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## Biodegradation: Updating the Concepts of Control for Microbial Cleanup in Contaminated Aquifers

**Meckenstock, Rainer U.; Elsner, Martin; Griebler, Christian; Lueders, Tillmann; Stumpp, Christine; Aamand, Jens; Agathos, Spiros N.; Albrechtsen, Hans-Jørgen; Bastiaens, Leen; Bjerg, Poul Løgstrup; Boon, Nico; Dejonghe, Winnie; Huang, Wei E.; Schmidt, Susanne I.; Smolders, Erik; Sørensen, Sebastian R.; Springael, Dirk; van Breukelen, Boris M.**

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## Feature

## Biodegradation: Updating the concepts of control for microbial clean-up in contaminated aquifers

Rainer Udo Meckenstock, Martin Elsner, Christian Griebler, Tillmann Lueders, Christine Stumpp, Winnie Dejonghe, Leen Lucia Bastiaens, Dirk Springael, Erik Smolders, Nico Boon, Spiros N. Agathos, Sebastian R. Sorensen, Jens Aamand, Hans Jorgen Albrechtsen, Poul Logstrup Bjerg, Susanne Schmidt, Wei E. Huang, and Boris Maurijn Van Breukelen

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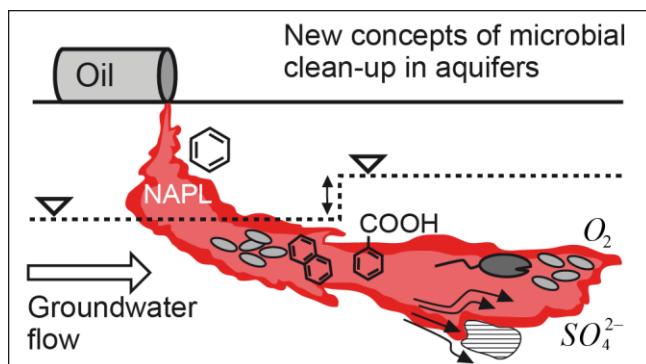
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5 Abstract

6 Biodegradation is one of the most favored and sustainable means of removing organic pollutants  
7 from contaminated aquifers but the major steering factors are still surprisingly poorly understood.  
8 Growing evidence questions some of the established concepts for control of biodegradation. Here,  
9 we critically discuss classical concepts such as the thermodynamic redox zonation, or the use of  
10 steady state transport scenarios for assessing biodegradation rates. Furthermore, we discuss if  
11 absence of specific degrader populations can explain poor biodegradation. We propose updated  
12 perspectives on the controls of biodegradation in contaminant plumes. These include the plume  
13 fringe concept, transport limitations, and transient conditions as currently underestimated processes  
14 affecting biodegradation.

15

16 Abstract Art



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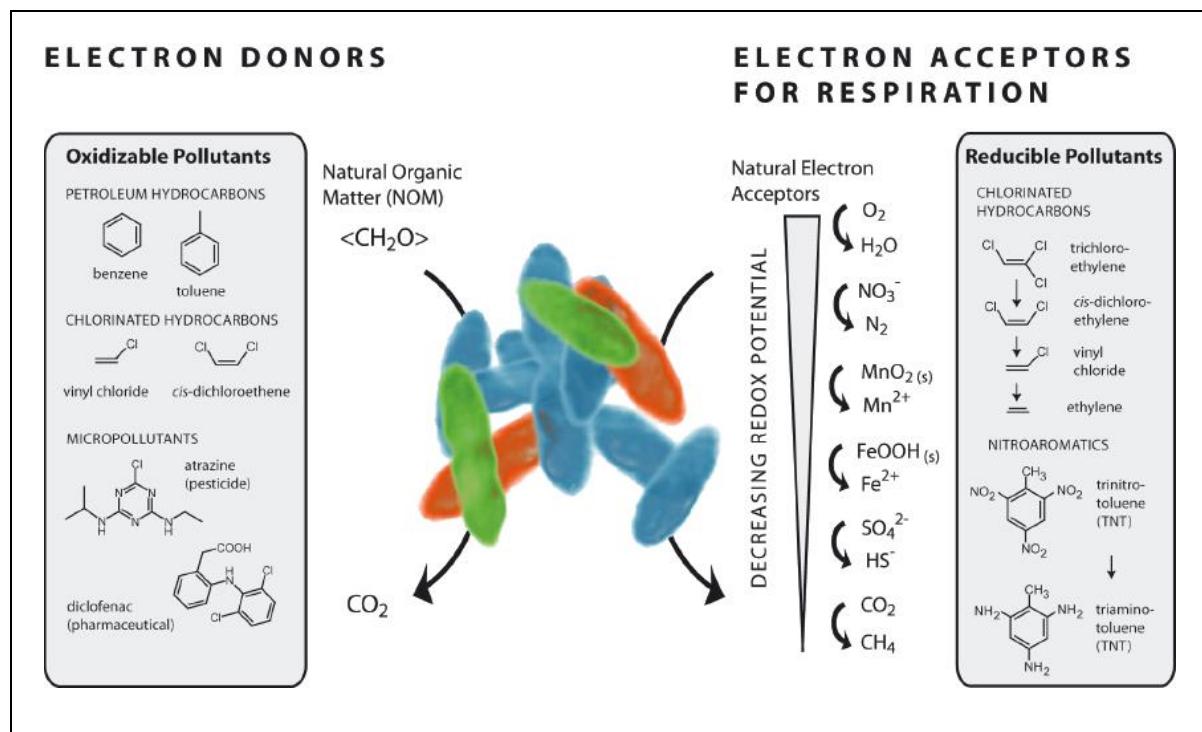
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## 19 1. Introduction

20 Over the last 150 years, the number of organic chemicals released into the environment has  
21 increased dramatically<sup>1</sup>, leaving an unprecedented chemical footprint on earth. Many groundwater  
22 contaminations result from point sources, originating from accidents or contaminations at industrial

23 sites. These contaminations typically form plumes with high concentrations of pollutants ( $\mu\text{g/L}$  to  
 24 mg/L range). Alternatively, chemicals may enter groundwater via widespread application in  
 25 agriculture or release from sewage treatment into rivers. Here, pesticides, pharmaceuticals, or  
 26 consumer care products are introduced as non-point sources and typically occur in much smaller  
 27 concentrations (micropollutants in ng/L to  $\mu\text{g/L}$  range)<sup>2</sup>.

28 For what seems at first sight a daunting perspective, nature fortunately has a remedy in place:  
 29 biodegradation. Microorganisms can oxidize organic pollutants to  $\text{CO}_2$  while reducing electron  
 30 acceptors such as molecular oxygen, nitrate, Fe(III) (and other metal oxides), or sulfate (Fig. 1).  
 31 Alternatively, some pollutants such as chlorinated solvents may serve as electron acceptors (Fig. 1,  
 32 right side).



33 **Fig. 1.** Contaminants can serve as electron donors or acceptors for aquifer micro-organisms.

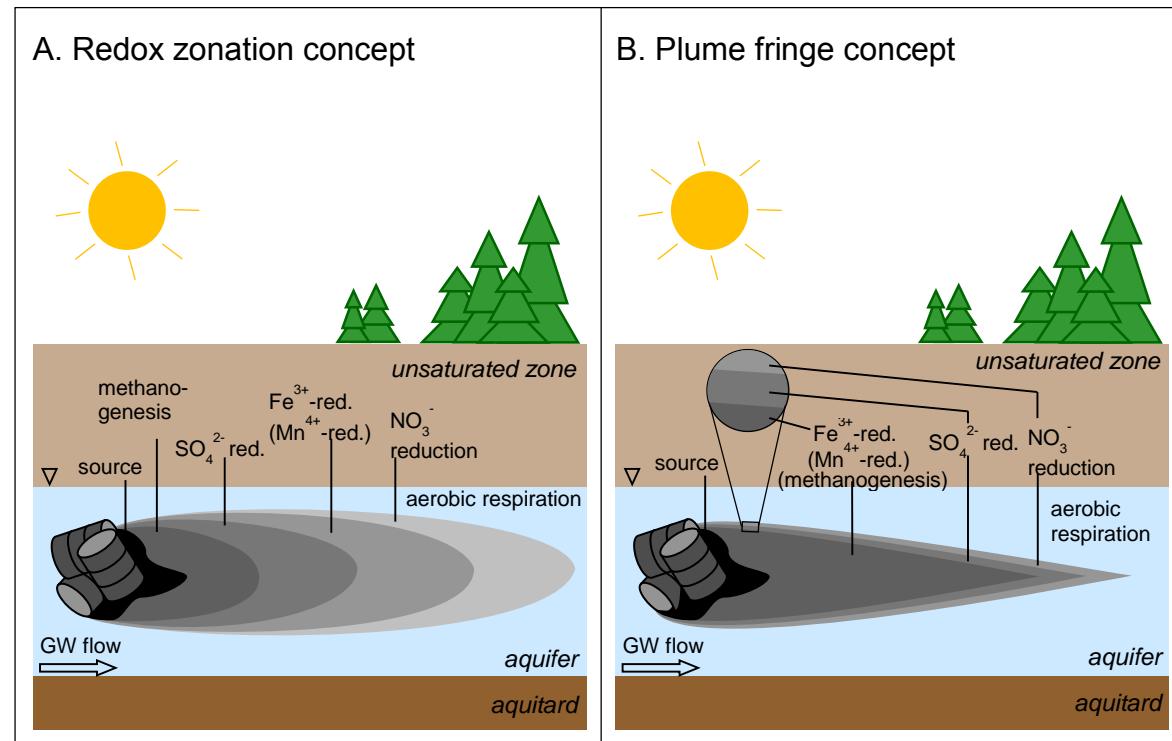
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35 However, despite decades of biodegradation research, the true drivers governing contaminant  
 36 degradation are still poorly understood. This article revisits and challenges current concepts on the  
 37 controls and limitations of biodegradation in aquifers. It critically discusses (i) whether  
 38 biodegradation is primarily governed by thermodynamics (i.e., redox zonation) at contaminated  
 39 sites, (ii) if biodegradation can be adequately predicted by considering the subsurface as one  
 40 reactive compartment and applying terms of environmental engineering (residence time, reaction

time), and (iii) the biological controls of biodegradation. We argue that groundwater ecosystems are much more heterogeneous and dynamic than currently perceived. Furthermore, we suggest that kinetic controls of biodegradation have been largely overlooked. Current concepts rely to a large part on thermodynamic considerations and steady state assumptions, while processes are frequently dynamic. In many cases, the steering parameters were not considered at appropriate spatial and temporal scales. However, the crucial controls of biodegradation discussed below provide potential for changing the future design of scientific projects, monitoring campaigns, or remediation strategies.

## 49 2. Revisiting redox zonation in contaminated aquifers

50 In highly polluted aquifers (e.g. petroleum spills with hydrocarbon concentrations of up to 100 mg/L), an excess of dissolved electron donors (hydrocarbons) prevails over acceptors (Fig. 1). In such 51 contaminant plumes, electron acceptors are readily depleted which is widely accepted as a major 52 limitation of biodegradation<sup>3,4</sup>. A longitudinal sequence of redox processes is assumed: 53 methanogenic degradation close to the contaminant source, followed by sulfate reduction, 54 manganese(IV) and iron(III)-oxide reduction, nitrate reduction, and finally aerobic processes towards 55 the distal end of the plume<sup>3-7</sup> (Fig. 2 A). However, this redox zonation concept is challenged in the 56 following.



68 **Fig. 2.** Comparison of the longitudinal redox zonation concept (A) and the plume fringe concept (B),  
69 both describing the spatial distribution of electron acceptors and respiration processes in a  
70 hydrocarbon contaminant plume. (B) Iron(III) reduction, manganese(IV) reduction, and  
71 methanogenesis may occur simultaneously in the core of the contaminant plume.

72

73 Dissolved electron acceptors depleted at the source zone cannot be readily replenished in the  
74 downstream plume due to laminar flow and the limited transversal dispersion in porous media (Fig.  
75 2 B). Accordingly, methanogenic degradation or reduction of insoluble iron(III) and manganese (IV)  
76 phases would be the only electron-accepting processes possible in the source zone or the  
77 downstream plume core. Recent field evidence supports this theoretical concept showing electron  
78 acceptor depletion in the plume center<sup>8,9</sup>. This is an evident contradiction to the classical concept of  
79 reverse longitudinal redox zonation (Fig. 2A). If dissolved electron acceptors such as sulfate or  
80 nitrate are consumed already at the source, they cannot become available again downstream  
81 allowing for sulfate or nitrate reduction. Even if not all electron acceptors are depleted during the  
82 passage through the source zone, a downstream redox succession should develop, where first  
83 nitrate and sulfate reduction take place, followed by methanogenesis, and not *vice versa*. Such  
84 spatial distributions have indeed been found along contaminant plumes when sampling was  
85 performed at appropriate resolution<sup>10,11</sup>.

### 86 **2.1. Is thermodynamics alone determining microbial competition in contaminant plumes?**

87 The theory behind every redox zonation model is that microorganisms reducing a  
88 thermodynamically more favorable electron acceptor can gain more energy (Fig. 1), e.g. nitrate- vs.  
89 sulfate-reducing bacteria<sup>12</sup>. In electron donor-limited systems such as aquifers with only little  
90 contamination, nitrate reducers should therefore be able to consume organic substrates to  
91 threshold concentrations no longer permissive for the activity of thermodynamically less favored  
92 respiratory guilds, which consequently become outcompeted<sup>13</sup>. However, in highly contaminated  
93 aquifers, electron donors are present in excess over the oxidation capacity of all electron acceptors  
94 and at concentrations much higher than where competition for electron donors (i.e., available  
95 organic substrate) can occur. Consequently, respiration processes should rather occur  
96 simultaneously as long as respective electron acceptors are present and do not become limiting for a  
97 certain respiratory guild. Biodegradation activity thus becomes controlled by availability of specific  
98 electron acceptors, rather than by thermodynamics. This concept is supported by studies on

99 electron acceptor-limited chemostats where axenic cultures express all respiratory pathways  
100 simultaneously rather than only the energetically most favorable<sup>14-17</sup>.  
  
101 We propose that the reason why more favored respiratory guilds may nevertheless appear in higher  
102 abundance is a kinetic advantage which is based on higher energy conservation. Conserving more  
103 energy leads to higher growth yields ( $Y$ ) and, therefore, growth rates ( $\mu$ ) (Equations 1-3; where  $X$  is  
104 the total biomass,  $X_0$  the initial biomass,  $S$  the substrate concentration, and  $t$  the time of  
105 observation).

106 
$$\mu = \frac{dX}{dt} \times \frac{1}{X_0} \quad (1)$$

107 
$$\frac{dX}{dt} = -Y \times \frac{dS}{dt} \quad (2)$$

108 
$$\mu = -Y \times \frac{dS}{dt} \times \frac{1}{X_0} \quad (3)$$

109 Thus, nitrate reducers can grow faster and to higher cell numbers in a given plume compartment  
110 suggesting an apparent out-competing of inferior respiratory guilds by thermodynamics. We  
111 propose that in many cases this will be controlled by the availability of electron acceptors and not by  
112 thermodynamic competition between respiratory guilds. Recently, Hansel et al. reported that  
113 microbial sulfate reduction was dominant over iron-reduction in sediments despite the lower  
114 thermodynamic energy gain<sup>18</sup>. The study exemplifies the importance of bioavailability rather than  
115 merely the thermodynamic redox potential of the electron acceptor.

116 **2.2. Importance of processes at plume fringes**

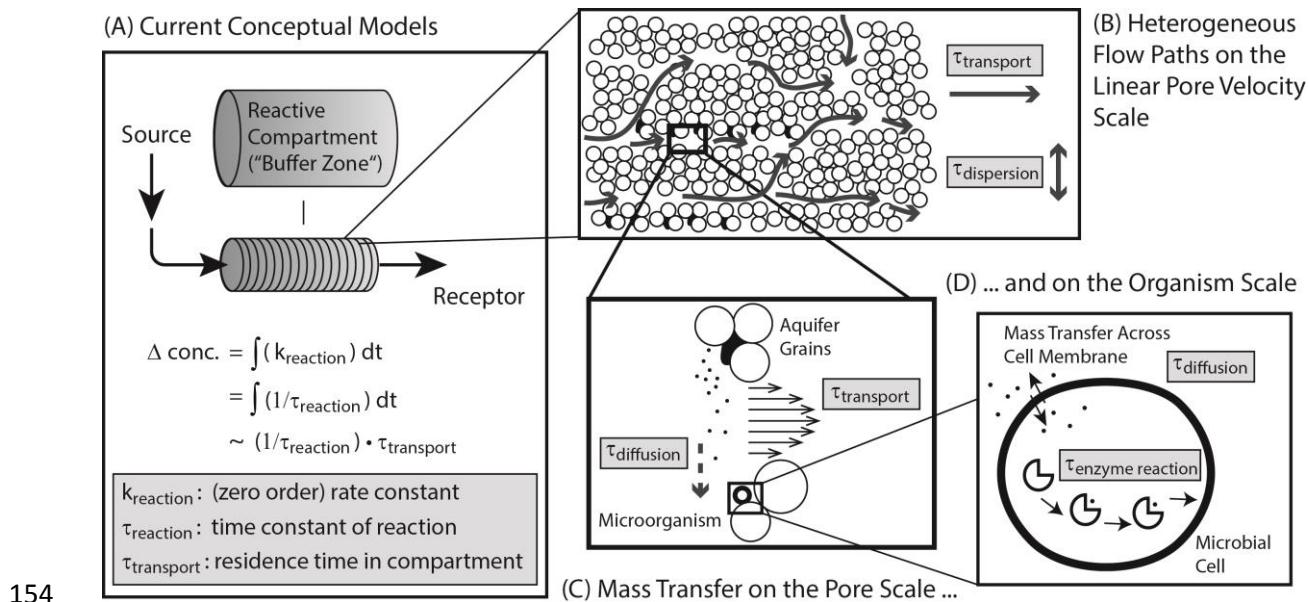
117 In recent years, it became apparent that biodegradation in contaminant plumes is much better  
118 explained by the ‘plume fringe concept’ than by the classical longitudinal redox zonation (Fig. 2)<sup>19</sup>.  
119 This concept is founded on the depletion of dissolved electron acceptors in the plume core.  
120 Biodegradation with oxygen, nitrate, or sulfate reduction can then only take place at the fringes of  
121 the plume, where electron acceptors are replenished from surrounding groundwater by dispersion  
122 and diffusion (“mixing in”) (Fig. 2 B)<sup>8,19-21</sup>. At an adequately high resolution of sampling, steep  
123 geochemical counter-gradients of electron donors and acceptors have indeed been observed in  
124 several contaminated aquifers thus verifying the ‘plume fringe concept’<sup>22-24</sup>. The concept also  
125 provides an appropriate explanation for the overall rather limited net biodegradation rates in  
126 hydrocarbon plumes: the small-scale dispersive mixing at plume fringes controls the mass transfer of  
127 electron acceptors and, thus, microbial activities. On the other hand, the concept predicts that the  
128 plume fringes are the true hot spots where biodegradation occurs *in situ*<sup>25,26</sup>.

129 The incomplete conceptual understanding of plume redox zonation brings about two fundamental  
130 caveats in many field investigations: (i) sampling at inappropriate scales and (ii) taking samples at the  
131 wrong spot<sup>27,28</sup>. Thus, many studies may actually have overlooked the most relevant processes and  
132 zones of biodegradation simply because groundwater sampling was done at meter rather than at  
133 centimeter resolution. While numerous studies reported on marked differences in overall microbial  
134 community assembly along plumes<sup>29-32</sup>, vertical heterogeneity of biodegradation activities has rarely  
135 been considered<sup>9,33,34</sup>. Consequently, appropriately high resolution monitoring and the limitations of  
136 dispersive mixing still await a better incorporation into conceptual models and study design. Future  
137 research should investigate the generic conditions affecting the processes at the plume fringes and  
138 the limitations of biodegradation.

139 **2.3. The plume core as a poorly understood compartment**

140 Even when all dissolved electron acceptors are depleted, methanogenesis and Fe(III)- or Mn(IV)-  
141 reduction may still drive biodegradation of hydrocarbons in the plume core (Fig. 2 B)<sup>35</sup>. Evidence for  
142 iron-reduction has indeed been reported for contaminated aquifers<sup>36-38</sup>. Very little studies, however,  
143 exist on methanogenic hydrocarbon degradation in laboratory incubations which is probably due to  
144 the extremely slow growth of such cultures<sup>39</sup>. Even less documented is methanogenic hydrocarbon  
145 degradation in aquifers<sup>10,11</sup>. Methanogenic hydrocarbon degradation may thus seem limited in  
146 spatial extent or relative contribution to the overall biodegradation<sup>40,41</sup>. An explanation might be  
147 provided by a study where agitation has been shown to impede methane production in soil slurries  
148<sup>42</sup>, most likely by disturbing close spatial interactions between syntrophic fermenters and  
149 methanogens. By analogy, groundwater flow may also interfere with efficient interspecies electron  
150 transfer in methanogenic plume zones by flushing away hydrogen or acetate and thus interfering  
151 with the energy fluxes needed for methanogenic activity<sup>43,44</sup>. Nevertheless, the true extent of  
152 methanogenic processes in contaminated aquifers requires further elucidation.

153 **3. Bottlenecks of degradation by mass transfer**



154

155 **Fig. 3.** Plug reactor or piston flow conceptual model of contaminated aquifers where contaminant  
 156 removal (time constant of reaction,  $\tau_{\text{reaction}}$ ) is inversely proportional to the average residence time  
 157  $\tau_{\text{transport}}$  (A). However, the distribution of flow paths on the linear pore velocity scale (B), mass  
 158 transfer on the pore scale (C), mass transfer through cell membranes on the organism scale, and  
 159 (bio)chemical enzymatic transformation on the molecular scale (D) can be important bottlenecks of  
 160 biodegradation not taken into account by the simplified model.

### 161 3.1. Average residence times ignore heterogeneous flow paths and flow velocities

162 Current conceptual models frequently treat natural sediments and aquifers like either completely  
 163 mixed or plug flow reactors (Fig. 3A)<sup>45,46</sup>. In these models adopted from chemical engineering, a  
 164 decrease in substrate concentration ( $\Delta \text{conc.}$ ) is proportional to the residence time ( $\tau_{\text{transport}}$ ) in the  
 165 reactive compartment (Fig. 3A). The relation of transport and degradation is frequently estimated  
 166 from the dimensionless Damköhler number Da

$$167 Da = \frac{\tau_{\text{transport}}}{\tau_{\text{reaction}}} = \frac{\text{reaction rate}}{\text{transport rate}} \quad (4)$$

168 where  $\tau_{\text{transport}}$  is the mean residence time ("how long does it take for a compound to pass the  
 169 compartment?") and  $\tau_{\text{reaction}}$  the time constant of the reaction ("how long does it take for the  
 170 compound to react?"). Note that the time constants are inversely correlated to the respective rates  
 171 (Fig. 3A and Equation 4). The larger Da, the more biodegradation can potentially take place. This  
 172 well-established concept might be a good proxy for identifying mass transfer as limiting factor in  
 173 steady state systems dominated by advection. However, it is challenged by the fact that  $\tau_{\text{transport}}$  and

174  $\tau_{\text{reaction}}$  are not well defined in natural systems because they depend on multiple parameters on  
175 different scales (Fig. 3). Black box approaches not considering multiple limitations will miss the  
176 opportunity for profound understanding of the steering parameters of such systems.

177 If transport is limiting on the pore velocity scale, increased flow velocities (decreasing residence  
178 times) may affect  $\tau_{\text{reaction}}$  and increase biodegradation. This counterintuitive observation can be  
179 explained when considering that increased flow velocities induce more heterogeneous flow due to a  
180 wider distribution of flow paths length. This results in higher transversal and longitudinal dispersion  
181 <sup>47,48</sup> and thus increases the apparent reaction rate by bringing reactants together. However, also the  
182 opposite can occur and contaminants may bypass reactive zones due to increased preferential flow.  
183 Furthermore, changes in flow velocities can create unfavorable growth conditions <sup>48</sup> due to shifts in  
184 nutrient fluxes and redox conditions (see section 4) adversely affecting degradation (longer  $\tau_{\text{reaction}}$ ).  
185 Concluding, the average residence time alone will not provide information on such ecological  
186 consequences. Rather, the distribution of residence times and the biogeochemical history along  
187 individual flow paths are governing mass transfer limitations in aquifers. This can be analyzed by  
188 high resolution monitoring which also allows for identifying dynamic flow fields and true solute  
189 fluxes <sup>49</sup>. However, there is a need to systematically investigate the influence of flow velocities and  
190 dynamic conditions on microbial degradation.

191 **3.2. Does diffusion limit bioavailability on the pore scale?**

192 In groundwater, most microorganisms are attached to sediments <sup>50</sup> where diffusion may become the  
193 dominant mode of substrate transport to cells at the pore scale <sup>51,52</sup> (Fig. 3C). If supply by diffusion is  
194 slow, biodegradation is availability-limited because microorganisms consume the substrate faster  
195 than it can be delivered <sup>53</sup>. The apparent  $\tau_{\text{reaction}}$  in a given environmental compartment is then  
196 determined by  $\tau_{\text{diffusion}}$  (Fig. 3C). Because diffusion to the cells takes place on the micrometer scale,  
197 steep diffusive gradients can create a situation in which much larger concentrations are observed in  
198 the surrounding water. Such limitations tend to be overlooked in conventional sampling. Whether or  
199 not such diffusion limitation is important, depends also on flow velocities because diffusion gets  
200 more important if water flow velocities are low <sup>54</sup>. This is exemplified in three scenarios regarding  
201 concentrations and the state of the system.

202 (i) At *high concentrations and steady state*, diffusive gradients between pore centers and sediment  
203 surfaces only build up if water flow velocities are low and if degradation rates are faster than the  
204 supply of contaminants. (ii) For *low concentrations and transient conditions* Langner *et al.* found that  
205 degradation rates of 2,4-dichlorphenoxyacetic acid were higher when water flow was slower, even

206 though the flow path was shorter<sup>55</sup>. This indicates that molecules needed sufficient time to diffuse  
207 into micropores and  $\tau_{\text{transport}}$  has to be sufficiently long compared to  $\tau_{\text{diffusion}}$ , since otherwise  
208 molecules were flushed through the pores without degradation. (iii) For *low to medium*  
209 *concentrations and at steady state*, one can expect that diffusive gradients become shallow if  $\tau_{\text{transport}}$   
210 is long and concentrations are low. Consequently, also the substrate supply by diffusive fluxes  
211 becomes slower according to Fick's law. Then, higher flow velocities would actually be advantageous  
212 because they replenish the substrate creating steeper gradients and increasing diffusive substrate  
213 supply to the organisms.

214 Thus, the pore water velocity is an important parameter contributing to diffusion limitations on the  
215 pore scale. However, both, too high and too small pore water velocities can induce limitations in  
216 bioavailability<sup>51</sup> which implies a need for a systematic elucidation of this topic.

### 217 **3.3. Thermodynamics, mass transfer, or enzyme kinetics: what is limiting on the organism scale?**

218 It is often observed that micropollutants are only incompletely degraded even when competent  
219 bacterial degraders are present<sup>56</sup>. This unsolved paradox of threshold concentrations might be due to  
220 different reasons. (i) Thermodynamic limitation for biodegradation is an often considered explanation,  
221 but can typically be excluded. For example at nM toluene concentrations, the Gibbs enthalpy of  
222 reaction  $\Delta G$  for aerobic degradation (-3890 kJ/mol) would be large enough to consume even the last  
223 toluene molecule. At high dilution, biodegradation is rather kinetically limited by mass transfer to the  
224 cell as explained above. (ii) An alternative explanation for incomplete degradation is a kinetic  
225 limitation by insufficient substrate uptake into the cell<sup>57</sup>. (iii) Furthermore, slow biochemical  
226 transformation rates (enzyme kinetics) might be due to the intrinsically difficult-to-degrade molecular  
227 structures of xenobiotics<sup>58</sup>. This is supported by comparably slow degradation rates of persistent  
228 compounds at higher but non-toxic concentrations in batch cultures<sup>59</sup>.

229 In natural systems, it was so far not possible to distinguish the different kinds of limitation of  
230 biodegradation on the organism scale, which opens future research fields.

### 231 **4. Microbial controls of biodegradation**

232 Absence of specific degrader populations is often assumed when insufficient biodegradation is  
233 observed at a given site. At organohalide-contaminated sites, bioaugmentation (amending  
234 respective degraders) of microbial consortia containing e.g. *Dehalococcoides* (Dhc) strains capable of  
235 reductive dechlorination of trichloroethylene (TCE) to ethene has been successful<sup>60-62</sup>. Similarly, the  
236 effectiveness of bioaugmentation in atrazine- and MTBE-contaminated (methyl-tert-butylether)  
237 aquifers has been demonstrated<sup>63-65</sup>. However, even highly specialized organohalide-respiring

238 bacteria are generally widespread in aquifers<sup>66-68</sup>. Thus for certain settings, it remains questionable  
239 whether respective degrader organisms were truly absent before bioaugmentation or only present  
240 at very low abundance.

241 **4.1. Limitation of biodegradation by microbial growth.**

242 In aquifers, a substantial fraction of the microbes is suggested to be in a status of low  
243 activity, inactive, or even dormant<sup>69</sup>. Moreover, microbial communities in aquatic systems exhibit  
244 growth efficiencies (yields) much lower than known from batch cultures and chemostats, with  
245 median values below 0.3 (g biomass / g substrate) for rivers, lakes, and oceans<sup>70,71</sup>. *In situ* growth  
246 rates are also low (equation 3) for aquifer systems, and doubling times can be in the range of  
247 months to years<sup>72</sup>. Under optimum conditions in the laboratory, the presence of one degrader cell  
248 at the moment of a hydrocarbon spill would allow aerobes to form notable biomass (e.g. 10<sup>5</sup> to 10<sup>6</sup>  
249 cells per liter groundwater) within a day, while e.g. sulfate reducers or organohalide reducers  
250 (doubling time: ~10 d) may require >100 d to establish a critical population size. Indeed, a fast  
251 response has been observed for an oxic aquifer system receiving a short contaminant pulse<sup>73</sup>, while  
252 anaerobic degradation coupled to denitrification established only over several weeks. For more  
253 recalcitrant compounds and pollutants, requiring anoxic conditions for degradation (such as  
254 halogenated solvents), it might take years before reasonable numbers of degraders have developed.  
255 Thus in aquifers, a slow community response might be misinterpreted as absence of degrader  
256 populations which needs to be verified in the future.

257 **4.2. Limitations of biodegradation by microbial physiology**

258 Total concentrations of dissolved organic carbon (DOC) in pristine groundwater are usually in the  
259 low mg L<sup>-1</sup> range (0.5-2 mg L<sup>-1</sup>), of which only 0.5 to 5% are readily assimilable organic carbon  
260 (AOC)<sup>74-76</sup>. This AOC consists of a plethora of individual compounds at extremely low individual  
261 concentrations, including organic micropollutants such as pesticides, pharmaceuticals, and many  
262 other low-level contaminants<sup>77</sup>. The latter are often present below the threshold concentrations of  
263 initial induction of catabolic genes and degradation pathways<sup>75</sup>. At very low concentrations in the  
264 environment, it is likely that microorganisms do not feed on only one substrate at a time. Mixed  
265 substrate utilization – where microbes can utilize a wide range of offered substrates simultaneously –  
266 has been observed in carbon-limited chemostats<sup>78</sup>. This leads to much lower threshold  
267 concentrations for individual compounds implying that the degradation of one compound “helps” to  
268 degrade another compound in energetic co-metabolism. However, at excess of substrate in  
269 hydrocarbon plumes, catabolite repression, competitive inhibition, or metabolic flux dilution might  
270 take place<sup>79,80</sup>.

271 Today, it is totally unclear how microbial metabolism is regulated in the environment. Such  
272 knowledge will be useful for designing strategies for removing micropollutants from drinking water  
273 in engineered systems or improving the licensing practice of pesticides<sup>77</sup>.

274 **4.3. Limitations of ecosystem response**

275 Low microbial growth rates imply that even under steady state conditions where organisms can  
276 develop without disturbance, long time spans are required to establish significant degrader biomass  
277 and thus biodegradation capacities. However in contaminated groundwater, conditions are not  
278 necessarily in steady state<sup>81-83</sup>. In fact, temporal hydraulic fluctuations may represent a major  
279 control of biodegradation in groundwater by repeatedly changing the environmental conditions  
280 encountered by degraders (e.g. sudden exposure of anaerobes to oxygen). This can be exemplified  
281 by plume fringes which are characterized not only as hot spots of biodegradation activity, but also by  
282 an apparently ‘specialized’ degrader microbiota<sup>25,9</sup> over only a few dm. If the prevailing geochemical  
283 conditions for microorganisms shift, degraders must continuously follow or re-establish in other  
284 strata. Geochemical shifts of the plume could thus represent a further, as-yet unrecognized kinetic  
285 limitation of biodegradation.

286 Moreover, transient supply of substrates by such fluctuations may not be sufficient to support  
287 growth of degrader populations. It can be speculated that under spatially and temporarily dynamic  
288 hydraulic conditions, degrader populations may never reach the biomass levels required to  
289 efficiently degrade substrate pulses. Once formed, however, degrader biomass may persist for  
290 months and perhaps even years after the source of contamination has disappeared<sup>73,84</sup>. Biomass  
291 established upon previous locations of the plume could sustain biodegradation capacities for future  
292 contaminations. Thus, aquifers could become preconditioned to efficiently degrade pollutants.

293 **4.4. Further research needs.**

294 The role of grazers (protozoa) and viruses (phages) in shaping prokaryotic degrader communities and  
295 influencing *in situ* degradation rates is totally overlooked to date<sup>85</sup>. While the influence of protozoa  
296 on bacterial community composition and *vice versa* has been shown also for contaminated  
297 groundwater habitats<sup>86,87</sup>, there is contradicting evidence on either the stimulation<sup>88,89</sup> or inhibition  
298<sup>90,91</sup> of biodegradation by protozoan grazing. Only a few studies are available on bacteriophages in  
299 groundwater<sup>92-94</sup> but their influence on degrader communities and activities have not been  
300 addressed, so far. Extrapolating recent advances from surface aquatic environments and marine  
301 systems, bacteriophages can be expected to play a significant role in controlling prokaryotic

302 production and diversity. With reference to the highly specialized degrader populations found in  
303 biodegradation hot spots, ecological concepts such as the ‘killing the winner’ hypothesis await to be  
304 tested. It predicts that when a given population grows beyond a critical density, grazers and viruses  
305 will decimate the population<sup>95</sup> affecting biodegradation.

306 **5. Conclusion and outlook**

307 Here we discuss several controls for biodegradation in contaminated aquifers that have been  
308 recognized in recent years, and call for an update of classical black box approaches in site  
309 assessment and restoration. New perspectives in groundwater research should include the plume  
310 fringe concept and mass transfer limitations as steering factors for biodegradation. On the organism  
311 scale, physiological properties of degraders and ecological drivers of degrader community structure  
312 have been identified to affect biodegradation. We propose that biodegradation in contaminated  
313 aquifers is largely controlled by kinetics. Different kinetic controls are interacting in complex ways  
314 and cannot be described by flow- or residence-time-dependent degradation rates alone. An  
315 important caveat is that many of these mechanisms act at the  $\mu\text{m}$ -to-cm scale, while sampling is still  
316 mostly conducted at the meter scale. To fully understand process limitations, samples have to be  
317 taken at adequate resolution, often including intact sediment cores or highly-resolved water  
318 sampling. Furthermore, temporal dynamics of processes demand for extended monitoring with  
319 more frequent sampling intervals in time and space.

320

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323

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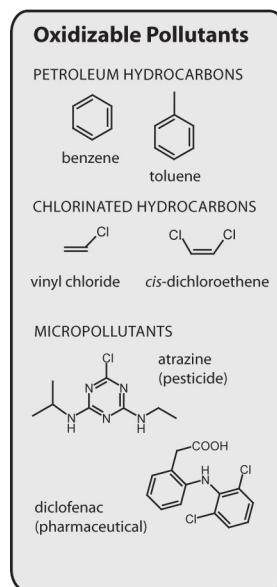
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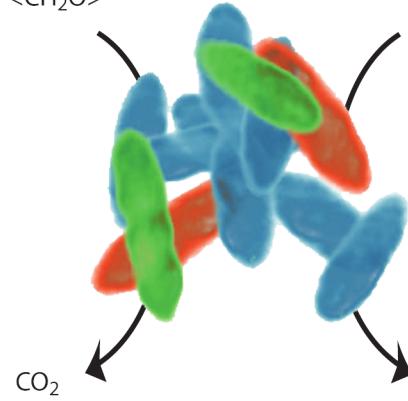
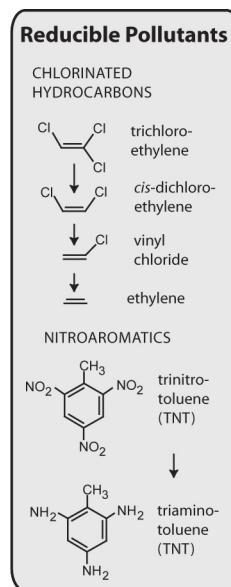
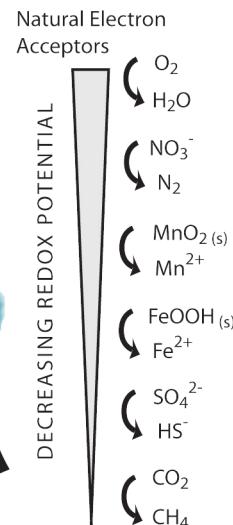
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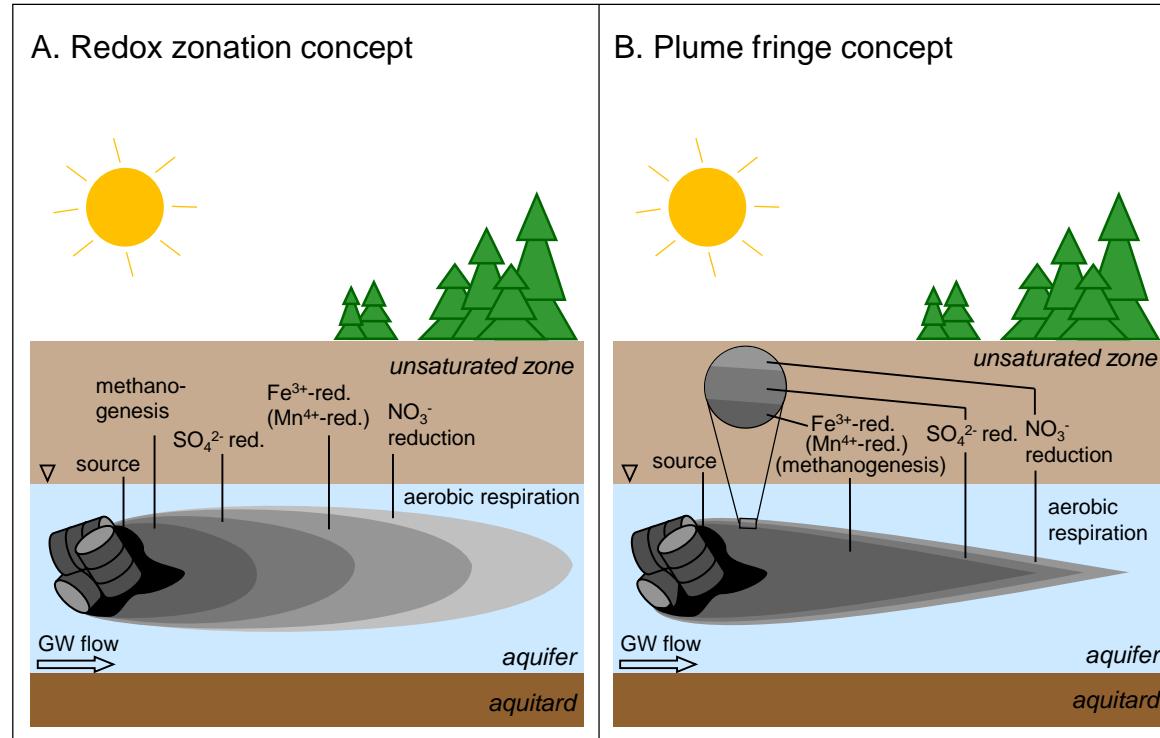
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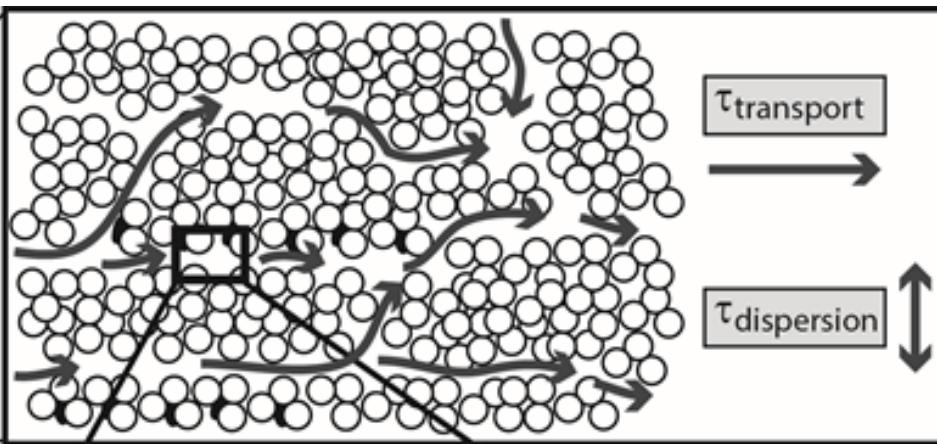
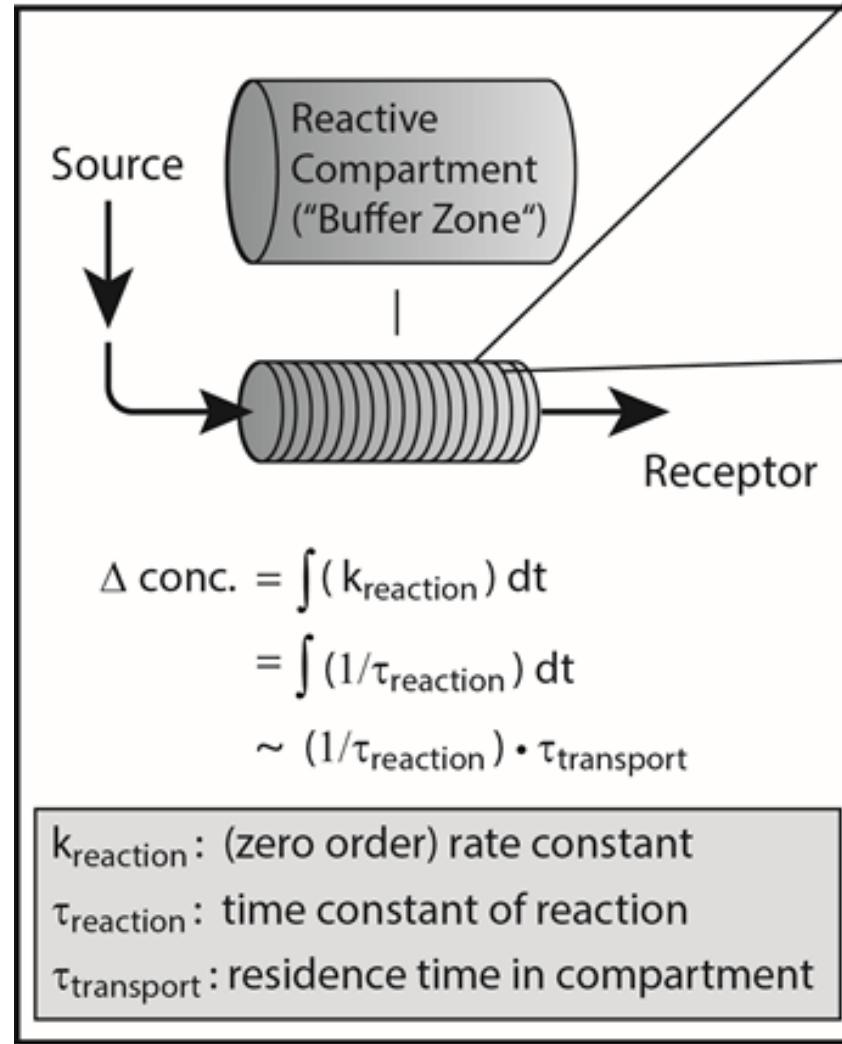
**ELECTRON DONORS**

Natural Organic Matter (NOM)

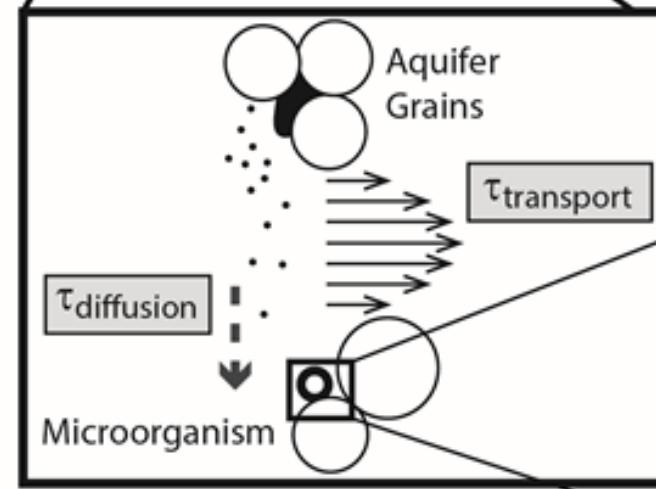
<CH<sub>2</sub>O>**ELECTRON ACCEPTORS FOR RESPIRATION**



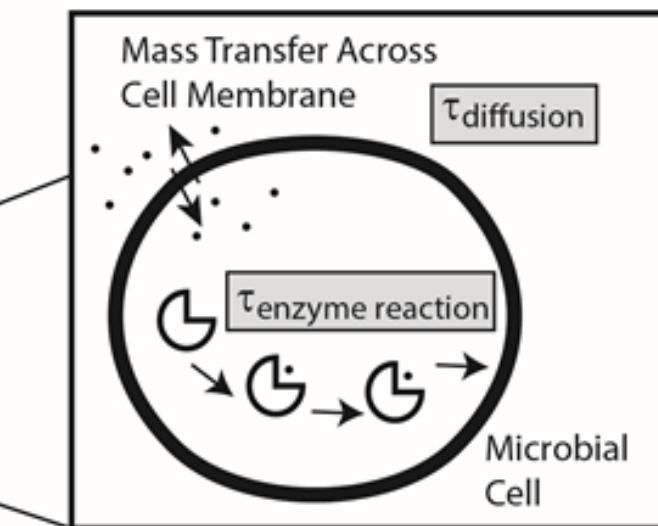
## (A) Current Conceptual Models



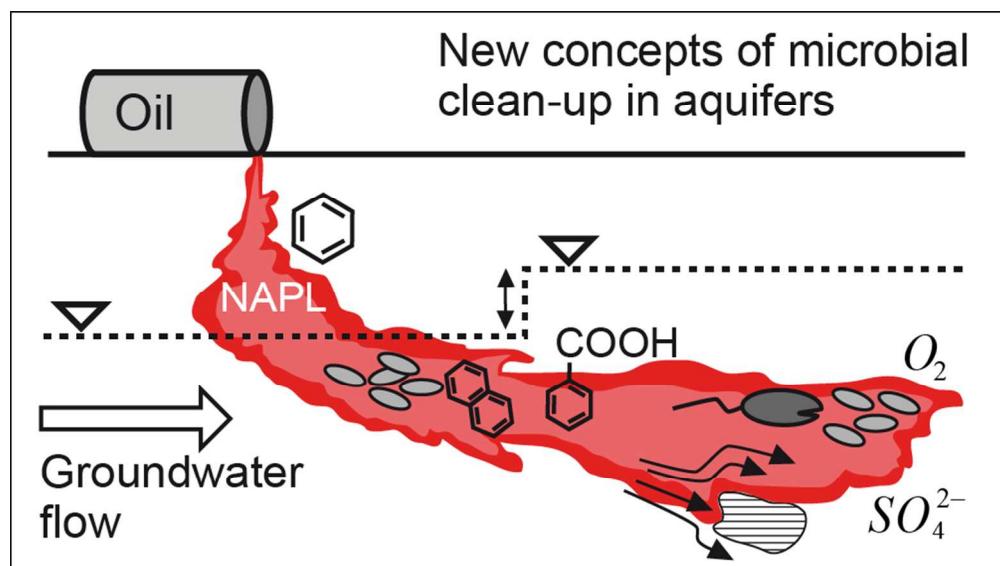
## (B) Heterogeneous Flow Paths on the Linear Pore Velocity Scale



## (D) ... and on the Organism Scale



## (C) Mass Transfer on the Pore Scale ...



85x47mm (300 x 300 DPI)