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Impact of chemical contaminants and process residues on biofilm formation of *Serratia marcescens*: implications for food and water system adulteration control

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ABSTRACT

Microbial biofilms are significant persistent contaminations in the food processing plants, drinking water distribution systems, pharmaceutical environments, and clinics. The role of chemical adulterants, residual disinfectants, nutrient supplements, and ambient contaminants is paramount in the modulation of microbial colonization, surface attachment, and long-standing persistence. Additionally, through the modulation of the physicochemical (pH, osmolarity, and nutrient) environment, these players also impact microbial interactions as well as help mediate cell wall-associated processes needed for attachment, thereby leading to biofilm formation. In addition, these compounds have inhibited EPS (extracellular polymeric substances) production and microbial metabolic activity temporally that are the stabilizing factors in biofilm development surrounding solid substrates. Understanding how chemical environments govern biofilm formation is key to a new class of effective strategies to control contamination. This study evaluated biofilm formation of *Serratia marcescens* (an opportunistic Gram-negative bacterium) recognized as an industrial fouler and water-borne contaminant in the presence of selected chemical agents (acetic acid, sodium hydroxide, ammonium chloride, dextrose, urea, and ethanol). Biofilm biomass (OD570 nm) was determined by crystal violet colorimetric and the planktonic growth spectrophotometric in a 600 nm static culture for 48 h; All experiments were performed as triplicate (n = 3 independent biological repeats). Statistical significance was analyzed using one-way ANOVA and Tukey's post-hoc test. Among the tested agents, acetic acid showed the most pronounced antibiofilm activity with about 40% increase in biofilm mass reduction from the untreated control. In contrast, ammonium chloride could enhance biofilm formation of 320% (over control). Biofilm growth is inhibited only to a moderate extent with nutrient supplements (urea and dextrose). At the tested concentrations, sodium hydroxide and ethanol showed very little inhibition. Furthermore, there was no significant correlation between planktonic growth and biofilm biomass ($r = 0.377$, $p > 0.05$) when analyzed using Pearson coefficients, suggesting that this effect is independent of the intercellular detection

system that bacteria can use to modulate their growth as a whole. These findings suggest that nutrient-rich adulterants and nitrogenous contaminants can promote survival of industrial biofilms, while acidic treatments are highly effective in preventing detachment. The study offers a quantitative framework that elucidates how chemicals affect microbial pollution risk to food and water environments.

Keywords: *Serratia marcescens*; Biofilm formation; Chemical contaminants; Food adulteration; Water system contamination; Crystal violet assay

1. INTRODUCTION

1.1. Biofilm as a Persistent Source of Contamination

A biofilm is an irreversibly adhered microspecies of microbiological community composed of bacterial cells growing upon biotic or abiotic surfaces surrounded by an extracellular polymeric substance (EPS) produced by the biofilm itself (Zhao et al., 2023). Exopolymeric substances (EPS matrix) are composed of polysaccharides, structural proteins, eDNA, and lipids with water channels; their protective barriers determine microorganisms' survival rates in various physiological states under environmental conditions (Kilic, 2025). The biofilm mode of growth provides several physiological advantages when compared to planktonic (free-floating) cells. Bacteria show distinct gene expression spatial patterns, metabolic heterogeneity, quorum–sensing–dependent intercellular communication, and collective stress responses in the biofilm microenvironment. All of these adaptations collectively support resistance to antimicrobial agents, disinfectants, desiccation, oxidative stress, and host immunity (Hernández-Jiménez et al., 2013).

In industrial and food-processing systems, the significance of biofilm is a major challenge to maintenance because biofilms can persist on differently textured surfaces at all stages of production and processing. Biofilms result in bio-fouling, corrosion of metallic surfaces, decreased heat-transfer efficiency, clogging of filtration systems, and deterioration of hygienic quality. Biofilms in food systems can offer natural niches for pathogenic/possibly pathogenic and opportunistic microorganisms that release toxins, spoilage enzymes, and virulence factors (Carrascosa et al., 2021). As a result, biofilm-associated contamination is identified as a source of microbial genera that can threaten the safety, shelf life, and regulatory compliance of your products. The persistence and adaptation of biofilms require a greater insight into the environmental conditions that favour their structure and function (Boldeanu et al., 2025).

1.2. Chemical Adulterants and Environmental Modulation

Chemical agents can enter food-processing and water-distribution systems through cleaning protocols, sanitization procedures, raw materials, and environmental inputs (Pakdel et al., 2023). Chemical contaminants are often cleaning-in-place (CIP) agents, such as sodium hydroxide and organic acids; residual salinity, nutrient supplements, such as vitamins or sugars; alcohol-based disinfectants; or nitrogen-containing compounds such as urea. This often includes the intentional application of these chemicals (e.g., sanitation, preservation, or processing), but can be persistent; later presence has a high probability to interfere with microbial physiology and biofilm adhesive competition (Sanawar et al., 2021).

Due to the ability of organic acids, including acetic acid, to lower the surrounding pH and compromise membrane integrity as well as intracellular metabolism, they are frequently used for preservation or an antimicrobial purpose (Elchemy, 2025). On the other hand, cleaning systems also employ sodium hydroxide and most alkaline compounds common in industry, but a reduced antimicrobial effect of sublethal concentrations can lead to adapted organisms that are less sensitive (Pinho et al., 2015) Nutrient-containing residuals (carbon compounds like dextrose, nitrogen derivatives such as urea and ammonium salts) might be accidentally nutritious for growing microbes or producing EPS (Ghanaim et al., 2025). These nutrients can increase metabolic activity, stimulate quorum-sensing pathways, and strengthen solid surface adhesion that is more resistant to external force. Considering that the components of the external environment play a key regulatory function in biofilm initiation, growth, and maintenance, it is, for practical purposes, important to gain an understanding of how this occurs. One has to know how these chemical contaminants influence the emergence of biofilms if there is any hope of coming up with more practical measures to prevent their formation (Kilic, 2025).

1.3. Relevance of *Serratia marcescens*

Serratia marcescens is a member of Gram-negative, facultatively anaerobic opportunistic pathogenic bacteria of the Enterobacteriaceae family. It is frequently present in soil, water, surfaces of plants, and food-processing environments and health care settings (Tavares-Carreon et al., 2023). This well-known organism has the ability to create powerful biofilms on several materials and surfaces, such as plastics, metals, glass, and medical devices. This environmental adaptability is associated with the production of extracellular enzymes,

surfactants that contribute to a characteristic red pigment, prodigiosin, and which may place this agent in an advantageous condition for stress resistance and complete survival (Ferguson et al., 2023). *S. marcescens* has also been associated with biofouling of pipelines, fluid store contamination in both industrial and water systems, and persistence of initial colonization of processing equipment. In clinical settings, it is responsible for urinary tract, wound, and bloodstream infections; it is of great importance as an opportunistic pathogen in immunocompromised patients. (Veggalam & Kandi, 2025). Given its ability to flourish under multiple chemical stresses and nutrient conditions, it is a suitable model organism for the analysis of chemical inhibition of biofilm-inducing compounds. So the study of *S. marcescens* biofilm development in the presence of environmental contaminants could shed light on contamination dynamics present in food and water systems (Boldeanu et al., 2025).

1.4. Study Objective

Herein, quantitative studies have been carried out to explore the influence of agrochemical toxins and nutrient-related adulterants on Serratia marcescens biofilm-forming ability in laboratory conditions. The focus of the study was to determine how organic acid (acetic acid), alkaline agent (sodium hydroxide), salt (ammonium chloride), carbon source (dextrose), nitrogen compound (urea), and organic solvent (ethanol) affected planktonic growth and biomass of surface-associated biofilms. A standardized, crystal violet colorimetric method in combination with statistical analysis was used to separate treatment effects that affect planktonic cell density from those that specifically impact biofilm formation, which only occurs after adherence to a surface. The goal was to determine chemical conditions that inhibit or promote bacterial colonization, as well as their potential relevance for contamination prevention and adulteration control of food-processing and water-distribution systems.

2. MATERIALS AND METHODS

2.1. Bacterial Strain and Culture Conditions

In the present investigation, *Serratia marcescens* was utilized as a test organism. The bacterial culture was kept in Luria–Bertani (LB) agar slant at 4 °C and had been sub-cultured before the experiment to verify purity and viability. For each experimental run, a new overnight culture was prepared by inoculating a single isolated colony into 10 mL rich LB broth and statically incubated at 37 °C for 24 hours. The turbidities of the cultures were adjusted to about 0.5 McFarland standard (10^8 CFU/mL) so that an inoculum density could be standardized for all treatments. The general equipment and reagents used were sterilized to avoid external contamination.

2.2. Preparation of Chemical Treatments and Experimental Design

The chosen chemical agents were of the type encountered in industrial processing, cleaning systems, and possible scenarios involving adulteration. As a strong representative of preservative and acidic cleaning environments, acetic acid (2% v/v) was used. Sodium hydroxide (2% w/v) was chosen as a model alkaline cleaning agent for this purpose owing to its common use in most cleaning-in-place (CIP) systems. Ammonium chloride (2% w/v) served as a model inorganic salt and nitrogen source, while dextrose (0.2 g per tube) and urea (2% w/v) were included as nutrient-associated compounds to evaluate common carbon and nitrogen adulterants in the tablet formulation. Ethanol (analytical grade) was selected as a representative organic solvent and disinfectant widely utilized in sanitation protocols.

The sterile glass test tubes were prepared for each treatment to contain 10 mL of sterile LB broth, 1 mL of a standardized bacterial inoculum, and 1 mL in volume of the respective chemical solution. The untreated control was LB broth containing the bacterial inoculum without a chemical additive. A blank of media alone containing LB broth was included to correct for background absorbance. To allow ample time for attachment of the cells to the surfaces and biofilm formation, all tubes were incubated at 37 °C under static conditions for 48 hours. All treatments were done in triplicate independent biological replicates (n=3).

2.3. Measurement of Planktonic Growth

After 48 h incubation, the planktonic bacterial growth was examined by aspirating the culture supernatant carefully but without disturbing the attached biofilm. Using a calibrated spectrophotometer, the optical density of the supernatant was measured through the 600-nm filter (OD 600). The LB blank was used to zero the instrument before measurements were made. The OD600 value of the supernatant is relative to the level of bacterial growth under this chemical treatment condition.

2.4. Biofilm Quantification by Crystal Violet Assay

Biofilm biomass was measured according to the standard crystal violet staining procedure. Subsequently, tubes were gently washed with sterile PBS three times to remove non-adherent cells after the planktonic culture removal. For biofilm size quantification, the attached biofilm remaining was stained with 3 mL of a crystal violet (0.1% (w/v)) solution and incubated for 15 min at room temperature. Excess stain was removed, and the tubes rinsed three to four times with sterile distilled water to remove unbound dye. The tubes were then allowed to air dry fully. Later, to solubilize the bound crystal violet, 3 mL of 30% (v/v) acetic acid was added and shaken for 15 min. Absorbance (OD570) was measured after transferring 1 ml of the solubilized solution from each well into a cuvette. The OD 570 readings, which were taken here, represent the total biofilm mass that formed via each treatment condition.

2.5. Calculation of Biofilm Inhibition and Statistical Analysis

Biofilm formation was normalized to 100% biofilm formation (untreated control). The relative percentage of biofilm was calculated as follows: (OD_{570}sample/OD_{570}control) × 100. The inhibition of biofilm was then calculated by subtracting the percentage of remaining biofilm from 100. Negative values of inhibition were considered a sign of increased formation of biofilm compared to the control.

Each experiment was performed in triplicate (n = 3) and expressed as mean ± standard deviation (SD). Question orthodoxy was used to calculate graphs, and one-way analysis of variance (ANOVA) for statistical comparison among groups. Pearson's correlation coefficient test was used to calculate the association between planktonic (OD600) and biofilm biomass (OD570), which enables distinguishing growth-dependent effects from biofilm-specific modulatory effects. P-values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Effect of Chemical Treatments on Planktonic Growth

The effect on the planktonic development of Serratia marcescens by chemicals was measured after 48 hours of incubation. For the measurement of bacterial growth, OD600 was recorded and is presented as mean ± standard deviation (n = 3) in Table 1 & Figure 1. Different types of chemicals affected bacterial growth differently. The highest growth was noticed in the case of dextrose (0.665 ± 0.015), suggesting that easily utilizable carbon sources in the medium have a positive effect on bacterial growth. Moderate growth (0.460 ± 0.010) was also observed with ammonium chloride, indicating that nitrogen in the medium may act positively on bacterial growth as well. The control group (0.445 ± 0.005) had normal bacterial growth and demonstrated that the bacteria were healthy in normal conditions. In acidic conditions, the growth of bacteria was significantly low (0.095 ± 0.005) at the concentration of acetic acid, showing an inhibition. Sodium hydroxide and ethanol also inhibited growth (0.065 ± 0.005), suggesting that alkaline conditions and solvent stress adversely impact bacterial proliferation. Urea had partial inhibition (0.355 ± 0.005), indicating that it affected bacterial growth, to some extent, less than dextrose in comparison with the control. In general, the present outcomes indicate exaggerated bacterial growth in the presence of nutrients such as dextrose, while acidic, alkaline, and solvent conditions reduced bacterial growth. All the above data are presented in Table 1.

Table 1. Effect of Chemical Treatments on Planktonic Growth of *Serratia marcescens* (OD600 nm, n = 3)

Treatment	Mean OD600 ± SD
Acetic Acid	0.095 ± 0.005
NaOH	0.065 ± 0.005
NH_4Cl	0.460 ± 0.010
Dextrose	0.665 ± 0.015
Urea	0.355 ± 0.005
Ethanol	0.065 ± 0.005
Control	0.445 ± 0.005
LB Blank	0

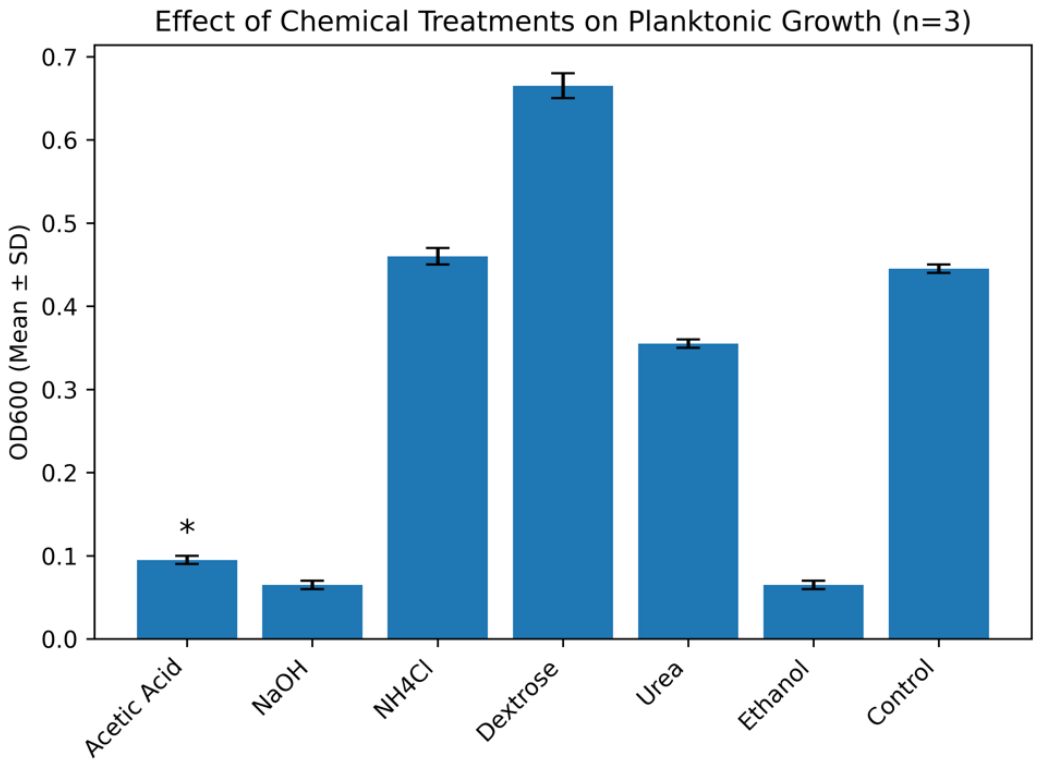

Figure 1. Effect of chemical treatments on planktonic growth (OD600 ± SD, n = 3).

3.2. Effect of Chemical Treatments on Biofilm Biomass

Using a crystal violet method for assessing biofilm biomass, absorbance was measured at 570 nm (OD570). The results are shown in Table 2 & Figure 2. Treatment groups had noticeable differences in biofilm formation. As for inhibitory effects on the formation of a biofilm, the most powerful was acetic acid. This resulted in the lowest biofilm biomass values (0.015 ± 0.005). This is lower than that of the value which is observed in the untreated control (0.025 ± 0.005), which suggests that the acidic conditions affect bacterial surface attachment or extracellular polymeric substances (EPS) stability. Biofilm biomass of sodium hydroxide and ethanol treatment was similar to that of the control (0.025 ± 0.005), therefore indicating minimal inhibition. Since ammonium chloride greatly promoted biofilm formation (0.105 ± 0.005), it also led to the largest biomass. This would suggest that its action is stimulating exopolysaccharide production or receptor binding so as to increase adhesion. Urea and dextrose both moderately improved biofilm mass (0.030 ± 0.010 and 0.030 ± 0.000, respectively), reflecting nutrient- promoted surface accumulation. However, these results were weaker than those seen with ammonium chloride. Yet they still as a whole indicate that nutrient availability contributes towards the building of biofilms. The chemical environment determines that under acidic stress, biofilm formation is restrained, and in a nutrient-rich environment, microorganisms can fasten onto surfaces via an attachment mechanism, which can be interpreted in Table 2.

Table 2. Effect of Chemical Treatments on Biofilm Biomass (OD570 nm, n = 3)

Treatment	Mean OD570 ± SD
Acetic Acid	0.015 ± 0.005
NaOH	0.025 ± 0.005
NH_4Cl	0.105 ± 0.005
Dextrose	0.030 ± 0.010
Urea	0.030 ± 0.000
Ethanol	0.025 ± 0.005
Control	0.025 ± 0.005
LB Blank	0

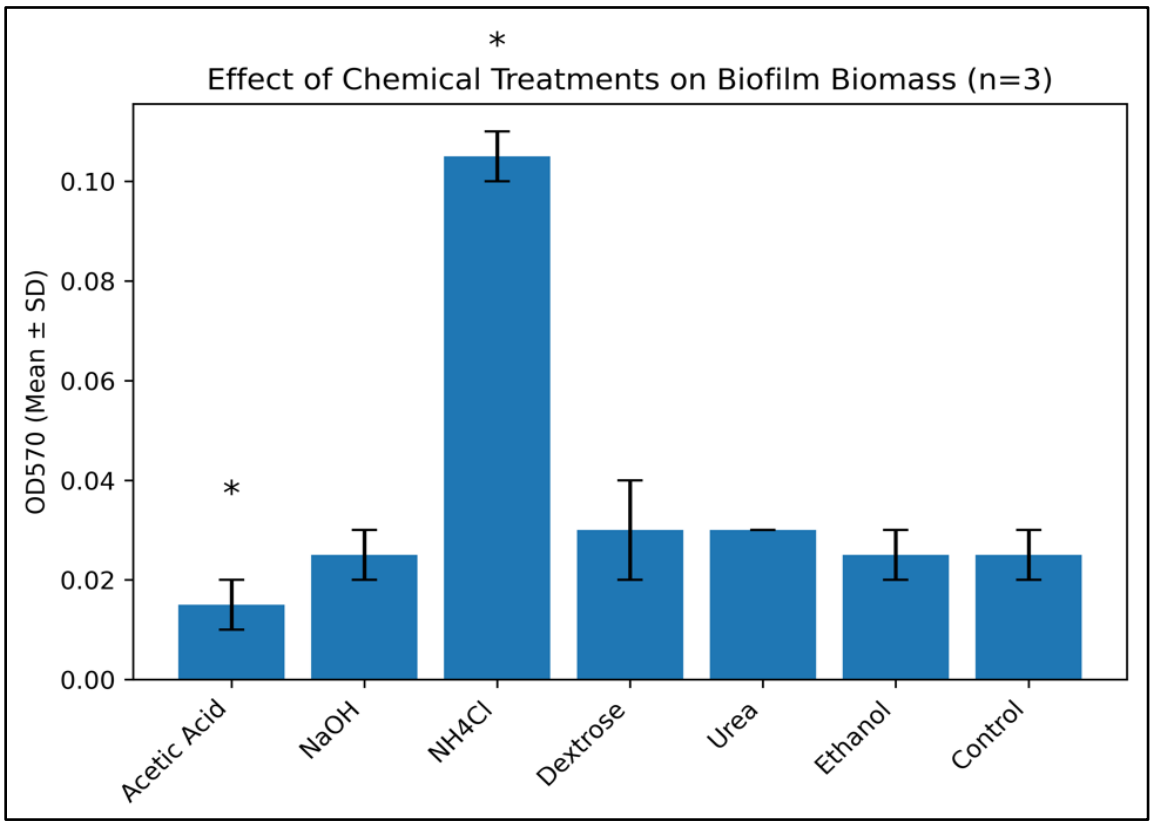

Figure 2. Quantitative biofilm biomass measured by crystal violet assay (OD570 ± SD, n = 3).

3.3. Percentage Biofilm Remaining and Inhibition

To compare how well the treatments worked, the percentage of biofilm remaining was calculated, as well as the percentage inhibition, based on an untreated control that was held equivalent to 100% for this purpose (Table 3 & Figure 3). Treatment with acetic acid brought biofilm back down to 60% of the control level, or 40% reduction. In contrast, sodium hydroxide and ethanol showed no detectable decrease in biofilm. Biofilm levels remained at 100%. Ammonium chloride, however, increased biofilm immensely, resulting in a 420% reading for remaining biofilm-this gives rise to 320% more compared to Control. Dextrose and urea slightly raised biofilm production, each reading 120 % biofilm remaining, indicating only a small increase in quantity. The negative values of inhibition observed for ammonium chloride, dextrose, and urea indicate that it was those three materials that raised biofilm formation rather than lowering it, as presented in Table 3.

Table 3. Percentage Biofilm Remaining and Inhibition (n = 3)

Treatment	% Remaining	% Inhibition
Acetic Acid	60%	40%
NaOH	100%	0%
NH_4Cl	420%	−320%
Dextrose	120%	−20%
Urea	120%	−20%
Ethanol	100%	0%
Control	100%	—

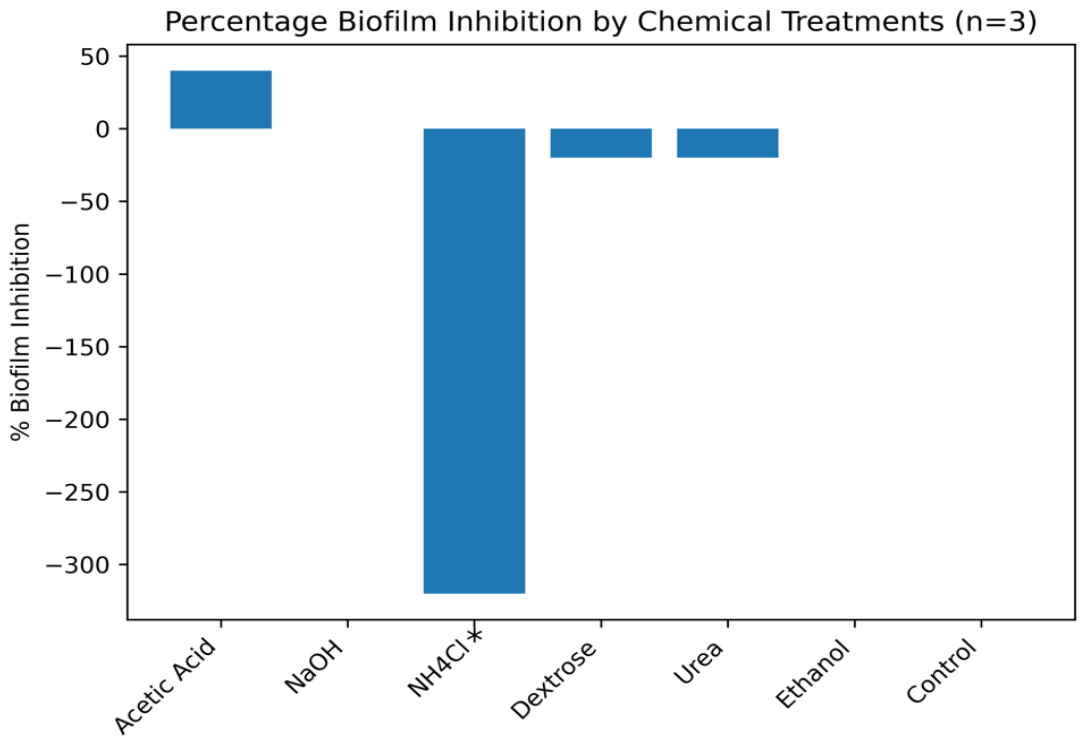

Figure 3. Percentage of the biofilm inhibition relative to the untreated control (n = 3).

3.4. Statistical Analysis

One-way ANOVA test indicated a significant difference in biofilm biomass by treatment groups ($p < 0.001$), whereas chemical impacts were very strong. Ammonium chloride significantly enhanced biofilm biomass when compared to acetic acid and control ($p < 0.01$) according to Tukey's HSD post-hoc analysis. while the addition of acetic acid significantly reduced biomass compared to ammonium chloride ($p < 0.01$). There were no statistically different findings between the sodium hydroxide, ethanol, and control groups. As indicated by the result of Pearson correlation analysis between planktonic growth (OD600) and biofilm biomass (OD570), $r = 0.377$, $p > 0.05$, it seems certain that the two variables would not directly correlate significantly via a linear relationship. This shows that biofilm modulation was not solely dependent on the overall bacterial population but also involved specific regulatory mechanisms that govern the surface attachment and EPS production.

4. DISCUSSION

The present study shows that *Serratia marcescens* biofilm formation is strongly regulated by the chemical environment. These results show how different classes of chemical agents have differential effects on the planktonic growth and the biofilm biomass, demonstrating a little bit of the complexity aspect of microbial responses towards environmental stressors or nutrient availability. Acetic acid reduced planktonic growth, as well as biofilm formation by 40%, indicating that an acidic environment interferes with surface adhesion and the stability of EPS. This effectively allows the undissociated form of the acid to diffuse across bacterial membranes, which results in intracellular acidification and collapse of the proton motive force, eventually resulting in inhibition of enzymatic activity and other processes (Khanashyam et al., 2023). Moreover, acidic stress could modify the structure of extracellular polymeric substances, leading to deteriorated biofilm architecture (Jyoti et al., 2024). However, strong suppression observed in this study supports freestanding acidic treatments as a potential tool for incorporation into contamination control strategies, including those of food-processing and water systems, where biofilm persistence is an issue.

While sodium hydroxide decreased the formation of planktonic cells, it was not able to significantly inhibit biofilm under our assay conditions. While alkaline-based cleaning agents are commonly used in the field of industrial sanitation, the sublethal levels of alkaline may not have sufficient action on preformed biofilm matrices (Fagerlund et al., 2020). Microbial cells in biofilms are often more tolerant to changes in the pH than planktonic microbes, due to the buffering effects of EPS and altered gene expression profiles. Likewise, ethanol only exhibited minimal antibiofilm activity, which implies that solvent stress does not effectively inhibit surface colonization at

the tested exposure levels (Segura et al., 2012). The addition of ammonium chloride significantly promoted biofilm formation, 320% higher than the control in biomass. The possible explanation for the effect is that ammonium, being a nitrogen substrate and an osmotic regulator, stimulates EPS production, promoting biofilm stability (Zhao et al., 2024). According to the enhancement effect, residual nitrogenous compounds in industrial or food systems can inadvertently enhance microbial persistence.

Dextrose supplementation resulted in high planktonic growth and moderate biofilm formation. The addition of an easily metabolizable carbon source probably enhanced metabolism and energy uptake, thus contributing to the release of EPS, which caused nucleation on the surface. Urea proved similar, with moderate biofilm outlining, probably related to its nitrogen contribution post-enzymatic hydrolysis. These data indicate that nutrient-poor environments potentiate microbiota adhesion and biofilm development (Sharma et al., 2023). Pearson correlation analysis also did not reveal any significant association between planktonic growth and biofilm biomass. This indicates that biofilm production is not just a marker of cell density, but instead governed by pathways regulating surface attachment and community formation. Divergence may be due to biofilm phenotype gene expression, quorum-sensing mechanisms, or environmental stress adaptation.

From an applied perspective, these findings carry important implications for food and water system adulteration control. Other possible factors can be related to nutrient-based adulterants and residual nitrogen compounds that could also promote the retention of microbes at the product/food interface when contamination potential arises (Haji et al., 2023). In contrast, acidic environments appear to be more efficacious in limiting biofilm formation. The data highlight the importance of extensive residue management and directed sanitation approaches to reduce biofilm-mediated cross-contamination. Thus, this study suggests that the various diverse sets of chemical environments in the environment at the same time can act as inhibitors or motors of microbial colonization. These interactions are important for informing evidence-based approaches to contamination prevention in industrial and public health environments.

5. IMPLICATIONS FOR FOOD AND WATER SYSTEM ADULTERATION CONTROL

The results of the current study have direct implications for contamination management and adulteration control at food-processing units, water-distribution systems, and industrial environments. However, microbial contamination associated with biofilms is being viewed as an underrecognized source of microbial adulteration because surface-attached microbiota can serve as long-term reservoirs for pathogens and spoilage microbes. These findings demonstrate the modulation of biofilm formation by environmental chemicals and emphasize the necessity of controlling residual chemical composition in processing systems.

The strong increase in biofilm formation by ammonium chloride shows that nitrogen-containing residues highly stimulate microbial persistence. Food systems can be contaminated with nitrogenous compounds like urea or ammonium salts through adulteration means, raw material contamination means, and sanitation actions that may not reach the standards. Upregulation of metabolic activity and extracellular matrix production in biofilms leads to stable surface colonization through the presence of these sessile microbes. This results in increased contamination risk even at sublethal nutrient residue levels, due to a more mature biofilm.

Similarly, the biofilm activity observed with the addition of dextrose in our study highlights the need to remove any carbohydrate residues from processing equipment. In most cases, sugar-based residues are not completely removed, and thus, it prevents biodeposits from microbial adhesion and EPS production, composing micro-environments which offer nutritional parts to microorganisms where they can actively grow by converting them. But that could increase the risk of chronic contamination and spoilage. On the other hand, acidic treatment displayed significant antibiofilm activity. Specifically, the decreased biofilm mass in acetic acid suggests that focused application of acidic sanitation processes, when applied effectively, may be more suitable to reduce biofilm formation with some environmental realities than their alkaline counterparts or solvent-based methods. Validity of the concentration and exposure parameters for disinfecting also has to be validated without endangering any material safety.

However, low levels of inhibition obtained with sodium hydroxide and ethanol in some experimental conditions indicate that these agents alone may not guarantee the elimination of biofilm persistence (i.e., residues left at sub-optimal concentrations); therefore, sanitation programs should evaluate combined or sequential chemical strategies, which should be implemented after removal of important nutrient residues before terminal disinfection processes. -This study emphasizes that contamination risk is determined not only by microbial load but also by the chemical milieu. Monitoring for chemical residues likely to activate microbial biofilms in all but the most favourable manner is critical to successful adulteration control at every stage of production.

6. LIMITATIONS AND FUTURE SCOPE

First, the experiments were performed in static in vitro conditions that may not accurately reflect the dynamic flow environments present within most industrial pipelines and water-distribution systems. The behavior of biofilm under shear stress and constant nutrient supply may vary from what we report here. Second, the assay was for biofilm biomass by crystal violet staining. Although this method provides a consistent estimate of attached biomass, it does not measure viable vs non-viable cells nor characterize structural/compositional-related data from the EPS matrix. However, this study is only able to provide qualitative adjuncts using basic light microscopy techniques, while understanding of structural modifications at a deeper level can be obtained using more advanced imaging technologies such as CLSM or SEM. Third, the study did not explore the molecular mechanisms that modulated biofilms. Gene expression analysis targeting quorum-sensing pathways led to further studies on regulation. EPS synthesis genes and stress response regulators that could clarify the regulatory basis of chemical-induced alterations in biofilm formation. Future studies may integrate more dynamic flow models, multi-species biofilm systems, and molecular approaches to obtain a better understanding of the mechanisms underlying chemical modulation of microbial attachment. Evaluation of combined chemical treatments and time-dependent exposure effects could also offer more realistic guidance for industry sanitation approaches.

7. CONCLUSION

In the current study, we show that the chemical environment modulates biofilm formation in Serratia marcescens. Nitrogen-containing compounds enhance biofilm biomass, including ammonium chloride; however, inhibit biofilm formation arising from acidic conditions (Especially acetic acid treatment). The combination of dextrose and urea additions not only promoted bacterial growth but also moderate surface biofilm formation. Both ethanol and sodium hydroxide had minimal in vitro antibiofilm activity at their screening concentrations.

Importantly, biofilm modulation was independent of planktonic growth, suggesting that surface-associated chemical signals encode unique information regarding microbial persistence.

These observations highlight the disturbing reality that nutrient-rich impurities and residual nitrogenous species in food and water systems could provide substrates that support the biofilm development potential of this seemingly uncommon organism classification, consequently increasing the likelihood of food- and/or water-borne diseases. Prime control strategies for blocking contamination are, therefore, a combination of microbial reduction practices and judicious treatment of chemical residues that can unwittingly promote biofilms. We offer a quantitative and reproducible framework for evaluating the effects of chemicals on microbial colonization, an important step towards developing improved sanitation and adulteration-prevention strategies not only in industrial settings but also for public health purposes.

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Additional Information

This work has not been published previously and is not under consideration elsewhere. The research was conducted in the Department of Microbiology, Faculty of Biochemistry, ZSCT's Thakur Shyamnarayan Degree College, Mumbai. The manuscript has not been presented at any previous conferences or scientific meetings.

Authors' Contributions

Conception and design of the study: Bhanupratap Harishchandra Vishwakarma.

The author conducted all experimental work, including bacterial culture preparation, chemical treatment assays, biofilm quantification, and data collection. The author also performed statistical analysis, interpreted the data and results, prepared figures and tables, and wrote and revised the manuscript. The author approved the final manuscript as submitted.

Informed consent

Not applicable.

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Ethical approval

In this article, the food and product ethical regulations are followed as per the ethical committee guidelines of Department of Microbiology, Faculty of Biochemistry, ZSCT's Thakur Shyamnarayan Degree College, Thakur Complex, Kandivali (East), Mumbai – 400101, Maharashtra, India; the authors observed the Impact of chemical contaminants and process residues on biofilm formation of *Serratia marcescens*. The "brand name" of the products are not mentioned in content and also the "brand image" not displayed as figure in the article. The food and product ethical guidelines are followed in the study for observation, identification & experimentation.

Conflict of Interest

The authors declare that they have no conflicts of interest, competing financial interest or personal relationship that could have influenced the work reported in this paper.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES

1. Boldeanu L, Boldeanu MV, Novac MB, Assani MZ, Radu L. *Serratia marcescens*: a versatile opportunistic pathogen with emerging clinical and biotechnological significance. *Int J Mol Sci* 2025;26(23):11479. doi:10.3390/ijms262311479
2. Carrascosa C, Raheem D, Ramos F, Saraiva A, Raposo A. Microbial biofilms in the food industry - a comprehensive review. *Int J Environ Res Public Health* 2021;18(4):2014. doi:10.3390/ijerph18042014
3. Elchemy. Acetic acid in food manufacturing: a cost-effective solution for food safety. *Elchemy Blog* 2025. Available at: https://elchemy.com/blogs/acetic-acid-in-food-manufacturing-a-cost-effective-solution-for-food-safety
4. Fagerlund A, Heir E, Møretrø T, Langsrud S. Listeria monocytogenes biofilm removal using different commercial cleaning agents. Molecules 2020;25(4):792. doi:10.3390/molecules25040792
5. Ferguson D, Ryder R, Lunsford R, Dash A, Kamali A, Kimura A, Crandall J, Mukhopadhyay R, Dowless H, Ortiz N, Zoorob R, et al. Serratia marcescens outbreak at a correctional facility: environmental sampling, laboratory analyses and genomic characterization to assess sources and persistence. Int J Environ Res Public Health 2023;20(17):6709. doi:10.3390/ijerph20176709
6. Ghanaim AM, Mohamed HI, El-Ansary AE. Production and characterization of exopolysaccharides from *Pseudomonas aeruginosa* AG01 with some medical potential applications. *Microb Cell Fact* 2025;24:107. doi:10.1186/s12934-025-02730-z
7. Haji A, Desalegn K, Hassen H. Selected food items adulteration, their impacts on public health, and detection methods: a review. Food Sci Nutr 2023;11(12):7534-7545. doi:10.1002/fsn3.3732
8. Hernández-Jiménez E, del Campo R, Toledano V, Vallejo-Cremades MT, Muñoz A, Largo C, Arnalich F, García-Rio F, Cubillos-Zapata C, López-Collazo E. Biofilm vs. planktonic bacterial mode of growth: which do human macrophages prefer? *Biochem Biophys Res Commun* 2013;441(4):947-952. doi:10.1016/j.bbrc.2013.11.012
9. Jyoti K, Soni K, Chandra R. Optimization of the production of exopolysaccharide (EPS) from biofilm-forming bacterial consortium using different parameters. Microbe 2024;4:100117. doi:10.1016/j.microb.2024.100117
10. Khanashyam AC, Shanker MA, Thomas PE, Babu KS, Nirmal NP. Phytochemicals in biofilm inhibition. In: Pati S, Sarkar T, Lahiri D, editors. Recent Frontiers of Phytochemicals. Elsevier; 2023:397-412. doi:10.1016/B978-0-443-19143-5.00018-9
11. Kilic T. Factors affecting biofilm formation and the effects of these factors on bacteria. In: Dincer S, Ozdenefe MS, Mercimek Takci HA, editors. *Exploring Bacterial Biofilms*. IntechOpen; 2025. doi:10.5772/intechopen.1008877
12. Pakdel M, Olsen A, Skjøndal Bar EM. A review of food contaminants and their pathways within food processing

facilities using open food processing equipment. *J Food Prot* 2023;86(12):100184. doi:10.1016/j.jfp.2023.100184

13. Pinho SC, Nunes OC, Lobo-da-Cunha A, Almeida MF. Inactivation of *Geobacillus stearothermophilus* spores by alkaline hydrolysis applied to medical waste treatment. J Environ Manage 2015;161:51-56. doi:10.1016/j.jenvman.2015.06.045
14. Sanawar H, Kim LH, Farhat NM, van Loosdrecht MCM, Vrouwenvelder JS. Periodic chemical cleaning with urea: disintegration of biofilms and reduction of key biofilm-forming bacteria from reverse osmosis membranes. *Water Res X* 2021;13:100117. doi:10.1016/j.wroa.2021.100117
15. Segura A, Molina L, Fillet S, Krell T, Bernal P, Muñoz-Rojas J, Ramos JL. Solvent tolerance in Gram-negative bacteria. Curr Opin Biotechnol 2012;23(3):415-421. doi:10.1016/j.copbio.2011.11.015
16. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. Microorganisms 2023;11(6):1614. doi: 10.3390/microorganisms11061614
17. Tavares-Carreon F, De Anda-Mora K, Rojas-Barrera IC, Andrade A. Serratia marcescens antibiotic resistance mechanisms of an opportunistic pathogen: a literature review. *PeerJ* 2023;11:e14399. doi:10.7717/peerj.14399
18. Veggalam S, Kandi V. Serratia marcescens as an uncommon cause of infection following craniectomy: a case report and literature review. Cureus 2025;17(6):e86673. doi: 10.7759/cureus.86673
19. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: from definition to treatment strategies. *Front Cell Infect Microbiol* 2023;13:1137947. doi:10.3389/fcimb.2023.1137947
20. Zhao ZC, Fan SQ, Lu Y, Tan X, Liu LY, Wang XW, Liu BF, Xing DF, Ren NQ, Xie GJ. Deep insights into the biofilm formation mechanism and nitrogen-transformation network in a nitrate-dependent anaerobic methane oxidation biofilm. Environ Res 2024;252(1):118810. doi: 10.1016/j.envres.2024.118810