfaces after incubation is shown in Fig. 5. Some indicative EPS compounds were associated with the main bands of the spectrum (Ojeda et al., 2008). The peaks in 1550, 1230 and 1055 cm^{-1} were assigned to the functional groups present in amide in proteins, carbohydrates containing phosphorus and polysaccharides and deformation of carbohydrate glycosidic bonds, respectively (Wang et al., 2018; Tugavora et al., 2017). According to Bosch et al. (2006) peaks in the spectrum that varied between 800 a 1000 cm^{-1} are probably related

to deformation of the glycosidic ring (C-O-C) in polysaccharide and asymmetric ring (C-C, C-O) of different groups of carbohydrates. The production of EPS by *P. fluorescens* suggests that the material is mainly formed by polysaccharides, proteins, phospholipids and other carbohydrates.

Figure 5 - FTIR-ATR spectrum of the biofilm of *P. fluorescens* in stainless steel after 24-hour incubation.



Source: Authors.

3.3 CIP procedure

Table 1 shows the values of the decimal reduction observed in each geometry and the final count of cells remaining on the surface. There were no significant differences in decimal reductions between pipe geometries in the form of a straight section, elbow, expansion and reduction. On the other hand, the tee section showed a lower decimal reduction of viable cells when compared to the other geometries ($p < 0.05$), due to the fluid dynamics of the sanitizing solution inside the tee.

Table 1 - Decimal reduction of viable *P. fluorescens* cells and final count for each geometry.

Geometry	Decimal reduction [-]	Final count [CFU·cm^{-2}]
Straight section (A)	4.40 ± 0.29[a]	< 1
Tee (B)	2.02 ± 0.23[b]	163 ± 16
Elbow (C)	4.25 ± 0.36[a]	<1
Expansion (D)	4.31 ± 0.26[a]	< 1
Reduction (E)	4.34 ± 0.24[a]	< 1

[a,b] Means followed by the same letter, in the same column, did not differ by Tukey's test (p > 0.05). Source: Authors.

The final count is an important parameter for microbiological quality of the food processing surface. The World Health Organization (WHO) and Pan American Health Organization (PAHO) admit maximum counts of 50 CFU·cm^{-2} for the surface to be considered sanitized. On the other hand, the *American Public Health Association* (APHA) presents a stricter recommendation with maximum counts of 2 CFU·cm^{-2} for sanitized surfaces. In this perspective, all geometries were sanitized, as they had counts less than 1 CFU·cm^{-2}, except the tee section which would not be properly sanitized and would require new interventions.

In the straight section, the flow occurred uniformly so that both faces experienced the same velocity of approximately 1.5 m·s^{-1} as shown in the velocity profile of Fig. 6A and in the velocity contour of Fig. 7A. In this configuration, the flow of the fluid produced a shear stress on the surface of approximately 6.5 Pa, which associated with the action of the sanitizer promoted the removal and destruction of *P. fluorescens* cells that were fixed on the stainless steel surface. Lelièvre et al. (2002) showed that the mean shear stress has a significant effect on the removal of *B. cereus* adhered to the stainless steel surface and that, in general, the straight section showed better levels of removal. Lemos et al. (2015) reported that greater shear stress associated with the sanitizing agent promoted greater removals of the *B. cereus* biofilm.

In the tee section it was noted the existence of a stagnation zone with fluid recirculation at low velocities (Fig. 7B), of the order of 0.3 m·s^{-1} as can be seen in the Fig. 6B. As a consequence, on that surface was applied about 0.9 Pa of shear stress. Jensen et al. (2007) explained that the flow generates a local tangential force acting on the liquid-surface interface and acts as a carrier for chemical agents. Paz et al. (2013) indicated the shear stress on the wall as a more significant parameter in the local removal than the velocity itself.

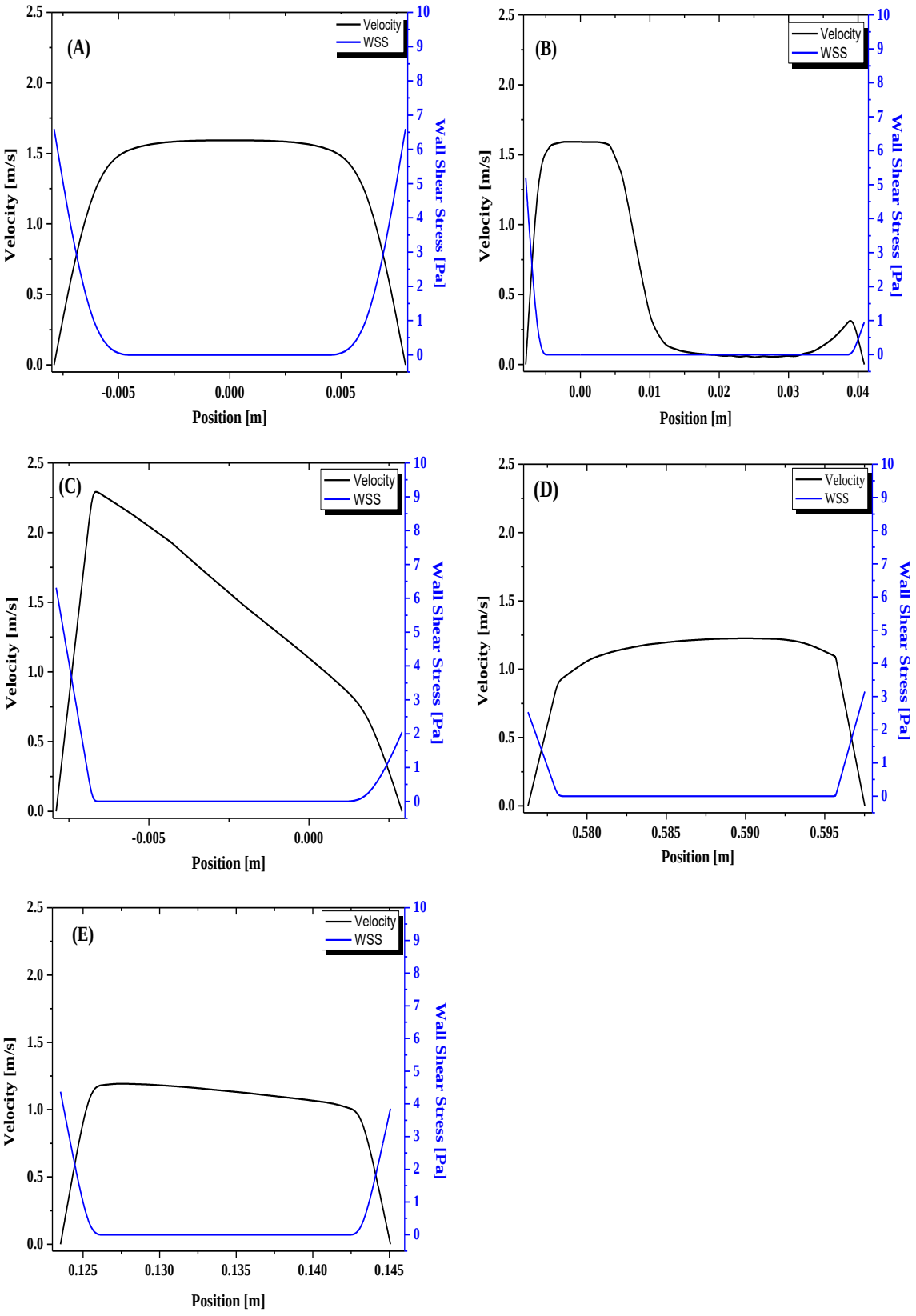
The constant recirculation zones are a known problem in the industry and in the practice of hygiene and are characterized by low and slow rates of fluid exchange compared to the main current and consequently are more difficult to sanitize (Li et al., 2019). Jensen e Friis (2005) also reported that the most difficult regions to clean are dead ends and cracks in the geometry that produce recirculation and stagnation areas. Associated with this, the low shear stress and less mass transfer of chemical agents leads to reduced efficiency.

However, it is important to notice that the tee section does not always produce areas of fluid recirculation and stagnation. Figueredo et al. (2009) showed in simulated CIP that the removal of *P. aerugionsa* cells was superior in the tee section compared to the straight section of the pipe. This difference is related to the layout of the pipe, the positioning of the geometries in the processing line and the flow that were applied.

In the elbow section, the formation of a preferential path on the left side was noted as shown in the velocity profile in Fig. 6C and Fig. 7C. At this point in geometry, the flow occurred with greater velocities of approximately 2.3 $m \cdot s^{-1}$ leading the shear stress to 6.3 Pa. The other region of the elbow was subjected to lower velocity of 0.8 $m \cdot s^{-1}$ (Fig. 7C) that produced a shear stress of 2.0 Pa. This flow behavior did not significantly influence the reduction of *P. fluorescens* cells in comparison to the straight tube, expansion and reduction.

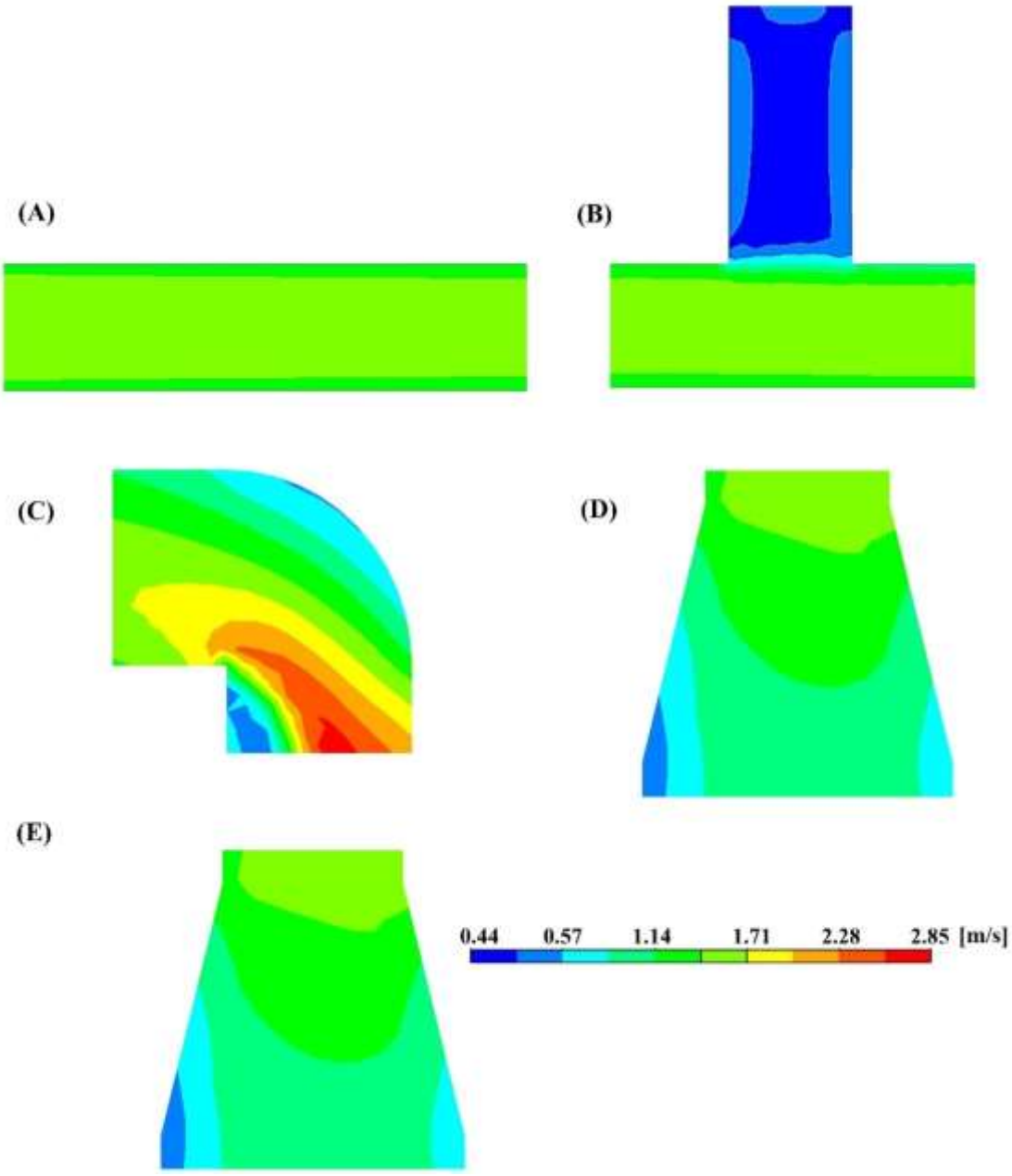
On the other hand, this effect is not extendable to all elbows of a piping system. Dev et al. (2014) worked on CIP procedure of a raw milk in milking pipe and reported that the elbow was the more difficult geometry to sanitize compared to the straight section. Figueiredo et al. (2009) also showed a significant difference in the removal of *P. aeruginosa* between the straight cylindrical geometries and the 90º elbow. Thus, it is understood that hygiene efficiency is a complex process and depends on numerous factors such as the system under study, the characteristics of microorganisms (presence of pili or flagella), biofilm structure and variability and physicochemical properties such as charge and the hydrophobicity of biofilm (Wu et al., 2012).

Figure 6 - Velocity and shear stress profiles inside the geometries (A) straight section, (B) tee, (C) 90º elbow, (D) expansion and (E) reduction.



Source: Authors.

Figure 7 – Velocity contour inside the geometries (A) straight section, (B) tee, (C) 90º elbow, (D) expansion and (E) reduction.



Source: Authors.

Expansion and reductions pipe sections are commonly used in the food industries (Blel et al., 2007) for adapting the pipeline diameter. In this study, such geometries showed a mean velocity of approximately 1.1 $m \cdot s^{-1}$ in the centerline region of the geometry as shown in Fig. 6D e 6E. The shear stress applied in this region ranged from 2.5 to 4.4 Pa and did not produce significant differences in the levels of hygiene produced by the CIP process when compared to straight and elbow pipes.

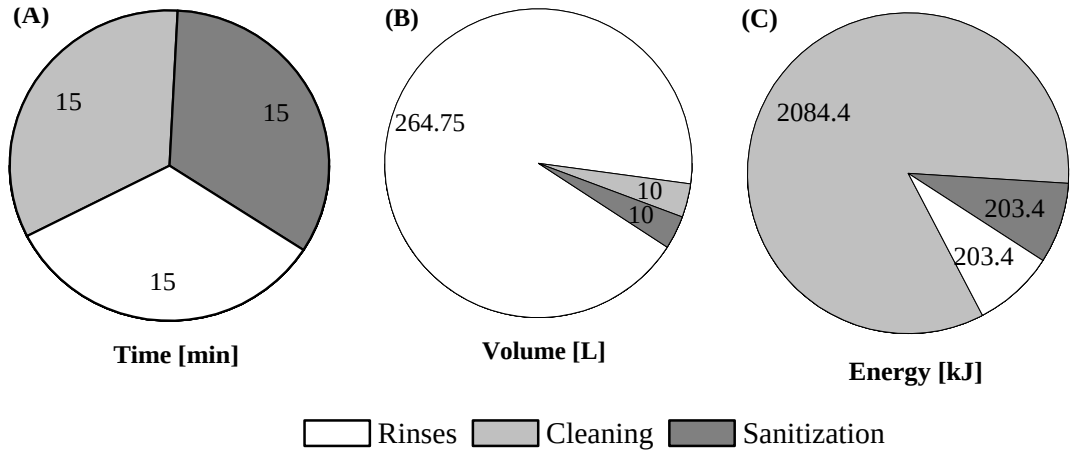
Despite this, the presence of a zone of fluid recirculation and stagnation close to the region of the larger diameter pipe was notable, as shown in Fig. 7D e Fig. 7E, considering that the low velocities occur where the fluid swirls in the conical section as also reported by Lelièvre et al. (2002). In addition, these authors classified these shapes as regions that are difficult to clean, since the shear stress in these localized points is low and not uniform throughout the geometry.

3.4 Process consumption

The three rinses of the CIP, the alkaline cleaning and the sanitization steps demanded equivalent times in the hygiene process as shown in Fig. 8A. Potable water consumption was considerably higher in the rinsing stages. Alkaline cleaning and sanitation steps consume the same amounts of water, since the process operates with the same amount of product and the circulation of these chemical agents is made in a closed circuit. Yang et al. (2018) reported that CIP hygiene processes, in general, consume relevant amounts of water, especially as rinsing steps for processing lines.

From an energetic point of view, the alkaline cleaning step consumed a greater amount of energy, since the product is applied hot and much of energy is used to heat the fluid together with the energy for circulating the detergent. The rinsing and sanitizing steps require equivalent amounts of energy since they operate in equivalent times, approximately 15 min.

Figure 8 - Consumption (A) time, (B) water volume and (C) energy at each stage in the CIP process.



Source: Authors.

4. Conclusion

This research investigated the contamination of stainless steel surfaces with *P. fluorescens*, the CIP cleaning of the pipe and its accessories, the fluid dynamics and the process inputs. A significant concern is related to the observation that in the typical period of operation of the processing unit it is possible to achieve high counts of cells on the surface. The computational fluid dynamics (CFD) proved to be useful in determining regions of difficult hygiene and pointed out the most problematic points of hygienic design of pipelines. All geometries showed microbiological safety at the end of the hygiene process, except for the tee section that presented a zone of fluid stagnation and recirculation, which impaired the quality of hygiene in that region. In addition, it was evident that the CIP process demands a significant amount of water for the rinsing steps, just as the alkaline cleaning step consumed considerable energy for heating the detergent. In this perspective, research is essential to improve the levels of hygiene in regions with difficult access and to optimize the CIP process.

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