Hydrogen Release Compound HRC®

HRC Performance Characteristics - Longevity

General Background

Since Hydrogen Release Compound (HRC®) is a time-release product, one of the central issues in site design and one of the most frequently asked questions is "How long does it last"? The short answer is that the basic HRC now sold, specifically defined as a formulation of Glycerol Polylactate (GPL), diluted with glycerol to a viscosity of 20,000 centipoise (cP), is estimated to stimulate reductive dechlorination within the aquifer for about 12 months. The longevity is a function of basic product chemistry and certain biological and geochemical features of the aquifer. HRC has been shown to have a direct effect on contaminants resulting from the lactic acid release and other secondary effects, related to the formation of other organic acids that "borrow hydrogen" and recycled biomass, that have a more prolonged effect on aquifer conditions.

Theoretical Considerations

Longevity as a Function of HRC Chemical Characteristics

Hydrogen Release Compound is a specific article of commerce as described. The "active ingredient", Glycerol (tri) Polylactate (GPL), is one of a family of polylactate esters, defined by our patent, that upon hydration break down to release lactic acid. The exact chemical nature of a specific polylactate ester, such as GPL, is a major factor in product longevity. In essence, the structure and degree of esterification determine viscosity and viscosity is a critical factor in longevity.

Structurally, an ester is the product of a reaction between an organic acid (COOH group) and an alcohol (OH group). In this reaction as shown in Figure 1, the two groups react and water drops out forming the ester linkage, noting that the amount of water removed in esterification can affect viscosity. The example used shows methyl alcohol reacting with lactic acid to form a simple lactate ester (methyl lactate).

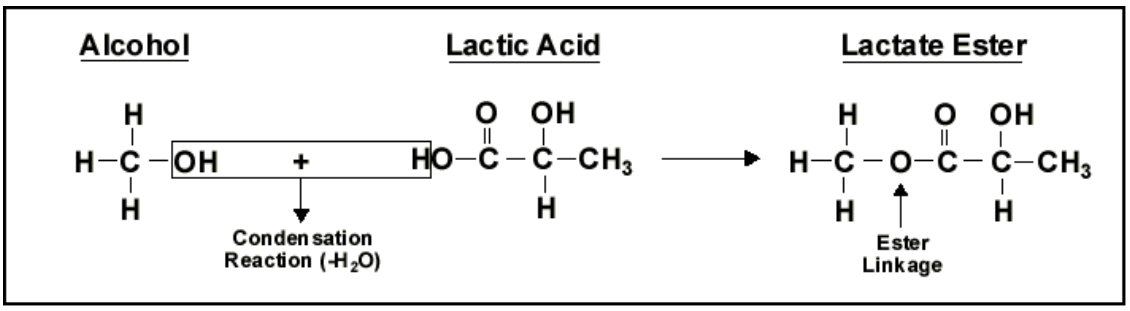

Figure 1. Formation of an Ester.

Polylactate esters are formed from the combination of certain alcohols with a unique lactic acid complex serving as the organic acid group. The alcohols used are compounds such as glycerol (3 OH groups), xylitol (5 OH groups) and sorbitol (6 OH groups). These "foundation" molecules are then esterified with a polylactic acid complex as illustrated in Figure 2.

One of the unique features of the polylactic acid complex is that lactic acid is esterified to itself. This is possible because lactic acid, as shown in Figure 1, has both an OH and a COOH group. As a result we can typically produce trimers or tetramers of lactic acid and create a "polylactic acid complex" or "polylactate complex", which is in turn esterified to the foundation OH donor as described.

The Glycerol (tri) Polylactate (GPL) component of the HRC in commerce is specifically the molecule in Figure 2. However, GPL itself can vary as a function of how many OH positions are actually esterified, so that one could have glycerol (tri) polylactate if all 3 are filled or glycerol (di)polylactate if just 2 are filled. The GPL in HRC is the Glycerol (tri) polylactate ester

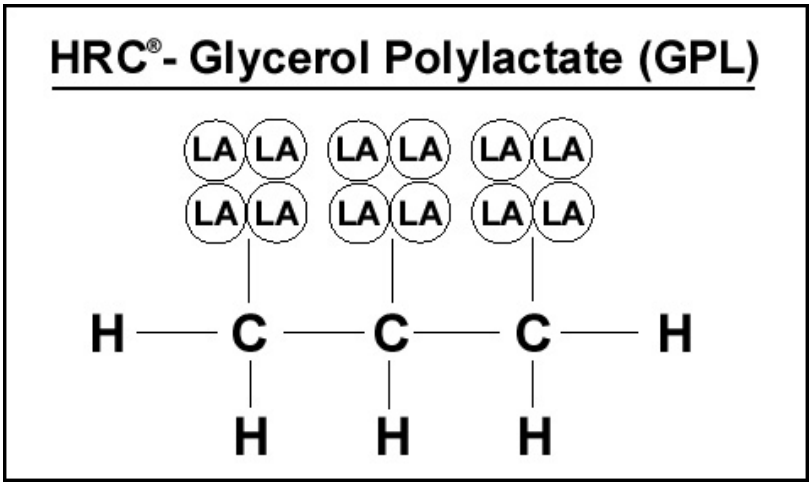

Figure 2. Structure of Glycerol (tri) Polylactate (GPL)

The point of this is to emphasize that the degree of complexity and esterification of the molecule control its viscosity and hence a major component of its reactivity. This in turn controls product longevity under a uniform set of conditions. For example, the (tri)polylactate form of GPL would be more viscous that the (di)polylactate form. Further, a molecule built with tetramers of lactic acid would be more viscous than one made with trimers. As a final example a sorbitol polylactate ester is more viscous than a glycerol polylactate ester, because it is based on 6 carbons rather than 3 carbons.

Viscosity becomes a dominant issue in longevity because it is a measurement of resistance to flow. As a result, viscosity controls the evolution of surface area over time and the speed at which HRC becomes soluble in water. Therefore, if a polylactate formulation becomes less viscous it will spread more, thus exposing more sites to chemical and biological attack. The latter case, involving the enzymatic action of microorganisms, is a particularly powerful mechanism and this will be expanded on later. In essence, "thicker lasts longer", which one would intuitively gather for the reaction of any solute with a solvent.

Returning to viscosity for a moment, we said that it is a measure of resistance to flow and can be measured in a viscometer. In a viscometer, the substance being tested is placed between two plates, one being stationary and one being movable. The amount of force applied to overcome resistance to the movement of the moveable plate is the key variable. Consequently, the units of viscosity in SI units are force-based, e.g., dyne-sec/cm^2 or Poise (P). Typically we measure the viscosity of HRC in centipoise (cP).

To be pumpable for push-point injection, HRC has to be about 20,000 cP (like honey). This is achieved by cutting raw GPL, which is about 200,000 cP (gel-like), with glycerol - by a factor of about 2, noting that the relationship between dilution and viscosity is non-linear. Therefore, we produce a form of HRC at 200,000 cP, as a function of the specific chemical structure, such that when it is cut about 50:50 with glycerol we achieve a pumpable 20,000 cP material.

Longevity as a Function of Aquifer Characteristics

The viscosity of the HRC is still only one side of the equation. The other important feature concerns the environment the HRC is placed into. An experimental model for the dynamics of HRC utilization in the bioremediation of TCE is published in Farone, Koenigsberg and Hughes (1999). Copies of this and other papers cited herein are available on request from Regenesis and it is convenient to use our web site at www.regenesis.com.

The model, which was calibrated with laboratory microcosm tests, clearly demonstrates that the nature and extent of microbial populations has a significant effect on the longevity of HRC. Simply put, most microbes - not just the kinds that ferment lactic acid into hydrogen or those that promote reductive dechlorination - will produce esterases and lipases that degrade HRC and release lactic acid.

Therefore, if an aquifer has a high microbial population it will metabolize a given mass of HRC at a faster rate than if the microbial counts are moderate to low. An example of this is presented in Figure 3 that shows a difference in lactic acid release rates at three different microbial concentrations. Note that this lab test represents a highly accelerated condition relative to field results, because everything is optimized and reacted in close proximity in the test tube environment.

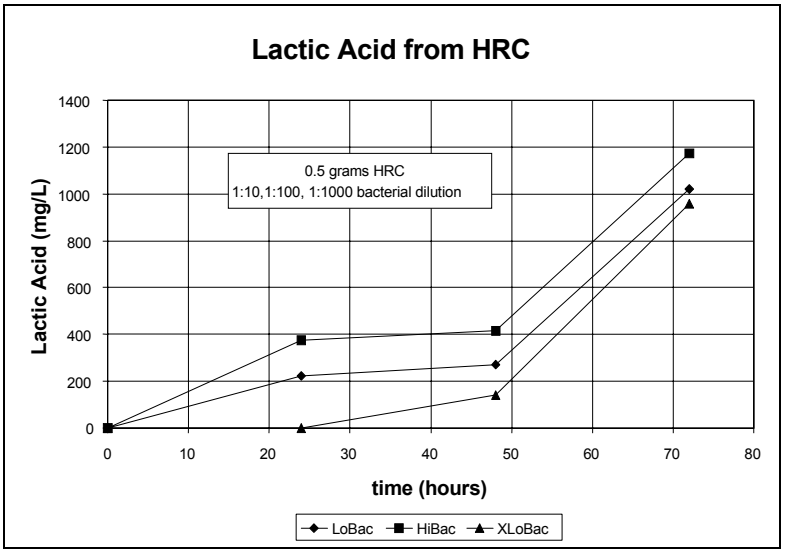

Figure 3. Lactic Acid Release Rates as a Function of Microbial Population.

Early Laboratory Work

Table 1 presents data that reflect a set of minimum times that standard HRC has lasted in the laboratory. Again, note that laboratory tests generally have high rates of activity relative to the field. The tests summarized below, were conducted to test the efficacy of HRC in remediating TCE and were not longevity tests per se. For economic reasons they were only run for about three months to collect the remediation rate data. Still, the tests supply some useful minimums for longevity. The tests were macrocosm studies conducted in Aquifer Simulation Vessels (ASVs) with a flow rate of about 1 ft/day. For a further discussion of the ASV type studies, please refer to TB 2.4.3.

Table 1. Macrocosm (ASV) Tests.

Product	Duration of Test	Presence of Product at Conclusion
GPL 52%/Glycerol 48%	84 days	Yes
GPL 52%/Glycerol 48%	80 days	Yes
GPL 52%/Glycerol 48%	80 days	Yes
GPL 52%/Glycerol 48%	94 days	Yes

Recent Laboratory Work

Recently an ASV study was established to specifically look at both longevity and diffusion issues regarding 20,000 cP HRC and 200,000 cP GPL. 15 g of each formulation was placed in the influent end of the ASV; there was no flow so that diffusion alone would be measured. While the main purpose of the test is covered in TB 2.4.3, it can be noted that, after 66 days, there is still considerable material left in the system of both products. The experiment will be continued until the product is exhausted. This is expected to be about one year for the 20,000 cP HRC and several years for the 200,000 GPL.

Field Results

Data from the Oldest Trials

The most powerful evidence for product longevity is of course the observations made in the field. As presented in Table 2 and graphically in Figure 4, the 20,000 cP HRC will last from 119 days to 580 days. It is important to note that this is a "living table" such that some of the applications are still in progress and the average longevity figures is getting larger. However, based on the range of this field experience and the fact that some tests are still in progress, we claim that 20,000 cP HRC degrades slowly, on average, for nine months (as modulated by certain features in the contaminated aquifer). However, just because the organic acids are gone does not mean the effects are over. Residual hydrogen will still be present and biomass accumulates which will later be available as fermentable carbon. The stimulated biomass is also more able to utilize native carbon as well. Therefore, ~20,000 cP HRC now sold is estimated to stimulate reductive dechlorination within the aquifer for at least 12 months.

Also, some work during the early phase of product development (not cited here) looked at the properties of a 1,000,000 cP sorbitol polylactate hard gel. In one test, the material physically lasted for at least two years at the point of application. In this experiment, which is presented in Dooley, Murray and Koenigsberg (1999), the SPL hard gel was placed in a canister with sufficient holes to allow it to ooze out slowly. It was placed in an injection well and the exposed surface was in contact with a fairly vigorous flow (relative to an aquifer velocity) of 0.25 gal/min. The active recirculation period ended at about 330 days, however, and the canister was retrieved after two years significant HRC was still present.

Regenesis is working on ways of delivering higher viscosity materials directly as "implants" using warmed GPL to lower the viscosity during application and special push-point injection and hollow stem auger techniques. These implants can be used as barriers or source treatments and can be expected to last for several years for very long-term plume management.

Table 2. Longevity Profile of GPL-HRC at 20,000 cP.

Site	Last Field Observation of Organic Acids- Lactic, Butyric, Propionic **(in days)**	Duration of Data Collection **(in days)**	Status
Site 1	150	364	Product exhausted before monitoring completed
Site 2	173	173	Product still present at conclusion of monitoring
Site 3	181	181	Product still present at conclusion of monitoring
Site 4	197	197	Product still present at conclusion of monitoring
Site 5	321	321	Product still present at conclusion of monitoring
Site 6	119	119	Product still present; monitoring on-going
Site 7	240	240	Product still present; monitoring on-going
Site 8	243	243	Product still present; monitoring on-going
Site 9	399	399	Product still present; monitoring on-going
Site 10	580	580	Product still present; monitoring on-going

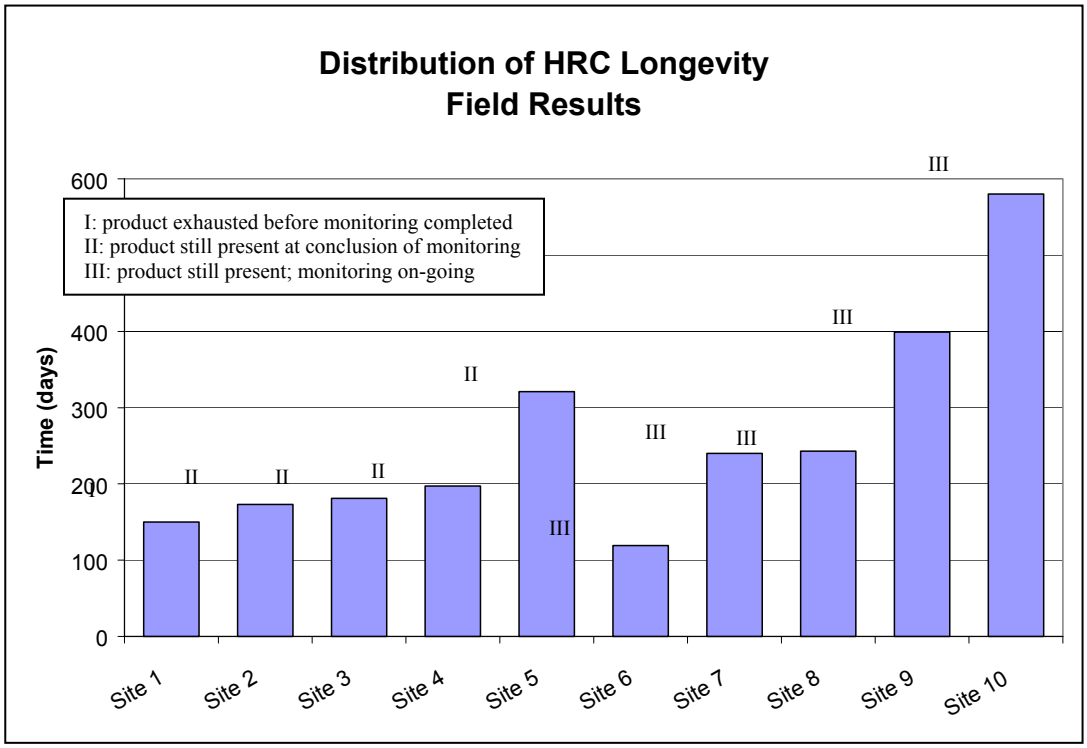

Figure 4.

Secondary Effects

Often we see remediation continuing after HRC breakdown products are no longer in the system. This observation led us to postulate that there may be more than one phase in the effect of adding HRC to the aquifer. Generally, we use the presence of elevated levels of organic acids as the metric, although we can also look at TOC data as well. However, sometimes after the footprint of the HRC is gone we can still see elevated rates of remediation. On further consideration we believe that the organic load to the aquifer that is provided by HRC is not a uni-directional event and that the biomass of microorganisms constructed from HRC can be recycled. This can cause an after effect as could native carbon in the system that might be more responsive to the elevated microbial population levels. While this is clearly not a dominant driver for reductive dechlorination relative to HRC, it apparently can have an impact. Lastly, at the formal exhaustion of the organic acids there is still hydrogen available.

To support this hypothesis we can look at an HRC injection as described in Sheldon, (1999). This experiment was a pilot test with a monitoring well (MW-8) that was clearly in the center of the HRC injection zone. Another well (MW-1) was in the experimental grid but was outside the migration zone for the HRC and its breakdown products during the course of the experiment. Figure 5 shows the nature of the organic acid profile over time and it should be noted that propionic acid is formed from lactic acid and then breaks down to release hydrogen. Acetic acid is generally considered to be a non-hydrogen evolving end point.

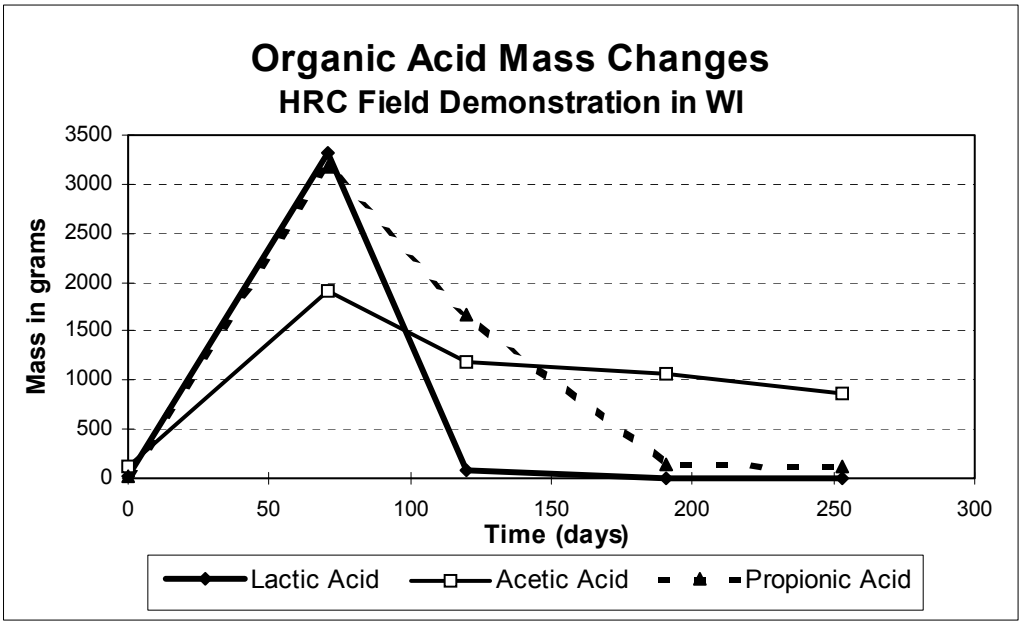

Figure 5.

The total mass of organic acids is calculated from a contouring exercise and the last appearance of lactic acid per se is between 70 and 120 days (the formulation was only about 10,000 cP in this early work). The disappearance of propionic acid between 120 and 190 days is further noted. With this background information we can now examine Table 3 and evaluate the remediation rate differences between the impacted and non-impacted wells (MW8 vs. MW1). It is clear that even after the organic acids are "gone" that there are still rate differences to be accounted for. At least two hypotheses can be presented.

- The HRC is still releasing slowly and is being consumed as fast as it is produced which would still allow remediation to proceed and cause a rate difference.

- The HRC is exhausted, but the organic material is being recycled and or native organic material is being mobilized by growing microbial populations - such that remediation continues.

Flow rates at this site are on the order of 0.5 ft/day and that is not sufficient to flush the system of HRC in the period involved in the study.

Table 3. TCE Degradation Rate Differentials at WI Site

Day	Half Life (d) Well TW01	Half Life (d) Well TW08	Ratio TW01/TW08
0-70	338	29	11.50
70-120	211	43	4.94
120-149	256	53	4.84
149-191	309	45	6.80
191-253	205	38	5.41

Summary

The main active ingredient in Hydrogen Release Compound is a polylactate ester called Glycerol (tri)Polylactate or GPL. Abiotic and microbially mediated hydrolysis of the ester linkage, between glycerol and the polylactate complex, releases the polylactate complex. The polylactate complex is a tetramer of lactic acid (4 lactic acid molecules esterified to themselves) and its degradation results in the release of individual lactic acid molecules which undergo fermentation to release hydrogen. These chemical features and the resultant viscosities, are an important component of longevity.

GPL is manufactured as a highly esterified thick gel and can be subsequently cut to a syrup-like consistency for injection. All other conditions being equal, product viscosity combined with the nature of microbial activity in the aquifer are the key determinants of product longevity. This is essentially a surface area argument. The thinner the material the more it will spread out and be exposed to chemical and biological degradation.

Based on the evidence presented in the laboratory and field, we can claim that ~20,000 cP HRC is estimated to stimulate reductive dechlorination within the aquifer for about 12 months. The longevity of this effect is a function of certain biological and geochemical features of the aquifer. HRC has been shown to promote reductive dechlorination resulting from the lactic acid release and possibly other indirect effects related in part to the formation of a nominal amount of recycling organic material that has a more prolonged effect on aquifer conditions. An "after effect" in the aquifer involving the recycling of carbon may extend the positive remediation effects beyond the formal longevity based on lactic acid release.

References

Dooley, M., W. Murray and S. Koenigsberg. 1999. "Passively Enhanced In Situ Biodegradation of Chlorinated Solvents". In: A. Leeson and B.C. Alleman (Eds.), *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination*, pp. 121-127. Battelle Press, Columbus, OH.

Farone, W.A., S.S. Koenigsberg and J. Hughes. 1999. "A Chemical Dynamics Model for CAH Remediation with Polylactate Esters". In: A. Leeson and B.C. Alleman (Eds.), *Engineered Approaches*

for In Situ Bioremediation of Chlorinated Solvent Contamination, pp. 287-292. Battelle Press, Columbus, OH.

HRC Technical Bulletin 2.4.3. Aquifer Simulation Vessel (ASV) Studies.

Sheldon, J.K., S.S. Koenigsberg, K.J. Quinn and C.A. Sandefur. 1999. "Field Application of a Lactic Acid Ester for PCE Bioremediation". In: A. Leeson and B.C. Alleman (Eds.), *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination*, pp. 61-66. Battelle Press, Columbus, OH.