Reproducing the non-mixed growth curve from the well-mixed data.

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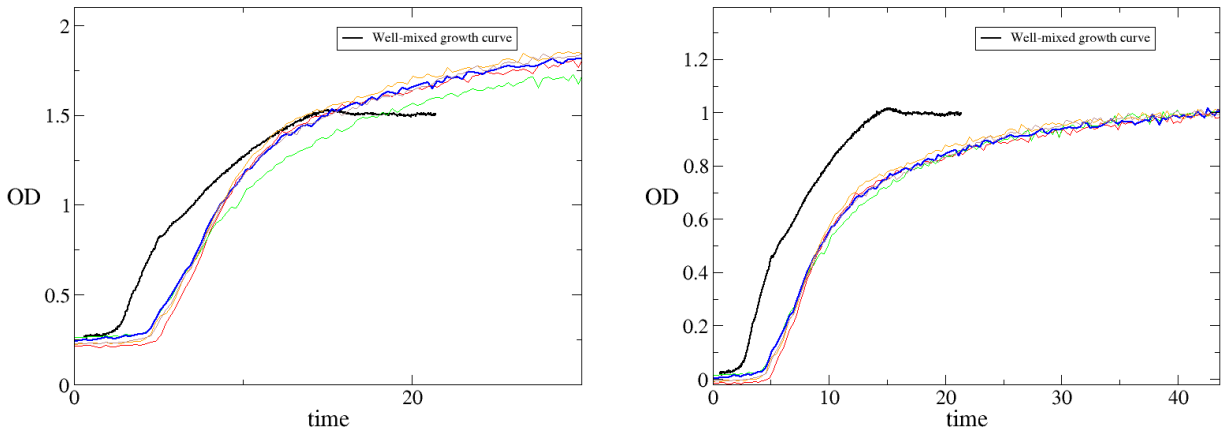
1 Summary

I have been trying to reproduce the experimental growth curve in a non-mixed single well using Bartek's well-mixed data. I have done this using 2 approaches: (1) using the full stochastic simulation where I model nutrient molecules explicitly and calculate local nutrient densities to determine the growth rate and (2) using a system of 2 coupled PDEs modeling bacteria and nutrient fields in 2D, solved using mathematica. Neither reproduces the experimental non-mixed growth curve. Note an assumption used in each: we assume a linear relation between bacterial and substrate density. That is, in determining the growth rate due to the local substrate concentration I have used Barteks growth function $g(n)$ but have replaced n with $(1-s)$. Think of the implications of this – what can I change?



(a) (b)

Figure 1: Bartek's "raw" experimental growth curves in isolated wells on LB growth medium (a). Black curve is a well-mixed experiment, all others are from non-mixed wells. Notice that the non-mixed curves all reached higher optical densities, perhaps suggesting the presence of oxygen? Should we be suspicious of this? Normally I will scale the y-axis to range from 0 to 1 as in (b). Note the slow growth for larger densities in the non-mixed experiments. Modelling the nutrient explicitly still fails to capture this aspect of the growth curve. See Section 5 for a discussion.

2 Stochastic simulation

2.1 Well-mixed: Simulation and experiment

Aside: one thing I have noticed is that the stochastic simulation and experimental growth curves do not actually match without me doing an appropriate scaling of time. Figure 2 shows this. This was due to the wrong choice of "max_growth_rate" which was 2.0 before. With the total propensity being (max_growth + max_death) and max_death = max_growth in Bartek's code even though there is no death in the model, I was a factor of 2 out in calculating the timesteps. This will also have been present in all of the resistance evolution simulations. Will it have affected wavespeeds or profiles!?). Changing this to 1.0 for $K = 10^5$ gave perfect fit with no rescaling. Also, the initial number of bacteria will affect if we need to shift the curve along the x-axis. I could use these fittings to determine the experimental initial innoculation size. In all of the plots that follow, to facilitate comparison I will have rescaled the y-axis (OD) to run from 0 to 1, and I may have rescaled the x-axis (time) by an arbitrary amount.

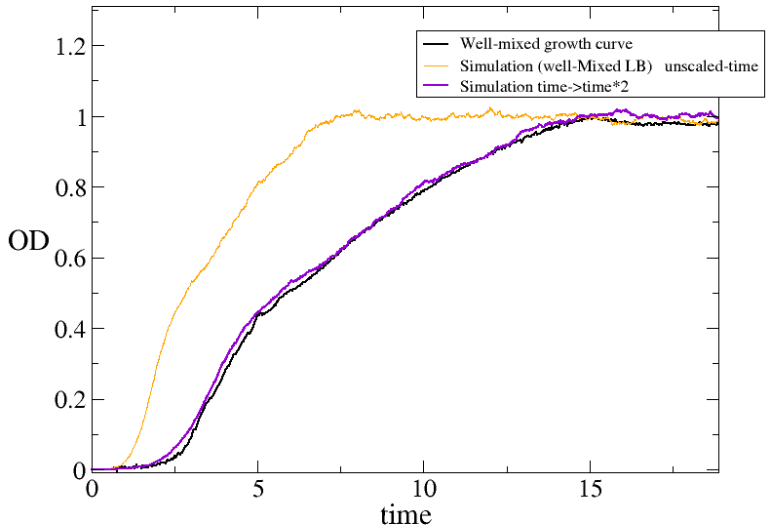
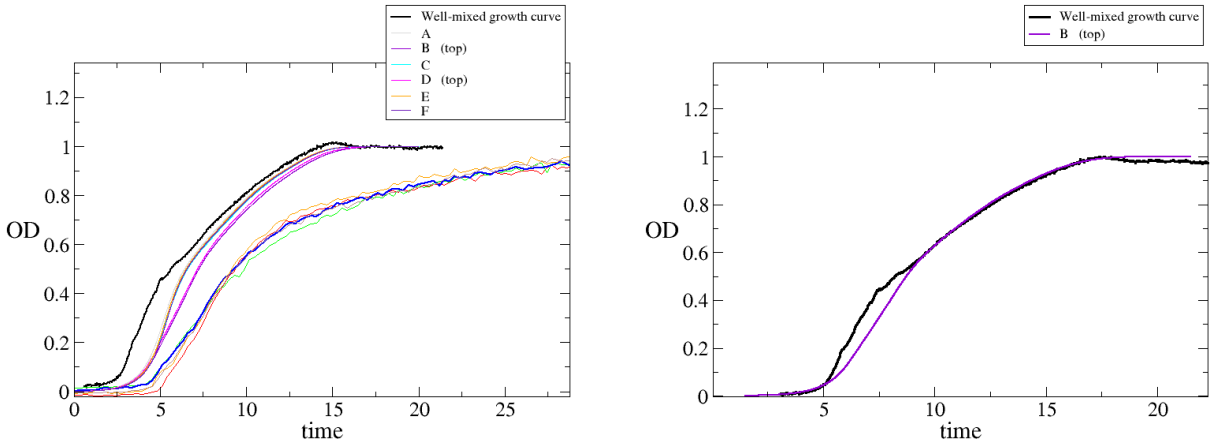


Figure 2: Comparing single-well growth curves from simulation and experiment. Black: scaled experimental growth curve; orange: full stochastic simulation; purple: orange curve with time re-scaled by a factor of 2. Good match between simulation and experiment in the well-mixed case.

2.2 Non-mixed



(a) (b)

Figure 3: The local nutrient stochastic simulations do not match the non-mixed experimental growth curve. (a) shows that the simulations including local nutrient densities (A–F) more closely resemble the experimental well-mixed curve (black) than the non-mixed growth curves (all others). I have simulated this system (A–F) for different diffusion constants of bacteria and nutrients, different initial conditions (bacteria evenly distributed, located at top of well, or located in centre of well) and each has produced a very similar growth curve. For example: curve A has bacterial and nutrient diffusion constants $D_B = 1.5$ and $D_S = 1.5$ mm h^{-1} with initial bacteria placed in the centre of the well; curve D assumed $D_B = 1.5$, $D_S = 0.7$ with initial bacteria restricted to the top 15% of the well. Nutrient was initially evenly distributed in all simulations.
(b) shows an overlay of the well-mixed experimental growth curve (black) and simulation B, illustrating that the local nutrient simulations do not strongly affect the shape of the growth curves.

3 Coupled PDEs in mathematica

$$\frac{\partial N(x,y,t)}{\partial t} = D_B \nabla^2 N + N g[1 - S(x,y,t)], \tag{1}$$

$$\frac{\partial S(x,y,t)}{\partial t} = D_S \nabla^2 S - N g[1 - S(x,y,t)], \tag{2}$$

where $g[S]$ is the well-mixed growth function from Bartek's experiments. To simulate the well-mixed system below I convert these to ODEs by removing the diffusion term.

3.1 Well-mixed

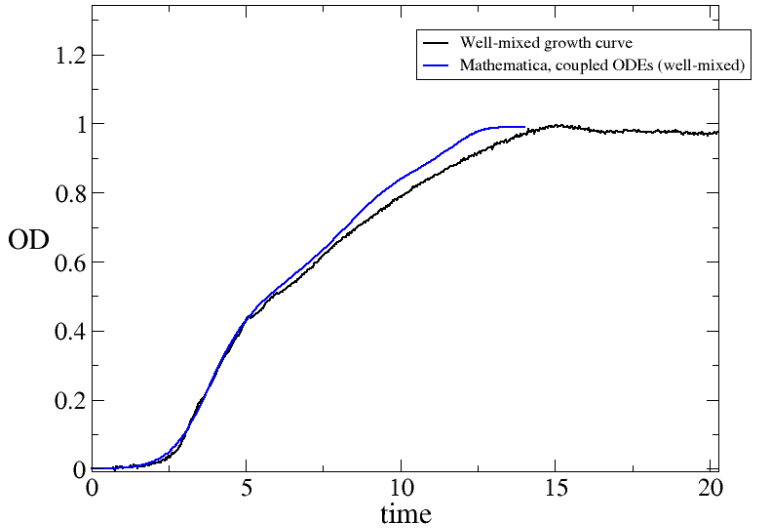


Figure 4: Well-mixed single-well growth curves. Experimental curve (black) vs coupled ODEs (i.e. well-mixed) (blue).

3.2 Non-mixed

I have tried various initial conditions, well-dimensions and diffusion constants and the outcome is always very similar to that found from my stochastic local-nutrient simulations.

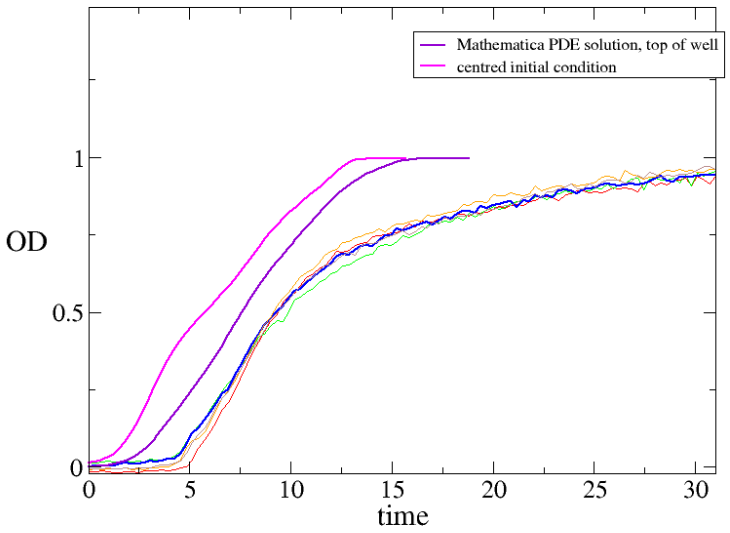


Figure 5: Non-mixed single-well growth curves in 2D. Coupled PDEs (violet and pink), experimental curves (all others). The initial condition used in the violet system was: $N(x, y, 0) = 1E^{-9}(xy)^2(L_x - x)^2(L_y - y^2)^2$ so that bacteria are initially located near the top of the well, and the nutrient was evenly distributed throughout the (2D) well. Parameters: $D_B = D_S = 0.2$, $L_x = 3.25$, $L_y = 11$. We see that the kinked shape of the well-mixed curve is lost but the match is poor – especially at higher bacterial densities. The pink curve had bacteria initially centred: $N(x, y, 0) = 0.000005(xy)^2(L_x - x)^2(L_y - y)^2 + 0.001$ with $D_B = D_S = 0.5$ and the shape of this growth curve resembles that of the well-mixed case. Thus we can alter the shape of the growth curve only slightly by altering the initial conditions as was the case in the full stochastic simulations.

4 Bacteria density-dependence

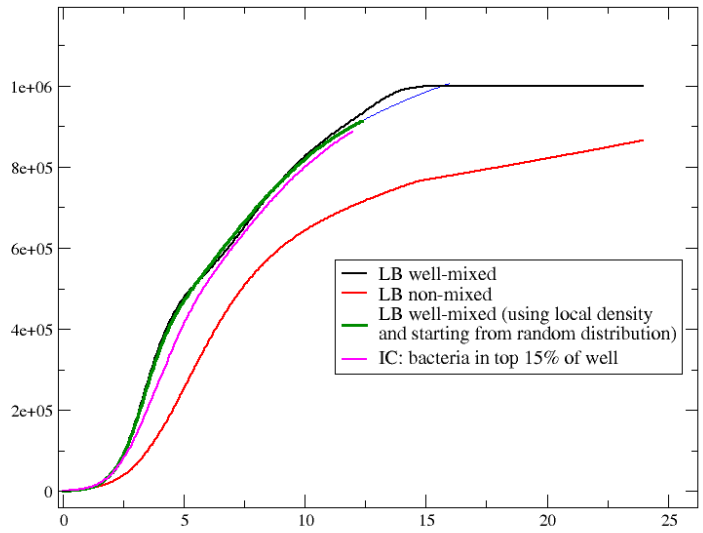


Figure 6: Rosalind's idea. This plot shows what I did before modelling nutrient molecules explicitly: nutrient is not explicitly measured but instead of calculating the bacterial density in the entire well I find the local density and use this to determine the growth rate. There are 2 plots using this local bacteria density (green starts from an initially even distribution of bacteria throughout well, while in the pink curve I place all initial bacteria in the top 15% of the well). I think what this shows is that for the given diffusion parameter ($D = 1.5$) the system essentially behaves as if it was well-mixed.

5 What does it mean?

The point of these calculations was to reproduce the non-mixed growth curve when using the well-mixed data. This was not the case, and fails worst when bacterial density is higher, not capturing the much slower growth in this regime observed in experiments. Ideas / possile problems:

- Our assumption that $g(n) \equiv g(1-s)$ may be an issue. Bartek performs an experiment to extract the growth curve $n(t)$. He then calculates from this, assuming $n \propto \text{OD}$, the functional form of $g(n)$ – that is, the growth rate depending on the density of bacteria. I then use this form in my simulations: I calculate the local density of substrate molecules s around a bacterium, and substitute $(1-s)$ for n in $g(n)$. So maybe it's not a surprise that the curves look very similar: since it seems that for realistic diffusion parameters we have behaviour close to the well-mixed case Fig. 6, perhaps the problem is the use of $(1-s)$. Plotting the form of $g(s)$, Fig. 7, clearly looks nothing like a standard substrate-dependence, such as a Monod function. Maybe this is just not a sensible thing to have done.
- Perhaps something is going on experimentally? That is, maybe there is something inconsistent between the well-mixed and non-mixed growth experiments? Perhaps the non-mixed case had more oxygen (since they all reached higher ODs) and as discussed with Bartek, this could lead to growth on the surface dominating until oxygen has been depleted, leading to 2 phases of growth. See Fig. 8
- I realise I have 5 non-mixed experimental growth curves but only 1 well-mixed curve. Are the well-mixed curves quite reproducible? Did they all stop at optical density ~ 1.5?

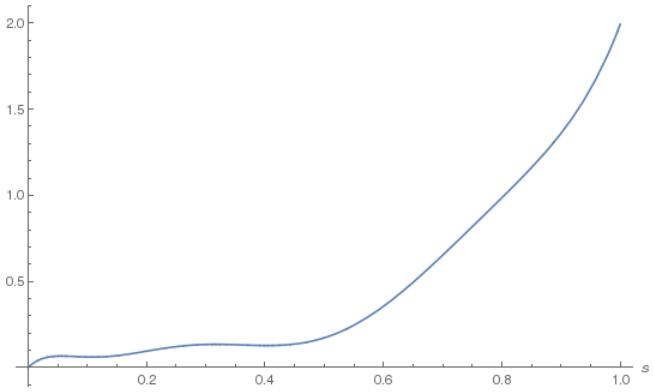


Figure 7: $g(s)$ vs s is clearly very different from the hyperbolic Monod function often assumed.

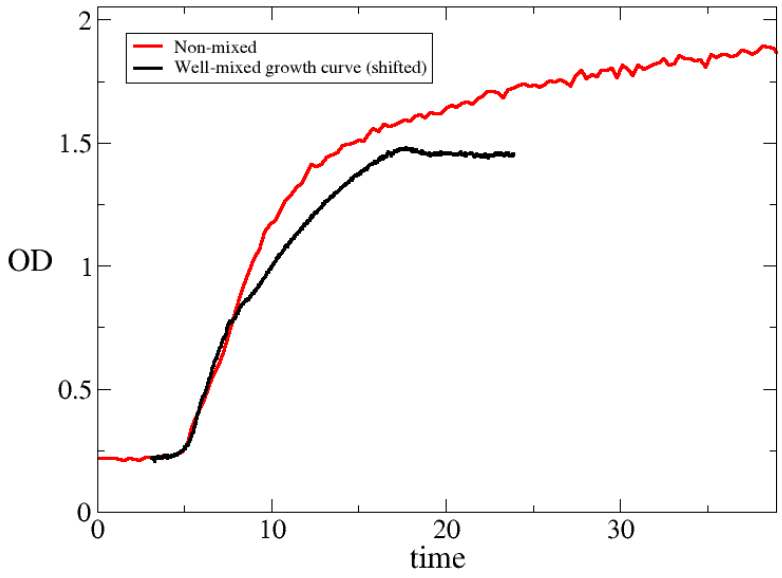


Figure 8: Consider these 2 "raw" growth curves, shifted by eye to overlap. Assuming that the same amount of LB nutrient was added to each, then what does the different final optical density mean? Let's assume it means there is more oxygen in the non-mixed (red) case: then from talking with Bartek, in the non-mixed case bacteria tend to accummulate at the top of the well, where they can access oxygen and grow faster. As the oxygen is depleted we would expect to see a gradually decreasing growth rate. In addition, as the thickness of the bacterial layer builds, the bacteria lower down will access less oxygen and so will grow slower. This might explain the smoothness of the non-mixed curve, potentially masking a "diauxic kink". In contrast, in the well-mixed curve (black) where we are assuming that there was less oxygen to begin with, we see the same initial increase (or maybe slightly faster since more bacteria on average can benefit from the oxygen), until the oxygen is depleted and we see a sharper transition to anaerobic growth. I.e. the "kink" in the LB curve is due to an aerobic-anaerobic transition rather than due to the different nutrients in the broth being utilized in a specific order? Can we be sure there was no oxygen the LB curve / that there was more in the non-mixed experiments?

6 TO DO

- Try simulations again but for much reduced diffusion, just to see what happens.
- Is it possible to agitate the wells in a travelling wave experiment, or would this just lead to spilling between wells?