Power System Technology
ISSN:1000-3673

Received: 06-05-2025 *Revised: 15-06-2025* *Accepted: 31-07-2025*

Microbial Adaptation in Fixed Submerged Bed Biofilms Under Extreme Conditions

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Abstract

Microbial adaptation in fixed submerged bed biofilms under extreme conditions is key to the effective treatment of wastewater, which comes from diverse sources such as domestic, industrial and agricultural. These waters have varied compositions that present challenges for their proper management. Biofilms formed by microorganisms in fixed submerged beds allow for the degradation of contaminants, but extreme conditions such as elevated temperatures, fluctuating pH and the presence of toxic contaminants can affect their effectiveness.

Microorganisms in biofilms adapt to these conditions through metabolic changes, genetic alterations and modifications in the biofilm structure, which ensures their survival and efficiency in treatment. A comprehensive approach that classifies wastewater according to its origin and composition, and that considers microbial adaptation mechanisms, is essential to develop more effective and sustainable solutions in wastewater management, protecting both public health and the environment.

Keywords: *Microbial adaptation, Biofilms, Fixed submerged beds, Extreme conditions, Microorganisms, Wastewater treatment, Adverse conditions, Adaptation mechanisms*

I. INTRODUCTION

Microbial adaptation in biofilms developed within fixed submerged beds under extreme conditions is an emerging field of study that intersects microbiology, environmental engineering, and biotechnology. This phenomenon refers to the ability of microorganisms to form structured communities—known as biofilms—on submerged support media, which is fundamental for the efficient treatment of wastewater and the removal of pollutants.

Biofilms are complex structures that allow microorganisms to adhere to solid surfaces, creating an environment where they can thrive and carry out critical metabolic functions. In fixed submerged bed systems, these microorganisms not only adapt to the aquatic environment but also develop survival strategies to withstand extreme conditions such as drastic fluctuations in temperature, pH, and salinity. This adaptability is essential for the operational efficiency of wastewater treatment systems, particularly in scenarios where environmental and process conditions are highly variable or unfavorable. Additionally, research has shown that the structure and composition of biofilms significantly influence their performance. Factors such

Power System Technology
ISSN:1000-3673
Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

as the type of carrier medium, hydraulic characteristics, and fluid dynamics affect both biofilm formation and stability.

In the food industry, microbial biofilm formation on surfaces and equipment presents a significant challenge, as these structures may act as reservoirs for undesirable microorganisms, including pathogens and spoilage agents. Biofilms offer enhanced resistance to conventional antimicrobial treatments, making their removal difficult and contributing to cross-contamination. This represents a serious threat to food safety and quality, emphasizing the need to develop effective prevention and control strategies. Nevertheless, under controlled conditions, biofilms may also offer beneficial applications—such as their use in fermentation processes in the food sector (Flemming et al., 2016).

II. THEORETICAL FRAMEWORK

Microbial Adaptation in Biofilms of Fixed-Bed Submerged Reactors Under Extreme Conditions.

Biofilms are multicellular formations of microorganisms that adhere to surfaces and become encapsulated within an extracellular matrix. They are of particular relevance in several fields, including wastewater treatment, bioremediation, and environmental biotechnology. In fixed submerged bed systems (known as LSSF for their initials in Spanish), biofilms play a critical role in the degradation of contaminants. These systems are often subjected to extreme conditions, such as variations in temperature, salinity, pH, high pollutant concentrations, or even the presence of toxic substances. These factors require the microbial communities within the biofilms to adapt in order to maintain their survival and optimal performance.

To understand the concept of biofilms, it is essential to recognize that these are microbial communities adhered to surfaces and surrounded by a matrix that provides protection from adverse environmental agents. This structural organization enables them to survive in environments where free-floating microorganisms could not.

In recent years, biofilms have gained considerable recognition for their applications in industrial settings, especially in wastewater treatment. This is due to their ability to concentrate microorganisms responsible for degrading pollutants, which is also critical for their resistance to antimicrobial compounds and tolerance to extreme conditions—such as acidic or alkaline pH, high salinity, and extreme temperatures.

Biofilms in Fixed Submerged Beds.

Biofilms are microbial communities that attach to surfaces through the production of an extracellular matrix composed mainly of polysaccharides, proteins, nucleic acids, and lipids. In fixed submerged bed systems, these biofilms develop on solid supports that facilitate microbial adhesion and growth while remaining immersed in a liquid medium (typically water

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ISSN:1000-3673
Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

or an aqueous solution). These systems are highly effective for biological wastewater treatment because they support high microbial densities and allow efficient contact between the substrate and the contaminated fluid.

Composition and Function of Biofilms.

- Biofilms are made up of an extracellular matrix that includes polysaccharides, proteins, nucleic acids, and lipids.
- This matrix serves multiple functions, such as acting as a protective barrier against environmental stressors.
- The biofilm structure supports the execution of key metabolic functions that aid in the degradation of both organic and inorganic contaminants.
- Biofilms have proven highly relevant in improving the efficiency of wastewater treatment systems (Mahto, Vandana, Priyadarshanee, & Das, 2022).

Key Characteristics:

- Structure: Biofilms are dynamic and multilayered, composed of various microorganisms (e.g., bacteria, fungi, algae) performing specific ecological roles.
- Function: The primary function of biofilms in submerged beds is the degradation of organic and inorganic compounds present in the water or flowing fluid.
- Challenges in extreme conditions: Biofilms in fixed submerged beds face variability in nutrient availability, pH shifts, temperature extremes, and the presence of toxic or inhibitory substances (Manrique, J., 2013).

Formation and Factors Influencing Biofilm Development.

- Biofilm development is a dynamic process influenced by physical, chemical, and biological factors.
- Nutrient availability, hydraulic flow, and substrate type determine biofilm growth and stability.
- In wastewater treatment, the type of carrier medium to which microorganisms adhere directly affects the performance of the biofilm.
- Environmental factors such as water pH, temperature, and dissolved oxygen levels impact biofilm structure and function, thereby influencing treatment efficiency (Saini, Tewari, Dwivedi, & *, 2023).

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ISSN:1000-3673
Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

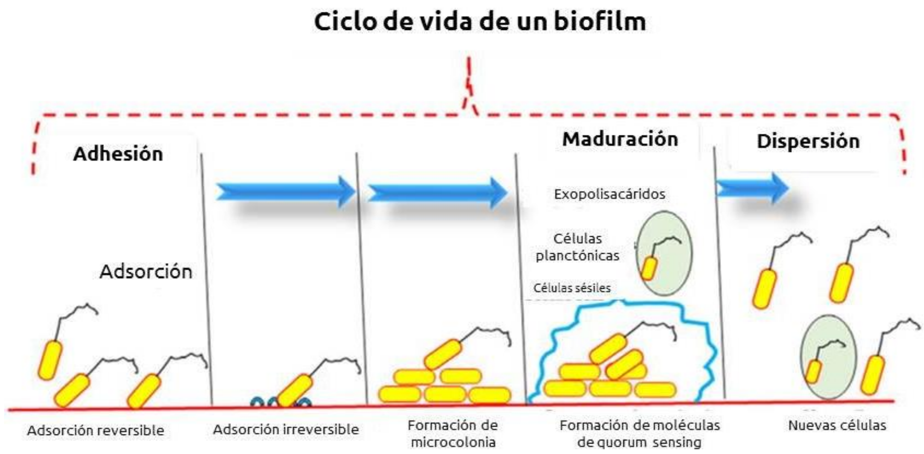

Figure 1. Life cycle of a biofilm: adhesion, growth, maturation, and dispersion. Source:). (Pavone, 2023).

Factors Contributing to Extreme Conditions.

- **Temperature**
- It significantly influences the growth rate and metabolism of microorganisms. Extremely high or low temperatures can affect the fluidity of cell membranes and enzyme activity. Thermophilic and psychrophilic microorganisms, adapted to extreme temperatures, may play a crucial role in the degradation of contaminants in extreme environments.
- **pH**

Fluctuations in pH affect the solubility of contaminants and the efficiency of metabolic reactions. Some biofilms exhibit remarkable capacity to adapt to extreme pH variations, such as acidophilic biofilms, formed by acid-loving bacteria, or alkaliphilic biofilms, which can survive in acidic or basic environments.

- **Salinity**

The salt content in water can impact the osmolarity of the environment. Halophilic bacteria thrive in high salinity conditions, and their presence in biofilms contributes to the system's adaptation to such environments.

- **Toxic Contaminants**

The presence of heavy metals, organic toxic compounds, or aggressive chemical substances can inhibit microbial growth. However, many bacterial species have developed tolerance or degradation mechanisms for these contaminants, which enables the formation of biofilms resistant to harmful substances.

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Microbial Adaptation in Extreme Conditions.

Microbial adaptation within biofilms under extreme conditions is essential for their performance in the treatment of industrial wastewater, where microorganisms inhabiting biofilms can resist stress factors such as high concentrations of heavy metals, abrupt pH shifts, and extreme temperatures. In this phase, thermophilic, acidophilic, and halophilic microbial species are especially suited to thrive in such environments, allowing them to degrade specific contaminants under extreme operating conditions. This type of adaptation is crucial for the treatment of industrial wastewater containing a complex mixture of pollutants, including aggressive chemical substances. (Koerdt, Gödeke, Berger, Thormann, & Albers, 2010).

Mechanisms of Microbial Adaptation in Biofilms.

Microorganisms present in biofilms can adapt to extreme conditions through various physiological and biochemical stress response mechanisms, including the production of protective compounds such as EPS (extracellular polymeric substances), which aid in the protection of microbial cells against environmental stress. These adaptation mechanisms allow some microorganisms to maintain vital functions in response to adverse conditions by enabling the formation of resistant biofilms. This is especially relevant in environments with high levels of toxic compounds, where tolerance mechanisms and genetic modifications enable these microorganisms to survive in environments with high salinity, extreme temperatures, or fluctuating pH.

- **Changes in Biofilm Composition.**

The structure of the biofilm is not homogeneous and can change in response to adverse conditions. Bacteria can modify the composition of their extracellular matrix, increase the production of polymers and other protective substances, or recruit different microbial species that are better adapted to environmental conditions.

- **Modification of the Extracellular Matrix.** Biofilms are not rigid structures; their composition may vary depending on environmental conditions. Under stress conditions, bacteria can increase the production of extracellular polymeric substances (EPS) to enhance protection against adverse factors, such as temperature fluctuations or the presence of contaminants. These changes allow for greater cohesion and mechanical resistance of the biofilm.

- **Spore Formation and Latent State.** Many bacterial species present in biofilms can form spores as a resistance strategy to extreme conditions. This mechanism is common in bacteria such as Bacillus and Clostridium, which can enter a latent state when nutrients are limited or when facing the unfavorable conditions of their environment. Tejero, J., & Santamaría, J. (2000).

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- **Symbiotic Interactions.**

Biofilms in fixed-bed submerged systems are often complex communities where different microbial species interact symbiotically. These interactions enable cooperation among different microorganisms for the degradation of complex contaminants, thereby increasing the efficiency of bioremediation processes and water treatment. For example, nitrifying and denitrifying bacteria collaborate in the nitrogen cycle, while metal-reducing bacteria can help detoxify environments contaminated with heavy metals.

- **Stress Resistance.**

Microorganisms within biofilms develop molecular mechanisms to resist environmental stress. These include the production of molecular chaperones that assist in the correct folding of proteins under thermal stress conditions, as well as the regulation of ion-pumping mechanisms to cope with pH and salinity variations.

Applications of Biofilms in Fixed-Bed Submerged Systems Under Extreme Conditions.

Biofilms in fixed-bed submerged systems are employed in various industrial and environmental applications, especially in environments where conditions are extreme:

Treatment of Industrial Wastewater: In chemical, petrochemical, and textile industries, biofilms adapt to the presence of toxic compounds and high concentrations of contaminants, enabling efficient purification.

Bioremediation of Contaminated Sites: Biofilms are essential in the remediation of wastewater and soils contaminated with heavy metals and other persistent toxic compounds. Microbial adaptations allow for the degradation or transformation of these pollutants under adverse environmental conditions. Tejero, J., & Santamaría, J. (2000).

Applications of Biofilms in Wastewater Treatment

Biofilms are extensively applied in the removal of pollutants from wastewater owing to their capacity to concentrate microorganisms involved in contaminant degradation. In this context, fixed-bed submerged systems play a crucial role in eliminating both organic and inorganic compounds, including phenols, detergents, heavy metals, and nutrients such as nitrogen and phosphorus. Additionally, biofilm-based systems have proven to be more efficient than traditional systems, such as activated sludge processes, due to their ability to withstand extreme conditions and maintain a high rate of pollutant degradation.

Various Environmental Applications of Biofilms.

Submerged bed systems stand out for promoting the formation of microbial communities like biofilms, which provide benefits by supporting the natural behavior of microorganisms. It is common for these organisms to be organized in this manner, which has sparked interest in their

Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

potential for biotechnological applications—especially in bioremediation. This technique enables interactions between biological systems and environmental contaminants, transforming them into less toxic or even beneficial compounds, thereby aiding the recovery of degraded environments. These processes have been successfully implemented in soils and water bodies, and their effectiveness fundamentally depends on the adaptive capabilities of the involved microorganisms to withstand contaminated environments.

Furthermore, the exploration of diverse biofilm applications in bioremediation processes highlights their use in bioreactors designed for treating large volumes of diluted aqueous solutions, such as industrial and municipal wastewater. The genetic improvement of microbial strains, the optimization of their metabolic pathways, and the increase in the copy number of genes encoding key enzymes for the degradation or transformation of pollutants could further enhance the efficiency of these biofilm-associated microbial strategies.

Biological Removal Techniques for Contaminants.

Toxic substances present in low concentrations can generate significant environmental effects.

Bioremediation allows the transformation of toxic organic and inorganic compounds into less harmful forms.

Microorganisms (bacteria and fungi), native plants, and their enzymes actively contribute to this process.

Metal Contamination and Biofilm-Based Removal

The toxicity of heavy metals is mainly attributed to their ionic charge, rather than the elemental metal itself.

Biofilms exhibit high tolerance to elevated concentrations of heavy meta

Microbial mechanisms to control contaminating metals include:

- Ion capture by specific cellular components.
- Efflux systems for cations and anions that help neutralize toxicity.
- Membrane transporters and enzymes that modify the redox state of metals, making them less toxic and suitable for biotechnological applications.

Types of Biofilm Systems Using Sisal Leaf Residues.

In Colombia, sisal leaves—used in the production of fiber and textiles—generate leachate waste during processing. These residues and leachates can cause environmental pollution and pose health risks.

Use of Anaerobic Biofilms in Bioreactors:

Received: 06-05-2025 *Revised: 15-06-2025* *Accepted: 31-07-2025*

Anaerobic bacterial biofilms have also been developed in bioreactors containing sisal leaf residues. In these systems, the anaerobic bacteria degrade the leachates while simultaneously producing biogas.

Stages of the Anaerobic Digestion Process:

1. First stage:

- Anaerobic bacteria decompose organic matter and produce methane (CH_4) and carbon dioxide (CO_2), the main components of biogas.

2. Second stage:

- The biodegradation of leachates is completed through biofilms adhered to the bioreactor bed, increasing the efficiency of the process.

III. METHODOLOGY.

To study microbial adaptation in biofilms within fixed-bed submerged systems under extreme conditions, a series of experimental steps and microbiological analyses were carried out to characterize the biofilms formed in these systems, evaluate the effects of extreme conditions, and determine the mechanisms of microbial adaptation.

1. Experimental Design

1.1. Selección del Sistema de Lecho Sumergido Fijo (LSSF)

- **Soporte sólido**: Se seleccionarán materiales con alta área superficial para la formación de biopelículas, como cerámica, plástico o piedra. Estos soportes deben ser resistentes a las condiciones extremas de temperatura, pH y salinidad que se inducirán en el experimento.

- **Fluido de cultivo**: El fluido en el que se sumergen los lechos será una mezcla de nutrientes diseñada para simular aguas residuales industriales o ambientes naturales, con una oncentración controlada de contaminantes (metales pesados, compuestos orgánicos,etc.).

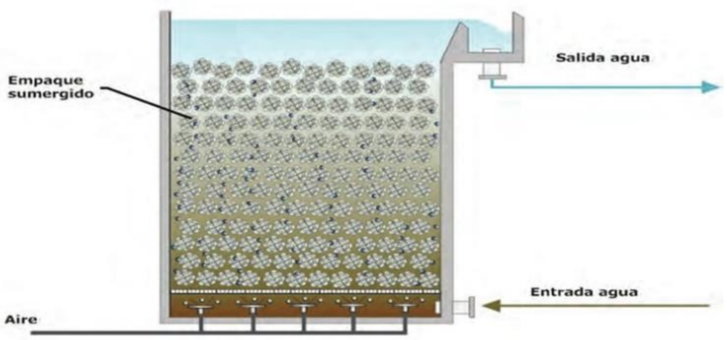

Figura 1 Esquema de un filtro sumergido aerobio. Fuente (Noyola, Morgan-Sagastume, & Güereca, 2023)

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Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

1.2. Experimental Conditions.
Extreme conditions will be modeled according to the type of stress to be studied. The main factors to be manipulated are:

- **Temperature**: Three temperature conditions (low: 4°C, medium: 25°C, high: 40–45°C) will be applied to simulate psychrophilic, mesophilic, and thermophilic environments.
- **pH**: The fluid's pH will be adjusted to extreme conditions (acidic, pH 3–4 and alkaline, pH 9–11) to assess the resistance of biofilms to pH fluctuations.
- **Salinity:** Salt concentrations ranging from 1% to 10% NaCl will be tested to simulate saline environments.
- **Contaminants**: Heavy metals (such as Pb^{2+}, Cu^{2+}, Cr^{3+}) and toxic organic compounds (such as phenols and detergents) will be introduced at concentrations representative of industrial effluents.

1.2. Controls

- **Non-stress control:** Optimal conditions will be maintained (25°C, neutral pH, low salinity) to observe the standard growth of the biofilms.
- **Biofilm-free control:** Conditions in which biofilm does not form will be used to evaluate the effect of isolated microorganisms, instead of the integrated microbial community.

2. Biofilm Formation and Growth

2.1. Inoculation of Microorganisms

- Local microbial consortia from wastewater or activated sludge will be used, containing a diverse microbial community capable of forming biofilms.

Specific strains of interest such as Pseudomonas putida, Bacillus subtilis, and Deinococcus radiodurans may also be used, as they are known for their ability to form biofilms and tolerate extreme conditions.

2.2. Biofilm Formation

- The fixed-bed submerged systems will be inoculated with the selected microorganisms and incubated under stress conditions of temperature, pH, and salinity previously defined.
- The time required for biofilm formation will be measured, which generally ranges from 1 to 3 weeks, depending on environmental conditions.

2.3. Biofilm Growth Monitoring

- Biomass measurement techniques will be used for biofilms, such as:
- Turbidity measurement (absorbance at 600 nm),

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- Dry weight determination of biofilms extracted from fixed-bed systems.
- The morphology and structure of the biofilm will be observed using scanning electronmicroscopy (SEM) and confocal laser scanning microscopy (CLSM) to assess cell distribution and viability Kirisits, M., et al. (2005).

3. Evaluation of Microbial Adaptation

- **Analysis of Microbial Composition**
- **Molecular techniques**: A molecular analysis of microbial diversity will be performed using 16S rRNA sequencing for bacteria. This will allow identification of the dominant species within the biofilm and their adaptation to extreme conditions.

3.1. PCR-DGGE techniques: Denaturing gradient gel electrophoresis (DGGE) will be used to compare microbial diversity in biofilms formed under different environmental conditions.

3.2. Evaluation of Metabolic Activity

- Microbial respiration rates will be measured by monitoring CO_2 production, providing an estimation of metabolic activity within the biofilm.
- Specific fluorescent probes will be used to evaluate enzymatic activity involved in contaminant metabolism, such as hydrolases, oxidoreductases, and enzymes related to the degradation of organic compounds or heavy metals.

3.3. Evaluation of Stress Resistance

- **Cell viability tests**: Propidium iodide and ethidium bromide staining techniques will be used to evaluate cell viability within the biofilms. The presence of live and dead cells will provide information on the ability of microorganisms to adapt and survive under extreme stress conditions.
- **Spore formation analysis**: Under extreme stress conditions, some bacterial species may form spores as a survival strategy. Specific staining techniques will be used to evaluate spore formation in biofilms.

3.4. Analysis of the Extracellular Matrix

The composition of the extracellular polymeric substances (EPS) in biofilms will be characterized using chromatography techniques (gel filtration and mass spectrometry) to identify the main polysaccharides, proteins, and nucleic acids present, as well as how these components vary under different environmental conditions.

4. Statistical Analysis

- **Analysis of Variance (ANOVA):** ANOVA will be used to compare microbial growth rates and metabolic activity under different experimental conditions (temperature, pH, salinity,

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ISSN:1000-3673
Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

contaminants).

- Differences in microbial diversity and biofilm structure will be analyzed using diversity indices (Shannon, Simpson) and Principal Component Analysis (PCA)

Additional tests may be conducted in pilot-scale fixed-bed submerged reactors to evaluate the scalability of the results obtained in the laboratory under industrial conditions or in contaminated natural environments. This step will involve the simulation of real effluent conditions and the assessment of treatment efficiency in wastewater purification.

IV. CONCEPTUAL FRAMEWORK

1. Biofilms

Biofilms are microbial communities whose formation is based on a communication system known as quorum sensing, in which they form a matrix that provides greater resistance to environmental stress; therefore, the study of microorganisms associated with biofilms is essential to understand their adaptive strategies in bioremediation processes.

Composition and Structure: Biofilms are primarily composed of extracellular polymeric substances (EPS), which include polysaccharides, proteins, nucleic acids, and lipids. This matrix protects the cells from environmental stressors such as changes in pH, temperature, the presence of contaminants, and nutrient deficiency.

Function: Biofilms have a fundamental ecological and metabolic role in the nutrient cycle. In wastewater treatment systems and bioremediation, they facilitate the degradation of pollutants by enhancing the concentration of microorganisms responsible for breaking down organic and inorganic compounds.

Los LechFixed-Bed Submerged Reactors (LSSF): Fixed-bed submerged reactors are a type of biological reactor used to treat wastewater through the application of biofilms. In these systems, microorganisms adhere to a solid support and are submerged in a liquid medium that provides nutrients and contaminants.

- **Solid support:** Fixed-bed submerged reactors employ materials with high surface area, such as ceramic, plastic, or stone, which promote biofilm formation. These materials facilitate microbial adhesion without being washed away by the liquid flow.

- **Advantages:** LSSF systems offer advantages over conventional biological treatment processes such as activated sludge reactors, due to improved separation between biomass and treated effluent, resulting in greater microbial population retention and system stability.

2. Microbial Adaptation

This refers to the ability of microorganisms to adjust to changes in their environment, allowing them to survive and continue performing their metabolic functions even under adverse

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conditions. In the context of biofilms, microorganisms exhibit a range of mechanisms to adapt to environmental stresses caused by extreme conditions.

Adaptation Mechanisms: The main adaptation mechanisms include

EPS Production: The extracellular polymeric matrix provides physical protection against environmental stress.

Spore Formation: Some microorganisms can form resistant spores when conditions are unfavorable.

Metabolic Changes: Microorganisms can modify their metabolism to utilize alternative energy sources or protect their enzymes and cellular structures from damage.

Stress Resistance: Microorganisms living in biofilms can develop resistance to thermal, chemical, osmotic, and other types of stress, which is essential for their survival in extreme environments**.** Iker De La Pinta Aresti (2019)

V. RESULTS

Biofilm Formation under Optimal and Extreme Conditions:

- Under optimal conditions (25 °C, pH 7, no salinity), the biofilms formed a biomass of 0.95 g/L with a thickness of 150 µm. At 4 °C (low temperature), biomass was 0.35 g/L, with a thickness of 80 µm.
- At 45 °C (high temperature), biomass was 0.55 g/L, and thickness reached 130 µm.
- Under acidic pH conditions (pH 4.0), biomass was 0.25 g/L with a thickness of 50 µm.
- At alkaline pH (11.0), the biofilms formed 0.85 g/L of biomass, with a thickness of 120 µm.
- In media with high salinity (10%), biomass was 0.45 g/L with a thickness of 90 µm, while low salinity (1%) showed results similar to the optimal conditions.

Table 1

Comparison of Biomass and Thickness under Different Temperature and pH Conditions

Condition	**Biomass (g/L)**	**Thickness (µm)**
Optimal conditions (25°C, pH 7)	0.95	150
Low temperature (4°C)	0.35	80

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ISSN:1000-3673
Received: 06-05-2025 Revised: 15-06-2025 Accepted: 31-07-2025

High temperature (45°C)	0.55	130
Acidic pH (pH 3-4)	0.25	50
Alkaline pH (pH 9-11)	0.85	120

Microbial Composition:

- Under optimal conditions, the biofilms were dominated by species such as Pseudomonas putida and Bacillus subtilis.
- Under extreme conditions, the biofilms were dominated by species adapted to each condition: Lactobacillus acidophilus under acidic pH, and Deinococcus radiodurans under high salinity.

EPS Production and Spore Formation:

- Biofilms formed under extreme conditions showed an increase in EPS production (up to 30% under acidic pH and high salinity), which contributed to cell protection.
- Spore formation was higher in biofilms developed at high temperature (45 °C) and acidic pH, reaching up to 40% spore content.

Table 2

EPS Production and Spore Formation under Extreme Conditions

Condition	EPS Production (%)	Spore Formation (%)
Acidic pH (pH 3-4)	30	40
High salinity (10%)	30	35
High temperature (45°C)	25	40

Cell Viability and Metabolic Activity:

- The microbial respiration rate (measured by CO_2 production) was lower under extreme conditions, with values of **0.15 µmol CO_2/h at 4 °C** and **0.25 µmol CO_2/h at 45 °C**.

Received: 06-05-2025 *Revised: 15-06-2025* *Accepted: 31-07-2025*

- Under acidic pH and high salinity conditions, cell viability decreased, reaching only 60–70% of viable cells, whereas under optimal conditions it exceeded 90%.

Table 3

Cell Viability and Respiration Rate in Biofilms

Condition	**Cell Viability (%)**	**Respiration Rate (µmol CO_2/h**
Optimal conditions (25°C, pH 7)	>90%	0,95
Low temperature (4°C)	60-70%	0,15
High temperature (45°C)	70-80%	0,25
Acidic pH (pH 3–4)	60-70%	0,20
Alkaline pH (pH 9–11)	70-80%	0,30
High salinity (10%)	60-70%	0,18
Low salinity (1%)	>90%	0,95

The results indicate that extreme conditions significantly affect the formation and growth of biofilms, reducing biomass and thickness compared to optimal conditions. However, microorganisms have developed adaptation strategies such as increased EPS production and spore formation, allowing them to better withstand environmental stress. These adaptations are crucial for the viability and function of biofilms in industrial and natural environments with extreme conditions, suggesting their potential application in wastewater treatment and bioremediation.

VI. DISCUSSION

Microbial adaptation in biofilms under extreme conditions was studied using a fixed-bed submerged system (LSSF). The results show that, despite unfavorable conditions (extreme temperatures, acidic or alkaline pH, and high salinity), biofilms were able to maintain their structure and functionality through several adaptive mechanisms.

Biofilm Formation under Environmental Stress:

Extreme conditions negatively impacted biomass production and the thickness of biofilms, with a significant reduction observed under low-temperature and acidic pH environments. These conditions inhibited microbial growth. However, biofilms formed under high temperature (45 °C) and alkaline pH showed remarkable structural resistance, highlighting the adaptive capacity of thermophilic and alkaliphilic microorganisms.

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Microbial Diversity and Specialization:

Microbial diversity decreased under extreme conditions, with dominant species being those best adapted to each specific environment. For instance, biofilms formed at acidic pH were dominated by acidophilic species such as *Lactobacillus acidophilus*, while those exposed to high salinity were characterized by halophilic species like *Deinococcus radiodurans*. This suggests that microbial communities in biofilms tend to specialize according to environmental stressors, which may enhance the stability and functionality of the biofilm under challenging conditions.

Adaptive Mechanisms: EPS and Spore Formation:

Extracellular polymeric substances (EPS) and spore formation were key adaptive strategies. Biofilms exposed to stress conditions exhibited an increase in EPS production, which provided structural protection against stressors. Spore formation was especially prominent at high temperatures and acidic pH, indicating that microorganisms utilize this mechanism to preserve their viability under prolonged stress.

Cell Viability and Metabolic Activity

Despite reductions in cell viability and metabolic activity under extreme conditions, the biofilms maintained sufficient microbial respiration rates to sustain their functionality. This indicates that, even when metabolic activity is affected, the biofilm remains viable and capable of carrying out essential metabolic functions, although at a reduced rate.

Implications for Industrial and Environmental Applications:

Resilient biofilms formed under extreme conditions have great potential in industrial applications, such as wastewater treatment in adverse environments, and in bioremediation processes in contaminated sites. The ability of microorganisms to adapt to extreme conditions through EPS production and sporulation suggests that biofilms could be effectively used in industrial environments with high salinity, extreme temperatures, or toxic contaminants.

Heavy Metal Removal Using Resilient Biofilms

One of the most remarkable advantages observed in this study is the high tolerance of biofilms to heavy metals such as lead, chromium, or copper. This is due to resistance mechanisms such as ion capture by cell components, efflux pumps, and enzymatic modifications that reduce metal toxicity, rendering them less harmful. These mechanisms strengthen the usefulness of biofilms in scenarios where inorganic contaminants pose a significant challenge.

Practical Application: Use of Sisal Leaf Residues in Anaerobic Biofilm Bioreactors

A successful example of implementing biofilms adapted to extreme conditions is the Colombian case using sisal leaf residues. In this system, anaerobic digestion was combined

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ISSN:1000-3673
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with biofilm formation on the reactor bed, achieving not only the removal of leachates but also biogas generation as a valuable by-product. This type of application demonstrates the added value of using biofilms in circular economy models in agro-industrial wastewater treatment.

Comparison with Other Treatment Systems: Biofilms vs. Activated Sludge

Compared to traditional activated sludge systems, fixed-bed biofilm systems offer greater operational stability, lower sludge production, and improved tolerance to changing loads and toxic substances. These findings suggest that in contexts where physicochemical parameters vary or are extreme, biofilm-based systems are not only more efficient but also more sustainable in the long term.

VII. CONCLUSION

This study provides an integrated view of microbial adaptation in biofilms formed in fixed-bed reactors under extreme conditions, revealing key mechanisms that enable microorganisms to survive and thrive in stressful environments. The results demonstrate that, despite limitations imposed by temperature, pH, and salinity stressors, microbial communities can adapt through various mechanisms such as EPS production and sporulation, contributing to cell protection against environmental stress.

The biofilms formed under extreme conditions exhibited reduced biomass and thinner structure compared to those formed under optimal conditions. This highlights the significant influence of environmental factors on the microbial growth capacity in biofilm-based systems.+

Future Perspectives

In the future, it would be of great interest to delve deeper into the identification of microbial strains that can be optimized for industrial applications, particularly in systems operating under extreme conditions. Moreover, studying the interaction between microbial species within biofilms and their relationship with environmental factors could provide valuable insights for the design of more efficient bioprocesses.

In summary, this study demonstrates that biofilms, although exposed to extreme environmental stressors, exhibit remarkable adaptive capacity through specific mechanisms. This knowledge is crucial for enhancing biotechnological processes, aiming to harness biofilms in industrial and environmental applications.

Additionally, the implementation of emerging technologies, such as genetic engineering of microorganisms to improve resistance to extreme conditions, could further enhance the performance of biofilms. Through genetic modification, it would be possible to optimize microbial capabilities to withstand heavy metals, pH fluctuations, and high salinity—thus making these biotechnological systems more robust and adaptable to a wider range of environmental conditions.

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Another important avenue of research is the exploration of synergistic interactions among different microbial species within biofilms. Understanding how microbial communities cooperate and communicate in these systems can enable the design of improved water treatment processes. Furthermore, the combination of such ecological insights with microbial genetic engineering could lead to more efficient and faster strategies for contaminant removal and ecosystem restoration.

REFERENCIAS

1. Flemming, H. C., & Wuertz, S. (2019). *The state of biofilm research: Challenges and opportunities*. Biofilms, 27(1), 9-12.
2. Manrique, J. (2013). Desarrollo de tratamientos aerobios en medio fijo. Recuperado de CMIC.
3. Tejero, J., & Santamaría, J. (2000). Tecnologías innovadoras para la depuración utilizando biopelículas. Recuperado de HISPAGUA.
4. Kirisits, M., et al. (2005). Estudio sobre formación y características funcionales de biopelículas. *Biblioteca Digital Univalle*
5. Vitaqua (n.d.). IFAS BIOFILT® - Reactores aerobios de lecho fijo sumergido. Recuperado de Vitaqua.
6. Ospina Pérez, M.C. (2008). Optimización del método de burbujeo para la remoción de biopelículas de
7. Pseudomonas aeruginosa MDC. Pontificia
8. Universidad Javeriana. Recuperado de Repositorio Javeriana.
9. Escuela Politécnica Nacional (2014). Estudio sobre medios plásticos como soporte para biopelículas en reactores aerobios. Recuperado de EPN.
10. c BARRIONUEVO, M. D., et al.,
11. (2009). Tratamiento biológico de efluentes industriales con contenido en metales: factores a tener en cuenta para un diseño eficiente. Buenos Aires: Química viva, 106-124.
12. BELOIN, C. ROUX, A. Y GHIGO,
13. JM. (2008). Escherichia coli biofilms. Rev. Curr Top Microbiol Immunol, Nº 322, 249-289. BETANCOURTH,
14. M. Botero, JE. Y RIVERA,
15. SP. (2004). Biopelículas: una comunidad microscópica en desarrollo.

Power System Technology
ISSN:1000-3673
Received: 06-05-2025 *Revised: 15-06-2025* *Accepted: 31-07-2025*

Colombia: Corporación editora médica del Valle, Rev. Colombia Médica, Vol. 35, Nº 3, 34-39.

16. Koerdt, A., Gödeke, J., Berger, J., Thormann, K. M., & Albers, S.-V. (2010). Crenarchaeal biofilm formation under extreme conditions.
17. Mahto, K. U., Vandana, Priyadarshanee, M., & Das, S. (2022). Biopelícula bacteriana y sustancias poliméricas extracelulares en el tratamiento de contaminantes ambientales: más allá del papel protector en la supervivencia. Revista de Producción Más Limpia. doi:https://doi.org/10.1016/j.jclepro.2022.134759
18. Noyola, A., Morgan-Sagastume, J. M., & Güereca, L. P. (2023). Selección de Tecnologías para el Tratamiento de Aguas Residuales Municipales. México.
19. Pavone, D. (2023). Biofilms: Peligros y beneficios de una comunidad bacteriana.
20. Saini, S., Tewari, S., Dwivedi, J., & *, V. S. (2023). Tratamiento de aguas residuales mediado por biopelículas: una revisión exhaustiva. doi:https://doi.org/10.1039/D2MA00945E