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Origin of temperature dependence of bacterial growth rate: Analogy with the viscosity of glass-forming liquids in inorganic materials

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Abstract. Based on a recent suggestion that the bacterial cytoplasm has a property similar to glass-forming liquids, we have proposed a new relation for the temperature dependence of the bacterial growth rate, $k = k_0\exp[-E_a/\mathrm{k}_B(T-T_c)]$ in the lower temperature range, where k_0 is a constant, E_a is the activation energy (eV), k_B is the Boltzmann constant, T is the absolute temperature (K), and T_c is the characteristic (frozen-in) temperature (K), resembling the temperature-dependent fluidity (inverse viscosity) observed in glass-forming liquids in inorganic materials. This monotonic behavior of bacterial growth breaks down at higher temperatures, that is, k decreases rapidly with T. This may be attributed to a rapid increase in the physiological cytoplasmic concentration above the critical temperature T_m. The finding on the temperature-dependent bacterial growth rate is analogous to that observed in glass-forming liquids in nonliving inorganic materials.

Keywords: bacterial growth rate, glass-forming liquid, glass transition, viscosity, free volume

Introduction

The bacterial cytoplasm is a liquid aqueous mixture crowded with macromolecules and organelles (Cossins et al. 2011; Golding, Cox 2006; Munder et al. 2016; Oyama et al. 2019; Perry et al. 2014; Trevors et al. 2013). In the bacterial cytoplasm, most metabolic activity occurs, as does small molecular diffusion. Diffusion within the bacterial cytoplasm is the dominant mechanism of molecular motion and is considered an integral part of bacterial life (Grimaldo et al. 2019; Munder et al. 2016; Perry et al. 2014; Smigiel et al. 2022). The bacterial cytoplasm has a glassy property similar to that of inorganic materials exhibiting glass transition (Lee et al. 2020; Nishizawa et al. 2017; Perry et al. 2014; Takatori, Mandadapu 2020). The study of intracellular glasses is of considerable interest as it can provide insight into the physical and chemical properties of the bacterial cytoplasm, which is a complex and dynamic environment that plays a vital role in cellular processes (Oyama et al. 2019). It is well known that a crucial environmental factor for a microorganism is temperature (Noll et al. 2020).

A recent study (Pinto, Shimakawa 2023) has investigated the temperature dependence of the dynamic bacterial growth rate to describe the bacterial growth rate at a glass transition temperature (low temperature) instead of the commonly used square-temperature-dependent law (Ratkowsky et al. 1983). However, the nonmonotonic behavior observed at high temperatures has not been discussed. In this paper, we propose a model that unifies the dynamics of the bacterial cytoplasm over the entire biokinetic temperature range. It is suggested that the free volume may dominate the overall features of temperature-dependent bacterial growth at low and high temperature.

Glass Dynamics in Bacterial Systems

Overview

Glass dynamics in the bacterial cytoplasm are related to temperature, viscosity, and molecular concentration (Lama et al. 2022; Lee et al. 2020; Nishizawa et al. 2017). The viscosity of the cytoplasm increases with the macromolecular concentration (Grimaldo et al. 2019; Nishizawa et al. 2017), leading to a decrease in the fluidity of metabolic activity at physiological concentrations. The diffusivity of molecules in the bacterial cytoplasm is also strongly related to intermolecular interactions, cell concentration, and free-volume availability (Cossins et al. 2011; Golding, Cox 2006; Oyama et al. 2019; Trevors et al. 2013). Moreover, at physiological concentrations, the diffusivity of molecules is limited owing to a decrease in free volume (Lee et al. 2020; Nishizawa et al. 2017; Ojovan 2008). The free volume of the bacterial cytoplasm decreases with temperature, which leads to vitrification (glass transition) (Pinto, Shimakawa 2023; Micoulaut 2021). The glass transition is a dynamical state change from a liquid to a glassy state that occurs via supercooling (Tanaka, Shimakawa 2021) or increasing the density (Ryabov et al. 2004) in nonliving inorganic materials. The concept of well-known inorganic materials has been applied to living bacteria (Balasubramanian et al. 2016; Berthier et al. 2019; Dauchot, Löwen 2019; Nishizawa et al. 2017; Pinto, Shimakawa 2023).

Recently, the present authors (Pinto, Shimakawa 2023) have proposed a model for the temperature dependence of the bacterial growth rate k (h^{-1}), which fits well at low temperatures:

$$k = k_0 \exp\left(-\frac{E_a}{K_B(T-T_c)}\right), \tag{1}$$

where k_0 is a constant, E_a is the activation energy (eV) for the temperature difference ($T - T_c$) (Micoulaut 2021; Pinto, Shimakawa 2023), T_c is the characteristic temperature (frozen-in) or glass transition temperature (K), and T is the ambient temperature (K). Note here that E_a is therefore not the same as the so-called activation energy in the Arrehenius plot. Equation (1) is similar to inverse viscosity, which is widely used for inorganic materials (Elliott 1990; Tanaka, Shimakawa 2021; Zallen 1983). There is a similarity between the bacterial growth rate and the fluidity of glass-forming liquids (Dauchot, Löwen 2019; Janssen 2019; Nishizawa et al. 2017; Pinto, Shimakawa 2023). Note that the most popular model was the square-root temperature dependence in which the square root of the growth rate k is given as (Ratkowsky et al. 1982)

$$\sqrt{k} = b(T - T_0)\ , \tag{2}$$

where b is a constant and T_0 is the hypothetical temperature, respectively. The present authors (Pinto, Shimakawa 2023) have suggested that equation (1) has more scientific insight than the square-root model, (equation (2)).

However, bacterial growth decreases at higher temperatures (Ratkowsky et al. 1982; 1983), indicating that the monotonic increase in the growth rate is disrupted. Therefore, the utility of phenomenological models has been discussed (Heitezer et al. 1991), and the following extended square root model (Ratkowsky et al. 1983) has been empirically introduced for the bacterial growth rate over a wide temperature range (Noll et al. 2020):

$$\sqrt{k} = b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\}\,, \tag{3}$$

where $T_{\min}$ and $T_{\max}$ are the minimum and maximum temperatures, respectively, b is the regression coefficient of the square root model of the growth rate (mentioned above), and c is an additional parameter that allows the model to fit the data for temperatures above the optimum temperature.

Although the fitting of equation (3) to the experimental data for the entire biokinetic temperature range is reasonably good (Heitezer et al. 1991; Noll et al. 2020; Ratkowsky et al. 1983), the model does not provide any physical insight. In our opinion, any model should have scientific basis.

Proposed model

Bacterial growth is highly dependent on the cytoplasm. The cytoplasm of bacterial cells plays a critical role in the survival and growth of the organism (Nishizawa et al. 2017). As already mentioned, the cytoplasm itself depends on the temperature (equation (1)) and is frozen-in at a low temperature. This is called the glass transition of the bacterial cell (Pinto, Shimakawa 2023). Other unique features of the bacterial cytoplasm include crowdedness (Fernandez-de-Cossio-Diaz, Vazques 2018; Lama et al. 2022; Munder et al. 2016; Nishizawa et al. 2017; Perry et al. 2014), with a high concentration of macromolecules occupying a relatively small volume. This type of crowding should have significant effects on the properties of the macromolecules, such as the diffusion rates and interactions with other molecules (Cossins et al. 2011; Grimaldo et al. 2019; Munder et al. 2016). Therefore, an increase in metabolic activity can lead to a decrease in the fluidity of the bacterial cytoplasm and eventually lead to a decrease in the growth rate (Trevors et al. 2013). If a particle is confined by the surrounding molecules, its movement is impeded (Fernandez-de-Cossio-Diaz, Vazques 2018; Yu et al. 2016). A recent report on the concentration dependence of viscosity has shown that viscosity increases with concentration (Nishizawa et al. 2017), indicating that the free volume decreases at physiological concentrations.

Therefore, we suggest that there are two types of freezing-in transitions of the cytoplasm in the bacterial growth process: one is the direct temperature effect (temperature-induced glass transition) (Pinto, Shimakawa 2023), and the other is the concentration-induced phenomena (Nishizawa et al. 2017). Accordingly, the bacterial growth rate k can be expressed by the conditional probability of these two events:

$$k = p_1 p_2 \ , \tag{4}$$

where p_1 is the probability of the freezing-in transition (equation (1)), and p_2 is the probability of the concentration-related freezing-in transition, which should also depend on the temperature, as will be discussed below.

Based on the composition-dependent glassy behavior of the bacterial cytoplasm, which is based on the Doolittle equation (Doolittle 1951), the conditional probability p_2 is assumed to be given as

$$p_2 = exp\left(-\frac{Ac}{c^{*}-c}\right), \tag{5}$$

where A is a constant, and c and c^* are the concentration and critical concentration of the cytoplasm, respectively. It is assumed here that the number of c obeys the Boltzmann law (Ryabov et al. 2004):

$$c^{*} = c_0 \exp(-\frac{E_\mathrm{b}}{k_\mathrm{B} T_m}) \ , \tag{6}$$

$$c = c_0 \exp(-\frac{E_\mathrm{b}}{k_\mathrm{B} T}) \ , \tag{7}$$

where E_b is the activation energy (eV), and T_m is the critical temperature (K).

Finally, we obtained the following universal equation for the temperature dependence of the bacterial growth rate k over the entire biokinetic temperature range:

$$\ln k = \ln k_0 - \frac{E_a}{k_B(T-T_\mathrm{c})} - \frac{A}{exp\left[-\frac{E_\mathrm{b}}{k_\mathrm{B}}\left(\frac{1}{T_\mathrm{m}}-\frac{1}{T}\right)\right]} \ . \tag{8}$$

Equation (8) consists of two thermodynamic behaviors: at a characteristic temperature T_c at low temperatures, the cytoplasmic movement is frozen-in (glassy state), which can be attributed to a decrease in thermal expansion (loss of free volume). Above T_c, the cytoplasm is in a liquid state and hence has sufficient free volume; hence, the metabolic activity is high, leading to a higher concentration of the cytoplasm. At a critical temperature T_m at high temperatures, the bacterial cytoplasm becomes more confined, and the molecular mobility slows, leading to a drastic reduction in mobility (or leading to cell death). This effect is not expected if the system has sufficient space. It is of interest to state in confined systems that a glass transition from the liquid state at high temperatures has been reported in nonliving inorganic materials (Ryabov et al. 2001; 2004; Tanaka, Shimakawa 2021; Zallen 1983).

Comparison with the experimental results

The solid circles in Fig. 1 show the experimental results of the logarithmic growth rate k (h^{-1}) against the reciprocal absolute temperature (K^{-1}) for four bacterial species: *Alteromonas* sp., *Aeromonas* sp., *Flavobacterium* sp., and *Moraxella* sp. (Ratkowsky et al. 1983) The solid line is given by equation (8). The logarithmic value of the bacterial growth rate k increases with the temperature up to a certain point, and this monotonic behavior breaks down (nonmonotonic) at higher temperatures. The fitting of equation (8) to the experimental data is good, with $R^2 > 0.9$; the physical parameters are presented in Table 1. The experimental results for other examples, namely, *Vibrio marinus*, *Thermus aquaticus*, *Escherichia coli,* and *Bacillus subtilis*, (Ratkowsky et al. 1983) are shown in Fig. 2. The fitting of equation (8) to the experimental data is also good, with $R^2 > 0.9$ (except for *E. coli* whose $R^2 = 0.689$). The physical parameters are listed in Table 2.

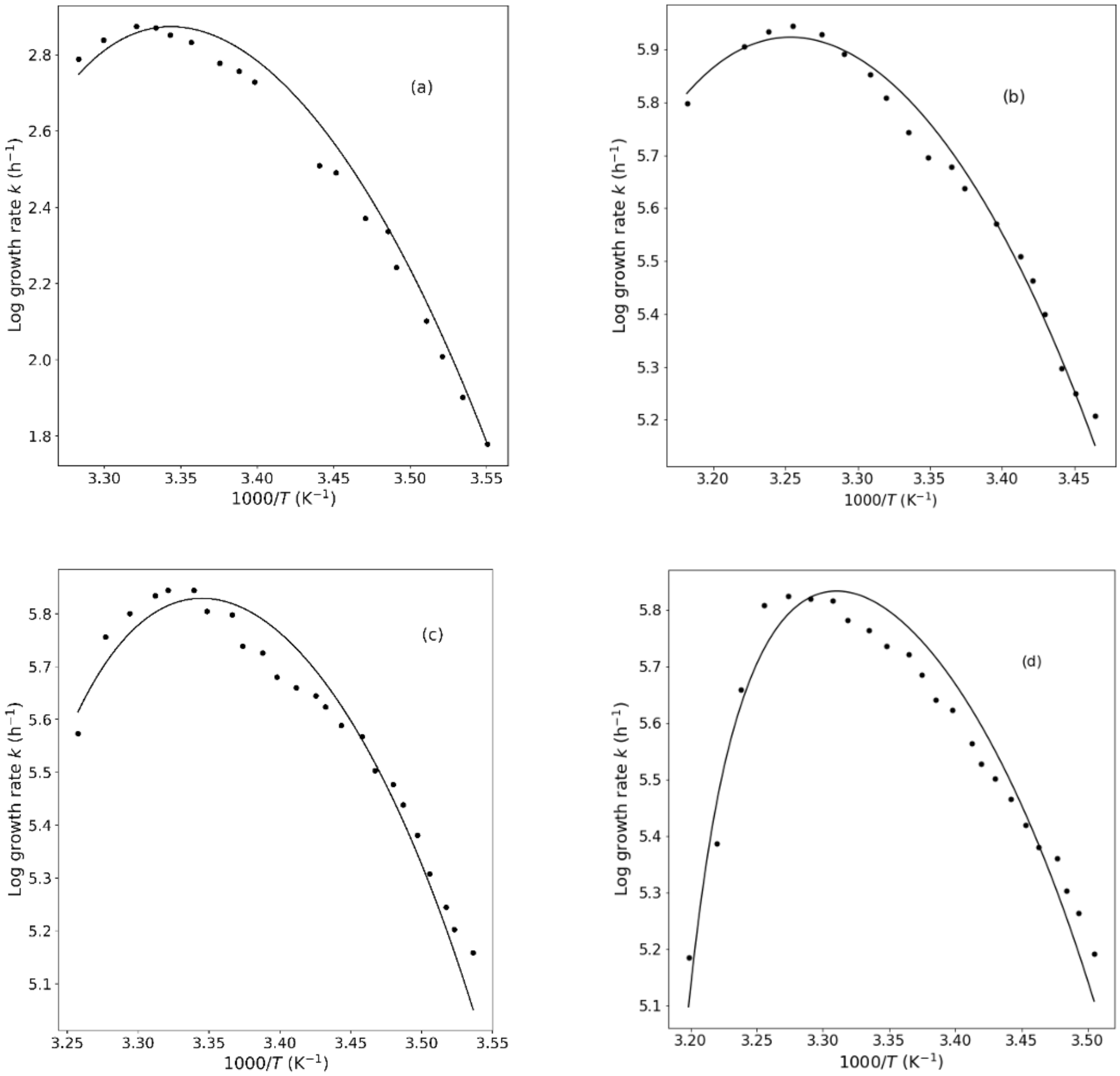

Fig. 1. Fitting of equation (8) to the experimental data (as indicated by the solid line) for a) *Alteromonas* sp., b) *Aeromonas* sp., c) *Flavobacterium* sp., and d) *Moraxella* sp. Experimental data were taken from (Ratkowsky et al. 1983)

Table 1. Fitting parameters for Fig. 1

Bacteria	T_c (K)	T_m (K)	E_a (eV)	E_b (eV)	R^2
Alteromonas sp.	240	334	0.025	0.08	0.978
Aeromonas sp.	250	353	0.015	0.079	0.983
Flavobacterium sp.	255	329	0.0086	0.004	0.946
Moraxella sp.	260	319	0.0057	0.0041	0.927

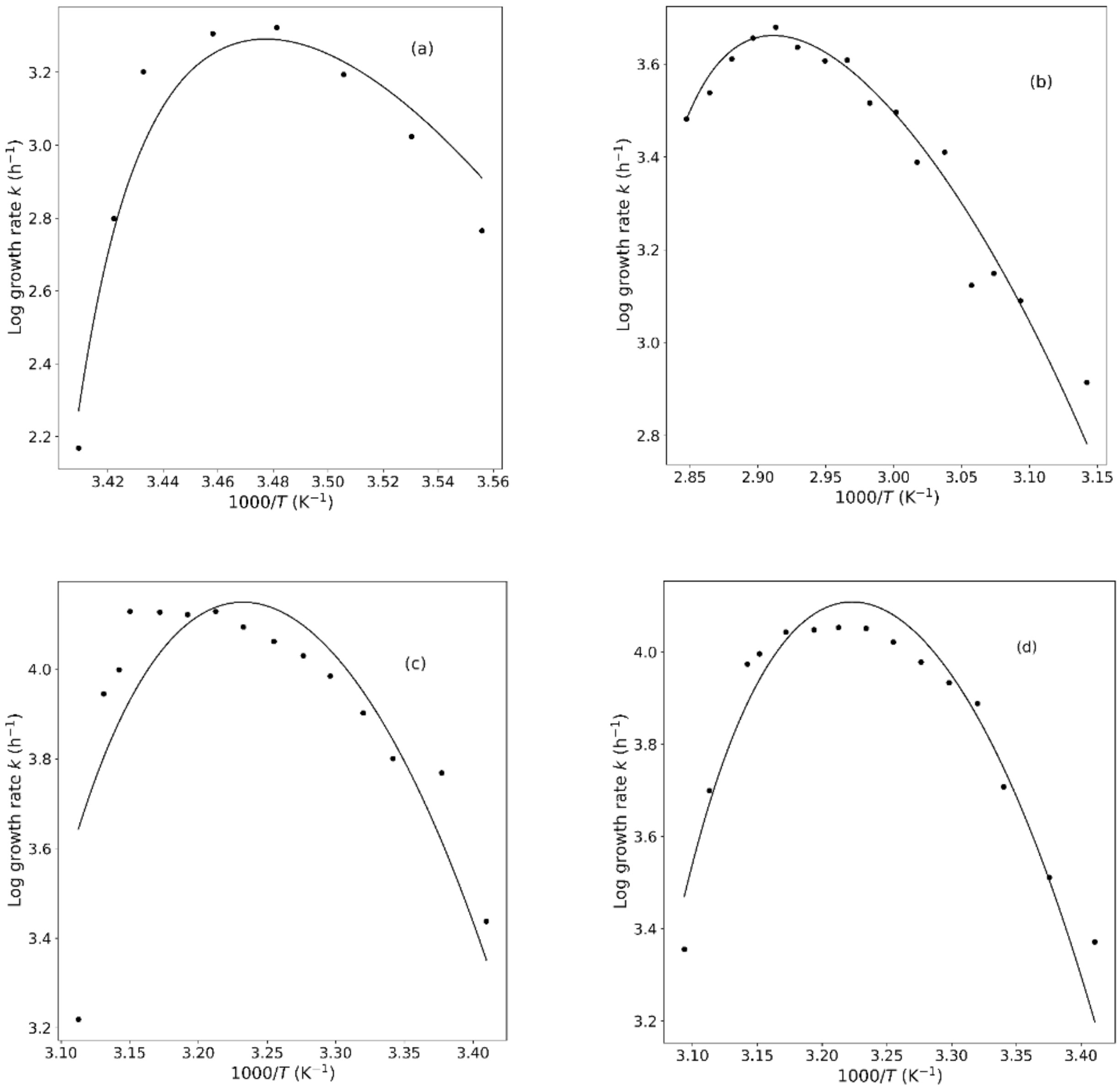

Fig. 2. Fitting of equation (8) to the experimental data (as indicated by the solid line) for a) *V. marinus*, b) *T. aquaticus*, c) *E. coli*, and d) *B. subtilis*. Experimental data were taken from (Ratkowsky et al. 1983)

Table 2. Fitting parameters for Fig. 2

Bacteria	T_c (K)	T_m (K)	E_a (eV)	E_b (eV)	R^2
Vibrio marinus	268	303	0.0048	0.081	0.921
Thermus aquaticus	244	393	0.053	0.05	0.941
Escherichia coli	248	352	0.022	0.056	0.689
Bacillus subtilis	252	351	0.022	0.055	0.919

This type of emergence of the glassy state in bacteria is of interest as bacteria characterized as glass-forming liquids can be vitrified at higher (T_m) and lower (T_c) temperatures. The metabolic activity of the cells ceases below T_c and above T_m (Arcus et al. 2016; Khonsari, Kollmann 2015; Lee et al. 2020). It is of interest to examine whether there is a correlation between T_c and T_m. Fig. 3 shows the correlation between T_c and T_m (Tables 1 and 2). There may be a weak correlation between T_m and T_c; T_m decreases linearly with T_c.

It should be noted that the energies E_a and E_b, as the fitting parameters, seem to be smaller than the inorganic glass-forming liquid. As already stated in the previous section (overview), the bacterial cytoplasm is in a low-temperature liquid state, and therefore thermal reaction-related energy is smaller than that of the popular inorganic glass-forming liquids. It should also be mentioned that the E_a itself is not the so-called activation energy (Micoulaut 2021; Pinto, Shimakawa 2023) (see section overview).

Conclusion

The glass transition can be defined as a state of reduced mobility ('frozen-in'), where the molecules can no longer move freely even in liquid states. We presented a new interpretation for the temperature dependence of the bacterial growth rate over the entire biokinetic temperature range: the bacterial cytoplasm exhibits two types of frozen-in transitions: at low (T_c) and high (T_m) temperatures. T_c is dominated by the free volume gained by thermal expansion, and T_m is dominated by the free volume corresponding to the physiological concentration in the cytoplasm. Below T_c and above T_m, the free volume collapses, inducing nonmonotonic temperature dependence of bacterial growth, which is phenomenologically analogous to that observed in glass-forming liquids in nonliving inorganic materials. It should be noted, however, that the loss of free volume above T_m in bacteria (lived matter) may lead to the death of the cytoplasm, but not in nonliving materials. Dynamics of the living system is very complex, and hence further careful discussion is required.

Conflict of Interest

The authors delare that there is no conflict of interest, either existing or potential.

Author Contributions

Data collection was performed by C. Pinto, and analysis was carried out by C. Pinto and K. Shimakawa. K. Shimakawa provided the information on the glass-forming liquid discussed in solid state physics (K. Shimakawa's field). The first draft of the manuscript was written by C. Pinto, and K. Shimakawa commented on the previous versions of the manuscript. Both authors read and approved the final manuscript.

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