Water Diffusion from a Bacterial Cell in Low-Moisture Foods

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Abstract: We used a Fick's unsteady state diffusion equation to estimate the time required for a single spherical shaped bacterium (assuming *Enterococcus faecium* as the target microorganism) in low-moisture foods to equilibrate with the environment. We generated water sorption isotherms of freeze-dried *E. faecium*. The water activity of bacterial cells at given water content increased considerably as temperature increased from 20 to 80 °C, as observed in the sorption isotherms of bacterial cells. When the water vapor diffusion coefficient was assumed as between 10^{-12} and 10^{-10} m^2/s for bacterial cells, the predicted equilibration times (t_{eq}) ranged from 8.24×10^{-4} to 8.24×10^{-2} s. Considering a cell membrane barrier with a lower water diffusion coefficient (10^{-15} m^2/s) around the bacterial cell with a water diffusion coefficient of 10^{-12} m^2/s, the t_{eq} predicted using COMSOL Multiphysics program was 3.8×10^{-1} s. This result suggests that a single bacterium equilibrates rapidly (within seconds) with change in environmental humidity and temperature.

Keywords: *Enterococcus faecium*, low-moisture foods, mathematical modeling, sorption isotherm, water activity

Introduction

Many pathogenic microorganisms such as *Salmonella* spp. can survive in low-moisture foods including spices, flours, food powders or dried nuts and fruits. In order to effectively inactivate microorganisms in a low-moisture environment, thermal energy may be applied. But microorganisms generally exhibit greater thermal resistance in a low-moisture environment than in high-moisture foods. Water activity (a_w), defined as the ratio of vapor pressure of water in a given system and that of pure water at a system temperature, is one of the most important factors determining the thermal resistance of microorganisms in a low-moisture environment other than temperature (Laroche and others 2005). Water activity is determined at the thermodynamic equilibrium state, at which its value is equivalent to the relative humidity of the surrounding environment (Murrel and Scott 1966). At a fixed water content, water activity of a material changes with temperature. The degree of such change depends on material composition (Syamaladevi and others 2016). Thus, when a low-moisture food contaminated with bacterial cells is heated to a high temperature, the water activity of the food may differ from that of the bacterial cells. As a result, water exchange will occur between the food and the microorganisms. Since mass of foods is significantly greater than that of microorganisms, the water activity of microorganisms may tend to adjust to the water activity of the foods through water diffusion. Industrial thermal processes are often designed for short treatment times in order to reduce thermal damages to foods. Thus, it is desirable to know the time taken by microorganisms to reach equilibrium a_w in a low-moisture environment when exposed to high temperatures. However, no systematic experimental or theoretical analyses have been reported so far to understand the approximate time required for moisture exchange between a bacterial cell and its surroundings.

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Specifically, we would like to gain knowledge of the equilibration time (t_{eq}), or the time required for microorganisms to achieve thermodynamic equilibrium (with similar temperature and water vapor pressure) with their environment. In this study, we considered the time required at the center of the bacteria to reach 99.9% of the environmental water vapor concentration to be the equilibration time. Mathematical modeling is a useful approach in the determination of equilibration for a single bacterial cell as its experiential determination is challenging because of the lack of commercially available diffusion measurement devices. Literature studies on mathematical modeling of water diffusion in bacterial cells are limited, possibly due to unknown values of water diffusion coefficients in bacterial cells (Sehy and others 2002). Further, a water sorption isotherm of bacterial cells at the environmental temperature is necessary to determine the water contents at equilibrium environmental relative humidity values, but is unavailable in the literature.

The objectives of this study were to (1) generate water sorption isotherms for bacterial cells (*Enterococcus faecium*) at 20 and 80 °C which was necessary for the mathematical simulation of water transfer in a bacterial cell and (2) estimate the equilibration time for a single bacterial cell using mathematical simulation when a bacterial cell is exposed to a changed environment. *Enterococcus faecium* was selected as the model bacteria in this study as they have been used as the potential surrogate for *Salmonella* in low-moisture foods (Jeong and others 2011; Ceylan and Bautista 2015).

Materials and Methods

Production of freeze-dried *E. faecium*

The stock culture of *E. faecium* NRRL B-2354 (ATCC® 8459™) was acquired from Dr. Linda Harris at University of California, Davis and was kept in a stock solution of trypticase soy broth (TSB) supplemented with 0.6% (w/v) yeast extract (YE) and 20% glycerol at −80 °C. The strain was taken from a stock with 20% glycerin at −80 °C, a loop was transferred into 9 mL of TSBYE, also 1% of the culture was transferred into 50-mL centrifuge tube and incubated for 24 h at 37 °C. The culture was centrifuged at 8000 × *g* for 15 min at 4 °C. The supernatant was removed and the remaining pellet was resuspended in 1 mL of

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sterilized double-distilled H_2O, washed for thrice, then centrifuged. The electrical conductivities of the washed water and pure distilled water were compared (4.97 μS/cm for the washed water and 4.86 μS/cm for pure distilled water), confirming that the bacteria pellets were free from media solutes. The bacteria pellets were frozen immediately in liquid nitrogen before freeze drying. The bacteria pellets were dried using a freeze dryer (FreeZone® Plus Freeze Dry Systems, 4.5 L Cascade, Labconco, Kansas City, Mo., U.S.A.). The temperature of the condenser was maintained at –70 °C and vacuum inside the freeze-drying vial connected to manifold was set at 5 Pa. After 48 h of freeze drying, the dried microbial cells were removed, sealed inside vials and kept at –20 °C. The viability of the freeze-dried bacteria was determined as approximately 10^{11} CFU/g. For scanning electron microscopy analysis, freeze dried *E. faecium* samples were fixed with glutaraldehyde and rinsed using 0.1 M phosphate buffer and then dehydrated using ethanol/water with increasing concentration of ethanol. An environmental scanning electron microscope (Quanta 200 ESEM, FEI Co, Hillsboro, Oreg., U.S.A.) was used to obtain images of *E. faecium*.

Water sorption isotherms of freeze-dried *E. faecium* at selected temperatures

An Aqualab vapor sorption analyzer (VSA) (Decagon Devices, Inc. Pullman, Wash., U.S.A.) using the dynamic vapor sorption (DVS) method (Yu and others 2008) was used to generate water sorption isotherms of bacterial cells at 20 °C. The VSA generates equilibrium relative humidity conditions between 3% and 90% (corresponding water activities of 0.03 to 0.90) with an accuracy of ± 0.005 at operating temperatures from 15 to 60 °C. The bacterial samples (approximately 0.2 g) were exposed to presented relative humdity conditions inside the VSA chamber until a constant sample mass was achieved. The water activity and water contents were recorded when the sample reached equilibrium. We selected a 10% increment change in relative humidity to achieve water activity intervals of 0.1 for the water sorption curve. The equilibrium water content values were recorded at each water activity level (Syamaladevi and others 2016). Average values of at least 2 replicates of water contents and water activities were determined.

To generate water sorption isotherms of bacterial cells at 80 °C, we used a newly developed sealed thermal cell containing a commercial relative humidity sensor (HX15-W, Omega Engineering, Inc.) (Syamaladevi and others 2016). We selected 80 °C for the isotherm testing, as this was used as a treatment temperature in several reported studies on pathogen control in low-moisture foods (Ha and others 2013; Syamaladevi and others 2016). The high temperature relative humidity sensor measured the relative humidity of the air headspace above the bacterial cells at 80 °C at equilibrium. The a_w of the sample (approximately 0.2 g, at least 2 replicates) is equivalent to the relative humidity of the headspace in thermodynamic equilibrium with the bacterial cells at the same temperature and pressure. We assumed that bacterial cells were exposed to 80 °C, hence water sorption isotherms of bacterial cells at 80 °C is necessary to model their equilibration time (explained in Sections "Isotherms for *E. faecium* cells" and "Equilibration time for a spherical bacterial cell with constant water vapor diffusion coefficient"). We calibrated this instrumental setup using the reported values of water activities of lithium chloride (13.41 M) and sodium chloride (6 M) solutions at different temperatures, obtained from their osmotic coefficients (Gibbard and Scatchard 1973; Gibbard and others 1974). The water sorption isotherm data of bacterial cells were fitted with Guggenheim, Anderson, and de Boer (GAB) model (Syamaladevi and others 2016). The GAB model is expressed as:

$$\frac{X}{X_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \quad (1)$$

where X is the dry basis water content of the material, X_m is the monolayer water content (dry basis), C and K are parameters which have physical meaning based on the multilayer adsorption of water. More details about these methods and modeling are described in Syamaladevi and others (2016).

Concept and mathematical formulation for 1D water transfer in a bacterial cell

In this study, we used 2 different methods for the estimation of equilibration time.

1. Consider a constant water vapor diffusion coefficient inside a bacterial cell
2. Consider cell membrane as an additional barrier for water vapor diffusion.

The water transport in bacteria is assumed as passive diffusion. It is a mass transfer phenomenon. We considered spherical shaped bacterium (such as *E. faecium*) exposed to a humid environment (Figure 1). One-dimensional Fick's equation can be used to formulate the problem of mass transfer in bacteria during equilibration.

Assumptions.

1. The bacteria is spherical in shape and isotropic (Figure 1)
2. Shrinkage of the system (bacteria) is neglected
3. Water vapor diffusion coefficient is constant throughout the process
4. Effect of intracellular macromolecules on water vapor diffusion is not considered

Governing equation for 1D water transfer in a bacterial cell. Based on the above assumptions, Fick's equation describing unsteady state diffusion in a spherical shaped bacterium with constant (infinite) source, (Figure 1) is given as:

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \quad (2)$$

where C is the water vapor concentration (kg/m^3), t is the time of equilibration (s), D is the diffusion coefficient of water vapor through bacterial cell (m^2/s), and r is the radial distance from bacterial cell center (m).

Initial and boundary conditions. Initially the bacterial cell is assumed to be at a uniform temperature, water content and water activity. For constant surface water concentration (C_s) at the radius of the spherical shaped bacterial cell (a), which was approximately 0.5×10^{-6} m (Figure 1), the following initial and boundary conditions apply:

$$C = C_o \qquad r = 0 \qquad t = 0, \quad (3)$$

$$C = C_s \qquad r = a \qquad t > 0, \quad (4)$$

$$C = C(r) \qquad 0 < r < a \qquad t > 0, \quad (5)$$

At the interface between air and bacterial surface, the water vapor concentration on 2 sides is assumed to be in local equilibrium.

Unsteady state diffusion of water vapor in bacterial cell. If the initial water vapor concentration in the spherical bacterial cell is C_o with constant surface concentration (C_s), the solution is (Crank 1975):

$$\frac{C(r) - C_o}{C_s - C_o} = 1 + \frac{2a}{\pi r}\sum_{n=1}^{\infty}\frac{(-1)^n}{n}\sin\frac{n\pi r}{a}\exp\left(-\frac{Dn^2\pi^2 t}{a^2}\right) \quad (6)$$

The water vapor concentration at the center can be expressed as:

$$\frac{C(0) - C_o}{C_s - C_o} = 1 + 2\sum_{n=1}^{\infty}(-1)^n\exp\left(-\frac{Dn^2\pi^2 t}{a^2}\right) \quad (7)$$

where $C(0)$ is the water vapor concentration at the center of the bacterial cell. The equilibration time depends on initial water vapor concentration in the bacteria (as a function of water activity and temperature), water vapor concentration of the environment (as a function of relative humidity and temperature of the environment), water vapor diffusion coefficient inside a bacterium, and radius of the bacterium. Here, the diffusion coefficient of water vapor is assumed as constant, similar in cell membrane and inside the bacterial body (cytoplasm). The equilibration time is considered as the time required when the center of the bacterium has the similar water vapor concentration as the surface/environment. Since complete equilibrium is impossible, we considered the time required for the center of the bacteria to reach 99.9% of the environmental concentration as the equilibration time (or 1.001 times of the environmental water vapor concentration if the bacterial water concentration is higher than the environmental water vapor concentration).

The Eq. 7 may also be written in terms of water contents as:

$$\frac{X(0) - X_o}{X_s - X_o} = 1 + 2\sum_{n=1}^{\infty}(-1)^n\exp\left(-\frac{Dn^2\pi^2 t}{a^2}\right) \quad (8)$$

where $X(0)$ is the water content (dry basis) at the center of the bacterial cell, X_o is the initial water content (dry basis) of the bacterial cell, which can be determined from the initial water activity using the water sorption isotherm of the bacterial cells. X_s is the surface water content (dry basis) of the bacterial cell which is in equilibrium with the external environment. X_s can be determined from the relative humidity of the external environment which is equivalent to the water activity of the bacterial cell. The water sorption isotherm of the bacterial cells at the external environment temperature was used to determine the value of X_s.

Results and Discussion

Isotherms for *E. faecium* cells

The water sorption (adsorption and desorption) isotherms for freeze-dried *E. faecium* at 20 and 80 °C (Figure 2) using a vapor sorption analyzer and a newly developed thermal cell with relative humidity sensor as discussed in Syamaladevi and others (2016). To the best of our knowledge, this is the first study, which generated the water sorption isotherm of freeze-dried bacterial cells at elevated temperatures (80 °C). At a same water content, the water activity of bacterial cells increased considerably as temperature increased from 20 to 80 °C. For instance, the water activity of bacterial cells was 0.1 for a water content of 0.1 kg water/kg dry solids at 20 °C, while it increased to 0.5 at 80 °C, where water content remains at 0.1 kg water/kg dry solids. The isotherm of bacteria at 20 and 80 °C were similar to the isotherms of protein and carbohydrate rich foods such as all-purpose wheat flour (Syamaladevi and others 2016).

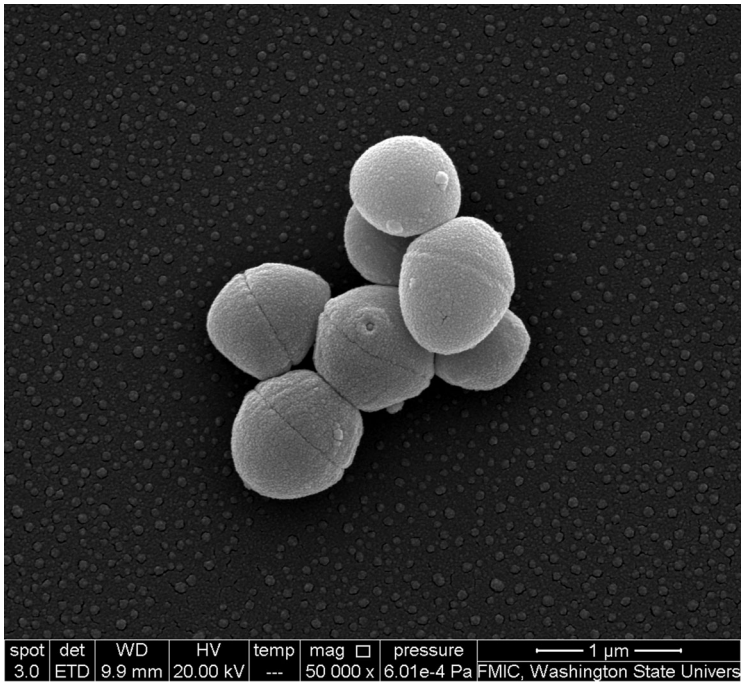

Figure 1–Scanning electron microscopy images of freeze-dried *E. faecium* (at least 2 replicates).

Equilibration time for a spherical bacterial cell with constant water vapor diffusion coefficient

The water sorption isotherm data of microorganisms at elevated temperatures was useful to predict water vapor diffusion and equilibration time of the microorganism. We considered a bacterial cell of spherical shape with initial water activity (a_w) of 0.1 at room temperature (approximately 20 °C) (Figure 1). Freeze-dried bacterial cells have typically low water activity in the range of 0.1 or less. The bacterium was assumed to be exposed to an environment with relative humidity of 30% at 80 °C. This condition was selected to mimic typical thermal processing condition for low-moisture foods. The water sorption isotherm of microbial cells relates the equilibrium relative humidity of the environment (or water activity of microbial cells) and equilibrium water content of the microbial cell surface at a specific environmental temperature. The initial water content of the bacterial cell corresponding to the initial a_w of 0.1 was determined from the water sorption isotherm of freeze-dried *E. faecium* at 20 °C to be 0.1 kg water/kg dry solids (X_o) (0.091 kg water/kg sample) (Figure 2). The bacterial cells are assumed to achieve an a_w of 0.3 after reaching equilibrium with the surrounding environment of 30% RH at 80 °C. The isotherm of freeze-dried *E. faecium* at 80 °C was used to determine the equilibrium water content at the surface of the bacteria (X_s) at 80 °C corresponding to the equilibrium relative humidity of the external environment of 30% or a_w of 0.3. The water content of bacterial cells corresponding to the a_w of 0.3 was determined as 0.075 kg water/kg dry solids (0.0698 kg water/kg sample) from the water sorption isotherm of bacterial cells at 80 °C. Hence, to reach equilibrium with surrounding environment, a. *E. faecium* cell would reduce its water content from original 0.1 kg water/kg dry solids to 0.075 kg water/kg dry solids. Water vapor diffusion occurs from the bacterial body to the environment as the water vapor concentration in the environment is smaller than that of bacterium. The equilibration time (t_{eq}) was determined using Eq. 8. The parameters used in the simulation are presented in Table 1.

One of the most important factors determining the equilibration time for bacteria to adjust to its changing environmental condition is the diffusion coefficient of water vapor inside the cell. The determination of diffusion coefficient of water vapor inside bacterial cell is experimentally challenging, and a few studies reported the diffusion coefficient of water vapor in microbial cells and seeds (Table 2). We assumed that the diffusion coefficient of water vapor ranged from 2×10^{-7} to 2×10^{-14} m^2/s to cover possible values of diffusion coefficients in a bacterial cell (Jasnin and others 2008). The influence of water vapor diffusion coefficient on equilibration time of bacteria was determined from Eq. 8 (Figure 3 and Table 3). The equilibration time was estimated to range between 8.24 and 8.24×10^{-7} s depending on the water vapor diffusion coefficient of the cells (Table 3). The quick equilibration of bacterial cells is attributed to the small size of the bacterial cell. However, there may be considerable difference in equilibration time, considering many number of single bacterium in colonies or in biofilm. More complexity is added to this concept if a bacterium inside a food environment is considered which is being investigated.

Table 1–Parameters used in simulation.

Parameter	Expression
Radius of bacteria, a (m)	0.5×10^{-6}
Initial water activity of bacteria, a_w	0.1
Initial temperature, T_1 (°C)	20
Relative humidity of air, RH (%)	30
Temperature of air, T_2 (°C)	80
Initial water content of bacterium from the isotherm at 20 °C (kg water/kg dry solids)	0.1
Water content of bacterium surface corresponding to a_w of 0.3 from isotherm at 80 °C (kg water/kg dry solids)	0.075

Water vapor diffusion through cell membrane of a spherical bacterial cell

Bacterial cells are surrounded by a cell membrane which may act as a diffusion barrier. It may be anticipated that the water transport across cell membrane consisting of lipid bilayers could be slow. Considering a cell membrane surrounding the spherical bacterial cell, the boundary condition for Eq. 2 at the boundary between bacterial cell and membrane is (Sehy and others 2002):

$$-D_c\frac{\partial C}{\partial r}=\frac{D_m}{d}\ (C_s-C_o)\qquad r=a\qquad t>0\qquad (9)$$

where D_c is the water vapor diffusion coefficient of the spherical bacterial cell, D_m is the water vapor diffusion coefficient of the

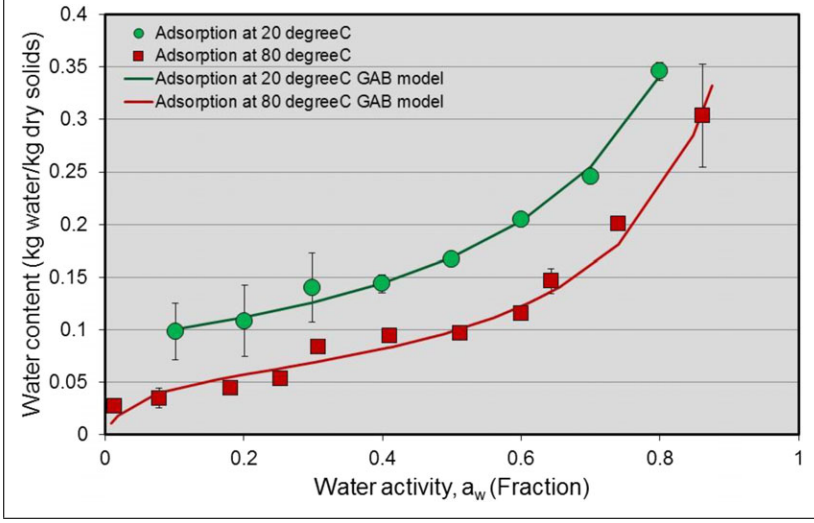

Figure 2–Water sorption isotherm of freeze-dried *E. faecium* at 20 and 80 °C.

Table 2–Reported diffusion coefficient of water vapor (*D*) values in microorganism/systems in the literature.

Microorganism/ system	*D* value (m^2/s)	Reference
Air	2.42×10^{-5}	Nobel (2005)
Eschericia coli (BLE21(DE3) strain	1.53 to 2.94×10^{-9}	Jasnin and others (2008)
Lentil hilium	approximately 10^{-9}	Tang and Sokhansanj (1993)
Lentil cotyledon	approximately 10^{-11}	
Lentil seed coat	approximately 10^{-14}	
Corn	approximately 10^{-11}	Muthukumarappan and Gunasekharan (1990)
Rice endosperm	approximately 10^{-11}	Steffe and Singh (1980)
Rice bran	approximately 10^{-12}	
Rice hull	approximately 10^{-12}	
Xanthan and deacetylated xanthan[a]	$<10^{-9}$	Hart and others. (1999)
Cellulose	approximately 10^{-10} to 10^{-11}	Fanta and others (2012)
Cellulose with pectin		
Cellulose with pectin and xyloglucan		

[a]Self and mutual diffusion coefficient of water.

Table 3–Relationship between diffusion coefficient of water vapor in a bacterial cell and the equilibration time determined using Eq. 8.

Diffusion coefficient of water vapor, *D* (m^2/s)	log *D*	Equilibration time, *t* (s)	log *t*
2×10^{-7}	− 6.7	8.24×10^{-7}	− 6.08
2×10^{-8}	− 7.7	8.24×10^{-6}	− 5.08
2×10^{-9}	− 8.7	8.24×10^{-5}	− 4.08
2×10^{-10}	− 9.7	8.24×10^{-4}	− 3.08
2×10^{-11}	− 10.7	8.24×10^{-3}	− 2.08
2×10^{-12}	−11.7	8.24×10^{-2}	−1.08
2×10^{-13}	− 12.7	8.24×10^{-1}	− 0.08
2×10^{-14}	− 13.7	8.24	0.916

cell membrane, d is the membrane thickness, C_s and C_o are the surface and initial water vapor concentration (mol/m^3) values of bacterial cell. We used COMSOL Multiphysics 4.2a (COMSOL, Inc. Burlington, MA 01803) to simulate the water transfer in bacterial cell (diameter of 1×10^{-6} m) with membrane (thickness of 1×10^{-10} m) at changed environmental conditions. The assumed values of D_c and D_m were 2×10^{-12} to 2×10^{-15} m^2/s, respectively (Goodman 2002). The C_s and C_o were considered as 1666.67 and 2200 mol/m^3, respectively based on the X_o and X_s values of 0.1 and 0.075 kg water/kg dry solids, respectively. The equilibration time (t_{eq}) as the time required between the bacterium and environment was estimated as 0.38 s considering above conditions. In this study, we considered simple spherical geometry of bacterial cell with radius of 0.5×10^{-6} m (Garrity and others 2005). The equilibration times (seconds) required for bacterium to adjust its water content and water activity were much shorter compared to the thermal treatment times (minutes) of dry foods. Hence it can be considered that bacterial cells in low-moisture foods exchange water quickly and within the duration of thermal treatments.

In this hypothesis study, we focused on determining the approximate water vapor equilibration times required for a single bacterial

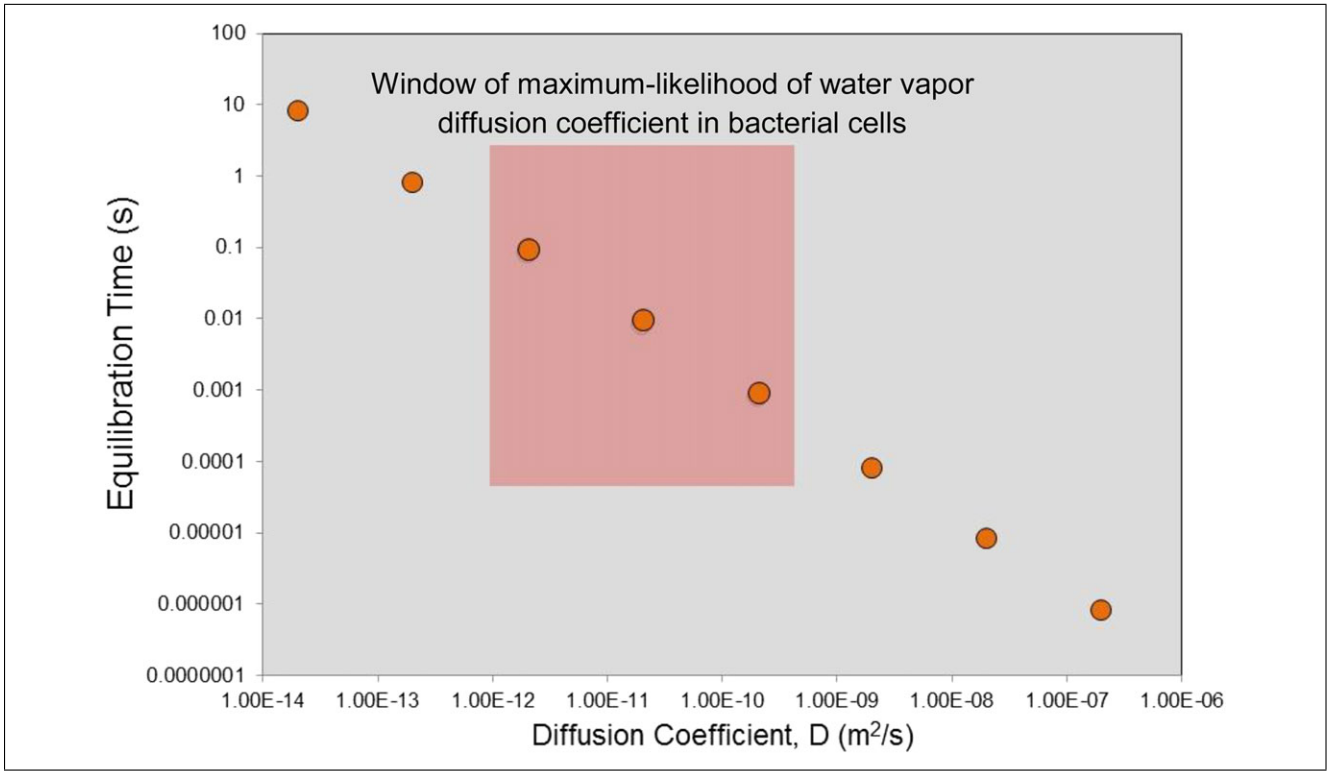

Figure 3–Relationship between water vapor diffusion coefficient and equilibration time for a spherical bacterial cell (the highlighted portion represents the maximum likelihood values of diffusion coefficients of water vapor in bacteria and corresponding equilibration time).

cell with respect to the surrounding environment. We used water vapor diffusion coefficient at room temperature (23 °C) to estimate the water vapor equilibration time for single bacterial cell. In reality the water vapor diffusion increases at high temperatures. Therefore, the real equilibration times may be shorter than the estimated values in this study. We compared the times required to achieve same average water vapor concentrations in spherical and with that of rod shaped (cylindrical) bacterial cells such as *Salmonella* with same diameter using Eq. 10 and 11.

For spherical cells:

$$\frac{C_m - C_o}{C_s - C_o} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{Dn^2\pi^2 t}{a^2}\right) \quad (10)$$

For cylindrical cells:

$$\frac{C_m - C_o}{C_s - C_o} = \sum_{n=1}^{\infty}\frac{4}{{\xi_n}^2}\exp\left(-\frac{D{\xi_n}^2 t}{a^2}\right) \quad (11)$$

where C_m is the average water vapor concentration in bacterial cell and ξ_n is the roots of the equation $J_o(x) = 0$, where $J_o(x)$ is the Bessel function of zero order.

For all the diffusion coefficients used in Table 3, the times required to achieve same average water vapor concentrations for cylindrical cells are about 65% longer than that of spherical cells of the same diameter. For example, if D was assumed as 1×10^{-12} m^2/s, the times required to achieve same average water vapor concentrations were 0.14 and 0.23 s for spherical and cylindrical cells (diameter of 0.5×10^{-6} m), respectively. Hence, the general conclusions regarding equilibrium times for spherical cells are similar for cylindrical cells.

We also considered desiccated bacterium in its metabolically inactive state as a physical system without considering any active transport of water through its membranes. Molecular level water channels or pores exist in cell membranes which enable water transport across cell membrane depending on concentration gradient (passive diffusion) with the help of membrane proteins called aquaporins (Calamita 2000).

This is the first study to establish an understanding of the complex phenomenon regarding moisture diffusion from bacterial cells. Future detailed studies are needed that consider shrinkage or expansion of bacterial cells due to water transfer, temperature dependent water vapor diffusion coefficient inside a bacterial cell and through cell walls and membranes.

Conclusions

In this hypothesis paper, we simulated water vapor diffusion in a single spherical bacterial cell (for example, *E. faecium*) in order to estimate the equilibration time required for the bacterial cell when exposed to changing environmental conditions, that is temperature and relative humidity. The simulation results show that bacterial cells quickly equilibrate to the environment, however, the equilibration time depends on the diffusion coefficient of water vapor inside the cell. We developed sorption isotherm of freeze-dried bacterial cells (*E. faecium*), which may be useful in predicting the equilibration time of bacterial cells. Experimental determination of water vapor diffusion coefficient in dehydrated bacterial cells is recommended in order to accurately predict the equilibration time. Also, bacterial equilibration experiments should be conducted to confirm the predicted equilibration time of bacterial cells. This study is important as it determined that the range of water vapor equilibration times required for the microorganism was considerably smaller (in seconds) than the thermal treatment times (in minutes), so microorganisms quickly equilibrates with conditions of the surrounding foods during thermal treatments.

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