

Research Article

Increasing the Critical Micellar Concentration (CMC) of Surfactants Enhances the Removal of Pathogenic Bacteria, *P. Aeruginosa*, and *S. Aureus* from Wastewater

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Abstract: Antimicrobial effects of increasing Critical Micellar Concentration (CMCn) (n varying from 1-12) on *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 over time of 5-, 10, and 20-minute exposures were evaluated. Three types of surfactants (Cetyltrimethylammonium Bromide (CTAB), Dodecyl Sulfate (SDS), and TX-100) were evaluated. The results showed that CTAB had the greatest impact on both bacterial strains' reduction. The best yield discharge (9.4 U-log) for *P. aeruginosa* was obtained with CTAB (1CMC); exposure time did not significantly improve the yield of bacteria removal. For *S. aureus*, CTAB yield discharge was optimised (9.4-Ulog) at 8CMC with time exposures of 20 minutes. The 12 CMC dose significantly impaired the antimicrobial activity of CTAB with *S. aureus*. Defining the effects of surfactant type, dose, and time exposure on *P. aeruginosa* and *S. aureus* discharge can improve advanced wastewater treatment when effluents are discharged into natural waterways. Methods of reducing the discharge of pathogenic bacteria using surfactants are valuable for environmental monitoring, food processing, or pharmaceutical manufacturing.

Keywords: Surfactant, Advanced Wastewater Treatment (AWWT), *Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, Critical Micellar Concentration (CMC), Bacterial Pathogens

Introduction

Contamination of foods, drugs, and wastewater with Pathogenic Bacteria (PB) presents a challenge to public health globally. Health risks increase with the emergence of antibiotic-resistant PBs (Ventola, 2015). Some PB have the ability to develop antibiotic resistance over time under specific environmental conditions (Sambaza and Naicker, 2023; Ahmed et al., 2024). Among the challenges to low-income countries like Tunisia is to develop a realistic, efficient approach to water quality improvement and to implement multiple strategies for effective wastewater treatment (OECD, 2014). Worldwide, surfactants are already used for the removal of a wide range of PB (Gill et al., 2023), as well as the removal of chemical pollutants. Previous research on the

use of surfactants in water treatment employed a maximum dose of 1 CMC for surfactants. The surfactant CMC is defined as the concentration required for reaching the lowest surface or interfacial tension. Surfactant-inducing cell lysis mechanisms are dependent on several factors, including type and concentration of the surfactant, as well as physicochemical characteristics of the bacterial cells (Manargadoo-Catin et al., 2016; Lu et al., 2021). This research aims to examine the type, dose, and exposure time of known surfactants in sequestering PB to identify the conditions for bacterial cell lysis. At relatively high surfactant concentrations, micelles form aggregates that can encapsulate the bacterial cell walls (Partearroyo et al., 1990). Over the past decades, efforts to develop antimicrobial surfactants have used a dose of 1 CMC (Zhou et al., 2020).

Surfactant molecules are altered in solutions by monomers following bacterial cell lysis. Monomers re-lysed in solution may affect the interfacial tension and change the CMC (Manaargadoo-Catin et al., 2016). In addition, lysis of bacterial cells contributes to the change in environmental media and likely adds internal bacterial components to the medium. Cell lysis can liberate some internal bacterial surfactants such as rhamnolipids and phospholipids (De Rienzo et al., 2016). Each liberated molecule following bacterial lysis may interact with the treatment surfactant to change bacterial removal. The design and operation of an efficient surfactant-disinfection system requires defining the dosage of surfactant based on operational conditions for a target pathogen. In a previous communication, we assessed the effectiveness of UV light (Saidi et al., 2011), phages (Grami et al., 2022), nanoparticles (Chemingui et al., 2019), and membranes (Ounifi et al., 2019) combined with the operational conditions of water treatment for a specified bacterial discharge. The central objective of this research is articulated in the Sustainable Development Goals (SDGs) framework (specifically SDG3 and SDG6) to develop an effective approach to water disinfection.

This research is a comparative study between three surfactants and CMC variables to understand the reduction in PB, to elucidate both the type of surfactant and the appropriate dosages to decrease PB based on the surfactant exposure time. The long-term goal of this research is to define methods to reduce the discharge of *P. aeruginosa* and *S. aureus* bacteria in sewage effluent with precise doses of the three surfactants (CTAB, SDS, and TX-100).

Table 1: List of surfactants with composition and properties

Surfactant	Classification	Chemical formulae	Effect on the cell wall
Triton X-100 (TX100),	Non-ionic Giant molecules with polymeric –CH ₂ –CH ₂ –O– structure as the hydrophilic end	(C ₈ H ₁₇ C ₆ H ₄ (OC ₂ H ₄) _n OH, (n = 9-10))	
Sodium dodecyl sulphate (SDS)	Anionic Strong detergent	C ₁₂ H ₂₅ OSO ₂ ONa	Disturb cell walls and denature proteins
Cetyltrimethyl ammonium bromide (CTAB).	Cationic surfactant (CS) quatarnary ammonium surfactant.	(C ₁₆ H ₃₃) N(CH ₃) ₃ Br;	Enhance cell permeability Increase hydrophobicity and zeta potential

The surfactant stock solution (12 CMC) was made using the following weights: 28.358 g of SDS, 2.444 g of CTAB, and 1.724 g of TX100. To prepare stock solutions, each quantity of surfactant was then dissolved in 1 L of ultra-purified, sterilized water. The mixtures were then stirred to obtain transparent solutions at 12 CMC

Table 2: Experimental design and composition of surfactant solutions

Final CMC	Volume of water (ml)	Volume of surfactant stock solution (12 CMC) (ml)
1	45.84	4.16
2	41.67	8.33
4	33.34	16.66
6	25	25
8	16.67	33.33
10	8.34	41.66
12	0	50

Materials and Methods

Bacteria *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 strains were exposed to surfactants as follows: Bacteria-TX-100, bacteria-CTAB, and bacteria-SDS. Surfactants were applied in the following CMC_n where n is equal to 1, 2, 4, 6, 8, 10, and 12 (Table 1).

Bacterial Strains and Growth Conditions

P. aeruginosa ATCC 27853 and *S. aureus* ATCC 29213 are reference strains, used throughout the study as representatives of Gram-negative and Gram-positive PB, respectively. The two strains were grown at 37°C for 24h in Tryptic Soy broth medium (Biokar, France). The bacterial pellets were prepared by removing 10 mL of each bacterium's overnight culture, then these samples were centrifuged at 4500 tr/min for 15 minutes. The supernatant was discarded, and the pellet was resuspended in 5 mL of physiological water. (9/1000) NaCl). After incubation at 37°C for 24h, colonies were counted using the dilution method of Wiegand et al. (2008), and results were expressed in colonies forming units per milliliter (CFU/mL).

Surfactant Solution

The surfactant stock solution (12 CMC) was first made using the following weights: 28.35 g of SDS, 2.44 g of CTAB, and 1.72 g of TX-100. To prepare stock solutions, each quantity of surfactant was then dissolved in 1 L of autoclaved distilled water. The mixtures were then stirred to obtain transparent solutions at 12 CMC. Diluted solutions of each surfactant were prepared as noted in Table 2.

Experiment Design

Serial dilutions were prepared from the stock solution CMC (12), over a range of 1, 2, 4, 6, 8, 10, and 12 CMC. After 5-, 10-, and 20-minute exposure of the bacteria with the surfactant suspension, a volume of 10 mL was sampled from each assay tube, and then was transferred to a sterile test tube. Each tube received 100 µl from the bacterial suspension previously prepared. Then, the mixture was serially diluted with 100 µl of physiological water (9/1000 weight to volume NaCl). After 5 minutes of contact, a volume of 100 µl of each dilution was spread on the surface of Petri dishes containing TSA. The Petri dishes were then incubated at 37°C. The same steps were carried out successively after exposure to 10- and 20-minute exposures to the different surfactant CMCn. After 24 hours of incubation, the colonies on the agar medium surface were counted. The PB removed or sequestered by the surfactants resulted in a lower colony count. A control without surfactant was included, and results were expressed with the following formula:

$$N = \text{Log} (N/N_0)$$

Where N is the bacterial count in the treated sample with surfactant, and N_0 is the bacterial count in the control test.

Statistical Analysis

The assays were performed in triplicate, and data were expressed as means \pm standard deviations. The

statistical analysis was done by using SPSS 20 software and the Newman-Keuls test. The statistical significance of the different colony counts inferred bacteria reduction values between the different types of surfactants and between values obtained over increasing CMCn of the same surfactant, using one-way analysis of variance at a $p < 0.01$ level of significance.

Results

Effects of exposure time at increasing CMCn surfactant on the growth of *P. aeruginosa* 27853.

Five-Minute Exposure

After five minutes of contact between *P. aeruginosa* 27853 and the 3 surfactants, results showed that the type of surfactant affects the efficiency of bacterial sequestration. CTAB (9.4-Ulog) sequesters more bacterial cells with a significant difference at $p < 0.01$ compared with SDS (2.0, 2.3-Ulog, minimum and maximum) and TX-100 (2.3, 2.6-Ulog; minimum and maximum). For all surfactants, the yield reduction values were not significantly different among increasing CMCn (Figure 1A).

Ten- and Twenty-Minute Exposure Times

The results indicated no bacterial reduction improvement with the increase in time exposure for *P. aeruginosa* when exposure time increased from 10 to 20 minutes (Figure 1B).

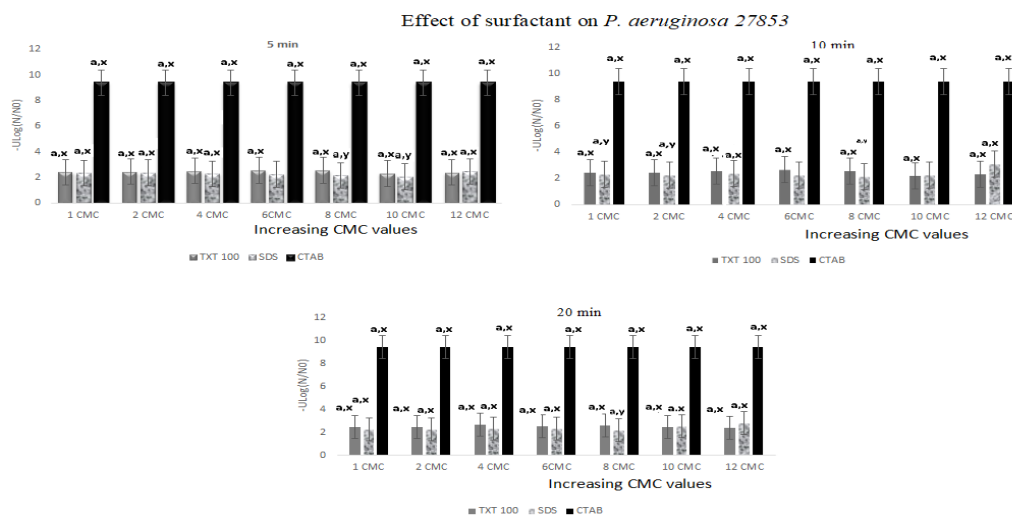


Fig. 1: Effect of surfactant (TX100, SDS, and CTAB) with increasing CMC on the discharge of *P. aeruginosa* 27853 expressed in logarithmic units (A): for 5 minutes contact (B): for 10 minutes contact (C): for 20 minutes contact. The number of bacteria removed or sequestered by the surfactant is represented by colony counts. A control without surfactant was considered, and results were expressed with the following formulae: $N = \text{Log} (N/N_0)$, where N is the number of bacteria determined in the sample treated with surfactant, and N_0 is the number of bacteria saved in the control test

Effects of Exposure Time at Increasing CMCn Surfactant on the Growth of *S. aureus*

Five-Minute Exposure

S. aureus after CTAB exposure during 5 minutes showed a significant difference ($p < 0.01$) for bacteria reduction between CTAB CMC4 (4.1-Ulog) compared with CTAB (CMC6-CMC8; 4, 9 -5.0 U-log) Fig. 2 A. In contrast, there wasn't a significant difference between CTAB 1CMC (3.1-Ulog) and CTAB CMC2 (3.6-Ulog). CTAB with higher CMC, such as CMC10 and CMC12, reduced *S. aureus* with values of 1.6 and 1.3-Ulog, respectively, not significantly different. For the SDS, the statistical study of *S. aureus* after 5 minutes surfactant exposure showed three different statistical subgroups, the first one, which provided the best reduction efficiency with values in the range 3.8 and 4.3-Ulog when CMCs were situated between 4 and 10, respectively. The second subgroup concerned the low CMC of 1 and 2, where *S. aureus* reduction was in the range 3.2, 3.4-Ulog, respectively. However, the third subgroup CMC12 reduced the load of bacteria only by 2.4-Ulog, which presents the lowest reduction value compared with the other CMCn. Concerning TX-100, results can be statistically classified into three subgroups; the strongest one includes only 1CMC with a reduction of 3.8-Ulog. The subgroup that encompasses reduction values of 3.3 and 3.2-Ulog was recorded with 2 and 6 CMC. The

weakest efficiencies not significantly different were recorded with 4, 8, 10, and 12 CMC, which scored sequestrations of 2.8- 2.5-2.7 and 2.6 -Ulog, respectively.

Ten-Minutes Exposure

Extending the time exposure of *S. aureus* from 5 to 10 minutes, CTAB showed no significant difference in value ($p < 0.01$). In fact, concentration of 1, 2, and 4 CMC, which scored values of 3.6, 3.4- 3.7 -Ulog reduction showed no significant different value ($p < 0.01$)- while for CMC6 and 8, there is no significant difference ($p < 0.01$) between both within values of 5.8-6.2-Ulog, respectively. The reduction values of 1.6-0 were assigned to the lowest subgroup in terms of reduction and were attributed to CMC10 and CMC12. For SDS, we distinguish the presence of 3 subgroups. The highest reduction value is for 6CMC, which marked a reduction of 4.5, followed by a second subgroup which included 2, 4, 8, 10, and 12 with reduction values of 3.2-2.6, 2.9-3.0-3.1, respectively, not significantly different. The average reduction value of 3.4 is obtained with 1CMC. For TX-100, there are 4 significantly different subgroups, the lowest subgroup for 8 and 12 CMC with sequestration values of 2.2 and 2.0, respectively. The medium subgroup includes 4 and 10 CMC with values of 2.8 and 2.7-Ulog. A third class of 3.3 and 3.2-Ulog for CMC of 2 and 6, and the highest reduction value for TX-100 surfactant is obtained for 1 CMC with a value of 3.8-Ulog.

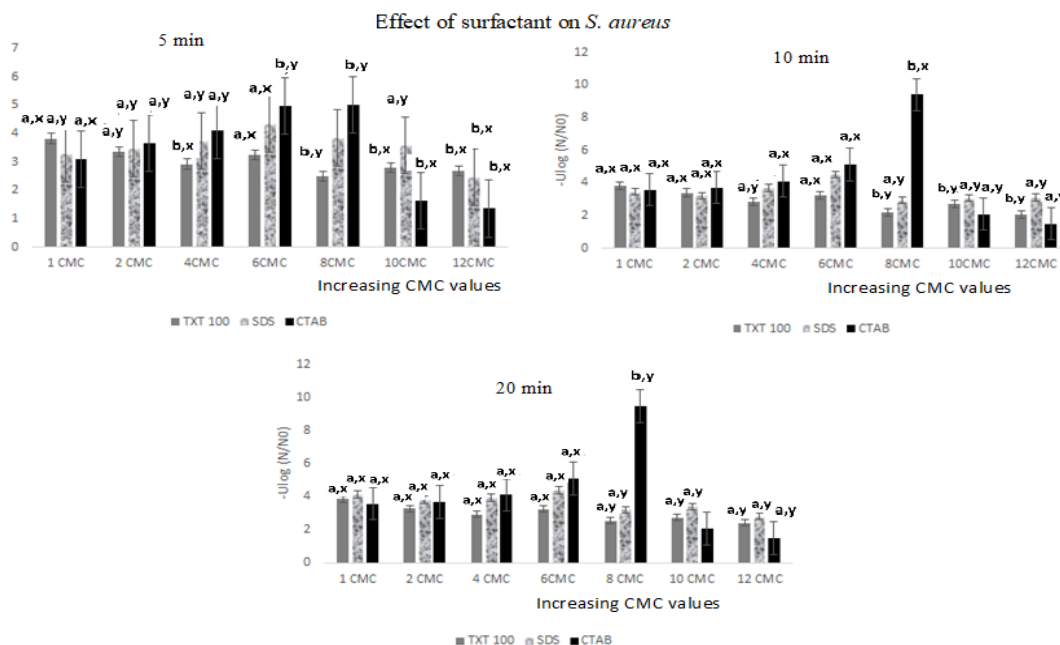


Fig. 2: Effect of surfactant (TX100, SDS, and CTAB) with increasing CMC on the discharge of *S. aureus* expressed in logarithmic units (A): for 5 minutes contact (B): for 10 minutes contact (C): for 20 minutes contact. The number of bacteria removed or sequestered by the surfactant is represented by colony counts. A control without surfactant was considered, and results were expressed with the following formulae: $N = \text{Log} (N/N_0)$, where N is the number of bacteria determined in the sample treated with surfactant, and N_0 is the number of bacteria saved in the control test

20-Minute Exposures

Extending the exposure time of the CTAB surfactant to *S. aureus* 29213 to 20 minutes showed there were 6 significantly different results based on CMC (Figure 2C). The first one showed the highest value of 9.4-Ulog applying 8 CMC; a reduction average of 5.1-Ulog was recorded with 6 CMC. Results of 4.1-Ulog were associated with the treatment of 4 CMC. By applying 1 CMC, we obtained a non-significant difference ($p < 0.01$); with 2 CMC, where values of 3.5 and 3.7-Ulog were recorded, respectively.

Figure 3 shows a radial curve including both tested PB strains, viewing the non-linear relationship between CMC and sequestration for the *S. aureus* strain. Also, Figure 3 shows the optimum of 9 Ulog value discharge

of *P. aeruginosa* and its constancy even when CTAB CMCn were increased from 1 to 12. The Ward method is used to analyze the variance in the distances between clusters in cluster analysis (Figure 4). A pattern was sought in the data set by grouping for each strain observations (CMCn) into clusters (based on time exposure evolution). For *P. aeruginosa*, results showed only two clusters: The first one included CTAB with increasing CMC and over time exposure, and the second cluster included both SDS and TX-100 at increasing CMC and increasing time exposure. Our findings show clustering of *S. aureus* results in four classes, confirming the non-linearity between *S. aureus* discharges and increasing CMC values.

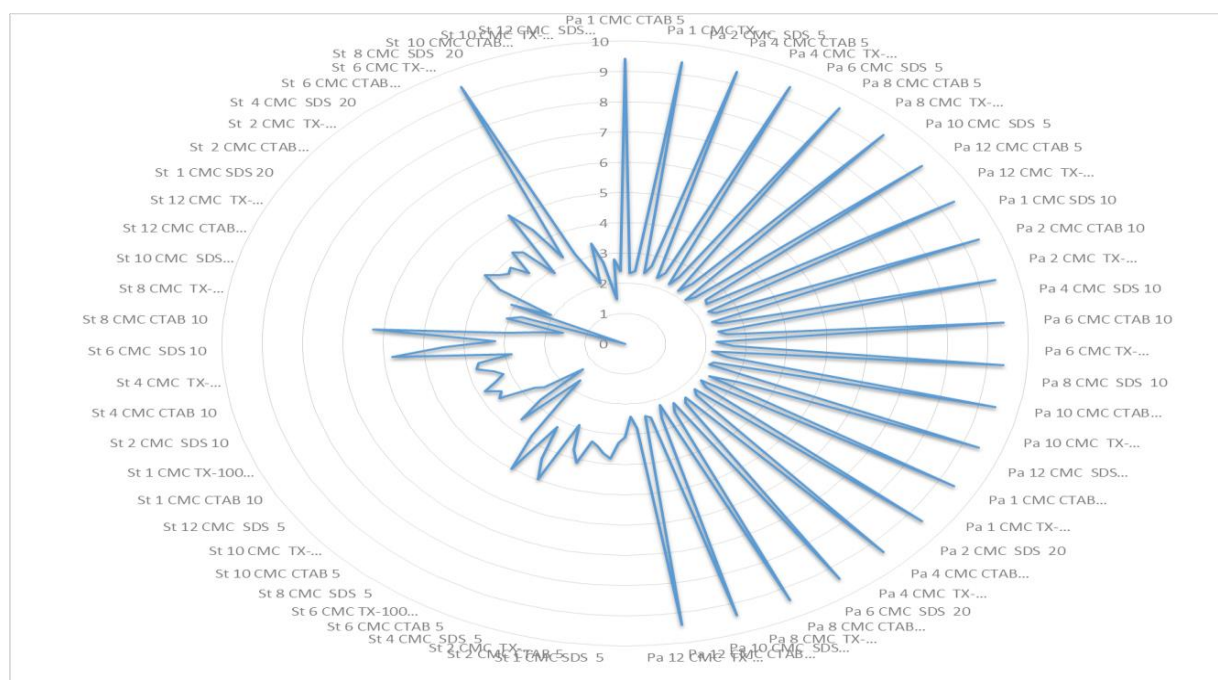


Fig. 3: Comparative study between the yield of *P. aeruginosa* and *S. aureus*

Discussion

The sequestration of PB from Advanced Wastewater Treatment (AWWT) secondary treatment would likely reduce environmental risk and allow water re-use in semi-arid climates. The two PB strains were chosen for their ubiquitous presence in wastewater as well as resistance to disinfectants, antibiotics, and biocides (Sanchez et al., 2024; Kuznetsova et al., 2025). Recent research has examined emerging technologies for the removal of resistant PB in wastewater using ultrasound (Naddeo et al., 2009), ultraviolet light (Sarkhoskhalat et al., 2024), hybrid ultrasound treatment with ozone (Rossi et al., 2021), and phages (Grami et al., 2022). The responses of *P. aeruginosa* and *S. aureus* strains to the

three surfactants at varying CMCn and exposure times were evaluated, and showed positive results for PB removal by only one surfactant: CTAB.

In fact, results showed clearly that the nature of surfactant affected the efficiency of PB reduction; CTAB was significantly more effective than SDS and TX-100. CTAB reduced *P. aeruginosa* by 9.4-Ulog when compared with SDS (2.1-3.1-Ulog) and TX-100 (2.2-2.6 Ulog). These differences in surfactant effectiveness in removing *P. aeruginosa* may be explained by the nature of the hydrophobic component of the surfactants. Phospholipids, the building block of biological membranes, are unique to each bacterial strain (Thomas, 2003) and may interact differently with the cationic surfactant, CTAB, compared to the anionic SDS and

non-ionic TX-100 surfactants. The interaction between each bacterial strain and each surfactant may differ as the three tested surfactants are different in charge, in length, and in conductivity of their hydrophobic components, and these parameters are also influenced by any change in the environmental medium (Begum, 2016). The chemical structure of biosurfactants consists of phospholipids, glycolipoproteins,

glycolipids, and lipopeptides. Molecules reacting towards surfactant may have unique perceptiveness towards bacteria. When bacteria are lysed, endogenous molecules and membrane destruction contribute to the destabilization of micelles at each CMC. A new equilibrium may be established and contribute to the formation of new micelles' formation Fig. 4.

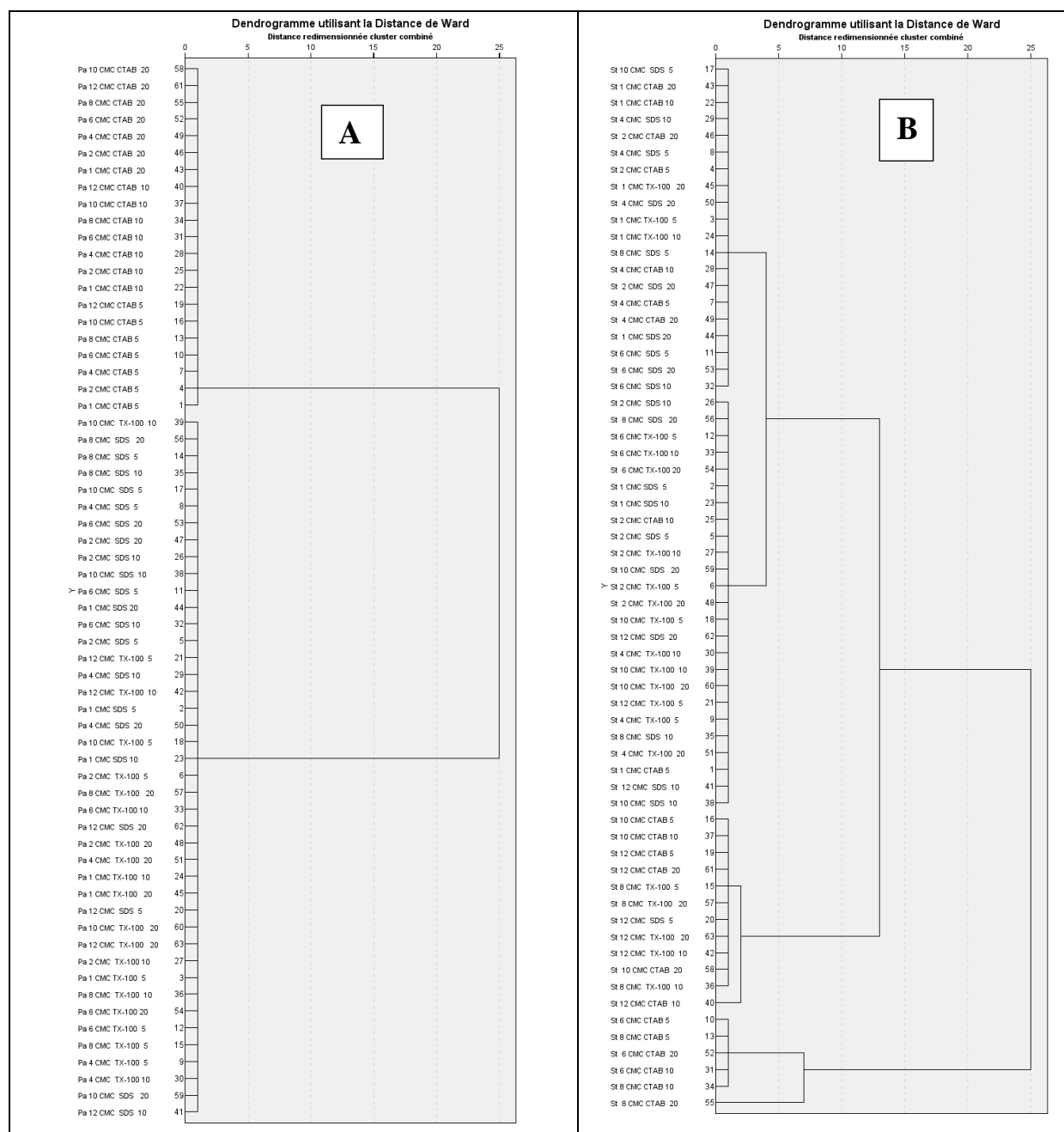


Fig. 4: Relationship between surfactant behaviour and micelles formation at each CMC considered varying from 1CMC to 12 CMC, a new equilibrium was established

For example, excess SDS may interact via ionic bonding to cationic amino acid side-groups and sulfate groups produced from intracellular bacterial cells. At concentrations below the CMC, the permeability of the bacterial membrane increases significantly (Kalmanzon et al., 1992). The outer surface of the cell lipid bilayer is considered the primary point for surfactant action; SDS can interact directly with both membrane phospholipids and bound enzymes (Singer and Tjeerdema, 1993). Lundahl et al. (1986) showed that the head group of SDS plays a significant role in the mechanism of bacterial lysis through the change of protein charge. Following lysis, the environmental medium changes when exposed to internal bacterial cell contents. Indeed, SDS molecules cause an expansion of the bilayer and form negatively charged pores, resembling inverted micelles, that act as cation channels. Kinetic studies showed that release was concentration-dependent below, and concentration-independent above the CMC.

The optimized effect of CTAB compared to the other tested surfactants may be related to its quaternary ammonium structure. The positive charge of the CTAB molecule allows it to denature the molecules contributing thus to bacteria lysis (Clarke, 2009). The anionic groups on the cell lysate act on anionic amino acid residues, and sulphate ions provide abundant adsorption sites for the small polar head of the CTAB ion. Under specific conditions, the maximum adsorption capacity for the surfactant is high. Palmer and Hatley (2018) showed that CTAB was the most effective surfactant to decrease the cell adsorption rate on hydrophilic surfaces. Hu et al. (2004) revealed that the adsorption mode for all surfactants (except SDS) is probably linked to hydrophilic interactions, as the adsorption increased the cell surface hydrophobic properties. Adsorption processes of surfactants are controlled by the nature of the adsorbent and adsorbate, noting that the composition of the medium may change following the lysis of the bacterial cell, and a novel equilibrium has to be established. Theoretically, the CMC is calculated in relation to the medium used, and any change in the environment may lead to a change in CMC. The driving force of the surfactant adsorption on the cell may include the diffusion force, electrostatic attraction or repulsion, Van Der Waals force, hydrogen bonding, dipolar force, as well as the hydrophobic interaction (Paria and Khilar, 2004). The predominant interaction determines the nature of the adsorption process and can be affected by relevant environmental conditions.

The surfactants specifically sequestering chemical pollutant molecules have been studied in many references considering heavy metals (Chauhan and Bordes, 2025), hydrocarbons and plastics. Surfactant acting on bacterial cells leads to their lysis, and the lysis of the bacterial cell alters the dynamic cell structure,

composition of the bilipid layer, and the presence of biosurfactant will alter the environmental medium and change the chemical balances.

Optimization of Treating Both Pathogens in a Wastewater Flow

In the present study, CTAB gives the best result for both Gram-positive and Gram-negative PB. In this present work, we have targeted two PB that commonly occur in the effluent of wastewater, surface waters, and even drinking water reserves. *P. aeruginosa* may alter *Staphylococcus aureus* transcriptome, antibiotic resistance, and internalization into epithelial cells (Briaud et al., 2019). Other authors showed that *P. aeruginosa* and *S. aureus* often display synergistic interactions that can enhance their colonization, virulence, or persistence (DeLeon et al., 2014). Gram-negative bacteria possess the peptidoglycan two-dimensional cell wall sandwiched between the inner membrane and outer membrane (OM), while in Gram-positive bacteria such as *S. aureus*, the cell wall contains the highly charged anionic polyelectrolytes, teichoic acid (TA), which may constitute up to 60% of the wall's mass. When lysed, the internal content released from the two bacteria, Gram-positive or Gram-negative, is different, and the types of molecules and the quantities are not similar. All these characteristics influence the environment of the surfactant and the dynamism of the new chemical equilibria. A new equilibrium that is established contributes to newer cell debris and released endogens and cell components such as organics (polymer: DNA, RNA, proteins), phospholipids, or anion and cation present in bacterial environment such as (Na^+ , K^+ , Mg^{2++} , Ca^{2++}); this molecular debris influences micelle surfactant equilibria. As demonstrated previously, there is an interaction between the departures of anions from the bacterial cell that allows the mobility of cations in the intercellular space. These electrochemical elements and their release will certainly allow a new interaction with the excess of surfactants. The present work demonstrates clearly that not in all cases is 1CMC the best concentration for bacteria sequestration, as it was recommended by the literature. The CMC can determine the micelle stability at a given state. The CMC of a surfactant varies within several factors, such as its chemical structure (Jahan et al., 2020), its ionic nature (Lechuga et al., 2016), temperature (Mohajeri and Noudeh, 2012), and the concentration of organic compounds (Edwards et al., 1992). Size variation of the hydrophobic region of the surfactant is also an important factor. In general, CMC decreases with an increase in the hydrophobic character of the surfactant (Becher, 1965; Haigh, 1996).

All the tested CMC of CTAB significantly reduced *S. aureus*, the bacteria being totally sequestered in the

micelles; specifically, when it was used, an 8 CMC dose resulted in *S. aureus* removal by 9.4 U-Log compared with 1CMC saving only 2 U-Log. For the removal of *P. aeruginosa*, increasing CMC values did not significantly improve bacterial sequestration compared to 1 CMC for CTAB, SDS, and TRX-100.

Effect of Time Exposure to Surfactants

A prolonged exposure time did not significantly improve the rate of *P. aeruginosa* removal, which remained constant after 10 minutes. TX-100 is a non-ionic detergent considered mild and relatively non-denaturing, as it breaks lipid–lipid interactions and lipid–protein interactions rather than protein–protein interactions (Levental and Lyman, 2023). This allows *P. aeruginosa* membrane proteins to be solubilized in TX-100. As a result, the adsorption capacity of TX-100 is low, and it takes a short time for the adsorption to reach equilibrium. This may explain the low kinetics obtained in the present work and the difficulty in improving the rate of bacteria sequestration over time. In contrast, Palmer and Hatley (2018) showed that the adsorption kinetics of the surfactants on the cell followed the second-order law. The adsorption of TX-100 was characterized by a short equilibration time. Koley and Bard (2010) found that any CMC of TX-100 at or below its CMC (0.18 to 0.24 mM) in the solution is fatal to the cells. Damage to the cell membrane was irreversible when the cells were exposed to TX-100 above CMC for only 10 to 30 s. However, no such effect on cell membrane permeability was observed at a concentration significantly lower (Koley and Bard, 2010). TX-100 applied; with all considered, CMC achieves at least 2 U-Log in terms of *S. aureus* sequestration. The results obtained can be explained by the stimulation of TX-100 in *S. aureus* for acylated lipoteichoic acid (LTAs) production, a putative endogenous regulator of autolysin enzymes (Komatsuzawa et al., 1994). In addition, Morath et al. (2001) showed that LTAs have been proposed as putative Gram-positive counterparts to Gram-negative bacteria, where LPS was implied instead of LTAs. The results found in this study considered high levels of TX-100 doses compared with those considered by Raychaudhuri et al. (1985), who showed that *S. aureus* growth was inhibited by only the use of 0.02% of CMC TX-100.

Conclusion

The best yield discharge (9.4 U-log) for *P. aeruginosa* was obtained with CTAB (1CMC) compared to SDS and TX-100 2.4-Ulog discharge. Time exposure did not significantly improve the yield discharge of *P. aeruginosa*. However, for *S. aureus* exposed to CTAB, the yield of 9.4 U-Log discharge occurred when 8CMC was applied compared to 1CMC, saving only 2-ULog. The 12 CMC

significantly impaired the antimicrobial activity of surfactant towards *S. aureus*. Optimising the yield discharge of both PB is of great interest in other water treatment applications, such as environmental monitoring, food preparation, or pharmaceutical production. This study was conducted in a controlled and sterilized aqueous environment. In future work, we will be interested in applying the results in real wastewater matrices to evaluate the impact of organic load, pH, and other interferers.

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Author's Contributions

Imen Laadouze: study conception, design, data collection, and analysis and interpretation of results. EG: Analysis and interpretation of results, improvement of figures, and discussion.

Rihab Hedhiri: Draft manuscript preparation.

Kathleen Sullivan Sealey and Neila Saidi: Were the supervisors of all the work and editors of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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