

Discovery of the Features of Salinity on Natural Gas Condensate Degradation in Marine and Estuarine Environments

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Abstract

The investigation into the impact of salinity on the degradation of Natural Gas Condensate in saltwater environments was comprehensively conducted, highlighting the pivotal role salinity plays in both biodegradation and the chemical transformation of these hydrocarbons within marine and estuarine ecosystems. This study delves into the dynamic interplay between varying salinity levels and their effects on degradation kinetics, as well as the compositional alterations of natural gas condensates under saline conditions. A detailed kinetic model was developed to elucidate how different concentrations of salinity both low and high affect the degradation processes of Natural Gas Condensate. Attention was directed towards understanding the inhibitory effects that salinity exerts on biological and chemical degradation mechanisms. The Natural Gas Condensate, sourced from an esteemed oil-servicing firm in Port Harcourt, while thorough microbial analysis of the surrounding water samples facilitated the identification, isolation, and characterization of the diverse microorganisms present in the ecosystem. A comparative analysis of the degradation rates of individual hydrocarbon components within the saline medium was performed, drawing on empirical data obtained from the experimental setups. The findings underscored the significant influence of salinity on the degradation rates, solubility, emulsification behaviors, and pathways of microbial degradation of Natural Gas Condensate. It was observed that halophilic microbial communities played a crucial role in accelerating the breakdown of lighter alkanes, which demonstrated greater susceptibility to biodegradation. Conversely, the heavier hydrocarbon fractions displayed a marked persistence, attributed to their reduced bioavailability in saline conditions. The study also revealed that salinity-induced alterations in interfacial tension and oxidative processes were critical factors contributing to the observed variations in degradation rates. A deep understanding of these interactions is essential for accurately predicting the environmental fate of condensate spills and for refining remediation strategies in saline aquatic environments. Ultimately, this research provides valuable insights into the geochemical and microbial mechanisms that govern hydrocarbon degradation in high-salinity settings, with significant implications for environmental management, oil spill remediation, and the strategic development of

bioremediation techniques. Additionally, the degradation parameters K_s (degradation rate constant) and R_s (yield coefficient) were calculated from Lineweaver-Burk plots, establishing a foundational framework to enhance existing kinetic models. These parameters also offer a roadmap for future pilot-scale bioremediation interventions designed by environmental engineers.

Keywords: *Salinity, Natural Gas Condensate, Biodegradation, Marine Environmental Remediation*

1. Introduction

This paper presents a comprehensive theoretical and experimental examination of the biodegradation of petroleum hydrocarbon pollutants in aquatic environments. The primary objective of this investigation is to develop a kinetic model that accurately predicts the rate of individual hydrocarbon degradation while accounting for the inhibitory effects of varying salinity levels. Kinetic modeling involves the intricate study of physical, chemical, and biological systems that evolve over-time, represented through a system of differential equations interlinked with algebraic equations that define state variables and rate laws pertinent to a given system. Essentially, the model serves as a quantitative framework that articulates the underlying principles governing real-world processes, embodying the fundamental concepts of cause and effect and grounded in the descriptions of the physiochemical mechanisms that underpin the biological system under scrutiny (Rajiv, 2018). Research focused on the bioremediation of petroleum hydrocarbon contaminants has underscored the significant challenges posed by the degradation of individual hydrocarbon components. This complexity arises from the phenomenon of inhibition, where the degradation of one hydrocarbon can be adversely influenced by the presence of other compounds in the mixture, as indicated by experiments conducted by various research teams. Among the various remediation strategies, bioremediation stands out as the most effective method for treating petroleum hydrocarbon contaminants and restoring environmental health (Roger, 1999). Its relative cost-effectiveness, particularly when addressing substantial quantities of pollutants, makes it a preferable choice. While alternative chemical and physical treatment methods may expedite the decomposition of petroleum hydrocarbons, the detrimental by-products generated can severely harm aquatic ecosystems, leading to the death of numerous marine organisms. Since the successful application of bioremediation techniques during the cleanup of the Exxon Valdez oil spill in 1989 in Prince William Sound, there has been a marked increase in interest surrounding the topic of biodegradation (Amadi *et al*, 2017). It has been observed that rising salinity levels can lead to a significant, yet temporary, decline in the growth rates of microorganisms. This decline, however, is often offset over time as microbial cultures adapt and recover from the adverse conditions. Quantifying this effect poses challenges, as it is both transient and applicable to mixed microbial cultures. Inhibition can be elucidated through the analogy of an enzyme interacting with a substrate; this interaction may slow down, pause, or entirely halt subsequent enzyme-substrate

reactions, reflecting the complexities of biochemical processes in the context of hydrocarbon degradation (ASTM, 1999). This paper adopts both theoretical and experimental methodologies to explore the biodegradation of individual hydrocarbons and their mixtures in saline aqueous environments. Key parameters influencing microbial activity during the remediation of these contaminated samples are thoroughly examined. The development of a kinetic model addressing reaction rates, the degradation of petroleum hydrocarbons, decay rates, and product inhibition comprises a substantial focus of this paper.

The degradation of natural gas condensate in marine and estuarine environments is influenced by salinity, microbial activity, and environmental conditions. Understanding these interactions is crucial for assessing hydrocarbon pollutants in aquatic ecosystems. Salinity affects hydrocarbon degradation by altering microbial community composition. (Ross *et al*, 2022) found that salinity fluctuations impact bacterial diversity and degradation efficiency in the southeastern Mediterranean Sea. (Liu *et al*, 2020) identified methanogenic microbes capable of degrading hydrocarbons in varying salinity conditions. Microbial Communities and Degradation Pathways. Halophilic and halotolerant microbes are essential for biodegradation in saline environments. Bacosa *et al*, (2022) noted that microbial consortia play a significant role in hydrocarbon breakdown, with increased salinity influencing their activity. Gray *et al* in 2015 identified isoprene-degrading bacteria that may have similar roles in estuarine environments. Environmental Factors Influencing Degradation. Sunlight, temperature, and oxygen also impact hydrocarbon degradation. Wiegner & Seitzinger (2005) demonstrated that sunlight accelerates the breakdown of organic matter in estuaries, suggesting that photodegradation works alongside microbial activity to naturally attenuate gas condensates.

2. Materials and Methods

2.1 The Model of Microbial Growth Kinetics

Microbial cultures utilize substrates as energy sources, incorporating these substrates into their cellular structures while also being involved in the synthesis of various products. It is crucial to acknowledge the principle of conservation of mass, as this law underpins the relationship between the quantities of materials consumed and those produced in reactions (Ukpaka, 2011). The yield coefficient serves as the ratio of the mass of the product synthesized to the mass of substrate consumed, typically regarded as a constant value. However, in the context of this study, the yield coefficient warrants a more nuanced definition, as it may not remain constant but rather become a function of time along with the physicochemical environment. This variability can result from the dynamic composition of the microbial cells involved, a phenomenon that can be elucidated through sensitive experimental designs. Mathematically, the yield coefficient can be expressed in terms of concentration as follows:

$$Y = \frac{\Delta x}{\Delta s} \quad (1)$$

where Δx = change in the biomass concentrate x , ΔS = change in substrate concentration.

The equation (1), originally formulated by Richardson and Peacock, plays a crucial role in understanding the dynamics of biodegradation processes. The negative sign in the equation signifies a reduction in substrate or nutrient concentration, which is directly associated with an increase in biomass concentration. This relationship underscores the fundamental principle that as microorganisms break down hydrocarbons, they consume available nutrients, leading to a depletion of the substrate. In the context of hydrocarbon biodegradation, particularly when involving microbial action, it is essential to recognize that the process generates several end products, including carbon dioxide (CO₂), water, and new biomass. These outcomes not only indicate the effectiveness of the biodegradation process but also introduce the concept of yield coefficients. Specifically, there are typically two distinct yield coefficients to consider. The first yield coefficient pertains to the conversion efficiency of the substrate—the hydrocarbons being broken down—into new microbial biomass. This indicates how much biomass can be produced from a given amount of hydrocarbon. The second yield coefficient relates to the quantity of carbon dioxide and other metabolites generated as a result of the biodegradation of individual hydrocarbons. This reflects the balance between the biomass produced and the byproducts released into the environment. Overall, understanding these coefficients and their implications helps to provide insights into the efficiency and outcome of the biodegradation process, and it highlights the intricate relationship between microbial activity and nutrient dynamics in the ecosystem. The yield coefficient for biomass:

$$Y_{x/s} = \frac{\Delta x}{\Delta S} \quad (2)$$

The yield coefficient is a critical parameter in understanding the biodegradation process of petroleum hydrocarbons. It quantifies the efficiency with which microbial organisms convert petroleum compounds into usable biomass during their metabolic processes. Specifically, the yield coefficient expresses the amount of cellular biomass produced per unit of substrate consumed, allowing researchers to assess the effectiveness of bioremediation strategies. Understanding this coefficient is essential for optimizing microbial activity in contaminated environments, as it provides insight into the dynamics of nutrient utilization, microbial growth rates, and the overall impact of bioremediation on restoring ecosystems affected by petroleum spills.

$$Y_{p/s} = \frac{\Delta x}{\Delta s} \quad (3)$$

In instances of multi-substrate biodegradation involving various products, specifically labeled as 1, 2, ..., i , ..., N , each product generated through microbial activity is associated with an individual yield coefficient. This yield coefficient plays a critical role in quantifying the efficiency with which microorganisms convert substrates into specific metabolic products. The relationship between the substrates and the resulting products can be described in greater detail using equation (4), which outlines the mathematical representation of these coefficients. Understanding these coefficients is essential for evaluating the overall effectiveness of the

biodegradation process and the dynamics of substrate utilization by the microbial community.

$$Y_{p/s} = \frac{\Delta P_i}{\Delta S} \quad (4)$$

The law of conservation of matter states that in any closed system, the total mass of substances remains constant over time, regardless of the processes occurring within the system. This principle implies that matter cannot be created or destroyed; it can only change forms through physical or chemical reactions (Schroll, 2014). For example, when wood burns, it transforms into ashes, gases, and heat, but the total mass of the wood before combustion is equal to the mass of the products after combustion. This fundamental concept is critical in fields such as chemistry, physics, and environmental science, as it underpins the understanding of reaction stoichiometry, mass balance in ecosystems, and the efficiency of material usage in industrial processes (Stater, 2003),

$$\sum_{i=1}^n Y_i/S = 1 \quad (5)$$

It is important to clarify that when calculating yield based on the mass of both the product and the substrate that has been consumed, the apparent balance of the yield may seem to be violated. In such scenarios, yield coefficients greater than one may be observed. This phenomenon occurs because the reaction may incorporate additional substances into the final product beyond the initial substrate, thereby effectively increasing the mass of the product relative to that of the substrate. As detailed in the subsequent sections of the paper, the yield coefficient associated with biomass production will be measured through experimental methods. This approach aims to isolate the biomass contribution from the overall yield coefficient. By doing so, we can return to the conventional yield coefficient value of unity (1.0), which reflects a more accurate representation of the efficiency of the substrate use in producing the desired product. This careful distinction will enhance our understanding of the factors influencing yield and provide a clearer framework for interpreting experimental results

$$Y_G = \frac{\Delta x}{\Delta S_G} \quad (6a)$$

The term YG, or yield coefficient, represents the efficiency at which feed or substrate is converted into biomass, specifically the mass of cells produced during the growth process. This coefficient is often referred to as the true growth yield and is a critical parameter in bioprocessing and microbial growth studies. To understand this concept further, it's essential to consider the material balance for substrate consumption. This balance accounts for the amount of substrate consumed during the growth process in relation to the biomass produced. It involves quantifying the initial amount of substrate, the mass of cells generated, and any byproducts formed. By applying this balance, one can assess the efficiency of the conversion process and gain insights into optimizing conditions for maximum cell yield.

$$\left[\begin{array}{l} \text{Total consumption} \\ \text{of substrate} \end{array} \right] = \left[\begin{array}{l} \text{Substrate used} \\ \text{for growth} \end{array} \right] + \left[\begin{array}{l} \text{Substrate used} \\ \text{for maintenance} \end{array} \right]$$

This means that

$$\frac{dS}{dt} = \frac{dX / dt}{Y_G} + mX \quad (6b)$$

The variable (m) represents the specific requirements needed for maintenance. To explore this relationship further, let's manipulate the equation by dividing both sides by (X). This operation will help isolate (m) and clarify how the maintenance requirements vary in relation to (X). By doing so, we can gain a deeper understanding of the factors influencing maintenance and better identify the necessary adjustments or actions required to meet these specifications.

$$\frac{\frac{dS}{dt}}{x} = \frac{\frac{dX / dt}{Y_G}}{x} + \frac{mX}{x} \quad (7)$$

$$\frac{1}{x} \frac{dS}{dt} = \frac{1}{X} \frac{dX}{dt} \frac{1}{Y_G} + m \quad (8)$$

Where $\frac{1}{x} \frac{dX}{dt}$ = specific growth rate of the biomass. This is usually denoted by μ . Dividing through the

equation with $\frac{1}{x} \frac{dX}{dt}$ and substituting into the equation (9) we have

$$\frac{\frac{dS}{dt}}{\frac{dx}{dt}} = \frac{1}{Y_G} + \frac{m}{\mu} \quad (9)$$

$Y_{x/s}$ is usually called the observed yield.

2.2 Decay Rate Model

The mathematical model that describes the decay rate of a microbial culture, which will be examined in the upcoming experiment, is founded on the crucial concept of doubling time. This concept is central to understanding the exponential growth pattern exhibited by microbial populations. In essence, the growth rate of these microorganisms is directly proportional to the current population size, illustrating how a larger population can lead to an even more rapid increase. Typically, in practical applications, the variable representing cell number (N) is

substituted with the more relevant metric of cell mass (X). The exponential growth of microbes can be accurately represented by a specific mathematical equation, capturing the dynamic nature of their proliferation under optimal conditions. The exponential growth of microbial populations can be mathematically expressed by the equation:

$$\frac{dX}{dt} = \mu X \quad (10)$$

where (X(t)) is the cell mass at time (t), (X₀) is the initial cell mass, (r) is the growth rate constant, and (e) is the base of the natural logarithm. This model highlights that, under optimal conditions, the population can grow rapidly, leading to significant increases in biomass over time. Understanding this exponential growth is crucial for our experiments, as it sets the foundation for analyzing the decay rates of microbial cultures under various environmental conditions. By applying this model, we can predict how changes in factors such as nutrient availability, temperature, and pH may impact the overall growth and decline of the microbial population.

The equation (10) is known as the Malthus law. Hence in line with the separable differential law, we have

$$\frac{dX}{dt} = \mu dt \text{ Integrating } \int_{x_0}^x \frac{dx}{x} = \int_0^t \mu dt \quad (11)$$

$$\ln \left(\frac{x}{x_0} \right) = \mu t \quad (12)$$

$$\frac{x}{x_0} = e^{\mu t} \quad (13)$$

$$X = X_0 e^{\mu t} \quad (14)$$

Exponential growth in microbial cultures occurs during a specific phase of development, characterized by a rapid increase in the population size. This phase is typically short-lived and is contingent upon the availability of a limited supply of nutrients in the environment. As microorganisms reproduce at an accelerated rate, they consume available resources, leading to optimal growth conditions. However, this growth is not sustainable in the long term, as the depletion of nutrients and the accumulation of waste products ultimately impose constraints that hinder further expansion. Therefore, understanding the dynamics of microbial growth requires a consideration of both the available resources and the environmental factors that influence these biological processes.

2.3 Effect of Salinity on Microbial Growth Rate

An increase in salinity can lead to a significant and immediate decrease in the growth rate of microbial populations. This initial decline, however, is often temporary; many microbes can adapt to their more saline environment, allowing their growth rates to recover over time. Despite this potential for recovery, quantifying the precise effects of salinity on microbial growth remains challenging. The impact of elevated salinity levels is typically transient and particularly relevant in mixed cultures, where different microbial species may react differently to changes in salt concentration. To illustrate the relationship between salinity and the maximum specific growth rate of microbial biomass, a model developed by Park and Marchland provides valuable insights into these dynamics.

$$\mu = \frac{(\mu_m - I_s)s}{K_s + S + (S^2 / k_1)} \quad (15)$$

Where, I_s = salinity inhibition constant (h^{-1}), K_1 = substrate inhibition constant ($mg l^{-1}$)

$$I_s = \frac{I_s(\% NaCl)}{0.01 + \% NaCl} \quad (16)$$

Where I_s = constant depending on culture.

In this study, we consider the relationship between salinity and the maximum specific growth rate of marine organisms, drawing from the modified model proposed by Park and Marchland. This revised model simplifies certain parameters to elucidate the connection more clearly. For our analysis, we established a reference point using a salinity level of 1% and a corresponding maximum specific growth rate of $0.01 h^{-1}$. Additionally, we utilized an estimated density of seawater at $1.027 g/cm^3$. It's important to note that these figures serve as a foundational hypothesis that will be rigorously tested through the experimental segment of this paper. The dynamics of salinity reveal a unique relationship: it is directly proportional to the density of seawater while simultaneously being inversely proportional to the maximum specific growth rate. This interaction is articulated through equation (17), highlighting the complex interplay between environmental conditions and biological growth metrics.

$$S\alpha \propto \rho \propto \frac{1}{\mu_m} \quad (17)$$

$$\text{Where, } S\alpha = \text{salinity level (\% } g/cm^3), \rho = \text{density of seawater, } S\alpha = D\alpha \frac{\rho}{\mu_m} \quad (18)$$

Where $D\alpha$ = constant for the salinity inhibition of maximum specific growth rate. Fixing the values

$$0.01 = \frac{D\alpha(1.027)}{0.01} \therefore D\alpha = 0.000009737(h^{-1}) \quad (19a)$$

$$\text{Considering that the salinity inhibits the process, thus } I_s = Da \quad (19b)$$

Hence, substituting equation 919a) into equation (15) we have

$$\mu = \frac{(\mu_m - D\alpha)S}{K_s + S + (S^2 / K_1)} \quad (20)$$

2.4 Henry's Equation

Henry's equation is a crucial tool utilized to calculate the masses and volumes of volatile hydrocarbons, which exist in both gas and liquid phases within a bioreactor environment. Understanding the behavior of these hydrocarbons is essential, as the microbial growth rate is influenced solely by the concentration of substrates in the liquid phase. Additionally, the biomass yield is determined by the total mass change of the substrate over time. These relationships highlight the significance of accurately measuring the volatile hydrocarbons to optimize microbial activity and overall bioprocess efficiency. The mathematical representations of these dynamics can be found in equations (21) and (22), which detail the specific interactions between substrate concentration, mass transfer rates, and the resultant impact on microbial growth and biomass production. By leveraging Henry's equation, researchers can obtain valuable insights into the system's performance, ensuring that the conditions within the bioreactor are conducive to effective microbial metabolism and a successful bioprocess.

$$M_{\text{ror}} = M_1 + MG \quad (21)$$

$$M_{\text{ror}} = M_L \left[1 + \left(\frac{H}{RT} \right) \left(\frac{VG}{V_L} \right) \right] = M_L \alpha \quad (22)$$

In scenarios where both the temperature and volume of the liquid hydrocarbon remain constant—conditions that may be achieved during certain experimental setups—the rate at which the substrate is consumed can be accurately described by the equation (22) provided below. This implies that, under these stable conditions, the dynamics of substrate consumption are predictable and can be quantified using the specified equation. Such constancy is critical for ensuring that external factors do not influence the outcomes, allowing for a clearer analysis of the underlying processes at

$$\text{play. } \alpha \frac{dS_L}{dt} = - \frac{\mu(S_L)x}{Y_{x/s}} \quad (23)$$

2.5 Product Kinetic Model

Microbial products arise from a complex interplay of biochemical processes occurring within the cells of a microbial culture. In certain applications, the microbial cell itself may serve as the target product, while in others, it is the metabolic byproducts generated during the cell's growth and metabolic activities that are of primary interest. It's crucial to recognize that many significant microbial products are classified as non-growth-associated. These products are synthesized through specific biochemical pathways that can operate independently of cell proliferation. Such mechanisms may involve secondary metabolism, where resources are diverted from primary growth processes to produce metabolites that serve various ecological functions, such as defense mechanisms or signaling molecules. In this context, the yield coefficient ($Y_{p/s}$), which quantifies the amount of product generated per unit of substrate consumed, may not provide a reliable indication of the product accumulation dynamics in batch cultures. This is particularly true in non-growth-associated product formation, where factors such as nutrient availability, metabolic state of the cells, and the presence of specific inducers can significantly influence productivity rates. Therefore, the overall productivity of a complete batch operation should be considered for a more accurate understanding of product formation kinetics. This encompasses considerations of the growth phase of the culture, substrate usage, and potential factors that control the expression of pathways leading to product synthesis. Understanding these dynamics is essential for optimizing microbial processes and improving yields in biotechnological applications. Soji Adeyinka J. (2001)

$$\frac{dP}{dt} = \delta \frac{dX}{dt} + \varepsilon X \quad (24)$$

where, quantities δ and ε are pH — dependent, $\delta \frac{dX}{dt}$ is growth-associated, εX is cellular activity due to maintenance functions. Dividing the equation though by X (biomass concentration) gives the relationship in terms of specific rates, as seen in equation (25);

$$\frac{1}{X} \frac{dP}{dt} = \delta \frac{1}{X} \frac{dX}{dt} + \varepsilon \quad (25)$$

$$\text{Substituting } \mu \text{ for specific growth rate } \frac{1}{X} \frac{dX}{dt} \quad \frac{1}{X} \frac{dP}{dt} = \delta \mu + \varepsilon \quad (26)$$

The model outlined above will be referred to as the Product Formation Model (PFM). Its validity has been rigorously evaluated through experiments detailed in the forthcoming paper. In these experiments, the parameters represented by the values of δ and ε can be estimated by analyzing the slope and intercept derived from the plotted data of the specific rate of product formation. This detailed approach ensures a comprehensive understanding of the model's effectiveness in

predicting product yields. $\left(\frac{1}{X}\right)\left(dt \frac{dP}{dt}\right)$ against μ . The second model for product formation kinetics can be tested based on developed theory.

$$\frac{dP}{dt} = \sum_1 A_i e^{-k_i t} \quad (27)$$

Where, τ = cell age (hours), A_i and K_i = positive constants. Concentration of product accumulated in the broth over the lifetime of the cell/unit mass of the cell =

$$\rho_1 = \int_0^t \sum_i A_i e^{-k_i \tau} d\tau \quad (28)$$

In the case of a range of cells of all possible ages, $[\xi(t)]$ and culture time = t . Then: Overall cell concentration $Xt = \int_0^t \xi(\tau) d\tau$ (29)

Overall product concentration in the fermenter is given as

$$P = \int_0^t \xi(\tau) \left(\int_0^{\tau} \sum_i A_i e^{-k_i \tau} d\tau \right) \quad (30)$$

2.6 Batch Reactor Equations

In the context of biochemical processes, a batch reactor is a closed system where a predetermined number of microorganisms and nutrient materials are combined to facilitate growth and metabolic activity. This process typically starts with the introduction of a small quantity of microorganisms, such as bacteria or yeast, or their spores, into a specific volume of nutrient medium within a suitable vessel, often referred to as a bioreactor. The primary goal in operating a batch reactor is to create optimal conditions—such as temperature, pH, and oxygen levels—for the microorganisms to thrive. During the batch growth phase, microorganisms consume the available nutrients and convert them into biomass, generating by-products in the process. To quantify this growth and the resulting changes in the system, material balance equations are employed. These equations account for the inputs, outputs, and accumulation of biomass and metabolites over time, providing a comprehensive framework for analyzing the efficiency and yield of the fermentation process. The material balance for batch growth can be expressed with equations that include rates of nutrient consumption, growth rates of microorganisms, and by-product formation. Understanding these equations is crucial for optimizing reactor conditions and maximizing microbial production efficiency (Umesi *et al*, 2017)

$$\left[\begin{array}{l} \text{Formation by} \\ \text{biochemical} \\ \text{reacion} \end{array} \right] = \left[\begin{array}{l} \text{Accumulation} \\ \text{within the} \\ \text{system} \end{array} \right] \quad (31)$$

Therefore, in the case of the biomass

$$R_x V = \mu V X = \frac{dx}{dt} V \quad (32)$$

For the substrate

$$R_s V = \frac{dS}{dt} V \quad (33)$$

The yield is used to relate to the equations above $Y_{x/s} = \frac{\Delta X}{\Delta S}$ Therefore

$$Y = \frac{\frac{dx}{dt}}{\frac{dS}{dt}}$$

$$\text{From the yield equation, which is } \frac{dx}{dt} = \frac{1}{Y_{x/s}} + \frac{m}{\mu} \frac{dS}{dt} = \frac{dx}{dt} \frac{1}{Y} \therefore Y = \frac{dS}{dt} = \frac{dX}{dt} \quad (34)$$

Citing the original yield equation (2), then this new equation becomes

$$Y \frac{dS}{dt} = - \frac{dX}{dt} \quad (35)$$

From Malthus' theory in equation (10)

$$\frac{dX}{dt} = \mu X \text{ substituting it into equation (35)}$$

$$Y R_s = - \mu X \quad (36)$$

$$\therefore R_s = - \frac{\mu X}{Y} \quad (37)$$

$$\frac{dS}{dt} = - \frac{\mu X}{Y} \quad (38)$$

$$\text{Since, } Y = - \frac{\Delta X}{\Delta S} \text{ Then } \therefore Y = \frac{x - x_o}{S_o - S} \quad (39)$$

$$S_o - S = \frac{x - x_o}{Y} \text{ Rearranging the equation } S = S_o - \frac{X - X_o}{Y} \quad (40)$$

If the growth rate follows the Monod model, then in the equation $\frac{dX}{dt} = \mu X$, μ will be substituted for the Monod equation. Therefore

$$\frac{dX}{dt} = \left(\frac{\mu_m S}{K_s + S} \right) X \quad (41)$$

Substituting the equation (40) into (41) will give

$$\frac{dX}{dt} = \left[\frac{\mu_m \left(S_0 - \frac{x - x_0}{Y} \right)}{K_s + \left(S_0 - \frac{x - x_0}{Y} \right)} \right] X \quad (42)$$

Integrating this equation using the separable differential principle

$$\int_{x_0}^x \frac{K_s + \left(S_0 - \frac{x - x_0}{Y} \right)}{\mu_m \left(S_0 - \frac{x - x_0}{Y} \right)} \frac{dX}{x} = \int_0^t dt \quad (43)$$

The resulting equation will be

$$\frac{(K_s Y + S_0 Y + x_0)}{\mu_m (S_0 Y + x_0)} \ln \left(\frac{x}{x_0} \right) + \frac{K_s Y}{\mu_m (S_0 Y + x_0 - x)} = t \quad (44)$$

Note that equation 944) above represents the biomass
For the case of the substrate concentration

$$\frac{(K_s Y + S_0 Y + X_0)}{\mu_m (S_0 Y + X_0)} \ln \left(1 + \frac{(S_0 - S)Y}{X_0} \right) + \frac{K_s Y}{\mu_m (S_0 Y + X_0)} \ln \left(\frac{S}{S_0} \right) = t \quad (45)$$

2.7 Correlation with Mono Model

The Monod model serves as a fundamental framework in understanding microbial growth dynamics, particularly in relation to substrate concentration and growth rate. In this research, we have established a correlation between the Monod model and the functional parameters derived from our developed equation. This correlation is particularly important for analyzing how the dilution rate interacts with various kinetic parameters. In applying the Monod model, we consider the dilution rate (D) to be equivalent to the specific growth rate (μ), as defined by the Monod equation. Consequently, this relationship implies that the equation (μ_1) can be reformulated to incorporate the effects of substrate concentration and limiting factors. By integrating the Lineweaver-Burk plot concept, we can further elucidate the dynamics of this relationship, providing a clearer understanding of how changes in dilution rates can influence microbial efficiency and metabolic responses. This detailed analysis not only enhances our understanding of microbial kinetics but also facilitates the optimization of bioprocess parameters for improved performance in industrial applications (Siddique *et al*, 2003),

$$\frac{dx}{dt} = \left(\frac{\mu m S}{K_s + S} \right) X \quad \text{Where} \quad \frac{1}{x} \frac{dx}{dt} = \mu = D \quad (46)$$

Therefore, the above expression can be written in terms of dilution rate as:

$$D = \left(\frac{\mu m S}{K_s + S} \right) \quad (47)$$

In terms of Lineweaver Burk plot

$$\frac{1}{D} = \frac{K_{sd}}{\mu m} \cdot \frac{1}{S} + \frac{1}{\mu m} \quad (48)$$

$$\frac{1}{\mu} = \frac{K_s}{\mu m} \cdot \frac{1}{S} + \frac{1}{S} + \frac{1}{\mu m} \quad (49)$$

In terms of the rate of salinity we have

$$\frac{1}{R_{sa}} = \frac{K_{sa}}{R_{sam}} \cdot \frac{1}{S} + \frac{1}{R_{sam}} \quad (50)$$

2.8 Experimental Producers

2.8.1 Sample collection

Samples were systematically collected from a saltwater environment in Bayelsa State, located within the Niger Delta region of Nigeria, which has been notably affected by various contaminants. To ensure the integrity of the samples, each was gathered in sterile 4-liter plastic containers specifically designed for environmental sampling. During the collection process, in situ measurements of several physicochemical parameters were conducted, including pH levels and temperature, to provide baseline data on the environmental conditions at the time of sampling. These parameters are critical for understanding the ecological context of the samples and assessing the degree of pollution. Following collection, the samples were promptly transported to the Department of Petroleum and Gas Engineering Laboratory at Federal University Otuoke Bayelsa State, for further analysis. Concurrently, microbial sampling was performed in the Department of Microbiology Laboratory within the same university to isolate and identify any microbial populations present, facilitating a comprehensive examination of the impacts of contamination in the saline ecosystem.

2.8.2 Physicochemical Analysis

The following physicochemical analysis methods were employed to assess the quality of the water samples: 1. pH Level: The pH was measured using method APHA 4500H+B, ensuring accurate determination of acidity or alkalinity in the samples. 2. Turbidity: Turbidity levels were assessed in nephelometric turbidity units (NTU) using standardized procedures outlined by the American Public Health Association (APHA). 3. Temperature: The temperature of the water samples was recorded using a calibrated thermometer, providing essential context for other

analytical results. 4 Electrical Conductivity: Conductivity was measured in micro siemens per centimeter ($\mu\text{S}/\text{cm}$) according to the methodology referenced in document number 1, offering insight into the ion concentration in the water. 5. Total Hardness: Total hardness was quantified in milligrams per liter (mg/l) using method APHA 2340C, which evaluates the concentration of calcium and magnesium ions. 6. Chloride Concentration. The chloride ion concentration in the samples was determined in milligrams per liter (mg/l) by applying method APHA 4500Cl-B. 7. Total Alkalinity: Total alkalinity was measured in milligrams per liter (mg/l) following ASTM standards indicated in document number 2, which assists in evaluating the buffering capacity of the water. 8. Sodium Content: Sodium levels were analyzed in milligrams per liter (mg/l) using methods APHA 3111B and ASTM D3561, providing insights into potential salinity issues. 9. Total Iron: The concentration of total iron was also evaluated in milligrams per liter (mg/l) using APHA method 3111B, which is crucial for assessing potential corrosion and water quality concerns. These analyses collectively provide a comprehensive understanding of the water's physicochemical properties, essential for evaluating its suitability for various applications.

2.8.3 Microbial Analysis

Total microbial counts were determined using a standard plate count technique, which is a widely accepted method for quantifying viable microorganisms in a given sample. Specifically, we employed the use of dipco plate count agar, a specialized medium designed to support the growth of bacteria while inhibiting the growth of unwanted microbial flora. This method, as outlined by the American Public Health Association (APHA, 1998), involves diluting the sample, inoculating the agar plates, and incubating them under controlled conditions to allow for colony development. The resulting colonies were then counted to provide an accurate measure of the total microbial population present in the sample. This approach is instrumental in assessing the sanitation and microbiological quality of various environments and products.

2.8.4 Contaminants Analysis

The extraction/spectrophotometer method was used as recommended by ASTM.

3. Results and Discussion

The data obtained from the investigation re presented in tables and figures as shown below:

Table 1: Some physicochemical parameters of the salt water environment

S/no	Component	Unit	Concentration of salt water	WHO standard
1	pH		6.46	65-8.5
2	Temperature	Oc	28	-
3	Turbidity	NTU	20.0	5
4	Conductivity	Ms/cm	9240	1000
5	Hardness	Mg/l	1042	150
6	Chroide	Mg/l	1465	250
7	Total alkalinity	Mg/l	5.08	-
8	Sodium	Mg/l	945	30
9	Total iron	Mg/l	0.44	0.01

Table 2: Experimental analysis result on substrate microbial concentration salinity consultation and theoretical computation of some functional parameters

Time (day)	Substrate conc S(ng/lg)	Microbial conc M(cfu/ml)	Salinity conc Sa(mg/l)	Dilution rate	1/S (day) ⁻¹	1/Sa (mg/l) ⁻¹	Specific rate Rs(mg/l)/day	1/D (day) ⁻¹	1/Rs (mg/l)/day ⁻¹	Rsa(mg/l ⁻¹)/day	1/Rsa (Mg/l-1/day) ⁻¹
0	100	1.72 x 10 ⁴	936	0.00	0.0100	1.068 x 10 ⁻³	-	00.0	-	-	-
1	100	4.31 x 10 ²	935	0.01	0.0100	1.0700 x 10 ⁻³	-	1000.0	-	-	1.00
2	87	2.0 x 10 ⁵	590	0.02	0.0115	1.695 x 10 ⁻³	13	50.0	7.692 x 10 ⁻²	1.000	2.899 x 10 ⁻³
3	50	8.53 x 10 ¹⁰	217	0.03	0.0200	4.608 x 10 ⁻³	37	33.3	4.762 x 10 ⁻²	345	2.681 x 10 ⁻³
4	29	4.6 x 10 ¹⁰	214	0.04	0.0345	4.673 x 10 ⁻³	21	25.0	6.250 x 10 ⁻²	373	0.333
5	13	5.97 x 10 ¹⁰	206	0.05	0.0769	4.854 x 10 ⁻³	16	20.0	2.500 x 10 ⁻¹	3	0.125
6	9	5.97 x 10 ¹⁰	200	0.06	0.1111	5.000 x 10 ⁻³	4	16,7	1.000	8	0.167
7	8	3.30 x 10 ⁶	185	0.07	0.1250	5.405 x 10 ⁻³	1	14.3	0.200	6	0.067
8	3	1.01 x 10 ⁵	153	0.08	0.3333	6.536 x 10 ⁻³	55	12.5	4.545 x 10 ⁻¹	15	0.031
9	0.8	3.6 x 10 ⁴	127	0.09	1.2500	7.874 x 10 ⁻³	2.2	11.1	1.667	32	0.038
10	0.2	8.2 x 10 ³	123	0.10	5.0000	8.130 x 10 ⁻³	0.6	10	1.667	4	0.25

The result obtained from the investigation are presented in figures and tables as shown below

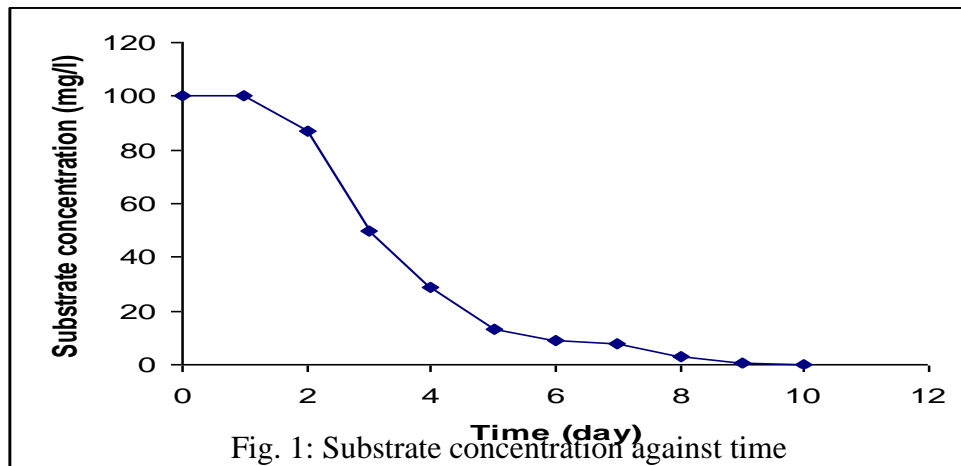


Fig. 1: Substrate concentration against time

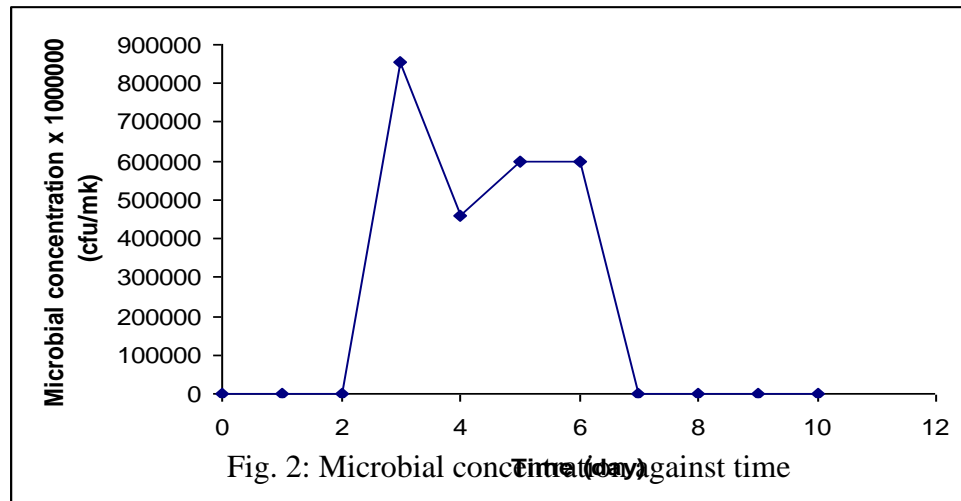
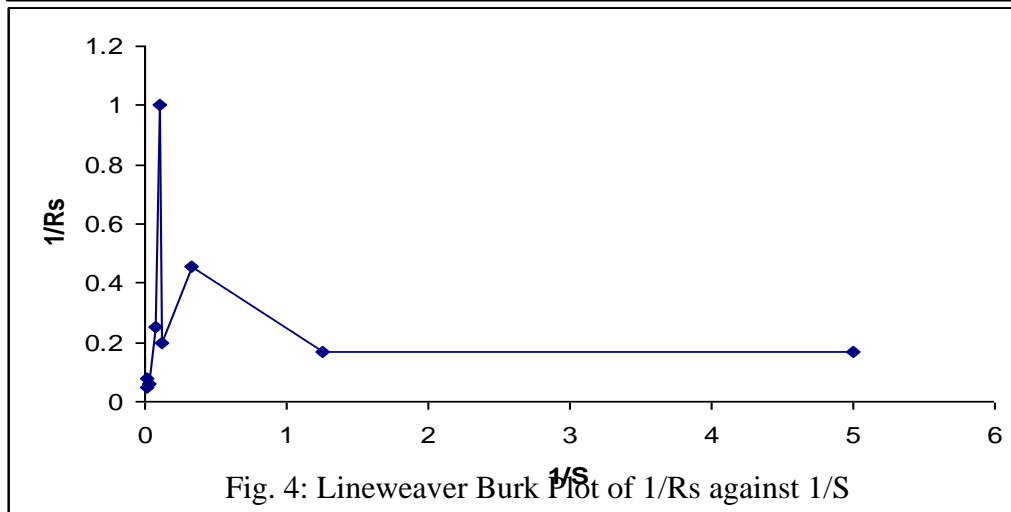
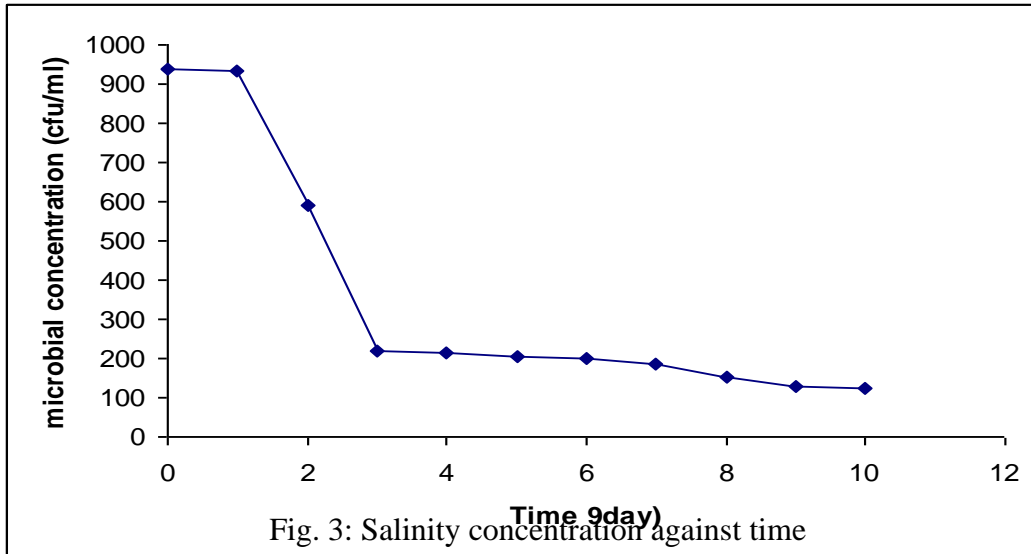


Fig. 2: Microbial concentration against time

Figure 1 illustrates the relationship between substrate concentration and time of exposure. An observable decrease in substrate concentration correlates with an increase in exposure time. This variation in substrate concentration can be directly attributed to the extended duration of exposure, suggesting that temporal factors play a crucial role in the bioavailability of the substrate for microbial activity. Furthermore, Figure 2 delineates the relationship between microbial concentration and time, highlighting distinct phases of microbial growth dynamics. The results demonstrate a typical growth pattern characterized by several key phases: lag phase, acceleration phase, progressive or exponential phase, stationary phase, retardation phase, and decline phase. Specifically, the data reveal that a notable decrease in microbial population occurs within the following timeframes: from 0 to 2 days (lag phase), from 2 to 5 days (acceleration, progressive, or exponential phase), from 5 to 6 days (stationary phase), from 6 to 8 days (retardation phase), and beyond 8 days (decline phase). This microbial population variation can be linked not only to the passage of time but also to fluctuations in salinity within the bioreactor system.



The investigation into the effects of salinity on the bioreactor system reveals that salinity concentrations tend to decrease as exposure time extends, as illustrated in Figure 3. The diminishing salinity appears to facilitate microbial growth within the system, concurrently resulting in a reduction in substrate concentration, as shown in Table 1. This variation in salinity is influenced by multiple factors, including time, microbial growth, substrate concentration, dilution rate, and other environmental conditions. The findings emphasize that salinity constitutes a critical parameter affecting the biodegradation processes occurring in saline environments. Elevated salinity levels can inhibit the active sites of microorganisms, thus reducing their metabolic activity. This alteration impacts the production of metabolic byproducts, such as increases in biomass concentration, heat generation, carbon dioxide release, and the production of water.

In conducting bioremediation efforts within saltwater environments, it is imperative to account for the salinity of the surrounding conditions, as it plays a significant role in shaping microbial activity and substrate degradation. Figure 4 presents the Lineweaver-Burk plot of $1/R_s$ (the reciprocal of the specific rate of substrate consumption) against $1/S$ (the reciprocal of the substrate concentration). This plot allows for the determination of the maximum specific rate of substrate utilization (μ_{max}) and the equilibrium constant (K_s), providing essential insights into the kinetics of substrate degradation.

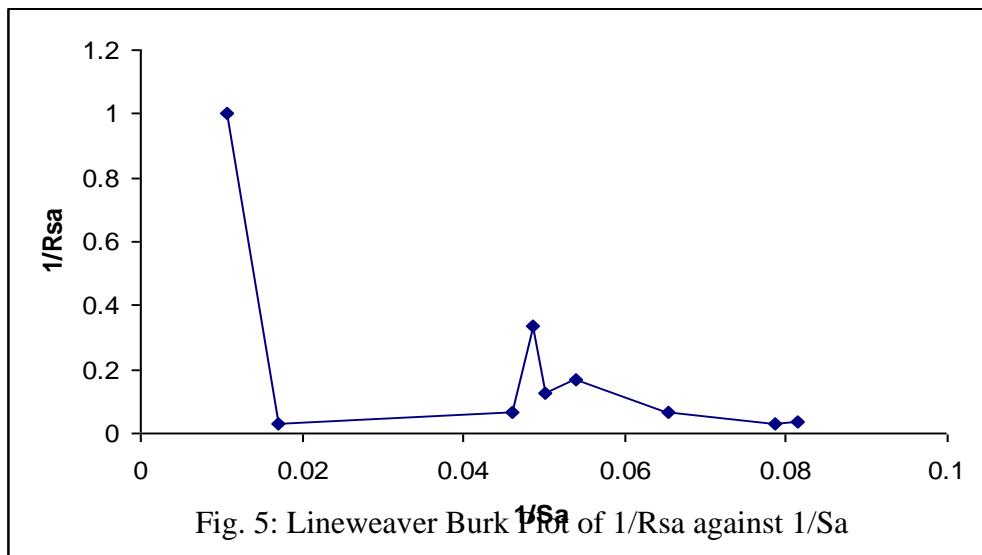


Fig. 5: Lineweaver Burk Plot of $1/R_{sa}$ against $1/S_a$

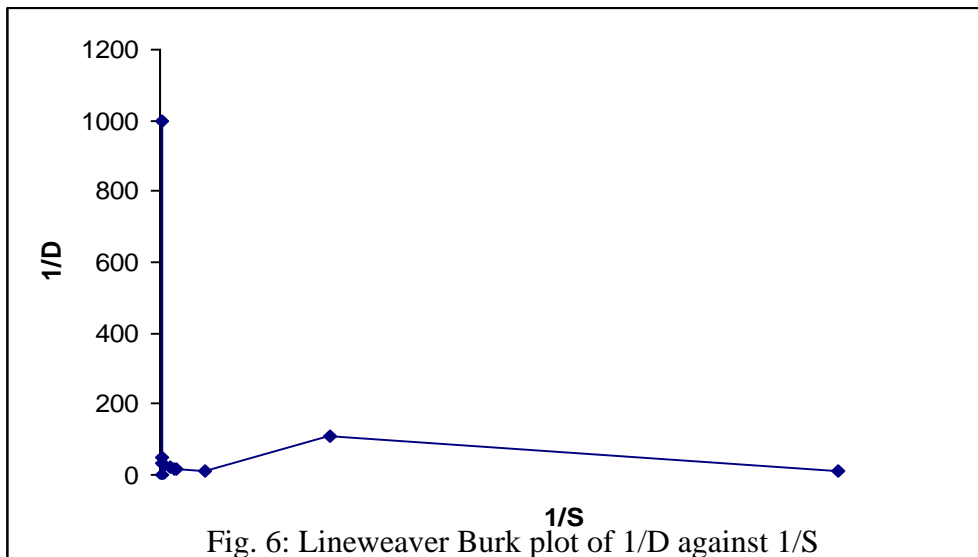


Fig. 6: Lineweaver Burk plot of $1/D$ against $1/S$

Similarly, Figure 5 illustrates the Lineweaver-Burk plot of $1/R_{sa}$ against $1/S_a$, focusing on the reciprocal relationship of salinity concentration. This plot enables the assessment of the maximum specific rate of salinity impact on microbial activity and the corresponding equilibrium constant. The variations in the reciprocal rates of both substrate and salinity

concentrations can be attributed to a myriad of factors, including time, microbial concentration, dilution rate, and other environmental conditions. The effect of dilution rate on microbial metabolism was explored further through the application of Lineweaver-Burk plot concepts, as depicted in Figure 6. This plot, which displays the relationship between $1/D$ (the reciprocal of the dilution rate) and $1/S$ (the reciprocal of the substrate concentration), facilitates an understanding of how dilution rates influence microbial growth dynamics. By analyzing the behavior of the curve, researchers can ascertain the maximum specific dilution rate and the corresponding equilibrium constant, thus enhancing our comprehension of microbial responses to varying environmental conditions in bioreactor systems.

4. Conclusion

This research has led to several significant conclusions regarding the impact of salinity on the biodegradation of contaminants in saline environments. **Salinity's Influence on Biodegradation:** It is evident that salinity levels significantly affect the biodegradation processes of various contaminants within salt water ecosystems. The unique chemical and biological properties of saline environments necessitate a tailored approach to remediation. **Microbial Activity at Varying Salinities:** A decrease in salinity has been shown to enhance certain microbial activities. Lower salinity levels create a more favorable environment for the proliferation of specific microbial populations that are essential for effective biodegradation. **Optimal Conditions for Degradation:** The degradation of contaminants occurs more efficiently when salinity levels are within a range that supports microbial growth. This optimal salinity fosters an environment conducive to microbial metabolism, leading to more effective contaminant breakdown. **Controlled Salinity in Bioremediation:** Implementing controlled salinity in bioremediation efforts has proven to be a crucial factor in enhancing effectiveness. By optimizing salinity levels, we can promote microbial health and maximize the degradation process, leading to more successful remediation outcomes. **Impact on Microbial Kinetics and Growth Phases:** Changes in salinity not only influence microbial viability but also affect the kinetics of microbial processes and their growth phases. Understanding these dynamics is critical for predicting and enhancing biodegradation rates in saline environments. **Cost Implications of Salinity Management:** The influence of salinity on bioremediation processes can lead to increased project costs. Managing salinity levels effectively may involve additional measures and resources, impacting the overall budget of environmental remediation projects. **Inhibition of Microbial Activity:** High salinity concentrations can inhibit the active sites of microbes, reducing their ability to function effectively. This inhibition can result in decreased rates of contaminant degradation and poses a challenge for bioremediation efforts. **Toxicity of High Salinity Substrates*:** Elevated salinity not only affects microbial activity but can also render certain substrates more toxic. This increased toxicity can further complicate the remediation process, necessitating careful consideration of salinity levels in project planning. Overall, these findings underscore the intricate relationship between salinity and microbial processes in the biodegradation of contaminants. Understanding and managing salinity is vital for optimizing bioremediation strategies in salt water environments.

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