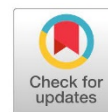


Research Article

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Exploring the Impact of Pipe Material and Environmental Conditions on the Bacterial Adhesion to the Surface of the Drinking Water Distribution System

Anad M. Alshaybani ^{1*}, Ebtessam Abdulhadi ², Abdallah I. Abdallah ³ and Hanin Bzizi ⁴

*Corresponding author:

aafhaima2011@my.fit.edu,

Department of Chemistry, Faculty of Sciences, Sirte University, Libya.

² Department of Zoology, Faculty of Sciences, Sirte University, Libya.

³ Department of Medical Microbiology, Faculty of Medicine, Sirte University, Libya.

⁴ Department of Biomedical Science, Western Michigan University, Kalamazoo, MI 49008, USA.

Received:

19 November 2024

Accepted:

27 December 2024

Publish online:

31 December 2024

Abstract

Bacterial adhesion to surfaces is a complicated process influenced by several factors. Key factors are the physical properties of the materials, characteristics of the bacteria, and environmental conditions. In this study, the effects of the type of water distribution pipe material, water temperature, water flow speed, and contact time on the rate of bacterial adhesion to the pipe walls were evaluated. Two species of bacteria, gram-negative and gram-positive, were selected to study their tendency to adhere to the surfaces of two types of materials (galvanized iron and Polyvinyl Chloride (PVC)) used in the manufacture of water distribution pipes. The results showed that elevated temperature and contact time contributed to increased adhesion of Gram positive bacteria to galvanized iron, whereas no discernible effects were observed for Gram negative bacteria. These findings also demonstrated that positively charged bacteria exhibited a higher capacity to adhere to mutually galvanized iron and PVC surfaces than Gram negative bacteria. Moreover, the rate of adhesion of Gram positive bacteria increased with increasing water flow rate, and there was no clear effect of water flow rate on the extent of adhesion of Gram negative bacteria on both surfaces. This study underscores the necessity of selecting appropriate pipe materials, factoring in operational temperatures and water flow dynamics to effectively manage bacterial biofilm development in distribution systems.

Keywords: Polyvinyl Chloride (PVC); Galvanized Iron; Bacterial Adhesion; Biofilm Formation; Water Distribution Systems; colony-forming units (CFUs).

INTRODUCTION

Biofilm growth considerably affects interactions between microbes and pipe surfaces during the initial adhesion stage. Nearly one billion people worldwide face health risks due to waterborne pathogens, predominantly facilitated by biofilm proliferation within drinking water distribution systems (DWDS) (Sharma et al., 2023; Adelodun et al., 2021; Owens et al., 2020). One of the primary avenues for these infections is through DWDS in which biofilms are frequently held within pipelines (Waqas et al., 2023; Gomez & Aggarwal, 2019; Zhang et al., 2018). Biofilms, which are resilient communities of microbes embedded within a polymer matrix, possess heightened resistance to disinfectants and environmental stressors (Rhoads et al., 2020).

The central problem revolves around understanding the influence of different pipe materials on bacterial adhesion and subsequent biofilm formation. This process is governed by physicochemical



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forces between the microbes and substrates, which have been elucidated through thermodynamic principles (Bain et al., 2014; Fulaz et al., 2019). The key contributing factors include surface charge, hydrophobicity, and hydrophobicity (Oh et al., 2018; Danchik et al., 2021). Microbial appendages, such as pili, play a crucial role in overcoming repulsive forces and facilitating enduring bonds with surfaces (Costerton et al., 1995). Additionally, environmental parameters, such as water temperature, pH, disinfectant levels, and nutrients significantly influence biofilm growth (Erdei-Tombor et al., 2024; Goldberg et al., 2002; Peng et al., 2002; Stefan et al., 2023). Significantly, the material of the pipes influences the stimulation of the biofilm and the strength of adhesion (Goraj et al., 2021; Schwering et al., 2013; Zhang et al., 2022).

Commonly used distribution pipes, such as concrete, galvanized iron pipes, and Polyvinyl Chloride (PVC), may possess varying properties that effect on stimulate biofilm formation (Learbuch et al., 2021; Chen et al., 2020). However, a comprehensive comparative analysis of these different pipe types under standardized conditions is currently lacking. Addressing this issue involves rigorous exploration of the bacterial adhesion strength across various commonly used pipe materials at different temperatures. By quantifying these adhesion strengths and evaluating biofilm growth under controlled conditions, this study aims to provide actionable insights. These insights can guide the selection of pipe materials and inform strategies for managing temperature fluctuations, ultimately curbing biofilm formation, and reducing the persistence of pathogens within water distribution systems. Minimizing microbial contamination in these systems is a pivotal step toward mitigating waterborne infections globally. The primary objective of this study was to investigate the impact of pipe material, temperature variations, water flow rate, and contact time on the strength of bacterial adherence and the subsequent development of biofilms along water pipes. Through controlled experiments, this study sought to unravel the interplay between these factors and elucidate their combined influence on biofilm formation in drinking water distribution systems.

Background & Related Work

Most studies have approached adhesion through a narrow lens by focusing on selected parameters (see Table 1). A deeper and more integrated understanding requires analyses that bridge the multiple domains. However, the study lacked a comprehensive explanation of the differences between various materials, leaving room for ambiguity.

(Lorenzetti et al., 2015) focused on the study of nano TiO_2 coatings and their effect on bacterial attachment. By employing a green fluorescent protein-expressing *Escherichia coli* strain, this study demonstrated reduced bacterial adhesion. However, the study did not establish a clear relationship with ζ potential, emphasizing the need for future studies with diverse materials to validate and generalize the findings.

(Liu and Tay, 2002) explored bacterial adhesion on different plastics, utilizing a microscopic cell counting chamber. Their findings indicate the effective role of surface hardness in bacterial adhesion. Nonetheless, the study mentioned that hydrophobicity did not influence bacterial adhesion, as shown by the complexity of the interactions involved.

(Bohinc et al., 2016) investigated the effect of surface roughness on bacterial adhesion using the crystal violet staining method.

This study indicated increased adhesion with increased surface roughness, although the absence of a study on extracellular polymeric substances (EPS) is a crucial factor that has not been explored. (Chik et al., 2018) investigated the factors affecting metallic surface adhesion using laser ablation and polishing. Their study revealed reduced adhesion after the laser treatment of metal surfaces, highlighting a potential method for deterring bacterial attachment.

Table :(1). Related Work

Author	Objective	Method	Outcome	Limitations
Yoda et al. (2014)	Investigate roughness impact on bacterial adhesion	Field emission scanning electron microscope (SEM)	Lower adhesion on hydrophobic surfaces, needs refinement	Lack of a comprehensive explanation of differences among materials.
Lorenzetti et al. (2014)	Study nano TiO ₂ coatings on bacterial attachment	Green fluorescent protein expressing E. coli strain	Reduced bacterial adhesion, no relation with ζ potential	There is a need for diverse materials for validation in future studies.
Liu et al. (2023)	Explore bacterial adhesion on different plastics	Microscopic cell counting chamber	Surface hardness influential on bacterial adhesion	Hydrophobicity not dominant in bacterial adhesion
Bohinc et al. (2016)	Investigate surface roughness on bacterial adhesion	Crystal violet staining method	Increased adhesion with roughness, EPS not studied	Lack of EPS as a key factor for adhesion.
Chik et al. (2018)	Study factors affecting metallic surface adhesion	Laser ablation and polishing	Reduced adhesion after laser treatment on metal surfaces	This study primarily examined initial adhesion rates, which may not consider long-term biofilm development and stability.
Oh et al. (2018)	Analyze hydrophobicity and zeta potential effect	Scanning electron microscope (SEM) micrographs	Greater adhesion on hydrophilic substrates	Adhesion was examined only under controlled laboratory conditions.
Fink et al. (2015)	Examine liquid flow and temperature on detachment	New liquid flow chamber	Turbulent flow and higher temperature increase detachment	Further experiments are required to gain a deeper understanding.
Oder et al. (2015)	Investigate material roughness and temperature	Spectrophotometric method	Positive relation between roughness and adhesion	Ineffectiveness in detecting survivors at specific temperatures.
Zeraik & Nitschke (2012)	Assess culture media and temperature on adhesion	Crystal violet staining technique	Adhesion varied with temperature and media	Hydrophobicity is insufficient to predict bacterial adhesion.

MATERIALS AND METHODS

Bacterial Strains Isolation and identify

The bacterial strains used in this study were isolated from tap water samples collected from a local water-supply system. A mixture of 1 ml of sterile tap water and 1 ml of local tap water was incubated in nutrient broth overnight at 37°C. The following day, one colony from each freshly cultured bacterial strain was inoculated onto artificial media, specifically R2A agar (Reasoner's 2A agar). The mixture was then incubated overnight at 37°C. Finally, the isolated bacterial strains were differentiated. The distinctions among the bacterial strains were based on the shape and size of their colonies, as well as their reaction to Gram staining, categorizing them as either gram-positive or gram-negative using the Gram stain technique (Paray et al., 2023).

Preparation of Pipe Material Samples

Pipe samples were obtained from materials normally used in water distribution systems. Polyvinyl Chloride (PVC) and galvanized iron were cut into 2 × 2 cm coupons. These samples were thoroughly cleaned and sterilized using a combination of 70% ethanol and UV irradiation. For the investigational setup, every pipe coupon was strongly fixed inside a separate 150 ml beaker using metal clips as shown in fig. 1.

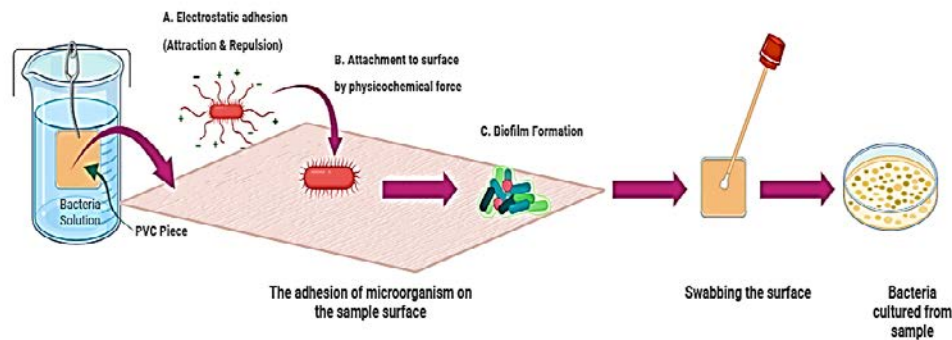


Figure: (1). The steps of experiment.

Preparation of bacterial Samples for Adhesion Assay Protocol

Approximately 10^9 colony-forming units of overnight cultured bacteria were diluted in 9 ml of sterilized tap water. One milliliter of the prepared bacterial water sample was transferred to a beaker containing 150 ml of sterilized water. Fixed polyvinyl chloride (PVC) and galvanized iron coupons were then immersed in bacterial water samples at various temperatures (5, 25, 35, and 45°C), contact times (30 and 60 min), and water flow speeds (2 and 4 rpm). After each experiment, the pipe coupon samples were gently rinsed to remove non-adherent cells.

Detachment and quantification of adhered cells on materials sample Surface

To quantify bacterial adhesion, all the bacteria that adhered to the sample coupon surface were swabbed using a swab stick. A swab stick was then immersed in 10 ml of distilled water and vortexed for 2 min to detach biofilm cells. One milliliter of each suspension was plated on R2A agar and cultured in a bacterial incubator at 37°C. After 24 h, the colony-forming units (CFUs) were counted.

Quantification of the strength of cell adhesion to PVC substrate using a centrifugation assay

The adhesion strength of the bacterial cells to the PVC coupon surfaces was evaluated using centrifugation. First, microbial cells were seeded onto the PVC coupon surfaces. The seeded PVC coupons were then transferred to a centrifuge test tube and covered with 1 ml of distilled water. The test tube was placed in a laboratory centrifuge and the bacteria were harvested by centrifugation for 2 min, applying six different dislodgement forces ranging from 0 to 3500 g. The resulting suspensions were then cultivated on R2A agar for 24 h, after which the colony-forming units (CFUs) were counted. The number of bacteria removed was plotted against the applied forces to create adhesion profile characteristics of the bacterial cells adhering to the surface.

EXPERIMENTAL DESIGN & STATISTICAL ANALYSIS

Data Description

This study applied a comprehensive dataset encapsulating various attributes related to bacterial adhesion and biofilm formation on different pipe materials under varying conditions. The data in Table 2 include categorical variables, such as pipe material, temperature, contact time, water flow speed, and bacterial type, as well as a numerical variable representing bacterial density. Each attribute plays a crucial role in analyzing the impact of environmental and experimental conditions on biofilm development.

Table :(2). Data Description

Attribute ID	Variables	Type	Description
1	Pipe material	Categorical	Two levels: Galvanized Iron and PVC
2	Temperature	Categorical	Five levels: 5°C, 15°C, 25°C, 35°C, and 45°C
3	Contact time	Categorical	Two levels: 30 minutes and 60 minutes.
4	Water flow speed	Categorical	Two levels: 2rpm and 4rpm
5	Species of bacteria	Categorical	Two Species: Gram positive and Gram negative
6	Bacteria density	Numerical	-

Experimental Design

The experimental design incorporates a set of experiments under various conditions to systematically assess the influence of pipe material, bacterial type, contact time, and temperature on bacterial adhesion. Specifically, trials used two types of pipes: galvanized iron and PVC, which represent commonly utilized distribution materials. These were tested across a range of five temperatures from 5°C to 45°C, which reflect the variable conditions in drinking water systems. Additionally, flow speeds of 2 rpm and 4 rpm were used to mimic the common water flow speeds in the pipe distribution system. Contact times of 30 min and 1 h revealed both the initial cell attachment and early biofilm development stages. By measuring adhered cell densities across these combinations of factors, the experiments enabled detailed modeling of how bacterial surface colonization depends on key physical and chemical properties. Statistical tests, such as two-way ANOVA, were performed to discern the significant main effects of each parameter and any interaction effects.

RESULTS

Assessment of bacteria adhesion

The aim of this study was to test the impact of surface type, temperature, and water flow speed on the rate of bacterial adhesion. Our results indicate that the adhesion of Gram positive bacteria onto both galvanized iron and PVC surfaces is influenced by temperature, as shown in Figure 2. We noted that a gradual increase in temperature resulted in a notable increase in bacterial adhesion, and the optimum bacterial growth temperatures were 25°C & 45°C for Gram positive bacteria. Our results are consistent with those of previous studies.

(Rode et al., 2007) have indicated that the rate of adhered *S. aureus* (Gram-positive bacteria) is promoted when bacteria incubate at optimal temperatures. (Da Silva-Meira et al. 2012) reported that the maximum intensity of biofilm formation on stainless steel and polypropylene was observed at 28 °C. (Roy et al., 2021) concluded that the high temperatures (25 to 42°C) lead to increased biofilm formation on food industry surfaces. (Oder et al., 2015; Ana Eliza et al., 2012) pointed out that there is a positive correlation between temperature and the intensity of bacteria adhering to the surface, with the highest adhesion number of bacteria found at a higher temperature of 35 °C and the lowest at 4 °C. In contrast, in our study, no significant effect was observed on the adhesion of the Gram negative bacteria to both galvanized iron and PVC surfaces.

According to (Bohinc et al., 2016) the most hydrophobic bacteria (*S. aureus* ŽMJ 72 and *P. aeruginosa* ŽMJ 87) adhere more to stainless steel surfaces. This effect is in agreement with the outcome of our study, which showed that the Gram positive bacteria have a greater ability to adhere to mutually galvanized iron and PVC surfaces. This can be attributed to the fact that bacteria that are more hydrophobic tend to adhere to more hydrophobic surfaces. These findings are in good agreement with those of (Doyle, 2000 ; Roosjen et al., 2006).

These studies revealed that microorganisms tend to attract more hydrophobic surfaces because of the inhibition of the formation of hydrogen-bonded water molecules. Our findings are also consistent with the common rule of bacterial adhesion that bacteria adhere to more hydrophobic surfaces (Katsikogianni and Missirlis, 2004). Additionally, the present study found that compared to PVC, the galvanized iron substratum was shown to promote the development of high-intensity adhered bacteria, as shown in Figure 2.

This result was consistent with the findings of (Niquette et al., 2000 ; Zhu et al., 2014). This difference in the number of adhered bacteria to the two types of material can be explained by the fact that microorganisms tend to adhere to rougher surface materials, as suggested by (Kerr et al., 1998).

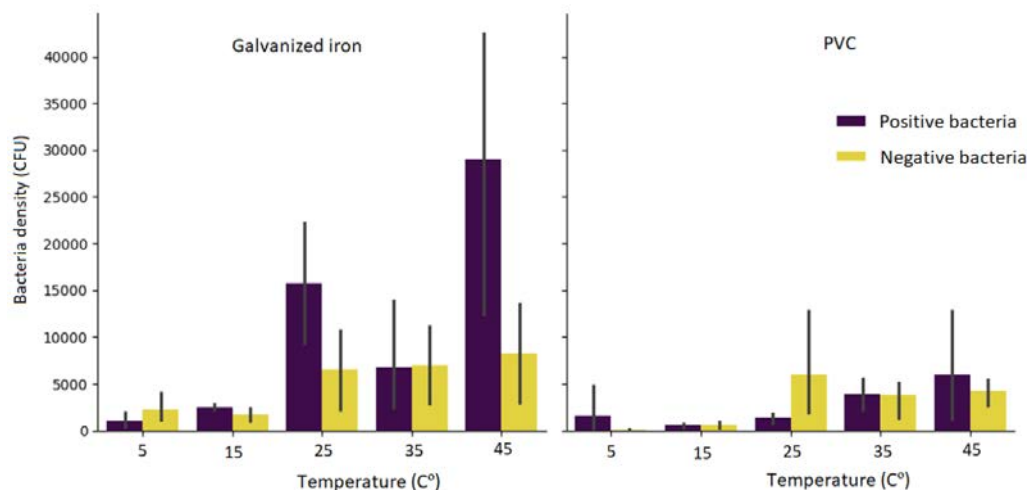


Figure (2). Adhesion assays of bacterial suspensions to galvanized iron coupon surfaces and PVC coupon surfaces at different temperatures

The impact of the water flow speed (at constant temperature and time) on bacterial adhesion to the surfaces is shown in Figure 3. The results revealed an increase in the counts of adhered- Gram positive bacteria on galvanized iron with an increased flow rate. (Liu et al. 2002; Chambless JD & Stewart PS 2007) suggested that increasing the flow of bacteria towards or parallel to the surface results in an increased rate of bacterial adhesion of microorganisms due to higher mass transport. However, the number of adhered Gram negative bacteria to galvanized iron decreased slightly with increasing flow rate because the fluid flow rate exceeded a critical value, which may prevent bacteria from having a suitable contact time to adhere and accumulate on the surface. Meanwhile, the water flow rate had no significant effect on the adhesion of bacteria to both the galvanized iron and PVC surfaces.

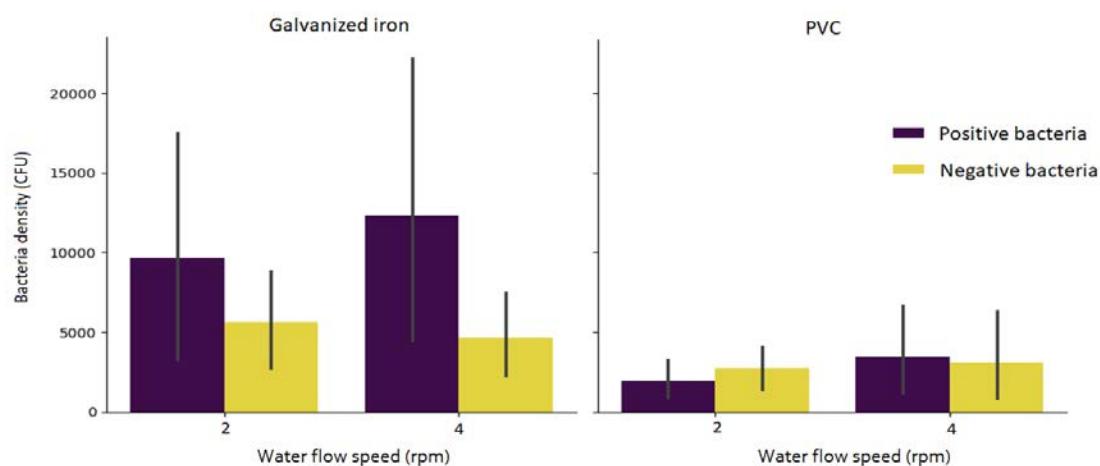


Figure: (3). Density of bacteria on galvanized iron coupon surfaces and PVC coupon surfaces at different rotational speeds (2rpm and 4rpm)

The impact of contact time on the rate of bacterial attachment to both the galvanized iron and PVC surfaces (at a constant temperature and flow rate) is shown in Figure 4. The results indicate that the number of adhered Gram positive bacteria on galvanized iron surfaces increases with increasing contact time; in contrast, there was no clear influence of contact time on the adhesion of both bacterial types to the PVC surface.

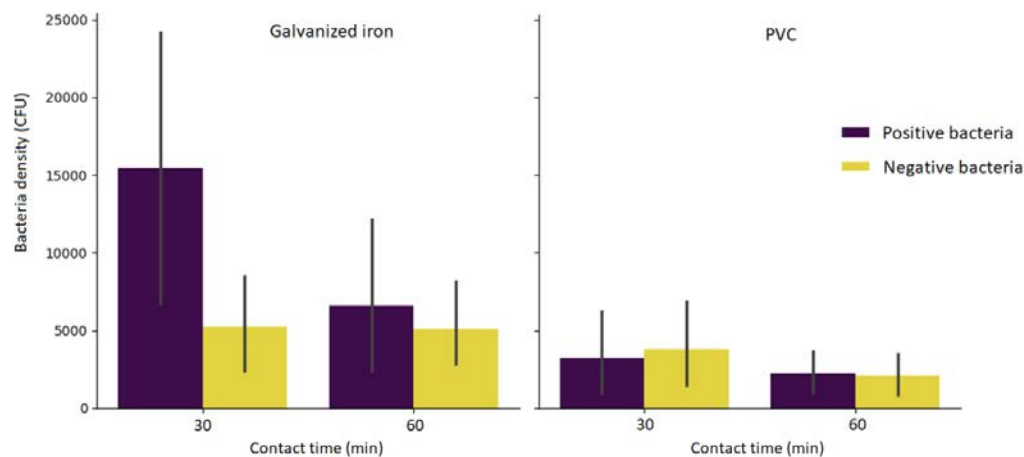


Figure (4). Density of bacteria on galvanized iron coupon surfaces and PVC coupon surfaces at different incubation times (30 min and 60 min).

To obtain further information about the interaction strength between the adhered cells and substrates, the strength of bacterial cell adhesion to the PVC substrate was evaluated using a centrifugation assay. The number of bacterial cells removed after application of the controlled force was counted.

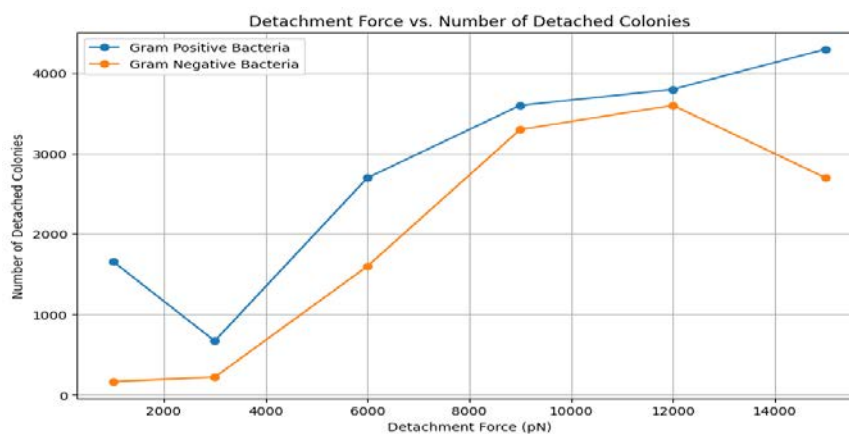


Figure (5). The relation between removal of bacteria cells and applied force.

The plot of the number of detached cells against the applied detachment force shows that the number of cells removed from the PVC surface increased exponentially as the applied force increased, as shown in Figure 5. The leftward shifts in this sigmoid curve with increasing applied force indicate that most of the bacteria were bound to the surface with a high adhesion force. This increase in the applied rupture force provided clear evidence that the bacteria adhered strongly to the surface even after short-term bacterial attachment (30 min and 60 min), which is required for the formation of biofilms on surfaces. The shape of the curve obtained is in agreement with that obtained by Reyes and García, 2003, who showed that increasing the applied force led to an increase in the number of bacteria detached from the surface.

STATISTICAL ANALYSIS

Description of Statics

A mean temperature of 20°C represents a pivotal point for bacterial behavior, as temperature is a key environmental factor influencing microbial growth and survival. A standard deviation of 11.269°C indicates substantial variability in temperature conditions. Quartile analysis showed 50% of observations below 27.5°C, with a significant portion residing in higher ranges that could exacerbate bacterial adherence and biofilm formation.

Table :(3). Description of Static Values

Factors	Mean	Standard Deviation	25%	50%	75%
Temperature	20°C	11.269	12.50	20	27.5
Water flow speed	3rpm	1.008	2	3	4
Contact Time	45min	15.119	30	45	60
Material surface type	0.500	0.504	0	0.5	1
Bacteria Density	3627.34	5302.63	682.5	2840	4350

As shown in Table 3, a mean material value of 0.500 and a standard deviation of 0.504 demonstrated heterogeneity in the pipe properties. With 50% of the observations at or below 0.5, this underscores variations in material composition and subsequent implications for bacterial-surface interactions and water contamination. Understanding these material-specific dynamics is imperative for the development of mitigation strategies. The mean water flow speed of 3 rpm and standard deviation of 1.008 rpm indicated variability in the flow dynamics modulating bacterial transport and adherence. Distinct quartile ranges reveal nuanced spreads linking flow velocity to biofilm development. This multifaceted relationship between the key factors of temperature, pipe material, and flow rate underscores the complex interplay that governs microbial water quality.

Three-way ANOVA

In this study, the relationship between environmental factors and bacterial adherence increase was a subject of great significance. A statistical analysis, ANOVA, was performed to understand the impacts of temperature, bacterial type, and material surface type on bacterial density. The analysis was structured methodically to consider the separate and combined effects of these variables. To perform a multifactorial ANOVA that included more than two factors, we used a model that assessed the main effects of each independent variable (temperature, water flow speed, contact time, material surface type, and bacterial species) and their interactions with the dependent variable (bacterial density). Both two-way interactions (such as Temperature with Speed, Temperature with Material) and higher-order interactions (such as Temperature with Speed with Material) were applied.

Impact of Temperature, Species of Bacteria, and Material on Bacterial Density

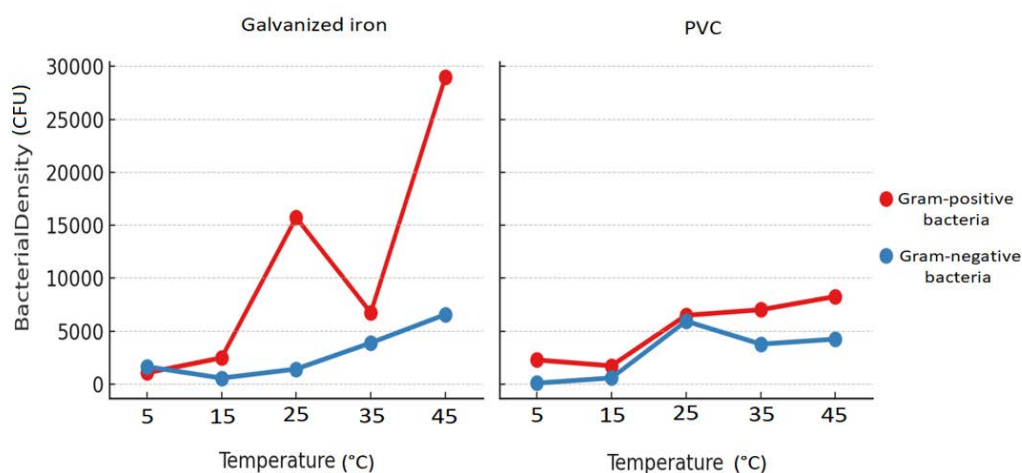
An extensive analysis of variance (ANOVA) was performed to resolve the effects of temperature, bacterial species, and material surface on bacterial density, as shown in Table 4. Follow-up analyses revealed precise differences in bacterial adhesion to galvanized iron at 5°C and 25°C. Regression modeling based on the entire dataset predicted cell adhesion rates for novel combinations beyond those explicitly tested. The ANOVA results are presented in Table 3. The effect of temperature on the bacterial density was significant ($p < 0.000002$), indicating that temperature is a critical factor for bacterial growth in pipes. This discovery is associated with essential knowledge that bacteria are highly sensitive to their thermal environment, with their metabolic processes being regulated by temperature fluctuations.

The species of bacteria was another influential factor ($p < 0.000101$), showing the diversity that exists, including the different bacterial species. This indicates that each bacterial type possesses exclusive attributes that shape its growth patterns, displaying the changes they have undergone to survive and thrive in their respective ecological niches. The role of the material as a growth substrate was also notable ($p = 0.025257$), indicating that the physical and chemical properties of the surface can influence the density of bacterial colonies. This finding has implications in materials science, where the development of antibacterial surfaces or materials designed to promote bacterial growth is an active area of research. An interesting interaction between temperature and bacterial species was observed ($p = 0.013825$), indicating that the effect of temperature on bacterial density varies across different bacterial species. This interaction suggests that certain bacterial species may be more resilient or better adapted to specific temperature ranges than others.

Table :(4). ANOVA Summary for Temperature, Type of Bacteria, and Material

Source	Sum of Squares	df	F Value	p Value
Temperature	1.301e+09	4	10.417	0.000002
Species of Bacteria	5.417e+08	1	17.347	0.000101
Material	1.644e+08	1	5.266	0.025257
Temperature: Species of Bacteria	4.274e+08	4	3.422	0.013825
Temperature: Material	3.897e+08	4	3.120	0.021292
Species of Bacteria: Material	1.770e+08	1	5.667	0.020480
Temperature: Species of Bacteria: Material	3.590e+08	4	2.874	0.030283
Residual	1.874e+09	60	-	-

The interaction between temperature and material also proved to be significant ($p = 0.021292$), suggesting that the influence of the material on bacterial density is temperature dependent, as shown in Figure 6. This indicates that the properties of certain materials may change with temperature, thereby affecting bacterial adhesion and growth.

**Figure (6).** Interaction between temperature and type of bacteria across material levels.

The interplay between bacterial species and material was a significant factor ($p = 0.020480$), suggesting that certain materials may selectively favor the growth of specific bacterial species. This finding has practical implications in various fields such as medical device manufacturing and environmental biotechnology. Finally, a significant three-way interaction among temperature, bacterial species, and material was observed ($p = 0.030283$), highlighting the intricate nature of the microbial ecosystem, where multiple factors converge to determine bacterial density.

In this study, we explored the impact of material type, contact time, bacterial strain, temperature, and speed on bacterial adherence using galvanized iron and PVC. Our results revealed that temperature, material composition, and bacterial strain significantly influenced bacterial adherence, with distinct effects observed at different temperatures and with different materials.

Effect of Speed, Species of Bacteria, and Material on Bacterial Density

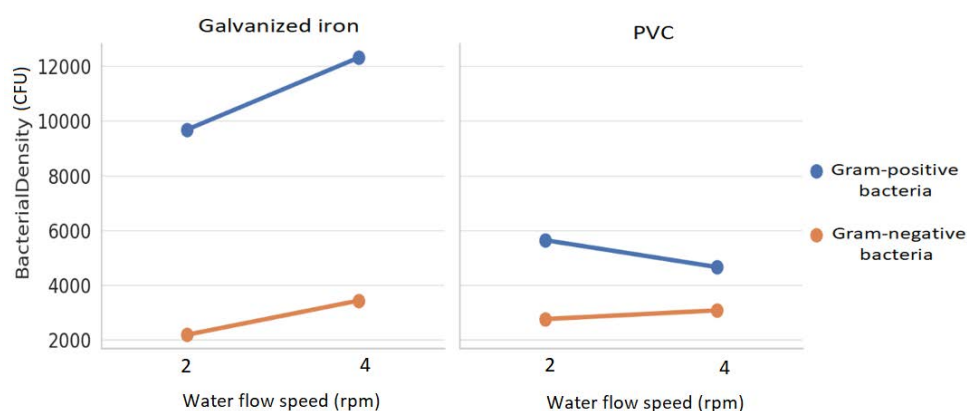
In an exploratory study examining factors influencing bacterial density, ANOVA was conducted to assess the impacts of water flow speed, species of bacteria, and material surface type, as shown in Table 5. This study aimed to elucidate the separated and interactive effects of these variables on bacterial population density.

Table (5). ANOVA Summary for Water flow speed, Species of Bacteria, and Material

Factors	Sum of Squares	df	F Value	p Value
Water flow speed	1.288e+07	1	0.215	0.643908
Species of Bacteria	5.417e+08	1	9.063	0.003595
Material	1.644e+08	1	2.751	0.101518
Water flow speed: Species of Bacteria	8.446e+03	1	0.000141	0.990548
Water flow speed: material surface type	2.569e+07	1	0.430	0.514200
Species of Bacteria: material surface type	1.770e+08	1	2.961	0.089601
Water flow speed: Species of Bacteria: material surface type	8.971e+06	1	0.150	0.699579
Residual	4.303e+09	72	-	-

The main effect of water flow speed on bacterial density was found to be non-significant ($p = 0.644$), suggesting that variations in water flow speed within the tested range did not significantly influence the density of bacteria, as shown in Figure 7. This finding indicates that, at least within the parameters of this experiment, the bacterial density remained unaffected by changes in water flow speed. However, the species of bacteria had a significant effect on the bacterial density ($p = 0.0036$). This result indicates a distinct difference in how two species of bacteria proliferate or sustain their populations, underscoring the biological diversity between bacterial species and their unique responses to environmental conditions. Material surface type as a factor did not demonstrate a significant main effect ($p = 0.102$). However, the relatively low p-value prompts consideration of further inquiry. This indicates the possibility of material composition exerting a subtle influence on bacterial density, a hypothesis that may require additional data or a broader scope of material types for a comprehensive evaluation. The interaction between speed and type of bacteria, when explored, did not yield statistical significance ($p = 0.991$). Similarly, the interaction between the water flow speed and material also failed to show significance ($p = 0.514$).

These outcomes suggest that the interplay between these factors does not meaningfully contribute to variations in bacterial density, at least not in the context provided by the current dataset. An interesting near-significant result emerged from the interaction between the two species of bacteria of bacteria and material type ($p = 0.090$). This near-threshold finding suggests a nuanced relationship in which the material type may influence bacterial density differently depending on the two species of bacteria. This interaction warrants further investigation, as it could reveal material-specific preferences or aversions unique to each two species of bacteria.

**Figure (7).** Interaction between speed of water and type of bacteria across material levels.

Finally, the three-way interaction between speed, species of bacteria, and material was found to be non-significant ($p = 0.700$). This indicates that the combined influences of these factors do not interact in a way that significantly deviates from their individual effects on bacterial density.

DISCUSSION

In the field of microbiology, understanding the factors that influence bacterial adherence is crucial for advancing both theoretical knowledge and practical applications. (Oh et al., 2018) analyzed the effects of hydrophobicity and zeta potential on bacterial adhesion using scanning electron microscope (SEM) micrographs. Their findings indicate greater adhesion on hydrophilic substrates, contributing to the growing understanding of the surface characteristics that influence bacterial attachment. (Fink et al., 2015) examined the influence of liquid flow and temperature on detachment by introducing a new liquid flow chamber. This study observed that turbulent flow and higher temperatures increased the detachment, signifying the need for further experiments to deepen our understanding of these dynamics. (Oder et al., 2015) investigated the relationship between material roughness, temperature, and bacterial adhesion using spectrophotometry.

Their study identified a positive correlation between roughness and adhesion, although it was noted to be ineffective in detecting survivors at specific temperatures, indicating potential limitations. (Zeraik & Nitschke 2012) assessed the impact of culture media and temperature on bacterial adhesion using the crystal violet staining technique. This study revealed that adhesion varies with temperature and media, emphasizing that hydrophobicity alone is insufficient to predict bacterial adhesion. Several limitations and gaps have been identified in studies on bacterial adhesion. First, there is a lack of comprehensive explanations for the differences observed among various materials. For instance, (Yoda et al., 2014) discovered lower adhesion on hydrophobic surfaces but failed to provide a thorough understanding of the variations in bacterial adhesion across different materials. Additionally, there are unexplored mechanisms and variations associated with different metals, as highlighted by (Chik et al., 2018).

While their study found reduced adhesion on metal surfaces after laser treatment, it did not delve into the underlying mechanisms or investigate potential variations with different metals. The incomplete understanding of how zeta potential contributes to bacterial adhesion is another notable gap identified by (Oh et al., 2018). Although they identified higher adhesion on hydrophilic substrates, a comprehensive understanding of the role of zeta potential in bacterial adhesion is lacking. Fink et al., 2014 emphasized the need for further experiments to understand the specific mechanisms under varied flow and temperature conditions. Although their study showed that turbulent flow and higher temperatures increased detachment, it underscored the necessity for additional experiments to gain a deeper understanding of the specific mechanisms involved. The ineffectiveness of detecting survivors at specific temperatures, as established by (Oder et al., 2015), reveals a positive relationship between material roughness and adhesion. However, these techniques are unable to detect survivors at certain temperatures, indicating the need for improved methods to capture the nuances of bacterial adhesion under different temperature conditions (Oder et al., 2015). Finally, the inadequacy of relying solely on hydrophobicity as a predictive factor for bacterial adhesion was highlighted by (Zeraik & Nitschke 2012). Their findings indicated that changes in temperature and media influence bacterial adhesion, emphasizing the limitations of using hydrophobicity alone as a predictive factor. Addressing these limitations and filling these gaps through further research and experimentation are crucial for developing a more comprehensive understanding of bacterial adhesion.

This study seeks to juxtapose the findings of our 2024 study with previous research, shedding light on the methodological innovations introduced, variables considered, and consequential insights derived from our investigation. Previously, research on bacterial adherence has often been segmented, with studies typically isolating single variables, such as temperature or material type, to discern their effects, as shown in Table 6. Such approaches, while foundational, offer a limited view of the multifaceted nature of bacterial behavior. In contrast, our study adopted a holistic methodology that integrates a spectrum of variables, including temperature, material type, contact time, bacterial

strain, and flow rate. This comprehensive approach not only corroborates the findings of previous studies but also unveils the complex interdependencies between these factors, thereby enriching our understanding of bacterial adherence in environments that closely mirror natural conditions.

Table :(6). Compassion for previous studies and our research.

Ref.	Temp	Time	Speed	Bacteria Type	Outcomes
da Silva et al., 2010		✓		✓	Increase in adhered Gram positive and negative bacteria on polyethylene and stainless steel at 20°C compared with lower temperatures.
Meylheuc et al., 2001	✓	✓		✓	The adhesion behavior of the bacterial cells on PTFE and AISI 304 stainless steel increased at 20 °C.
Ana & de, 2012	✓	✓		✓	The adhesion behavior was altered by temperature changes.
Fletcher, 1977	✓	✓		✓	The highest number of bacterial cells adhering to quartz was recorded at sub-optimal temperatures (20°C, 25°C, and 30°C).
Morisaki, 1991	✓			✓	The adhesion behavior of the bacterial cells on polystyrene decreased with decreasing temperature.
Christersson et al., 1988		✓	✓	✓	The number of adhered bacterial cells on the titanium nanostructures decreased with increasing flow rate.
Senevirathne et al., 2022	✓		✓	✓	Sigmoidal increase in cell detachment with increased liquid velocity.
Our Research, 2024	✓	✓	✓	✓	Temperature, type of material, and type of bacteria had a greater effect on bacterial adherence to PVC and galvanized iron.

Prior investigations have consistently underscored the significant role of temperature and material type on bacterial adherence. Many studies have delineated how these factors individually affect bacterial behavior. Our research corroborates these findings but extends the inquiry to examine the synergistic effects of temperature, material type, and bacterial strains. This exploration unearthed nuanced dynamics, revealing that specific combinations of these variables can lead to markedly distinct adherence outcomes, thereby illuminating the intricate mechanisms underlying bacterial adherence. The influence of liquid velocity and flow rate on bacterial detachment and adherence has been highlighted in previous studies (Christersson et al., 1988; Senevirathne et al., 2022). However, these studies often limited their scope to single bacterial strains or a narrow selection of bacterial types. Building on this foundation, our study expands the scope of investigating the impact of varying flow rates across multiple bacterial strains.

This wider investigation revealed that the effect of speed on bacterial adherence is related to the bacterial type, challenging the main assumption of a uniform answer to flow rate changes and suggesting a more nuanced approach to managing bacterial adherence. While previous research has provided valuable insights into the individual factors affecting bacterial adherence, the findings have often been piecemeal. Our 2024 study synthesizes these disparate strands of knowledge and presents a unified framework that captures the complex interplay of multiple factors. The discovery of significant three-way interactions among temperature, material type, and bacterial strain not only advances our theoretical understanding, but also heralds new possibilities for targeted bacterial management strategies. Our study represents a significant leap forward in the field of microbiology by bridging the gaps left by previous research. By adopting a more integrated and comprehensive

approach, we unveiled the complex web of interactions that govern bacterial adherence. These findings not only enrich our theoretical knowledge, but also open new horizons for practical applications, from healthcare to industrial and environmental management, paving the way for innovative strategies to harness or mitigate the effects of bacteria in various contexts.

CONCLUSION

In this study, two species of materials (galvanized iron and PVC) used in the manufacturing of water distribution pipes were subjected to bacterial isolation from drinking water under various conditions of temperature, water flow speed, and contact time. The findings revealed that increasing the temperature, contact time, and flow rate led to a gradual increase in the adhesion rate of Gram positive bacteria to galvanized iron, while there was no clear effect of these factors on the Gram negative bacteria. The significant three-way interaction suggests that the influence of temperature on bacterial density cannot be fully understood without considering both the type of bacteria and material, as these factors jointly affect bacterial growth. The significant main effects and interactions observed in this study provide valuable insights for applications where bacterial density is a critical concern, such as designing antibacterial surfaces, optimizing growth conditions in bioreactors, and understanding natural bacterial colonization patterns in various environments.

ACKNOWLEDGEMENT

We sincerely thank the Chemistry and Zoology Departments, Faculty of Science, Sirte University, Libya for their essential resources and support. We also thank the Department of Medical Microbiology, Faculty of Medicine, Sirte University, Libya for their valuable collaboration and technical assistance.

Duality of interest: The authors declare no duality of interest associated with this manuscript.

Author contributions: All authors contributed to the studies conception and design. Material preparation and data collection were performed by [Anad M. Alshaybani], [Ebtesam Abdulhadi], and [Abdallah I. Abdallah], data analysis was done by [Anad M. Alshaybani] and [Hanin Bzizi], and first draft of the manuscript was written by [Anad M. Alshaybani]. All authors read and approved the final manuscript.

Funding: There are no sources of financial funding and support.

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