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Activation Energy, Temperature Coefficient and Q_{10} Value Estimations of the Growth of an SDS-degrading Bacterium

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ABSTRACT

This research delves into how temperature affects the growth rates of bacteria in relation to the breakdown of sodium dodecyl sulfate (SDS) by types of microbes. A graph resembling a Chevron pattern, which plots the growth rate against temperature (1/T), revealed a change at 32.19 °C, indicating a crucial temperature for optimizing bacterial growth. Through regression analysis, activation energies were determined to be 65.10±10.83 kJ/mol for temperatures between 20 30°C and 36.31±0.97 kJ/mol for temperatures between 35 45°C showing that metabolic demands are higher at temperatures. The theta value was calculated to be 1.06, which closely aligns with known values for processes hinting at moderate temperature sensitivity. These results highlight the impact of temperature on microbial metabolic efficiency, requiring energy at lower temperatures due to increased enzyme activity demands while operating more efficiently at higher temperatures with lower energy needs. This study emphasizes incorporating temperature-related factors like Q10 and theta values into models that predict bioremediation strategies. Understanding these dynamics can improve the success of bioremediation efforts by ensuring breakdown in diverse environmental conditions. The insights gained from this research can inform management practices. Advance the development of biotechnological applications sensitive to temperature variations.

INTRODUCTION

When conducting a study on the bacteria responsible for the process of chemical decomposition, temperature is an essential component to consider. Applying the temperature function, the Arrhenius model is usually practiced to calculate the apparent activation energy, H*, which is believed to be present for either growth or decay on different metabolic substrates. The temperature function Arrhenius model is gaining popularity as a tool for analyzing the growth and decay rates of bacteria, and this trend is expected to continue in the foreseeable future [1–9]. The value of delta H, denoted by ΔH*, remains rather stable during the vast portion of the temperature gradient. When applied to extremely high temperatures, this value can change by a factor of three or four, depending on the temperature range [10]. In some studies, the model may be incorrect when applied to the entire temperature range of the bacterial process [11].

Arrhenius' model is frequently employed for simulating temperature effects; however, when the temperature ranges are vast, this model is utilized significantly less frequently [12]. The Arrhenius plot may also show a previously identified transition, which is a fast shift in activation energy [13]. Because Arrhenius' model includes the fewest parameters, researchers widely accept it [12]. Because of its biological underpinnings and non-linear behavior this rival Ratkowsky model is also based on the linear growth premise but lacks constant development [14]. This indicates that the Arrhenius models are necessary to determine

how temperature influences the development of bacteria. In order to acquire an estimate of the Arrhenius parameter, an Arrhenius plot must first be plotted, and then linear regression must be performed on the data. Arrhenius plot analysis and the effect of temperature on SDS-degrading bacterium's SDS growth were the subject of a similar study a number of years ago [4]. This study revealed something previously unknown: a bacterium can break down SDS at several different activation energies. This information will be beneficial in predicting how SDS breakdown and transport will occur during bioremediation, as it will help determine how SDS will be broken down and transported.

MATERIALS AND METHODS

Growth and maintenance of Bacterium and measurement of the Activation energy of growth on SDS

Enterobacter sp. strain Neni-13 growth and characterization on SDS has been published previously and stored in the university's culture collection unit [15]. The growth data obtained from the Bacterium that degrades SDS was analyzed by transforming the growth rates, which are dependent on temperature, into natural logarithms.

The Arrhenius equation [16] is as follows,

$$\mu = Ae^{-\frac{E_a}{RT}} \qquad \text{[Eqn. 1]}$$

Where R signifies universal gas constant (0.008314 kJ/molK^{-1}), T signifies absolute temperature (Kelvin = ºC + 273.15), E_a signifies activation energy (kJ/mol) and A physically signifies the rate constant at which all the participating molecules possess sufficient energy prior reaction (E_a = 0). A linearized form is given via the plot of log normal growth rate against 1/T and the equation is as follows;

$$\ln \mu = \ln A - \frac{E_a}{R} \cdot \frac{1}{T} \qquad \text{[Eqn. 2]}$$

Coefficient of Q_{10} estimation

The Q_{10} value is estimated via the following equation.

$$Q_{10} = e^{\left(\frac{Ea}{R}\right)\left(\frac{10}{T_2 T_1}\right)} \qquad \text{[Eqn. 3]}$$

Subsequent rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R}\right)\left(\frac{1}{T_1 T_2}\right) \qquad \text{[Eqn. 4]}$$

The coefficient of temperature, often known as the theta (θ) value, is another important biological constant that may be calculated by substituting the supplied values into the equation for reaction rates, which is regulated by the Q10 rule (simplified Arrhenius temperature coefficient);

$$kT = k20\Theta\,(T-20) \qquad \text{[Eqn. 5]}$$

RESULT AND DISCUSSION

The temperature affects the pace of bacterial growth. The highest rate of bacterial growth on SDS was observed to occur at the theoretical temperature of 35, and this rate decreased as the temperature rose (**Fig. 1**). A graph that looked like a Chevron was produced when the logarithmic growth rate (per day) was compared to the temperature in the form of 1/T. This graph showed that there was a break in the curve over the whole temperature range (**Fig. 2**). The breaking point was located at 32.19 degrees Celsius. According to the results of the regression analysis presented in **Table 1**, the activation energy required for growth on SDS in the range 20-30 °C and 35-45 °C were 65.10±10.83 and 36.31±0.97 kJ/mol, respectively. Based on the data, from **Table 1** regression analysis, it was observed that the energy needed for growth on SDS varied notably between temperature ranges of 20 30°C and 35 45°C. Specifically the energy required for growth at 20 30°C was measured at 65.10±10.83 kJ/mol while at 35 45°C it stood at 36.31±0.97 kJ/mol. These results imply that microbial activity demands energy at temperatures (20 to 30°C) compared to higher temperatures (35 to 45°C).

The higher activation energy needed at temperatures can be explained by the increased metabolic needs necessary to sustain functions and enzyme operations in more relaxed environments. Enzymes responsible for breaking down SDS are less effective in climates requiring energy input to maintain metabolic processes than in warmer conditions. This aligns with enzyme kinetics principles, where enzymatic reaction rates typically drop with temperatures due to reduced movement and interactions between enzymes and substrates [12]. Conversely, the decreased activation energy required at temperatures signifies efficient microbial activity as enzymes involved in SDS breakdown function optimally under warmer circumstances.

These results support the accepted idea that many enzymes produced by microorganism's work within specific temperature ranges, enhancing their ability to drive metabolic processes more efficiently. These discoveries carry implications for bioremediation initiatives in settings prone to temperature changes. Recognizing the energy requirements at temperatures can assist in forecasting how effectively SDS breakdown will occur in environmental conditions. It also underscores the importance of regulating temperature in bioremediation approaches to ensure function and pollutant decomposition.

The results of this study using the Arrhenius model indicate that the activation energies fall within a range, which is what has been reported in the literature for various biodegradation processes (refer to Table 2). This implies a relationship between temperature increase and reduced energy needed for activity. Many studies on activation energy typically present a value across a temperature range rather than identifying multiple activation energies at different ranges. This is often due to the need for research to accurately determine these values. The estimation of activation energy heavily relies on the metabolic process rates at varying temperatures. Some research suggests that activation energy is higher at temperatures while other studies show the trend with higher values at lower temperatures. These differences can be attributed to metabolic pathways and the microbial communities involved in biodegradation processes. The results of this study support the idea that activation energy is influenced by temperature and varies across ranges (see Table 2).

The findings from this study emphasize how intricate microbial metabolism is and how temperature impacts biodegradation efficiency. The discovery of varying activation energies, across temperature ranges underscores the importance of conducting investigations to comprehend microbial processes

comprehensively. It also highlights the importance of customizing bioremediation approaches to accommodate temperature fluctuations and maximize activity and contaminant breakdown. In summary, the activation energies derived from the Arrhenius model in this research align with the figures documented in existing literature on biodegradation processes.

The link between rising temperatures and reduced energy consumption implies that optimizing temperature conditions can boost the effectiveness of bioremediation endeavors. More investigations are needed to fully grasp the temperature-related aspects of activation energies and formulate strategies for remediation (**Table 2**). An example is the growth of *Bacillus* sp. JF8 on the xenobiotic polychlorinated biphenyl (PCB) with an activation energy from 20 to 46 ºC of 12.1 Kj/mol and from 50 to 70 ºC the activation energy is 31.4 Kj/mol [17]. A contrasting study shows the growth on phenol from 15 to 30 ºC by *Pseudomonas* sp. AQ5-04 of 38.92 Kj/mol and from 35-45 ºC, the activation energy is 11.34 Kj/mol [18].

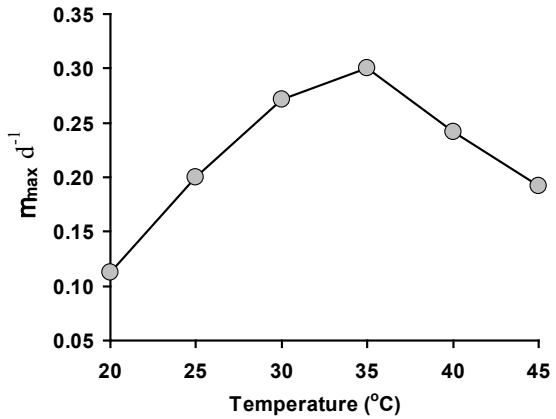

Fig 1. The effect of temperature on the specific growth rate of *Enterobacter* sp. strain Neni-13 on SDS.

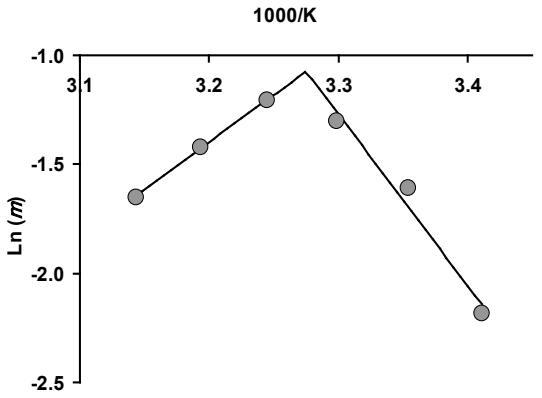

Fig 2. Growth rate of SDS by *Enterobacter* sp. strain Neni-13 in the form Arrhenius plot.

Table 1. The Arrhenius plot of the biodegradation rate of SDS by *Enterobacter* sp. strain Neni-13 subjected to regression analysis.

Distribution of the experimental points	Three points to the left, three points to the right
	Left part
Temperature range ºC	35 to 45
Regression equation	y = 4.361 - 15.379
Coefficient of determination	0.999280553
tan α ± Standard error	4.37 ± 0.12
E_a ± Standard error, kJ mol^{-1}	36.31 ± 0.97
t-Statistic	37.27
Degrees of freedom	2
	Right part
Temperature range ºC	20 to 30
Regression equation	y = -7.8341x + 24.582
Coefficient of determination	0.973062077
tan α ± Standard error	-7.83 ± 1.30
E_a ± Standard error, kJ mol^{-1}	65.10 ± 10.83
t-Statistic	-6.01
Degrees of freedom	2
	Break point data
Intersection coordinates, (x, y)	3.275
Break point temperature ºC	32.19

When it comes to how bacteria behave, temperature plays a role, in influencing aspects of cell functions. Temperature impacts not the speed of metabolic processes but also the folding, structure and stability of biomolecules. These temperature related effects are essential for the survival and adjustment of microbes in their surroundings. Microorganisms need to be able to detect and react to temperature changes to regulate their metabolic activities accordingly. Pathogenic bacteria specifically have developed ways to sense the presence of a host and adjust their metabolism as necessary to deal with fluctuations in temperature organisms have evolved sensing mechanisms that indirectly perceive these shifts enabling them to adjust gene expression as required. This regulation of genes based on temperature is vital for survival and pathogenicity. For instance, responses like heat shock and cold shock are strategies that bacteria employ to handle temperature variations. During a heat shock event bacteria might accumulate ribosomes while a cold shock can cause proteins to aggregate into a state. Both responses are critical for sustaining functions under conditions.

The capacity to detect and respond to temperature changes isn't exclusive to pathogenic bacteria. Many environmental bacteria also possess mechanisms that help them thrive in different, ever-changing environments. Understanding these processes is crucial for creating strategies to manage growth in environmental settings. For example, the heat shock response triggers the production of heat shock proteins (HSPs) that aid in repairing damaged proteins and shielding cells from heat related harm [36]. On the hand during shock bacteria might generate cold shock proteins (CSPs) to help stabilize nucleic acids and ribosomes ensuring proper cell function [37]. In essence regulating temperature plays a role in physiology by influencing metabolic rates, protein stability and overall cell function. The capability of bacteria to detect and react to temperature variations is essential for their survival and adaptation to environments. This understanding is crucial, for developing targeted strategies to manage growth and tackle challenges associated with infections and environmental pollution [2,3,38–43].

Table 2. Arrhenius temperature characteristics for growth on numerous xenobiotics.

Microorganisms	Temperature range (°C)	Substrate	*ΔH**apparent activation energy (Kj.mol^{-1})	Q_{10}	Theta	Ref
Enterobacter sp. strain Neni-13	20-30 35-45	SDS	65.10±10.83 36.31±0.97	2.23	1.06	This study
activated sludge	10–20	phenol	39.0			[19]
Selanastrum capricornutum	20–28	phenol	28.4			[20]
aerobic fluidized-bed reactors (FBRs)	14-16.5	2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), and pentachlorophenol (PCP)	TCP and TeCP 126-194 PCP 59-130			[21]
Pseudomonas putida Q5	10–25	phenol	61.6			[12]
Acclimated cultures	15-30	nonylphenol	42.7		1.06	[22]
Pseudomonas putida MTCC 1194	15-30	phenol	57.74			[23]
Bacillus sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20–46 °C) 31.4 (50–70 °C)			[17]
Pseudomonas sp. AQ5-04	15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	1.834		[18]
Pseudomonas sp. Strain DrYJ7	10-20	SDS	14.96	2.17	1.03	[4]
Cupriavidus sp. strain CNP-8	20-40	2-chloro-4-nitrophenol	75.16 88.71			[24]
Escherichia coli BL21	20-50	Chromate	28.01			[25]
Ochrobactrum intermedium	25-35	Chromate	120.69			[26]
Shewanella oneidensis·MR-1	25-40	Selenate	62.90 (Control system) 47.33 (TPPS-supplemented system)			[27]
anaerobic sludge	30-55	Reactive Red 2	22.9			[28]
activated bacterial consortium	20-37	Remazol Black B	48.8			[29]
Enterobacter sp. strain (GY-1)	20-35	Reactive Black 5 (RB 5)	35.56			[30]
Escherichia coli NO3	20-45	Reactive red 22	27.49			[31]
Pseudomonas aeruginosa	15-45	Reactive Black 39 and Acid Red 360 by	61.89 (RB39) 81.18 (AR360)			[32]
Pseudomonas sp. LPM-410	20-28	EDTA	91.2			[33],
Cupriavidus sp.	20–25 30–40	2-chloro-4-nitrophenol	88.71 (20-25) 75.16 (30-40)			[5]
Gram positive unidentified bacterium	20-27 30-42	2,4-dinitrophenol	41.72 (20-27) 84.72 (30-42)	2.905	1.11	[34]
Bacillus circulans strain Neni-10	20-30 35-45	Metanil Yellow	62.07 (20-30) 30.93 (35-45)	1.46	1.04	[35]

Note: (TPPS) Meso-tetrakis (4-sulfonatophenyl) porphyrin mediator

Molecular thermosensors can be created using switches as elements. These sensors are capable of sensing changes, in temperature through mechanisms. One method involves modifying the structure of molecules to cause functioning. Another technique involves using proteins that react to temperature changes or membrane alterations due to fluidity shifts. These proteins are sensitive to fluidity and play a role in preserving functions across varying temperatures. Maintaining temperature is crucial for processes and its significance cannot be emphasized enough. This concept holds promise for temperature-controlled biotechnology applications, where precise temperature regulation can optimize metabolic functions and improve processes. Temperature variations trigger a variety of metabolic adjustments, many of which are interconnected and can be seen as part of a process due to their association.

It is essential to develop models that explain how biological processes adapt with temperature changes to understand these relationships. Such models aid in simplifying our comprehension of these processes and their interdependencies. In the 1900s, Svante Arrhenius devised a model called the Arrhenius equation to illustrate how biological reactions react to fluctuations in temperature. This "primary temperature model" offers a framework for forecasting the behavior of reactions under diverse thermal conditions. Arrhenius's theory suggests that as temperature rises, the speed of a reaction also increases significantly until it reaches a point where the reaction rate stabilizes or decreases due to factors like enzyme damage or temperature constraints. This theory has explained how temperature impacts physiological functions and remains an essential tool in studying thermodynamics within biological systems. In essence, utilizing switches in thermosensors shows promise for detecting changes in temperature within biological systems. It is vital to comprehend and model the impact of temperature on processes as illustrated by the Arrhenius equation further to advance our understanding and application of temperature-controlled biotechnology. [39–43].

Bacteria require more energy when the activation energy is higher because they must break down more complex xenobiotics, leading to an increased activation energy. According to the findings of this study, the activation energies of various microbial species capable of degrading xenobiotics fall within the ranges reported for both temperature ranges in this investigation, as shown in **Table 2**. These findings align with the broader literature, which indicates that the activation energies for different microbial species involved in xenobiotic degradation are comparable to those observed in this study. In contrast, the

activation energies of typical mesophilic bacteria generally range from 33.50 to 50.30 kJ/mol. This suggests that the bacteria studied here may be well adapted to breaking down more complex compounds, requiring higher activation energies for effective degradation.

Understanding these activation energy requirements is crucial for optimizing bioremediation processes, as it highlights the importance of selecting microbial species that are not only capable of degrading specific xenobiotics but also effective under given temperature conditions. The higher activation energies observed in some bacteria underscore the metabolic demands placed on these organisms when degrading more complex pollutants, which can inform strategies to enhance bioremediation efficiency by manipulating environmental conditions or employing consortia of microbial species with complementary metabolic capabilities. In summary, the study's findings on activation energies provide valuable insights into the metabolic challenges faced by bacteria degrading xenobiotics.

The observed ranges of activation energies align with those reported in the literature for mesophilic bacteria, offering a basis for further research and application in environmental biotechnology [44]. This research noted that the activation energy varied at temperature ranges, showing that some ranges had activation energies, then others. This discovery aligns with a study by [39] indicating that activation energy fluctuates with temperature rather than staying constant. Due to these fluctuations, it is recommended to use the model as a predictive tool since it cannot consider all the simultaneous interactions in diverse biological systems. To better understand activation energy, it is crucial to view it within the context of a microorganism's temperature response than just as the energy needed for chemical reactions. This broader perspective can offer insights into how microorganisms adapt and operate in varying conditions. By focusing on temperature responses, researchers can enhance their understanding of metabolism complexities and the factors that affect activation energy.

This method emphasizes the changing nature of responses to temperature variations. Stresses the significance of considering environmental and biological variables when studying activation energy. It also suggests that future models should integrate an array of factors to predict behavior more accurately in various thermal settings. It's essential to grasp these connections for use in biotechnology as adjusting conditions for function can boost activities like bioremediation. To sum up the differences in activation energy, at temperature levels highlight the importance of taking an approach to investigating how microbes react to temperature. By considering activation energy within the context of temperature reactions we can gain insight into microbial adjustments and enhance the precision of models applied in both research and real world scenarios [45].

Notwithstanding these limitations, the model is nevertheless used in a wide variety of contexts. In many different contexts, including the decolorization of different dyes by different organisms, temperature has been shown to affect the activation energy of microorganisms. The results of other trials corroborate this (Table 2). Two possibilities could account for the alteration, even though the exact mechanism that triggers it is yet unknown. Here are the choices: Changes in water properties accompany state transitions, and one idea, the "bottleneck," proposes that these changes occur rapidly and concurrently [46]. Given the results of several observations regarding the temperatures at which the Arrhenius break point occurs, it appears that the first theory is not correct. There are a lot of different reasons why it's so hard to prove the "bottle-neck" hypothesis, as stated by the "bottle-neck" concept. Any time the temperature changes, it will create a shift in the cell membrane due to the effect of temperature on the membrane [47]. Many scholars continue to believe in the "bottleneck" concept [13,48].

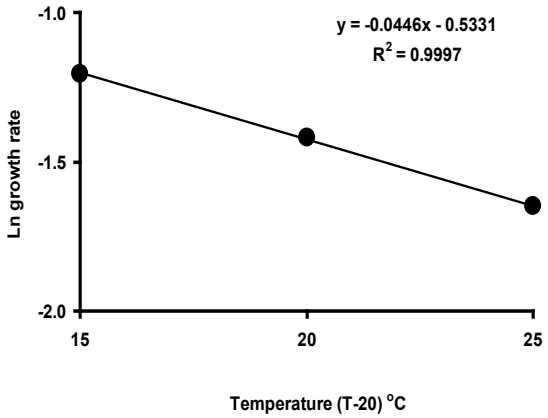

Fig. 3. Ln growth rate vs temperature plot for estimating theta.

One alternative is to measure the growth rates at a variety of incubation temperatures that varied by ten degrees. Another is to use the Arrhenius plots to calculate the Q_{10} values [49]. When plotting bioreduction and growth rates logarithmically versus temperature in a temperature logarithm form, the resulting curve is known as the Arrhenius curve (Kelvin) (**Fig. 1**). For temperatures between 30 and 42 degrees Celsius, a Q_{10} value of 2.223 was observed (Fig. 3). Nevertheless, the dynamic nature of biological processes results in the existence of several Q_{10} values within any given temperature range. A value of 2.038 was observed in the conversion of molybdate into molybdenum blue [50], while a Q_{10} value of 2.31 was found in *Morganella* sp, yet another molybdenum reducer. GPT

To accurately credit the development process to a particular biological activity, it is crucial to comprehend the activation energy and its corresponding temperature response. Degradation rates for other petrochemical compounds affected by salinity were reported to exhibit a Q_{10} value of 2.2 [51]. For example, the Q_{10} value, which represents the rate at which biological activities change with a 10°C rise in temperature, offers an understanding into microbial activity across various thermal conditions. A previous study investigating oil breakdown in a column of beach gravel found a Q_{10} value of 2.7. This indicates that the rate of oil degradation multiplied by 2.7 for every 10°C increase in temperature, emphasizing the substantial influence of temperature on microbial degradation processes.

A comprehensive understanding of these parameters is essential for effectively modeling and optimizing bioremediation techniques. High Q_{10} values, such as those seen in studies on oil degradation, indicate that even small temperature increases can greatly stimulate microbial activity, resulting in more effective degradation of contaminants. Hence, including Q_{10} and other temperature-related characteristics in predictive models can enhance the development of more efficient environmental management and remediation strategies [52]. Nevertheless, another study on decane and toluene-contaminated soil reported a Q10 value of 2.2 for microbiological process [53]. Declining temperatures frequently lead to an increase in the Q_{10} value [54,55]. The Q_{10} value for the phenol degradation by

Pseudomonas sp. strain AQ5-04 was 1.834 [18] while a Q_{10} value of 2.17 was calculated for the growth rate of this organism on SDS. A lower Q_{10} value of 2.17 is reported in another study on acrylamide biodegradation by the Antarctic Bacterium *Pseudomonas* sp. strain DRYJ7 [4].

The theta value was determined to be 1.06 (**Fig. 3**), which is close to the theta value of 1.08 found for molybdenum reduction by *Serratia* sp. strain HMY1 [50]. In contrast, a lower theta value of 1.03 is reported for the growth rate on acrylamide by the Antarctic Bacterium *Pseudomonas* sp. strain DRYJ7 [4]. While there are reports of xenobiotics being broken down with theta values as high as 16.2, theta values typically range between 1.1 and 1.7, which is within the normal range for many biological processes. This range indicates that most biological reactions experience moderate increases in reaction rates with rising temperatures, consistent with the metabolic activities of various microorganisms. The theta value is a crucial parameter for understanding the temperature sensitivity of microbial processes. A theta value of 1 suggests that the biological activity is relatively stable across the studied temperature range. In comparison, higher theta values indicate greater sensitivity to temperature changes, which can significantly impact the efficiency of biodegradation processes [5]. Understanding these values helps predict and optimize microbial activity in various environmental and biotechnological applications.

CONCLUSION

This research emphasizes how temperature significantly affects the growth rate of bacteria, especially when breaking down substances, like SDS. The graph resembles a Chevron pattern, which plots the growth rate against temperature (1/T) and shows a point at 32.19°C that optimizes bacterial growth. Through regression analysis, it was found that the energy needed for growth on SDS varies significantly across different temperature ranges: 65.10±10.83 kJ/mol for 20 30°C and 36.31±0.97 kJ/mol for 35 45°C. These results suggest that bacterial metabolism requires energy at temperatures due to increased demands to maintain enzyme activity in colder conditions, while at higher temperatures, microbial activity becomes more efficient with reduced activation energy. The study also identified a theta value of 1.06, which closely matches values seen in processes and indicates moderate sensitivity to temperature changes. This aligns with reactions where reaction rates tend to increase moderately as temperatures rise. Understanding these factors is essential for enhancing bioremediation processes by guiding the selection of species and environmental conditions to improve degradation efficiency. The relationship between temperature and activation energy highlights the importance of customizing bioremediation approaches to accommodate changes in temperature. By integrating factors like Q_{10} and theta values into models we can improve the success of bioremediation initiatives. This thorough grasp of how microbes respond to temperature will assist in creating environmental management strategies and enhancing breakdown across different environments.

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