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Activation energy, Temperature Coefficient and Q_{10} Value Estimations of the Growth of *Alcaligenes* sp. YLA11 on Diesel

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ABSTRACT

Several models can be used to mimic the temperature-dependent growth rate of microorganisms on different media. Arrhenius is a popular model because of its small number of parameters. Microbial growth and metabolic activity on their substrates are generally affected by temperature. Microbes are vulnerable to temperature changes because of their small size. With a chevron-like discontinuous graph of apparent activation energy and a breakpoint, growth on diesel by *Alcaligenes* sp. YLA11 is described showing a breakpoint at 28.05 °C. Regression analysis resulted in two activation energies: 20–27 °C and 30–42 °C with the activation energies of 41.72 kJ/mol and 84.72 kJ/mol, respectively. For the examined temperature range (30-42 °C), a Q_{10} value of 2.905 and a theta value of 1.11 was calculated. Predicting the breakdown of diesel and its movement during bioremediation is an output that can be predicted by parts of this study.

INTRODUCTION

Physical, chemical, and biological methods can all be used to clean up a diesel-contaminated environment. Physicochemical methods can be expensive, as it necessitates the use of a lot of specialized equipment and especially in soil-contaminated areas. Even though the chemical method of treating diesel contamination is widely accepted, it still leaves chemical residues or sludge behind that must be cleaned up. If chemical residues are not handled properly, they can have additional negative effects on ecosystems, including environmental pollution, health issues for animals and humans, and loss of biodiversity. Both physical and chemical treatment have their drawbacks, and biological treatment is seen as a promising solution. Hydrocarbon polluted soil and oil spills in the ocean can also be treated with this method [1–9].

Diesel can be degraded by microorganisms using it as a carbon source. A common bioremediation agent for diesel is bacteria, which are microscopic organisms. Diesel can be completely broken down by a variety of bacteria into harmless outputs such as CO_2 and H_2O. Diesel-degrading bacteria isolated from diesel-contaminated areas are said to have a different metabolism that makes them more efficient. Determining the mechanism by which bacterial isolates degrade diesel fuel may lead to improved application in real-world contaminated settings. We can achieve a high removal efficiency by learning about the factors that influence the treatment process, including microorganism type, diesel solubility, pH (pH), temperature (temperature), oxygen (oxygen), nutrients (nutrients), and the level of salinity (salinity) [10–15].

Because of their small size, microorganisms are especially vulnerable to extreme temperatures. Temperature has an effect on physiology, allowing organisms to better adapt to their ever-changing environments as a result. The temperature at which a substance decomposes is a critical factor to consider. It has been widely used in the study of bacterial growth and rates and is frequently used to measure the apparent activation energy, ΔH^*, which is thought to exist for either growth or decay in different metabolic substates. The value of delta H (ΔH^*) is nearly constant in most temperature ranges. " However, depending on the range of temperatures being examined, this number can vary by three or four times when dealing with extreme temperature variations [16]. When applied to the entire bacterial process temperature, the model may be inaccurate, according to some studies [17]. The Arrhenius model is frequently used for

simulating temperature effects in a narrow temperature range, but it is rarely used for larger temperature ranges [18]. When plotting activation energy, the Arrhenius curve may show a previously discovered transition in activation energy [19]. In terms of parameters, the Arrhenius model is the simplest and most widely accepted by scientists [18]. When it comes to the Ratkowsky model's linear growth assumptions and non-linear behavior due to its biological foundations, the Ratkowsky model falls short [20]. That is to say that because of this, the Arrhenius models are used to better understand how temperature affects bacterial growth. The Arrhenius parameter estimate is obtained by plotting an Arrhenius plot and then performing a linear regression on the data. Arrhenius plot analysis and the effect of temperature on *Pseudomonas* sp. strain DRYJ7's acrylamide growth were the subject of a similar study a number of years ago [21].

A bacterium's ability to break down diesel can be activated by a wide range of energies. To understand the principles behind bioremediation, as well as the breakdown of diesel and the movement of diesel, it is fascinating.

MATERIALS AND METHODS

Activation energy of growth on diesel

Biodegradation rate data from *Alcaligenes* sp YLA11 previously isolated [22] was processed by transferring the growth values at each temperature to natural logarithm. The Arrhenius equation [23] is as follows,

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

Where R is the universal gas constant (0.008314 kJ/molK^{-1}), T is the absolute temperature (Kelvin = °C + 273.15), E_a is the activation energy (kJ/mol) and A actually indicates the rate constant prior to which the molecules involved have sufficient energy to carry out the 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;

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

Following rearrangement,

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

An additional important biological constant, theta (Θ), can be obtained by substituting the obtained values into the reaction rates equation under the Q_{10} rule, and it is a simple Arrhenius temperature coefficient;

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

RESULT AND DISCUSSION

At 27 °C, the yeast's growth rate reaches its maximum, and as the temperature rises, the yeast's growth rate diminishes. (**Fig. 1**).We obtained a Chevron-like graph when we plotted ln μ_m versus 1/T, revealing a discontinuous curve throughout the entire temperature range (**Fig. 2**). An interesting finding was the presence of break point at 28.05 °C. Regression analysis results shown in **Table 1** suggest that in the lower temperature range of 20–27 °C, growth on diesel had an activation energy of 41.72 kJ/mol, whereas at the higher temperature range of 30–42 °C, it had an activation energy of 84.72 kJ/mol. It was previously found that *Pseudomonas* sp. DrYJ7's growth rate on another xenobiotic; acrylamide had an activation energy of 14.96 kJ/mol between 10 and 20 °C [21], which is much lower. For various xenobiotic biodegradations, our Arrhenius model activation energy was within the published literature's range (**Table 2**).

Breaking the bonds seems to take more effort. It takes less energy to raise the thermostat setting. Most reports on activation energy based on metabolism rate only mention the presence of a single activation energy that is consistent across a wide temperature range. In one study, activation energy is higher at higher temperatures than at lower temperatures, whereas in another study, activation energy is lower at higher temperatures than at lower temperatures (**Table 2**). An example is the growth of *Bacillus* sp. JF8 on polychlorinated biphenyl (PCB) where the activation energy was 31.4 kJ/mol from 50 to 70 °C and 12.1 kJ/mol from 20 to 46 °C and [24]. In another contrasting study, the growth on phenol by *Pseudomonas* sp. AQ5-04 shows an activation energy of 38.92 kJ/mol from 15 to 30 °C and 11.34 kJ/mol from 35-45 °C [25].

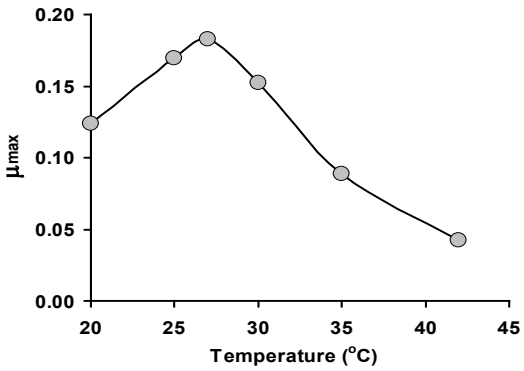

Fig 1. The effect of temperature on the specific growth rate of *Alcaligenes* sp. YLA11 on diesel.

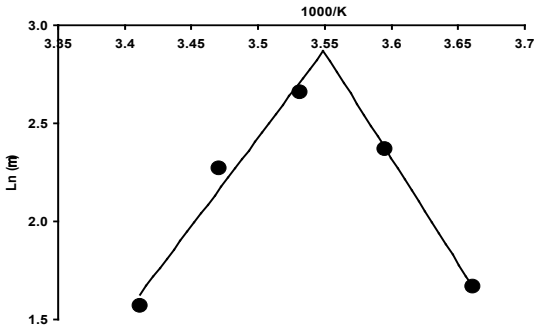

Fig. 2. Arrhenius plot of the diesel biodegradation rate by *Alcaligenes* sp. YLA11.

Table 1. The two-part linear regression analysis for the Arrhenius plot of diesel biodegradation rate by *Alcaligenes* sp. YLA11.

Distribution of the experimental points	Three points to the left, three points to the right
	Left part
Temperature range °C	30, 35 and 42
Regression equation	y=10.195x - 35.508
Coefficient of determination	0.97
tan a ± Standard error	10.19±0.05
E_a ± Standard error, kJ/mol	84.72±0.41
t-Statistic	205.01
Degrees of freedom	2
	Right part
Temperature range °C	20, 25 and 27
Regression equation	y = -5.0207x + 15.043
Coefficient of determination	2
tan a ± Standard error	-5.02±0.47
E_a ± Standard error, kJ/mol	41.72±3.89
t-Statistic	-10.72
Degrees of freedom	2
	Break points data
Intersection coordinates, (x, y)	3.32, -1.642
Break point temperature °C	28.05

Table 2. Arrhenius temperature characteristics for growth on phenol and phenolics.

Microorganisms	Temperature range (°C)	Substrate	*ΔH**apparent activation energy (kJ.mol^{-1})	Ref
activated sludge	10–20	phenol	39.0	[26]
Selanastrum capricornutum	20–28	phenol	28.4	[27]
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	[28]
Pseudomonas putida Q5	10–25	phenol	61.6	[18]
Acclimated cultures	15-30	nonylphenol	42.7	[29]
Pseudomonas putida MTCC 1194	15-30	phenol	57.74	[30]
Bacillus sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20–46 °C) 31.4 (50–70 °C)	[24]
Pseudomonas sp. AQ5-04	15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	[25]
Pseudomonas sp. Strain DrYJ7	10-20	acrylamide	14.96	[21]
Cupriavidus sp. strain CNP-8	20-40	2-chloro-4-nitrophenol	75.16 88.71	[31]
Escherichia coli BL21	20-50	Chromate	28.01	[32]
Ochrobactrum intermedium	25-35	Chromate	120.69	[33]
Shewanella oneidensis·MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[34]
anaerobic sludge	30-55	Reactive Red 2	22.9	[35]
activated bacterial consortium	20-37	Remazol Black B	48.8	[36]
Enterobacter sp. strain (GY-1)	20-35	Reactive Black 5 (RB 5)	35.56	[37]
Escherichia coli NO3	20-45	Reactive red 22	27.49	[38]
Pseudomonas aeruginosa	15-45	Reactive Black 39 and Acid Red 360 by	RB39 61.89 AR360 81.18	[39]
Pseudomonas sp. LPM-410	20-28	EDTA	91.2	[40],

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

It takes a lot of energy for the bacterium to metabolize phenolics at high activation energies. Activation energies for numerous xenobiotic degradation processes are within this study's temperature range, as shown in **Table 2**. Microbial species. Mesophilic bacteria's activation energy, on the other hand, ranges from 33.5 to 50.3 kJ/mol [41], Indicating that one of the temperature ranges tested in this study has a higher activation energy. With temperature, activation energy was discovered to change rather than being a constant [42]. The model can be thought of as an observational model, despite the fact that we are unable to provide an accurate estimation of all of the interacting complex biological processes that are occurring at the same time. Therefore, one should not think of activation energy as the activation energy that is used in chemical processes; rather, one should think of activation energy as the total temperature response of the microorganism [43].

Despite all of these issues, the model is still widely used all over the world. It has been shown in a variety of conditions using a variety of substrates, such as the deolorization of various dyes, that the activation energy, which is dependent on the change in temperature, plays an important role in the metabolic activity of microorganisms. This has been demonstrated both theoretically and experimentally [19,35–38], chromate reduction [32,33] and phenolics biodegradation [18,25–28] and acrylamide [21]. Although the specifics of the process that underlies the shift are not yet known, there are two hypotheses that offer two possible explanations for the phenomenon. The first hypothesis is that the properties of water shift as it passes through different states, and the "bottle neck" hypothesis postulates that only a small number of events can take place at the same time and in quick succession [44]. According to a number of different Arrhenius break point temperatures that have been measured, the first theory does not appear to be accurate [19]. According to the "bottle-neck" concept, it is impossible to verify the "bottle-neck" hypothesis because each chained enzyme has its own distinct thermal characteristics. This is in accordance with the "bottle-neck" idea. When the surrounding temperature is taken into account, the cell membrane will exhibit a range of different behaviors [45]. The so-called "bottleneck" theory is still widely accepted in the academic community [19,46]. As an alternative, the Arrhenius plots can be used to estimate the Q_{10} values, or the Q_{10} values can be obtained by monitoring the growth rates at varied temperatures with a range of ten degrees [47]. Logarithmic against 1000/Kelvin plot is the Arrhenius curve plot for the bioreduction and growth rates against the Arrhenius curve (**Fig. 1**). Regarding the temperature spectrum that was investigated (30-42 °C), a Q_{10} value of 2.905 was obtained (**Fig. 3**). However, due to the dynamic nature of biological processes, it is possible that more than one Q_{10} value can be assigned to a particular temperature range that is being researched.

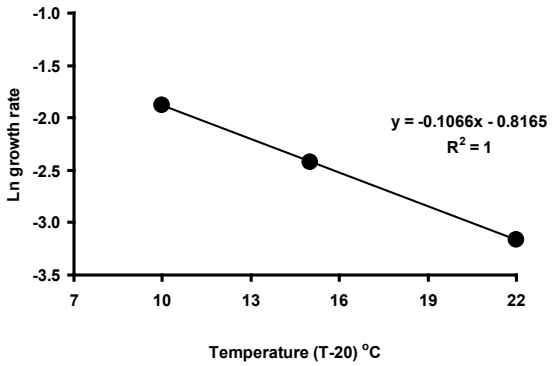

Fig. 3. Ln growth rate vs temperature plot for estimating theta.

The value 2.038 was found to be obtained during the reduction of molybdate to produce molybdenum blue [48] while in a another molybdenum reducer; *Morganella* sp, a Q_{10} value of 2.31 was obtained. This value is essential in determining whether or not a growth process can be attributed to a specific biological activity. Previous research found that the Q_{10} value for oil biodegradation in a beach gravel column was calculated to be 2.7 [49]. On the other hand, a different study on soil that was contaminated with decane and toluene found that it had a Q_{10} value of 2.2 [50]. Degradation by bacteria and temperature's effect on it were both found to have a Q_{10} value that was equal to or greater than 2 [51]. The Q_{10} value of acrylamide, which is produced by immobilized bacterial systems at temperatures ranging between 25 and 45 Celsius, is 2.8 [52]. It is commonly the case that decreasing temperatures result in an increase in the value of Q_{10} [53,54]. According to the findings of another study, the Q_{10} value produced by *Pseudomonas* sp. strain AQ5-04 was 1.834. [25] whereas a Q_{10} value of 2.17 was determined to be appropriate for the growth rate of this organism when fed diesel. In another study on the biodegradation of acrylamide, the Q_{10} value was reported to be 2.17, which is lower [21]

According to our calculations (**Fig. 3**), the theta value of the bacterium's molybdenum reduction was equivalent to that of our previous theta value estimate of 1.08 for *Serratia* sp. strain HMY1 [48]. *Pseudomonas* sp. strain DRYJ7 grew at a rate of 1.03 on acrylamide, according to a study. Theta was found to be a value of 1.03 [21]. There have been reports of xenobiotic degradation theta values as high as 16.2, however the theta value here is between 1.1 and 1.7, which is common for many biological processes [55].

CONCLUSION

For the first time, an Arrhenius plot shows a broken profile with two activation energies for the activation energy required for yeast to biodegrade diesel. Because this investigation was the first of its sort, this discovery was achievable. There is a general effect that temperature has on microbial growth and metabolic activities. Microbes are very sensitive to temperature changes because of their small size. Distinct chevron-like lines are utilized to represent the apparent activation energy of *Alcaligenes* sp.YLA11 growth on diesel fuel at 28.05 °C. Regression analysis resulted in two activation energies: 20–27 °C and 30–42 °C with the activation energies of 41.72 kJ/mol and 84.72 kJ/mol, respectively. The temperature range that was looked at (30-42 °C) resulted in the calculation of a Q_{10} value of 2.905 and a theta value of 1.11. The quantum is relatively higher than the typical energies seen in mesophilic microorganisms, particularly in the temperature range of 30 to 42 °C. It is hypothesized that the activation energy required to break an amide bond is significantly higher. To determine the effects of temperature on growth kinetics, additional research and investigation is currently being conducted, particularly on the parameters themselves. The values that were found through this research fall within the typical range for a variety of biological processes. The values that were found through this research fall within the typical range for a variety of biological processes.

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