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Arrhenius Plot Analysis, Temperature Coefficient and Q10 Value Estimation for the Effect of Temperature on the Rate of Molybdenum Reduction by *Serratia marcescens* strain DRY6

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ABSTRACT

Molybdenum is a micronutrient that serves as a co-factor for over 50 enzymes and catalyses a variety of hydroxylation and redox transfer activities in animal and plant physiology. When its concentration is elevated, it poses a great danger as it can interfere with the processes of spermatogenesis and oogenesis in several animals including fish. Its remediation using molybdenum-reducing bacterium is an emerging important tool. One of the parameters that are important in governing molybdenum reduction is temperature. Several models may be used to simulate the growth rate of microorganisms on various media as a function of temperature. Arrhenius is one of the very popular models since it has minimal parameters. Temperature generally affects microbial growth and metabolic activity on their substrates. The small nature of microbes makes them susceptible to change in the surrounding temperature. Growth on molybdenum by *Serratia marcescens* strain DRY6 is described, with a discontinuous chevron-like graph of apparent activation energy with a breakpoint at 26.25 °C. Regression analysis resulted in two activation energies: 15-25 °C and 30–40 °C with the activation energies of 52.7 kJ/mol and 13.33 kJ/mol, respectively. For the examined temperature range (15-25 °C), a Q_{10} value of 2.08 and a theta value of 1.02 was calculated. This is study is very useful in predicting the breakdown of molybdenum and the movement of molybdenum during bioremediation.

INTRODUCTION

It is now our activities that are putting our environment in peril. Heavy industry, urbanisation, and agriculture have all contributed to environmental destruction due to the rise in the global population. A combination of overuse of natural resources and ignorance of natural laws compounds the problems. Due to hydrocarbon and metal ion-based pollution, the overall level of pollution has increased over time. Occupational and environmental high-exposure circumstances have been related to many acute and chronic toxicity cases due to toxins generated from metals and their compounds. Naturally occurring heavy metals are present in the environment [1–6]. There has been a significant increase in heavy metal levels due to human activities, as shown by pre-industrial measurements. In parallel with increasing population and industrial activity, a large and indiscriminate release of poisons into the environment is happening. Harmful effects on human health and biota may arise if heavy metal levels increase over the critical load. In addition to being toxic in their elemental forms, several metals are nondegradable, such as cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, silver, and zinc. The metal build-up in the food chain may represent a major environmental threat. Due to the increased global attention on heavy metal pollution, it is essential to remove them from the environment [7–12].

Molybdenum is a micronutrient that serves as a co-factor for over 50 enzymes. It catalyses a variety of hydroxylation and redox transfer activities in animal and plant physiology [13–18]. The exposure of the general population to molybdenum's toxicity has risen in conjunction with industrial ceramics, glass, contact lens solution, metallurgical processes, lubricants, pigment, catalyst, electronic products, and as a colouring ingredient in cosmetics [19–25]. 0.5 mg/L of molybdenum in groundwater at mining sites has been observed to be greater than the WHO guideline of 0.07 mg/L in drinking water. Drinking water or foraging for plants containing molybdenum is a greater risk factor for hypocuprosis and molybdenosis in animals who have had direct contact with the element [21].

Molecules are sensitive to molybdenum breakdown at certain temperatures because of their small size. As temperatures change, organisms are better equipped to adapt to their environments. When it comes to biodegrading chemicals, the temperature is of critical importance. The Arrhenius model has been used to estimate the apparent activation energy for bacterial growth and is also often used in studying bacterial growth and metabolism. For most temperature ranges, the value of delta H (ΔH*) is approximately constant. However, when looking at extreme ranges of temperature, this figure may deviate by three or four times [26]. Some studies show that the model may not be an accurate representation of the bacterial process when applied across the board.

The Arrhenius model is less commonly applied to wider temperature ranges [27,28]. Another previously unknown transition could be shown on the Arrhenius graphic. This Arrhenius model contains the fewest parameters, which makes it easy for researchers to believe in. The Ratkowsky model is based on the premise of linear growth, but because it's grounded on biology, it shows non-linear behaviour [29,30]. In other words, the Arrhenius models are utilised in understanding how temperature affects bacterial development because of this. The Arrhenius parameter estimate is calculated by drawing a linear regression on the Arrhenius plot. Several years ago, similar research looked at Q_{10} value estimates of Arrhenius plot analysis and the impact of temperature on molybdenum growth done by *Pseudomonas* sp. strain DRYJ7 [31].

This study revealed that there were many potential activation energies for the breakdown of molybdenum by a bacterium, which is a previously discovered phenomenon. It is fascinating in terms of principles, and it will also be very useful in predicting the breakdown of molybdenum and the movement of molybdenum during bioremediation.

MATERIALS AND METHODS

The activation energy of growth on molybdenum

Molybdenum reduction rate data from *Serratia marcescens* strain DRY6 previously isolated as a was processed [32] by transferring the growth values at each temperature to the natural logarithm and calculating the value of the slope, which is equivalent to the specific growth rate.

The Arrhenius equation [33] is as follows,

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

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

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

The coefficient of temperature or theta (Θ) value (simplified Arrhenius temperature coefficient) is another important biological constant obtained from the substitution of the obtained values into the reaction rates equation governed by the Q10 rule;

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

RESULTS AND DISCUSSION

The effect of temperature on the growth rate of the bacterium on molybdenum shows an increasing growth rate leading to a maximum rate between 25 and 30 °C and a decrease of growth rate at higher temperatures (**Fig. 1**). When plotting ln □m versus 1/T, we got a Chevron-like graph, which showed a discontinuous curve across the entire temperature range (**Fig. 2**). An interesting finding was the presence of a breakpoint at 26.25 °C. Regression analysis results shown in **Table 1** suggest that in the lower temperature range of 15-20 °C, growth on molybdenum had an activation energy of 52.7 kJ/mol, whereas, at the higher temperature range of 30–40 °C, it had an activation energy of 13.33 kJ/mol. A previous study on the growth rate of *Pseudomonas* sp. strain DrYJ7 between 10 and 20 °C on molybdenum showed activation energy of 14.96 Kj/mol [31], which is much lower.

The calculated activation energy was determined according to the Arrhenius model and was found to be in the same range of activation energy for different biodegradation of xenobiotics as reported (**Table 2**). It seems that these relationships are harder to break apart. Using less energy is better when the temperature is increased. The limited number of studies reporting on metabolic activation energy as a function of temperature focuses mostly on just one activation energy covering a wide range of temperatures. One research finds greater activation energy, whereas the other reports the opposite: lower activation energy at higher temperatures (**Table 2**). A case in point is the growth of *Bacillus* sp. JF8 on polychlorinated biphenyl (PCB) where the activation energy was 12.1 Kj/mol from 20 to 46 °C and 31.4 Kj/mol from 50 to 70 °C [34]. In another contrasting study, the growth of phenol by *Pseudomonas* sp. AQ5-04 shows activation energy of 38.92 Kj/mol from 15 to 30 °C and 11.34 Kj/mol from 35-45 °C [35].

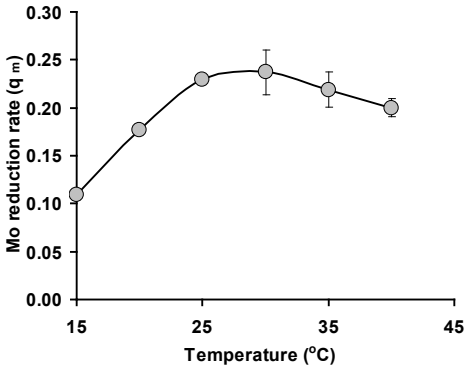

Fig 1. The effect of temperature on the specific growth rate of *Serratia marcescens* strain DRY6 on molybdenum. Error bars represent mean ± standard deviation (n=3).

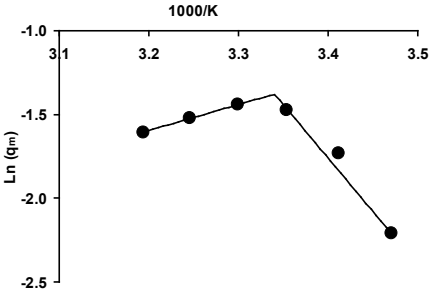

Fig 2. Arrhenius plot of the molybdenum reduction rate by *Serratia marcescens* strain DRY6.

Table 1. The two-part linear regression analysis for the Arrhenius plot of molybdenum reduction rate by *Serratia marcescens* strain DRY6.

Distribution of the experimental points	Three points to the left, three points to the right
	Right part
Temperature range °C	15, 20, 25
Regression equation	y = -6.3505x + 19.863
Coefficient of determination	0.98
tan a ± Standard error	-6.35±1.04
E_a ± Standard error, kJ mol^{-1}	52.77±8.63
t-Statistic	-6.11
Degrees of freedom	2
	Left part
Temperature range °C	30, 35, 40
Regression equation	y = 1.6037x - 6.7283
Coefficient of determination	0.99
tan a ± Standard error	1.60±0.06
E_a ± Standard error, kJ mol^{-1}	13.33±0.53
t-Statistic	24.92
Degrees of freedom	2
	Breakpoints data
Intersection coordinates, (x, y)	3.34, -1.38
Break point temperature °C	26.25
Q_{10} (15-25 °C)	2.08
Theta	1.02

Table 2. Arrhenius temperature characteristics for metal reduction.

Microorganisms	Temperature range (°c)	Substrate	Δ*H**apparent activation energy (kJ.mol^{-1})	Ref
Ochrobactrum intermedium BCR400	25-35	Chromate	120.69	[36]
Arthrobacter sp. SUK 1201	25-60	Chromate	36.21	[37]
Aspergillus niger	30-60	Chromate	8.56	[38]
Bacillus sp.	25-40	Chromate	22.0	[39]
Thermus scotoductus SA−01	65	Chromate	35 (membrane bound enzyme) 40.3 (soluble)	[40]
Thermus scotoductus SA−01	60-65	Iron	30	[40]
Shewanella profunda LT13a	4-37	Iron	50.3	[41]
β-Proteobacteria	15-40	Vanadate	36	[42]
Shewanella oneidensis·MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[43]
Serratia marcescens strain DRY6	15-40	Molybdate	52.7 (15-25 °C) 13.33 (30-40 °C)	This study

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

The higher the activation energy, the more energy the bacterium needs to use to metabolize xenobiotics. Based on **Table 2**, the values obtained in this study for both temperature ranges are within the activation energy for numerous xenobiotic degradations by microbial species. However, the activation energy for the typical mesophilic bacteria is between 33.5 and 50.3 kJ/mol [44], indicating that the activation energy for one of the temperature range studied in this study was relatively a bit higher. The low activation energy for the higher range of temperature was within the range reported for several chromate-reducing microbes (**Table 2**).

In the current study, we found that the activation energy is not constant, rather it depends on the temperature chosen [45]. While we can't accurately estimate all of the interacting complex biological processes that are taking on at the same time, the model functions as an observational model. Activation energy thus should not be thought of as the activation energy utilised in chemical processes, but rather the total temperature response of the microorganism [46].

The greater activation energy required to digest xenobiotics means higher energy consumption. Table 2 provides the results for both temperature ranges and reveals that the experimentally determined values for both are within the activation energy for many xenobiotic degradations by microbial species. The average mesophilic bacterium will have an activation energy of between 33.5 to 50.3 kJ/mol [30,47–50], chromate reduction [36,51] and phenolics biodegradation [27,35,52–54] and molybdenum [31].

The details of the process that causes the change are still unknown, but two hypotheses provide two plausible explanations. The first is that water characteristics change as it transitions and a hypothesis of "bottleneck" hypothesises that a limited number of events occur simultaneously in rapid succession [55].

Based on various measured Arrhenius breakpoint temperatures, the first theory does not seem to be correct [30]. Following the "bottle-neck" idea, since each of the chained enzymes has its unique thermal characteristics, it is impossible to verify the "bottle-neck" hypothesis. When taking into consideration the ambient temperature, the cell membrane will also vary [56]. The “bottleneck” theory continues to hold strong among academics [30,57].

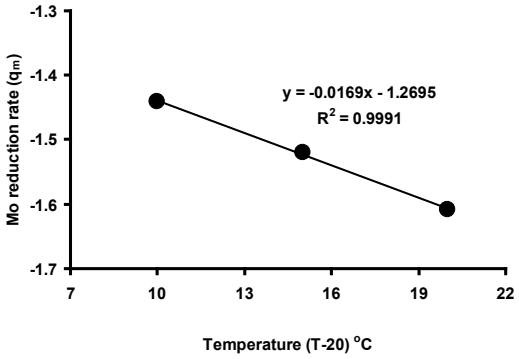

Fig. 3. Ln growth rate vs temperature plot for estimating theta.

Alternatively, the Arrhenius plots may be used to estimate the Q_{10} values, or they can be calculated by measuring the rates of growth for different incubation temperatures with ten degrees of variation [58]. When the bioreduction and growth rates have been logarithmically plotted against 1000/temperature (Kelvin), the Arrhenius curve is the slope of the resulting plot (**Fig. 1**).

For the examined temperature range (15-25 ºC), a Q_{10} value of 2.08 was obtained. However, since biological processes are dynamic, there may be more than one Q_{10} value for a distinct temperature range being investigated. In the reduction of molybdate to molybdenum blue, a 2.038 value was obtained [59] while in another molybdenum reducer; *Morganella* sp, a Q_{10} value of 2.31 was obtained. When attributing the growth process to a distinctive biological activity, this value is essential. Q_{10} was calculated to be 2.7 for oil biodegradation in a beach gravel column in previous studies [60]. However, different research on soil polluted with decane and toluene shows a Q_{10} value of 2.2 [61]. Both bacteria's ability to break down petrochemicals and the effects of temperature on it were determined to have a Q_{10} of 2.2 [62], while, immobilised bacterial systems at temperatures ranging from 25 and 45 degrees Celsius produce molybdenum and its Q_{10} value is 2.8. [63]. Increasing the value of Q10 as the temperature decreases is often true [64,65]. In another research, *Pseudomonas* sp. strain AQ5-04 produced a Q_{10} value of 1.834 [35] while a Q_{10} value of 2.17 was calculated for the growth rate of this organism on molybdenum. A lower Q_{10} value of 2.17 is reported in another study on molybdenum reduction [31]

A theta value of 1.02 was calculated (**Fig. 3**), which was similar to a theta value of 1.08 calculated for the molybdenum reduction by the bacterium *Serratia* sp. strain HMY1 [59]. In the growth rate on molybdenum by the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7, a theta value of 1.03 was obtained [31]. The theta value is also within the range for many biological processes that are from 1.1 to 1.7 although higher values of up to 16.2 have been reported for the degradation of other xenobiotics [66].

CONCLUSION

This is the first study demonstrated that the activation energy needed for the bioreductionof molybdenum by a bacterium which displays a broken profile with two activation energies observed in the Arrhenius plot. Temperature generally affects microbial growth and metabolic activity on their substrates. The small nature of microbes makes them susceptible to change in the surrounding temperature. Growth on molybdenum by *Serratia marcescens* strain DRY6 is described, with a discontinuous chevron-like graph of apparent activation energy with a breakpoint at 26.25 ºC. Regression analysis resulted in two activation energies: 15-25 ºC and 30–40 ºC with the activation energies of 52.7 kJ/mol and 13.33 kJ/mol, respectively. For the examined temperature range (15-20 ºC), a Q_{10} value of 2.08 and a theta value of 1.02 was calculated. The quantum, especially in between 15 and 20 ºC, is relatively a bit higher than the typical energies observed in mesophilic microorganisms. The amide bond is postulated to hold much higher activation energy to be broken. Additional work is under investigation, particularly on parameters themselves, to determine the effects of temperature on growth kinetics. The values obtained in this work are within the normal range for many biological processes. The values obtained in this work are within the normal range for many biological processes.

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