

The Effect of Temperature on the Specific Growth Rate of *Bacillus circulans* strain Neni-10 on Metanil Yellow: Determination of Activation energy, Temperature Coefficient and Q₁₀ Value

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HISTORY

Received: 24th April 2023
Received in revised form: 28th June 2023
Accepted: 14th July 2023

KEYWORDS

Metanil Yellow
Bacillus circulans
Activation energy
Arrhenius plot
Breakpoint

ABSTRACT

Bioremediation is a sustainable process. To better understand the effect of temperature to a bioremediation process, analyzing the effect of temperature to the specific growth values of a remediate microbes on a particular xenobiotic will yield important information that can be translated into the field in future studies. Due to their tiny size, microbes are particularly very sensitive to variations in temperature. Temperature typically influences the growth and metabolic activity of bacterium on the substrates they are grown upon. There are several models that may be utilized to mimic the rate of growth of microbes on a numerous plethora of substrate or media at varying temperatures. Among the most often adopted models is the Arrhenius model, in part because to its minimal parameter number. To study the effect of temperature on *Bacillus circulans* strain Neni-10 growth on Metanil Yellow, a chevron-like graph with a discontinuous apparent activation energy was observed. The graph's exhibited a breakpoint at 31.91 °C. Per the regression analysis obtained, the activation energy needed for growth on Metanil Yellow at temperatures ranging between 20 and 30 °C was 62.07 kJ/mol. At temperatures ranging from 35 to 45 °C, its activation energy reduces to 30.93 kJ/mol. At a temperature of 35 °C, the maximum growth rate of the bacterium on Metanil Yellow occurred, and this rate was found to be reduced as the temperature was increased.

INTRODUCTION

Approximately one million tonnes of diazo dyes and direct basic dyes are generated yearly. The Ecological and Toxicological Association of the Dyestuff Manufacturing Industry has indicated that some of the dyes exhibited elevated levels of toxicity (LD₅₀) to numerous animal models. Being highly pigmented and water-soluble, these reactive and acid dyes are often unaffected by standard water treatment processes [1]. In addition, their elevated Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), color, pH, and concentration of harmful metal ions pose a serious ecological danger [2]. Given the fact that 10–15 percent of dyes are lost in the effluent during the dyeing process, it is common knowledge

that the textile industry is one of the greatest contributors to water contamination. Absorption by mouth induces cyanosis and leads to toxic methaemoglobinemia in humans. Animals administered Metanil Yellow by intraperitoneal, oral, or intratesticular methods had testicular abnormalities, most likely due to injury to the seminiferous tubules, according to toxicity testing. The final outcome is a drop in the rate of spermatogenesis [3]. Exposure with the colorant causes severe dermatitis. Other studies have demonstrated that Metanil Yellow possesses carcinogenic effects in human subjects [3].

Throughout the majority of the difference in temperature, the value of delta H, indicated by H*, is very steady. Depending on the temperature range, this figure can vary by a multiplier of

3 or 4 if exposed to extreme temperatures [4]. During conducting research on the microorganisms responsible for the chemical breakdown process especially in biodegradation works, temperature is a key factor to consider. Typically, the Arrhenius model is used to determine the value for the apparent activation energy, H^* , that is assumed to be present for either growth or decay on distinct metabolic substrates. This trend is predicted to continue in the near future [5–13]. In certain research, applying the concept to the whole temperature range of the bacterial process could be inaccurate [14]. Constructing the Arrhenius plots may potentially reveal a possible early transition characterized by a rapid change in the value of the activation energy [15].

The Arrhenius' model is widely used to simulate temperature effects; however, when the temperature ranges are broad, this model is adopted much less often [16]. Due to the wide range of biological foundations and behavior that is nonlinear, this competing Ratkowsky model is similarly built on the concept of growth that is linear [17]. Since the Arrhenius model has the fewest parameters, it is broadly acknowledged among scientists [16]. This shows that Arrhenius model is required to determine whether temperature affects the bacterial growth. To obtain an estimate of the Arrhenius parameter, first an Arrhenius plot must be constructed, followed by applying linear regression to the data [8]. This research reveals a heretofore unrecognized fact: a bacterium may degrade Metanil Yellow at with two values of the activation energy. This knowledge would be incredibly valuable for forecasting the breakdown and fate of Metanil Yellow during the process of bioremediation.

MATERIALS AND METHODS

Growth and maintenance of bacterium and measurement of the Activation energy of growth on Metanil Yellow

The Metanil Yellow-degrading bacterium growth and growth characterization on Metanil Yellow has been published previously [18]. Growth data from Metanil Yellow-degrading bacterium was processed by converting the temperature-dependent growth rates to natural logarithms (Fig. 1).

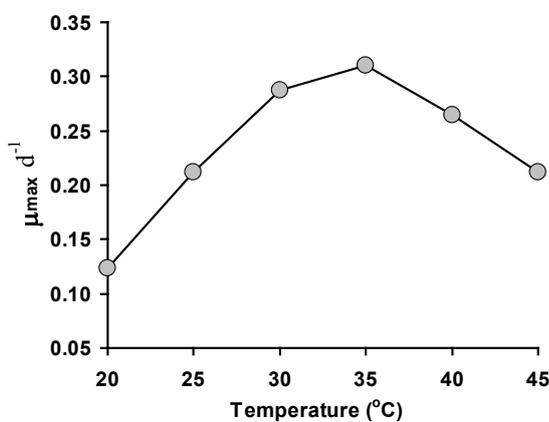


Fig 1. The effect of temperature on the specific growth rate of the bacterium on Metanil Yellow.

The Arrhenius equation [19] is as follows,

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

Where T represents absolute temperature (Kelvin = °C + 273.15), R is the universal gas constant (0.008314 kJ/molK⁻¹), E_a represents activation energy (kJ/mol) and A kinetically represents the rate constant at which all the interacting molecules have adequate energy prior to a reaction ($E_a = 0$). Using the plot of log normal growth rate against 1/T, the following linearized form is derived:

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

Coefficient of Q₁₀ estimation

The Q₁₀ value can be estimated via the following equation;

$$Q_{10} = e^{\left(\frac{E_a}{R}\right)\left(\frac{10}{T_2 T_1}\right)} \quad [\text{Eqn. 3}]$$

Following rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R}\right)\left(\frac{1}{T_1 T_2}\right) \quad [\text{Eqn. 4}]$$

The coefficient of temperature, or theta (θ), is another key biological constant determined by putting provided values into the reaction rates equation governed by the Q₁₀ rule. The equation is as follows;

$$kT = k20\theta(T-20) \quad [\text{Eqn. 5}]$$

RESULT AND DISCUSSION

When the logarithmic growth rate (per day) was compared to the temperature in the form of 1/T, a Chevron-shaped graph is formed. This plot revealed a break in the curve over the whole temperature range (Fig. 2). Completely unexpected was the discovery of a breaking threshold at 31.91 degrees Celsius. Utilizing the Arrhenius model yielded activation energies which fell within the range of activation energies described in the research journals for a number of degradation processes (Table 2). As per the regression analysis given in Table 1, the activation energy needed for growth on Metanil Yellow at temperatures between 20 and 30 degrees Celsius was 62.07 kJ/mol. At temperatures ranging from 35 to 45 degrees Celsius, its activation energy was reduced to 30.93 kJ/mol.

The maximum rate of bacterial growth was seen on Metanil Yellow at 35 degrees Celsius, and this rate was reduced as the temperature increases (Fig. 2). In lieu of mentioning the existence of two activation energies, several studies on the determination of activation energy for biodegradation processes merely mention a single value of an activation energy that spans a wide temperature range. It appears that the results of two activation energies will involve more work on in the future. There is a relationship between an increase in temperature and a reduction in energy use. This is due to the fact that disclosing the occurrence of two activation energies would necessitate more investigation. According to the results of one study, activation energy is greater at higher temperatures than at lower temperatures. In contrast, the findings of the second research indicate that the phenomena happen in the other way. This is due to the fact that the activation energy is proportional to the observed temperature (Table 2). This study indicates that the

activation energy is higher at lower temperatures than at higher temperatures. Nevertheless, both cases are supported by the findings of the other study in the literature. This is due to the fact that the activation energy is proportional to the observed temperature (Table 2).

A similar trend to this study was observed in another study involving the growth on phenol from 15 to 30 °C by *Pseudomonas* sp. AQ5-04 of 38.92 KJ/mol and from 35-45 °C, the activation energy is 11.34 KJ/mol [20]. A contrasting study was discovered in the biodegradation of polychlorinated biphenyl (PCB) by the bacterium *Bacillus* sp. JF8 exhibited an activation energy of 12.1 KJ/mol from the temperature range 20 to 46 °C and 31.4 KJ/mol from the range 50 to 70 °C [21].

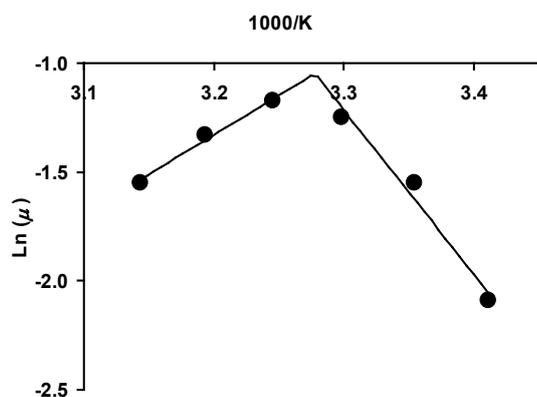


Fig 2. Growth rate of the bacterium in the form Arrhenius plot.

Table 1. The Arrhenius plot of the growth rate of Metanil Yellow-degrading bacterium was subjected to regression analysis.

Distribution of the experimental points	Three points to the left, three points to the right
Temperature range °C	Left part 35 to 45
Regression equation	$y = 3.7215x - 13.236$
Coefficient of determination	0.988
$\tan a \pm$ Standard error	± 0.41
$E_a \pm$ Standard error, kJ mol ⁻¹	± 3.40
t-Statistic	9.09
Degrees of freedom	2
Temperature range °C	Right part 20 to 30
Regression equation	$y = -7.4687x + 23.426$
Coefficient of determination	0.9778
$\tan a \pm$ Standard error	± 1.13
$E_a \pm$ Standard error, kJ mol ⁻¹	± 9.35
t-Statistic	-6.64
Degrees of freedom	2
Intersection coordinates, (x, y)	Break points data 3.278
Break point temperature °C	31.91

Whenever considering bacteria, temperature is a highly important variable to take into account. Temperature controls the rate during which metabolic activities occur, virtually every element of the structure, folding, and biomolecules stability is also governed. This consists of the rate at which metabolic activities occur. A bacterium's ability to continue surviving in its surroundings leverage on its ability to change its metabolic activity accordingly.

Table 2. Activation energy values for bacterial growth on numerous xenobiotics.

Microorganisms	Temperature range (°C)	Substrate	ΔH^* apparent activation energy (KJ.mol ⁻¹)	Ref
activated sludge	10-20	phenol	39.0	[22]
<i>Selanastrum capricornutum</i>	20-28	phenol	28.4	[23]
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	[24]
<i>Pseudomonas putida</i> Q5	10-25	phenol	61.6	[16]
Acclimated cultures	15-30	nonylphenol	42.7	[25]
<i>Pseudomonas putida</i> MTCC 1194	15-30	phenol	57.74	[26]
<i>Bacillus</i> sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20-46 °C) 31.4 (50-70 °C)	[21]
<i>Pseudomonas</i> sp. AQ5-04	15-45	phenol	38.92 (15-30 °C) 11.34 (35-45 °C)	[20]
<i>Pseudomonas</i> sp. Strain DrYJ7	10-20	SDS	14.96	[8]
<i>Cupriavidus</i> sp. strain CNP-8	20-40	2-chloro-4-nitrophenol	75.16 88.71	[27]
<i>Escherichia coli</i> BL21	20-50	Chromate	28.01	[28]
<i>Ochrobactrum intermedium</i>	25-35	Chromate	120.69	[29]
<i>Shewanella oneidensis</i> MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[30]
anaerobic sludge	30-55	Reactive Red 2	22.9	[31]
activated bacterial consortium	20-37	Remazol Black B	48.8	[32]
<i>Enterobacter</i> sp. strain (GY-1)	20-35	Reactive Black 5 (RB 5)	35.56	[33]
<i>Escherichia coli</i> NO3	20-45	Reactive red 22	27.49	[34]
<i>Pseudomonas aeruginosa</i>	15-45	Reactive Black 39 and Acid Red 360 by EDTA	RB39 61.89 AR360 81.18 91.2	[35]
<i>Pseudomonas</i> sp. LPM-410	20-28			[36]
<i>Pseudomonas</i> sp. AQ5-04	15-45	phenol	38.92 (15-30 °C) 11.34 (35-45 °C)	[20]
<i>Cupriavidus</i> sp.	20-25 30-40	2-chloro-4-nitrophenol	88.71 (20-25) 75.16 (30-40)	[9]
<i>Pseudomonas</i> sp. strain DRYJ7	10-20	Acrylamide	14.96	[8]
<i>Bacillus albus</i> DD1	25-45	Reactive Black 5	34.46	[37]
<i>Bacillus circulans</i> strain Neni-10	20 to 30 35 to 45	Metanil Yellow	62.07 30.93	This study

Note: (TPPS) Meso-tetrakis (4-sulfonatophenyl) porphyrin mediator

Organisms like bacterium can adjust the expression of the gene expression according to variations of temperature as they have specific sensory systems capable of indirectly sensing fluctuations of temperature. This allows the organisms to adapt correctly to environmental temperature fluctuations. The bacterium is therefore able to respond appropriately. Both a cold and heat shocks condition have the ability to result in the aggregation of dormant ribosomes, but only a cold shock does have the capacity to result in the aggregation of inactive proteins. The buildup of inactive ribosomes is also a possible outcome of heat shocks [6,7,38–43].

Molecular switching might be used to construct thermosensors at the molecular level. Changes in immediate sensing purposes can take on a number of distinct forms. As an example, consider the modification of molecular structures to induce malfunctioning activity. Further illustrations incorporate regulatory proteins that respond to the fluctuations of temperature resulting in the destruction of the integrity of the lipid membrane affecting the flexibility or fluidity of the membrane. In addition, there are regulatory proteins that are fluidity responsive. Regarding the control of biochemical functions, it is nearly hard to overestimate the significance of temperature. Application areas in the realm of temperature-controlled biotechnology may employ this approach.

Adjustments in temperature induce a vast array of metabolic alterations, the bulk of which are intricately interconnected leading to a single process due to their tight interaction. Considering this, the creation of models that illustrate how the progression of a living process diverges as a function of fluctuations in temperature is essential if we wish to streamline matters and get a deeper knowledge of how the whole thing is interconnected. Arrhenius devised his "primal temperature model," a mathematical explanation of how living responses responded to high or low ranges of temperatures. The Arrhenius model represented mathematically when biological responses respond to high or low temperatures. Arrhenius's model shows how the behavior of biological responses may alter as a function of fluctuating temperature [39–43].

When the activation energy is higher, bacterium requires more energy as they must degrade more complicated xenobiotics. This increases the quantity of the activation energy. Values of the activation energies of a number of bacterial species competent of biodegrading toxic chemicals descent within the arrays given in this analysis for both range of temperature, as determined by the results of this experiment. The outcomes are given in Table 2. Generally, the activation energy of typical mesophilic bacteria vary between 33.50 and 50.30 kJ/mol [44]. There is always a possibility that one part of the studied range of temperature generated findings with a larger activation energy than the others.

In this study, it was shown that the value of the activation energy is not consistent but instead fluctuates with fluctuating in temperature [45]. Because the model cannot account for all of the concurrent interfaces that occur linking the several biological systems, it is advised that it be used as an experimental model instead than an extrapolative one. Rather than considering activation energy in conditions of the activation energy necessary for biochemical reactions, one must think of the activation energy as a result of the microorganism's entire response to temperature fluctuations.

This will help us gain a greater understanding of the value of the activation energy obtained [46]. Regardless of the fact that the specific process that produces the transformation is still unidentified, there are two possible explanations for the change. These choices include: The water properties vary throughout the transition through one state to the other, so there is a notion known as the "bottleneck" that states that the same processes are occurring concurrently and rapidly [47]. A substantial sample sizes on the temperatures at which the Arrhenius break point occurs indicate that the first theory is not valid. According to the "bottle-neck" concept, the "bottle-neck" hypothesis is infamously hard to demonstrate for a variety of diverse causes. Cell membrane is sensitive to fluctuation of temperature and variations occurs as the temperature changes [48]. The "bottleneck" perception is still extensively embraced in academia [15,49].

Also, there is the option of estimating the Q_{10} values using Arrhenius graphs or by monitoring the rate of growth at a range of ten-degree-variable temperature ranges. The gradient of the graph of Arrhenius curve follows when the growth rates or bioreduction are plotted using the logarithmic scale (Kelvin) [50] (**Fig. 1**).

It was found that a Q_{10} value of 1.46 follows the temperature range of 35 to 45 degrees Celsius (**Fig. 3**). Owing to the energetic nature of biological activities, however, for each range of temperature investigated, many Q_{10} values might well be obtained. The bioreduction of sodium molybdate into molybdenum blue exhibited a Q_{10} value of 2.038 [51]. A Q_{10} value of 2.31 was discovered in a molybdenum reducer. This value is vital for correctly assigning the growth activity to a particular biological reduction activity. Prior studies determined Q_{10} to be 2.7 for oil breakdown in a column of beach pebbles [52]. In spite of this, another investigation on decane- and toluene- biodegradation in polluted soil revealed a Q_{10} value of 2.2 for the microbiological-aided process [53].

It was observed that the Q_{10} value for other petrochemical compounds influenced by salinity was 2.2 [54], and the degradation of acrylamide between 25 and 45 degrees Celsius in a bacterium in an immobilized form yielded a Q_{10} value of 2.8 [55]. Typically, reducing the temperatures produces in an expansion in the Q_{10} value [56,57]. The Q_{10} value in the phenol-degrading *Pseudomonas* sp. strain AQ5-04 was 1.834 [20] while its growth rate on another toxicant; SDS, yielded a Q_{10} value of 2.17. Another research on acrylamide biodegradation by the Antarctic bacteria *Pseudomonas* sp. strain DRYJ7 reports a lower Q_{10} value of 2.17 [8].

In this study, the theta value was 1.04 (**Fig. 3**). In one study, the molybdenum reduction by *Serratia* sp. strain HMY1 was found to be near to the theta value of 1.08 [51]. The bacterium *Pseudomonas* sp. strain DRYJ7, an Antarctic bacterium, had a reduced theta value of 1.03 for its growth rate on acrylamide [8]. There have been examples of xenobiotics being degraded with theta values as high as 16.2, however theta values are normally between 1.1 to 1.7, which is the typical range for various biological activities [58]. However, lower values from the typical range has been reported such as during the nonylphenol biodegradation by soil bacteria, were a theta value of 1.06 was recorded [25].

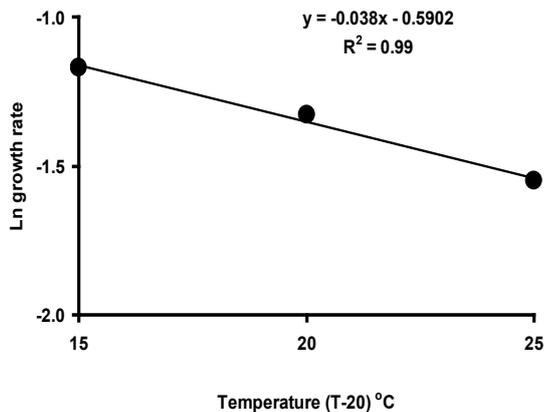


Fig. 3. Ln growth rate vs temperature plot for estimating theta.

CONCLUSION

For the biodegradation of Metanil Yellow, an activation energy calculated based on the Arrhenius plot revealed the presence of two activation energies. This study's findings are the first for biodegradation of Metanil Yellow. The influence of temperature on the growth development of bacteria and the metabolic activity of the substrates on which they fed is substantial. Temperature plays an essential role in each of these processes. Due to their small size, the tiniest changes in temperature can tremendously affect microbes. On the presented plot depicting apparent activation energies, the apparent activation of Metanil Yellow shows a break point at 31.91 degrees Celsius. Growth on Metanil Yellow at temperatures between 20 and 30 degrees Celsius exhibited an activation energy value of 62.07 kJ/mol. However, activation energy was reduced to 30.93 kJ/mol at temperatures ranging from 35 to 45 degrees Celsius. The maximum rate of bacterial growth was seen on Metanil Yellow at 35 degrees Celsius, and this rate was reduced as the temperature increases. Another important parameter is the Q_{10} value. For the tested temperature range, the value of Q_{10} was determined to be 1.46, while the theta value was determined to be 1.04 (35 to 45 degrees Celsius), both values are well within the typical range for biological processes. It is well acknowledged that a significantly larger activation energy is required to successfully break a strong bond like the azo bond. In order to have a deeper knowledge of the relationship between the effect of temperature on bacterial growth kinetics, more study and investigation are presently under study. This is being done to have a greater comprehension of the relationship between temperature and growth kinetics.

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