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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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...
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 is found via drinking water or while foraging for plants are more prone [23]. Animals who have had direct contact with molybdenum (WHO) recommended limit of 0.07 mg/L in drinking water [24], which is greater than the World Health Organization equivalent to specific growth rate.

The Arrhenius model [35] is as follows,

$$\ln \mu = \ln A - \frac{E_a}{R} \frac{1}{T}$$

[Eqn. 2]

Coefficient of Q₁₀ estimation

The Q₁₀ value is estimated via the following equation;

$$Q_{10} = e^{\left(\frac{E_a}{R} \left(\frac{10}{T_{27}}\right)\right)}$$

[Eqn. 3]

Following rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R} \left(\frac{1}{T_{27}}\right)\right)$$

[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;

$$kT = k20\Theta (T-20)$$

[Eqn. 5]

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,

$$\mu = Ae^{-\frac{E_a}{RT}}$$

[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;

$$\ln \mu = \ln A - \frac{E_a}{R} \frac{1}{T}$$

[Eqn. 2]

Another competing model, the Ratkowski, 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 Q₁₀ value estimates of Arrhenius plot analysis and impact of temperature on molybdenum growth done by Pseudomonas sp. strain Dr.Y17 [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, $\Delta H^*$, 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.
A previous study on the growth rate of *Pseudomonas* sp. strain DrY17 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].

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

<table>
<thead>
<tr>
<th>Distribution of the experimental points</th>
<th>Three points to the left, three points to the right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range °C</td>
<td>20, 25, 30</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = -7.4712x + 22.847$</td>
</tr>
<tr>
<td>Coefficient of determination</td>
<td>0.99</td>
</tr>
<tr>
<td>tan α ± Standard error</td>
<td>-7.47 ± 0.65</td>
</tr>
<tr>
<td>$E_a$ ± Standard error, kJ mol$^{-1}$</td>
<td>62.09 ± 5.37</td>
</tr>
<tr>
<td>t-Statistic</td>
<td>-11.57</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>2</td>
</tr>
<tr>
<td>Temperature range °C</td>
<td>37, 40, 45</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 7.8283x - 27.158$</td>
</tr>
<tr>
<td>Coefficient of determination</td>
<td>0.99</td>
</tr>
<tr>
<td>tan α ± Standard error</td>
<td>7.83 ± 0.42</td>
</tr>
<tr>
<td>$E_a$ ± Standard error, kJ mol$^{-1}$</td>
<td>65.05 ± 3.49</td>
</tr>
<tr>
<td>t-Statistic</td>
<td>18.65</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2. Arrhenius temperature characteristics for metal reduction.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Temperature range (°C)</th>
<th>Substrate</th>
<th>$\Delta H^{apparent}$ activation energy (kJ mol$^{-1}$)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ochrobactrum intermedicum</em></td>
<td>25-35</td>
<td>Chromate</td>
<td>120.69</td>
<td>[38]</td>
</tr>
<tr>
<td><em>Arthrobacter</em> sp. SUK 1201</td>
<td>25-60</td>
<td>Chromate</td>
<td>36.21</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>30-60</td>
<td>Chromate</td>
<td>8.56</td>
<td>[40]</td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>25-40</td>
<td>Chromate</td>
<td>22.0</td>
<td>[41]</td>
</tr>
<tr>
<td><em>Thermus scotoductus</em> SA-01</td>
<td>65</td>
<td>Chromate</td>
<td>35 (membrane bound enzyme)</td>
<td>[42]</td>
</tr>
<tr>
<td><em>Thermus scotoductus</em> SA-01 LT13a</td>
<td>60-65</td>
<td>Iron</td>
<td>30</td>
<td>[42]</td>
</tr>
<tr>
<td><em>Shewanella profunda</em></td>
<td>4-37</td>
<td>Iron</td>
<td>50.3</td>
<td>[43]</td>
</tr>
<tr>
<td><em>ß-Proteobacteria</em></td>
<td>15-40</td>
<td>Vanadate</td>
<td>36</td>
<td>[44]</td>
</tr>
<tr>
<td><em>Shewanella oneidensis</em> MR-1</td>
<td>25-40</td>
<td>Selenate</td>
<td>62.90 TPSS-supplemented system 47.33</td>
<td></td>
</tr>
</tbody>
</table>

*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

![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).](image)

![Fig 2. Arrhenius plot of the molybdenum reduction rate by *Enterobacter* sp. strain Dr.Y13.](image)
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 °C) and (37–45 °C), $Q_{10}$ 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 $Q_{10}$ 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 °C) and (37–45 °C), 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].

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 °C. Regression analysis results 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. For the examined temperature range (20–30 °C) and (37–45 °C), $Q_{10}$ 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 °C,
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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