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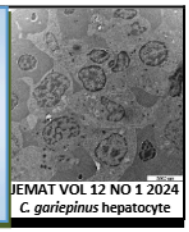

Activation Energy, Temperature Coefficient and Q_{10} Value Estimations of the Growth of *Rhodotorula* sp. Strain MBH23 on Acrylamide

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ABSTRACT

Various models can be used to predict how microorganisms grow at different temperatures, with the Arrhenius model being one of the most commonly used due to its simplicity and requiring only a few parameters. Temperature is a key factor that impacts microbial growth and their metabolic activities on various substrates. Because of their small size, microorganisms are very sensitive to the changes in environmental temperatures. The growth behavior of *Rhodotorula* sp. strain MBH23 on acrylamide reveals a unique chevron-like pattern in apparent activation energy, with a notable breakpoint at 28.05°C. The analysis revealed two distinct ranges for activation energy: 32–40°C with a value of 60.66 kJ/mol and 20–27°C with 51.60 kJ/mol. Within the 32–40°C range, the Q_{10} value, which measures the rate of reaction change with temperature, was found to be 2.15, while the theta value, indicating the temperature coefficient, was 1.08. These findings offer important insights into how acrylamide breaks down and moves, particularly in the context of bioremediation efforts. Study provides valuable insights for predicting acrylamide degradation and its movement during bioremediation processes.

INTRODUCTION

Acrylamide is a dangerous chemical that can cause cancer and damage the nervous system. It is created in foods that have a lot of carbohydrates, especially when these foods are cooked at high heat. This process is called the Maillard reaction, which involves the reaction between sugars and amino acids that eventually produces acrylamide [1] [2]. Other chemicals with carbonyl groups can also help make acrylamide [3]. In countries like Sweden and Norway, acrylamide has been found in the water of some rivers, and it has been linked to deaths of fish and cows [4]. Polyacrylamide (PAM), a polymer that is used in products like glue, plastics, and printers, is the most common form of acrylamide. Because acrylamide keeps being produced, the polyacrylamide that is made in factories is also contaminated with this toxic chemical. As a result, it has entered the food chain. Acrylamide can also be found in the herbicide Roundup, which contains 30% polyacrylamide [5]. This situation makes it clear that there is a need to find biological methods to remove acrylamide from our environment.

A number of microorganisms, including different types of bacteria, are able to degrade acrylamide. Examples of these include *Enterobacter aerogenes* [6], *Burkholderia* sp. [7], *Pseudomonas* sp. [8], *Bacillus cereus* [8] (Shukor et al., 2009b), the yeast *Rhodotorula* [9], an Antarctic bacterial strain [8], and the fungus *Aspergillus oryzae* [10]. Due to their small size, microbes are highly sensitive to temperature changes, which play a major role in acrylamide degradation. Temperature affects microbial physiology, helping them adapt to environmental shifts and playing a key role in the biodegradation process. The Arrhenius model is often used to study bacterial growth and reaction rates, as it helps calculate the apparent activation energy, ΔH*, which is associated with either microbial growth or the breakdown of substrates.

For most temperature ranges, the value of ΔH* tends to remain constant. However, depending on the studied temperature range, this value can significantly vary at extreme temperatures, increasing by three or four times [11]. Certain research suggests that the model may not be accurate when applied to the entire temperature range of the bacterial process [12]. The Arrhenius model is typically more effective for simulating the effects of a small temperature range rather than a larger one [13]. Additionally, a recently found transition—a quick shift in the activation energy—may be visible in the Arrhenius figure [14]. Because it includes the fewest parameters, Arrhenius's model is mostly

agreed upon by researchers [13]. The Ratkowsky model is another alternative that relies on linear growth as its premise; nevertheless, this model displays non-linear behavior and lacks constant development because of its biological basis [15]. Put simply, the Arrhenius models are employed to comprehend the impact of temperature on bacterial development for this reason. Linear regression on the Arrhenius plot allows for the estimation of the Arrhenius parameters. It was previously known that yeast could break down acrylamide [9]), but this work showed that there were several possible activation energies. Theoretically intriguing, it will also out to be highly helpful in bioremediation prediction of acrylamide breakdown and movement.

MATERIALS AND METHODS

Activation energy of growth on acrylamide

The activation energy for growth on acrylamide was determined using biodegradation rate data from *Rhodotorula* sp. strain MBH23 (KCTC 11960BP), a yeast previously isolated for its ability to degrade acrylamide [9]. The growth values at each temperature were divided by the incubation time, and these values were then converted to natural logarithms for further analysis.

The Arrhenius equation [16] is as follows,

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

In this equation, T represents the absolute temperature (in Kelvin, where Kelvin = °C + 273.15), R is the universal gas constant (0.008314 kJ/mol·K^{-1}), Ea is the activation energy (in kJ/mol), and A is the pre-exponential factor, which reflects the rate at which molecules have sufficient energy to react when Ea equals 0. The linearized form is derived by plotting the logarithm of the normal growth rate against the inverse of temperature (1/T), as shown in the following equation:

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

Coefficient of Q_{10} estimation

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

$$Q_{10} = e^{\left(\frac{Ea}{R}\right)\left(\frac{10}{T_2T_1}\right)} \qquad \text{[Eqn. 3]}$$

Following rearrangement,

$$\ln Q_{10} = \left(\frac{E_a}{R}\right)\left(\frac{1}{T_1T_2}\right) \qquad \text{[Eqn. 4]}$$

A further significant biological constant, the theta (Θ) value, which is the simplified Arrhenius temperature coefficient, is derived by substituting the values obtained into the reaction rates equation controlled by the Q_{10} rule.

$$kT = k20\Theta\,(T-20) \qquad \text{[Eqn. 5]}$$

RESULT AND DISCUSSION

The growth rate of yeast on acrylamide increases with temperature, reaching a maximum at 30°C, and at elevated temperature, the growth rate declined (**Fig.** 1). A graph plotting ln μm against 1/T displayed a chevron-like shape, indicating a discontinuous curve across the entire temperature range (**Fig.** 2). A clear breakpoint was observed at 29.88°C.

The regression analysis in Table 1 demonstrates that the activation energy for growth on acrylamide, at temperatures between 22 and 27°C, was recorded at 51.60 kJ/mol.The value increases to 60.66 kJ/mol at higher temperature range (32–40°C). Previously, a study conducted on the ability of *Pseudomonas* sp. strain DrYJ7 in degrading acrylamide revealed a significantly lower activation energy of 14.96 kJ/mol when growing at temperatures ranging from 10 to 20°C [17]. The activation energy values derived from the Arrhenius model align with those reported in the literature for different xenobiotic biodegradation processes (Table 2). Conducting a linear regression on the Arrhenius plot allows for the estimation of the Arrhenius parameters.

It appears that more energy is required to break these connections apart, while higher temperatures actually reduce the energy needed. Several studies indicate activation energy in relation to metabolic rates at different temperatures; however, few recognise the possibility of two distinct activation energies, frequently opting to present a single average value over a wide temperature range. Current research indicates two distinct patterns: one study observes an increase in activation energy at elevated temperatures, whereas another study presents a contrary trend (refer to Table 2).For example, in the case of *Bacillus* sp. JF8 growing on polychlorinated biphenyl (PCB), the activation energy was 12.1 kJ/mol between 20–46°C, but it increased to 31.4 kJ/mol between 50–70°C [18].In addition, the activation energy values of *Pseudomonas* sp. AQ5-04 grown on phenol at temperatures ranging from 15 to 30°, and 35 and 45°C were found at 38.92 Kj/mol and 11.34 Kj/mol, respectively [19].

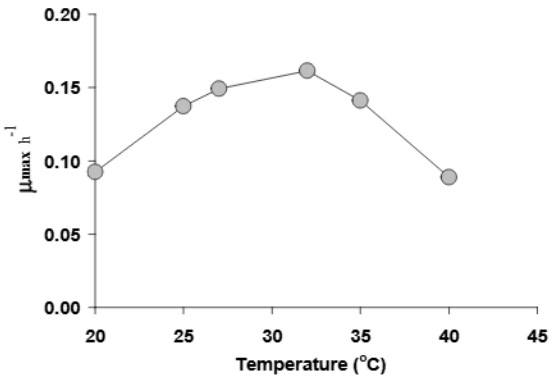

Fig 1. Influence of temperature on the specific growth rate of *Rhodotorula* sp. Strain MBH23 on acrylamide.

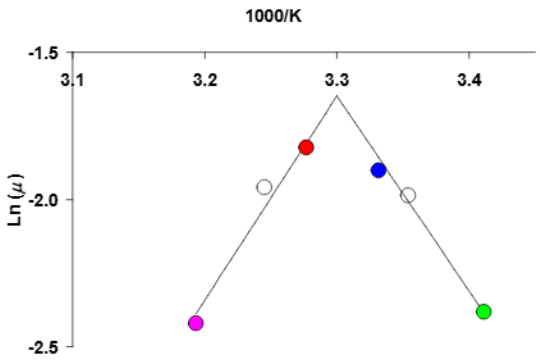

Fig 2. The Arrhenius figure shows the rate of growth on acrylamide by *Rhodotorula* sp. Strain MBH23.

Table 1. The Arrhenius plot showing the growth rate on acrylamide involving a two-part linear regression analysis by *Rhodotorula* sp. Strain MBH23.

Distribution of the experimental points	Three points to the left, three points to the right
	Left part
Temperature range °C	32, 35 and 40
Regression equation	y = 7.2999x - 25.709
Coefficient of determination	0.97
tan a ± Standard error	7.30±1.24
E_a ± Standard error, kJ mol^{-1}	60.66±10.32
t-Statistic	5.88
Degrees of freedom	2
	Right part
Temperature range °C	20, 25 and 27
Regression equation	y = -6.2098x + 18.81
Coefficient of determination	0.99
tan a ± Standard error	-6.21 ± 0.68
E_a ± Standard error, kJ mol^{-1}	51.60±5.67
t-Statistic	-9.10
Degrees of freedom	2
	Break points data
Intersection coordinates, (x, y)	3.3
Break point temperature °C	29.88
a Θ (theta)	1.08
Q_{10}	2.15

A higher activation energy indicates that more energy is required for a bacterium to metabolize phenolics. As shown in Table 2, the activation energy values from this study for both temperature ranges are consistent with those observed in various microbial species that degrade xenobiotics. However, the activation energy for typical mesophilic bacteria usually falls between 33.5 and 50.3 kJ/mol [39], which suggests that the activation energy for one of the temperature ranges in this study was greater. Our results suggest that activation energy is not fixed, but rather varies depending on the temperature selected for analysis [40]. While it is difficult to precisely account for all the complex biological processes happening at once, the model provides a valuable observational framework. As such, activation energy should be considered not in the same way as it is in chemical reactions, instead as an indication of the microorganism's overall temperature response [41]. Despite all of this complexity, the model is still widely used globally. The activation energy, which varies with temperature, serves as crucial for microbial metabolic activity. It has been demonstrated under various conditions using different substrates, such as the decolorization of dyes [14,29–32], chromate reduction [26,27], phenolic biodegradation [13,19–22], and also growth on acrylamide by a bacterium [17].

Table 2. Arrhenius temperature characteristics for growth on xenobiotics.

Microorganisms	Temperature range (°C)	Substrate	ΔH^*apparent activation energy (Kj.mol^{-1})	Ref
activated sludge	10–20	phenol	39.0	[20]
Selanastrum capricornutum	20–28	phenol	28.4	[21]
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	[22]
Pseudomonas putida Q5	10–25	phenol	61.6	[13]
Acclimated cultures	15-30	nonylphenol	42.7	[23]
Pseudomonas putida MTCC 1194	15-30	phenol	57.74	[24]
Bacillus sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20–46 °C) 31.4 (50–70 °C)	[18]
Pseudomonas sp. AQ5-04	15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	[19]
Pseudomonas sp. Strain DrYJ7	10-20	acrylamide	14.96	[17]
Cupriavidus sp. strain CNP-8	20-40	2-chloro-4-nitrophenol	75.16 88.71	[25]
Escherichia coli BL21	20-50	Chromate	28.01	[26]
Ochrobactrum intermedium	25-35	Chromate	120.69	[27]
Shewanella oneidensis·MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[28]
anaerobic sludge	30-55	Reactive Red 2	22.9	[29]
activated bacterial consortium	20-37	Remazol Black B	48.8	[30]
Enterobacter sp. strain (GY-1)	20-35	Reactive Black 5 (RB 5)	35.56	[31]
Escherichia coli NO3	20-45	Reactive red 22	27.49	[32]
Pseudomonas aeruginosa	15-45	Reactive Black 39 and Acid Red 360 by	RB39 61.89 AR360 81.18	[33]
Pseudomonas sp. LPM-410	20-28	EDTA	91.2	[34],
Rhodotorula sp. Strain MBH23	32—40 20—27	acrylamide	60.66 51.60	This study

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

The exact mechanisms behind the observed changes remain unclear, but two hypotheses offer possible explanations. The first suggests that water properties change during the transition, while the second, the "bottleneck" hypothesis, proposes that a limited number of events occur rapidly in succession [35]. Based on various measured Arrhenius breakpoint temperatures, the first hypothesis does not appear to be accurate (Angelova et al., 2008). According to the "bottleneck" theory, each enzyme in the chain has distinct thermal characteristics, making it difficult to verify the hypothesis directly. Additionally, when considering ambient temperature, the cell membrane properties will also vary [36]. Despite these challenges, the "bottleneck" theory remains widely accepted in academic discussions [14,37]. A chevron plot on an Arrhenius graph is a distinctive feature often observed in the study of microbial growth or biodegradation on toxic substrates. This type of plot typically exhibits a discontinuous, chevron-like shape, which suggests the presence of more than one activation energy across different temperature ranges. This phenomenon appears when microbial growth or biodegradation progresses distinct metabolic pathways or mechanisms at different tempera-

tures. In such cases, two or more activation energies can be recognised, each matching to different stages of microbial activity or different enzyme systems being activated at specific temperature ranges. The chevron pattern is particularly relevant when studying the biodegradation of toxic substances, such as acrylamide or heavy metals, because it reflects how the microorganism's metabolic activity alters to the toxicity and environmental conditions [36].

At lower temperatures, microorganisms may exhibit a lower activation energy, demonstrating slower or less efficient metabolic activities. As the temperature increases, metabolic rate speed up, heading to a higher activation energy. Nevertheless, at even higher temperatures, the plot may show a reduction in metabolic productivity due to thermal stress or enzyme denaturation, ensuing in another breakpoint or change in the activation energy. Generally, the chevron-shaped Arrhenius plot offers intuitions into the complex interactions between temperature, microbial growth, and the degradation of toxic substances [14,37].

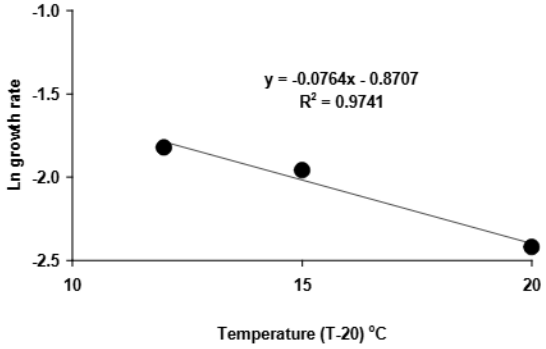

Fig. 3. Ln growth rate vs temperature plot for estimating theta.

Q_{10} values may also be estimated through Arrhenius plots, which are derived from measuring growth rates at varying incubation temperatures with a ten-degree increment [38]. The logarithmic plot of bioreduction and growth rates against 1000/temperature (in Kelvin) yields a slope that correlates with the Arrhenius curve (**Fig. 1**). A Q_{10} value of 2.905 was determined for the temperature range of 30–42°C (**Fig. 3**). Since biological processes are dynamic, different Q_{10} values may be observed across different temperature ranges. For instance, a Q_{10} value of 2.038 was found in the reduction of molybdate to molybdenum blue [39], while a Q_{10} value of 2.31 was reported for another molybdenum-reducing species, *Morganella* sp. The significance of this value emerges when linking growth processes to particular biological activities. In previous studies, a Q_{10} value of 2.7 was determined for oil biodegradation in a beach gravel column [40].

A separate study on decane and toluene-contaminated soil reported a Q_{10} value of 2.2 [41]. This value was observed for both the bacterial ability to degrade petrochemicals and the effect of temperature on this ability [42]. In contrast, immobilized bacterial systems producing acrylamide at temperatures between 25 and 45°C had a Q_{10} value of 2.8 [43]. It is often observed that Q_{10} values increase as the temperature decreases [44,45]. In another the study, *Pseudomonas* sp. strain AQ5-04 exhibited a Q_{10} value of 1.834 [19], whereas the growth rate of this organism on acrylamide was recorded at a Q_{10} value of 2.17. A separate investigation into acrylamide biodegradation indicated a lower Q_{10} value of 2.17 [17].

A theta value of 1.11 was determined (**Fig. 3**), which is comparable to the theta value of 1.08 observed for molybdenum reduction by *Serratia* sp. strain HMY1 [39]. The growth rate of the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7 on acrylamide yielded a theta value of 1.03 [17]. The breakdown of various xenobiotics has been documented resulting in theta values up to 16.2, while the range for numerous biological processes typically lies between 1.1 and 1.7 [46]. The theta (θ) value serves as a significant parameter in biodegradation research, especially for estimating the significance of temperature on microbial growth and the degradation of toxic compounds. It is taken from the Q_{10} value, which evaluates the temperature sensitivity of a biological process, such as biodegradation. The theta value is often used to illustrate how the rate of a biological process shifts in response to temperature over a specific range. In the context of biodegradation, the theta value echoes the change in the metabolic activity of microorganisms as the temperature shifts.

A theta value of 1 signifies that temperature has little to no effect on the degradation rate, while values greater than 1 advocate that the biodegradation process speed up as temperature increases. Conversely, values less than 1 may indicate a reduced rate of biodegradation at higher temperatures, potentially due to enzyme denaturation or other thermal stresses. Theta values are especially useful in understanding the temperature dependencies of microbial processes when dealing with toxic substrates, such as pollutants or hazardous chemicals. In these cases, microbial efficiency in breaking down toxic compounds often varies with temperature, and the theta value helps quantify this relationship. By examining theta values, researchers can optimize environmental conditions for bioremediation processes, ensuring that microorganisms function most effectively in degrading harmful substances at different temperatures [44,45].

CONCLUSION

This study is the first to show that the activation energy for acrylamide biodegradation by yeast has a broken profile, revealing two distinct activation energies in the Arrhenius plot. Temperature plays a key role in microbial growth and metabolic activity, and due to their small size, microorganisms are especially sensitive to temperature changes. When *Rhodotorula* sp. strain MBH23 grows on acrylamide, its apparent activation energy follows a discontinuous, chevron-like pattern, with a notable breakpoint at 29.88°C. The regression analysis identified two separate activation energies: one for the 22–27°C range and another for 32–40°C. In the 30–42°C range, a Q_{10} value of 2.15 and a theta value of 1.08 were calculated. These values align with the typical energy ranges seen in mesophilic microorganisms. Ongoing research is investigating additional factors to deepen our understanding of how temperature influences microbial growth dynamics. The results from this study are consistent with the energy patterns observed in other biological processes.

AI AND AI-ASSISTED TECHNOLOGIES DECLARATION

Throughout the first phases of our research and paper preparation, we made extensive use of powerful AI-based technologies, including Grammarly, ScholarAI, and ChatGPT. These tools were not directly involved in making scientific conclusions or interpreting data; rather, they served as supplemental resources that helped with data collecting, analysis, and manuscript editing. Scholarly work, including interpretations, conclusions, and the presentation and consistency of arguments, is entirely the writers' duty.

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