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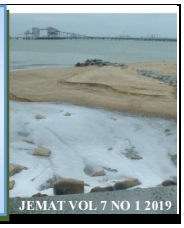

Arrhenius Plot Analysis, Temperature Coefficient and Q_{10} Value Estimation for the Effect of Temperature on the Growth Rate on Acrylamide by the Antarctic Bacterium *Pseudomonas* sp. Strain DRYJ7

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ABSTRACT

Several models are available to model the effect of temperature on the growth rate of microorganism on substrates. One of the models is Arrhenius and is very popular due to it having few parameters. An apparent activation energy based on the Arrhenius plot on the growth on acrylamide by *Pseudomonas* sp. strain DRYJ7 on acrylamide is reported for the first time. The bacterium was grown in minimal salts media supplemented with 1000 mg/L of acrylamide as a nitrogen source and glucose as the carbon source. The plot of ln μ_m (specific growth rate) against 1/T for growth on acrylamide was carried out spanning the range of temperature from 10 to 35 °C. Regression analysis from 10–20 °C results in an activation energy of 14.96 kJ mol^{-1}. A relative 10 °C increase in the surrounding temperature, usually results in doubling the reaction rate, with corresponding Q_{10} value of 1.8, which is the approximate value for a number of biological reactions. The Q_{10} values, determined from the Arrhenius plot of 2.17 and a theta value of 1.03 obtained in this work, are within the normal range for many biological values. This is the first time that values for the activation energy, Q_{10} and theta for the growth of a bacterium on acrylamide is reported.

INTRODUCTION

The majority of microbes, because of their minute size are strongly influenced by the ambient temp, as they are ectothermic. The outcome of temperature fluctuations influences microorganism adaptation and physiology via the modulation of their biochemical pathway. For this reason, temperature is certainly an important aspect that must be taken into account when understanding the biodegradation of toxic chemicals by microorganisms. The Arrhenius version is extremely popular to understand the actual end result of the effect of temperature in regards to the growth of harmful bacteria and has been utilized for the approximation of the apparent activation energy or ΔH^*, for growth or degradation on toxic substances. The values are

sometimes assumed to be constant, despite the conditions to the temperature range selected for bacterial processes can diverge either 3- or 4 times [1].

Likewise, the usefulness of the model over the comprehensive temperature range of bacterial metabolisms has been found to be limited and could be not even right in accordance with to literature reports [2]. Having said that, it is often reported that the Arrhenius model is very popular in modelling temperature effect to biological processes carried out by microorganism's in just a restricted range of temperature [3].

In general, there is limited information on the effect of temperature on the growth rate of bacterial cells on acrylamide especially from Antarctica. We have previously report on the isolation and characterization of an acrylamide-degrading bacterium from Antarctica [4]. One of the hallmarks of this paper is the demonstration of a breakpoint in the Arrhenius plot where it is very difficult to find a similar phenomenon reported in acrylamide biodegradation by bacteria. This breakpoint is important because it addresses the issue of the possible existence of more than one possible value for the activation energy of a biodegradation process in general and acrylamide in particular, especially when a wide range of temperature is studied. The Arrhenius' activation energy values are not only interesting fundamentally, but they will be very useful in predicting the fate and transport of acrylamide during bioremediation works.

MATERIALS AND METHODS

Growth and maintenance of acrylamide-degrading bacteria

The acrylamide-degrading bacterium *Pseudomonas* sp. strain DRYJ7 was previously isolated from Antarctica [4]. The growth and maintenance of the bacterium was carried out in growth medium as before [4].

Determination of specific growth rates on acrylamide

The maximum specific growth rate of the bacterium, μ_m was studied using a batch culture of the bacterium grown in MSM supplemented with acrylamide [4]. The modified Gompertz model was utilized to obtain the specific growth rates [5–7] (**Eqn. 1**) using a nonlinear regression software (CurveExpert Professional software, Version 1.6).

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad \text{[Eqn. 1]}$$

Activation energy of growth on acrylamide

Bacterial growth rate on acrylamide is affected by temperature and can be modelled according to the Arrhenius equation [8] as follows,

$$\mu = Ae^{-\frac{E_a}{RT}} \quad \text{[Eqn. 2]}$$

Where R (8.314 J $mol^{-1}K^{-1}$) is the universal gas constant, T (Kelvins or K = °C + 273.15) is the absolute temperature, E_a (kJ mol^{-1}) is the activation energy and A has a physical meaning in that it signifies the rate constant of which all of the participating molecules possess sufficient energy before reaction can occur (Ea= 0). The linearized form the equation is obtained by plotting the normal logarithms of the growth rate against 1/T as follows;

$$\ln \mu = \ln A - \frac{E_a}{R}.\frac{1}{T} \quad \text{[Eqn. 3]}$$

A two-part least-squares linear regression was utilised for the analysis of the breakpoint, regression coefficients estimation and statistical evaluation of the resultant Arrhenius plot [9].

Coefficient of Q_{10} estimation

The temperature dependence of biological reaction often reported as Q_{10} value, is the number of times that a 10 °C change in temperature results in changing the rate of the said reaction. The Q_{10} value relates to the activation energy via the following equation;

$$Q_{10} = e^{\left(\frac{Ea}{R}\right)\left(\frac{10}{T_2 T_1}\right)} \quad \text{[Eqn. 4]}$$

Following rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R}\right)\left(\frac{1}{T_1 T_2}\right) \quad \text{[Eqn. 5]}$$

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 Q_{10} rule;

$$k_T = k_{20}\Theta^{(T-20)} \quad \text{[Eqn. 6]}$$

RESULTS AND DISCUSSION

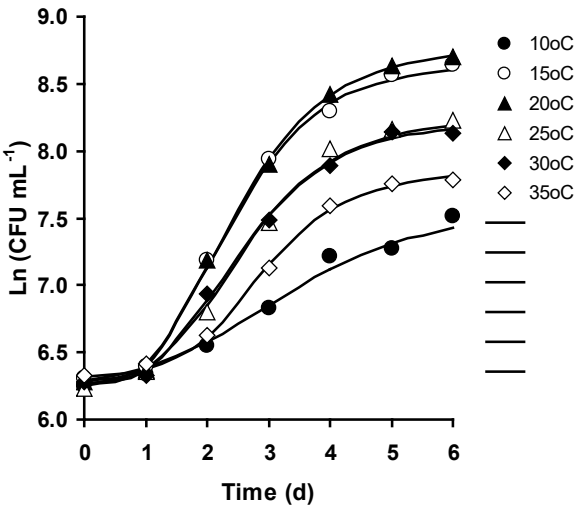

Fig. 1. Growth profiles of *Pseudomonas* sp. strain DRYJ7 grown at various temperatures.

The Arrhenius model has the fewest number of parameters and hence fare better parsimonously 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 [3].

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 [10]. 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. Initially, values of the specific growth rate; μ_m, were obtained from a modelling exercise using the modified Gompertz model (**Fig. 1**). The plot of ln μ_m against 1/T shows a discontinuous chevron-like graph for the whole temperature range studied (**Fig. 3**). An important observation is the existence of an inflection point located near the midrange at 21.12 ºC. At the temperature range below this point (10–20 ºC), the regression analysis result which is summarised in **Table 2** indicates that growth on acrylamide exhibited a lower activation energy (14.958 kJ mol^{-1}) than for the next temperature range studied (25–35 ºC) at 19.994 kJ mol^{-1}. A similar breakpoint of the Arrhenius plot was reported in *Bacillus* sp. JF8, a thermophilic polychlorinated biphenyl (PCB) degrader where two activation energies; 12.1 kJ mol^{-1} and 31.4 kJ mol^{-1} were reported for the temperature ranges from 50–70 ºC and 20–46 ºC, respectively [11]. However, this work shows for the first time, the breakpoint for growth on acrylamide.

The lower activation energy in between 10 and 20ºC compared to between 25 and 35 ºC indicates that a lesser energy is needed to break the amide bond in between 10 and 20 ºC. This is not surprising since higher energy allows for the increase in reaction rates. However, it is expected that at higher temperatures growth on acrylamide will be arrested as the denaturation of protein and enzymes will take place. The calculated apparent Arrhenius activation energy for growth on acrylamide for the temperature range from 10 to 35 ºC has not been reported before for acrylamide-degrading bacterial works but is within the range for values reported in the literature for other xenobiotic-degrading bacteria such as several phenol- and nonylphenol-degrading bacteria ranging from 28.4 to 57.74 kJ.mol^{-1} (**Table 2**).

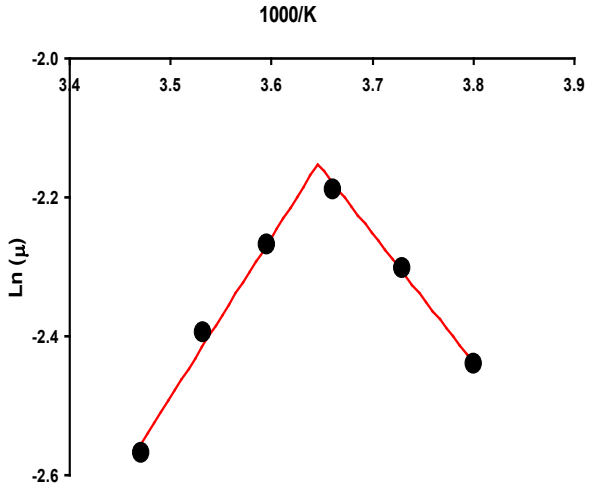

Fig 2. Arrhenius plot of growth rate on acrylamide.

In general, the higher the activation energy means the bacterium must spend more energy in metabolizing substances. Based on **Table 2**. 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} [16] 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 [17]. 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 [18]. 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 [19]. Notwithstanding these issues, the model continues to be very popular among researchers globally.

Table 1. The two-part linear regression analysis for the Arrhenius plot of growth rate on acrylamide.

Distribution of the experimental points	three points to the left, three points to the right
	Left part
Temperature range ^{O}C	10-25
Regression equation	y = 2.3994x - 10.886
Coefficient of determination	0.99
tan α ± Standard error	-10.885 ± 0.849
E_a ± Standard error, kJ mol^{-1}	19.94±1.99
t-Statistic	-22.87
Degrees of freedom	2
	Right part
Temperature range ºC	30-40
Regression equation	y=-1.8x+4.4036
Coefficient of determination	0.99
tan □ ± standard error	4.403± 0.294
E_a ± standard error, kJ mol^{-1}	14.958±0.653
t-Statistic	14.99
Degrees of freedom	2
	Break points data
Intersection coordinates, (x, y)	(3.646, -2.15)
Break point temperature ºC	21.12

Table 2. Arrhenius temperature characteristics for growth on xenobiotics.

Microorganisms	Temperature range (ºc)	Substrate	Δ*H**apparent activation energy (kJ.mol^{-1})	Ref
Activated Sludge	10–20	Phenol	39.0	[12]
Selanastrum Capricornutum	20–28	Phenol	28.4	[13]
Pseudomonas Putida Q5	10–25	Phenol	61.6	[3]
Acclimated Cultures	15-30	Nonylphenol	42.7	[14]
Pseudomonas Putida Mtcc 1194	15-30	Phenol	57.74	[15]
Bacillus sp. jf8	20-70	Polychlorinated Biphenyl (Pcb)	12.1 (20–46 ºc) 31.4	[11]

The abrupt transition or break points of the activation energy upon a change of temperature is a known occurrence in the physiology of microorganisms and have been reported in various processes using various substrates such as dye biodegradation [9] nitrification rate of *Nitrosomonas* cells [20], bacterial growth on glucose [21], ethanol [22] and EDTA [23], yield of biomass [22,24] and microbial cells thermal inactivation process [21,25]. It is still unknown what causes the transition, but there are two hypotheses that have been suggested. The first is that the transition is caused by a change in the physical properties of the water taking place at 15 ^{o}C interval [21], and a ''bottle neck'' hypothesis that exhibits limiting rate reaction in a succession of a chained of enzymatic reactions. The fact that the Arrhenius break point temperatures have been observed to be truly diverse seems not to support the first hypothesis [9]. As each of the chained enzymatic reactions will have unique thermal characteristics, the "bottle neck" hypothesis is difficult to be proven. Also, the cellular membrane will change according to the surrounding temperature, and this has to be taken into account as well [26]. To date, the "bottle neck" hypothesis remains popular among researchers [9,22].

The Q_{10} 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 [27]. 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 Ea. Conversion of Q_{10} from Ea value is then calculated according to **Eqn. 4**. The Q_{10} value of 2.17 obtained in this work, is within the normal range of 2 to 3 for many biological values. The theta value of 1.03 is also within the range for many biological processes. Values range from 1.1 to 16.2 have been reported for the degradation of other xenobiotics [28]. Until present, the Q_{10} value for growth on acrylamide has not been reported.

The validity of Q_{10} value holds for a range of studied temperature, though biological process may have more than one Q_{10} values for a range of different temperature under investigation. For example, a Q_{10} value of 2.7 was obtained for the biodegradation of oil in a beach gravel column [29] while a bioventing study on decane and toluene contaminated soil exhibits a Q_{10} value of 2.2 [30]. Similarly, the effect of temperature on bacterial degradation of petroleum showed a Q_{10} value of 2.2 [31]. Whereas, acrylamide production by an immobilized bacterial system at temperature range between 25 and 45 ^{o}C gives a Q_{10} value of 2.8 as calculated for the free and immobilized cells [32]. Generally, Q_{10} value increase with decrease in temperature [33,34].

The Q_{10} value of this bacterium is within the range of biological activities, reported for the first time for growth on molybdate medium. This value is important in assigning the growth process to a characteristic biological activity.

CONCLUSION

The activation energy needed for growth on acrylamide by *Pseudomonas* sp. strain DRYJ7 shows a discontinuous profile with two activation energies as seen in the Arrhenius plot. The activation energy of between 10 and 20 ^{o}C was much less than between 25 and 35 ^{o}C. More works are currently being explored to assess the effects of temperatures on the growth kinetics especially on the parameters themselves.

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