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Activation energy, Temperature Coefficient and Q_{10} Value Estimations of the Growth of *Serratia marcescens* strain DRY6 on SDS

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ABSTRACT

Sodium dodecyl sulfate, occasionally known as SDS, is a widely used anionic surfactant globally as a foaming component in a wide variety of cleaning products, including shampoos, toothpaste, and detergents. Large quantities of SDS are released into the environment despite the fact that they are hazardous and have the ability to create foam. This can lead to problems in sewage treatment facilities. There are a few different models that may be utilized to mimic the growth rate of microbes on a variety of different medium at various temperature. Arrhenius is one of the most often used models partly since it has a small number of parameters. Temperature frequently influences the progress and metabolic activities of microbes on the substrates they are growing on. Because of their small size, microbes are very sensitive to variations in temperature. In order to describe the development of *Serratia marcescens* strain DRY6 on SDS, a discontinuous apparent activation energy with a chevron-like graph was used, and the graph's break point was set at 28.05 degrees Celsius. Following the completion of the regression study, two activation temperatures were established: 20-27 degrees Celsius and 30-42 degrees Celsius, with respective activation energies of 41.72 and 84.72 kilojoules per mole. Within the temperature range that was taken into account, it was projected that the Q10 value would be 2.905, and the theta value would be 1.11. (30-42 °C). This study is especially helpful in projecting SDS breakdown and migration during bioremediation because of its comprehensive nature.

INTRODUCTION

Detergents have been shown to be harmful to marine life. According to previous findings, anionic surfactants are toxic to a wide spectrum of aquatic creatures from 0.0025 to 300 mg/L [1]. It altered the life cycle of aquatic species and caused behavioral changes. Another study found that the oyster digestive gland is susceptible to SDS exposure, creating a detrimental disruption in the nutritional and metabolic activities of the oyster, resulting in reduced oyster survivorship [2]. As more anionic surfactants are discharged into bodies of water, pollution from these substances will increase the deleterious effects on invertebrates and crustaceans. SDS, commonly known as Sodium Lauryl Sulfate, is the most often used anionic detergent in home items such as toothpastes, shampoos, bubble baths, cosmetics, shaving foams, and detergents [3]. However, in the industry, it is employed as a wool cleaning agent, a de-inking agent in the paper industry, a leather softening agent, a penetrant, a flocculating agent, and a key component of fire-fighting equipment, engine degreasers, floor cleaners, and car wash soaps [4–6]. The existence of SDS in the environment is mostly due to its presence in home and industrial effluents, as well as its direct discharge from some applications. Because of this, SDS remediation is critical. Microorganisms are recognized for their capacity to degrade organic substances such as SDS [7–11], and their usage as bioremediation agents is economically critical for the removal of xenobiotic pollutants. One of the first reports of an SDS-degrading bacteria was *Pseudomonas* sp. strain C12B biodegrading anionic surfactant under aerobic conditions [12].

It is not very common to find research on bacteria that display the ability to degrade numerous xenobiotics. However, because polluted sites typically include a wide variety of inorganic and organic contaminants, isolating such unique bacteria is absolutely necessary. Because of their microscopic nature, SDS decomposition is sensitive to temperature changes. The physiology of an organism may be modulated by temperature, which enables the organism to better adapt to

changing environments. When researching the process of chemical breakdown by microbes, temperature is an important factor to take into consideration. It is common practice to use the temperature function Arrhenius model to compute the apparent activation energy, H*, which is thought to be present for either growth or decay on distinct metabolic substates. The temperature function Arrhenius model has become increasingly popular for use in the investigation of the growth and decomposition rates of bacteria [13–21].

Throughout the majority of the temperature range, the delta H (H*) value is nearly constant. For extreme temperatures, this value can vary by three or four times depending on the temperature range [22]. In some studies, the model may be incorrect when applied to the entire temperature range of the bacterial process [23]. When modeling temperature effects, Arrhenius' model is frequently used, but it is less frequently used when the temperature ranges are large [24]. The Arrhenius plot may also show a previously identified transition, which is a fast shift in activation energy [25]. Because Arrhenius' model includes the fewest parameters, it is pretty widely accepted by researchers [24].

Because of its biological underpinnings and non-linear behavior this rival Ratkowsky model is also based on the linear growth premise but lacks constant development [26]. This means that the Arrhenius models are essential for figuring out how temperature affects bacterial growth. The Arrhenius parameter estimate is obtained by plotting an Arrhenius plot and then performing a linear regression on the data. Arrhenius plot analysis and the effect of temperature on *Pseudomonas* sp. strain DRYJ7's SDS growth were the subject of a similar study a number of years ago [16]. This study found that a bacterium can break down SDS at a number of different activation energies, which was previously unknown and will be extremely useful in predicting how SDS breakdown and transport will occur during bioremediation.

MATERIALS AND METHODS

Growth and maintenance of bacterium

Serratia marcescens strain DRY6 growth and characterization on SDS has been published previously and stored in the university's culture collection unit [27]. The SDS as sole carbon source medium composition (g L^{-1}) was as follows; KH_2PO_4, (1.36), KNO_3, (0.5), $MgSO_4$ (0.01), $CaCl_2$ (0.01), Na_2HPO_4 (1.39) and $(NH_4)_2SO_4$ (7.7). The final concentration of the standard trace elements was 0.01 mg L-1 in the medium. Sodium dodecyl sulfate, filter-sterilized, was added to the medium as a carbon source. Sodium dodecyl sulphate, a carbon source, was added to the medium at a final concentration of 1.0 g/L via filter-sterilization (Dhouib et al. 2003). The bacterium was grown on nutrient agar plates treated with SDS at the same dose for 5 days at 30 °C. The colony count method was used to measure the growth of microorganisms.

Measurement of the Activation energy of growth on SDS

Biodegradation growth data from *Serratia marcescens* strain DRY6 previously isolated as an SDS-degrading bacterium was processed [27] by converting the temperature-dependent growth rates to natural logarithms.

The Arrhenius equation [28] is as follows,

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

Where R is the universal gas constant (0.008314 $kJ/molK^{-1}$), T is the absolute temperature (Kelvin = $^{\circ}C$ + 273.15), E_a is the activation energy (kJ/mol) 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;

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

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,

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

Another essential biological constant derived by substituting the given values into the reaction rates equation regulated by the Q_{10} rule is the coefficient of temperature or theta () value (simplified Arrhenius temperature coefficient);

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

RESULT AND DISCUSSION

Bacterial growth rate is affected by temperature. Plotting ln growth rate (per day*)* vs 1/T yielded a Chevron-like graph, revealing a discontinuous curve across the entire temperature range **(Fig. 2**). It was surprising to find a break point at 28.05 °C. At temperatures ranging from 20 to 27 °C, growth on SDS showed an activation energy of 41.72 kJ/mol, with a rise in activation energy to 84.72 kJ/mol at temperatures between 30 and 42 °C, as shown in regression analysis results in **Table 1**. As the temperature rises, the maximum rate of bacterial growth on SDS is found to be 27 °C, and the maximum rate drops as the temperature increases (**Fig. 1**).

On acrylamide, a previous study found an activation energy of 14.96 Kj/mol for *Pseudomonas* sp. strain DrYJ [16], which is definitely much lower. It was found that the activation energy obtained using the Arrhenius model was within the range of activation energies reported in the literature for diverse biodegradation processes (**Table 2**). Dismantling the connections appears to necessitate more effort. Energy savings can be achieved by raising the temperature. Many publications on activation energy computed from metabolic process rates at various temperatures report only one activation energy across a wide temperature range, rather than reporting on the occurrence of two activation energies. One study shows a higher activation energy at higher temperatures than at lower temperatures, whereas the other shows the opposite occurrence in the opposite direction (**Table 2**). It was found that the activation energy obtained using the Arrhenius model was within the range of activation energies reported in the literature for diverse biodegradation processes (**Table 2**). Dismantling the bonds appears to necessitate more effort. Energy savings can be achieved by raising the temperature. Many publications on activation energy computed from metabolic process rates at various temperatures report only one activation energy across a

wide temperature range, rather than reporting on the occurrence of two activation energies. One study shows a higher activation energy at higher temperatures than at lower temperatures, whereas the other shows the opposite occurrence in the opposite direction (**Table 2**). An example is the growth of *Bacillus* sp. JF8 on the xenobiotic polychlorinated biphenyl (PCB) with an activation energy from 20 to 46 °C of 12.1 Kj/mol and from 50 to 70 °C the activation energy is 31.4 Kj/mol [29]. A contrasting study shows 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 [30].

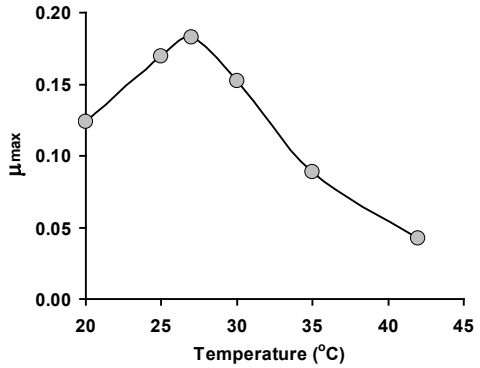

Fig 1. The effect of temperature on the specific growth rate of *Serratia marcescens* strain DRY6 on SDS.

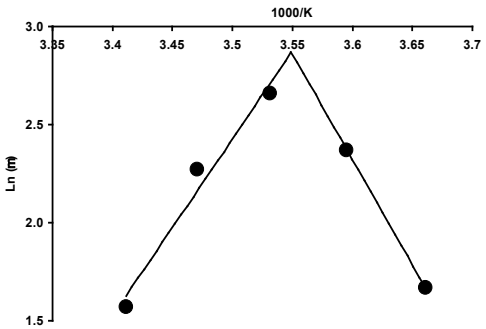

Fig 2. Growth rate of SDS by *Serratia marcescens* strain DRY6 in the form Arrhenius plot.

Table 1. Regression analysis was performed on the Arrhenius plot of the SDS biodegradation rate by *Serratia marcescens* strain DRY6.

Distribution of the experimental points	Three points to the left, three points to the right
	Left part
Temperature range °C	30, 35 and 42
Regression equation	y=10.195x - 35.508
Coefficient of determination	0.97
tan a ± Standard error	10.19±0.05
E_a ± Standard error, kJ/mol	84.72±0.41
t-Statistic	205.01
Degrees of freedom	2
	Right part
Temperature range °C	20, 25 and 27
Regression equation	y = -5.0207x + 15.043
Coefficient of determination	2
tan a ± Standard error	-5.02±0.47
E_a ± Standard error, kJ/mol	41.72±3.89
t-Statistic	-10.72
Degrees of freedom	2
	Break points data
Intersection coordinates, (x, y)	3.32, -1.642
Break point temperature °C	28.05

Table 2. Arrhenius temperature characteristics for growth on numerous xenobiotics.

Microorganisms	Temp-erature range (°C)	Substrate	*DH** apparent activation energy (Kj.mol^{-1})	Ref
activated sludge	10–20	phenol	39.0	[31]
Selanastrum capricornutum	20–28	phenol	28.4	[32]
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	[33]
Pseudomonas putida Q5	10–25	phenol	61.6	[24]
Acclimated cultures	15-30	nonylphenol	42.7	[34]
Pseudomonas putida MTCC 1194	15-30	phenol	57.74	[35]
Bacillus sp. JF8	20-70	polychlorinated biphenyl (PCB)	12.1 (20–46 °C) 31.4 (50–70 °C)	[29]
Pseudomonas sp. AQ5-04	15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	[30]
Pseudomonas Strain DrYJ7	sp. 10-20	SDS	14.96	[16]
Cupriavidus strain CNP-8	sp. 20-40	2-chloro-4-nitrophenol	75.16 88.71	[36]
Escherichia coli BL21	20-50	Chromate	28.01	[37]
Ochrobactrum intermedium	25-35	Chromate	120.69	[38]
Shewanella oneidensis·MR-1	25-40	Selenate	Control system 62.90 TPPS-supplemented system 47.33	[39]
anaerobic sludge	30-55	Reactive Red 2	22.9	[40]
activated bacterial consortium	20-37	Remazol Black B	48.8	[41]
Enterobacter strain (GY-1)	sp. 20-35	Reactive Black 5 (RB 5)	35.56	[42]
Escherichia coli NO3	20-45	Reactive red 22	27.49	[43]
Pseudomonas aeruginosa	15-45	Reactive Black 39 and Acid Red 360 by	RB39 61.89 AR360 81.18	[44]
Pseudomonas LPM-410	sp. 20-28	EDTA	91.2	[45],
Pseudomonas AQ5-04	sp. 15-45	phenol	38.92 (15–30 °C) 11.34 (35–45 °C)	[30]
Cupriavidus sp.	20–25 30–40	2-chloro-4-nitrophenol	88.71 (20-25) 75.16 (30-40)	[17]
Pseudomonas strain DRYJ7	sp. 10-20	Acrylamide	14.96	[16]

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

The temperature is an essential factor to take into account while thinking about bacteria. Nearly every aspect of the folding, structure, and stability of biomolecules, in addition to the rate at which metabolic reactions occur, are all influenced. The ability of microbes, such as pathogens, to sense the presence of a host and adapt their metabolic processes accordingly is essential to the microbe's ability to live on in its environment. In order to modify their gene expression in response to changes in temperature, organisms have created specialized sensing systems that are capable of detecting temperature fluctuations in an indirect manner. Both a heat shock and a cold shock can result in the aggregation of aggregated proteins, but only a cold shock can result in the accumulation of stopped ribosomes [14,15,46–51]. Molecular thermosensors could be built using molecular switches as the building blocks. Changes in molecular architecture that result in dysfunctional activity are one example of direct temperature sensing. Other examples include temperature-responsive regulatory proteins and alterations in lipid membrane integrity in relation to fluidity. It is impossible to exaggerate the

importance of temperature in the regulation of biological processes. Applications in the field of temperature-controlled biotechnology could also stand to profit from this method. Changes in temperature bring to a plethora of metabolic modifications, many of which are closely connected with one another. In light of this, models that illustrate how the progression of a biological process varies as a function of temperature are an absolute must if we want to simplify things and get a better understanding of how everything is connected. As early as the 1900s, Arrhenius provided a mathematical account of how biochemical reactions respond to high or low temperatures in the form of his "primal" temperature model [47–51].

Bacteria need more energy to break down more complex xenobiotics when the activation energy is higher. Several xenobiotic-degrading microbial species have activation energies within the ranges observed in this study for both temperature ranges, as shown in **Table 2**. In contrast, the activation energies of typical mesophilic bacteria range from 33.5 to 50.3 kJ/mol [52]. It's possible that one of the temperature ranges examined had a higher activation energy. The activation energy was found to vary with temperature in this study, rather than being a constant [53]. The model is useful as an observational model because it can't account for all of the simultaneous interactions among the various biological processes. Instead of thinking in terms of the activation energy used in chemical reactions, we should consider activation energy to be the total temperature response of a microorganism [54].

These drawbacks notwithstanding, the model continues to see widespread use. Temperature affects the activation energy of microbes, which has been demonstrated in a variety of settings, such as the decolorization of various colors by various organisms (**Table 2**). Although the exact mechanism causing the transformation is still a mystery, two hypotheses can account for it. When transitioning from one state to another, the water features change and a "bottleneck" theory suggests the same processes occur simultaneously and quickly [55]. Based on numerous observations of Arrhenius break point temperatures, the first hypothesis does not appear to be valid. There are many reasons why it is difficult to prove the "bottle-neck" hypothesis, according to the "bottle-neck" theory. Because of the effect of temperature on the cell membrane, it will also change [56]. Academics continue to believe in the "bottleneck" theory [25,57].

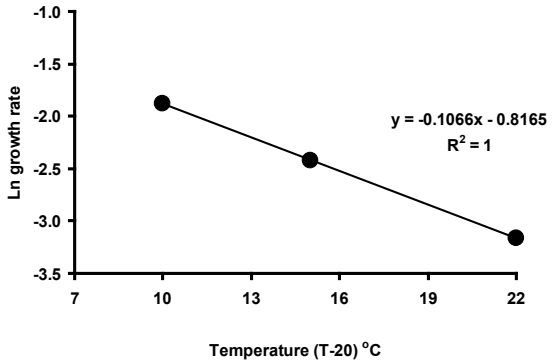

Fig. 3. Ln growth rate vs temperature plot for estimating theta.

It is also possible to calculate the Q_{10} values using the Arrhenius plots, or by monitoring growth rates at various incubation temperatures with ten degrees of variation [58]. The Arrhenius curve is the slope of the resultant plot when the bioreduction and growth rates are logarithmically plotted against 1000/temperature (Kelvin) (**Fig. 1**). For the temperature range of 30 to 42 °C, a Q_{10} value of 2.905 was obtained (**Fig. 3**). However, due to the dynamic nature of biological processes, many Q_{10} values may be found for each investigated temperature range. The conversion of molybdate to molybdenum blue yielded a 2.038 value [59]. A Q_{10} value of 2.31 was found in *Morganella* sp, yet another molybdenum reducer in nature. In order to properly attribute the growth process to a specific biological activity, this value is essential. For oil degradation in a beach gravel column, previous research found Q_{10} to be 2.7 [60]. Nevertheless, a Q_{10} value of 2.2 for microbiological process was reported in another study on decane and toluene-contaminated soil [61]. Degradation rates for other petrochemical compounds affected by salinity was reported to exhibit a Q_{10} value of 2.2 [62], while acrylamide degradation between 25 and 45 °C in immobilized bacterial systems reported a Q_{10} value of 2.8 [63].

Declining temperatures frequently lead to an increase in the Q_{10} value [64,65]. The Q_{10} value for the phenol degradation by *Pseudomonas* sp. strain AQ5-04 was 1.834 [30] while a Q_{10} value of 2.17 was calculated for the growth rate of this organism on SDS. A lower Q_{10} value of 2.17 is reported in another study on acrylamide biodegradation by the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7 [16]. The theta value was determined to be 1.11 (**Fig.** 3), close to the theta value found of 1.08 for molybdenum reduction by *Serratia* sp. strain HMY1 [59]. A lower theta value of 1.03 is reported for the growth rate on SDS by the Antarctic bacterium *Pseudomonas* sp. strain DRYJ7 [16]. There are reports of xenobiotics being broken down with theta values of up to 16.2, but theta values are typically between 1.1 and 1.7, which is within the typical range for many biological processes [66]. In the biodegradation of nonylphenol, a theta value of 1.06 was observed [34].

CONCLUSION

SDS biodegradation requires an activation energy that can be seen in an Arrhenius plot with two activation energies. This is the first study to demonstrate this. Temperature has a significant impact on microbial growth and metabolic activity on their substrates. Because they are so small, microorganisms are extremely sensitive to temperature changes in their environment. An apparent activation energy graph was presented, with a break point at 28.05 °C showing the apparent activation of *Serratia marcescens* DRY6 on SDS. There were two different activation temperatures: 20–27 °C and 30–42 °C, both with activation energies of 41.72 and 84.72 kJ/mol, respectively, based on the regression analysis results. A Q_{10} value of 2.905 and a theta value of 1.11 were calculated for the studied temperature range (30-42 °C). More than three times as powerful as mesophilic bacteria at temperatures between 30 and 42 °C, the quantum. Breaking an amide bond is thought to require a much higher activation energy. Additional research and inquiry into the parameters themselves are being conducted in order to better understand the relationship between temperature and growth kinetics.

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