



PETROLEUM REMEDIATION AND PETROFIX

Frequently Asked Questions (FAQs)



PetroFix Performance for Bio-Remediating Petroleum Impacted Sites - FAQ

Todd Herrington and Paul Erickson from REGENESIS presented a webinar on how to apply and evaluate the effectiveness of PetroFix™, a dual-action treatment approach consisting of micron-scale activated carbon for contaminant adsorption and electron acceptors to stimulate biodegradation. This activated carbon remediation technology is easily injected in-situ under low pressure, distributes in the subsurface without fracturing, and includes electron acceptors to stimulate biodegradation of petroleum contaminants in soil and groundwater. Along with design considerations, the presentation by Dr. Erickson and Mr. Herrington discussed a former bulk petroleum

storage facility where PetroFix was injected in an area with over 50 ppm total petroleum hydrocarbons. Multiple lines of evidence were used to evaluate performance: contaminant concentrations to evaluate contaminant adsorption and removal from groundwater, geochemical parameters to document microbial utilization of added electron acceptors, and QuantArray®-Petro and mRNA analyses to evaluate biodegradation of BTEX and other hydrocarbons.

The following Q&A include questions from the audience, with answers from Dr. Erickson and Mr. Herrington of REGENESIS, as well as Dora Taggart, President of Microbial Insights, Inc.



Paul Erickson, PhD
Senior Research Scientist, REGENESIS

Dr. Paul Erickson is a Senior Research Scientist with REGENESIS and currently specializes in research and development of new environmental solutions to address complex remediation challenges. In his research and development (R&D) role, Dr. Erickson led the development of the PetroFix Design Assistant application and played a central role in advancing PetroFix as a treatment technology. Dr. Erickson earned a BS degree in Chemistry from Florida State University, a Masters Science in Chemistry from the University of Minnesota, and a PhD in Environmental Chemistry from ETH Zurich.



Todd Herrington
Global PetroFix Product Manager, REGENESIS

Todd Herrington is Global PetroFix Product Manager for REGENESIS. Mr. Herrington collaborates with sales, operations, and R&D departments at REGENESIS in order to provide environmental solutions. Mr. Herrington has over 20 years of environmental remediation experience and has been with REGENESIS since 2004. He earned his B.S. in Civil Engineering from Colorado State University and his Masters of Science in Environmental Engineering from the University of Cincinnati.



Dora Taggart
President, Microbial Insights, Inc.

Dora Taggart is the President of Microbial Insights, Inc. in Knoxville, Tennessee. She received a Biomedical Engineering degree from Vanderbilt University and has focused on the optimization and implementation of molecular tools for environmental remediation, microbiologically-influenced corrosion and microbial source tracking. Since joining Microbial Insights in 2001, she has developed and commercialized over 60 different nucleic acid-based analyses. Under her direction, Microbial Insights has become a worldwide provider of molecular tools for leading consulting firms, government agencies and academia. Ms. Taggart runs national and international workshops on these tools. She has more than 20 peer reviewed co-authored publications and is often invited to speak at conferences around the world.



PetroFix and Bioremediation

Q1: Can this product be combined with your product ORC® or have results shown to be more favorable sticking with anerobic degradation?

There is no incompatibility using ORC Advanced with PetroFix. The only limitation to this approach would be the additional cost for ORC Advanced and the need to inject the material in separate injection points because they are incompatible to be injected together. However, once injected through separate grid injection points the combination of ORC Advanced plus PetroFix with provided nitrate and sulfate electron acceptors would be a very aggressive and effective remediation approach because the stimulation and growth of aerobic bacteria would happen quickly as well as subsequent aerobic oxidation of a broad range of fuel hydrocarbons. As oxygen is the preferred natural electron acceptor for hydrocarbon bioremediation based on the standard free energy available for oxidation it would be used preferentially and nitrate and sulfate use would be suppressed until oxygen was depleted. Following depletion of oxygen, nitrate and sulfate would be utilized. The combined use oxygen followed by nitrate and sulfate electron acceptors would be expected to improve syntrophic processes under anaerobic conditions because you have increased the total electron acceptor capacity and are bio-stimulating a more diverse range of bacteria capable of handling the more recalcitrant VOCs such as benzene. We do want to emphasize that while we would be highly confident on the combined use of ORC Advanced with nitrate and sulfate, we still believe that for most sites the provided nitrate and sulfate are sufficient for stimulating fuel biodegradation.

**Q2: Why does PetroFix utilize/stimulate anaerobic bioremediation?
Is it therefore not effective in shallow, well oxygenated aquifers?**

We agree with your question and statement in theory, but in practice we find that oxygen replenishment to aquifers from the atmosphere or surface water infiltration are insufficient to stimulate aerobic biodegradation in the time frames needed. If your aquifer already is anaerobic then we believe the use of the provided nitrate and sulfate a wise choice. However, if you evaluate the levels of dissolved oxygen and oxidation/reduction potential (ORP) of your aquifer and find that it is indeed well oxygenated and aerobic then injecting PetroFix would still be beneficial by allowing the PetroFix to sorb and remove dissolved phase contamination while having the naturally high oxygen concentrations stimulate aerobic biodegradation of sorbed contaminants. The use of the additional electron acceptors provided in separate white buckets is optional.

Q3: But why is acetate inhibitory? I know it can be for nitrate-reducing organisms, but not for most others. Is it strictly a matter of an abundance of "simple" hydrocarbons that drive either a preference of the degradation of those compounds rather than the hydrocarbons, or because it stimulates methanogenesis and methanogenic conditions? Also, is there a pH-related component due to the buildup?

In short, it comes down to chemical equilibrium and when the overall syntrophic process is thermodynamically favorable. As fermentation end-products (acetate, H₂) accumulate, the entire process yields less energy for the microbes. As long as these intermediates are being removed by sulfate reducers and/or methanogens and the pH doesn't increase too much (which can slow things down) the process remains energetically favorable.

Q4: How do you know that benzene is being biodegraded versus only sorbed? And how do I know PetroFix is working since it removes hydrocarbons from the groundwater and I can't tell total reductions from simply monitoring groundwater?

From lab studies we know hydrocarbons, and specifically benzene is biodegraded by doing full-bottle extractions on batch experiments. After incubating with benzene, activated carbon, soil and water, an entire bottle is extracted with another solvent and we see all benzene is gone. In control experiments like this (killed/sterile bottles) the benzene can be recovered from the activated carbon at very high recovery percentages, showing biodegradation is the removal process. *REGENESIS PlumeStop technical bulletin 3.1* is available on regenesisc.com showing a post-sorption contaminant biodegradation lab study with benzene that documents benzene biodegradation on the activated carbon (PlumeStop and PetroFix use the same diameter carbon and this lab study is representative for PetroFix as well). In the field, we rely on multiple lines of evidence to show ongoing biodegradation after adsorption. The utilization of added nitrate and sulfate in the right pattern (nitrate first, sulfate second) is the first line.

Q5: You have mentioned three inhibition mechanisms - can you discuss the effect of temperature, specifically low temperature around 4°C?

Temperature will certainly affect the expected rate of biodegradation. At 4°C biodegradation will be slower, but still occurring. Many deep lakes in cold climates have sediments at this temperature, yet still experience natural methane emissions from organic carbon being biodegraded. The effect is loosely comparable to how quickly food would spoil inside vs. outside of a refrigerator. Despite this, PetroFix would still be a viable treatment option at low temperatures.

Q6: Has any isotope analysis been completed to document actual biodegradation occurring?

We have plenty of lab data confirming biodegradation is occurring, and did not see the need to use isotopic tests as a part of initial field product testing. With that said, when using PetroFix compound specific isotope analysis (CSIA) has limited field use because groundwater contaminant levels commonly drop to ND after treatment. Stable isotope probing (SIP) is a method offered by Microbial Insights where a labelled compound is placed on a bio trap (activated carbon beads) and its removal, as well as incorporation into microbial biomass, can be followed.

Q7: Since most enzymes are intracellular thereby needing the COC to cross the cell membrane, can you speak to how the TPH is bioavailable on the PetroFix?

It is true that microbes must uptake TPH rather than rely on extracellular enzymes, but there are other potential ways for microbes to actively uptake TPH, including biofilm and/or biosurfactant production. While the exact mechanisms are not clear, we conclusively know (see answer 13) adsorbed compounds can be degraded.

Q8: What is the PlumeStop polymer that bacteria reportedly degraded resulting in the unwanted consumption of electron acceptors?

The formulation of PlumeStop is proprietary, but the point is the polymer is a source of carbon for the microbes much the same as the hydrocarbons are. The added organic polymer must be biodegraded along with the PHCs and this process will consume electron acceptors.



PetroFix and Optimal Use

Q9: You stated the PetroFix is not suitable for light non-aqueous phase liquid (LNAPL). However, you also mentioned you can use PetroFix to address source area smear zones, which is actually separate phase but immobile LNAPL. Is the issue just bulk floating petroleum?

Yes, the issue is bulk floating petroleum. We believe using a rule-of-thumb that the presence of continuously measured floating LNAPL is an indicator of high mass that usually results in an exceedance of the desired performance range of PetroFix. High amounts of LNAPL could both overwhelm sorption sites on the PetroFix carbon and the rates of anaerobic degradation. Our goal for PetroFix is to provide sustained multiple-order of magnitude reductions of BTEX and TPH and to let our customers know of site conditions where we think attaining this goal would be challenged. If uncertain, we advise that you order a single drum of PetroFix and pilot test at your site.

Q10: Can you explain more on using PetroFix in sand vs clay?

The particle size of PetroFix is 1 to 2 μm in diameters which is below the pore-throat size of fine sands and silts. However, PetroFix cannot be injected into tight clay without high-pressure fracturing which we try and avoid. We realize that sorbed LNAPL often is present as a source in clay and PetroFix is not designed to directly remediate LNAPL bound in clay. Instead, PetroFix is designed to infiltrate all permeable transport zones above, below, or interbedded in clays that lead from a source to downgradient receptors. By focusing on liquid delivery in these zones you will stop migration of contaminants, particularly by mitigating the impact of contaminant back diffusion into these zones, and reduce site risk which opens up possibilities for closing sites by this method alone or in conjunction with other source treatment approaches.

Q11: It was presented that PetroFix both be used for a site with comingled petroleum and chlorinated hydrocarbon plumes?

PetroFix is specifically formulated for hydrocarbon plumes and while it could sorb both solvents and petroleum, its formulation will not promote anaerobic destruction of chlorinated products. In fact, PetroFix injected with its electron acceptors in an area targeted for reductive dechlorination treatment of solvents would be expected to repress reductive dechlorination until they are depleted and this would negatively delay solvent treatment. What we advise is that if you are concerned about overlap that you contact Regenesys for design assistance to evaluate an optimal treatment scenario.

Q12: Presenter showed how PetroFix attaches to soil particles to provide long term localized effectiveness. How do the electron acceptors remain effective and not just get flushed down gradient?

It is true water soluble electron acceptors can flush out of a system, while PetroFix will remain positionally stable. We see this as an issue for a minority, not majority of projects and keep in mind that there will be some co-migration of contaminants with electron acceptors, albeit at different rates. For nitrate, its utilization is so fast that we predict it will not migrate significantly before it is utilized in most aquifers. Sulfate utilization is slower and generally dependent on nitrate being depleted first and may move out of the system in high velocity groundwater environments. If you are concerned about washout it may be possible to replenish the aquifer with additional soluble electron acceptors through further injections, drip system, etc. One option is to co-inject ORC Advanced within or upgradient of the PetroFix application to supply continuous oxygen (ORC Advanced material doesn't migrate with groundwater).

Q13: Does the treatment of soil with one of these products restrict one's ability to excavate and reuse this soil in the future, as surficial soil, for example.

We don't see an issue with using soil once treated with PetroFix as long as treatment concentrations have achieved acceptable use concentrations. Aesthetically, treated soil will be black which might be a consideration on their use.

Q14: Does Petrofix work well with high end petroleum hydrocarbon fractions F1 to F4?

Yes, PetroFix will work well with treating dissolved phase contamination in those ranges. For readers who may not be familiar with Canadian F1 to F4 ranges they are: F1 (C6-C10); F2 (>C10-C16); F3 (>C16-C34), and F4 (>34). The Indiana case study we showed presented both TPH-GRO (C6-C10, or F1 fractions) and TPH-DRO (C10-C28; or F2 and partial F3 ranges) ranges each having reductions of +2 OOM and +1 OOM reductions, respectively. We don't have direct experience stimulating the biodegradation of F4 fractions at this point, but know that both the susceptibility and biodegradation potential are related to respective solubility of hydrocarbons. Components for F3 or F4 that are water soluble and mobile would be expected to be sorbed and treated by PetroFix using our mixed electron acceptor approach.

Q15: Is there a threshold on the upper level for benzene where this product would not be utilized? Early in the presentation you had mentioned that this is not designed for use at sites with high levels of benzene but your pilot test sites did have elevated concentrations.

When treating hydrocarbon sites we hope to achieve one or more order-of-magnitude (OOM) reductions of contaminants sustained, if not better, for BTEX and TPH ranges. Reductions will happen quickly upon contact with hydrocarbon followed by biodegradation. The Indiana case study we presented was a challenging site that performed well at high concentrations and we will continue to monitor for performance. We believe that upper limits depending on soil type is probably in the 30 to 50 mg/L range total hydrocarbons before you might need to consider future, supplemental electron acceptor injections. Keep in mind that we believe that a single injection of PetroFix is probably suitable for most sites and any additional rebound can be addressed with the injection of desired electron acceptor and/or nutrient blends. The caveat to this is if you suspect that aquifer distribution was not achieved and that might warrant supplemental PetroFix injections to fill in the gaps.

Q16: Can PetroFix be injected at the same event as other REGENESIS products such as RegenOx®, or would it require an additional injection event?

We believe that PetroFix is compatible with RegenOx and PersulfOx® chemistry and could be co-injected in the same areas as these products. However, these material should not be injected simultaneously in the same hole. Other constraints to consider would be the ability to get total volumes from both ISCO products and PetroFix in the ground simultaneously since both require high volumes and high effective pore space filled. We see PetroFix as a very strong option for post ISCO polishing as either a second subsequent injection or possibly simultaneous injection during a single mobilization. The practitioner would need to determine the optimal timing when PetroFix should be injected to coincide with the mass reduction goals of the ISCO injections and at which injection event PetroFix should be paired. Regenes is happy to discuss such options and make recommendations.

Q17: Is it possible to apply this technology in deep aquifers?

Yes, this product can be applied in deep aquifers as long as injection wells were designed for that purpose. Achieving distribution would have to take into consideration the limited ROI and distribution potential for pressure injection of PetroFix or consider pump-and-pull well arrangements if the aquifer were highly permeable to enhanced distribution. Please also see question 21.

Q18: Has PetroFix been tested at a fractured bedrock site and if so, what were the results?

We have had a project where PetroFix was distributed in a bedrock aquifer and distribution was better than expected because effective pore space is less than in soil. As long as fractures are interconnected and the aperture sizes of those fractures are large enough you would expect to be able to push PetroFix into the formation, potentially using larger ROI's than what we normally estimate for direct push. In a fractured bedrock site with sufficient interconnectivity you also have the option to use a pump-pull arrangement using different wells to facilitate distribution in different directions. We would recommend a small pilot test using a drum of material to verify the deliverability of the product and validation of ROI.

Q19: Could PetroFix be used in a clay-rich environment that has been fractured to increase permeability? The thought is that the fractured zones could serve as a hydrocarbon sink to reduce BTEX concentrations in the impacted soils over time through diffusion from the soils into the groundwater within the fractures.

We advise caution and proper design in these situations since we are trying to avoid uncontrolled or partial placement of PetroFix in an aquifer. PetroFix as shipped and undiluted would possibly meet density/viscosity requirement as a frack fluid and possibly could be paired with a sand proppant. Hence, PetroFix fracturing could be performed and it's also entirely feasible that PetroFix fractures could be replenished with additional electron acceptor blends if they were connected to re-injection wells. We also have evidence to believe that PetroFix may also diffuse into clays beyond the fracture. Furthermore, the amount of carbon and electron acceptors in PetroFix would probably be insufficient for direct remediation of LNAPL in clay. The ideal application of PetroFix is in the high flux zones where the material can control future back diffusion from clays into permeable zones to control risk. It is worth considering that many sites, despite having tightly bound contamination in clay, may achieve closure simply by injecting PetroFix into conductive zones to reduce off-site flux to levels sufficient to protect human health and environment. We leave it up to the practitioner to determine the specific need for source treatment in clay and perspectives on answering such a question was covered recently by Dr. John Wilson in a webinar entitled "Defining Cleanup Success for Groundwater Remediation" which can be accessed at regenesi.com.

Q20: Can additional nutrients be added to PetroFix for injection?

Under most circumstances there is no need to add anything besides what is delivered with PetroFix. If needed or desired, small amounts of other nutrients like diammonium phosphate can be added to PetroFix for injection as it is water soluble and doesn't interfere with the chemistry.

Q21: Have you had any success delivering PetroFix via injection wells?

Yes, this product can be applied in injection wells and more so for sites where the wells were specifically designed for pressure injection at targeted intervals. However, achieving distribution would have to take into consideration the limited ROI and distribution potential for pressure injection of PetroFix and whether a large number of injection wells makes economic sense over direct push.

We realize there may be situations where distribution in deep aquifers or the hybrid use of both direct push injection and injection wells may be desired at a site. Ideal well construction would have 30-slot screen with 8/16 or 6/9 sand pack and if using schedule 40 PVC you would have a maximum operating pressure of 166 psi for a 2-inch well. We anticipate that most injections would require pressures well under this maximum operating pressure. However, it is possible that depending on depth and type of soils higher pressures could be realized without initiating fracturing and you may want a higher rated well material such as schedule 80 PVC which has a maximum operating pressure of 243 psi for a 2-inch well. We recommend that the injection screens be positioned at the target injection zones and that the screen doesn't go above the water table. We also recommend strategic use of solid sections of pipe and bentonite grout seals as needed to maintain discrete injection intervals and pressures. Given the relatively high cost to install injection wells it would be worth performing a well injection pilot test before full scale. For higher permeability environments one could consider pump-and-pull well arrangements for enhanced distribution.

Q22: Is PetroFix only suitable for groundwater contamination, or is it applicable to strictly soil contamination above aquifers?

PetroFix, when properly designed, will prevent any contamination from passing through the flux zone where it is placed. As such, it is ideally suited for saturated environments and mostly applicable for groundwater contamination. We cannot rule out the innovation of our clients who might find ways to inject the material in flux zones above the groundwater to prevent downward migration which would pass into PetroFix zones, which is feasible, but far more challenging to accomplish. Any such approaches should be pilot tested at small scale in the field.

Q23: Do you have rules-of-thumb for how many pounds of PetroFix are needed per pound of hydrocarbon?

We do not have rules-of-thumb for how many pounds of PetroFix are needed per pound of hydrocarbon. Because we use independent carbon isotherms for each hydrocarbon compound and because different soil types alter carbon loading calculations using our isotherms we perform independent calculations for each site to get the best estimate for PetroFix dosage.



PetroFix and General Questions

Q24: What is the difference between the PetroFix technology and PlumeStop/ISCO?

The difference between PetroFix and PlumeStop, both carbon injectates, was explained in the webinar. Key points would be that PetroFix has the advantage of having more carbon than PlumeStop, but at the cost of decreased radius of influence (in part due to a lack of the polymer coating that PlumeStop has). Using ISCO to reduce mass would benefit subsequent application of either PetroFix or PlumeStop. One key advantage of PetroFix if used with ISCO is that the treatable concentration range for PetroFix is much higher than for PlumeStop and if tight injection spacing is not an issue, PetroFix would be a good polishing choice offering high performance. Also, since most aggressive ISCO source mass reductions involve two or more back-to-back injections of oxidant, in some situations the higher ranges that PetroFix can treat could eliminate an oxidation injection event, thereby lessening overall field activity and saving money.

Q25: Does the "black" GAC coated soil color remain permanent in the ground forever?

Yes, since PetroFix permanently sorbs to the surface of soil it is expected to stay in place and color the soil "black" permanently.

Q26: How many years of data do you have to show that the carbon continues to adhere where it is injected?

We have lab and field evidence that show us the activated carbon remains in place indefinitely. We have performed hundreds of laboratory column experiments with our activated carbon products in a variety of soils to understand their transport and deposition. Our lab data conclusively show the activated carbon remains in place under most typical aquifer conditions. In the field we have site data going back about four years showing expected performance that can only be possible if the carbon remains in place. On occasion we see grey water in downgradient monitoring wells for a few months after an application. This indicates a small amount of the injected material remains mobile and it usually completely stops within three months.

Q27: What countries and states have approved the use of PetroFix so far?

At the time of this writing (March 2019) and only 5 months into product launch PetroFix has been used or approved for pending applications in CA, CO, CT, IN, KY, MA, PA, WA, FL and NJ as well as parts of the EU and Canada. In addition, we are actively engaged in the approval process for PetroFix in the state of Florida and believe this state will be added to the list soon.

Q28: Any kick back from regulators when PetroFix gets in surrounding monitor wells?

We have not had projects stopped by concerns of PetroFix coming into contact with monitoring wells and we are aware of the reasons why it might be a concern. In comparison to high pressure fracking of PAC products, the activated carbon particles in PetroFix are very small and which allows for distribution through soil and aquifer pores under natural flow conditions. By simply applying a PetroFix suspension through wells or direct push points, the PetroFix particles simply flow with groundwater through existing flux zones, coating the aquifer material surface with PetroFix particles. No artificial preferential pathways are formed which could irreversibly pack the well with powered activated carbon. During injection to the subsurface PetroFix may flow into nearby monitoring wells, as evidenced by sampling the well and finding the water color to be black. This occurrence is not uncommon and is simply the result of the PetroFix material flowing out into the natural flux zones of the subsurface.

The injection of PetroFix is intended to coat the contaminated aquifer matrix with the PetroFix particles. Much of this process occurs immediately as the process of having all particles drop from suspension and binding to the aquifer matrix may take up to 1-3 months. To ensure that PetroFix entering monitoring wells at the time of injection does not drop from suspension within a monitoring well bore and filter pack, the material can be flushed from impacted wells upon completion of injection activities. This simply pushes any PetroFix suspended within the monitoring wells back out into the aquifer where the particles will drop from suspension. This step helps to preserve the integrity of the monitoring well and ensures that water samples obtained from the well in the future are representative of groundwater within the aquifer itself. REGENESIS can provide specific instructions for the flushing of monitoring wells.

Q29: Carbon is added as a 1-2 µm diameter suspension. What about the electron acceptors, in terms of particle size?

The electron acceptors are either water soluble (nitrate and much of the sulfate) or added as a 325 mesh fine powder that serves as the slow-release sulfate source. This powdered calcium sulfate is present in a low enough amount to not cause injection/transport issues.

Q30: Do you see an opportunity in the Oil and Gas Field for well-site remediation?

Yes, we are actively exploring new fields where PetroFix would be useful. Addressing groundwater contamination at current or prior operation E&P facilities falls within the potential scope of this product.

Q31: Can I have REGENESIS develop a PetroFix design for me instead of my own involvement? When can REGENESIS do this?

PetroFix ideally is designed for self-design and self-application of the product. However, there are situations where the combined use of PetroFix with other technologies is appropriate and in those situations REGENESIS can become more involved in the design process. An example would be if preliminary ISCO activities were proposed ahead of PetroFix for mass reduction of LNAPL followed by PetroFix as a polish. REGENESIS can provide design services for these scenarios. Please contact REGENESIS if you have questions.

Q32: How do you recommend addressing data from PetroFix-impacted monitoring wells?

As long as free PetroFix is visibly suspended within the well water it is best to wait until the well clears up naturally. This can be facilitated by flushing clear water into the well which will push free particles back into the aquifer. Wait a reasonable period of time before sampling and purging to ensure that the sample volume collected is indicative of aquifer waters. In the event that you cannot wait to sample before clarification, it is possible to filter PetroFix out of samples to get a true dissolved phase concentration. Please contact Regenesis if you need help with this.

Furthermore, PetroFix is not like powdered activated carbon products that disrupt the filter pack around the well screen, irreversibly filling the filter pack pores with powdered activated carbon. PetroFix particles are small enough to move freely into and out of the filter pack with the natural flow of groundwater. PetroFix entering the filter pack material will coat that portion of the filter pack adjacent to the flux zone with a thin layer of PetroFix particles. In many cases, due to the much lower surface area available within the filter pack material, however, the amount of PetroFix retained there should be substantially less than would have been left behind on the native aquifer material had the well not been present. Please also refer to the answer to question 28.

Q33: How do you derive your design calculations?

Once values are entered into the PetroFix Design Calculator the amount of PetroFix is calculated in this order:

1. Treatment volumes and effective porosity are estimated based on soil type;
2. PetroFix activated carbon isotherms are used to estimate carbon loading rates for each individual hydrocarbon or hydrocarbon range to achieve two order-of-magnitude groundwater reductions. Most of the isotherms that REGENESIS uses are determined from our own laboratory experiments.
3. Estimated sorbed mass is calculated using textbook values of Koc and foc for respective contaminants and soil types.
4. For application, an estimated total injection volume is chosen to achieve appropriate effective pore space filled.
5. The required product quantity is rounded to the nearest drum.

Q34: What is the price per pound or gallon of PetroFix?

Unit rates for PetroFix start at \$3.95/lb or lower depending on amount ordered. Proper unit rates are calculated when you complete a PetroFix design in the PetroFix Design Assistant at www.petrofix.com/design.

For PetroFix designs and pricing in Europe, please contact your local REGENESIS representative or email europe@regenesis.com.



PetroFix and Case Study Questions

Q35: Why aren't there any measurements of soil concentrations before and after?

We might have a chance to do this on the Indiana site. We collected a few baseline core samples that we can compare with.

Q36: Has REGENESIS quantified mass removal by biodegradation (vs sorption) on the 2 examples provided in the presentation? This question is in relation to forecasting a probable timeframe for the site to meet applicable criteria (groundwater or soil).

We have not done this in the field but we have extensive lab data like this. We set product dosing to a level that should remove hydrocarbon contaminants from the groundwater and surrounding soil and keep groundwater concentrations low or undetectable for the length of time needed for full contaminant biodegradation. As pointed out already our microbial and geochemical data support in situ biodegradation is occurring.

Q37: Were qPCR cell counts adversely affected by the sorption of bacterial DNA/RNA to PetroFix? Were qPCR tests run on groundwater in the absence of PetroFix to evaluate this potential?

We have exclusively measured groundwater samples. All samples sent to Microbial Insights for post-PetroFix application analysis were free of activated carbon, so this was not a concern.

Q38: For the South Bend site, the table shows that the contaminants were showing up after treatment. What is the possible cause of that? Does that mean that the PetroFix used was not enough?

The Indiana site was very contaminated by most consultants standards and exceeded 50 mg/L total BTEX, TPH-G and TPH-D. The location chosen for the PetroFix injection was within a greater plume area and prone to additional influx of contaminants. Despite this, we did achieve significant reductions across the board. What we have is a situation where the amount of PetroFix injected and its ability to sorb and the rates of anaerobic biodegradation are being exceeded by the flux of incoming contamination from upgradient and even combined with back diffusion from within the treatment area as well. The treatment could be improved by treating upgradient contamination, which will actually be the case for this site which will have PetroFix applied full-scale later this year. One of the positive features of PetroFix is that the solution isn't necessarily to inject more PetroFix. The solution can be to supply additional electron acceptors (ORC-A or additional nitrate/sulfate blend) to accelerate biodegradation and regenerate the PetroFix carbon.

Q39: How would you characterize the Eh trend through the full in situ treatment process? I ask because this insoluble metals (such as arsenic) can be released due to changing Eh conditions.

The ORP does become more positive following PetroFix treatment or any other remedial efforts that remove the chemical and biological oxidant demand associated with the contamination. Unless the site is co-contaminated with metals or metals were a problem prior to petroleum contamination being present, treatment with PetroFix will not create a problem.

Q40: Have you conducted any testing of the solid media in the injection areas to evaluate the COC concentrations in that media over time?

From lab studies we know hydrocarbons, and specifically benzene is biodegraded by doing full-bottle extractions on batch experiments. After incubating with benzene, activated carbon, soil and water, an entire bottle is extracted with another solvent and we see all benzene is gone. In control experiments like this (killed/sterile bottles) the benzene can be recovered from the activated carbon at very high recovery percentages, showing biodegradation is the removal process. *REGENESIS PlumeStop technical bulletin 3.1* is available on regenesisc.com showing a post-sorption contaminant biodegradation lab study with benzene that documents benzene biodegradation on the activated carbon (PlumeStop and PetroFix use the same diameter carbon and this lab study is representative for PetroFix as well). In the field, we rely on multiple lines of evidence to show ongoing biodegradation after adsorption. The utilization of added nitrate and sulfate in the right pattern (nitrate first, sulfate second) is the first line.

We have not done this in the field but we have extensive lab data like this. We set product dosing to a level that should remove hydrocarbon contaminants from the groundwater and surrounding soil and keep groundwater concentrations low or undetectable for the length of time needed for full contaminant biodegradation. As pointed out already our microbial and geochemical data support in situ biodegradation is occurring.

Q41: Did the bacterial cell counts increase following the addition of the PetroFix? If so what were the cell counts before and after injection? ¶The slides showing bacterial marker trends didn't seem to support bacterial growth as a result of the addition of PetroFix, but rather that the addition of PetroFix didn't adversely affect the existing populations and/or markers.

In the short term, we see that PetroFix does not adversely affect the microbial population. This is significant by itself because we are removing most or all of the petroleum hydrocarbons from the groundwater. If the adsorbed hydrocarbons were not bioavailable we would expect to see drastic drops in cell/gene counts, which does not occur. Over time on the Indiana site we have seen the cell/gene counts rise above what they were at baseline in some cases even with dissolved TPH reduced by 1 OOM.

Q42: What are the starting concentrations of sulfate and nitrate in the PetroFix suspension injected into the groundwater and what concentrations were achieved in groundwater post injection?

On the Indiana site sulfate and nitrate were injected at around 1400 mg/L. We filled (roughly) half the effective pore space and through dilution and mixing the measured sulfate concentration a month post-application was roughly 500 mg/L. Nitrate was never measured this high because it is consumed so rapidly, and was already about 70 mg/L.

Q43: How was daylighting of treatment suspensions at previous injection bore holes prevented given the tightly spaced injection grid of 5-7 feet on center?

As a standard practice we recommend using a granular bentonite seal (not chip) to seal prior injection holes to prevent surfacing.

PetroFix: The technology at a Glance

PetroFix[™] is a cost-effective, dual-functioning activated carbon solution designed to remediate petroleum spills and provide immediate results for gas station and UST sites, including domestic oil spill sites.

A Dual-Functioning, Activated Carbon Remedial Technology for Treating Petroleum Hydrocarbons

PetroFix has a dual function: it removes hydrocarbons from the dissolved phase by adsorbing them on to activated carbon particles and then stimulates hydrocarbon biodegradation by adding electron acceptors. PetroFix is a highly concentrated water-based suspension consisting of micron-scale activated carbon and biostimulating electron

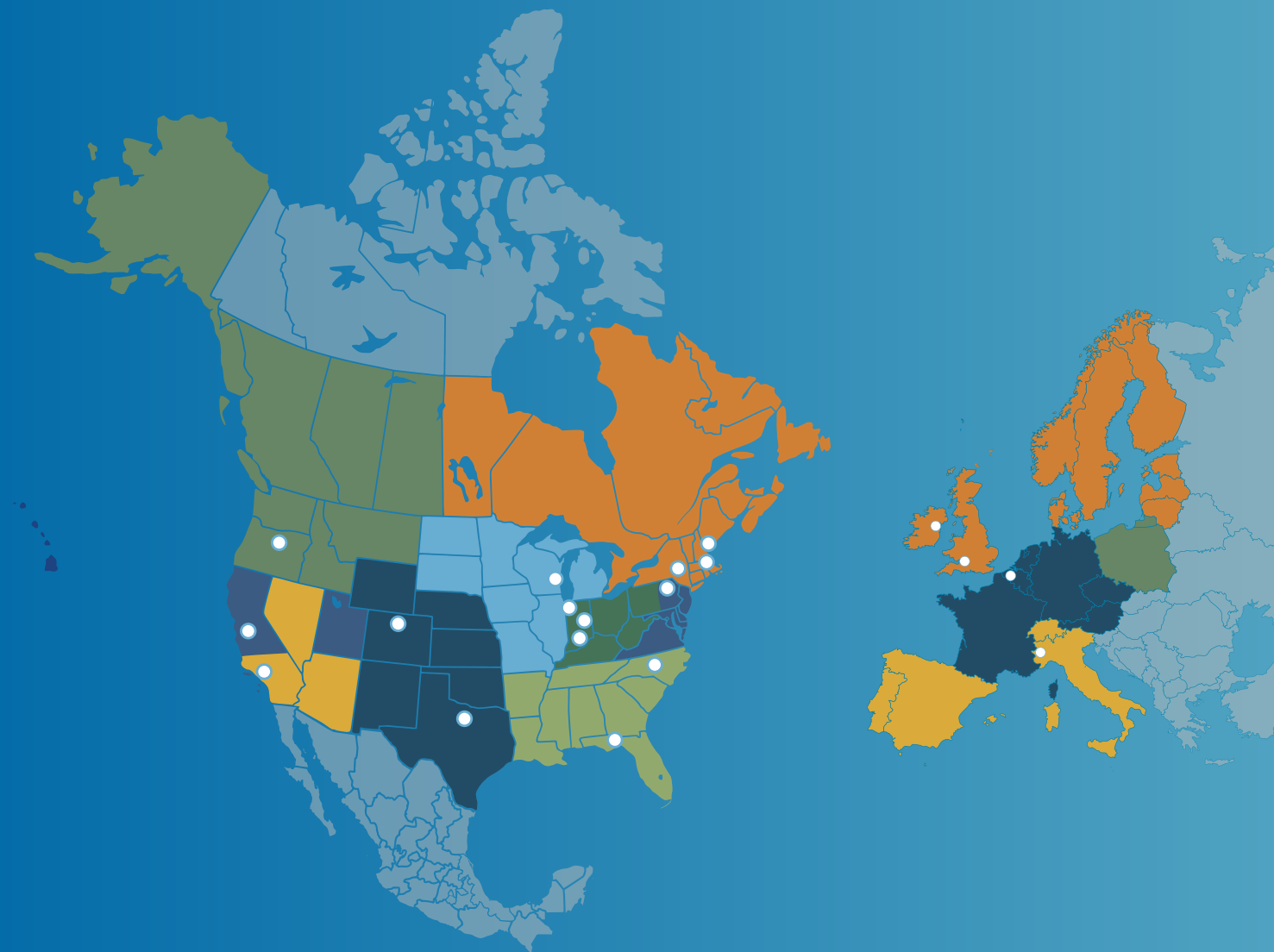
acceptors. The environmentally-compatible formulation of micron-scale activated carbon (1-2 microns) is combined with both slow and quick-release inorganic electron acceptors. Practitioners can select between a sulfate and nitrate combination blend (recommended) or sulfate only for the additional electron acceptors required.



- Affordable and reliable
- Applied under low pressure
- Tailored site design



WE'RE READY TO HELP YOU FIND THE RIGHT SOLUTION FOR YOUR SITE



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