MicroChat: Elements of critical thinking

Bottlenecks and Rate limiting parameters

(*Kenneth Timmis*)

Jess is the boy in the centre of the image whereas Kate is on the right
(created by Fran Jebok; https://www.theabsurdmicrobe.com/recurring-characters/)

Jess: Farmer Giles, what are you putting on the fields today?

Farmer Giles: My boy, I am adding fertilizer, so that the wheat will grow well and you will have freshly-baked bread for your breakfast.

Jess: What does fertiliser do and why do plants grow better with it?

Farmer Giles: Well, a plant needs nitrogen to grow – its cells are full of proteins and other bits and pieces that contain a lot of nitrogen and that carry out the things that cells need to do – metabolism and stuff – and grow and produce more cells. Soil does not generally contain enough nitrogen to satisfy a plant's needs, so it cannot grow as well as it would like.

And, more importantly, as well as I would like.

A bottleneck or rate limiting parameter is something that restricts a process, no matter whatever else you do to speed it up. Like when your mum drives you to school when you are late because you slept in: you can both be frantic and want to go faster, her car can be as powerful as anything, she can put the very best quality petrol in the tank, and the road sign can say that the speed limit

is 50 miles per hour, but you cannot move faster than the slow moving garbage truck in front of you: the garbage truck is at that moment the rate limiting parameter for your school run. The only way you can get to school on time is if the truck goes down a side street, gets out of your way, and is no longer your rate limiting parameter.

Nitrogen availability is a *rate-limiting parameter* for plant growth. So I add more nitrogen to the soil with fertilizer and this allows plants to grow more and faster. Which is good because the more people there are on the planet, the more food we farmers have to produce to feed them, so we need our crop plants to provide the best yields possible.

Jess: But my sister Kate also adds fertiliser to her pot plant but it hardly grows at all and in fact is looking rather sick right now. How can that be?

Farmer Giles: Of course, there can be several different reasons why a plant does not grow, and a fertiliser is just one solution to one problem, but not others. For example, plants also need water because the plant cell, like the cells of our own bodies, consists mostly of water and, if there is not enough, the plant cannot produce new cells and so cannot grow. Thus, water availability can be a rate limiting parameter and we can appreciate this every summer when we see water sprinklers watering the crops on the farms: here, water not nitrogen is the key issue, so we need to supply more water in order to get maximum growth. In fact, poor water availability is a major global rate-limiting parameter for food production and security.

But Kate's plant may be experiencing other problems and I'd have to look at it before deciding why it is not doing well. Would you like me to come over for a cup of tea and a slice of your mum's delicious fruit cake in order to advise Kate on the best course of action?

Jess: Oh, yes please!

But our teacher told us that the fertiliser farmers put on the fields also causes our lakes to go green with algae which are sometimes toxic and use up all the oxygen, which then kills the fish and other organisms living there. Is this true?

Farmer Giles: Yes, your teacher is perfectly correct. Some of the fertiliser we add for the benefit of the plants gets washed out of the soil into our rivers and lakes when it rains. The thing is: nitrogen is also a rate limiting parameter for the growth of microalgae, especially cyanobacteria, that live in rivers, lakes, ponds and ditches, so when the concentration of nitrogen in water bodies increases, the microalgae can grow much more and produce so-called *blooms*.

The problem with fertiliser-caused blooms, so-called *eutrophication*, is that the normal ecological checks and balances of the natural food web are disturbed, the microalgae are not eaten as fast as they should be because the predators that normally feed on them are too few (the predators of the cyanos become the rate limiting parameter in the normal food web), so they grow almost out of control, exhausting all the nutritional resources of their environment. And then they die, in vast quantities. And when they die, other microbes degrade them, using up all the oxygen and creating oxygen minimum zones which can no longer support oxygen-requiring organisms like fish. Oxygen becomes the rate-limiting parameter for such organisms.

Yes indeed: feeding the growing world population comes at a significant ecological cost.

Jess: But that is terrible! What can be done?

Farmer Giles: Well, on one hand, we have to feed the world, and this means increasing the level of the rate limiting parameter nitrogen in soil. On the other hand, we must reduce the use of chemical fertilisers that are mobile in water and get into local water bodies. So what we are often doing is to use biological fertilisers.

Certain types of plant, such as the legumes that include plants which produce peas and beans, and clover of course, naturally add nitrogen to the soil by taking it from the atmosphere – air is 80% nitrogen. In fact, it is not the plants themselves but the microbes that grow around and within the plant roots, usually in so-called root nodules. These microbes are called symbionts because they form partnerships with plants: they supply the plant with nitrogen – its growth rate-limiting parameter – and the plant feeds the microbes with sugar, which is the growth rate-limiting parameter of the microbes. But the symbionts not only feed the plant with nitrogen but also accumulate it in root nodules in which they live. When we harvest the crop, the root nodules are left behind and slowly rot, slowly releasing their nitrogen to feed the next crop of plants. Importantly, because this nitrogen is, unlike chemical fertilisers, not mobile, it stays in the soil and is not washed by rain into ditches.

This is why we alternate crops needing added nitrogen with crops that add nitrogen to the soil, which significantly reduces the amount of chemical nitrogen we need to add. In fact, some farmers have stopped adding chemical nitrogen to their fields altogether.

Jess: Well, that is great: microbes to the rescue!

Farmer Giles: Indeed! In fact, microbes help us in many ways. For example, they also help plants obtain phosphorus from soil, another chemical that plants need and that can become rate limiting if there is enough nitrogen. And they degrade many environmental pollutants.

Jess: But I thought environmental pollutants were poisonous! Why would microbes eat them if they are poisonous?

Farmer Giles: Well, microbes are always hungry and looking for things to eat. Which is good for us because they eat our wastes and prevent them from accumulating. And they are very creative, and have evolved not only ways and means of eating many different pollutants but also of resisting their toxic activities.

Jess: But surely, if this were the case, we would not have environmental pollution?

Farmer Giles: Ah, you are of course correct, and this brings us back to the issue of rate-limiting parameters, because pollutants have a way of ending up in situations that do not favour their rapid biodegradation. Imagine: if we release a pollutant into the environment at a rate which is faster than the rate at which microbes can degrade it, it will accumulate. And some pollutants are difficult to degrade, so may persist in the environment for a very long time, such as in so-called *superfund sites*. Rate limiting parameters of biodegradation include insufficient oxygen, for those microbes using oxygen for degradation, nitrogen or water, as we have already discussed, and for some really difficult pollutants that cannot be degraded in a way that provides the microbes with nutrition, an additional source of food which is called a co-substrate.

If pollutants persist in the environment, that is, the natural activities of microbes are insufficient to remove them, or remove them fast enough to avoid significant ecological damage, we need to get involved by helping the microbes. This sector of biotechnology, the use of microbes to get rid of pollutants in the environment, is called *bioremediation*. And the trick in bioremediation is to identify the biodegradation rate-limiting parameter in order to increase its level and so increase biodegradation activity. This is called *biostimulation* and the job of clever engineers who design and create processes to increase the level of the rate-limiting parameter.

Jess: Wow: rate limiting parameters are really important!

Farmer Giles: Absolutely! Occasionally, all the usual necessary components needed for degradation are present in adequate amounts but it still does not proceed fast enough. In such cases, it may be that the microbes with the degradation ability are too few or indeed not present at all. In these cases, the microbe itself is the rate-limiting parameter, so the microbiologist needs to grow large quantities of these special microbes in a fermenter and then transport and add them to the site. This is called *bioaugmentation*.

However, these microbiologists-engineers need also to be microbial ecologists, and make sure that the natural predator:prey relationships are maintained: addition of too many microbes just attracts predators which not only eat up all the added microbes but also any of those that were there before the addition, which produces a situation worse than before. Microbes are typically present in polluted sites at concentrations of 1 million to 1 billion per cubic centimeter (cc). Biodegraders dealing perfectly well with pollutants are typically present at concentrations of 10,000 to 100,000/cc, so only represent 10%, but usually much less than 0.1% of the total population. Adding more than 10,000-100,000/cc is not only wasteful but mostly counterproductive, because the predators sweep them up.

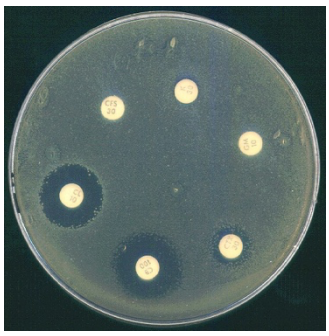
Jess: How do we know all this?

Farmer Giles: well, in the past one or two scientists have made the mistake of ignoring ecological common sense and simply threw degraders into soil without first determining whether or not they were needed, i.e. without considering the principle of rate limitation. Scientists call this bad experimental design. Unsurprisingly, addition of these bugs had no benefit. Surprisingly, the scientists came to the incorrect conclusion that addition of bugs cannot help bioremediation. But most microbiologists have ecological common sense and design experiments properly, and some showed that addition of the right bugs when needed is highly beneficial, in some cases essential.

Jess: Well: we really do need to stay as close to nature as possible!

Farmer Giles: Absolutely!

But a rate-limiting parameter is not always something that is too little, it can also be something that is too much. Remember the garbage truck example I just gave you: it was not the absence of the truck, but its presence that slowed you down. When nutrients or oxygen are plentiful in contaminated soil, and the microbes still don't degrade the pollutant very much, it might be that it is too poisonous and inhibits microbial growth. In this case, the rate-limiting factor is an inhibitor whose concentration needs to fall in order not to be rate-limiting any more. We can readily see this on an agar plate spread all over with microbes and having a disk on the surface containing an antibiotic: the microbes grow everywhere on the plate except around the disk where the antibiotic concentrations limit growth. In fact, this test is the one used in all hospitals and clinical microbiology labs the world over to determine the antibiotic resistance spectrum of pathogenic microbes in samples taken from patients, in order to discover which antibiotic may be prescribed by the doctor to treat an infection.



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Jess: So: rate limiting parameters control all aspects of life?

Farmer Giles: Well, my boy: that is a very profound conclusion and in many ways true. I see that my chats with you are paying off – you are a fast learner.

But, like us, microbes are also very clever and try to control the conditions of their environment, just as we try to stay warm or cool by adding or removing warm clothing, a process we call self-regulating. Microbes rarely accept a rate-limiting parameter and try where possible to swim towards the source of something that promotes their growth or away from something that inhibits their growth. To do this, they have systems that detect concentration-differences of the substance as they swim around and use this information to decide the direction they need to swim: this is the process of taxis: movement towards or away a chemical (chemotaxis), oxygen (aerotaxis), and so forth. This is of relevance to a farmer because soil microbes swim to plant

roots which can supply them rate-limiting foods. In return, root microbes can supply the plant with rate limiting nutrients, like nitrogen and phosphorus (and also help fend off pathogens). Taxis and self-regulation lie at the heart of some important microbial activities but, in order to understand this properly, we'll have to have another chat sometime about gradients and things.

Jess: Gosh: you do know a lot of microbiology stuff!

Farmer Giles: Yes: all farmers need to know their microbiology because microbes influence so many aspects of farming, especially how well plants and animals grow, and how fertile the soil is. We are the stewards of the land, just as you may be one day, and to do our job properly, we need to understand what the invisible life is doing as well as the visible life.

So, young Jess: as you go through life, and want to increase the rate of some activity or other, be it your running speed (you need to train harder to build up your leg muscles) or speed of completing your homework (you need to focus more by reducing the distraction of social media), always ask the question: what is the relevant bottleneck/rate-limiting parameter (some people also call this the pinch point)? This will identify what you need to change in order to achieve your goal.

Now: let's go round to your house and see if your mum has been baking: her rate limiting parameter is probably that there are not enough hours in the day!

Class-/homework for individuals/groups/the class: think about and discuss examples of different situations in which rate limiting parameters are obviously important, identify them and discuss how to reduce/circumvent/remove them.
Some examples:

- what is the rate limiting parameter hindering your favourite football/baseball/hockey team from reaching the top of its league?
- What is the rate limiting parameter hindering you personally from doing better at mathematics/geography/etc?
- We all need to help with household chores, right? What is the rate limiting parameter hindering your contributing more help?