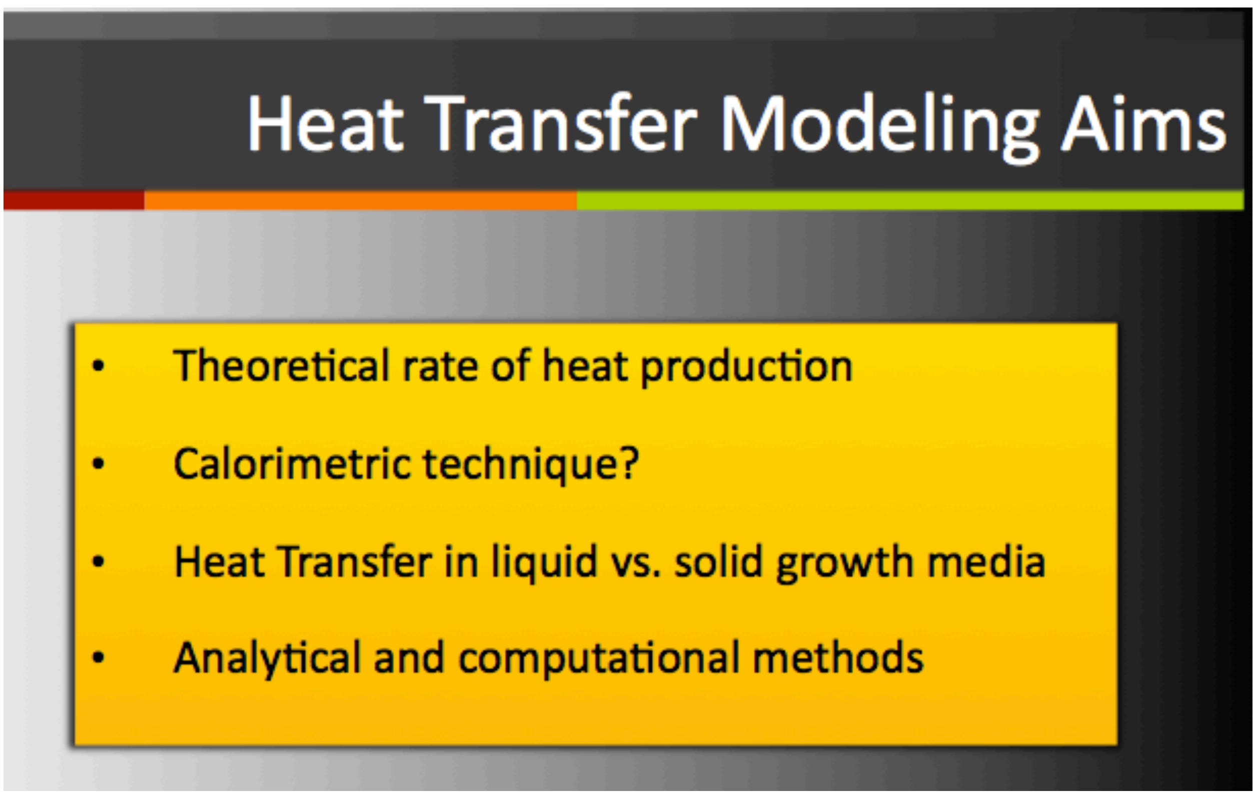
MODELING BACTERIAL HEAT PRODUCTION
DUE TO AOX PATHWAY

AOX pathway is responsible for thermogenesis in various organisms. But to what extent it would be responsible for heat production in genetically engineered bacteria remains an interesting question. Georgia Tech modeling team aimed at theorizing an answer to this question using both analytical and computational methods. The primary goal was to suggest a calorimetric technique with optimal sensitivity, as well as to compare heat transfer in liquid culture and bacterial colonies.



We Knew…

1. The non-growth rate of ATP production is 7.6 mmol per gram of biomass per hour (E. coli W3110) at 38C. This rate is slightly higher in growth-associated conditions (13 mmol/g/h). (Varma & Palsson, 1994)

2. AOX branches from the cytochrome pathway at the level of the ubiquinone pool and couples the oxidation of ubiquinol to the four-electron reduction of oxygen to water. (Albury, Elliott & Moore, 2009)

3. Electrochemical potential gradient difference between Uq and O_2 is 800 mV (Ingledew & Poole, 1984)

4. Two electrons are required per production of 1 ATP molecule

5. The dry mass of one E. coli cell = 7*10^-13 g (http://bionumbers.hms.harvard.edu/bionumber.aspx?s=y&id=103904&ver=9)

Theoretical Rate of Heat Production

The amount of energy released by 4 electrons is given by:

$$\text{Volt} = \frac{\text{Joule}}{\text{Coulomb}} \Rightarrow$$

$$800 \times 10^{-3} = \frac{\text{Joules}}{(4 \text{ electrons})(1.6 \times 10^{-19}\ C)}$$

$$\text{Energy released} = 5.12 \times 10^{-19} \text{ Joules}$$

The amount of energy per cell per second is given by:

$$\frac{7.6 \times 10^{-3} \text{ mol ATP}}{3600 \text{ sec} \times \text{g. biomass}} \times \frac{2 \text{ mol elec}}{\text{mole ATP}} \times \frac{6.0223 \times 10^{23} \text{ electrons}}{\text{mol elec}} \times \left(\frac{5.12 \times 10^{-19}\ J}{4 \text{ electrons}}\right) \times 0.7$$

$$= 0.2278 \ \frac{\text{J}}{\text{g biomass} \times \text{sec}} = 1.6 \times 10^{-13} \frac{\text{J}}{\text{cell} \times \text{sec}}$$

0.7 is an efficiency factor representing approximately 70% conversion of electrons in the pathway in sacred lotus cells [Applied and Environmental Microbiology, Oct. 1994, p. 3724-3731 Vol 60 No 10]

By way of comparison, the specific heat of water is 4.187 J per ml (when 1 ml = 1 gram).

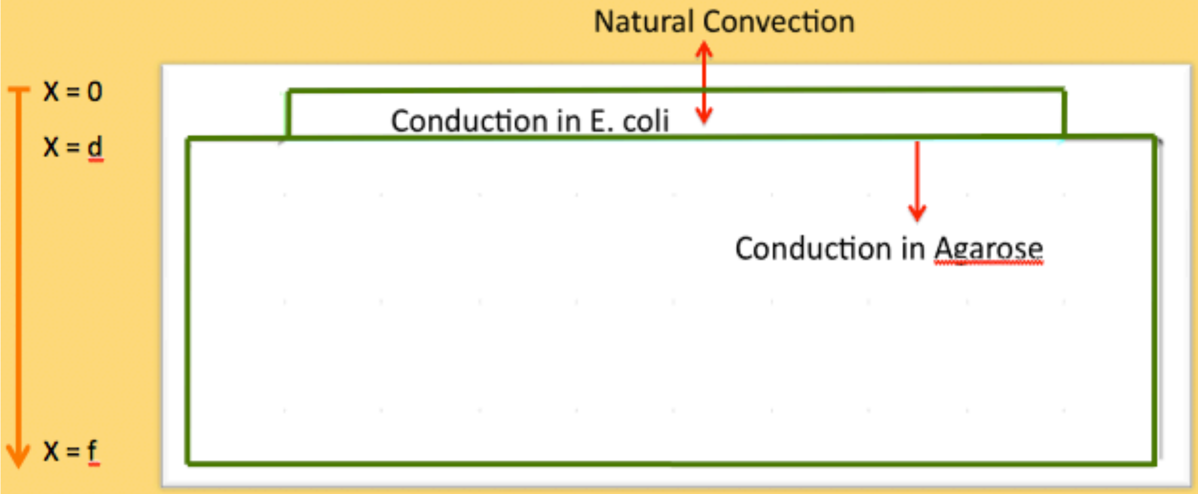
Heat Transfer in Liquid Media

- Simplifying Assumptions
  1. Liquid solution can be assumed water
  2. Complete insulation
  3. Heat accumulation within system
  4. Homogeneous mixture
  5. No work done on or performed by the system
- Density of bacterial culture can vary by 2 orders of magnitude
- Temperature of system can be raised by 1K in 4 – 40 min.

$$1\ \text{ml} \times 1\times10^{10}\ \frac{\text{cells}}{\text{ml}} \times 1.6\times10^{-13}\ \frac{\text{J}}{\text{cell}\times\text{sec}} = 1.6\times10^{-4}\ \frac{\text{J}}{\text{sec}}$$

$$4.187\frac{\text{J}}{\text{ml}} \times \frac{\text{seconds}}{1.6\times10^{-4}\ \text{J}} = 2{,}417\ \text{seconds} \times \frac{1\ \text{min}}{60\ \text{sec}} = 40.3\ \text{minutes}$$

Heat Transfer in Solid Growth Media



Assumptions:

1. Petri dish is completely insulated, and kept at 288K
2. Ambient temperature is 288K
3. Conduction through E. coli is similar to that in water
4. Constant coefficients for conductivity in both media, constant convective coefficient for air
5. Aspect ratio : width of colony >> height of colony

Steady-State Temperature Profile

1D Control-Volume (E. coli) using Rectangular Coordinates

Control-Volume 1: E. coli

- The general energy equation: No shaft or viscous work, no accumulation, steady-state

$$\cancel{\frac{\partial Q}{dt}} - \cancel{\frac{\partial W_s}{dt}} - \cancel{\frac{\partial W_\mu}{dt}} = \iint_{c.s.} (e + \frac{p}{\rho})\rho(v \bullet n)dA + \frac{\partial}{dt}\iiint_{c.v.} e\rho dV$$

- Incompressible fluid, without velocity, constant k, 1-Dimensional heat transfer

$$\alpha\nabla^2 T = -\frac{Q}{\rho c_p} \quad \text{Where} \quad k = \alpha\rho c_p \quad \text{and} \quad \nabla^2 T = \frac{d^2T}{dx^2} + \cancel{\frac{d^2T}{dy^2}} + \cancel{\frac{d^2T}{dz^2}}$$

- Integrating Poisson Equation

$$\frac{d^2T}{dx^2} = -\frac{Q}{k} \rightarrow \frac{dT}{dx} = \frac{-Q}{k}x + C_1 \rightarrow \boxed{T(x)_{Ecoli} = \frac{-Q}{2k}x^2 + C_1 x + C_2}$$

Steady-State Temperature Profile

1D Control Volume (Agarose) using Rectangular Coordinates

Control-Volume 2: Agarose

- The general energy equation is simplified to Laplace equation.
- Integrating Laplace Equation:

$$\frac{d^2T}{dx^2} = 0 \quad \rightarrow \quad \frac{dT}{dx} = C_3 \quad \rightarrow \quad \boxed{T(x)_{Agarose} = C_3 x + C_4}$$

- It is not a quadratic function of X : no heat generation term.

A more detailed derivation of temperature profiles…

Control volume 1: E. coli

$$\frac{dQ}{dt}-\frac{dW_s}{dt}-\frac{dW_\mu}{dt}=\iint_{C.S.}(e+\frac{p}{\rho})\rho(v\bullet n)dA+\frac{d}{dt}\iiint_{C.V.}e\rho dV$$

$$\frac{dW_s}{dt}=0$$

$$\frac{dW_\mu}{dt}=0$$

$$\frac{dQ}{dt}=(k\frac{dT}{dx}\Big|_{x+\Delta x}-k\frac{dT}{dx}\Big|_{x})\Delta y\Delta z+Q(\Delta x\Delta y\Delta z)$$

……..

$$\nabla k\nabla T+Q+\phi=\rho c_p\frac{DT}{dt}$$

$$\rho c_p\nabla T+Q=k\nabla^2T$$

$$\frac{dT}{dt}=\alpha\nabla^2T+\frac{Q}{\rho c_p}$$

$$\because\frac{dT}{dt}=0$$

$$\therefore\alpha\nabla^2T=-\frac{Q}{\rho c_p}$$

$$\nabla^2T=\frac{d^2T}{dx^2}+\frac{d^2T}{dy^2}+\frac{d^2T}{dz^2}$$

$$\because\frac{d^2T}{dz^2}=\frac{d^2T}{dy^2}=0$$

$$k=\alpha\rho c_p$$

$$\therefore\frac{d^2T}{dx^2}=-\frac{Q}{k}$$

$$\frac{d^2T}{dx^2}=\frac{-Q}{k}$$

$$\frac{dT}{dx}=\frac{-Q}{k}x+C_1$$

$$T(x)=\frac{-Q}{2k}x^2+C_1x+C_2$$

Control Volume 2: Agarose

$$\nabla k \nabla T + Q + \phi = \rho c_p \frac{DT}{dt}$$

$$\because [Q = 0] and [\phi = 0]$$

$$\rho c_p \nabla T = k \nabla^2 T$$

$$\frac{dT}{dt} = \alpha \nabla^2 T$$

$$\because [\frac{dT}{dt} = 0]$$

$$\therefore [\alpha \nabla^2 T = 0]$$

$$\nabla^2 T = \frac{d^2T}{dx^2} + \frac{d^2T}{dy^2} + \frac{d^2T}{dz^2}$$

$$\because [\frac{d^2T}{dz^2} = \frac{d^2T}{dy^2} = 0]$$

$$\therefore [\frac{d^2T}{dx^2} = 0]$$

$$\frac{dT}{dx} = C_3$$

$$T(x) = C_3 x + C_4$$

Analytical Solutions

Solving for Boundary Conditions (BCs)

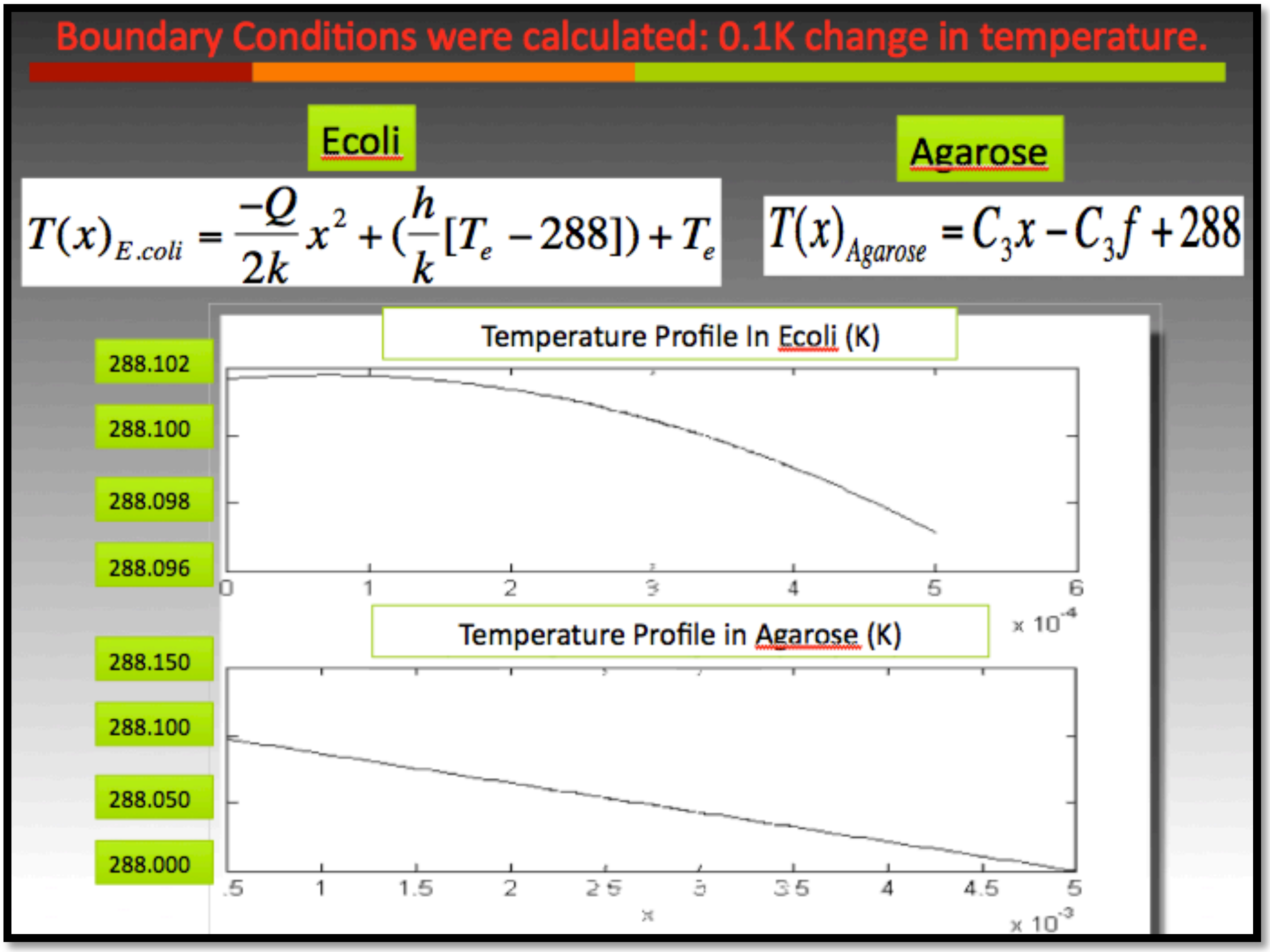
$$T(x)_{Ecoli} = \frac{-Q}{2k}x^2 + C_1x + C_2 \quad \longleftrightarrow \quad T(x)_{Agarose} = C_3x + C_4$$

- Solving for C_1, C_2, C_3, and C_4 :
  - ✓ Q: volumetric flow of heat generated by Ecoli
  - ✓ k : conductive coefficient of water at 288K
  - ✓ h : convective coefficient of air at 288K
  - ✓ $T_{ambient}$: 298 K
  - ✓ Measurements of height of colony and agarose
  - o $C_2 = T_e$, Temperature at Ecoli- air boundary (unknown)
- Heat flux at the E. coli - air boundary was equal to the convective heat flux (x = 0)
- Heat flux and temperature were equated at E. coli - agarose boundary (X = d)

Constants

- Q = 29106.00 J/m^3 ; the heat produced per unit volume on an agarose plate as determined by the theoretical rate of heat production assuming 10^11 cells per colony
- d = 0.0005m ; height of E. coli colony
- h = 20 W/(m^2 * K); convective heat transfer coefficient of air
- f = 0.005m; height of the agarose plate

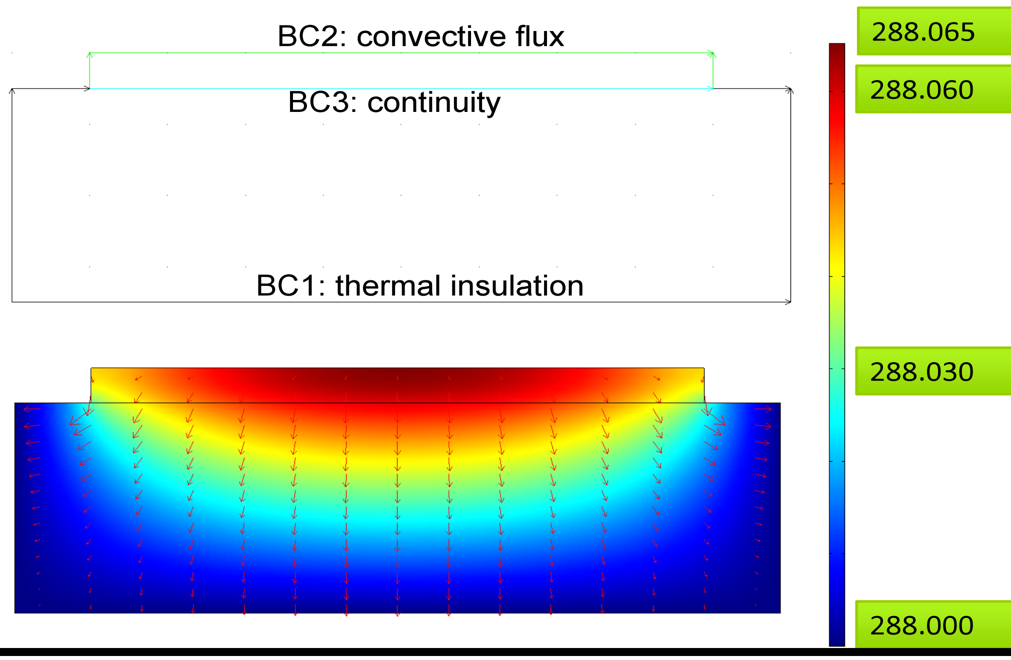
Solving both temperature profiles simultaneously in MATLAB:

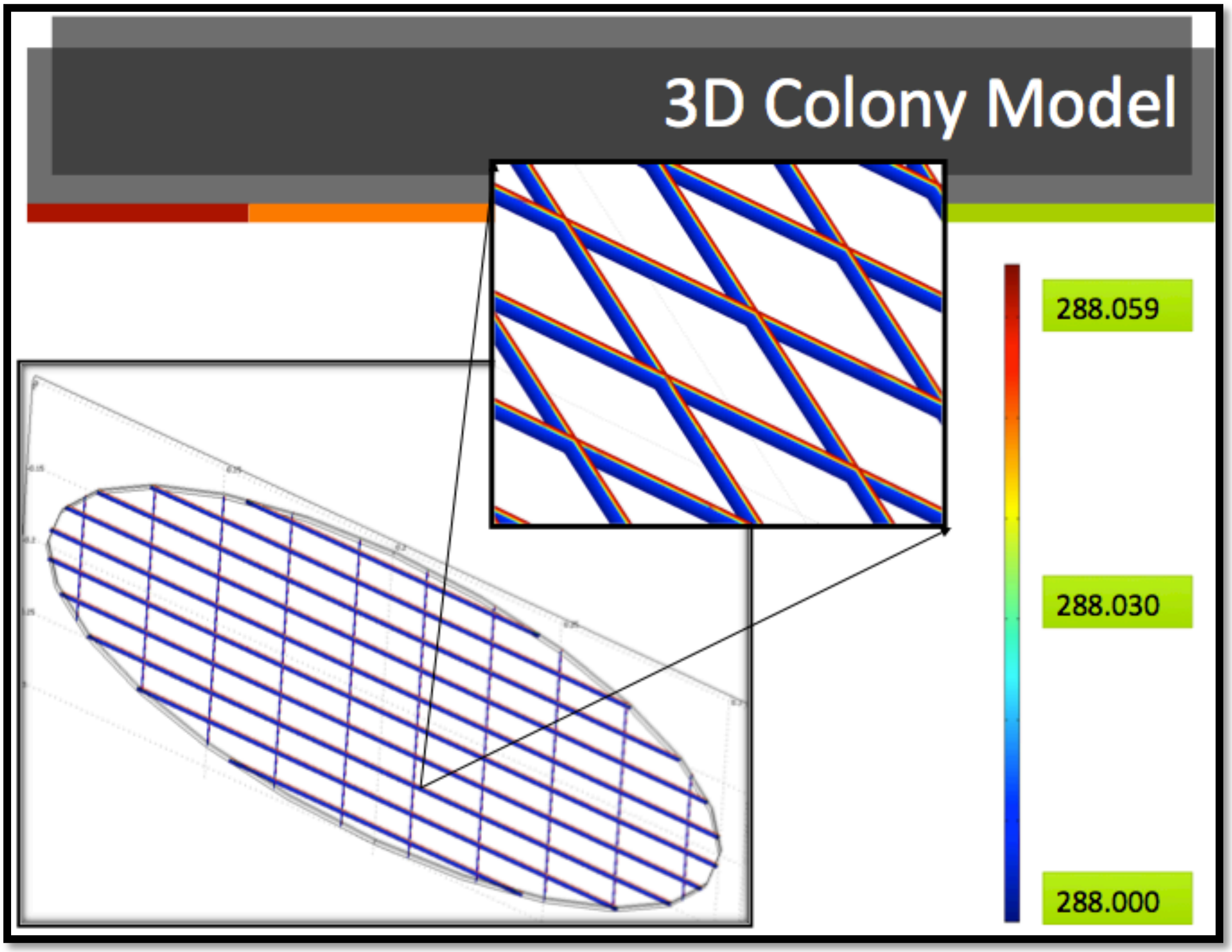


This figure shows the temperature profile of bacteria and the solid growth media as a function of height. In E. coli, temperature drops quadratically, and it drops linearly in agarose. This is because of the heat generation term included within the Poisson equation developed to describe heat transfer in E. coli. The total drop of temperature at steady state across the height of bacterial colony and agarose is approximately 0.1 K.

Computational Approach:

Radial Direction Temperature Profile in COMSOL





This figure as well as the previous were developed in COMSOL. They depict 2D and 3D heat transfer in bacterial colony and agarose. The difference between peak temperatures in both scenarios did not differ by more than 0.006K which indicates that a 2D control volume may provide sufficiently accurate representation. In a 2D control volume, heat is transferred radially to the environment, which may explain the difference in peak temperature values derived analytically and computationally (288.102 K and 288.065K, respectively). The difference however is not more than 0.037K, which suggests that radial heat transfer may in fact be an important consideration. If higher aspect ratio is implemented, as in case of a uniform stretch of bacterial colony formed on a petri dish, then 1D control volume will be sufficient.

Conclusions on Modeling

- Within solution 1K change in temperature in 4 – 40 minutes
- On agar, steady state temperature profile derived analytically matches closely with those found computationally using COMSOL.
- Using 1D control volume is a good assumption, since 3D temperature profile was not considerably different.
- Derived analytically and computationally, the change in temperature due to AOX expression should be approximately 0.1 K (on solid growth media).
- Due to better accumulation of energy in liquid media, characterization of heat production may be easier in liquid media.
- A highly sensitive (at least 0.1K) thermal imaging camera will be essential for measuring heat production of bacterial colony in both liquid and solid growth media.