Arrhenius Plot Analysis, Temperature Coefficient and Q_{10} Value Estimation for the Effect of Temperature on Molybdenum Reduction Rate by *Pantoea* sp. strain HMY-P4

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ABSTRACT

The process of microbial molybdenum reduction to molybdenum blue precipitate is a biological phenomenon exploited in the bioremediation of molybdenum pollutant. This process is impacted by a change of the surrounding temperature. In general, to model the impact of small temperature ranges on the growth rate of microorganisms on substrates, several models are available. Arrhenius is one of the models and is very common because it has few parameters. An apparent activation energy based on the Arrhenius plot on the rate of molybdenum reduction by *Pantoea* sp. strain HMY-P4 is reported in this study. A linear plot of ln q_{max} against 1/T at the temperature range studied (25–40 °C) resulted in an activation energy value of 45.69 kJ per mol (95% Confidence Interval or C.I., 32.04 to 59.35). The plot also yields the Arrhenius frequency factor (A) which was calculated as 2.71×10^7 (95% C.I., 1.25×10^5 to 5.86×10^9). The corresponding Q_{10} value of 2, which represents a relative 10 °C increase in the surrounding temperature and a doubling of the reaction rate. The Q_{10} value of 1.82 (95% C.I., 1.52 to 2.18) and a theta value of 1.06 (95% C.I., 1.05 to 1.08) was calculated. The values obtained in this work are within the normal range for many biological processes.

INTRODUCTION

Metals occurred naturally in the environment at a relatively low concentration, in soils, water, rocks and biota. They provide the living systems with the essential minerals necessary for survival, and cause no toxicity in trace amount. However, current industrial revolution, has made heavy metals one of the main sources of environmental pollution globally. In addition to industrial and mining activities, landfill leaching, fertilisers and pesticides used in agricultural processes, burning of waste and fossil fuels, and municipal waste treatment have tremendously increased heavy metal released into the environment [1]. These metals are not degradable, and eventually will accumulate in plants, animals, and humans. At elevated concentrations heavy metals caused toxicity to humans [2,3].

The less report on molybdenum (Mo) toxicity compared to other metals such as chromate, lead, mercury and selenate makes its study at first appearing less important. The metal is however very toxic to ruminant, as it causes tissue copper depletion. A number of animal studies have indicated molybdenum compounds to inhibit spermatogenesis and cause spermatogenic necrosis by modulating the complex testicular oxidative stress processes. Mo may inhibit copper or copper-binding proteins functions in the testis thereby decreasing the fertility index as well as testicular function [4]. Rats exposure to sodium molybdate resulted in a dose-dependent degeneration of the testicular morphology and function associated with decline in sperm concentration, motility, normal morphology [5].

In recent years, microbial-based detoxification and remediation of environmental pollutants has drew much interest as a promising innovative technology [6–8]. The astonishing catabolic capacity of microbes to abolish, transform or attenuate pollutants into less hazardous and often useful products along with CO_2, water, inorganic salts and microbial biomass, has been exploited [9]. However, inorganic toxicants for example heavy metals are not biodegradable, as such biologically encoded changes in the redox state and active metabolic capacity of the

microbe will determine their bioremediation. In general, heavy metals are essentially necessitated by most microorganisms for life processes. However, the adsorption capacity requires both of total microbial biomass and system's geochemistry. There are number of metals oxyanions which don't interact with microorganisms thus their bioremoval is then centred on enzyme catalyzed redox conversion to their precipitable forms [10].

For over a century, bacterial molybdenum reduction to Mo-blue had become an unsolved puzzle among scientists. This phenomenon was only discovered and proved to be enzymatic rather than abiotic recently. Previous works on molybdenum reduction focus on isolating molybdenum-reducer that has higher Mo-blue production capacity as a tool in bioremediation, until the work of Halmi *et al.* (2013) that reported the isolation multi-tolerant bacteria with ability to reduce molybdenum as well degrade SDS. However, as most polluted sites contained mixed contaminants from organic and inorganic origins, an effective remediation thus had become a complex one. Currently, the attention has shifted towards isolating microorganisms with multi-reduction and/or degrading potentials which could be used to remediate co-contaminated areas. Till recent years, about eight molybdenum-reducing bacteria with the potential to degrade other organic contaminants had been isolated. Therefore, further understanding of the reduction mechanism and kinetics of Mo-reducing enzyme through various optimization processes will help in solving the phenomenon of molybdenum reduction to Mo-blue. Thus, becomes an important step towards effective translation of laboratory findings to the field practice.

The temperature of incubation is one of the major factors affecting microbial growth and toxicity degradation [12,13]. Owing to their limited size and ectothermic nature, the vast majority of microorganisms directly suffer from environmental temperature. As a result, temperature affects the physiology and adaptation of microbes in their cellular biochemical pathways to the introduction to the new environment. In biological detoxification of xenobiotics, temperature regulation is of special significance.

Arrhenius feature may have defined the influence of temperature on the highest growth rate of bacteria on their substrates in modeling and design. Currently, a UTD or universal temperature dependence theory is proposed to set a small range of values for all metabolic activities, ranging from 57.9 to 67.5 kJ/mol Arrhenian activation energy (E_a). Similarly, it has been identified that the utility of the model over the extensive temperature spectrum of bacterial metabolisms is limited. [14]. After saying this, Arrhenius model is frequently reported to be very common for modeling temperature effects in micro-organism biological processes within only a limited range of temperature [15]. When translated into Q_{10} values, this range falls between 2.3 and 2.7. But the debate about the adoption of this set is heated [16–18] as it was found that less than 20% of reported works on microbial degradation of xenobiotics have Q_{10} values that fall within this range [18].

In biological reaction, Q_{10} values normally ranges between 2 and 3. The reaction rate doubles resulting in an average Q_{10} value of 2 for each 10 °C increase in temperature. A Q_{10} reaction value of less than 2 implies that the concentrations at a certain temperature are greater than what the Q_{10} equation predicts. In this study the above values will be estimated for the rate of reduction of molybdenum to Mo-blue by the bacterium *Pantoea* sp. strain HMY-P4.

MATERIALS AND METHODS

The specific growth rates for molybdenum reduction at various temperatures on low phosphomolybdate medium (LPM) were studied in a batch culture of the bacterium grown in low phosphomolybdate (LPM) broth containing 10 mM sodium molybdate as before [19].

The Arrhenius equation [20] 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 mol^{-1}K^{-1}), E_a is the activation energy (kJ mol^{-1}) 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;

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

[Eqn. 2]

Coefficient of Q_{10} estimation

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

$$Q_{10} = e^{\left(\frac{E_a}{R}\right)\left(\frac{10}{T_2 T_1}\right)}$$

[Eqn. 3]

Following rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R}\right)\left(\frac{1}{T_1 T_2}\right)$$

[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]

RESULTS AND DISCUSSION

The Arrhenius model has the fewest number of parameters and hence far better parsimoniously compared to other models such as Schoolfield, Eyring and Urry and Sharpe and DeMichele, and due to this, it is widely accepted by numerous researchers [15]. Another candidate model is the Ratkowsky. It appears that a biological basis for the model is absence and is based on an empirical observation on the linear relationship between the temperature and the square root of the specific growth rate [21]. This explains the popularity of the Arrhenius models in describing the effect of temperature on bacterial growth rate. Estimated Arrhenius parameter is obtained from the linear regression of the Arrhenius plot. The plot of ln q_{max} against 1/T shows a linear curve for the temperature range studied (**Fig. 1**). At the temperature range studied (25–40 °C), the regression analysis resulted in an activation energy value of 45.69 kJ per

mol (95% C.I., 32.04 to 59.35). The calculated apparent Arrhenius activation energy for the rate of molybdenum reduction by *Pantoea* sp. strain HMY-P4 for the temperature range from 25 to 40 °C has not been reported before for molybdenum-reducing bacterial works but is within the range for values reported in the literature for other microorganisms' metal-reducing studies (**Table 1**). The plot also yields the Arrhenius frequency factor (A) which was calculated as 2.71×10^7 (95% C.I., 1.25×10^5 to 5.86×10^9). The Arrhenius frequency factor represents the frequency of collisions between reactant molecules at a standard concentration.
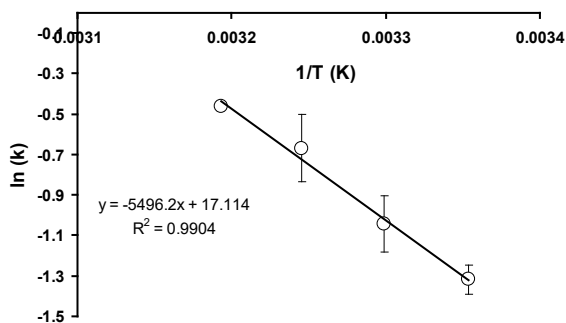

Fig. 1. Arrhenius plot of the rate of molybdenum reduction by *Pantoea* sp. strain HMY-P4.

In general, the higher the activation energy means the bacterium must spend more energy in metabolizing substances. Based on **Table 1**, the activation energy reported in this work, is lower than the activation energies for microbial processes in general, which spans the range from 33.5 to 50.3 kJ mol^{-1} [22] but since this is Antarctic bacterium the difference in value from the norm is not unusual since lower activation energies of between 10 and 20 kJ mol-1 for Antarctic bacterial proteases compared to mesophilic organisms have been reported [23]. Criticism to the Arrhenius models include the observation that the apparent activation energy, E_a or ΔH^* is usually presumed a constant value when in fact it is not; and is contingent to the range of temperature chosen as observed in this work [24]. Furthermore, the model is an empirical model and does not readily be interpretable when dealing with the complex biological systems having thousands of reactions occurring simultaneously. Strictly speaking, the E_a value is not the activation energy observed in chemical reactions, but it is the measurement of the microbial community's temperature response in totality. It is also known as the temperature characteristic [25]. Notwithstanding these issues, the model continues to be very popular among researchers globally.

Table 1. Arrhenius temperature characteristics for biological metal reduction.

Microorganisms	Temperature range (°C)	metal	ΔH^*apparent activation energy (kJ.mol^{-1})	Ref
Ochrobactrum intermedium BCR400	25-35	Chromate	120.69	[26]
Arthrobacter sp. SUK 1201	25-60	Chromate	36.21	[27]
Aspergillus niger	30-60	Chromate	8.56	[28]
Bacillus sp.	25-40	Chromate	22.0	[29]
Thermus scotoductus SA−01	65	Chromate	35 (membrane bound enzyme)	[30]
Thermus scotoductus SA−01	60-65	Iron	40.3 (soluble) 30	[30]
Shewanella profunda LT13a	4-37	Iron	50.3	[31]
β-Proteobacteria	15-40	Vanadate	36	[32]
Pantoea sp. strain HMY-P4 (this study)	25-35	molybdenum	45.69 (95% C.I., 32.04 to 59.35)	This study

The Q10 values can be determined either from the Arrhenius plots or as a ratio of growth rates measured at various incubation temperatures with ten degrees difference [33]. In the earlier case, the logarithmic value of the growth or bioreduction rates is plotted against 1000/temperature (Kelvin), while the slope of the Arrhenius curve is the value of the E_a (**Fig. 1**). Conversion of Q10 from E_a value is then calculated. The Q10 value of 1.82 (95% C.I., 1.52 to 2.18) obtained in this work, is within the normal range of 2 to 3 for many biological values. Until present, the Q10 value for molybdenum bioreduction has not been reported. This value is important in assigning the growth process to a characteristic biological activity. The validity of Q10 value holds for a range of studied temperature, though biological process may have more than one Q10 values for a range of different temperature under investigation. As far as molybdenum reduction is concern, a previous study demonstrates a similar Q10 value of 2.038 [34]. In other works, a Q10 value of 2.7 was obtained for the biodegradation of oil in a beach gravel column [35] while a bioventing study on decane and toluene contaminated soil exhibits a Q10 value of 2.2 [36]. Similarly, the effect of temperature on bacterial degradation of petroleum showed a Q10 value of 2.2 [37]. Whereas, acrylamide production by an immobilized bacterial system at temperature range between 25 and 45 °C gives a Q10 value of 2.8 as calculated for the free and immobilized cells [38]. Generally, Q10 value increase with decrease in temperature [39,40]. In another study, the growth of *Pseudomonas* sp. AQ5-04 on phenol gave a Q10 value of 1.834 [41] while the Q10 value for the growth rate on acrylamide by the Antarctic bacterium Pseudomonas sp. strain DRYJ7 is estimated as 2.17 and a theta value of 1.03 was obtained [42].

A theta value of 1.06 (95% C.I., 1.05 to 1.08) was calculated (**Fig. 2**), which was similar to a theta value of 1.08 calculated for the molybdenum reduction by the bacterium *Serratia* sp. strain HMY1 [34]. In the growth rate on acrylamide by the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7, a theta value of 1.03 was obtained [42]. The theta value is also within the range for many biological processes that is from 1.1 to 1.7 although higher values of up to 16.2 have been reported for the degradation of other xenobiotics [18].
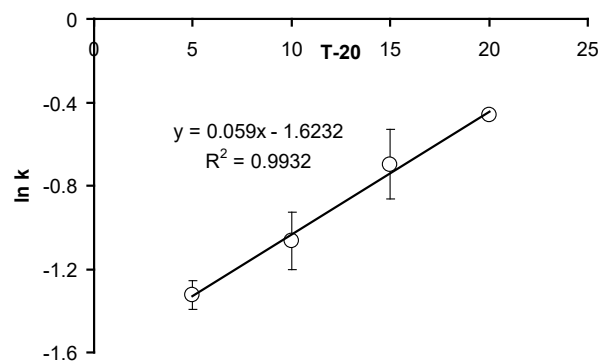

Fig. 2. Growth rate vs temperature plot for estimating theta.

CONCLUSION

Temperature generally affects microbial growth and metabolic activity on their substrates. The small nature of microbes makes them susceptible to change in surrounding temperature. The plot of ln q_{max} against $1/T$ shows resulted in an activation energy value of 45.69 kJ per mol (95% C.I., 32.04 to 59.35), an Arrhenius frequency factor (A) of 2.71×10^7 (95% C.I., 1.25×10^5 to 5.86×10^9). In addition, the Q10 and theta values for molybdenum bioreduction, estimated as 1.82 (95% C.I., 1.52 to 2.18) and a theta value of 1.06 (95% C.I., 1.05 to 1.08) which was determined from the Arrhenius plots has not previously been reported. The values obtained in this work, are within the normal range for many biological values.

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