THE ROLE OF PLANTS AND BACTERIA IN PHYTOREMEDIATION - KINETIC ASPECTS

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Abstract: Phytoremediation is the common name for cleaning techniques for polluted soils, sediments, and wastewaters using plants. It has been shown repeatedly that several types of pollutants, e.g., petroleum products and solvents, are degraded faster in the presence of plants. A couple of processes are known to influence the elimination of pollutants, among them transpiration of water, oxygen transport, biological stimulation in the root zone and plant uptake of chemicals. However, it is frequently unclear whether the plants directly metabolise the pollutants, or whether they only play an indirect role by supporting microbial action.

The metabolism kinetics of plant enzymes is mathematically described by the *Michaelis-Menten* kinetics. This means, that at low substrate concentrations, the degradation is first order, whereas it is linear and therefore limited at high substrate concentrations. Bacteria use the substrate for growth, and grow better at higher substrate availability. This is described by the *Monod* kinetics. Therefore, bacteria have a limited degradation capacity at low substrate concentrations. This often prohibits the biodegradation of polluted sites down to required levels. The combination of plants with bacteria might be a successful method to overcome these short-comings.

Key words: Metabolism; Michaelis-Menten; Monod; kinetics; phytoremediation

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1. INTRODUCTION

Phytoremediation is an engineering technique for remediating polluted soils, but also wastewater and sediments, by use of plants. In principle, phytoremediation is a kind of "enhanced natural attenuation", because it uses the natural clean-up mechanisms of soil, which are supported by a vegetation cover. Plants contribute to the removal of soil pollutants by a number of processes. Aside from uptake of compounds with subsequent metabolism, plants frequently participate indirectly by changing the soil conditions so that soil microorganisms can degrade pollutants (Trapp & Karlson 2001). Phytoremediation is carried out by the "team" of higher plants, bacteria and fungi, and depends on several biological, physical and chemical processes. This article discusses the kinetic aspects of the roles of bacteria and plants.

2. BASIC MATHEMATICS

2.1 Plants

2.1.1 Ecology of Plants

An estimate of the global biomass is 1841×10^9 tons worldwide (excluding bacteria), hereof are 1837×10^9 tons on continents, and 99% phytomass, 0.9% is fungi, and about 0.1 % only is animal biomass. Of the 1837×10^9 tons terrestrial biomass, more than 92% are forests, and hereof, approximately 95% is wood (all data Sitte *et al.,* 1991).

Compared to other life forms, plants have the largest genomes, with some species exceeding 10^{11} base pairs (bacteria $<10^8$) (Voet *et al.,* 1998). This corresponds with the very complex secondary metabolism of plants. More than 80 000 secondary metabolites are known today, with many more to be identified (Richter, 1998). However, only limited knowledge on the degradation pathways and rates of xenobiotics is available. There are indications that enzymes targeting endogenous plant compounds also co-metabolize xenobiotics (Messner *et al.,* 2003).

Most plants are autotrophic organisms, which means that they can form complex carbon compounds from simple inorganic precursors (carbon dioxide, water) and with sunlight as energy source. Thus, plants do not rely on organic compounds as substrate for growth. Although some pollutants, e.g., cyanide, can be used by the metabolism of plant cells (Larsen *et al.,*

2004), most xenobiotics may be expected to be either inert to the plant cell metabolism or have a negative effect.

The metabolism of xenobiotics, which may occur in plant cells, mainly targets detoxification. For the detoxification of xenobiotics, such as herbicides, cytochrome P-450 monooxygenases and glutathione-S-transferases (GST) seem to be the most important enzyme types (Pflugmacher and Schröder, 1995). P-450 enzymes catalyze phase I transformation reactions, frequently hydroxylation, but also sulfoxidation, and *N*-and *O*-dealkylation. GST are responsible for phase II conjugation reactions, which play a central role in detoxification of herbicides in plants. Unlike animals, plants cannot excrete conjugates formed via urine. Instead, phase III of plant xenobiotic metabolism involves storage and compartmentation of soluble conjugates in the vacuole and of insoluble conjugates in the cell wall (Komossa *et al.,* 1995). This may lead to so-called "bound residues".

2.1.2 Growth and Metabolism Kinetics of Plants

The growth velocity of plants depends on factors such as the availability of the resources sunlight, nutrients, and water and on environmental constraints (e.g., temperature). Growth rates of plants are between 0.2 d^{-1} (doubling time 3 days, e.g., maize under favorable conditions) and 0 (ecosystems in equilibrium conditions). A meadow in Central Europe has growth rates of about 0.035 d^{-1} (doubling time of about three weeks). However, these growth rates are related to the exponential growth phase of plants. Towards ripening, the growth of plants stops, and the plant mass may even be decreasing, due to wilting.

The growth of (annual) plants is usually described by a sigmoid curve of the general form

$$M_P = \frac{K}{1 + b \times e^{-rt}}$$

where M_P is the mass of the plant, K is the maximal plant mass, b and r are kinetic parameters and t is time.

Plants do not use xenobiotics as growth substrate, their growth velocity is in most either cases not affected or slowed down by exposure to xenobiotics. Figure 1 shows an illustrative example of plant growth - and of a reduced growth, due to toxic impact of the chemical.

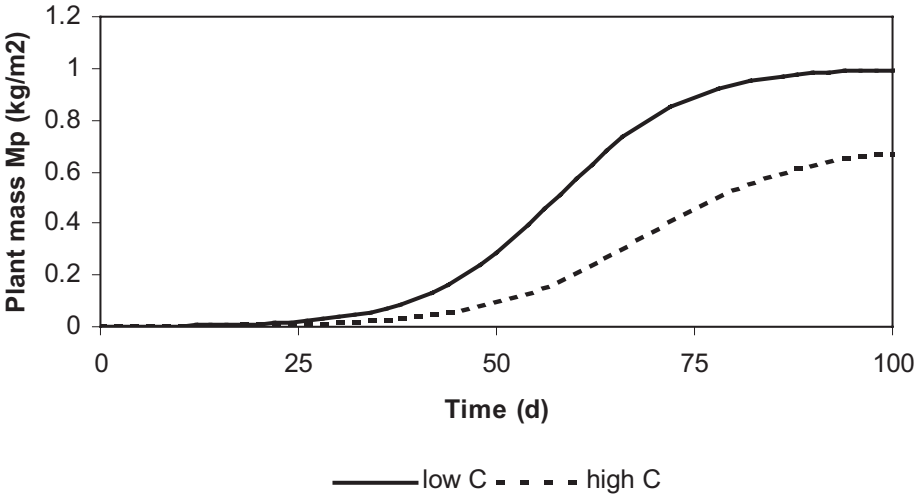


Figure 1. Plant mass vs. time at different xenobiotics concentrations in soil.

The metabolism kinetics of enzymatic reactions can be described by the *Michaelis-Menten* kinetics (Cornish-Bowden 1995):

Equation 1:

$$v = \frac{v_{\max} \times C}{K_M + C}$$

where v [mg (kg plant)$^{-1}$ d^{-1}] is the removal rate per plant mass of the substrate concentration C (mg/L), v_{max} is the maximal removal velocity and K_M (mg/L) is the half-saturation constant.

The overall removal velocity of xenobiotics by plants therefore depends on

– the mass of plant
– the velocity of uptake of the xenobiotic
– the enzymatic reaction rate

The plant mass has an upper limit, K, and the velocity of the enzymatic reaction, too (v_{max}). If follows that there will always be an upper limit for xenobiotics' degradation by plants, which is

Equation 2:

$$\frac{dm_{\max}}{dt} = -\frac{v_{\max} \times C}{K_M + C} \times K$$

where K is the maximum plant mass.

If C >> K_M, this reduces to the constant rate

$$\frac{dm_{\max}}{dt} = -v_{\max} \times K$$

2.2 Bacteria

2.2.1 Ecology of Bacteria

Most bacteria are heterotrophic organisms, that means, they need an organic substrate to feed on. This substrate can be xenobiotics, which are then used as nutrient source by degrader bacteria.

Bacteria have developed a wide range of enzymes that can chemically alter xenobiotics. Xenobiotics can hereby be used as electron acceptor, electron donator, as energy source or as precursor for other molecules (Schlegel 1993).

2.2.2 Growth and Metabolism Kinetics of Bacteria

The growth of bacteria depends on the availability of substrate. The bacterial growth or decay is described by the *Monod* kinetics plus a decay term:

Equation 3:

$$\frac{dB}{dt} = \frac{\mu_{\max} \times C \times B}{K_S + C} - k_{death} \times B$$

where B is the bacterial mass (kg), μ_{max} is the maximal growth rate of the bacteria, C is the substrate concentration (mg/L), K_S is the half-growth concentration (i.e., the concentration where the growth is half of the maximum) and k_{death} is a first order rate describing the death of bacterial cells by arbitrary events, e.g., by grazing protozoa. The growth curve for bacteria may be negative (i.e., the number of bacteria declines) when the death rate is higher than the growth rate. Because the growth depends on

the substrate concentration, but not the death, the number of degrader bacteria will increase at high substrate concentrations, but decline when the substrate is no more available. High substrate concentrations may also have a inhibition effects (Edwards 1970), which is not considered here.

During growth, the bacteria metabolize the substrate. The kinetics of the enzymatic reaction can again be described by the *Michaelis-Menten* kinetics.

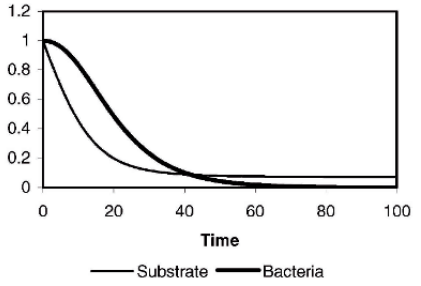
The mass balance equation for the substrate mass *m* (mg) is then

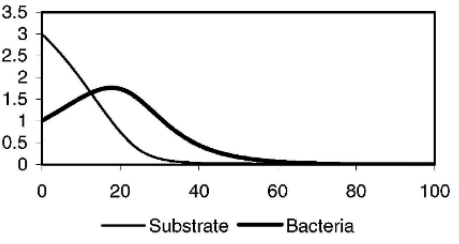
Equation 4:

$$\frac{dm}{dt} = -\frac{v_{\max} \times C}{K_M + C} \times B$$

where v_{max} has the unit mg (kg bacteria)$^{-1}$ d^{-1}. As for plants, this enzymatic reaction velocity has an upper limit. However, the loss of mass has no upper limit, because the number of bacteria increases, as long as substrate is available (and some other resources, such as nutrients etc.). Therefore, the reaction velocity of the bacterial degradation has (mathematically) no upper limit. In reality, there might be a inhibitation of the bacterial growth at higher xenobiotics' concentrations.

However, from an inspection of the bacterial growth equation it can be seen that the degradation by bacteria has a *lower* limit: If the substrate concentration is from the beginning too low to allow a growth of degrader bacteria, the number of bacteria will decline, and thus also the bacterial degrader capacity.





Figures 2 and 3. Bacterial population *B* and substrate concentration *C* at low (left) and high (right) initial *C*.

Therefore, higher xenobiotics pollution might be degraded faster and more complete, than low contamination. This is illustrated in the Figures 2 and 3, which show solutions of Equation 3 and 4 for a start concentration C = 1 mg/L and C = 3 mg/L. All other parameters remained unchanged. As can be seen, at C = 1 mg/L, the bacterial population decreases from the start. After 60 days, the population is erased, but a rest C of about 0.1 mg/L is still present. When starting at C = 3 mg/L, the bacterial population grows, and the contamination is completely degraded - then the bacteria die, too.

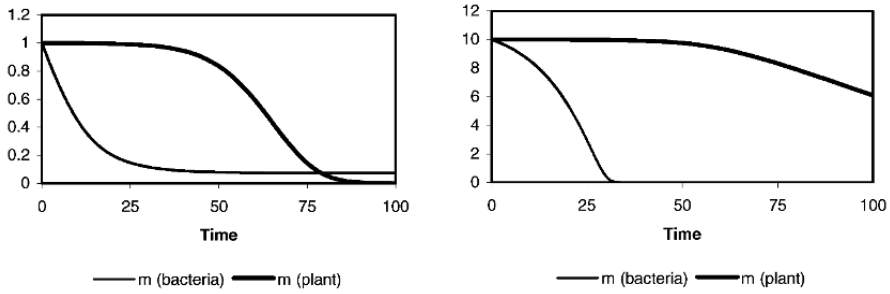
2.3 Comparison of Mass Balances

It is an interesting exercise to compare the degradation of a xenobiotic, which can be metabolized by both bacteria and plants. The next two Figures show the mass (or the concentration) remaining in soil for a low starting concentration (as before), and for a high starting concentration. The *Michaelis-Menten* parameters for plant and bacteria are the same (v_{max} 0.1 mg per kg and per d and K_M was 0.5 mg/L). The growth of plants for low C is taken from Figure 1, for bacteria from Figure 2.

For a low start concentration (C = 1 mg/L), Figure 4, bacteria initially degrade a fraction of the pollution, before the degradation stops (bacteria have starved). For bacteria, this is the same case as depicted in Figure 2. Initial degradation by plants is very low, because the plant mass is very low (see Figure 1). With time, when the plants have established most of their final size, and the absolute amount of chemical removal is peaking (after 64 days, dm/dt = -0.03 mg/d). From then on, the degradation is rapid, and after 100 days, the pollution has been degraded completely.

The picture changes completely for a higher initial concentration (C = 10 mg/L), Figure 5. The simulated bacteria can well grow at this pollution level, and soon a bacterial population is established, which degrades the pollutant completely. However, plants are less effective. Even when the vegetation cover is established, the degradation is too slow, compared to the level of pollution: the maximum degradation is dm/dt = - v_{max} x plant mass = - 0.1 mg (kg plant)$^{-1}$ d^{-1} x 1 kg plant = - 0.1 mg/d. Therefore, after 100 days, more than 50% of the pollution is still present.

If the pollution level was even higher, e.g., initial C = 100 mg/L, bacteria would need about 50 days for a complete degradation (all other parameters constant). Plants would probably die from toxic effects, but even if not, their contribution to degradation would be absolutely negligible.



Figures 4 and 5. Substrate concentration *C* at low (1 mg/L, left) and at high initial *C* (10 mg/L); degradation either by plant or by bacteria.

3. CONCLUSIONS

Even though the simulations made above were not based on real data, some conclusions can be made:

– It is not only the presence of degrader pathways in plants or bacteria, that decides about the role the organisms play in phytoremediation. Kinetic aspects need to be considered, too.

– Even if plants are able to detoxify a xenobiotic substrate, plants always have an *upper limit* for their detoxification capacity.

– Bacteria, which depend on the availability of substrate for their growth, have a *lower limit* for their degradation capacity. Below this limit, a growth on that substrate is no more possible.

– Plants are not suited to treat "hot spots" of pollution: First, because toxic effects are to be expected; second, because their metabolism is limited and slow at high pollution levels.

– Bacteria are well-suited to treat "hot spots". However, at low substrate concentrations, e.g., pesticides in the nanogram/L level in groundwater, bacteria may fail to degrade to "null"-levels (Toräng *et al.,* 2003).

– Plants might be favorable for low contamination levels (e.g., after initial clean-up of a site, as final polishing step), because their metabolic capacity does not decrease with the pollution level.

– A combination of bacteria and plants might be most useful - e.g., in form of the ENDEGRADE concept (Barac *et al.,* 2004).

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