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Activation Energy, Temperature Coefficient and Q_{10} Value Estimations of the Growth of 2,4-dinitrophenol-degrading Bacterium on 2,4-dinitrophenol

Hafeez Muhammad Yakasai[1]* Noor Suffiah Md. Zin[2] and Mohd Yunus Shukor[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Science, Bayero University Kano, PMB 3011, Nigeria.
[2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Hafeez Muhammad Yakasai,
Department of Biochemistry,
Faculty of Basic Medical Sciences,
College of Health Science,
Bayero University Kano,
PMB 3011,
Nigeria.
Email: hmyakasai.bch@buk.edu.ng

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ABSTRACT

In addition to its use as a pesticide, the chemical compound known as 2,4-dinitrophenol (2,4-DNP) is also utilized in the creation of wood preservatives and colors. Symptoms of acute (short-term) exposure to 2,4-DNP in humans include nausea or vomiting, sweating, headaches, disorientation, and a loss of weight. This type of exposure occurs when the substance is consumed orally. There are a few different models that may be used to simulate the growth rate of microorganisms on a variety of different medium at a range of temperatures. These models can be found online. One of the models that is employed the most frequently is the Arrhenius model, in part because it has a limited number of parameters. The development and metabolic activities of bacteria on the substrates they are grown on are commonly influenced by temperature. Temperature can also affect the growth of germs. Microbes are extremely sensitive to changes in temperature because of their small size. A discontinuous apparent activation energy with a chevron-like graph was used in order to describe the growth of a 2,4-dinitrophenol-degrading bacterium on 2,4-dinitrophenol. The break point of the graph was set at 28.05 °C. This was done so that the development of the bacterium could be described. Following the conclusion of the regression analysis, two temperature ranges for activation were determined. These temperature ranges were 20-27 °C and 30-42 °C, and their associated activation energies were 41.72 and 84.72 kilojoules per mole, respectively. It was anticipated that the Q_{10} value would be 2.905 and that the theta value would be 1.11 when considering the temperature range that was considered (30-42 °C). Due to the all-encompassing scope of this study, it is particularly useful for forecasting the effect of temperature on the breakdown and fate of 2,4-dinitrophenol during bioremediation.

INTRODUCTION

Nitrophenols are one of the xenobiotic chemicals that can be found in nature in the greatest abundance. Nitrophenols, also known as nitroaromatic chemicals, can be found in any and all parts of the environment. In most cases, the synthesis of insecticides, dyes, explosives, herbicides, and pharmaceuticals requires the use of these molecules as the building blocks [1] and precursors of arylamines that are commonly utilized in manufacturing [2]. Because of this, nitrophenols can be found as contaminants in waste water, rivers, groundwater (in soils that have been treated with pesticides), and even the atmosphere [3]. Nitroaromatic compounds are pollutants that can be harmful. Some examples include 2,4-dinitrophenol (2,4-DNP) and 2,4,6-trinitrophenol. Because they are carcinogenic, these substances provide a substantial danger to one's health. Bioremediation of phenol and phenolics including 2,4-dinitrophenol is amongst the emerging techniques in remediation of these toxic compounds. Microorganisms degrading 2,4-dinitrophenol and other nitrophenols are abundantly reported [4–7] including *Burkholderia* sp. strain KU-46, *Trichosporon cutaneum*, *Sphingomonas* sp. UG30, *Nocardioides simplex*, and several *Rhodococcus* spp. such as *Rhodococcus erythropolis*, *Rhodococcus imtechensis* and *Rhodococcus koreensis* [8–24],

and their characterization including temperature effect on degradation is an important parameter.

When conducting study on the bacteria that are responsible for the process of chemical decomposition, temperature is an essential component to take into mind. To calculate the apparent activation energy, H*, which is believed to be present for either growth or decay on different metabolic substates, it is usual practice to apply the temperature function Arrhenius model. The temperature function Arrhenius model is gaining in popularity as a tool for analyzing the growth and decay rates of bacteria, and this trend is expected to continue in the foreseeable future [25–33].

The value of delta H, denoted by H*, remains rather stable during the vast portion of the temperature gradient. This value, when applied to temperatures that are extremely high, can change by a factor of three or four, depending on the temperature range [34]. In some studies, the model may be incorrect when applied to the entire temperature range of the bacterial process [35]. Arrhenius' model is frequently employed for simulating temperature effects; however, when the temperature ranges are vast, this model is utilized significantly less frequently [36]. The Arrhenius plot may also show a previously identified transition, which is a fast shift in activation energy [37]. Because Arrhenius' model includes the fewest parameters, it is pretty widely accepted by researchers [36]. 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 [38].

This indicates that the Arrhenius models are necessary for the purpose of determining how temperature influences the development of bacteria. In order to acquire an estimate of the Arrhenius parameter, first an Arrhenius plot must be plotted, and then linear regression must be performed on the data. Arrhenius plot analysis and the effect of temperature on 2,4-dinitrophenol-degrading bacterium's 2,4-dinitrophenol growth were the subject of a similar study a number of years ago [28]. This study revealed something that was previously unknown: a bacterium can break down 2,4-dinitrophenol at a number of different activation energies. This information will be extremely useful in predicting how 2,4-dinitrophenol breakdown and transport will occur during bioremediation, as it will help determine how 2,4-dinitrophenol will be broken down and transported.

MATERIALS AND METHODS

Growth and maintenance of bacterium and measurement of the Activation energy of growth on 2,4-dinitrophenol

The 2,4-dinitrophenol-degrading bacterium growth and characterization on 2,4-dinitrophenol has been published previously and stored in the university's culture collection unit [4]. Growth data from 2,4-dinitrophenol-degrading bacterium was processed by converting the temperature-dependent growth rates to natural logarithms. The Arrhenius equation [39] is as follows,

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

Where R represents universal gas constant (0.008314 kJ/molK^{-1}), T represents absolute temperature (Kelvin = °C + 273.15), E_a represents 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;

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

Coefficient of Q_{10} estimation

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

$$Q_{10} = e^{\left(\frac{Ea}{R}\right)\left(\frac{10}{T_2T_1}\right)} \qquad \text{[Eqn. 3]}$$

Following rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R}\right)\left(\frac{1}{T_1T_2}\right) \qquad \text{[Eqn. 4]}$$

Another essential biological constant derived by substituting the given values into the reaction rates equation regulated by the Q_{10} rule is the coefficient of temperature or theta (θ) value (simplified Arrhenius temperature coefficient);

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

RESULT AND DISCUSSION

Temperature has an effect on the pace of bacterial growth (**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**). It came as a complete surprise when a breaking point was located at 28.05 degrees Celsius. According to the results of the regression analysis presented in **Table 1**, the activation energy required for growth on 2,4-dinitrophenol was 41.72 kJ/mol at temperatures ranging from 20 to 27 degrees Celsius. This activation energy increased to 84.72 kJ/mol at temperatures ranging from 30 degrees Celsius to 42 degrees Celsius. The highest rate of bacterial growth on 2,4-dinitrophenol was observed to occur at a temperature of 28.05 degrees Celsius, and this rate decreased as the temperature rose (**Fig. 2**).

It was discovered that the activation energy that was obtained through the utilization of the Arrhenius model fell within the range of activation energies that were reported in the scientific literature for a variety of biodegradation processes (**Table 2**). It would appear that removing the connections will require you to put in further effort on your end. There is a correlation between an increase in temperature and a decrease in the amount of energy that is consumed. Instead of reporting on the occurrence of two activation energies, many papers on activation energy only mention one activation energy spanning a large temperature range. This is because reporting on the presence of two activation energies would require more research. This is because an estimate of the activation energy is dependent on the rates of the metabolic process at varying temperatures. The findings of one study suggest that the activation energy is greater at higher temperatures than they are at lower temperatures. However, the findings of the other study suggest that the opposite is true, and that the phenomenon occurs in the opposite direction. This is because the activation energy is proportional to the temperature at which it is measured (**Table 2**). It was discovered that the activation energy that was obtained through the utilization of the Arrhenius model fell within the range of activation energies that were reported in the scientific literature for a variety of biodegradation processes (**Table 2**). It would appear that severing the ties will need a greater amount of work

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Table 1. The Arrhenius plot of the biodegradation rate of 2,4-dinitrophenol by 2,4-dinitrophenol-degrading bacterium was subjected to regression analysis.

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

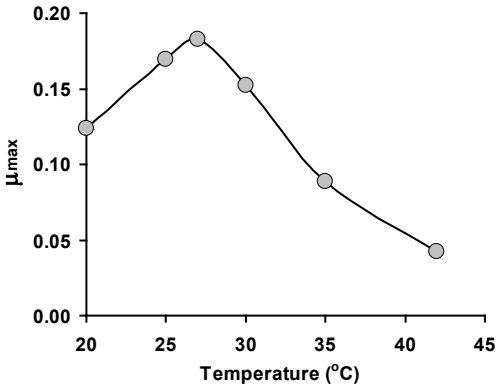

Fig 1. The effect of temperature on the specific growth rate of 2,4-dinitrophenol-degrading bacterium on 2,4-dinitrophenol.

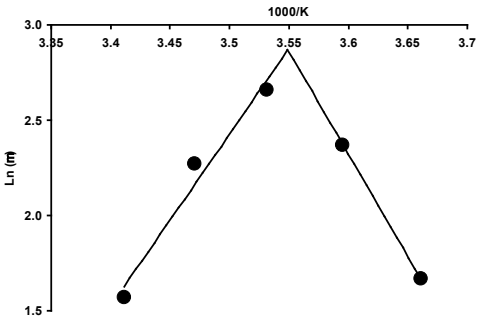

Fig 2. Growth rate of 2,4-dinitrophenol by 2,4-dinitrophenol-degrading bacterium in the form Arrhenius plot.

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

Microorganisms	Temperature range (°C)	Substrate	□*H**apparent activation energy (kJ.mol^{-1})	Ref
activated sludge	10–20	phenol	39.0	[42]
Selanastrum capricornutum	20–28	phenol	28.4	[43]
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	[44]
Pseudomonas putida Q5	10–25	phenol	61.6	[36]
Acclimated cultures	15-30	nonylphenol	42.7	[45]
Pseudomonas putida MTCC 1194	15-30	phenol	57.74	[46]
Bacillus sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20–46 °C) 31.4 (50–70 °C)	[40]
Pseudomonas sp. AQ5-04	15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	[41]
Pseudomonas sp. Strain DrYJ7	10-20	SDS	14.96	[28]
Cupriavidus sp. strain CNP-8	20-40	2-chloro-4-nitrophenol	75.16 88.71	[47]
Escherichia coli BL21	20-50	Chromate	28.01	[48]
Ochrobactrum intermedium	25-35	Chromate	120.69	[49]
Shewanella oneidensis·MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[50]
anaerobic sludge	30-55	Reactive Red 2	22.9	[51]
activated bacterial consortium	20-37	Remazol Black B	48.8	[52]
Enterobacter sp. strain (GY-1)	20-35	Reactive Black 5 (RB 5)	35.56	[53]
Escherichia coli NO3	20-45	Reactive red 22	27.49	[54]
Pseudomonas aeruginosa	15-45	Reactive Black 39 and Acid Red 360 by	RB39 61.89 AR360 81.18	[55]
Pseudomonas sp. LPM-410	20-28	EDTA	91.2	[56],
Pseudomonas sp. AQ5-04	15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	[41]
Cupriavidus sp.	20–25 30–40	2-chloro-4-nitrophenol	88.71 (20-25) 75.16 (30-40)	[29]
Pseudomonas sp. strain DRYJ7	10-20	Acrylamide	14.96	[28]

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

When thinking about bacteria, temperature is a very significant factor to take into consideration as a part of the equation. In addition to the rate at which metabolic events take

place, practically every aspect of the folding, structure, and stability of biomolecules, in addition to the pace at which they take place, are all regulated. This includes the rate at which metabolic processes take place. In order for a microbe to be able to continue living in its environment, it is absolutely necessary for the microbe to have the capability of sensing the presence of a host and adjusting its metabolic activities in response to that feeling. One type of bacteria that have this ability is referred to as a pathogen. In order for organisms to modify their gene expression in response to differences in temperature, they have created specialized sensing systems that are capable of detecting temperature fluctuations in an indirect manner. This enables organisms to respond appropriately to environmental changes. Because of this, the organisms are able to respond in the appropriate manner. Both a heat shock and a cold shock have the potential to cause the accumulation of ribosomes that have become inactive, but only a cold shock has the potential to cause the aggregation of proteins that have already become inactive.

Heat shocks also have the potential to cause the accumulation of ribosomes that have become inactive [26,27,57–62]. Molecular thermosensors could be built using molecular switches as the building blocks. There are a few different guises that direct temperature sensing variations can assume. One illustration of this would be the alteration of molecular architecture in order to generate dysfunctional activity. Additional examples include regulatory proteins that react to shifts in temperature, as well as shifts in the integrity of lipid membranes in response to fluidity. Other examples include fluidity-responsive regulatory proteins. When it comes to the regulation of biological processes, the importance of temperature is virtually impossible to overstate. The temperature is of the utmost importance.

Applications in the field of temperature-controlled biotechnology could make use of this mechanism in the future. Temperature shifts bring about a wide range of metabolic alterations, the majority of which are intricately intertwined with one another and can even be construed as a single process due to their close relationship. In light of this, the development of models that describe how the evolution of a biological process varies as a function of temperature is an absolute necessity if we want to simplify things and have a better understanding of how everything is connected. In the early 1900s, Arrhenius established a mathematical account of how biological reactions respond to high or low temperatures in the form of his "primal temperature model." Arrhenius's model was a mathematical representation of how biological reactions respond to high or low temperatures. The model developed by Arrhenius explains how the behavior of biological reactions might change depending on the temperature [58–62].

Bacteria have a greater need for energy when the activation energy is higher because they must break down more complex xenobiotics. This results in a higher activation energy. According to the findings of this inquiry, the activation energies of a variety of different microbial species that are capable of degrading xenobiotics fall within the ranges reported in this investigation for both temperature ranges. These results can be shown in **Table 2**. On the other hand, the activation energies of typical mesophilic bacteria can range anywhere from 33.50 to 50.30 kJ/mol [63]. There is a chance that a temperature range that was tested yielded results that showed a higher activation energy than the other temperature ranges. In this particular research endeavor, it was discovered that the activation energy is not a constant but rather varies with the temperature [64]. It is recommended to make use of the model as an observational model rather than a predictive one because the model is unable to take into account all of the simultaneous interactions that take place between the many biological systems. Instead of thinking about activation energy in terms of the activation energy required for chemical reactions, we should consider activation energy in terms of the overall temperature response of a microorganism. This will assist us in developing a deeper comprehension of activation energy [65].

In spite of all of these drawbacks, the model is nonetheless utilized in a considerable number of different settings. Temperature has been found to have an effect on the activation energy of microorganisms in a variety of various circumstances, such as the deolorization of a variety of colors by a variety of different organisms. This has been demonstrated through a number of different experiments (**Table 2**). Despite the fact that the particular process that causes the shift is still a mystery, there are two alternatives that have the ability to explain the change. These options are: When transitioning from one condition to another, the water characteristics change, and there is a theory that is referred to as the "bottleneck," which posits that the same processes are occurring simultaneously and quickly [66].

The first hypothesis does not appear to be accurate in light of the findings of a significant number of observations concerning the temperatures at which the Arrhenius break point takes place. The "bottle-neck" hypothesis, according to the "bottle-neck" idea, is notoriously difficult to show for a number of distinct reasons. Because of the influence temperature has on the cell membrane, it will change whenever the temperature does [67]. The "bottleneck" notion is still widely held in the academic community [37,68].

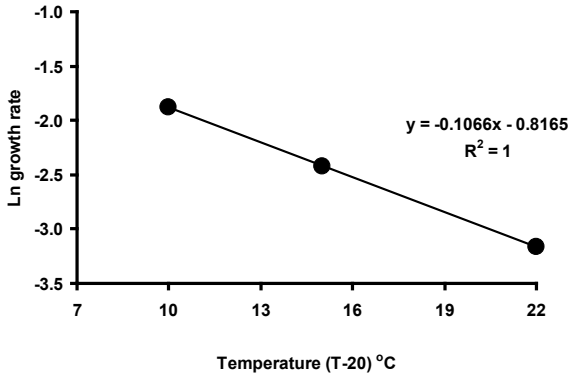

Fig. 3. Ln growth rate vs temperature plot for estimating theta.

There is also the possibility of calculating the Q_{10} values by utilizing the Arrhenius plots or by measuring the growth rates at a range of incubation temperatures that varied by ten degrees [69]. The Arrhenius curve is the slope of the plot that is produced when the bioreduction and growth rates are plotted logarithmically versus temperature in the form of a temperature logarithm (Kelvin).

A Q_{10} value of 2.905 was found to be appropriate for the temperature range of 30 to 42 degrees Celsius (**Fig. 3**). On the other hand, as a result of the dynamic nature of biological processes, numerous Q_{10} values may be discovered for each temperature range that is explored. A value of 2.038 was obtained from the transformation of molybdate into molybdenum blue [70]. A Q_{10} value of 2.31 was found in *Morganella* sp, yet another molybdenum reducer in nature. In order to properly attribute the growth process to a specific biological activity, this value is essential. For oil degradation in a beach gravel column, previous research found Q_{10} to be 2.7 [71]. Nevertheless, a Q_{10} value of

2.2 for microbiological process was reported in another study on decane and toluene-contaminated soil [72].

Degradation rates for other petrochemical compounds affected by salinity was reported to exhibit a Q_{10} value of 2.2 [73], while acrylamide degradation between 25 and 45 degrees Celsius in immobilized bacterial systems reported a Q10 value of 2.8 [74]. Declining temperatures frequently lead to an increase in the Q_{10} value [75,76]. The Q_{10} value for the phenol degradation by *Pseudomonas* sp. strain AQ5-04 was 1.834 [41] 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 [28]. The theta value was determined to be 1.11 (**Fig. 3**), close to the theta value found of 1.08 for molybdenum reduction by *Serratia* sp. strain HMY1 [70]. A lower theta value of 1.03 is reported for the growth rate on acrylamide by the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7 [28]. There are reports of xenobiotics being broken down with theta values of up to 16.2, but theta values are typically between 1.1 and 1.7, which is within the typical range for many biological processes [77]. In the biodegradation of nonylphenol, a theta value of 1.06 was observed [45].

CONCLUSION

For the biodegradation of 2,4-dinitrophenol, an activation energy is required, and this requirement may be seen in an Arrhenius plot with two different activation energies. The findings of this study are the very first to suggest that this might be the case. Temperature has a considerable impact on both the growth of microorganisms and the metabolic activity of the substrates that they feed on. Temperature is a crucial factor in both of these processes. Due to the fact that they are so little, microorganisms are extremely sensitive to even the most minute changes in temperature that can take place in the environment in which they are found. On a graph displaying apparent activation energy for apparent activation, which was provided, the apparent activation of Serratia marcescens DRY6 on 2,4-dinitrophenol was shown with a break point at 28.05 degrees Celsius. Additionally, the graph displayed apparent activation energy for apparent activation. The results of the regression analysis showed that there were two separate temperature ranges for the activation process. The first range was 20–27 degrees Celsius, while the second range was 30–42 degrees Celsius. The activation energies, which were x and 41.72 kJ/mol, respectively, were determined for each temperature range. The value of Q_{10} was determined to be 2.905 for the temperature range that was investigated, while the value of theta was determined to be 1.11. (30-42 degrees Celsius). At temperatures ranging from 30 to 42 degrees Celsius, the quantum has an efficacy that is larger than three times that of mesophilic bacteria. It is generally accepted that a substantially higher activation energy would be necessary in order to successfully break an amide bond. Additional research and investigation into the parameters themselves are currently being carried out in order to get a better understanding of the connection that exists between temperature and growth kinetics. This is being done in order to get a deeper understanding of the connection that exists between temperature and growth kinetics.

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