

Arrhenius Plot Analysis of the Temperature Effect on the Biodegradation Rate of 2-chloro-4-nitrophenol

Mohd Yunus Shukor

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia
43400 UPM Serdang, Selangor, Malaysia
Email: yunus.upm@gmail.com/mohdyunus@upm.edu.my

ABSTRACT. Several models are available to determine the effect of temperature on the growth rate of microorganisms on substrates. An example is Arrhenius model, which is very popular because it has few parameters. For the first time, a discontinuous chevron-like graph of apparent activation energy based on the Arrhenius plot on the growth of 2-chloro-4-nitrophenol by *Cupriavidus* sp. is reported. The plot of $\ln m_m$ against $1/T$ shows a discontinuous chevron-like graph for the entire investigated temperature range with an inflection at 27.75°C. This indicates that the existence of 2 activation energies for growth on 2-chloro-4-nitrophenol ranges from 20 to 40°C. Furthermore, a regression analysis from 20–25°C and 30–40°C results in activation energies of 88.71 kJmol⁻¹ and 75.16kJ mol⁻¹, respectively. This is probably the first time a Chevron-like graph was observed for the Arrhenius plot on the effect of temperature on the growth rate of 2-chloro-4-nitrophenol.

Keywords: Arrhenius plot; breakpoint; *Cupriavidus* sp.; temperature; 2-chloro-4-nitrophenol-degrading

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INTRODUCTION

Even at low doses, the toxicity of phenols and phenolic compounds is well known and their existence as toxins in the atmosphere is progressively recorded (Christen *et al.*, 2012; Luo *et al.*, 2012; Zulkharnain *et al.*, 2013; Affandi *et al.*, 2014; Bay *et al.*, 2014; Ghazali & Johari, 2015). Meanwhile, there are some physicochemical methods for the removal of these toxins, these include biodegradation, which is still being studied to date as an environmentally friendly process (Saravanan *et al.*, 2008; Hasan & Jabeen, 2015).

Furthermore, the typical representatives of chlorinated nitroaromatics include chloronitrophenols, which are commonly used in the synthesis of dyes, pesticides, fungicides, medicines, colorants, and others (Fuentes *et al.*, 2013; Arora *et al.*, 2018; Kasana & Pandey, 2018). The most common CNP isomer that is used for the manufacture of the herbicide dicapthone and the fungicide nitrofungin is 2-chloro-4-nitrophenol (Ghosh *et al.*, 2010; Arora & Jain, 2011; Pandey *et al.*, 2011). This isomer has caused significant degradation to agricultural soils and water supplies due to its solubility and high mobility, which results in the extreme negative health effects on humans

and animals. Therefore, the removal of 2-chloro-4-nitrophenol from the environment has recently aroused wide biodegradation concern, which had been presented as a potential environmentally friendly method as several degrading bacterium have been isolated (Ghosh *et al.*, 2010; Arora & Jain, 2011; Pandey *et al.*, 2011). Virtually all microorganisms are specifically affected by temperature due to their size. By regulating the internal biochemical pathways, temperature affects and influence microorganism physiology as an adaption tool to suit new environments. Therefore, temperature is a significant consideration which must be taken into account when studying the biodegradation of compounds. Furthermore, the Arrhenius model is a popular method in investigating the effects of temperature on bacterial growth rates. It is also used to quantify the apparent activation energy or ΔH^* , for growth or degradation on substrates.

The values are normally falsely assumed to be constant, even though ΔH^* diverges either three or fourfold but conditional to the range of temperature being studied (Singh *et al.*, 2008). Likewise, its applicability over the entire temperature of the bacterial process is

constrained and may not be reliable (Reardon *et al.*, 2000). However, this model is commonly used in the simulation of the temperature effect in a small range (Onysko *et al.*, 2000). Furthermore, Arrhenius plot show a significant but an unknown breakpoint, which is a sudden shift of the activation energy (Angelova *et al.*, 2008) when the growth rate is tested over a wide temperature.

In contrast to other models such as the Schuelefield, Eyring and Urry, Sharpe and DeMichele, Arrhenius includes the least amount of parameters and therefore is generally accepted by most investigators (Onysko *et al.*, 2000). Ratkowsky is another model competitor, however, its biological foundation tends to be insufficient and relies on the analytical study of the linear relationship between the temperature and the square root of the growth rate (Zwietering *et al.*, 1991). Therefore, Arrhenius models are commonly used in explaining the effects of temperature on bacterial growth. The estimation of its parameter is derived from the linear regression of Arrhenius plot.

In this analysis, activation energies for the degradation of 2-chloro-4-nitrophenol by a bacterium demonstrated a breakpoint indicating that it has more more than one possible value. Furthermore, the activation energy is not only fundamentally interesting, but it is very helpful in predicting the biodegradation and transport of phenols during bioremediation works.

MATERIALS AND METHODS

Activation energy of growth on 2-chloro-4-nitrophenol. The data on the rate of biodegradation in Table 1 (Min *et al.*, 2018) at various temperatures was processed using Webplotdigitizer 2.5 (Rohatgi, 2018). Furthermore, Arrhenius equation (Arrhenius, 1889) was utilized to model the effect of temperature on biodegradation rate as follows,

$$\mu = Ae^{-\frac{E_a}{RT}} \quad [1]$$

Where,

T (Kelvins or K = °C + 273.15) is the absolute temperature, R (8.314 J mol⁻¹K⁻¹) is the universal gas constant, E_a (kJ mol⁻¹) is the activation energy while the physical meaning of

A relates to the rate constant of which all of the participating molecules acquires enough energy before the reaction (E_a= 0). The linearized form the equation is attained by plotting the normal logarithms of the growth rate against 1/T as follows;

$$\ln \mu = \ln A - \frac{E_a}{R} \cdot \frac{1}{T} \quad [2]$$

The analysis of the breakpoint, statistical evaluation of the resultant Arrhenius plot, and regression coefficients estimation were carried out using the two-part least-squares linear regression (Angelova *et al.*, 2008; Gafar *et al.*, 2019).

RESULTS AND DISCUSSION

A Chevron-like graph was obtained when plotting $\ln \mu_m$ against 1/T showing a discontinuous part for the whole temperature range studied (Fig. 1). The presence of an inflection point is an important observation found near the midrange at 27.75°C. At 20–25°C, the regression analysis result, which is summarized in Table 1 indicates that the growth on 2-chloro-4-nitrophenol exhibited an activation energy of 75.165 kJ mol⁻¹ while at 30–40°C, it was 88.711 kJ mol⁻¹. The overlapped 95% confidence interval between these two values indicate that there is no significant difference in terms of activation energy value. Furthermore, the apparent Arrhenius activation energy for growth on 2-chloro-4-nitrophenol for the temperature studied is within the range of the reported values in the literature for halogenated phenol and is much higher than several phenol-degrading bacteria, which ranged from 28.4 to 57.74 KJ.mol⁻¹ (Table 2). It appears that more energy is needed to break the halogenated bonds. Moreover, increasing the temperature reduces the energy needed. This is probably the reason for the lower activation energies observed in the degradation of polychlorinated biphenyl (PCB) by the thermophilic *Bacillus* sp. JF8, where two activation energies; 12.1 kJ mol⁻¹ and 31.4 kJ mol⁻¹ were reported for 50–70°C and 20–46°C, respectively (Mukerjee-Dhar *et al.*, 2005). However, this study shows the breakpoint for growth on 2-chloro-4-nitrophenol for the first time.

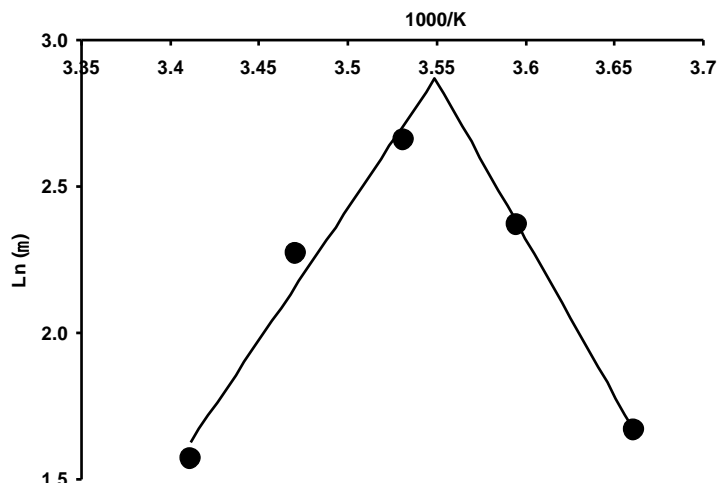


Fig. 1. Arrhenius plot of 2-chloro-4-nitrophenol biodegradation rate

The higher the activation energy, the more energy is required by the bacterium to metabolize phenolics. Based on Table 2, *Pseudomonas* species tend to be more energetic than other organisms in degrading phenol. However, this does not mean a better phenol degradation efficiency compared to other bacteria. As mentioned above, the activation energy, which ranges from 33.5 to 50.3 kJ mol⁻¹ are far higher than those needed by mesophilic (Tchobanoglous & Schoeder, 1985).

Furthermore, the criticism of Arrhenius model is based on the observation that the activation energy, is generally believed to be a constant value when it is actually not, and it is dependent on the temperature selected as observed in the present study (Ratkowsky *et al.*, 1982). Therefore, the model is observational, which is not easily understood as the complicated interacting biological processes occurs simultaneously in thousands at the same time. Strictly speaking, the activation energy value is not used in chemical reactions but is the measurement of the overall temperature response of the microbe (Melin *et al.*, 1997). Despite these challenges, the model is still popular among scientists worldwide.

The sudden transition or breaking point of the activation energy as temperature shift is an integral physiology of microorganisms and has been documented using different substrates. For instance, the biodegradation of dye (Angelova *et al.*, 2008), *Nitrosomonas* cells nitrification rate (Benyahia & Polomarkaki, 2005), bacterial utilization of glucose (Kuhn *et*

al., 1980), utilization of ethanol (Mutafov & Minkevich, 1986), biodegradation of EDTA (Minkevich *et al.*, 2006), biomass yield (Chistyakova *et al.*, 1983; Mutafov & Minkevich, 1986), and the thermal inactivation of microorganisms (Verrips & Kwast, 1977; Kuhn *et al.*, 1980).

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

Distribution of the experimental points	Three points to the left, two points to the right
Temperature range °C	30, 35 and 40
Regression equation	y=9.0452x-29.23
Coefficient of determination	0.970
tan θ ± Standard error	9.045±1.602
E _a ± Standard error, kJ mol ⁻¹	75.165 ± 13.312
t-Statistic	65535
Degrees of freedom	2
	Right part
Temperature range °C	20 and 25
Regression equation	y=-10.675x+40.75
Coefficient of determination	1.00
Tan θ ± Standard error	-10.675±0.000
E _a ± Standard error, kJ mol ⁻¹	88.711±0.00
t-Statistic	5.64
Degrees of freedom	1
	Break points data
Intersection coordinates, (x, y)	(3.549,2.868)
Break point temperature °C	27.75

Table 2. Arrhenius temperature characteristics for growth on phenol and phenolics

Microorganisms	Temperature range (°C)	Substrate	ΔH^* apparent activation energy (Kj.mol ⁻¹)	Ref
activated sludge	10–20	phenol	39.0	Benedek & Farkas, 1970
<i>Selanastrum capricornutum</i>	20–28	phenol	28.4	Reynolds <i>et al.</i> , 1974
aerobic fluidized-bed reactors (FBRs)	14-16.5	2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), and pentachlorophenol (PCP)	TCP and TeCP 126-194 PCP 59-130	Melin <i>et al.</i> , 1998
<i>Pseudomonas putida</i> Q5	10–25	phenol	61.6	Onysko <i>et al.</i> , 2000
Acclimated cultures	15-30	nonylphenol	42.7	Jahan <i>et al.</i> , 2008
<i>Pseudomonas putida</i> MTCC 1194	15-30	phenol	57.74	Bandyopadhyay <i>et al.</i> , 1981
<i>Bacillus</i> sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20–46°C) 31.4 (50–70°C)	Mukerjee-Dhar <i>et al.</i> , 2005
<i>Pseudomonas</i> sp. AQ5-04	15-45	phenol	38.92 (15–30°C) 11.34 (35–45°C)	Aisami <i>et al.</i> , 2017
<i>Pseudomonas</i> sp. Strain DrYJ7		acrylamide	14.96 (10–20°C)	Gafar <i>et al.</i> , 2019
<i>Cupriavidus</i> sp. strain CNP-8	20-40	2-chloro-4-nitrophenol	75.16 (30-40°C) 88.71 (20-25°C)	This study

What triggers the transformation is still unclear, however, two theories are proposed. The first is that a difference in the physical properties of water is induced during the transition at 15°C (Kuhn *et al.*, 1980). The second is the theory of "bottle neck", which indicates a small rate reactions across a series of enzyme in succession. The fact that the Arrhenius breakpoint temperatures have been observed to be truly diverse seems not to support the first hypothesis (Angelova *et al.*, 2008). Since each of the chained enzyme has special thermal properties, it is difficult to prove the "bottle-neck" theory. Moreover, the cell membrane varies depending on the surrounding temperature, and this need to be taken into account (Ceuterick *et al.*, 1978). From the many hypothesis, the "bottle neck" continues to be prevalent among scholars (Mutafov and Minkevich, 1986; Angelova *et al.*, 2008).

CONCLUSION

This is the first study to demonstrate that the required activation energy for the biodegradation of 2-chloro-4-nitrophenol by

Cupriavidus sp. strain CNP-8 displays a broken profile with the two observed in the Arrhenius plot. This discontinuous activation energy covers the temperature range of 20 to 25 and 30 to 40°C with values that are not significantly different. However, the quantum are much higher than the typical energies observed in mesophilic microorganisms. Furthermore, the halogenated bond is postulated to hold much higher activation energy to be broken. Finally, further investigations are been carried out particularly on the use of parameters to determine the effects of temperature on growth kinetics.

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