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Research Article

Role of the *Enterococcus Faecalis* Growth Phase in the Purification of Water by Non-Activated Charcoal

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Abstract

This study investigates the influence of growth phase on the adhesion of *Enterococcus faecalis* to non-activated charcoal in aquatic environments, a potential low-cost solution for microbial water decontamination. *E. faecalis*, a fecal indicator bacterium, was isolated from polluted water in Cameroon and cultured in peptone water to determine growth phases (exponential phase: 4 to10 hours, stationary phase: 10 to 34 hours). Adhesion experiments were conducted using charcoal fragments from *Cylicodiscus gabunensis* wood, sterilized and immersed in bacterial suspensions under varying pH (4, 7, 10), temperatures (7°C, 23°C, 37°C, 44°C), and organic matter concentrations (0, 500, 1000 mg/L). Results showed that adhesion was significantly higher in the exponential phase (118.12 ± 2.34 to 148.69 ± 2.01×10^5 CFU/cm^2) compared to the stationary phase (5.2 ± 1.65 to 9.55 ± 2.14×10^5 CFU/cm^2), attributed to increased cellular activity and exopolymer synthesis. Maximum adhesion occurred later in the exponential phase (12 to 24 hours) versus early in the stationary phase (3hours), reflecting differences in adhesion mechanisms: reversible electrostatic interactions dominated the stationary phase, while irreversible exopolymer-mediated binding prevailed in the exponential phase. The kruskal wallis « H » test reveals a highly significant difference ($P < 0.01$) in the adsorption of bacterial cells across the various growth phases considered.

The Freundlich adsorption coefficient (Kf) ranged from 3.63 to 50463.22 cells/cm^2, with the highest values observed at pH 4 and 37°C. Adhesion rates reached 20.343 ± 2.15×10^5 CFU/cm^2/h, suggesting a microbial load reduction of 10%, below WHO standards for standalone water treatment. The study highlights non-activated charcoal's potential as a supplementary filtration material in multi-stage systems, though further optimization is needed to meet purification targets.

Keywords: *Enterococcus faecalis*; Bacterial Adhesion; Non-Activated Charcoal; Growth Phase; Water Decontamination

Introduction

A key component of the WHO and UNICEF's preventive strategy to combat diarrheal diseases is household water treatment using low-cost technologies and locally available materials capable of improving the microbiological quality of water [1,2].

Several studies have shown that the adhesion of microorganisms to rock fragments (basalt, granite) or non-activated charcoal immersed in water leads to a more or less significant reduction in the microbial load of the water [3-5]. These findings open a pathway for applying bacterial adhesion to the field of microbial water decontamination. However, harnessing this potential requires a thorough understanding of the mechanisms governing the adhesion process, particularly factors related to the bacteria.

According to [6], bacterial adhesion to solid surfaces occurs in two stages: first, reversible initial adsorption driven by electrostatic or hydrophobic forces; second, irreversible binding mediated by the synthesis of exopolymers that anchor the cells to solid surfaces. The production of these exopolymers is triggered by the initial adsorption and can increase fivefold when the cell is attached to a surface [6].

It has also been demonstrated that bacterial adhesion is a reversible process that evolves over time depending on cellular activity and changes in the properties of the bacterial cell wall [7-9]. The stability of this adhesion depends on the number of sites and functional groups present on the cell wall surface, which may vary with the physicochemical characteristics of the environment [10]. The influence of the growth phases helps us to understand how the physiological state of bacteria and the expression of their genes affect their ability to form biofilms and colonise surfaces.

During the exponential growth phase, bacterial cells grow at their maximum rate and produce numerous surface structures necessary for primary adhesion (initial contact). During the stationary phase, growth ceases due to the depletion of nutrients or the accumulation of waste products. A phenotypic change is observed, along with matrix synthesis and increased resistance of the bacterial cells [15].

Furthermore, the production of cell wall exopolymers, the number of surface functional sites, and bacterial activity can vary depending on the physiological state of the cells [6,9]. Given that physiological changes occur in bacteria during their growth phases, it is plausible that the growth phase influences the bacterial adhesion process. However, this hypothesis has not been sufficiently explored, and data on species such as *Enterococcus faecalis* are currently unavailable.

E. faecalis is a species found in the gastrointestinal tracts of humans and animals, including mammals, birds, insects, and reptiles [11,12]. Its presence in water indicates fecal contamination and renders the water unsuitable for human consumption. *E. faecalis* can cause urinary tract infections, wound infections, and soft tissue infections [11,13]. It is also associated with bacteremia, which can lead to endocarditis in the presence of pre-damaged heart valves [13].

Previous studies have highlighted biological changes in *E. faecalis* that occur depending on the growth stage [14,15]. Specifically, differences have been observed in the morphology of *E. faecalis* cells, the biochemical composition of peptidoglycan, and cell wall polymers between viable but non-culturable cells and cells in the exponential and stationary growth phases [14,15]. Additionally, [16] demonstrated that changes in the physicochemical properties of the cell wall surface can affect the adhesion behavior of *E. faecalis*.

This raises a fundamental question: Does the growth phase influence the adhesion of *E. faecalis* to surfaces? While an answer to this question can be inferred from previous work, no experimental study has yet been conducted to test this hypothesis. The aim of this study is to investigate the influence of growth phases on the adhesion of *E. faecalis* to non-activated charcoal immersed in water containing a controlled microbial load.

Materials and Methods

Isolation and identification of E. faecalis

The bacterial species used, *E. faecalis*, was isolated from a polluted natural aquatic environment (surface water called Olezoa) in Yaoundé, Cameroon (Central Africa). The water sample was collected in a sterile glass bottle and transported to the laboratory in a refrigerated container (5°C), following the recommendations of [17,18]. Subsequently, 100 µL of the sample was spread onto Bile-Esculin-Azide (BEA) agar plates. After incubation (37°C, 24h), presumptive *E. faecalis* colonies (translucent with a black halo) were subcultured onto standard agar and examined fresh and after Gram staining. Biochemical identification tests were then

performed to confirm the species [19]. This work was conducted at the Hydrobiology and Environment Laboratory of the University of Yaoundé 1 (Cameroon, Central Africa).

Experimental determination of E. faecalis growth phase durations

The durations of *E. faecalis* growth phases were determined experimentally in microcosms using strains isolated from the environment.

Three series of 16 test tubes, each containing 10 mL of sterile peptone water, were used. The tubes in each series were labeled t0, t2, t4, t6, t8, t10, t12, t14, t16, t18, t20, t22, t24, t26, t~28~, and t30. A 10 mL suspension of *E. faecalis* with a density equivalent to the 0.08–0.1 McFarland standard (10^8 CFU/mL) was prepared and distributed into each of the 16 tubes (100 µL/tube).

The bacterial suspensions in the three tubes labeled t0 were immediately analyzed, and their cell concentrations were determined by spreading 100 µL of the suspension onto BEA agar. Plating was performed in triplicate, and the plates were incubated at 37°C for 24h. The suspensions in the tubes labeled t2, t4, t6, ..., t30 were first incubated at 37°C for 2, 4, 6, ..., and 30 hours, respectively. Their cell concentrations were then determined by spreading 100 µL onto BEA agar. The plates were incubated at 37°C for 24h. Plating was performed in triplicate, and the average colony-forming units (CFU) were calculated after each incubation period. The durations of the cell growth phases (lag, exponential, stationary, and decline) were determined as 0–4h, 4–10h, 10–34h, and 34–48h, respectively. For purposes of this study, we will be working with cells arrested in the exponential phase and those arrested in the stationary phase, as these are the phases in which cellular activity is most pronounced.

Preparation of non-activated charcoal fragments

For this study, non-activated charcoal was chosen as the adhesion substrate due to its economic and technical accessibility. Activated charcoal is expensive and requires complex carbonization and activation techniques (physical or chemical), which vary depending on the raw material used (coconut shells, bituminous coal, peat, etc.) [20]. The non-activated charcoal used in this study was derived from the wood of *Cylicodiscus gabunensis* (commonly known as "Okan" or "Adoum"), a heavy wood from the *Fabaceae* family widely used in construction. This wood is also known for its antimicrobial properties, making it effective against bacterial, fungal, or parasitic attacks. It is also used therapeutically for digestive benefits, such as facilitating intestinal transit and acting as an effective antispasmodic [21].

The choice of this wood was based on its widespread use for cooking charcoal, making it accessible to all social strata.

The charcoal fragments were cut into rectangular parallelepipeds with a surface area of 22 cm^2 using a metal saw. The substrates were sterilized in an autoclave (121°C for 15 minutes) before adhesion testing. The total surface area (A) of each fragment was calculated as A = 2(S1 + S2 + S3), where S1, S2, and S3 are the areas of the different faces of the fragment (Figure 1).

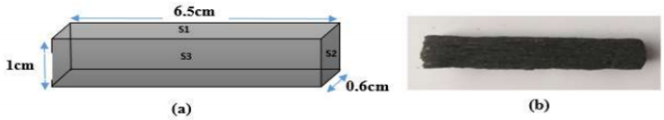


Figure 1: (a) – Representation of the different areas of a charcoal fragment; (b) – Photograph of a fragment of non-activated charcoal (Okan).

To verify the sterility of the charcoal fragments, they were placed on the surface of standard agar in 15 Petri dishes (90 mm diameter). The dishes were divided into three groups of five plates each. The first group was incubated at 42°C for 2 days, the second at 37°C for 3 days, and the third at room temperature (23 ± 2°C) for 5 days. The absence of colony-forming units after incubation confirmed the sterility of the charcoal fragments.

Evaluation of E. faecalis cell adhesion to charcoal fragments

Preparation of bacterial suspensions at desired growth phases

The protocol for determining *E. faecalis* growth phase durations allowed us to deduce the incubation times required to maintain the cells in non-renewed peptone water at the desired growth phases. In this study, adhesion tests were performed with cells blocked in the exponential and stationary growth phases. The time intervals were 4–10h for the exponential phase and 10–34h for the stationary phase. These two growth phases are the most significant, as major physiological changes in the cells occur during these periods.

Two bacterial suspensions were prepared as described above, one blocked in the exponential phase and the other in the stationary phase. Their cell densities were adjusted to the 0.08–0.1 McFarland standard (10^8 CFU/mL).

To simulate certain environmental conditions, each suspension was prepared at different pH levels (4, 7, and 10), temperatures (4°C, 23°C, 37°C, and 44°C), and organic matter concentrations (0 mg/L, 500 mg/L, and 1000 mg/L).

Immersion of charcoal fragments in bacterial suspensions

A 1 mL volume of each bacterial suspension (exponential or stationary phase) was pipetted into sterile glass flasks containing 100 mL of sterile physiological saline (0.85% NaCl). The flasks were gently agitated to homogenize the suspension. The pre-sterilized charcoal fragments were then introduced horizontally into the flasks. The immersion times were 3h, 6h, 9h, 12h, and 24h, which were the intervals required to monitor adhesion.

Removal of charcoal substrates and detachment of adhered bacterial cells

After each immersion period, the charcoal fragments were removed from the flasks using sterile forceps and placed in test tubes containing 10 mL of sterile physiological saline.

The detachment of adhered bacterial cells was achieved by agitating the fragments in 10 mL of sterile physiological saline (0.85% NaCl) using a Bunsen AGT-9 tube agitator at increasing speeds (30–50 rpm) for approximately 20 seconds. The agitation was repeated six times for each substrate, with each agitation performed in a new tube containing 10 mL of physiological saline. The final volume containing the detached cells from each fragment was 60 mL, which was used to determine the number of cells adhered to the substrate.

The detached (adhered) cells were enumerated by spreading onto BEA agar [19]. The Petri dishes were incubated at 37°C for 24h. Results were expressed as CFU per unit surface area of the immersed charcoal fragment (CFU/cm^2).

The planktonic cells (those not initially adhered to the substrate) were enumerated in the remaining suspension in the 100 mL flask after the charcoal fragment was removed.

Data analysis

Variations in adhered cell abundances under each experimental condition were illustrated using histograms.

Determination of cell adhesion rates

The adhesion rates of microorganisms to the charcoal fragments were evaluated using Excel by calculating the linear regression equations of adhered cells at each 3-hour incubation interval ($y = ax + b$). In this equation, "y" represents the number of adhered cells (CFU) per incubation period, and "x" represents the incubation period in hours (3h). The slope "a" of the equation corresponds to the cell attachment rate when positive and the detachment rate when negative. Results were expressed as the number of adhered or detached cells/cm^2/hour.

Modeling of E. faecalis adhesion

The adhesion experiment data were analyzed using the Freundlich model. This isotherm was chosen due to the number and relevance of the insights it provides into actual adsorption mechanisms and its remarkable ability to adapt adsorption doses. The Freundlich isotherm is described by the following equation:

$$Cs = K_f \cdot C^{1/n}$$

Where:

- Cs: Quantity of bacteria adsorbed per adsorbent unit.

- C: Concentration of planktonic cells at equilibrium.

- K_f: Freundlich adsorption coefficient.

- 1/n: Linearity coefficient; "n" represents adsorption intensity.

Relations between the analysed variables

The degree of correlation between the variables used, were assessed using Spearman's « r » correlation test under each experimental condition. Comparisons of means were carried out using the Kruskal-Wallis « H » test. All these analyses were perfomed using SPSS version 16.0.

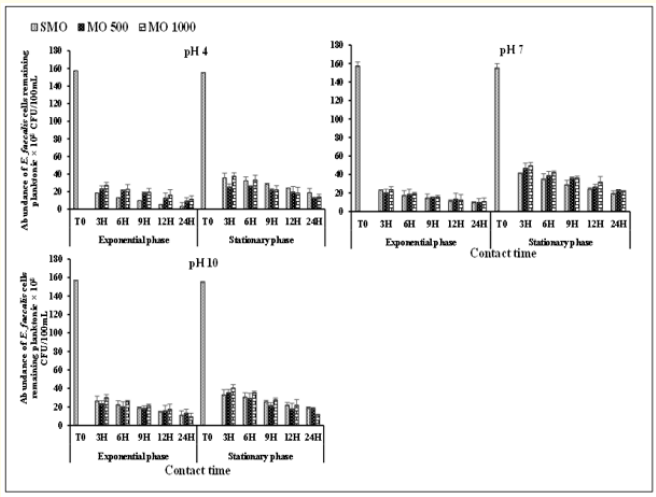
Results

Abundances of planktonic *E. faecalis* cells

The abundances of planktonic cells were evaluated after 3h, 6h, 9h, 12h, and 24h of incubation. The initial cell densities at t0 were adjusted to 10^8 CFU/mL.

Planktonic cell abundances at 7°C for each growth phase, pH, and organic matter concentration

Figure 2 shows the concentrations of planktonic *E. faecalis* cells at 7°C for each growth phase and pH condition. The concentration varied between 3.26x10^8 CFU/100 mL and 49.58x10^8 CFU/100 mL.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 2: Planktonic *E. faecalis* cell abundances at 7°C for each growth phase, pH, and organic matter concentration.

At pH 4, planktonic *E. faecalis* cell densities in the exponential phase ranged from 3.26×10^8 CFU/100 mL (after 24h immersion without organic matter) to 27.22 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 13.52×10^8 CFU/100 mL (after 24h immersion at 500 mg/L organic matter) to 37.58×10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

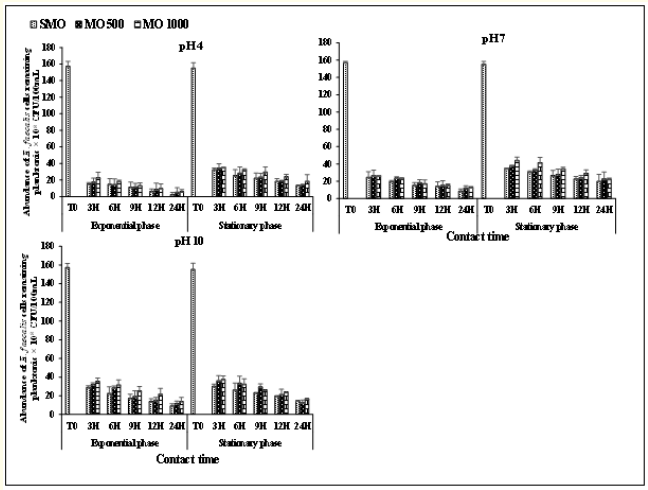
At pH 7, planktonic cell densities in the exponential phase ranged from 9.87×10^8 CFU/100 mL (after 24h immersion without organic matter) to 23.65×10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 19.36× 10^8 CFU/100 mL (after 24h immersion without organic matter) to 49.58 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

At pH 10, planktonic cell densities in the exponential phase ranged from 9.36×10^8 CFU/100 mL (after 24h immersion at 1000 mg/L organic matter) to 30.12×10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 11.58×10^8 CFU/100 mL (after 24h immersion at 1000 mg/L organic matter) to 40.52×10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

The minimum abundance of planktonic *E. faecalis* cells (3.26×10^8 CFU/100 mL) was observed in the exponential phase at pH 4 after 24h immersion without organic matter. The maximum abundance (49.58×10^8 CFU/100 mL) was observed in the stationary phase at pH 7 after 3h immersion at 1000 mg/L organic matter.

Planktonic cell abundances at 23°C for each growth phase, pH, and organic matter concentration

Figure 3 shows the concentrations of planktonic *E. faecalis* cells at 23°C for each growth phase and pH condition. The concentration varied between 2.56 x 10^8 CFU/100 mL and 44.12 x 10^8CFU/100 mL.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 3: Planktonic E. faecalis cell abundances at 23°C for each growth phase, pH, and organic matter concentration.

At pH 4, planktonic *E. faecalis* cell densities in the exponential phase ranged from 2.56 x 10^8 CFU/100 mL (after 24h immersion without organic matter) to 22.85x10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 13.51 × 10^8 CFU/100 mL (after 24h immersion at 500 mg/L organic matter) to 35.12 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

At pH 7, planktonic cell densities in the exponential phase ranged from 8.59 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 23.29 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 19.94 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 44.12 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

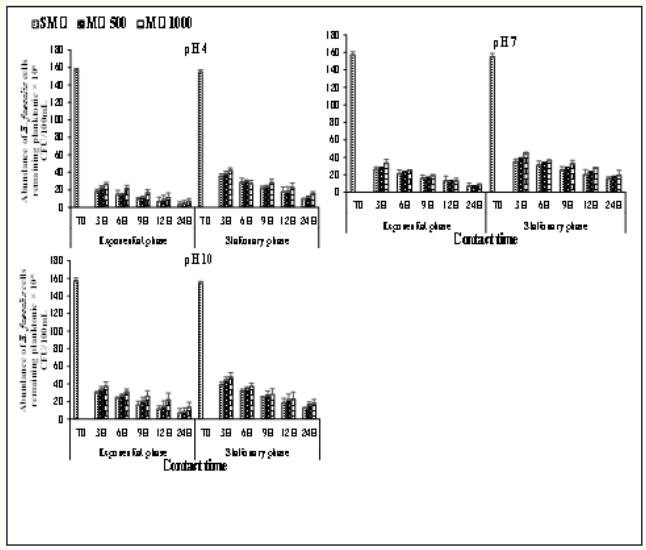
At pH 10, planktonic cell densities in the exponential phase ranged from 9.11 × 10^8 CFU/100 mL (after 24h immersion at 1000 mg/L organic matter) to 30.56 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 12.68 × 10^8 CFU/100 mL (after 24h immersion at 500 mg/L organic matter) to 37.45 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

The minimum abundance of planktonic *E. faecalis* cells (2.56 × 10^8 CFU/100 mL) was observed in the exponential phase at pH 4 after 24h immersion without organic matter. The maximum abundance (44.12 × 10^8 CFU/100 mL) was observed in the stationary phase at pH 7 after 3h immersion at 1000 mg/L organic matter.

Planktonic cell abundances at 37°C for each growth phase, pH, and organic matter concentration

Figure 4 shows the concentrations of planktonic *E. faecalis* cells at 37°C for each growth phase and pH condition. The concentration varied between 3.24 x 10^8 CFU/100 mL and 47.86 x 10^8 CFU/100 mL.

At pH 4, planktonic *E. faecalis* cell densities in the exponential phase ranged from 3.24 x 10^8 CFU/100 mL (after 24h immersion without organic matter) to 26.81x10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 9.96 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 42.23 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 4: Planktonic *E. faecalis* cell abundances at 37°C for each growth phase, pH, and organic matter concentration.

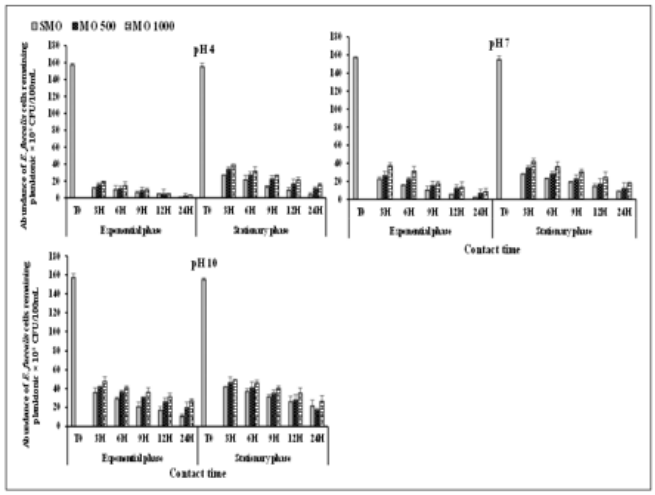
At pH 7, planktonic cell densities in the exponential phase ranged from 7.45 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 33.69 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 15.84 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 44.76 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

At pH 10, planktonic cell densities in the exponential phase ranged from 7.22 × 10^8 CFU/100 mL (after 24h immersion at 1000 mg/L organic matter) to 37.45 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 13.26 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 47.86 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

The minimum abundance of planktonic *E. faecalis* cells (3.24 × 10^8 CFU/100 mL) was observed in the exponential phase at pH 4 after 24h immersion without organic matter. The maximum abundance (47.86 × 10^8 CFU/100 mL) was observed in the stationary phase at pH 7 after 3h immersion at 1000 mg/L organic matter.

Planktonic cell abundances at 44°C for each growth phase, pH, and organic matter concentration

Figure 5 shows the concentrations of planktonic *E. faecalis* cells at 37°C for each growth phase and pH condition. The concentration varied between 1.22 x 10^8 CFU/100 mL and 49.66 x 10^8 CFU/100 mL.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 5: Planktonic E. faecalis cell abundances at 44°C for each growth phase, pH, and organic matter concentration.

At pH 4, planktonic *E. faecalis* cell densities in the exponential phase ranged from 1.22 x 10^8 CFU/100 mL (after 24h immersion without organic matter) to 19.63 x 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 4.27 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 39.66 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

At pH 7, planktonic cell densities in the exponential phase ranged from 2.58 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 37.52 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 9.42 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 42.15 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

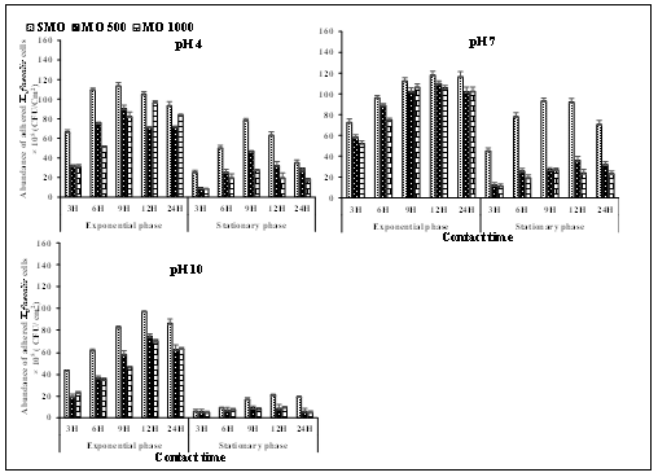
At pH 10, planktonic cell densities in the exponential phase ranged from 11.01 × 10^8 CFU/100 mL (after 24h immersion without organic matter) to 47.91 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter). In the stationary phase, they varied from 17.71 × 10^8 CFU/100 mL (after 24h immersion at 500 mg/l organic matter) to 49.66 × 10^8 CFU/100 mL (after 3h immersion at 1000 mg/L organic matter).

The minimum abundance of planktonic *E. faecalis* cells (1.22 × 10^8 CFU/100 mL) was observed in the exponential phase at pH 4 after 24h immersion without organic matter. The maximum abundance (49.66 × 10^8 CFU/100 mL) was observed in the stationary phase at pH 10 after 3h immersion at 1000 mg/L organic matter.

Abundances of *E. faecalis* cells adhered to charcoal substrates

Adhered cell abundances at 7°C for each growth phase, pH, and organic matter concentration

Figure 6 shows the concentrations of adhered *E. faecalis* cells at 7°C for each growth phase and pH condition. The concentration varied between 5.12 x 10^5 CFU/100 mL and 118.12 x 10^5 CFU/100 mL.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 6: Adhered *E. faecalis* cell abundances at 7°C for each growth phase, pH, and organic matter concentration.

At pH 4, adhered *E. faecalis* cell densities in the exponential phase ranged from 31.56 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 113.66 × 10^5 CFU/cm^2 (after 9h immersion without organic matter). In the stationary phase, they varied from 8.75 × 10^5 CFU/cm^2 to 78.36 × 10^5 CFU/cm^2 under the same conditions.

At pH 7, adhered cell densities in the exponential phase ranged from 52.36 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 118.12 × 10^5 CFU/cm^2 (after 12h immersion without organic matter). In the stationary phase, they varied from 11.42 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 93.26 × 10^5 CFU/cm^2 (after 9h immersion without organic matter).

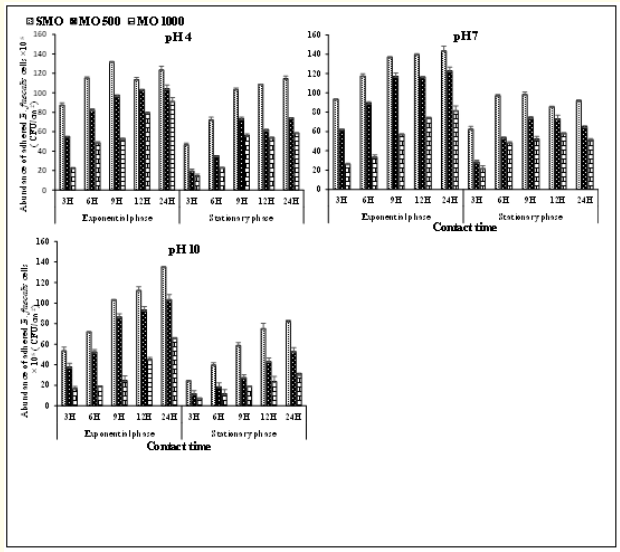
At pH 10, adhered cell densities in the exponential phase ranged from 19.65 × 10^5 CFU/cm^2 (after 6h immersion at 500 mg/L organic matter) to 97.45 × 10^5 CFU/cm^2 (after 12h immersion without organic matter). In the stationary phase, they varied from 5.12 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 19.85 × 10^5 CFU/cm^2 (after 9h immersion without organic matter).

The minimum adhered cell abundance (5.12 × 10^5 CFU/cm^2) was observed in the stationary phase at pH 10 after 3h immersion at 1000 mg/L organic matter. The maximum adhered cell abundance (118.12 × 10^5 CFU/cm^2) was observed in the exponential phase at pH 7 after 12h immersion without organic matter.

Adhered cell abundances at 23°C for each growth phase, pH, and organic matter concentration

Figure 7 shows the concentrations of adhered *E. faecalis* cells at 23°C for each growth phase and pH condition. The concentration varied between 7.52 x 10^5 CFU/100 mL and 143.41 x 10^5 CFU/100 mL.

At pH 4, adhered *E. faecalis* cell densities in the exponential phase ranged from 22.85 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 123.52 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 14.74 × 10^5 CFU/cm^2 to 114.58 × 10^5 CFU/cm^2 under the same conditions.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 7: Adhered *E. faecalis* cell abundances at 23°C for each growth phase, pH, and organic matter concentration.

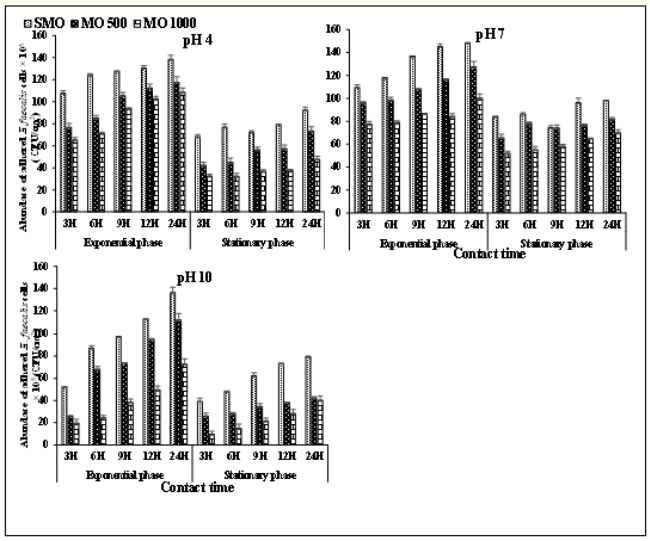
At pH 7, adhered cell densities in the exponential phase ranged from 26.29 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 143.41 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 21.76 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 98.32 × 10^5 CFU/cm^2 (after 9h immersion without organic matter).

At pH 10, adhered cell densities in the exponential phase ranged from 16.89 × 10^5 CFU/cm^2 (after 3h immersion at 500 mg/L organic matter) to 135.32 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 7.52 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 82.41 × 10^5 CFU/cm^2 (after 24h immersion without organic matter).

The minimum adhered cell abundance (7.52 × 10^5 CFU/cm^2) was observed in the stationary phase at pH 10 after 3h immersion at 1000 mg/L organic matter. The maximum adhered cell abundance (143.41 × 10^5 CFU/cm^2) was observed in the exponential phase at pH 7 after 24h immersion without organic matter.

Adhered cell abundances at 37°C for each growth phase, pH, and organic matter concentration

Figure 8 shows the concentrations of adhered *E. faecalis* cells at 37°C for each growth phase and pH condition. The concentration varied between 9.55 x 10^5 CFU/100 mL and 148.69 x 10^5 CFU/100 mL.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 8: Adhered *E. faecalis* cell abundances at 37°C for each growth phase, pH, and organic matter concentration.

At pH 4, adhered *E. faecalis* cell densities in the exponential phase ranged from 65.22 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 138.45 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 32.44 × 10^5 CFU/cm^2 (after 6h immersion at 1000 mg/L organic matter) to 92.48 × 10^5 CFU/cm^2 (after 24h immersion without organic matter).

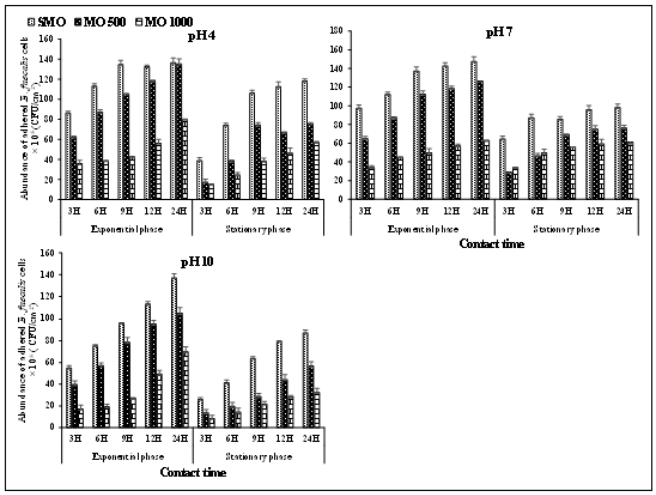
At pH 7, adhered cell densities in the exponential phase ranged from 77.42 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 148.69 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 52.11 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 98.45 × 10^5 CFU/cm^2 (after 24h immersion without organic matter).

At pH 10, adhered cell densities in the exponential phase ranged from 19.65 × 10^5 CFU/cm^2 (after 3h immersion at 500 mg/L organic matter) to 136.35 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 9.55 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 79.52 × 10^5 CFU/cm^2 (after 24h immersion without organic matter).

The minimum adhered cell abundance (9.55 × 10^5 CFU/cm^2) was observed in the stationary phase at pH 10 after 3h immersion at 1000 mg/L organic matter. The maximum adhered cell abundance (148.69 × 10^5 CFU/cm^2) was observed in the exponential phase at pH 7 after 24h immersion without organic matter (figure 8).

Adhered cell abundances at 44°C for each growth phase, pH, and organic matter concentration

Figure 9 shows the concentrations of adhered *E. faecalis* cells at 44°C for each growth phase and pH condition. The concentration varied between 8.36 x 10^5 CFU/100 mL and 147.36 x 10^5 CFU/100 mL.



SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Figure 9: Adhered *E. faecalis* cell abundances at 44°C for each growth phase, pH, and organic matter concentration.

At pH 4, adhered *E. faecalis* cell densities in the exponential phase ranged from 35.66 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 136.26 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 15.47 × 10^5 CFU/cm^2 to 118.25 × 10^5 CFU/cm^2 under the same conditions.

At pH 7, adhered cell densities in the exponential phase ranged from 34.23 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 147.36 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 34.11 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 98.22 × 10^5 CFU/cm^2 (after 24h immersion without organic matter).

At pH 10, adhered cell densities in the exponential phase ranged from 17.24 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 136.98 × 10^5 CFU/cm^2 (after 24h immersion without organic matter). In the stationary phase, they varied from 8.36 × 10^5 CFU/cm^2 (after 3h immersion at 1000 mg/L organic matter) to 87.32 × 10^5 CFU/cm^2 (after 24h immersion without organic matter).

The minimum adhered cell abundance (8.36 × 10^5 CFU/cm^2) was observed in the stationary phase at pH 10 after 3h immersion at 1000 mg/L organic matter. The maximum adhered cell abundance (147.36 × 10^5 CFU/cm^2) was observed in the exponential phase at pH 7 after 24h immersion without organic matter (figure 9).

Apparent adhesion rates of *E. faecalis* to charcoal fragments

The apparent adhesion rates of *E. faecalis* to charcoal substrates varied depending on the growth phase (Table 1).

		Adhesion speed "a" (x10^5) and coefficient of determination (R^2)											
		7°C			**23°C**			**37°C**			**44°C**		
		SMO	**MO 500**	**MO 1000**	**SMO**	**MO 500**	**MO 1000**	**SMO**	**MO 500**	**MO 1000**	**SMO**	**MO 500**	**MO 1000**
pH 4	Exponential phase	4.92	7.50	14.97	7.05	11.90	16.81	6.77	10.88	11.82	11.98	17.70	10.60
		(0.17)	(0.30)	(0.78)	(0.44)	(0.83)	(0.97)	(0.89)	(0.95)	(0.95)	(0.79)	(0.99)	(0.86)
	Stationary phase	3.28	4.67	1.97	17.28	13.67	11.89	5.06	7.48	3.64	19.73	14.51	10.50
		(0.06)	(0.30)	(0.21)	(0.89)	(0.78)	(0.82)	(0.76)	(0.92)	(0.84)	(0.88)	(0.80)	(0.99)
pH 7	Exponential phase	10.98	10.91	13.09	12,26	14.82	15.13	10.51	7.97	5.05	13.03	15,40	6,93
		(0.82)	(0.70)	(0.74)	(0.86)	(0.84)	(0.97)	(0.95)	(0.97)	(0.79)	(0.91)	(0.92)	(0.98)
	Stationary phase	6.49	4.96	2.81	4.75	9.24	6.94	3.84	3.14	4.56	7.59	12.26	6.38
		(0.27)	(0.77)	(0.55)	(0.26)	(0.60)	(0.60)	(0.39)	(0.65)	(0.98)	(0.81)	(0.88)	(0.85)
pH 10	Exponential phase	11.92	12.42	11.57	20.34	17.24	12.43	19.41	20.07	13.14	20.2	16.80	13.53
		(0.79)	(0.80)	(0.89)	(0.98)	(0.94)	(0.88)	(0.97)	(0.94)	(0.95)	(0.99)	(0.98)	(0.90)
	Stationary phase	3.93	0.20	0.23	15.16	10.63	6.00	10.61	4.29	7.47	16.11	11.14	6.23
		(0.86)	(0.04)	(0.03)	(0.98)	(0.98)	(0.99)	(0.98)	(0.99)	(0.98)	(0.99)	(0.97)	(0.99)

Table 1: Adhesion rates of *E. faecalis* cells to charcoal.

SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

In the exponential phase, the minimum adhesion rate was 4.9 × 10^5 CFU/cm^2/h (at 7°C, pH 4, without organic matter), and the maximum was 20.34 × 10^5 CFU/cm^2/h (at 37°C, pH 10, without organic matter). In the stationary phase, the minimum adhesion rate was 0.19 × 10^5 CFU/cm^2/h (at 7°C, pH 10, 500 mg/L organic matter), and the maximum was 19.73 × 10^5 CFU/cm^2/h (at 37°C, pH 4, without organic matter).

Freundlich adsorption isotherms of *E. faecalis*

The Freundlich adsorption coefficient (Kf) ranged from 3.63 to 50463.22 adhered cells/cm^2. The lowest value (3.63 adhered cells/cm^2) was recorded in the stationary phase at pH 10, 7°C, and 500 mg/L organic matter, while the highest (50463.22 adhered cells/cm^2) was recorded in the exponential phase at pH 4, 37°C, without organic matter (Table 2).

The linearity coefficient (1/n) ranged from -0.006 to 2.59. The lowest value (-0.006) was recorded in the stationary phase at pH 4, 44°C, and 1000 mg/L organic matter, while the highest (2.59) was recorded in the exponential phase at pH 4, 37°C, without organic matter (Table 2).

			7°C			23°C			37°C			44°C		
			SMO	MO 500	MO 1000	SMO	MO 500	MO 1000	SMO	MO 500	MO 1000	SMO	MO 500	MO 1000
pH 4	Exponential phase	Kf	11.88	5.31	6.39	49.54	14.97	6.36	50463.22	147.47	49.89	253.89	46.53	16.84
		1/n	0.82	0.399	0.006	1.42	0.93	0.32	2.59	1.74	1.42	1.88	1.47	1.29
	Stationary phase	Kf	4.55	4.03	4.49	9.59	7.78	5.12	472.39	25.12	16.65	12.98	5.74	5.99
		1/n	-1.83	1.51	-0.49	0.35	-0.26	-0.78	2.03	1.22	1.13	0.81	-0.17	-0.01
pH 7	Exponential phase	Kf	26.53	8.49	9.98	56.92	12.33	5.86	613.39	2013.64	1098.6	1706.5	30.22	28.19
		1/n	1.11	0.38	0.52	1.35	0.59	-0.04	1.94	2.19	2.16	2.17	1.17	1.37
	Stationary phase	Kf	7.96	5.85	5.69	9.17	6.53	6.29	42.74	111.49	60.41	47.85	8.47	11.68
		1/n	-0.01	-0.31	-0.31	0.18	-0.27	0.44	1.27	1.67	1.51	1.38	0.37	0.62
pH 10	Exponential phase	Kf	10.42	4.58	6.92	14.79	9.89	5.65	21.96	7.68	6.48	18.97	9.33	5.87
		1/n	0.47	-0.63	0.27	0.78	0.49	0.099	1.02	0.24	0.079	0.88	0.21	-0.61
	Stationary phase	Kf	4.25	3.63	4.21	5.84	5.41	4.61	15.78	13.49	5.39	6.93	6.14	5.44
		1/n	-0.92	-0.46	-1.62	-0.23	-0.17	-0.56	0.89	1.02	-0.13	-0.202	-0.15	-0.76

Table 2: Freundlich adsorption coefficient (Kf) and linearity coefficient (*1/n*) of *E. faecalis.*

SMO: without organic matter, MO 500: organic matter concentration 500 mg/L, MO 1000: organic matter concentration 1000 mg/L.

Correlations between the variables under consideration

Correlation between the densities of adherent bacterial cells and organic matter concentrations

According to the table below, there is a highly significant negative correlation (P<0,01) between adherent cells and organic matter concentrations.

	E. faecalis
Growth phase	-0,494**

Table 3: Spearman's corrélation coefficient « r » between bacterial densities and organic matter concentrations.

** : P < 0,01; dof = 359.

Correlation between the densities of adherent bacterial cells and pH

The table below shows that there is a highly significant negative correlation (P<0,01) between the number of adherent cells and the various pH values used.

	E. faecalis
Growth phase	-0,206**

Table 4: Spearman's corrélation coefficient « r » between bacterial densities and pH levels.

** : P<0,01; dof = 359.

Correlation between the densities of adherent bacterial cells and incubation times

According to the table below, there is a highly significant correlation (P<0,01) between the number of adherent cells and incubation times.

	E. faecalis
Growth phase	0.375**

Table 5: Spearman's corrélation coefficient « r » between bacterial densities and Incubation times.

** : P<0,01; dof = 359.

Correlation between the densities of adherent bacterial cells and incubation temperatures

A highly significant correlation (P<0,01) was observed between adherent cells and incubation temperatures.

	E. faecalis
Growth phase	0.202**

Table 6: Spearman's corrélation coefficient « r » between bacterial densities and incubation temperatures.

** : P<0,01; dof = 359

Comparison of average densities of adherent bacterial cells at differnt growth phases

A comparison of average abundances of adherent bacterial cells at different growth phases was carried out using the Kruskal Wallis « H » test, and the results are presented in the table below.

This table shows that the mean abundances of *E.faecalis* cells adhering to okan during the exponential phase differ significantly from those adhering to the same species during the stationary phase.

	E. faecalis
Growth phase	P = 0.0001

Table 7: Comparison of the mean abundances of adherent bacteria from different growth phases.

Significant Difference at p< 0,05 dof = 1

Discussion

This study is one of the first to explore the adhesion of *Enterococcus faecalis* to non-activated charcoal, a locally accessible material with potential for microbial water decontamination. The results demonstrated that non-activated charcoal can serve as a substrate for *E. faecalis* adhesion, with adhered cell densities ranging from 5.2×10^5 CFU/cm^2 to 148.69×10^5 CFU/cm^2. Adhesion mechanism of *E. faecalis* to non-activated charcoal has not yet been described. However, it is established that the cell wall of *E. faecalis* is negatively charged due to the presence of molecules such as teichoic acids and lipoteichoic acids [14]. The initial approach of cells to the substrate is likely controlled by electrostatic interactions. When the cell and the support are close, forces such as van der Waals interactions or hydrophobic interactions may contribute to the adsorption process of *E. faecalis* to non-activated charcoal [6]. These forces initiate a reversible adhesion phase due to their ease of disruption. Subsequently, *E. faecalis* cells synthesize exopolymers or surface proteins such as gelatinase, which firmly anchor the bacterial wall to the substrate [22, 23]. This second phase of adhesion is irreversible.

This study highlighted the influence of growth phase on *E. faecalis* adhesion. The cell densities adhered during exponential phase ranged from 118.12×10^5 CFU/cm^2 to 148.69×10^5 CFU/cm^2, whereas during stationary phase, adhered cell densities fluctuated between 5.2×10^5 CFU/cm^2 and 9.55×10^5 CFU/cm^2. Bacterial adhesion was more significant in the exponential growth phase. This result can be explained by increased cellular activity and the synthesis of adhesion molecules. [14 15] reported biological and biochemical modifications in *E. faecalis* cell wall of when cells are in different growth phases. The morphology of the cells, as well as the biochemical composition of the peptidoglycan and the exopolymers of the wall, undergo remodeling. This could account for the observed differences in adhesion between the stationary and exponential phases. The influence of the growth phase on adhesion is evident not only with the adhered cell densities but also with the adhesion rate. The maximum cell densitie adhered during the stationary phase was achieved 3 hours after immersing the charcoal fragments in the microbial suspension, whereas during the exponential phase, it was achieved between 12 and 24 hours. This suggests that maximum adhesion occurs relatively late in the exponential phase and early in the stationary phase. This difference could be attributed to the dominant mechanisms governing

adhesion in the two growth phases. It is established that adhesion is a process that occurs in two steps: a reversible attachment phase, which occurs early and controlled by electrostatic and van der Waals or hydrophobic forces, and an irreversible attachment phase, which occurs later and controlled by the cell wall exopolymers synthesis [6]. This could explain why adhesion in the stationary phase is primarily governed by electrostatic and van der Waals forces, while adhesion in the exponential phase is more likely governed by the synthesis of adhesive proteins.

The results of this study show differences in adhesion behaviors of *E. faecalis* when compared to other microorganisms and substrates reported by other authors. [5] found that the maximum adhered densitie of *Vibrio cholerae* and *Escherichia coli* to non-activated charcoal were 53.7×10^7 CFU/cm^2 and 53.2×10^7 CFU/cm^2, respectively, during the exponential phase, under the same microcosm conditions as this study (maximum adhesion: 148.69×10^5 CFU/cm^2). The relatively lower adhesion of *E. faecalis* compared to *V. cholerae* and *E. coli* on non-activated charcoal could be attributed to the cellular morphology and/or to the absence of locomotive elements. *E. faecalis* is a coccus lacking flagella and pili, whereas *V. cholerae* and *E. coli* are bacilli equipped with these structures. The rod-shaped cell morphology and the presence of pili may facilitate the adhesion process [24, 25]. Bacteria such as *E. faecalis*, which lack pili, adhere less readily than bacilli that possess them [24, 25]. Additionally, other studies exploring the adhesion of *Staphylococcus aureus* to different substrates, such as granite and basalt, reported maximum adhered cell abundances of 75.3×10^3 CFU/cm^2 [4]. These abundances appear lower than those observed in this study. Given that *S. aureus* shares similar characteristics with *E. faecalis* (coccus lacking pili), the difference could be explained by the nature and composition of the substrate surface [7]. The texture and mineralogical composition of granite and basalt differ from those of non-activated charcoal derived from plant pyrolysis.

The slope values of the regression lines "a," corresponding to the speed of cell movement (attachment or detachment from the substrate), were all positive. This suggests that during the immersion of the charcoal in the microbial suspension, adhesion was the predominant process; detachment being negligible. This result may seem surprising because adhesion is considering sometimes as a reversible process [26, 27, 25].According to [26], the extent of reversibility depends on the nature of the substrate and the interactions between the adhesive structures of the cell wall and the substrate surface. Some substrates may offer less reversibility than others. Based on this hypothesis, the non-activated charcoal (Okan) used in this study may exhibit lower reversibility. This property could be advantageous for water purification applications, as it would reduce the release of adhered bacteria into the water. The adhesion rate of *E. faecalis* to non-activated charcoal reached up to 20.343×10^5 CFU/cm^2/h, indicating the material's potential for reducing microbial loads in water. Considering the initial bacterial load of 10^8 CFU/ml in the prepared microbial suspensions, the estimated microbial reduction rate is approximately 10%. However, the reduction rate (10%) remains below WHO standards for household water treatment [28].

Conclusion

E. faecalis cells adhere to non-activated charcoal, with adhesion parameters (adhered cell density, adhesion rate, and Freundlich coefficient Kf) varying significantly with growth phase. Exponential-phase cells exhibited higher adhesion than stationary-phase cells. While non-activated charcoal shows promise for microbial load reduction, its current efficiency is insufficient for standalone water purification. However, it could be integrated into multi-stage treatment systems to enhance cost-effectiveness.

Credit Authorship Contribution Statement

- **Aurelie Dzenga Njeunkam:** Writing - review and editing, writing - original draft, Methodology, Investigation, Formal analysis, Data curation.
- **Jean Samuel Eheth:** Writing - review and editing, writing - original draft, Data curation.
- **Yves Yogne Poutoum:** Methodology, Investigation, Formal analysis, Data curation.
- **Luciane Marlyse Moungang:** Writing - review and editing, writing - original draft, Visualization, Validation, supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.
- **Adams Ibn Rabiou Mouhama Sani:** Methodology, Investigation, Formal analysis, Data curation.
- **Moïse Nola:** Supervision, Investigation.

Declaration of Competing Interest

Authors declare no conflict of interest regarding the publication of this paper entitled "Role of the *Enterococcus faecalis* growth phase in the purification of water by non-activated charcoal".

Data Availability

We will be made available on request.

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