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The Effect of Temperature on the Rate of Molybdenum Reduction by *Enterobacter* sp. strain Dr.Y13: Arrhenius Plot Analysis, Temperature Coefficient and Q_{10} Value Estimation

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ABSTRACT

Molybdenum is a micronutrient that is needed a co-factor for many hydroxylation and redox transfer activities in animal and plant physiology. The greatest risk of overexposure is its ability to interfere with the sperm production and egg-production processes in a variety of species, including fish. It is only beginning to be used as a remediation technique for molybdenum-reducing bacteria. Temperature is one of the factors that influence molybdenum reduction. Many models may be used to predict the growth rate of microorganisms on different medium, depending on the temperature. The Arrhenius model is popular because it has few parameters. Temperature generally affects microbial growth and metabolic activity on their substrates. The small nature of microbes makes them susceptible to change in surrounding temperature. Growth on molybdenum by *Enterobacter* sp. strain Dr.Y13 is described, with a discontinuous chevron-like graph of apparent activation energy with a breakpoint at 32.66 ºC. Regression analysis results suggest that in the lower temperature range of 20-30 ºC, growth on molybdenum had an activation energy of 62.09 kJ/mol, whereas at the higher temperature range of 37–45 ºC, it had an activation energy of 65.05 kJ/mol. For the examined temperature range (20-30 ºC) and (37-45 ºC), Q_{10} values of 2.32 and 2.21 and theta values of 1.09 and 1.08 were obtained, respectively. This is study is very useful in predicting the breakdown of molybdenum and the movement of molybdenum during bioremediation.

INTRODUCTION

Our own activities are now putting our environment at jeopardy. Heavy industry, urbanization, and agriculture have all caused havoc on the environment as the world's population continues to rise. Natural resource overexploitation, as well as men's ignorance of natural rules, aggravate the problems. Pollution caused by hydrocarbons and metal ions has been rising throughout the globe in recent years. Metals and their compounds have been related to a range of acute and chronic toxicity cases in occupational and environmental high-exposure settings. Heavy metals are naturally present in the environment. Heavy metal levels have skyrocketed in recent years as a consequence of human activities dating back to pre-industrial times [1–6].

Toxins are being released into the environment in large and indiscriminate amounts in parallel with the increasing population and intensity of industry. When heavy metal levels exceed the critical load, it may have a negative effect on human health and biota. Arsenic, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, silver, and zinc are all toxic in their elemental forms and different combinations, and they are also non-biodegradable. Metal accumulation in the food

chain may represent a major environmental risk due to its carcinogenic and mutagenic properties. Heavy metal pollution has become a global public health problem in recent years, making it essential to remove them from the environment [7–14].

Molybdenum is a trace element that functions as a micronutrient and is needed as a cofactor for over 50 enzymes. It stimulates cellular activity in animal and plant physiology by catalyzing a variety of hydroxylation and redox transfer activities [15–20]. Molybdenum's extensive usage in the industrial manufacture of ceramics, glass and contact lens solution, metallurgical processes, lubricants, pigment, catalyst, electronic products, and as color additives in cosmetics has raised the risks to individuals exposed to its toxicity [21–27]. It has been claimed that there has been an increase in the quantity of molybdenum in groundwater around mining sites of up to 0.5 mg/L, which is greater than the World Health Organization (WHO) recommended limit of 0.07 mg/L in drinking water [23]. Animals who have had direct contact with molybdenum via drinking water or while foraging for plants are more prone to develop hypocuprosis or molybdenosis over time [17].

For most temperature ranges, the value of delta H (ΔH*) is approximately constant. However, for extreme ranges of temperature, this number may diverge three or fourfold depending on the range of temperatures being examined [28]. according to some studies, the model may not be accurate when used across the whole bacterial process temperature [29]. Although being frequently employed in simulating the temperature impact in a limited temperature range, the Arrhenius model is less often used to larger ranges [30]. The Arrhenius plot may also display a previously discovered transition which is a rapid change in the activation energy [31]. Arrhenius's model has the fewest parameters, making it relatively universally accepted by researchers [30].

Another competing model, the Ratkowsky, is also built on the assumption of linear growth, but due to biological foundations, this model suffers from a lack of steady development and exhibits non-linear behavior [32]. In other words, the Arrhenius models are utilized 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 impact of temperature on molybdenum growth done by Pseudomonas sp. strain Dr.YJ7 [33]. Due to their size, microorganisms are particularly vulnerable to molybdenum breakdown at certain temperatures. Temperature influences physiology to enable organisms to better adapt to changing surroundings. Temperature is an important factor to take into account while biodegrading substances. The Arrhenius model has proven very popular in the research of bacterial growth and rates, and it is often used to measure the apparent activation energy, ΔH^*, which is thought to exist for either growth or decay on different metabolic substrates.

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 *Enterobacter* sp. strain Dr.Y13 previously isolated as a was processed [34] by transferring the growth values at each temperature to the natural logarithm and calculating the value of the slope, which is equivalent to specific growth rate.

The Arrhenius equation [35] 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 substitution of the obtained values into the reaction rates equation governed by the Q10 rule;

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

RESULT 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 at 30 °C and a decrease of growth rate at higher temperatures (**Fig. 1**). When plotting ln μm 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 break point at 32.66 °C. Regression analysis results shown in **Table 1** suggest that in the lower temperature range of 20-30 °C, growth on molybdenum had an activation energy of 62.09 kJ/mol, whereas at the higher temperature range of 37–45 °C, it had an activation energy of 65.05 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 [33], which is much lower. Activation energy estimated using the Arrhenius model was within the published literature's range of activation energy for various biodegradation of xenobiotics (**Table 2**). The connections seem to require more energy to break apart. Increasing the temperature uses less energy. Of the many reports on the activation energy calculated from rates of metabolic process at various temperatures, very few works report on the presence of two activation energies opting instead to report for only one activation energy spanning a large range of temperature. Of the reports, two contrasting difference is seen wherein one study, a higher activation energy is reported at higher temperatures compared to a lower range of temperature while in another study, an opposite phenomenon is observed (**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 [36]. 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 [37].

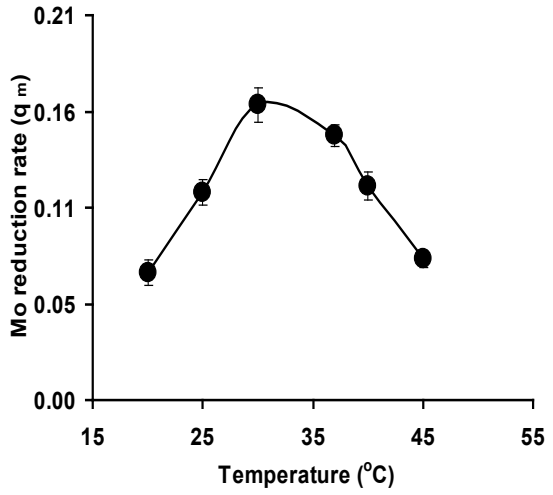

Fig 1. The effect of temperature on the specific growth rate of *Enterobacter* sp. strain Dr.Y13 on molybdenum. Error bars represent mean ± standard deviation (n=3).

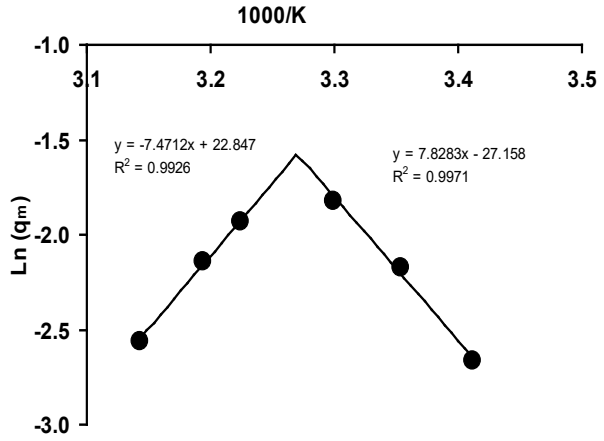

Fig 2. Arrhenius plot of the molybdenum reduction rate by *Enterobacter* sp. strain Dr.Y13.

Table 1. The two-part linear regression analysis for the Arrhenius plot of molybdenum reduction rate by *Enterobacter* sp. strain Dr.Y13.

Distribution of the experimental points	Three points to the left, three points to the right
	Right part
Temperature range °C	20, 25,30
Regression equation	y = -7.4712x + 22.847
Coefficient of determination	0.99
tan a ± Standard error	-7.47±0.65
E_a ± Standard error, kJ mol^{-1}	62.09±5.37
t-Statistic	-11.57
Degrees of freedom	2
	Left part
Temperature range °C	37,40,45
Regression equation	y = 7.8283x - 27.158
Coefficient of determination	0.99
tan a ± Standard error	7.83±0.42
E_a ± Standard error, kJ mol^{-1}	65.05±3.49
t-Statistic	18.65
Degrees of freedom	2
	Break points data
Intersection coordinates, (x, y)	3.34, -1.38
Break point temperature °C	32.66
Q_{10} (20-30 °C)	2.32
Theta (20-30 °C)	1.09
Q_{10} (37-45 °C)	2.21
Theta (37-45 °C)	1.08

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	[38]
Arthrobacter sp. SUK 1201	25-60	Chromate	36.21	[39]
Aspergillus niger	30-60	Chromate	8.56	[40]
Bacillus sp.	25-40	Chromate	22.0	[41]
Thermus scotoductus SA−01	65	Chromate	35 (membrane bound enzyme) 40.3 (soluble)	[42]
Thermus scotoductus SA−01	60-65	Iron	30	[42]
Shewanella profunda LT13a	4-37	Iron	50.3	[43]
β-Proteobacteria	15-40	Vanadate	36	[44]
Shewanella oneidensis·MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[45]
Enterobacter sp. strain Dr.Y13	20-45	Molybdate	62.09 (20-30 °C) 65.05 (37-45 °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 [46], 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 [47]. 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 utilized in chemical processes, but rather the total temperature

response of the microorganism [48]. Even with these problems, the model is in use worldwide. The activation energy, which depends on the temperature change, plays an important role in the metabolic activity of microorganisms, and it has been shown in a variety of conditions not limited to metal-reducing activity and include processes such as the decolourization of various dyes [31,49–52], chromate reduction [38,53] and phenolics biodegradation [30,37,54–56] and molybdenum [33].

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" hypothesizes that a limited number of events occur simultaneously in rapid succession [57]. Based on various measured Arrhenius breakpoint temperatures, the first theory does not seem to be correct [31]. 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 [58]. The “bottleneck” theory continues to hold strong among academics [31,59].

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 [60]. 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. 2**).

For the examined temperature range (20-30 ºC) and (37-45 ºC), Q_{10} values of 2.32 and 2.21 were obtained, respectively. 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 [61] 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 [62].

However, different research on soil polluted with decane and toluene shows a Q_{10} value of 2.2 [63]. 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 [64], while, immobilised bacterial systems at temperatures ranging from 25 and 45 degrees Celsius produce molybdenum and its Q_{10} value is 2.8. [65]. Increasing the value of Q10 as the temperature decreases is often true [66,67]. In another research, *Pseudomonas* sp. strain AQ5-04 produced a Q_{10} value of 1.834 [37] 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 [33]

For the examined temperature range (20-30 ºC) and (37-45 ºC), theta values of 1.09 and 1.08 were obtained, respectively (**Fig. 3** and **Fig. 4**), which was similar to a theta value of 1.08 calculated for the molybdenum reduction by the bacterium *Serratia* sp. strain HMY1 [61]. In the growth rate on molybdenum by the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7, a theta value of 1.03 was obtained [33]. 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 [68].

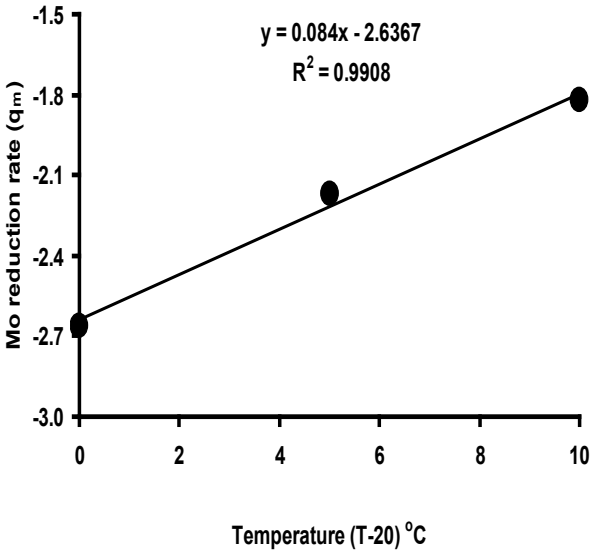

Fig. 3. Estimation of theta value for rate of Mo-reduction within the temperature range of 20 to 30 ºC.

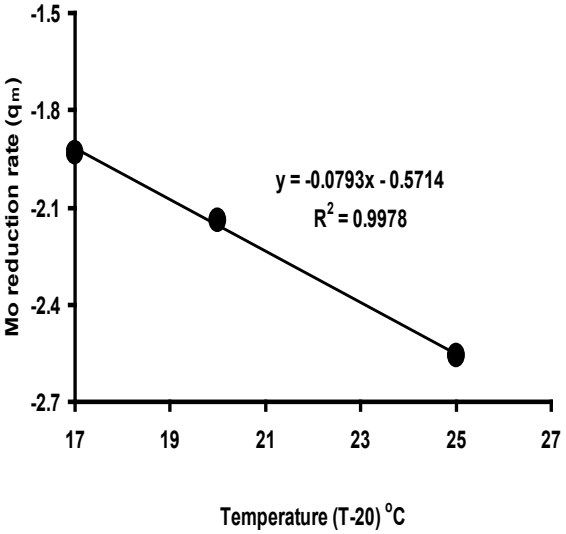

Fig. 4. Estimation of theta value for rate of Mo-reduction within the temperature range of 37 to 45 ºC.

CONCLUSION

This is the first study demonstrated that the activation energy needed for the biodegradation of 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 *Enterobacter* sp. strain Dr.Y13 is described, with a discontinuous chevron-like graph of apparent activation energy with a breakpoint at 32.66 ºC. Regression analysis results suggest that in the lower temperature range of 20-30 ºC, growth on molybdenum had an activation energy of 62.09 kJ/mol, whereas at the higher temperature range of 37–45 ºC, it had an activation energy of 65.05 kJ/mol. For the examined temperature range (20-30 ºC) and (37-45 ºC), Q_{10} values of 2.32 and 2.21 and theta values of 1.09 and 1.08 were obtained, respectively. 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.

CONFLICT OF INTEREST

"The authors declare that there is no conflict of interests regarding the publication of this article."

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