DEQ
Montana Department of Environmental Quality

Memo

TO: Tom Livers, Director

THRU: Jenny Chambers, WMRD Division Administrator
Tom Stoops, Federal Superfund Bureau Chief

FROM: David Bowers, Montana Pole State Project Officer

DATE: April 2, 2018

SUBJECT: **Palmer Letter (October 20, 2017) Re: Montana Pole and Treating Plant – More Consideration and Evaluation of Alternatives**

The release of the *Fourth Five-Year Review Report for the Montana Pole and Treating Plant*, finalized June 30, 2017, by the Environmental Protection Agency (EPA), and subsequent Department of Environmental Quality (DEQ) public presentations last year in August and September evoked a comment letter (Exhibit 1) from Dave Palmer, Chief Executive, Butte-Silver Bow Council of Commissioners. In November 2017, all but one of the questions and/or comments in the letter were addressed in a formal response (Exhibit 2) from DEQ to Chief Executive Palmer.
The remaining comment/question requested that DEQ "validate, explain and communicate to the public, the [sic] dioxin cannot be "re-treated" and remediated to meet acceptable standards . . .." DEQ appreciates these concerns and stresses that when selecting a cleanup for a Comprehensive Environmental Cleanup and Responsibility Act (CECRA; a.k.a. State Superfund) or Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; a.k.a. Federal Superfund) site, DEQ follows EPA's Guidance for Conducting Remedial Investigations and Feasibility Studies (RI/FS Guidance) Under CERCLA (October 1988). By carefully and routinely following the RI/FS guidance, DEQ's State Project Officers have become very familiar with proven technologies. Our consultants and technology vendors regularly update DEQ Project Officers on emerging remedial technologies. The technology review and selection component of the RI/FS process ensures that technologies are selected based on merit and not arbitrary and/or careless actions.

Regardless, DEQ is committed to providing the necessary information to validate, explain and communicate why it is not technically advisable or feasible to "re-treat" the remaining dioxins (dioxins) at the Montana Pole and Treating Plant (Montana Pole) site. The limitations and challenges found in screening and selecting dioxin technologies used at wood treating sites such as Montana Pole are presented in the following sections:

1. Initial Alternatives Screening Document (IASD) for a Wood-Treating Waste Site in Montana;
2. Feasibility Study for a Wood-Treating Waste Site in Montana;
3. Biological Remediation (Fungal-Based) for Wood-Treating Waste Sites;
4. Tetra Tech National Corporate Inquiry for New Technologies at Wood-Treating Waste Sites.

INITIAL ALTERNATIVES SCREENING DOCUMENT FOR A WOOD-TREATING WASTE SITE IN MONTANA

A helpful start to understanding the current state of dioxin remediation is to examine a recently developed Initial Alternatives Screening Document (IASD) for a Montana site containing pentachlorophenol (PCP), petroleum constituents, and dioxins. The S&W Sawmill site (S&W site) in Darby, Montana, is being addressed by DEQ under State Superfund and, by adhering to the RI/FS guidance, an IASD was completed in September 2017.

International Paper is identified as the lead responsible party for cleanup of the S&W site. From 1961 to 1978, portions of the S&W site operated as a wood treatment plant that used PCP mixed with diesel as a wood preservative, the same process as Montana Pole, and therefore useful for comparison purposes. The chemical mixture and its by-products (dioxins) contaminated the soil and groundwater. The S&W Sawmill is currently not in use (S&W Sawmill Fact Sheet; Exhibit 3).

An IASD is the first step in the RI/FS alternatives identification and selection process. The purpose of the IASD is to develop a comprehensive list of potential treatment and disposal technologies and screen them based on effectiveness, implementability, and relative cost. The S&W site IASD (Exhibit 4) provides a current list of treatment technologies used for PCP, petroleum constituents, and dioxin remediation. From this list, selected alternatives were carried forward to be screened again. For the second screening, the technologies are evaluated for effectiveness, implementability, cost, availability, and reliability and maintainability. Currently underway, this process will result in a short list of alternatives that will be retained for the detailed evaluation that is performed in the feasibility study.

The S&W site IASD includes 42 potential treatment technologies listed for soil treatment, as well as additional technologies for groundwater. Most of these technologies are not stand-alone and will be retained for possible use in conjunction with other technologies. In fact, the only technologies considered to be stand-alone are incineration and excavation for off-site hazardous waste disposal. The lack of stand-alone technologies is, in large part, due to the challenges with remediating dioxin contamination to meet cleanup levels.

FEASIBILITY STUDY FOR A WOOD-TREATING WASTE SITE IN MONTANA

We also examined the 2015 feasibility study report (FS; DEQ, 2015; <http://deq.mt.gov/Portals/112/Land/StateSuperfund/Documents/MWPS/FinalFSReport2015_2complete.pdf>) for the White Pine Sash (WPS) site in Missoula, a historic wood treatment site also contaminated by PCP and dioxins. The WPS FS represents the current technology selection process for wood treating wastes. The following soil remediation technologies were retained for a detailed evaluation in the FS:

1. No Action;
2. Excavation and offsite disposal;
3. Excavation and Onsite Ex-Situ Treatment (enhanced bioremediation, chemical oxidation, incineration);
4. Excavation and Onsite Spreading (methane-containing soil only);
5. In-Situ Treatment (enhanced bioremediation, chemical oxidation, soil flushing, soil vapor extraction); and
6. Containment.

No Action
The no-action alternative will not achieve cleanup objectives; however, it provides a baseline for comparison with other options and alternatives (DEQ, 2015). Including the no-action alternative is standard procedure when performing an alternatives analysis.

Excavation and Off-site Disposal
Off-site incineration for wood treating waste, which is considered a hazardous waste when it contains PCP, is a standard disposal option. A Resource Conservation and Recovery Act (RCRA) permitted hazardous waste incineration facility would perform the incineration. Note: Soil containing dioxins, but not PCP, is considered nonhazardous waste and can be disposed of at a local solid-waste landfill (DEQ, 2015).

Excavation and On-site Ex-Situ Treatment (enhanced bioremediation, chemical oxidation, incineration)

Enhanced Bioremediation
Bioremediation is a presumptive remedy for organics associated with wood treating sites in soil (EPA, 1995). For the purpose of evaluation, bioremediation was considered for all contaminated soils containing PCP, petroleum compounds, and dioxins. Experience with this technology at other sites indicates that degradation is incomplete for dioxins. Therefore, dioxins-contaminated soil co-located with PCP that is not treated to cleanup levels through bioremediation would need to be addressed in another manner. (DEQ, 2015).

Treated soil that meets all cleanup levels can be used as backfill onsite (e.g. in the original excavation), and is included in the cost estimate. However, as described above, treated soil may not meet cleanup levels for dioxins; therefore, it may need to be disposed at a local solid-waste landfill (provided it meets RCRA hazardous waste requirements) or placed into an onsite dioxins repository (DEQ, 2015).

Chemical Oxidation

Chemical oxidation is becoming a proven technology for many contaminants. Chemical oxidants are intended to destroy contaminants of concern (COCs) and some oxidants are generally accepted as being effective in oxidizing organic chemicals such as PCP and petroleum hydrocarbons. However, the ability of chemical oxidants to oxidize dioxins is less certain (DEQ, 2015). In the FS, various chemical oxidation laboratory-scale treatability studies and field-scale pilot tests were conducted for the White Pine Sash site, as well as other similar facilities (Davis Post Yard site; S&W Sawmill site; and the Kalispell Pole & Timber, Reliance Refinery, and Yale Oil site) in Montana (DEQ, 2015). The chemical oxidation of dioxins was much less effective (30 to 45% decrease) than in soil under ideal laboratory (bench-scale test) circumstances (complete saturation and thorough mixing). In the field test that followed, the reduction in dioxin concentrations was even less than the bench-scale test with an average concentration reduction of 5 percent. As in the bench-scale tests, oxidation of dioxins was much more limited than the oxidation of PCP, and field conditions appear to have hampered dioxin oxidation even further compared to the ideal conditions of the bench-scale test (DEQ, 2015).

On-site Incineration

There are significant uncertainties associated with an on-site incinerator, including the ability to meet cleanup levels and meet Clean Air Act regulations. Additionally, a RCRA treatment, storage, and disposal permit would be required for this alternative, which would require significant time and expense to obtain. It is unlikely such a permit could be acquired for this location because it is in City limits next to a residential area (DEQ, 2015).

Excavation and Onsite Spreading (methane-containing soil only)

Applies only to methane-containing soils and, therefore, does not apply to Montana Pole.

In-Situ Treatment (enhanced bioremediation, chemical oxidation, soil flushing, soil vapor extraction)

Soil in-situ treatment technologies include methods that either separate and remove contaminants or degrade the contaminants in place (DEQ, 2015).

Enhanced Bioremediation

In-situ bioremediation of soil is inherently more difficult, and usually less effective, than ex-situ bioremediation due to the variable nature of soil and uncertainties in the ability to deliver amendments uniformly throughout the entire soil matrix (EPA, 1991). In-situ

bioremediation of soil can be used to treat contaminated soils that could not be feasibly excavated and would be applicable to areas containing PCP and petroleum hydrocarbons (DEQ, 2015).

Chemical Oxidation

Chemical oxidants are intended to destroy COCs and some oxidants are generally accepted as being effective in oxidizing organic chemicals such as PCP and petroleum hydrocarbons. However, the ability of chemical oxidants to oxidize dioxins is less certain (DEQ, 2015).

Soil Flushing

While soil flushing was considered for other contaminants at the White Pine Sash site, it had never been applied at wood-treating facilities or involved PCP or dioxins. Soil flushing (using solvents) also comes with considerable risk of mobilizing contaminants into uncontaminated areas (DEQ, 2015).

Soil-Vapor Extraction (SVE)

Per EPA's presumptive remedy guidance for wood-treating contamination, SVE is not capable of directly remediating PCP or dioxins due to their extremely low volatility relative to other VOCs such as petroleum constituents. However, by removing the carrier (petroleum constituents), the mobility of PCP and dioxins may be reduced, since both are much more soluble in petroleum than in water. In addition, removing the petroleum carrier and introducing oxygen to the subsurface may enhance the ability of PCP to biologically degrade in situ (EPA, 1992; DEQ, 2015).

Containment

Per the WPS FS,

> *Soil barriers, such as a horizontal cap, can be used to minimize exposure, prevent vertical infiltration of water and leachate, contain waste while treatment is being applied, control vapor and odor emissions, or to create a land surface that is suitable for the intended reuse of the property. Capping is the most common form of barrier remediation because it is generally less expensive than other technologies and may effectively manage the human health risk* (DEQ, 2015).

Containment can be implemented in numerous ways including a soil cap, a concrete/asphalt cap, and/or a clay/geosynthetic membrane. Caps can eliminate the direct-contact exposure pathway and eliminate leaching of contaminants through the subsurface soil to groundwater as a result of precipitation infiltration (DEQ, 2015).

Per the FS,

> *. . . containment was retained for more detailed analysis – applicable to soil on the SSLLP property containing dioxins at concentrations that exceed SSCLs* (cleanup levels)*, as well as soils from the residential area, and the former treating and AST areas and treated soil that may meet SSCLs for all COCs except dioxins* (DEQ, 2015).

The practice of capping treated soils (containment) was very much in line with the cleanup of wood-treating sites throughout the United States, as noted in a September 1995 report from the U.S. Congress, Office of Technology Assessment titled "Cleaning Up Contaminated Wood-Treating Sites," *(OTA-BP-ENV-164 Washington DC: U.S. Government Printing Office, September 1995):*

> *Even after the best cleanup of a wood-treatment site some contaminants will remain … Physically capping a site is particularly useful to complete the overall protection of a complete wood-treatment cleanup strategy.*

Due to the challenges and limitations with remediating dioxin contamination to meet cleanup levels, containment remains the preferred final-phase alternative at most wood-treating waste sites. It is important to remember that *institutional and/or engineering controls* (containment) *alone do not satisfy CERCLA's preference for achieving reductions of toxicity, mobility, or volume through treatment as a principal element of the remedy. However, the use of institutional and/or engineering controls* (containment) *after the treatment of a principal threat . . . can enhance the long-term reliability of the remedy* (EPA, 1994). At wood-treating sites throughout Montana, containment is being used as the final phase for various treatment trains to address residual dioxins contamination that exceeds cleanup levels.

BIOLOGICAL REMEDIATION (FUNGAL-BASED) FOR WOOD-TREATING WASTE SITES

Bioremediation and specifically fungal-based remediation have been the center of much discussion around the remediation at Montana Pole. While fungal-based remediation using white rot fungi (WRF) has shown promising results in the laboratory, limited field studies have shown it to be less effective. There are three key reasons why WRF is still considered an emerging technology:

1. Extremely low dioxins cleanup levels, often below 1 part per billion or 1 microgram per kilogram (ug/kg) are hard to achieve;
2. The variability of soil conditions found in the field is hard to replicate in the laboratory;
3. The cultivation and delivery of WRF is expensive.

The Montana Pole dioxins recreational cleanup level, per the October 2017 DEQ memorandum (Exhibit 5), is 100 parts per trillion (ppt) or 100 nanograms per kilogram (ng/kg). To better understand the realm of ppt, one ppt is equivalent to one second in nearly 32,000 years. Using this analogy, the Montana Pole recreational dioxin cleanup level is equivalent to 1 minute 40 seconds in 32,000 years.

The relationship between cleanup level and the mass of contamination found in the contaminated soils becomes crucial when considering the effectiveness of a cleanup technology. As an example, 30 dioxins samples were taken for the treated soils in the 2007 off-load at Montana Pole (Exhibit 6). The range in concentrations was 900 ng/kg to 9,100 ng/kg. Dioxin concentrations in this range would require a cleanup technology to achieve 99 percent removal

efficiency to meet the Montana Pole recreational cleanup level of 100 ng/kg. Contaminant removal efficiencies greater than 90 percent are daunting, even for the most effective cleanup technologies.

Examining the research and development of WRF as a cleanup technology helps clarify the relationship between contaminant mass and contaminant mass removal. One promising laboratory-scale evaluation of WRF effects on PCP and three specific dioxins congeners (HpCDF, HpCDD, OCDD) was performed in New Zealand by EarthFax Development Corp. (EarthFax) and University of Waikato for Carter Holt Harvey SPHERE (Exhibit 7).
The results of two laboratory-scale studies were very promising. The mean treatment percent decrease for WRF application rate of the three congeners in Study 1 ranged from 78.9 to 96.1 percent at a 10 percent WRF application rate. At a 20 percent WRF application rate the mean treatment percent decrease ranged from 75.8 percent to 95.9 percent.
In Study 2, using one (B101) of two fungi isolated from highly PCP-contaminated soil, the mean treatment percent decrease for the three congeners ranged from 63.6 to 94.7 percent. Using the second fungi (B102), the mean treatment percent decrease ranged from 53.3 percent to 93.5 percent.

The conclusions for the two WRF studies included:

- PCP/PCDD/PCDF contaminated soils had “rapid and extensive” decreases in contaminant concentrations;
- Treatment of soils contaminated with PCP/PCDD/PCDF has excellent potential.

It was also noted that further work was needed and/or underway at pilot scale with the goal of getting the technology to full scale and on a commercial basis. It’s important to note that even with these promising results, it is unlikely that the dioxin contaminate mass in the off-loaded soils at Montana Pole would be reduced to the recreational cleanup level of 100 ng/kg.
One of the reasons the New Zealand study is of interest is that EarthFax went on to perform large-scale production and field testing of WRF at the S&W Sawmill site in Darby, Montana (Exhibit 8). The field study began in August 2004. Five in-ground biocells were constructed with one of the cells acting as the control cell. In the other four cells, WRF was mixed into the contaminated soil at rates of 5 percent for two cells and 10 percent for two cells.

The results were very promising for the treatment of PCP with contaminant mass reductions of between 70 percent (5% WRF) and 90 percent (10% WRF). The contaminant mass reduction for dioxins at 10 percent WRF ranged from 25.7 to 36.3 percent, while the biocells with 5 percent WRF exhibited an apparent increase that ranged from 13 to 81.2 percent. The control biocell came in at 8.6 percent mass reduction of the dioxins. The increases in the biocells with 5 percent WRF were attributed to biopile soil variability. To improve the removal rate for dioxins, EarthFax suggested the addition of a surfactant (emulsified soybean oil) would help mobilize the dioxins out of the soil.

The overall results for the WRF technology were very promising, especially for PCP. However, the field testing also underscored that WRF treatment technology faced challenges for remediating dioxins. Cleanup levels would be difficult to achieve due to the soil variability and persistent hydrophobic nature of dioxins. WRF treatment for dioxins would require further research if the technology hoped to obtain a higher efficiency of removal.

The field test report also provided an estimate of cost for the WRF inoculum. In 2005 the cost for enough WRF to treat 1 ton of contaminated soil was estimated to be $29. Assuming a 1:1 ratio of cubic yards to tons, the cost to purchase enough WRF inoculum to treat 200,000 cubic yards of Montana Pole soil would be $5.8 million. Remaining tasks would include mixing batches of soil and placing each batch in the land treatment unit (LTU) for at least a full year. The cost to get the WRF on site and properly apply it to the 200,000 cubic yards of soil would be a large financial risk considering that containment/capping would likely still be required after treatment because the technology could not reduce the dioxins contaminant mass to the required recreational cleanup level.

A review by Dawen Gao, et. al, *A Critical Review of the Application of White Rot Fungus to Environmental Pollution Control* (Exhibit 9), identified another EarthFax Engineering project using WTF to remediate PCP and dioxins. The mass contaminant removal range was 61 to 87 percent for select congeners over a 282-day period.

The review goes on to identify limitations and technical challenges for use of WTF in bioremediation and concludes,

> *The development of biotechnologies using white rot fungi has been implemented to treat various refractory wastes and to bioremediate contaminated soils. Degradation of many hazardous chemicals and wastes has been demonstrated on a laboratory-scale, especially under sterile conditions. The technical challenge remains for the application including bacterial contamination and for the scale-up of the process. The white rot fungus P. ostreatus has been applied for scaled-up bioremediation in the field. More research and development is still needed for cost-effective and sustainable application.*

As a final note, a recent paper by Susana Rodriguez-Couto, *Industrial and Environmental Applications of White-rot Fungi* (Exhibit 10; Exhibit 10a) provides an update for WRF as a technology for bioremediation of environmental pollutants:

- WRF shows promising potential for the treatment of industrially-contaminated soils, but few recent attempts have been made to use WRF despite its clear advantages over bioremediation using bacteria.
- Numerous environmental variables (pH, nitrogen limiting conditions, WRF long growth cycle) make maintenance of WRF difficult in bioreactor applications.
- WRF at full scale on a commercial/industrial basis continues to be a technical challenge.

NATIONAL CORPORATE INQUIRY FOR NEW TECHNOLOGIES AT WOOD-TREATING WASTE SITES

As part of DEQ's efforts to identify any possible new technologies, it asked Tetra Tech, a company that performs remedial cleanups world-wide, to have its Remedial Strategies team to perform a query for dioxin cleanup methods alternative to containment/capping and incineration. Two responses in the memo (Exhibit 11) from the team were noted:

1. Depending on dioxin concentrations and hazardous waste determinations, incineration or landfilling was typically used.
2. The other response included incineration or landfilling, but also indicated that a project at the Vietnam Airport in Danang was using thermal desorption for dioxin removal.

No other replies were received and biodegradation of dioxin was not identified as an alternative. A note regarding thermal desorption. A quick search (Exhibit 12) found that it costs about $70 million to treat 200,000 cubic yards; it has the potential to be very effective at 96 percent removal and higher (still not a high enough rate to address all dioxin concentrations at Montana Pole); and the technology has had isolated issues with air emissions containing dioxins at unacceptable levels.

CONCLUSION

DEQ's routine oversight of the RI/FS alternatives identification and selection process at federal and state superfund sites keeps the agency closely involved with remedial technologies – emerging and proven. Careful examination of a recent IASD and a feasibility study for two Montana wood-treating waste sites demonstrates that dioxin remediation technologies continue to be limited. An update on the status of bioremediation, specifically the use of white rot fungi (WRF), identifies the challenges faced in developing WRF as a remedial technology for dioxins. Finally, Tetra Tech (a national company that performs environmental cleanups worldwide) was tasked to perform a query into remedial alternatives other than consolidation and capping, and incineration. The query produced limited results aside from consolidation and capping, and incineration.

DEQ is confident that it has thoroughly and carefully considered all available treatment alternatives for the Montana Pole Site, including bioremediation. In doing so, DEQ accomplishes the intent of this memo:

1. validate, explain and communicate that dioxin cannot be "re-treated" and remediated any further to meet Montana Pole cleanup levels;

2. validate, explain and communicate that containment (capping) will provide a protective solid barrier between buried, off-loaded/treated soils containing dioxin and the surface and its everyday users.

EXHIBITS

Exhibit 1: Butte-Silver Bow (Chief Executive Palmer) Letter to DEQ (Director Livers). October 20, 2017.

Exhibit 2/2a: DEQ (Director Livers) Response Letter to Butte-Silver Bow (Chief Executive Palmer) & Addendum to Response Letter. November 22, 2017.

Exhibit 3: S&W Sawmill Fact Sheet. December 2014.

Exhibit 4: S&W Sawmill Initial Alternatives Screening Analysis. September 19, 2017.

Exhibit 5: Montana Pole Direct Contact Cleanup Level 5-Year Review. October 3, 2017.

Exhibit 6: Montana Pole Removal Efficiencies Table. February 28, 2018.

Exhibit 7: EarthFax Development Corp WRF Laboratory Scale Study. < https://www.wasteminz.org.nz/pubs/evaluation-of-fungal-based-remediation-for-treatment-of-a-pcpdioxinfuran-contaminated-soil-from-several-former-wood-treating-facilities-in-new-zealand/> 2003.

Exhibit 8: EarthFax Development Corp WRF Field Study in Darby, MT. August 2005.

Exhibit 9: Critical Review of Application of WRF to Environmental Pollution Control. March 2010.

Exhibit 10: Industrial and Environmental Applications of WRF. 2017.

Exhibit 11: Tetra Tech Internal Query for Applied Dioxin Remedial Technologies. November 20, 2017

Exhibit 12: In-Pile Thermal Desorption of Dioxin Contaminated Soil and Sediment. March 20, 2014.

REFERENCES

Montana Department of Environmental Quality (DEQ). 2015. Final Feasibility Study Report: Missoula White Pine Sash Facility, Missoula, Montana. February.

U.S. Environmental Protection Agency (USEPA). 1991. Guide for Conducting Treatability Studies under CERCLA: Aerobic Biodegradation Remedy Screening. EPA/540/2-91/013A. July.

USEPA.1992. Evaluation of Soil Venting Application. EPA/540/S-92/004. April.

USEPA. 1994. Office of Solid Waste and Emergency Response, Presumptive Remedies for Soils, Sediments, and Sludges at Wood Treater Sites. EPA/540/R-95-128. December.

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October 20, 2017

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Subject: **Fourth Five-Year Review Report for the Montana Pole and Treating Plant Site**

Dear Mr. Livers and Mr. Vranka:

As Butte-Silver Bow Chief Executive, with the concurrence of the Butte-Silver Bow Council of Commissioners, and on behalf of all residents of the Butte community, I submit this letter and comments regarding the *Fourth Five-Year Review Report for the Montana Pole and Treating Plant Site, May 2017 (**Report**)*. Despite the fact that we have been informed that there is no formal comment period on the ***Report,*** we hope the Agencies will accept these comments as meaningful input regarding the status of the cleanup at the Montana Pole Site.

The comments presented in this letter are in response to the Agencies' staff presentations to the Council of Commissioners on August 16, 2017, as well as at two other public meetings held thereafter at the Butte Chamber offices and the Racetrack Fire Training Center. Further, Butte-Silver Bow would also ask the Agencies to address and consider the comments and input that Butte-Silver Bow has subsequently received from concerned citizens regarding this ***Report*** and the status of the Montana Pole Site.

Public Process

Butte-Silver Bow appreciates recent efforts made by the Agencies to do a better job of disseminating information about the status of what's going on at the MT Pole Site. Although we have several issues with how the Site has been managed and the disappointing results of the cleanup (to date), as reflected in the ***Report***, we have noticed a marked increase in the effort to at least inform the public. We hope the Agencies will continue to commit the time and resources to keep the local government and citizens, particularly those near the Site, well informed of next steps.

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That said, there has been a marked failure to communicate effectively with the public over the past two years regarding the results published in the ***Report***. After 20+ years of site characterization, active remediation/treatment and management, the ***Report*** reveals a troubling lack of certainty and apparent understanding of the contaminants at the Site and their long-term fate, for example, not knowing the exact presence or extent of contaminants on the north side of Silver Bow Creek, whether remnant contaminants are present beneath the LTU liner, and there appears to be no cleanup standard for protectiveness to groundwater.

The revolving door of Site Managers and resulting lack of continuity has eroded community confidence significantly. Add to that the questionable remedial actions taken over the years, for example, offloading soils from the Land Treatment Unit known to be contaminated with dioxin; building a Water Treatment Plant on top of contaminated ground; removing sheet piling designed to protect Silver Bow Creek without understanding the full effects on site groundwater flows, not dealing with the wastes below Interstate I-90/15 when the bridges were replaced, among others. What assurances do we have that DEQ staff and contractors will be more attuned to Site operations and community needs in the future?

Another, most recent example of disconnected communications: Over the past 24 months, the Montana Pole Site was being seriously considered as a viable site option for the County Shops, which is categorized as an industrial use. But the ***Report*** (dated May 2017) indicates, and Agency staff have confirmed, that the proposed alternative plan to manage the site will restrict industrial use, mostly due to the presence of dioxin. The community should have been long before Butte-Silver Bow and the State's own Natural Resource Damage Program spent valuable time and resources to hold public, land use planning workshops and perform other evaluations to determine whether the Site could be used for the County Shops.

Poor Results on Cleanup/More Consideration and Evaluation of Alternatives

With all due respect, for the ***Report*** and Agencies' staff to state that "revision to the cleanup plan is evidence of progress at the Site" rings quite hollow in the neighborhood of the Site and throughout the greater community of Butte. That is not progress. Again, after more than two decades of Site activity, there were and are reasonable expectations that the contaminants would be fully remediated; to be told in 2017 that the cleanup technology, i.e., bioremediation, has not been successful in treating "all" the hazardous waste is utterly disappointing.

The ***Report*** explains that the selected technology did work well enough to clean the pentachlorophenol (PCP), at least to acceptable levels that will protect human health and the environment, but that the remediation technology did not work for other contaminants, particularly dioxin. Therefore, the Agencies have decided (we hope preliminarily) that the only option is to propose an alternative plan for the Site: Essentially, to re-excavate, move and consolidate any soil materials with unacceptable levels of contaminants to an on-site repository, and build a cap over the repository that will protect groundwater and eliminate any human exposure to the wastes. Further, the Agencies state, this proposed plan will require use restrictions and covenants of the surface property, aka institutional controls.

It would appear to Butte-Silver Bow that the Agencies are proposing to select an alternative plan based on convenience and cost, and have failed to consider, or at least failed to present information about their consideration of other alternatives. For example, have the Agencies considered the use of other treatment technologies that can remediate the remaining dioxin? If such an alternative was implemented, could then the entire site be made available for industrial uses, in addition to open space/recreational uses? Butte-Silver Bow would ask for the State to consider alternatives.

If, for reasons the Agencies will validate, explain and communicate to the public, the dioxin cannot be "re-treated" and remediated to meet acceptable standards, and then subsequently, the Agencies determine that building a protective cap is absolutely necessary, Butte-Silver Bow would ask the Agencies to consider enlarging the footprint of the protective cap area, thus allowing for less land use restrictions on the site. It should be noted that the original land use plan for the Site (1996), in its post-remedial/cleaned up condition, was a mixture of open space/recreational on the east end, commercial along Greenwood Avenue, and the bulk of the west end for industrial uses. This original plan was also reviewed and updated in the fall 2014 (as part of the County Shops Re-location discussion). These local land use plans for the Site should be given full consideration when deciding on any changes to the remedial action plan and the resulting impacts to the future uses of the property.

Water treatment plant operations

Inconsistent statements in the ***Report*** would seem to reveal how little the Agencies appear to know about the groundwater and water treatment at the site, this despite 20 years of operation. For example, less than three years ago, the State DEQ confidently outlined for Butte-Silver Bow what seemed to be a very conclusive breakdown of future costs at the Site, in particular water treatment costs for around 20-25 years. But the ***Report*** states, in one section, that water treatment will now be needed for "the next 56 to 123 years;" yet, in another section of the ***Report,*** it states that ROD language of "beyond 30 years" is adequate. There is no reliability in the information, which is particularly troubling in the context of determining the long-term O&M costs of treatment.

Butte-Silver Bow would ask: Where is the plan to more aggressively deal with the contaminated groundwater, rather than just declaring 30-50 years of operations will be required? Mr. Bowers (DEQ) has alluded to "steps" that could be taken or different methodologies or technologies that could be used to accelerate the process; but to date, the DEQ has provided no details or assurance that alternatives are even under consideration.

Butte-Silver Bow has asked for and received a spreadsheet on DEQ's contract with Tetra Tech to operate the plant at $900K/year – we're reviewing the data now. But there has been no information provided about how that cost could be reduced. It was reported at one of the public presentation in 2017 that "a decision was made" to hire a second treatment plant operator. However, there has been no explanation or justification for this decision, nor any documentation/breakdown of the long-term cost implications of a second operator.

Trust Fund/Finances

During the public presentations on the ***Report***, too many questions have been addressed by Agencies' folks with answers that appear to limit options based on money, i.e., "there are not enough funds available to consider that." At the same time, there is in excess of $29 million in the Trust presently, plus interest earned on that principle, which appears to be a significant amount to comprehensively remediate the Montana Pole Site. Given that there was approximately $36 million in the Trust (when it was created in 1996), and DEQ has reported expenses over the past 20 years well in excess of $30-40 million, there would appear to be sufficient resources to deal with options for the water and soils treatments, especially when considering the time value of money, discount rates, etc. Butte-Silver Bow would ask for a full accounting of the expenses from the Trust since its inception, a status report on the Trust today, and whatever spreadsheets the Agencies have developed to complete remaining remedial actions and manage O&M life-cycle costs in the future.

Steps Forward

We have been informed by Agencies' staff that 1) there is a "data gap report" forthcoming that will provide critically needed information on the status of contaminants still remaining on the Site; 2) there is a design process underway to revise the cleanup plan for the Montana Pole site; and 3) there will be an Explanation of Significant Differences that will detail changes to the 1995 Record of Decision, including new cleanup levels and approaches.

We understand that there will be opportunities for formal public comment on these next steps. In advance, we would ask the Agencies to consider ALL alternatives (some of which are suggested in this letter) before making final decisions on the future of the Montana Pole site. Further, Butte-Silver Bow would ask that local government staff be included in ongoing or future design and decision-making meetings.

Thank you for the opportunity to present these comments. Better work is needed at this Site, and the Agencies need to design and execute revisions to the cleanup plan that secure long-term, permanent protection of human health and the environment.

Sincerely,

Dave Palmer
Chief Executive

CC: Butte-Silver Bow Council of Commissioners
Butte Legislative Delegation
Governor Bullock
Montana Natural Resource Damage Program
Montana Congressional Delegation
Other Interested Parties

DEQ
Montana Department
of Environmental Quality

November 22, 2017

Mr. Dave Palmer
Butte-Silver Bow Chief Executive
Courthouse, 155 Granite St., Suite 106
Butte, Montana, 59701-9256

Dear Chief Executive Palmer,

Thank you for your letter providing comment on the "Fourth Five-Year Review Report for the Montana Pole and Treating Plant Site." We have received and noted your comments and will refer to them going forward. They provide a helpful summary of citizen and local government concerns as we commence the final phase of cleanup at the Montana Pole Site.

As your letter noted, we are committed to keeping the local government and citizens well-informed of newly available information and next steps as we move forward with the Site. We recognize that historical communication/outreach has not been as effective as it should have been. We appreciate your recognition of our recent efforts and are committed to this effort in the future. DEQ has taken concrete steps to ensure more consistent public outreach efforts, including the hiring of a public information officer, Karen Ogden, dedicated to our Waste Management & Remediation Division. We're pleased that you have noticed improved communication over the past several months. Please don't hesitate to contact Karen any time you have questions or concerns or need information. She can be reached at 406-444-6360 or karen.ogden@mt.gov.

Regarding your questions on the future use of the Montana Pole Site, DEQ is open to consideration of enlarging the footprint of the protective cap area, as well as other options, to allow for fewer land use restrictions on the southern portion of the Site. We look forward to discussing this further with Butte-Silver Bow. DEQ understands that the local land use plan calls for a mixture of open space/recreational on the east end of the Site, commercial along Greenwood Avenue, and industrial use on the west end of the Site, and we recognize the desire to make as much of the Site as possible available for industrial/commercial use.

Your letter also referenced the Data Gap Investigation, which is now being finalized and which DEQ will present to you later this month. The Data Gap Investigation provides an updated understanding of the soil contaminant levels throughout the Site, and is appropriately timed as we enter the final cleanup phase of the soil media. . In addition, DEQ will begin a groundwater investigation on the northern portion of the Site after the Explanation of Significant Differences (ESD) is finalized. The groundwater investigation will re-baseline what we know about the groundwater plume and help determine if additional measures can be taken to expedite the groundwater cleanup. Routine operation and maintenance and groundwater monitoring will continue. The water treatment plant itself also will be evaluated to determine if measures can be taken to optimize performance while reducing annual costs.

The attached addendum provides detailed information on other specific topics addressed in your letter. We're happy to answer any further questions you have on any of these topics.

I look forward to meeting with you later this month to present the findings of the Data Gap Investigation and to explore the options for future industrial/commercial development on the southern portion of the Montana Pole Site. DEQ values our working relationship with the Butte-Silver Bow Commission, and we are committed to continued dialogue and frequent communication with both the Commission and the general public as we move forward with the final phase of cleanup at the Montana Pole Site.

Best regards,

Tom Livers

Tom Livers

Addendum to 11/22/17 response letter to
Butte-Silver Bow Chief Executive Dave Palmer

General Site Background & Information

The Record of Decision (ROD) for the Montana Pole Site cleanup was finalized in 1993 and the soil treatment process began in 1995. Pentachlorophenol (PCP) was the most prevalent contaminant at the Montana Pole Site. Federal law lists PCP-contaminated soil from wood treatment facilities as an F032 hazardous waste, which restricted cleanup options at the Montana Pole Site.

At the time of the Record of Decision use of biological treatments were considered state of the art and had been demonstrated to be effective against a wide range of hydrocarbon molecules. While biologic treatments remain an effective remediation tool, their efficacy against complex organics compounds, such as dioxins and furans, has not been deemed effective at reducing the concentrations at a meaningful rate.

The Land Treatment Unit (LTU) at the Montana Pole Site successfully treated more than 200,000 cubic yards of PCP-contaminated soil, meaning levels of PCP and associated PAH contaminants were reduced to below the cleanup level prescribed in the 1993 ROD. The biological treatment of soils removed 95% (over 266,000 pounds) of the PCP in the contaminated soils.

However, the soil also contained dioxin, a co-contaminant common to wood treatment facilities. Dioxin is problematic because it does not easily break down. What's more, treatment options for dioxin are limited at sites containing wood-treating waste because (as explained above) these soils are listed as hazardous waste and can only be managed on-site or placed at a special waste-handling facility. The ROD finalized in 1993 for the Montana Pole Site called for the treated soil to be placed under a protective cap, and that remains the plan today.

The practice of capping treated soils was very much in line with the cleanup of wood-treating sites throughout the United States at the time(as noted in a September 1995 report from the U.S. Congress, Office of Technology Assessment titled "Cleaning Up Contaminated Wood-Treating Sites," (OTA-BP-ENV-164 Washington DC: U.S. Government Printing Office, September 1995) and remains the cleanup alternative today:

"Physically capping a site is particularly useful to complete the overall protection of a complete wood-treatment cleanup strategy."

Consideration of other cleanup alternatives

When the ROD for the Montana Pole Site was finalized in the early '90s, alternative cleanup options, such as adding micro-organisms to the soil to break down dioxins, had not been proven to be successful at locations similar to Montana Pole. Even today, introducing micro-organisms to break down the dioxins at the Montana Pole Site would be considered an experimental remedy because the efficacy has not been proven in locations with conditions similar to those found at Montana Pole – i.e. climate, soil composition, hydrology, volume of contaminated soil, and co-contaminants.

Incineration of the contaminated soil, another option considered in the ROD, came with its own set of health and safety concerns, as well as opposition from the adjacent neighborhood. DEQ's initial estimate for moving the contaminated soil to the nearest hazardous waste facility, located in Utah, is

approximately $100 million for 10,000 truckloads of soil – far exceeding the budget determined by the $35 million settlement for the Site, even when interest is considered.

At the request of BSB and the community, DEQ is examining other potential treatment alternatives to ensure that the alternative screening process was thorough and complete. **Contaminated soil beneath the I-90/15 Bridge.**

During the Montana Department of Transportation's (MDT) design phase of the interstate bridge project, DEQ (using CDM as its contractor) looked very closely at the possibility of removing the remaining contamination under the bridge embankment.

Based on borings completed to better define the extent of contamination in this area, there remain approximately 40,000 cubic yards of contaminated soil beneath the embankment. MDT determined that it would take two years for contractors to complete the bridge replacement project, doing one lane each year, and that it would take the entire construction season of each year to conduct this work. Any additional work requested by DEQ would need to be worked into MDT's two-year construction schedule.

If DEQ had gone ahead and requested acceleration of MDT's construction schedule, all costs plus any cost or schedule over-runs would have been the responsibility of DEQ, taking money away from other aspects of the cleanup. The work would have entailed:

- Excavating the embankment down to the original ground surface plus another approximately 20 feet below to excavate the contaminated soils.
- Stabilizing the remaining roadway while accommodating active traffic.
- Dewatering and managing the groundwater, as the excavation would have extended into the groundwater.
- Backfilling and compacting the soils to the elevation needed for the interstate roadway.

Because of the time constraints and other complicating factors, the remediation would have required MDT to construct the entire bridge at one time, rather than one lane at a time. This would have required an alternate route for interstate traffic.

Removal of sheet piling designed to protect Silver Bow Creek

The sheet piling/Gundwall was in place to prevent the contaminated "oil" (or product) from migrating to Silver Bow Creek. The wall was removed during the Phase 1 ROD remedy implementation (1996-97) when the Near Highway and Near Creek Recovery Trenches were installed. This was after the north side excavation was completed (wherein the saturated contaminated soils were excavated, thus removing the source of the product). When the Near Highway Recovery Trench was installed, it included an HDPE barrier wall that performed the same function of containing or preventing contaminated product from migrating toward Silver Bow Creek, located further to the south and closer to the remaining contamination under the interstate.

Exact presence or extent of contaminants on north side of Silver Bow Creek

With regard to the contaminants north of the site (including the north side of Silver Bow Creek), the ROD states:

"*Once site remediation has effectively contained the contaminated groundwater and LNAPL (Light Non-Aqueous Phase Liquid), and releases to Silver Bow Creek have been effectively reduced or eliminated, it is expected that natural biodegradation and attenuation will effectively reduce the levels of organic contaminants in Silver Bow Creek, stream sediments and groundwater downstream (down-gradient) of the site. These natural mechanisms will be relied upon to address the low level contamination found in this area.*"

DEQ took on the task of implementing the remedy actions as defined in the ROD, including groundwater monitoring. DEQ monitors wells on the north side of Silver Bow Creek as part of its groundwater monitoring program. Regular monitoring in this area on a semi-annual (February/August) basis commenced in 2010 due to concerns about groundwater drawdown from the construction operations at the new municipal wastewater treatment plant, and we continue to monitor the situation today.

Not knowing whether remnant contaminants are present beneath the Land Treatment Unit (LTU) liner

Data from numerous samples from the Remedial Investigation (prior to the installation of the liner in the LTU) showed little to no PCP contamination in the eastern treated wood storage yard.

Nevertheless, the use of that area over the past 25 years to treat contaminated soils will require additional sampling once the demolition and disposal of the LTU liner is complete. Post-removal or confirmatory sampling is standard practice after removal of a liner to ensure that it was protective. At that time, DEQ will perform additional dioxin sampling as well to ensure that the area meets or exceeds the ROD cleanup standards.

Appears to be no cleanup standard for protectiveness to groundwater

The ROD did not include a cleanup standard for protectiveness to groundwater. In the ROD, protectiveness was established through containment and treatment of the groundwater, and implementation of institutional controls (controlled groundwater area) to prevent access to or impacts upon contaminated groundwater at the site. The fourth Five-Year Review identified the lack of protectiveness to groundwater cleanup level as a data gap for this year's design Data Gap Investigation. The data collected from this investigation will assist in determining a protection to groundwater cleanup standard, which will be included in the ESD.

Water treatment plant operations (concern about inconsistent statements)

Projecting groundwater remediation timeframes is very difficult, if not impossible in some instances. This is reflected in the 2016 evaluation that speculates that it will take an additional 50 to 120 years to remediate the inaccessible areas. Note that this timeframe also assumes that the treatment approach would not change. This is consistent with the ROD in that it lists "30+ years – groundwater, operations and maintenance."

In addition, the ROD states in Alternative 5A:

"*Total estimated costs for Alternative 5A assume that groundwater action would occur for a period of 30 years. Although groundwater remediation to cleanup levels is expected under this alternative, some inaccessible source areas (under the interstate highway) would remain and be treated in place. Therefore, actual costs and efforts associated with site monitoring, enforcement of institutional controls and operation and maintenance of the groundwater treatment system for inaccessible areas (under the interstate highway) may be incurred beyond 30 years.*"*

This is why DEQ is planning to re-baseline the groundwater plume and identify all contamination sources, including what is underneath the interstate highway and underneath the water treatment plant. Once this is complete, DEQ will examine alternatives that could effectively expedite the groundwater cleanup process.

***** Note: (This statement also applies to the chosen alternative – Alternative 5B – which, as the ROD notes, is the same as Alternative 5A with the exception of the soil treatment method (biological land treatment (5B), versus incineration (5A)).

Water treatment plant operations (hiring a second treatment plant operator)

The hiring and training of a second treatment plant operator was done to ensure that there would be an emergency backup and fill-in as necessary. Prior to the hiring, there wasn't anyone available who could step in and run the plant on a day-to-day basis in the event of an emergency. A graduate student at Montana Tech was selected for the position. Now employed by Tetra Tech, he is available for fill-in at the water treatment plant, but performs other duties as assigned elsewhere throughout Montana and the United States.

Steps forward (design process)

The design process is already underway (design Data Gap Investigation and Report) and is focused on the engineering requirements for the offload from the LTU, storm water run-on/run-off channel specifications, any additional removals to ROD cleanup levels, and final cap design. A 30% design should be ready to share sometime this winter. This design will be the basis for the bid package that will guide next year's construction.

Steps forward (Explanation of Significant Differences)

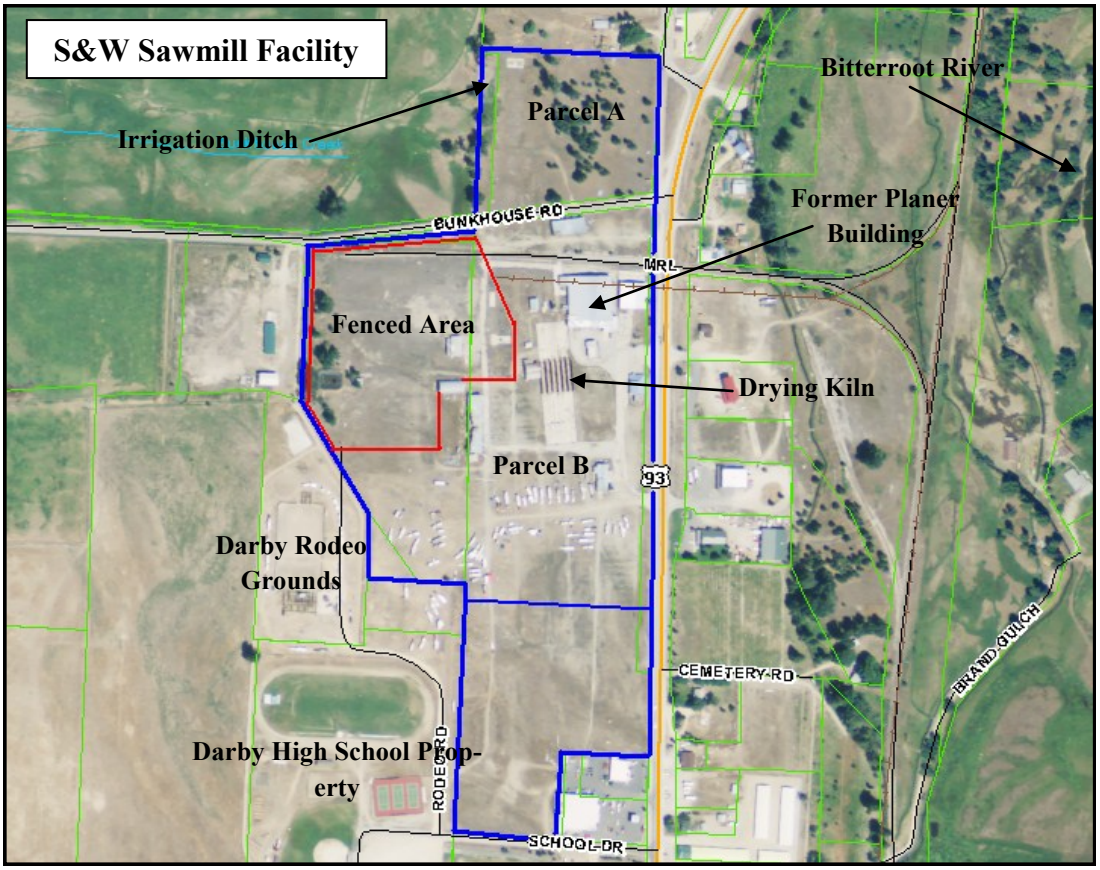
The ESD will update, if necessary, the cleanup standards in the ROD. Updating a cleanup number in a ROD requires a formal risk analysis to determine if the change is warranted. DEQ will perform this analysis as part of the ESD. The ESD also will explain why it is deemed necessary to move from just an earthen cover to an engineered cap for all of the offloaded/treated soils. The engineered cap will be designed to be protective of groundwater (leaching of PCPs) and will provide additional protection from dioxin exposure.

S&W Facility Facility Update

STATE SUPERFUND UNIT **DECEMBER 2014**

Background

The S&W Sawmill Facility, located approximately 1/2 mile north of the City of Darby at the intersection of US Highway 93 and Bunkhouse Road, is shown on the map below. The S&W Sawmill Facility is being addressed by the Montana Department of Environmental Quality (DEQ) under the authority of the state Superfund law, CECRA. International Paper (IP) is designated as the lead liable party for remediation, or cleanup, of the Facility. From approximately 1961 to 1998, portions of the Facility operated as a sawmill. From approximately 1961 to 1978, portions of the Facility also operated as a wood treatment plant that used the chemical pentachlorophenol (PCP) mixed with diesel as a wood preservative. The chemical mixture and its by-products (dioxins/furans) have contaminated the soil and groundwater at the Facility. The former Facility is currently not in use.



Contaminated Areas

The major contaminants of concern are PCP and dioxins/furans. IP completed a remedial investigation (RI) in November 2004 and confirmed contamination in both the soils and the groundwater. IP concluded a supplemental RI in September 2012. It focused on surface soils throughout the Facility and surrounding residential and commercial properties. The fenced area of the Facility contains the heaviest contamination of soils, with the groundwater plume stretching from the fenced area to properties across US Highway 93.

S&W Sawmill Facility Update

STATE SUPERFUND UNIT DECEMBER 2014

Recent Activities - 2012 through 2014

On-Going Groundwater Monitoring: IP evaluates the groundwater plume twice a year through the sampling of 28 groundwater monitoring wells. Five of these wells are residential drinking wells east of the Facility.

Irrigation Ditch Investigation: IP conducted a dioxin/furan investigation of the irrigation ditch running north away from the Facility. High levels of dioxins/furans were discovered in the ditch and sampling was performed between the northern boundary of the Facility (Parcel A) and Moles Lane. In total, approximately 2000 feet of the irrigation ditch was sampled. DEQ approved of IP's report documenting this investigation in October 2012, entitled Drainage Ditch Soil Sampling Investigation—April and May 2012.

Baseline Risk Assessment: In August 2014, IP submitted the Final Baseline Risk Assessment (BRA) to DEQ. A risk assessment is intended to estimate potential human health and environmental risks posed by current and potential future conditions assuming no further remediation of the Facility. This risk assessment calculated and documented the clean-up levels of soil, groundwater, and soil vapor to protect human health and the environment.

Fate and Transport Study: On March 7, 2014, IP submitted the Fate and Transport Data Package to DEQ. This document included details of the soil collection activities to obtain fate and transport data in October 2013. After DEQ approved the data package, IP submitted the March 21, 2014 Fate and Transport Study Work Plan (Work Plan) and the September 12, 2014 Fate and Transport Study Model Parameters Technical Memorandum (FTMP Memo). The Work Plan detailed the methods to be employed in modeling and calculating fate and transport clean-up levels. The FTMP Memo provided DEQ with the actual values of different model parameters that would be inputted into the fate and transport model conducted by IP. DEQ approved of these documents on October 6, 2014, and instructed IP to proceed with performing the modeling and submitting a Fate and Transport Study Report to DEQ. The main objective of the Fate and Transport Study is to develop facility-specific soil cleanup levels for the protection of groundwater. It is anticipated that this Study will be completed by the end of 2015.

Future Activities

With the expectation that the Fate and Transport Report will be completed in 2015, DEQ will require IP to build on this completed task and begin developing and evaluating remedial alternatives, to be documented in the Feasibility Study, during 2016.

DEQ Montana Department of Environmental Quality

If you have questions, please contact:
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For more information about the Facility, please visit:

http://www.deq.mt.gov/StateSuperfund/swsawmill.mcpx

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Soil								
No Further Action	None	**None**	Will not remove contamination or reduce risk to human health and the environment.	Inclusion of this option is required by DEQ procedures.	Low	Easy	Low	Yes
Institutional Controls	Land Use Controls	**Zoning, Deed Notices, Environmental Control Easement**	Protects human health by limiting site uses and related exposures to contaminated soil. Not protective for the leaching to groundwater pathway. Requires long-term maintenance and enforcement of land use controls.	USEPA (2012)	Moderate	Easy	Low	Yes
	Site Administrative Procedures	**Health and Safety Programs**	Protects human health by limiting exposures to contaminated soil through appropriate health and safety practices. Not protective for the leaching to groundwater pathway. Requires long-term program compliance.	USEPA (2012)	Moderate	Easy	Low	Yes
		Monitoring and Site Security Measures	Protects human health by limiting unauthorized site access and related exposures to contaminated soil. Not protective for the leaching to groundwater pathway. Requires long-term maintenance and enforcement.	USEPA (2012)	Moderate	Easy	Low	Yes
Monitored Natural Attenuation	Natural Attenuation/Long-Term Monitoring	**Natural Attenuation**	Effectiveness depends on site-specific conditions and COCs. Limited to no effectiveness for dioxins and furans. Requires long-term monitoring to assess the progress of natural attenuation.	USEPA (2012)	Moderate	Easy	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
Soil Containment	Physical Barrier	**Horizontal Barrier** (caps: asphalt/concrete, soil/bentonite/clay, multi-layer cover systems, Aquablok, apatite, and coke breeze in a laminate mat)	Prevents or reduces direct human contact with contaminated soil. May limit or eliminate leaching of contaminants to groundwater. Requires long-term maintenance.	FRTR (2007)	High	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
Soil Removal and Transport	Excavation	**Excavation** Contaminated material is removed and transported, either onsite for treatment (such as landfarming, composting, bioslurry treatment, and thermal desorption), or to permitted offsite treatment or disposal facilities. Some pretreatment of the contaminated media may be required to meet land disposal restrictions.	Proven technology. Excavation becomes more difficult to implement and more expensive with depth, particularly below the water table.	FRTR (2007)	High	Moderate to Difficult	Moderate to High	Yes
Ex Situ Soil Treatment	Bioremediation	**Land Farming** Contaminated soil is excavated and placed into lined beds; the soil is then mixed or tilled to stimulate aerobic degradation. Liners and other methods are used to control leaching of contaminants.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Likely requires physical separation of stones and rubble prior to treatment. Requires use of a liner and leachate collection system for PCP treatment. Requires a large area and management to prevent offsite migration or contaminant transport.	FRTR (2007), USDA (2002)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Composting Contaminated soil is excavated and mixed with bulking agents and organic amendments. Organic contaminants are degraded to innocuous stabilized byproducts.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Requires physical separation of stones and rubble prior to treatment. Requires use of a liner and leachate collection system for PCP treatment. Technology can be difficult to maintain and requires a large area.	FRTR (2007), USDA (2002)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Ex Situ Soil Treatment	Bioremediation	**Phytoremediation (e.g. poplar trees, grasses)** Phytoremediation is a process that uses plants to remove, transfer, stabilize, and destroy contaminants in soil and sediment.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Usually an *in situ* technology but can be applied *ex situ*. High concentrations of hazardous materials can be toxic to plants, limiting the effectiveness and increasing maintenance requirements. May be seasonal, depending on location. This alternative was not retained for further evaluation due to the fact that this technolgy can be more effectively implemented *in situ* and more effective *ex situ* technologies exist for treatment (e.g. land farming, compositing and biopiles). *In situ* phytoremediation (retained technology) is discussed below.	FRTR (2007), Hechmi et al. (2014), Mills et al. (2006)	Moderate	Easy to Moderate	Low to Moderate	No
		Biopiles A full-scale technology in which excavated soils are mixed with soil amendments and placed on a treatment area.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Requires leachate collection systems to control runoff. Requires use of a liner and leachate collection system for PCP treatment.	FRTR (2007)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Bioslurry (slurry-phase biotreatment, soil-slurry bioreactor) The controlled treatment of excavated soil in a bioreactor.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. This technology has been demonstrated at the laboratory scale for dioxins and furans. Requires physical separation of stones and rubble prior to treatment. Post-treatment soil fines require dewatering, generating wastewater that requires disposal. Process option not retained for further analysis due to the large amount of gravel in subsurface, limited applicability to dioxins and furans and petroleum constituents, and because there are more technically and economically feasible approaches to treating PCP and petroleum constituents (e.g., biopiles).	FRTR (2007), Kao and Wu (2000)	Moderate	Moderate	High	No
	Physical / Chemical Treatment	***Ex Situ* Soil Vapor Extraction (hot air vapor extraction, steam enhanced extraction)** A full-scale technology in which soil is excavated and spread over aboveground piping. Organic contaminants are volatized by applying a vacuum to the soil though the piping.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Prevention of off-gas emissions is required. Eliminated from further evaluation as it has limited applicability to Site COCs.	FRTR (2007)	Low	Moderate	Moderate	No
		Aeration (thermal aeration, mechanical soil aeration) Involves excavation of soil and arrangement into piles or rows; the hydrocarbons and other VOCs are then allowed to passively volatilize into the atmosphere.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. High maintenance process that requires specific controls to prevent other problems and can be difficult to obtain cleanup levels. Eliminated from further evaluation as it has limited applicability to Site COCs.	ODEQ (2006)	Low	Easy	Low to Moderate	No
		Soil Washing A water-based process for scrubbing soils to remove contaminants by dissolving or suspending them in the wash solution, or by concentrating them into a smaller volume of soil through particle size separation, gravity separation, and attrition scrubbing.	Potentially applicable to organic contaminants and inorganic contaminants, but a complex mixture of contaminants in the soil can make it difficult to remove different types of contaminants. Technology is often used as a pretreatment for, or in conjunction with, other treatment technologies as part of a treatment train. Water generated from soil washing requires treatment. It can be challenging to apply this technology to soils with high organic content (silts and clays). Pilot testing may be necessary.	CL:AIRE (2007), FRTR (2007), USDA (2002)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Ex Situ Soil Treatment	Physical / Chemical Treatment	**Solvent Extraction** A means of separating hazardous contaminants from soils, sludges, and sediments using an extracting chemical solvent. This technology can be implemented by liquid or liquefied gas solvent. Gaseous solvent is used under pressure (where it condenses) to extract contaminants.	Applicable to Site COCs. Physical separation is often needed before chemical extraction. Technology is often used as a pretreatment for, or in conjunction with, other treatment technologies. Liquefied/gas solvents are not effective for soils with high organic content; however, may be applicable at the Site. Soil type and moisture content may impair performance. In addition, traces of solvent may remain in the treated solids.	FRTR (2007), Saldana et al. (2005), Jonsson et al. (2010)	Moderate	Moderate	High	Yes (retained for possible use in conjunction with other technologies)
		Separation (physical separation, MAECTITE Process, precipitation/filtration, or electrokinetics) A physical separation process used for removing contaminated concentrates from soils, to leave relatively uncontaminated "treated" fractions. This process is usually conducted in conjunction with another technology.	Limited applicability to Site COCs. Various processes can be used, such as gravity separation, magnetic separation, and sieving. Commonly used in combination with other technologies (as pretreatment). Soil type and moisture content may impair performance. Physical separation has been retained in conjunction with other technologies.	FRTR (2007)	Low	Moderate	Moderate	Yes (physical separation retained for possible use in conjunction with other technologies)
		Immobilization (solidification/stabilization, chemical fixation/solidification) Process that physically binds contaminants within a stabilized mass, or in which chemical reactions are induced to reduce the mobility of contaminants.	Potential applicability to Site COCs. Requires treatability studies and long-term effectiveness has not been demonstrated for many contaminants. Has been applied at full scale for similar contaminants. May require physical separation of stones and rubble prior to treatment. Can result in significant increase in volume.	Bates et al. (2000), FRTR (2007), USEPA (2009)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)
		Chemical Reduction/Dechlorination (solvated electron technology, base-catalyzed dechlorination) Reduction chemically converts hazardous contaminants to non-hazardous or less toxic compounds that are more stable, less mobile, and/or inert.	Applicable to PCP; limited applicability to petroleum constituents and dioxins and furans. Treatability tests required to identify optimal reducing agents. The process is not cost-effective for high contaminant concentrations because of the large amounts of reducing agent required. Incomplete reduction or formation of intermediate contaminants may occur depending upon the contaminants and reducing agents used. Eliminated from further evaluation as it has limited applicability to dioxins and furans and petroleum constituents and there are more technically and economically feasible approaches to treating PCP.	FRTR (2007)	Low	Moderate	High	No
		Mechano-Chemical Destruction (Ball Milling) This is a potentially chemical-free technology that employs mechanical energy to initiate chemical reactions. The reactors consist of special hard-wearing cast rotors that make continuous contact with thousands of stainless steel balls to create continuous and repetitive particle collisions. Soil crystal damage caused by the vibration leads to the formation of highly reactive free radicals, which react with organic molecules in the vicinity (including any organic contaminants).	Treatment of a variety of recalcitrant compounds including pesticides, herbicides, polycyclic aromatic hydrocarbons, and dioxins has been demonstrated under both laboratory-scale and, to a limited extent, field-scale settings with minimal pre-treatment except for the drying of the contaminated materials. Prevention of off-gas and particulate matter/dust emissions is required. Pilot testing may be necessary. May be a cheaper destructive *ex situ* soil treatment technology than others.	USEPA (2010), Mudhoo et al. (2013)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Ex Situ Soil Treatment	Thermal Treatment	**Dehalogenation (flame reactor)** Replaces the halogen molecules or decomposes and partially volatizes the contaminants.	Limited applicability to Site COCs and technology is not readily available. The technology is more amenable to small-scale applications and can be very expensive for larger-scale applications. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Difficult	High	No
		Immobilization (vitrification) Vitrification uses heat to melt and then solidify harmful chemicals in a solid mass of glasslike material.	Potential applicability to Site COCs. Does not reduce the volume of contaminated material and can result in an increase in volume. Requires disposal of treated materials. Long-term effectiveness has not been demonstrated for many processes. Process option not retained for further analysis due to limited demonstration of long-term effectiveness.	FRTR (2007)	Moderate	Difficult	High	No
		Thermal Desorption Wastes are heated to volatilize organic contaminants; the gas-phase contaminants are treated in a gas treatment system. Can be categorized into two groups: high temperature thermal desorption (HTTD) and low temperature thermal desorption (LTTD).	Applicable to Site COCs. Process may not completely remove/destroy organic contaminants; disposal of residuals may be required.	Baker et al. (2006), USEPA (1997a), FRTR (2007), USDA (2002), Sorenson et al. (2011)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)
		Incineration/Thermal Destruction Uses high temperatures to volatilize and combust halogenated and other refractory organic compounds. Incineration can be conducted onsite or offsite; however, onsite incineration is heavily regulated.	Applicable to Site COCs. Off-gases and combustion residuals generally require treatment. A limited number of offsite incinerators are permitted to treat dioxin-bearing wastes.	FRTR (2007), USDA (2002)	High	Difficult	High	Yes
		Pyrolysis (advanced electric reactor, pyrovac vacuum pyrolysis) Chemical decomposition induced in organic materials by heat in the absence of oxygen; although it is not possible to achieve a completely oxygen-free atmosphere, and some oxidation will occur.	Applicable to Site COCs; however, technology is not readily available. Requires drying of the soil and can be difficult to maintain. Eliminated from further evaluation as there are more technically and economically feasible approaches for *ex situ* soil treatment.	FRTR (2007)	High	Difficult	High	No
		Hot-Gas Decontamination Contaminants are volatized, and the volatile constituents are then destroyed in an afterburner system.	Limited applicability to petroleum constituents and PCP; not applicable to dioxins and furans. The gas effluent from the material is treated in an afterburner system to destroy all volatilized contaminants. Costs are high. Eliminated from further evaluation as there are more technically and economically feasible approaches for *ex situ* soil treatment.	FRTR (2007)	Low	Moderate	High	No
		Solar Destruction Solar energy is used to thermally detoxify organic compounds after they have been removed from the contaminated medium.	Limited applicability to Site COCs. Pilot-scale technology and is not readily available. Eliminated from further evaluation due to limited applicability to Site COCs.	Doty et al. (1997)	Low	Easy	Moderate	No

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
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Soil Disposal	Offsite Disposal	**Solid Waste Landfill** Material is removed and transported to permitted off-site treatment or disposal facilities and will be subjected to Subtitle D restrictions.	Waste will be subject to landfill restrictions. Contaminated material is removed and transported to permitted offsite treatment or disposal facilities. Some pretreatment of the contaminated media may be required to meet land disposal restrictions.	FRTR (2007)	High	Easy	Moderate	Yes
		Hazardous Waste Landfill Material is removed and transported to permitted off-site treatment or disposal facilities and will be subjected to Subtitle C restrictions.	Will accept most waste not generally accepted by a solid waste landfill. Material will be subject to landfill restrictions.	FRTR (2007)	High	Easy	High	Yes
		Reclamation/Recycling (asphalt reprocessing) Petroleum-contaminated soils can be recycled into viable, safe construction materials. Two methods — encapsulation and bio-remediation — are used to convert these soils into environmentally safe products for use as construction base or as soil cover for landfills. The encapsulation process uses commercial emulsions to bind the petroleum materials, thereby preventing further migration. Bioremediation uses naturally occurring microbes to break down the petroleum into inert substances.	Applicable to petroleum constituents; limited to no applicability to PCP and dioxins and furans. Usually requires a state- and locally-permitted facility. Only soils contaminated with gasoline, fuel oil, or other petroleum products are accepted for recycling, and these soils must be tested to determine if the materials are classified as nonhazardous and therefore are amenable to the recycling process. Applicable for larger rocks.	Aggregate Industries US (2006)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)
	Onsite Disposal	**Waste Repository** Contaminated material is removed and transported to permitted onsite disposal facilities. Some pretreatment of the contaminated media usually is required to meet land disposal restrictions.	Applicable to Site COCs. Operation and maintenance last for duration of repository. DEQ may require treatment for some contaminants prior to placement.	FRTR (2007)	High	Moderate	High	Yes
		Backfill Excavations Soil is treated to meet applicable cleanup criteria and then used as clean fill to backfill the excavation.	Applicable after treatment to meet applicable federal and state cleanup criteria.	USEPA (1997b)	Moderate	Moderate	Low	Yes (retained for possible use in conjunction with other technologies)
In Situ Soil Treatment	Bioremediation	**Phytoremediation (e.g. poplar trees, grasses)** A process that uses plants to remove, transfer, stabilize, and destroy contaminants in soil and sediment.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. High concentrations of hazardous materials can be toxic to plants, limiting the effectiveness and increasing maintenance requirements. May be seasonal, depending on location. May not be effective for deeper subsurface contamination.	FRTR (2007), Hechmi et al. (2014), Mills et al. (2006)	Moderate	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
		***In Situ* Landfarm** Contaminated surface soil is treated in place by tilling to achieve aeration. Primarily used in association with other technologies.	Applicable to PCP and petroleum constituents; not applicable to dioxins and furans. Runoff collection facilities must be constructed and monitored. Dust control is an important consideration, especially during tilling and other material handling operations. Effectiveness is limited for deeper soils. Technology not retained as it may not be effective for soil contamination at depth and landfarming surface soils may enhance transport of subsurface soils to groundwater.	USACE (1999)	Moderate	Moderate	Low to Moderate	No
		Enhanced Bioremediation (aerobic) Enhancements include the addition of nutrients, oxygen, cultured microorganisms (bioaugmentation), or other amendments. Amended water is frequently circulated through the treatment zone to enhance mixing and contact.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Enhanced bioremediation is especially effective for low-level residual contamination after source removal. Pilot testing may be required as bioaugmentation is highly site specific and highly dependent on the ecology and physiology of the subsurface.	FRTR (2007)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)

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In Situ Soil Treatment	Bioremediation	**Enhanced Bioremediation (anaerobic)** An electron donor (molasses, HRC or similar product, or nitrate) is added to soil to increase the number and vitality of indigenous microorganisms involved in anaerobic bioremediation.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Anaerobic bioremediation occurs at a slower rate than aerobic bioremediation, and development of nitrate enhancement is still at the pilot scale. Many states prohibit nitrate injection into groundwater because nitrate is regulated through drinking water standards.	FRTR (2007)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Bioventing Stimulates the natural biodegradation of any aerobically degradable compounds in soil by providing oxygen via low-pull vacuum to the vadose zone.	Applicable to PCP and petroleum constituents; not applicable to dioxins and furans. A water table that is within several feet of the surface, saturated soil lenses, or low-permeability soils reduce the performance of bioventing.	FRTR (2007)	Moderate	Easy	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Bioslurping Combination of bioventing and vacuum-enhanced free-product recovery.	Not applicable to Site COCs as NAPL is non-mobile. The off-gas from the bioslurper system may require treatment before discharge. Bioslurper systems can extract large volumes of water that may need to be treated for discharge, depending on the concentration of contaminants in the process water. Process option not retained for further analysis as it is not applicable to Site NAPL.	FRTR (2007)	Low	Moderate	Moderate	No
	Physical / Chemical Treatment	**Soil Vapor Extraction** A full-scale technology that volatilizes organic compounds by applying a vacuum to the soil though subsurface extraction wells.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Handling of off-gases is required. Soil vapor extraction is not effective in the saturated zone. Process option not retained for further analysis due to limited applicability to PCP and petroleum constituents and it is not applicable to dioxins and furans.	FRTR (2007)	Low	Easy	Moderate	No
		Air Sparging An *in situ* technology that injects air through contaminated material. Injected air traverses horizontally and vertically in channels through the soil column, creating an underground stripper that removes contaminants by volatilization. Air sparging is typically used in conjunction with soil vapor extraction.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. This technology is demonstrated at numerous sites, though only a few sites are well documented. Air sparging has demonstrated sensitivity to minute permeability changes, and air flow through the saturated zone may not be uniform. Process option not retained for further analysis due to limited applicability to PCP and petroleum constituents and it is not applicable to dioxins and furans.	FRTR (2007)	Low	Easy	Moderate	No
		***In Situ* Chemical Oxidation (ISCO)** This process chemically converts hazardous contaminants to nonhazardous or less toxic compounds.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Contact of oxidizing agents with COCs in the vadose zone is difficult and may be improved with use of application using soil blending. The process is not cost-effective for high contaminant concentrations (NAPL or smear zones) because of the large amounts of oxidizing agent required. Technology retained if used in conjunction with groundwater ISCO treatment.	FRTR (2007)	Low	Moderate	Moderate to High	Yes (retained for possible use in conjunction with other technologies)
		Solidification / Stabilization The alternative reduces the mobility of hazardous substances and contaminants in the environment through both physical and chemical means.	Potential applicability to Site COCs. Requires treatability studies and long-term effectiveness has not been demonstrated for many contaminants. Can result in significant increase in volume. Processing contamination below the water table may require dewatering and disposal of wastewater.	Bates et al. (2000), FRTR (2007), USACE (1999)	Moderate	Difficult	Moderate	Yes (retained for possible use in conjunction with other technologies)

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In Situ Soil Treatment	Physical / Chemical Treatment	**Electrokinetic Separation (low-current DC)** Relies on application of direct current through the soil between electrodes. Metal ions, ammonium ions, and positively charged organic compounds move toward the cathode. Anions such as chloride, cyanide, fluoride, nitrate, and negatively charged organic compounds move toward the anode.	Not applicable to Site COCs. Oxidation/reduction reactions can form undesirable by-products. Process option not retained for further analysis as it is not applicable to Site COCs.	FRTR (2007)	Low	Moderate	High	No
		Soil Flushing (cosolvent flushing) Water that contains an additive to enhance contaminant solubility is applied to the soil or injected into the groundwater to raise the water table into the contaminated soil zone. Contaminants are leached into the groundwater, which is then extracted and treated.	Limited applicability to Site COCs. Contamination is deep and under the water table and there is potential for solvent to sorb to soils. Reliability and effectiveness are uncertain. Retained if used in conjunction with other technologies (e.g., cosolvent flushing for NAPL mobilization).	USACE (1999)	Low	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)
	Thermal Treatment	***In Situ* Thermal Desorption (ISTD)** Primarily an unsaturated (vadose) zone soil remediation technology that applies heat to the subsurface to volatilize organic contaminants. A vacuum is applied to the soil to induce the controlled flow of air and remove the volatilized contaminants.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Highly dependent upon the specific soil and chemical properties of the contaminated media. High costs. Retained for potential use in conjunction with other technologies.	FRTR (2007)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)
		***In Situ* Vitrification (ISV)** Uses an electric current to melt soil or other earthen materials at extremely high temperatures and thereby immobilize most inorganic constituents and destroy organic pollutants by pyrolysis.	Applicable to Site COCs; however, technology is at pilot-scale level and there have been few, if any, commercial applications. Process option eliminated from further evaluation as there are more technically and economically feasible approaches for *in situ* soil treatment.	FRTR (2007)	High	Difficult	High	No
NAPL and Groundwater								
No Further Action	None	**None**	Will not remove contamination or reduce risk to human health and the environment.	Included as a baseline	Low	Easy	None	Yes
Institutional Controls	Groundwater Use Restrictions	**Controlled Groundwater Area**	Protects human health by limiting exposure pathways and risk of future exposures. Limited effectiveness for contaminant removal or treatment until residual NAPL and dissolved phase has been addressed by natural attenuation. Requires public outreach and educating the community on the potential exposure pathways and risk of future exposures.	USEPA (2012)	Moderate	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
	Site Administrative Procedures	**Monitoring and Site Security Measures**	Protects human health by limiting exposure pathways and risk of future exposures. Limited effectiveness for contaminant removal or treatment until residual NAPL and dissolved phase has been addressed by natural attenuation or other remediation activities. Requires periodic inspections to ensure compliance with site administrative procedures.	USEPA (2012)	Moderate	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
Monitored Natural Attenuation	Monitored Natural Attenuation/Long-Term Monitoring	**Monitored Natural Attenuation**	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Effectiveness depends on site-specific conditions and completeness of source removal. Requires long-term monitoring to assess the progress of natural attenuation. Limited effectiveness for contaminant removal or reduction of risk to human health and the environment in the short term.	USEPA (2012)	Moderate	Easy	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)

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Monitored Natural Attenuation	Natural Source Zone Depletion	**Natural Source Zone Depletion** This approach evaluates the rate chemicals are being naturally lost from the source zone due to volatilization, dissolution, biodegradation, and sorption.	Applicable to Site COCs. Effectiveness depends on site-specific conditions. Requires monitoring to assess the rate at which the source zone is being depleted. Limited effectiveness for contaminant removal or reduction of risk to human health and the environment in the short term.	ITRC (2009)	Moderate	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
NAPL Collection, Reduction, and/or Treatment	Bioremediation	**Bioremediation** This approach enhances the natural biological activity in the subsurface to reduce contaminant concentrations and to degrade contaminants.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Enhanced bioremediation is especially effective for residual NAPL contamination. Pilot testing may be required as the need for bioaugmentation is highly site specific and highly dependent on the ecology and physiology of the subsurface.	USEPA (2005)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Bioventing/Biosparging Bioventing enhances the natural biological activity by supplying oxygen in the subsurface to reduce petroleum hydrocarbon mass in the vadose and smear zone.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Can be especially effective for low-level residual NAPL contamination. Pilot testing, including respirometry testing would be performed to evaluate the efficacy of bioventing and/or biosparging.	ITRC (2009), USEPA (2005)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
	Physical Removal	**Excavation** Contaminated material is removed and transported, either onsite for treatment (such as landfarming, composting, bioslurry treatment, and thermal desorption); or to permitted off-site treatment or disposal facilities. Some pretreatment of the contaminated media usually is required to meet land disposal restrictions.	Applicable to Site COCs. Well-documented and well-proven technology. Treatment through excavation deeper than 10 ft becomes more difficult to implement and more expensive with depth. Due to difficulty in implementing and the costs, this alternative is usually only appropriate in the removal of source material covering a limited area.	FRTR (2007)	High	Moderate to Difficult	Moderate to High	Yes
	Combined Physical Removal and Bioremediation	**Bioslurping/Enhanced Fluid Recovery (EFR)** Bioslurping/EFR reduces LNAPL in the subsurface through applied vacuum in conjunction with up to two pumps (e.g., a vacuum with a downhole stinger tube or vacuum applied in conjunction with a positive-displacement pump).	Not applicable to immobile Site NAPL. Site NAPL is considered immobile (Integral 2016). Baildown testing is typically performed to estimate NAPL transmissivity and evaluate the feasibility of mechanical recovery. Apparent NAPL thickness is currently below the threshold (i.e., minimum of 0.5 ft recommended by ITRC [0.2 ft by DEQ]) for completing a baildown test. Process option is not retained as NAPL is immobile and mechanical recovery is unfeasible.	ITRC (2009)	Low	Moderate	Moderate	No
		Air Sparging and Soil Vapor Extraction (AS/SVE) AS injects ambient air or other gases (e.g., oxygen) down well boreholes or trenches below the groundwater table, aerating groundwater and volatilizing LNAPL. SVE induces a vacuum that volatilizes LNAPL if present above the water table and removes LNAPL vapors from the subsurface.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. SVE extracts vapors from the unsaturated zone. As volatile NAPL constituents are stripped, NAPL can become more viscous, and more recalcitrant constituents can become more concentrated. A pilot study would be required to assess its treatment effectiveness and satisfy design requirements. Process option not retained for further analysis due to limited applicability to Site COCs.	ITRC (2009)	Low	Moderate	Moderate	No

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NAPL Collection, Reduction, and/or Treatment	Combined Physical Removal and Bioremediation	**Multi-phase extraction** Multi-phase extraction is a combination of bioventing and vacuum-enhanced free-product recovery. A high-vacuum system is applied to simultaneously remove various combinations of contaminated groundwater, separate-phase petroleum product, and hydrocarbon vapor from the subsurface.	Not applicable to immobile Site NAPL. Removes contaminants from above and below the water table. Exposes more of the vadose zone by lowering the water table around the well, exposing more contaminants to vapor extraction. Once above ground, the extracted liquids and vapors are separated and treated. Effective on VOCs and fuels (such as NAPLs). More effective than SVE for heterogeneous clays and fine sands, but not as effective on low-permeability formations. Can shorten the cleanup time for other technologies. Water (and possibly vapor) treatment is required. Process option is not retained as NAPL is immobile and mechanical recovery is infeasible (Integral 2016).	FRTR (2007)	Low	Moderate	Moderate	No
		Dual Pump Liquid Extraction (DPLE) DPLE uses two pumps to recover LNAPL (one dedicated to removing LNAPL and one dedicated to remove groundwater to cause cone of depression to increase LNAPL gradient).	Residual NAPL is no longer measureable in Facility groundwater wells and is considered immobile (Integral 2016). Process option is not retained as NAPL is immobile and mechanical recovery is unfeasible.	ITRC (2009)	Low	Moderate	Moderate	No
		Trenches/Drains Trenches and drains are the most hydraulically efficient means for removing fluids from the aquifer. May be used to recover mobile LNAPL at shallow depths (15 to 20 ft below ground surface). Trenches are excavated perpendicular to the direction of groundwater flow, and LNAPL is allowed to pool in the trench for recovery. The trench is sometimes lined on the back side to contain the LNAPL. Open trenches can be converted to drains by backfilling with permeable materials. Sumps or wells may be installed along the trench or drain to collect LNAPL.	NAPL is no longer measureable in Facility groundwater wells and is considered immobile (Integral 2016). Process option is not retained as NAPL is immobile.	USEPA (1995)	Low	Moderate	Moderate to High	No
		Hydraulic Pumps The technology involves pumping LNAPL from wells or trenches under ambient pressure. Groundwater can simultaneously be recovered to increase the hydraulic gradient to help induce the flow of LNAPL to the well or trench.	NAPL is no longer measureable in Facility groundwater wells and is considered immobile (Integral 2016). Process option is not retained as NAPL is immobile and mechanical recovery is unfeasible.	USEPA (2005)	Low	Moderate	Moderate	No
	Physical / Chemical Treatment	**Passive and Active Skimmers** (for example, belt skimmers, QED passive or active skimmer [by Enviroequip], or Blackhawk's LNAPL recovery attachment) Skimmers recover LNAPL by skimming under ambient pressure. They are often applied where LNAPL can be concentrated, such as in a trench with a vertical NAPL barrier. They can also be used in an extraction well.	NAPL is no longer measureable in Facility groundwater wells and is considered immobile (Integral 2016). Process option is not retained as NAPL is immobile.	USEPA (2005)	Low	Easy	Low	No
		Hot Water or Cosolvent Flushing Methods for enhancing oil recovery, such as injection of hot water or steam, cosolvents (for example, ethanol), surfactants, alkaline agents, and polymers, are being evaluated for remediation of LNAPL sites because typical LNAPL recovery systems (such as drains and pumping wells) will usually remove only 50 percent or less of the total LNAPL.	Limited applicability to Site COCs. Has typically been used in the oil production industry. However, there has been significant research and testing for use in the environmental industry in recovery of LNAPL and DNAPL. There are practical limitations to the effectiveness of enhanced oil recovery; however, the results from ongoing studies and field tests are favorable.	USEPA (1995)	Low	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)
		Radio-Frequency Heating (RFH) RFH energy is introduced into the subsurface via heating antennae. The subsurface is maintained at temperatures low enough to mainly influence the viscosity of the LNAPL. The mobilized LNAPL is recovered hydraulically.	Limited applicability to Site COCs. New technology and has not been widely used for NAPL remediation. Difficult to implement this technology due to concerns on the effective capture of mobilized soil vapor. Eliminated from further evaluation as there are more technically and economically feasible approaches to recovering residual NAPL.	ITRC (2009)	Low	Difficult	High	No

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NAPL Collection, Reduction, and/or Treatment	Physical / Chemical Treatment	**Thermal Remediation Using Electric Resistance Heating (ERH)** ERH is a polyphase electrical technique used to resistively heat soil and mobilize and volatilize LNAPL. Electrodes are installed through the contaminated zone and electrical current flows from each electrode to the other electrodes. The soil matrix is heated due to the resistance to electric flow. The mobilized LNAPL is recovered from extraction wells.	Limited applicability to Site COCs. Very difficult to implement this technology due to concerns associated with effective capture of mobilized soil vapor, high heat loss due to groundwater inflow to contaminated zone, electrical safety, and difficulties of electrodes installation. Eliminated from further evaluation as there are more technically and economically feasible approaches to recovering residual NAPL.	ITRC (2009)	Low	Difficult	High	No
		Thermal Remediation Using Thermal Conduction Heating (TCH) High *in situ* temperatures can be achieved by thermal conduction using superheated heater rods that are installed in vertical wells. Thermal energy is then conducted through the subsurface. NAPL recovery is enhanced via increased pressure gradients from groundwater boiling into steam, reduced viscosities caused by increased temperatures, and enhanced dissolution and volatilization of NAPL constituents, including VOCs, SVOCs, PCBs, dioxins/furans, and/or other organic contaminants, depending on the *in situ* temperature.	Can be applicable to all site contaminants, but for NAPL treatment, is likely only to be cost effective in source areas where mobile NAPL is concentrated and may be mobilized more easily. High energy requirements. Superheated temperatures greater than the boiling temperature of water can only be reached once dewatering is complete, so groundwater management is often necessary. Off-gas and condensate treatment is required. If applied, could also be used to provide *in situ* thermal desorption.	Sorenson et al. (2011), Baker et al. (2006), USEPA (1997a), FRTR (2007), USDA (2002)	High	Difficult	High	Yes (retained for possible use in conjunction with other technologies)
		***In Situ* Chemical Oxidation (ISCO)** Addition of oxidants generates free radicals that chemically convert hazardous organic contaminants to nonhazardous or less toxic compounds. The most common chemical oxidants include ozone, hydrogen peroxide, and sodium/potassium permanganate. Oxidation reactions occur in the dissolved phase.	Applicable to PCP and petroleum constituents; not applicable to dioxins and furans. ISCO is not typically applied for remediation of NAPL because of the high chemical demand required to destroy contaminants. The oxidant must be matched to the site conditions and project objectives. A treatability/pilot study would be required to assess its treatment effectiveness and satisfy design requirements. May be performed in conjunction with soil and/or groundwater ISCO.	ITRC (2009)	Low	Moderate	Moderate to High	Yes (retained for possible use in conjunction with other technologies)
Groundwater Containment	Physical Barrier	**Horizontal Barrier** (for example: asphalt/concrete cap, soil/bentonite/clay cap, multi-layer cover systems, Aquablok, apatite, or coke breeze in a laminate mat) These barriers are a horizontal cap intended to prevent infiltration of surface water through contaminated soil to groundwater.	Applicable to site COCs. Prevents, or reduces, migration of contaminants from infiltrating water. Does not actively remediate contaminants. Requires long-term maintenance. This alternative does not address the source; however, human contact with soil and groundwater is restricted. May be used in conjunction with a soil horizontal barrier.	FRTR (2007)	Moderate	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
		Vertical Barrier (for example: grouting, sheet piling, and slurry walls) Slurry walls utilize a vertically excavated trench that is filled with a slurry, grout, or other materials that support the trench and reduce the flow of groundwater.	Applicable to Site COCs. Prevents, or reduces, migration of contaminants and direct human contact. Does not actively remediate contaminants. Requires long-term maintenance. This alternative does not address the source; however, human contact with soil and groundwater is restricted.	FRTR (2007)	High	Moderate to Difficult	Moderate to High	Yes (retained for possible use in conjunction with other technologies)
	Groundwater Extraction Systems	**Trenches** Possible objectives of trenches include extraction of dissolved contaminants from the subsurface, and containment of contaminated groundwater to prevent migration.	Applicable to Site COCs; however, would be economically unfeasible for subsurface contaminants at depths greater than 10 ft. Issues could arise due to seasonal flux of groundwater levels. Requires long-term maintenance. Eliminated from further evaluation as there are more technically and economically feasible methods of hydraulic containment.	FRTR (2007)	High	Moderate to Difficult	Moderate to High	No

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Groundwater Containment	Groundwater Extraction Systems	**Extraction Wells** Possible objectives of wells include extraction of dissolved contaminants from the subsurface and containment of contaminated groundwater to prevent migration.	Applicable to Site COCs. Requires long-term maintenance. Process option would require use in conjunction with a groundwater treatment technology. Removing groundwater can result in longer treatment times. Groundwater pumping is not applicable to contaminants with high residual saturation or high sorption capabilities. Both extraction wells and injection wells can foul. The effectiveness and cost of wells are affected by the depth of the groundwater.	FRTR (2007)	High	Easy	Moderate	Yes (retained for possible use in conjunction with other technologies)
		Directional Wells Drilling techniques are used to position wells horizontally, or at an angle, to reach contaminants that are not accessible by direct vertical drilling.	Applicable to Site COCs. Specialized equipment is required. Wells can be difficult to position precisely, limiting their effectiveness. Installation of horizontal wells is typically costly. Requires long-term maintenance and the potential exists for the wells to collapse. Eliminated from further evaluation as there are more technically and economically feasible methods of hydraulic containment.	FRTR (2007)	High	Moderate	Moderate to High	No
		Fracturing (for example, pneumatic or hydraulic fracturing or blast enhanced) Cracks are developed in low-permeability and over-consolidated sediments by fracturing beneath the surface to open new passageways. Cracks are filled with porous media that improve pumping efficiency.	Applicable to Site COCs; however, Site soils are permeable and unconsolidated (Integral 2016). Process option not retained for further analysis as it is not applicable to Site soils.	USEPA (1994)	Low	Moderate	Moderate	No
Ex Situ Groundwater Treatment	Bioremediation	**Constructed Wetlands** This technology incorporates the principal components of wetland ecosystems; however, microbial activity is used to remediate contaminated groundwater.	Applicable to PCP and petroleum constituents; not applicable to dioxins and furans. Limited effectiveness in cold-weather climates. The long-term effectiveness of constructed wetlands is not well known. Requires long-term maintenance. Process option not retained for further analysis because of climate limitations and because it is not applicable to dioxins and furans.	FRTR (2007)	Low	Moderate	Moderate to High	No
		Land Treatment A full-scale bioremediation technology that applies contaminated groundwater to the soil surface. The soil is then tilled to aerate and facilitate bioremediation. Liners and other methods are used to control leaching of contaminants.	Applicable to PCP and petroleum constituents; not applicable to dioxins and furans. Typically is available for application to groundwater only when a soil landfarm is implemented. The site must be managed properly to prevent offsite migration or contaminant transport. Adequate monitoring and environmental safeguards are required. Requires long-term maintenance. Process option not retained for further analysis as it would concentrate dioxins and furans in the soils without effectively treating it.	FRTR (2007)	Moderate	Moderate	Moderate	No
		Engineered Bioreactors (applied within a tank) These bioreactors use attached or suspended growth biological systems to degrade contaminants in water. In suspended growth systems, contaminated groundwater is circulated in an aeration basin and is aerobically degraded with microbes. A sludge is formed and settled in a clarifier. In attached growth systems, microorganisms are grown on an inert support matrix such as a rotating disk contactor.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Very high contaminant concentrations may be toxic to microorganisms. Low ambient temperatures significantly decrease rates of biodegradation, resulting in longer retention time requirements. Residuals from sludge processes require treatment or disposal.	FRTR (2007)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)

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General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Ex Situ Groundwater Treatment	Physical / Chemical Treatment	**Air Stripping** This full-scale technology increases the area of the contaminated groundwater that is exposed to air, thereby removing VOCs.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Biological fouling of the equipment can occur. Effective only for groundwater with concentrations of VOCs or SVOCs with a dimensionless Henry's constant greater than 0.01. High energy costs. Off-gas treatment may be required. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Easy	Low to Moderate	No
		Adsorption Generally categorized as either physical adsorption or chemisorption. Weak molecular bonds drive physical adsorption. A chemical reaction forms a strong chemical bond between the compound and the surface of the solid in chemisorption. A full-scale technology that pumps groundwater through activated carbon or other sorbents (e.g., synthetic resins). Dissolved organic compounds adsorb to the sorbent.	Applicable to Site COCs. Effective on most organic contaminants and selected inorganic contaminants from liquid and gas streams. Adsorption by activated carbon has a long history of use in treating municipal, industrial, and hazardous wastes. The carbon can be regenerated when concentrations reach a high level,- and spent carbon may need to be transported and decontaminated and eventually disposed of. Adsorption of site compounds of concern is also feasbile via Macro Porous Polymer Extraction (MPPE) and synthetic resins. Fouling can occur. Costs are high if used as the primary treatment on waste streams with high contaminant levels.	FRTR (2007)	High	Easy	Low to Moderate	Yes
		Chemical/Ozone/UV Oxidation Addition of oxidants generates free radicals that chemically convert hazardous organic contaminants to nonhazardous or less toxic compounds. UV irradiation also generates free radicals that destroy organic contaminants and can be used to enhance chemical oxidation. A wide variety of organic contaminants are susceptible to destruction by chemical/UV oxidation, including petroleum hydrocarbons and chlorinated hydrocarbons.	Applicable to Site COCs. The most common chemical oxidants include ozone, hydrogen peroxide, and sodium/potassium permanganate. Handling and storing hydrogen peroxide require special safety precautions. Easily oxidized organic compounds, such as simple aromatic hydrocarbons, are rapidly destroyed. UV oxidation depends on how effectively UV light is transmitted to the dissolved contaminants. High turbidity of the water can cause interference. Pretreatment of the aqueous stream may be required. Energy requirements and costs can be very high.	FRTR (2007), Trach (1996)	High	Moderate to Difficult	Moderate to High	Yes
		Solar Oxidation (for example: solar detoxification, or photocatalytic destruction) Solar detoxification uses sunlight as the energy source for reactions that will break down contaminants in groundwater. The reactions are photochemical and use UV light from the solar spectrum. Photocatalytic destruction uses photocatalysts (such as hydrogen peroxide or ferrioxalate) that absorb UV light to power chemical reactions.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Emerging technology with limited full-scale application. Process option not retained due to limited applicability to Site COCs.	FRTR (2007)	Low	Moderate	Moderate	No
		Electrokinetics The technology relies on application of direct current through the media between electrodes. Metal ions, ammonium ions, and positively charged organic compounds move toward the cathode. Anions such as chloride, cyanide, fluoride, nitrate, and negatively charged organic compounds move toward the anode.	Not applicable to Site COCs. Intended to separate heavy metals and organic compounds from groundwater. Affects the migration of contaminants by imposing an electrical field via electroosmosis, electromigration, or electrophoresis. Effective on metals and most organic compounds but is difficult to implement and can result in high maintenance. Process option not retained for further analysis as it is not applicable to Site COCs.	Van Cawenberghe (1997)	Low	Difficult	Moderate	No
		Irradiation (electron irradiation) The treatment of substances with high energy electrons (E-beam). Electron irradiation generates free radicals, which destroy organic contaminants, similar to chemical and UV oxidation.	Limited applicability to Site COCs. Demonstrated on chlorinated solvents, fuels, and MTBE, but not well tested on other groundwater contaminants. Costs are typically high. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Difficult	High	No

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Ex Situ Groundwater Treatment	Physical / Chemical Treatment	**Ion Exchange** Removes ions from the aqueous phase by the exchange of cations or anions.	Not applicable to Site COCs. Material used may include synthetic resins or inorganic and natural polymeric materials. Typically implemented to remove dissolved metals from aqueous solutions. Other compounds that have been treated include nitrate, ammonia nitrogen, and silicate. Process option not retained for further analysis as it is not applicable to Site COCs.	FRTR (2007)	Low	Moderate	Moderate	No
		Precipitation/Coagulation/ Flocculation (metals precipitation, electrocoagulation) Precipitation has been a primary method for treating metals in industrial wastewater and has also been proven successful in treating groundwater that contains metals. In the precipitation process, coagulation and flocculation are used to increase particle size through aggregation and, therefore, the efficiency of the process. After the coagulants have increased particle size, flocculation is used to promote contact between the particles.	Not applicable to Site COCs. The three main types of coagulants are inorganic electrolytes (such as alum, lime, ferric chloride, and ferrous sulfate), organic polymers, and synthetic polyelectrolytes with anionic or cationic functional groups. Typically implemented to remove dissolved metals from aqueous solutions. Process option not retained for further analysis as it is not applicable to Site COCs.	FRTR (2007)	Low	Moderate	Moderate	No
		Membrane Technologies (reverse osmosis, pervaporation, ultrafiltration, microfiltration) Reverse osmosis is the process of pushing a solution through a filter that traps solute on one side and allows the solvent to pass through to the other side. This process is best known for its use in desalination. Micro- and ultrafiltration are similar to reverse osmosis but have larger pore sizes in the filter/membrane. Pervaporation is a new membrane process to remove and concentrate VOCs from contaminated water. Two different membrane configurations have been tested using hollow fibers. Pervaporation was described by a resistance-in-series model: a liquid film resistance, and a membrane resistance.	Applicable to Site COCs. Cost is very high and the presence of organic matter can foul the membrane, resulting in high maintenance costs. Reverse osmosis and electrodialysis are common technologies for treatment of contaminants such as arsenic and perchlorate in drinking water, but tend to be more expensive than other technologies. Membrane treatment technology may be appropriate for removal of suspended/colloidal solids on which organic contaminants may be adsorbed, but which may not otherwise filter out or adsorb to activated carbon.	FRTR (2007)	Moderate	Moderate	High	Yes
		Other Separation/Filtration Separation physically removes contaminants from groundwater through processes that include distillation or freeze crystallization.	Limited applicability to Site COCs. Used mainly as a pretreatment or post-treatment process to remove contaminants from wastewater. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Moderate	Moderate	No
		Volatilization Typically uses the pressurized distribution of water through a standard sprinkler irrigation system to volatilize VOCs from contaminated wastewater.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Effective on VOCs, SVOCs, fuels, explosives, and pesticides. Regulatory approval may be difficult to obtain because of the potential for direct release of contaminants to the atmosphere. Temperature may reduce the effectiveness. May be limited by Clean Air Act requirements. Process option not retained for further analysis as it is limited applicability to Site COCs.	FRTR (2007)	Low	Easy	Low to Moderate	No
Groundwater Discharge	Discharge of Treated or Untreated Groundwater	**Land Application** Water is spread out on the ground to either infiltrate or evaporate.	Applicable after groundwater is treated to meet relevant state and federal cleanup criteria.	FRTR (2007)	High	Easy	Low	Yes (retained for possible use in conjunction with other technologies)
		Injection Wells (shallow or deep injection wells) or Trenches	Applicable after groundwater is treated to meet relevant state and federal cleanup criteria. Sampling of the influent and effluent will likely be required.	FRTR (2007)	High	Moderate to Difficult	Moderate	Yes (retained for possible use in conjunction with other technologies)

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
Groundwater Discharge	Discharge of Treated or Untreated Groundwater	**Discharge to Surface Water**	Applicable after groundwater is treated to meet relevant state and federal cleanup criteria.		High	Easy	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Discharge to Stormwater Collection System	Process option not retained for further analysis as discharge to a stormwater collection system is not available at the Site.		High	Difficult	Moderate	No
		Discharge to a Municipal Sanitary Sewer	Process option not retained for further analysis as discharge to a municipal sanitary sewer is not available at the Site.		High	Difficult	Low	No
		Disposal Offsite	Applicable to Site COCs. The contaminated groundwater is pumped into a vacuum truck for disposal as contaminated water. Disposal must be in accordance with all applicable local, state, and federal rules. Offsite disposal can be expensive for large volumes of water.		High	Moderate	High	Yes
In Situ Groundwater Treatment	Bioremediation	**Enhanced Bioremediation (aerobic)** Enhancements include the addition of nutrients, oxygen, cultured microorganisms (bioaugmentation), or other amendments. Amended water is frequently circulated through the treatment zone to enhance mixing and contact.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Enhanced bioremediation is especially effective for low-level residual contamination after source removal. Pilot testing may be required as bioremediation is site specific and highly dependent on the ecology and physiology of the subsurface.	FRTR (2007)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Nitrate Enhancement Solubilized nitrate is circulated throughout groundwater contamination zones to provide an alternative electron acceptor for biological activity and enhance the rate of degradation of organic contaminants.	Limited applicability to Site COCs. Technology is still at the pilot scale. Many states prohibit nitrate injection into groundwater because nitrate is regulated through drinking water standards. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Difficult	Moderate	No
		Enhanced Bioremediation (anaerobic) An electron donor (molasses, HRC, or similar product) is added to soil to increase the number and vitality of indigenous microorganisms involved in anaerobic bioremediation.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Anaerobic bioremediation occurs at a slower rate than aerobic bioremediation. Pilot testing may be required as bioremediation is site specific and highly dependent on the ecology and physiology of the subsurface.	FRTR (2007)	Moderate	Moderate	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
		Phytoremediation (e.g. poplar trees, grasses) Phytoremediation is a process that uses plants to remove, transfer, stabilize, and destroy contaminants in groundwater.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. High concentrations of hazardous materials can be toxic to plants, limiting the effectiveness and reliability of the system. It involves the same mass transfer limitations as other biotreatments. It may be seasonal, depending on location. The effectiveness of the alternative depends on the depth to groundwater.	FRTR (2007), Hechmi et al. (2014), Mills et al. (2006)	Moderate	Easy	Low	Yes
		Bio-Sparging An *in situ* technology that injects air through a contaminated aquifer. Injected air traverses horizontally and vertically in channels through the soil column to promote bioremediation.	Applicable to PCP and petroleum constituents; not applicable to dioxins and furans. The need for bioaugmentation is highly site specific and highly dependent on the ecology and physiology of the subsurface.	Miller (1996)	Moderate	Easy	Low to Moderate	Yes (retained for possible use in conjunction with other technologies)
	Physical / Chemical Treatment	***In Situ* Chemical Oxidation (ISCO)** Addition of oxidants generates free radicals that chemically convert hazardous organic contaminants to nonhazardous or less toxic compounds. The most common chemical oxidants include ozone, hydrogen peroxide, and sodium/potassium permanganate.	Applicable to PCP and petroleum constituents; limited applicability to dioxins and furans. Costs increase with high contaminant concentrations (NAPL or smear zones) because of the large amounts of oxidizing agent required.	FRTR (2007)	Moderate	Moderate	Moderate	Yes (retained for possible use in conjunction with other technologies)

Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	
In Situ Groundwater Treatment	Physical / Chemical Treatment	**Passive/Reactive Treatment Wall/Zone** These barriers allow the passage of water while adsorbing, degrading, or removing contaminants. This could include injection of granular activated carbon or nano-scale iron, etc.	Applicable to Site COCs. Passive treatment walls may lose their reactive capacity, reducing the effectiveness and requiring replacement of the reactive medium.	USACE (1999), Birnstingl et al. (2016)	High	Moderate to Difficult	Moderate to High	Yes
	Physical / Chemical Treatment	**Air Sparging** An *in situ* technology that injects air through a contaminated aquifer. Injected air traverses horizontally and vertically in channels through the soil column, creating an underground stripper that removes contaminants by volatilization.	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Air flow through the saturated zone may not be uniform. Site groundwater is deep and technology will be difficult to implement. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Easy	Moderate	No
	Physical / Chemical Treatment	**In-Well Air Stripping or In-Well Aeration** Air is injected into a double-screened well, lifting the water in the well and forcing it out the upper screen. Simultaneously, additional water is drawn in the lower screen. Once in the well, some of the VOCs in the contaminated groundwater are transferred from the dissolved phase to the vapor phase by air bubbles. The contaminated air rises in the well to the water surface, where vapors are drawn off and treated by a soil vapor extraction system. Water can also be pumped through the well (as in a circulating well).	Limited applicability to PCP and petroleum constituents; not applicable to dioxins and furans. Biological fouling of the equipment can occur. Effective only for contaminated water with concentrations of VOCs or SVOCs with a dimensionless Henry's constant greater than 0.01. High energy costs. Off-gas treatment may be required. In-well air stripping may not be efficient in sites with strong natural flow patterns. Process option not retained for further analysis due to limited applicability to Site COCs.	FRTR (2007)	Low	Easy	Moderate	No

Notes:

COC = chemical of concern
DNAPL = dense, nonaqueous-phase liquid
LNAPL = light, nonaqueous-phase liquid
NAPL = nonaqueous-phase liquid
PCP = pentachlorophenol
VOC = volatile organic compound
SVOC = semivolatile organic compound

Shaded rows indicate technologies that have been retained.

The criteria used to evaluate the alternatives were selected from the Federal Remediation Technology Roundtable (FRTR) database: implementability, effectiveness, and cost. Implementability refers to how readily an alternative can be implemented at a site. Effectiveness refers to how well the alternative can address the contaminants of concern, taking into consideration site-specific conditions. Cost refers to the capital and operation and maintenance costs of an alternative.

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Table 1. Comprehensive List of Potential Treatment Technologies

General Response Action	Technology Type	Process Options	Evaluation/Comments	References	Initial Evaluation Factors			Retained
					Effectiveness	Implementability	Cost	

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Memorandum

To: David Bowers

From: Aimee Reynolds

Date: 10/3/2017

Re: Montana Pole Direct Contact Cleanup Level 5-Year Review

This memo is meant to supplement my June 3, 2016 memo regarding recalculation of the site-specific cleanup levels (SSCLs) for Montana Pole using updated exposure parameters and toxicity criteria as appropriate. The reason for this supplemental memo is that the EPA finalized toxicity information for benzo(a)pyrene in January 2017. The carcinogenic polycyclic aromatic hydrocarbon (PAH) toxicity is based upon the toxicity of benzo(a)pyrene. The calculated cleanup levels, including the updated PAH values are provided in the table below along with the preliminary remediation goals (PRGs) provided in Table 2 of the September 1993 Record of Decision (ROD). The dioxins/furans and PAHs concentrations are based upon toxicity equivalents (TEQs). I did not include residential PRGs or recalculate SSCLs since I understand that this usage is not being considered for the site. All concentrations are in milligrams per kilogram (mg/kg). I added SSCLs for construction worker exposure to both surface and subsurface soil in case they were needed since they were not provided in the ROD. Please see the June 3, 2016 memo for additional details.

Chemical (mg/kg)	Recreational		Industrial		Construction	
	PRG	SSCL	PRG	SSCL	PRG	SSCL
Pentachlorophenol	34	36	9	7	NA	77
Dioxins/Furans (TEQ)	0.0002	0.0001	0.00003	0.00004	NA	0.0004
Carcinogenic PAHs (TEQ)	4	14	0.7	3.9	NA	39

Mass Removal Efficiencies Based on Montana Pole Dioxin Concentrations

Dioxin (ng/kg) Concentration	99% Removal Efficiency	95% Removal Efficiency	90% Removal Efficiency	MT Pole Recreational Cleanup
1900.00	19.00	95.00	190.00	100 ng/kg
9100.00	91.00	455.00	910.00	100 ng/kg
2600.00	26.00	130.00	260.00	100 ng/kg
1600.00	16.00	80.00	160.00	100 ng/kg
1200.00	12.00	60.00	120.00	100 ng/kg
1900.00	19.00	95.00	190.00	100 ng/kg
1100.00	11.00	55.00	110.00	100 ng/kg
1300.00	13.00	65.00	130.00	100 ng/kg
1100.00	11.00	55.00	110.00	100 ng/kg
900.00	9.00	45.00	90.00	100 ng/kg
3600.00	36.00	180.00	360.00	100 ng/kg
2800.00	28.00	140.00	280.00	100 ng/kg
1800.00	18.00	90.00	180.00	100 ng/kg
2800.00	28.00	140.00	280.00	100 ng/kg
3700.00	37.00	185.00	370.00	100 ng/kg
2500.00	25.00	125.00	250.00	100 ng/kg
6000.00	60.00	300.00	600.00	100 ng/kg
1900.00	19.00	95.00	190.00	100 ng/kg
1000.00	10.00	50.00	100.00	100 ng/kg
1600.00	16.00	80.00	160.00	100 ng/kg
2500.00	25.00	125.00	250.00	100 ng/kg
4200.00	42.00	210.00	420.00	100 ng/kg
2300.00	23.00	115.00	230.00	100 ng/kg
1900.00	19.00	95.00	190.00	100 ng/kg
1000.00	10.00	50.00	100.00	100 ng/kg
2700.00	27.00	135.00	270.00	100 ng/kg
3700.00	37.00	185.00	370.00	100 ng/kg
3200.00	32.00	160.00	320.00	100 ng/kg
2000.00	20.00	100.00	200.00	100 ng/kg
2200.00	22.00	110.00	220.00	100 ng/kg

Exceeds MT Pole Recreational Cleanup Level

Evaluation of Fungal-BasedRemediation for Treatment of a PCP/Dioxin/Furan-Contaminated Soil from several former Wood-Treating Facilities in New Zealand

Richard T. Lamar	Director of Research and Applications	EarthFax Development Corp
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Roberta R. Farrell [1]	Professor, School of Science and Technology	University of Waikato
Joanne Thwaites	Research Scientist, School of Science and Technology	University of Waikato
Ian Davies [2]	Manager – Environmental science and engineering	Carter Holt Harvey SPHERE
Alan Blair	Project Manager - Remediation	Carter Holt Harvey SPHERE

INTRODUCTION

Fungal-based soil remediation employs the pollutant-degrading abilities of a group of wood decay fungi referred to as white-rot fungi (WRF). These fungi are saprophytes - they obtain their carbon for energy and biomass from dead organic matter - and thus are not human or plant pathogens. Indeed, common edible mushrooms including the oyster mushroom (*Pleurotus ostreatus*), the Shitake (*Lentinulus edodes*), and the white button mushroom (*Agaricus bisporus*) are all produced by WRF. White-rot fungi are ubiquitous in nature. Thus, local New Zealand strains of WRF species that possess pollutant-degrading abilities, can be used. These fungi, which require oxygen and grow best, in general, at 24ºC to 35ºC, are called WRF because they have the ability to degrade the darker colored material in wood called lignin, leaving behind the lighter colored cellulose. This gives WRF decayed wood a bleached appearance.

Carter Holt Harvey has joined with the University of Waikato and EarthFax, a specialist engineering and research & development company based in Utah in the United States, to explore the opportunities for degrading and treating pentachlorophenol (PCP) contaminated soil. PCP-contaminated soil is a legacy of the past use of a very effective antisapstain timber treatment chemical. The use of PCP as an antisapstain ceased in the early 1990's when the chemical was de-registered by the New Zealand Pesticides Board.

In early 2003, following almost 2 years of laboratory research, Carter Holt Harvey, the University of Waikato and EarthFax formed a collaborative venture to develop applications for the use fungal-based soil remediation technologies from the laboratory scale. This work has been undertaken at a facility on Carter Holt Harvey's Kinleith Industrial Park.

BACKGROUND

Fungal-based soil remediation consists of amending the contaminated soil with a fungal inoculum, using a predetermined application rate. Prior to soil treatment the most effective WRF, inoculum application rate and necessity of soil amendments (e.g. surfactants, bulking agents) are determined through a laboratory treatability study (usually a 2-month bench-scale study). The inoculum-soil mixture is then treated in a forced-aeration biopile. Forced aeration is necessary to prevent overheating of the piles, which can result from the large quantities of metabolic heat produced by WRF, to temperatures that would inhibit or kill the WRF. Throughout the treatment period, both pile temperature and moisture are closely monitored and regulated to control conditions that best suit fungal growth and pollutant-

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degrading activity. The inoculum is prepared on-site, a process that requires from 3-6 weeks, depending on ambient temperatures. The inoculum consists of a pure culture of the selected WRF grown on a lignocellulosic substrate. A variety of inexpensive agricultural and forestry residues (e.g., wood chips, cotton seed hulls, cotton gin trash, sawdust, straw) can be used.

Both direct (e.g. catalysis of pollutant oxidation by lignin-degrading enzymes) and indirect (e.g. pollutant mineralization during lignolysis) evidence indicates that the lignin-degrading systems of white-rot fungi are involved in pollutant degradation. Lignin has a heterogeneous, aromatic structure with many different types of subunit linkages. The enzyme systems and associated mechanisms that WRF have evolved to depolymerize (i.e. break apart) lignin and to further degrade the aromatic subunits are highly oxidative, extracellular and nonspecific (Kirk and Farrell, 1987).

In addition to oxidative agents, the fungi possess reductive agents that are also involved in the degradation of aromatic substructures of lignin that are produced from its depolymerization. Aromatic pollutants like pentachlorophenol (PCP), polynuclear aromatic hydrocarbon components of coal tar and creosote (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo dioxins (PCDDs) and dibenzo furans (PCDFs) closely resemble the aromatic substructures that are produced during lignin depolymerization. In liquid fungal culture, chlorinated phenols, including PCP have been shown to be degraded via a series of reactions that remove all the chlorines after which the aromatic ring is oxidatively cleaved and further degraded to CO_2 (Valli and Gold 1991, Joshi and Gold 1993). In soil, mineralization (i.e. degradation to CO_2 and H_2O) has been demonstrated to be a minor fate for PCP. Instead, a large fraction of the PCP becomes irreversibly associated with soil humic materials (i.e. it becomes part of the soil organic matter (Ruttiman-Johnsan and Lamar 1997). It is thus detoxified because it is no longer "bioavailable."

The objective of the described studies was to demonstrate the ability of fungal-based remediation, using New Zealand strains of WRF grown on locally available lignocellulosic substrates (e.g. radiata pine or eucalyptus wood chips), to decrease the concentration of PCP and a selected hepta- and octachlorinated dioxin/furan congeners in soil samples collected from three former wood-treating facilities in New Zealand. Technology performance was based on decreasing the concentrations of PCP and the dioxin/furan congeners to levels that were sufficient to indicate that regulatory goals could be met or exceeded under full-scale conditions.

MATERIALS AND METHODS

Approach

In two scientifically-controlled experiments, the ability of selected species of WRF, grown on either radiata pine or eucalyptus pulpwood chips, to decrease the concentrations of PCP and a 1,2,3,4,6,7,8-heptachlorodiebenzo furan (HpCDF), 1,2,3,4,6,7,8-heptachlorodibenzo dioxin (HpCDD), and octachlorodibenzo dioxin (OCDD) in contaminated soils from three former wood-treating facilities: the former Carter Holt Harvey sawmills at Whakatane, Brookside and Kinleith, were evaluated. A preliminary analysis of the soils revealed that these particular PCDD/PCDF congeners were present in the highest concentrations. In the first study the following factors were evaluated: fungal species, inoculum application rate,

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and surfactant addition, on the Whakatane soil. In the second study fungal species and inoculum application rate were evaluated on the Carter Holt Harvey Brookside and Kinleith soils. We evaluated inoculum substrates that are readily available in New Zealand (i.e. radiata pine and eucalytpus pulpwood chips). Solid inoculum was prepared by inoculating sterile substrates with mycelial slurries of the selected fungi. The slurries were prepared by homogenizing liquid fungal cultures.

Soils

Representative soil samples were obtained from the Carter Holt Harvey Whakatane, Brookside and Kinleith sites. The soils were air-dried, sieved to pass a 2-mm screen and thoroughly mixed. Soils were then stored dry, in sealed containers, until use. The concentrations of target chemicals were determined on soil subsamples using appropriate extraction and analytical techniques. Prior to the study, a moisture content to be used for each soil was determined. This was done by gradually adding water to soil and mixing until a good working consistency is reached. The moisture content was then determined gravimetrically.

Surfactant

Because the regulatory drivers in these soils are the PCDDs and PCDFs and these compounds are extremely hydrophobic, the effect of amending the Whakatane soil with a surfactant to enhance fungal degradation of PCDD/PCDF congeners was evaluated. Based on our past experience with PAH-contaminated soils, the surfactant evaluated was emulsified vegetable oil (EVO) which was applied at a rate of 3% (weight of oil to dry weight of soil). The EVO was mixed with the water that was used to adjust the moisture content of the soil to provide homogeneous distribution of the surfactant.

Fungi and Inoculum

A total of 5 WRF species were evaluated. Four New Zealand strains were provided by Dr. Roberta Farrell of Department of Biological Sciences in the School of Science and Technology at the University of Waikato. These were *Phanerochaete gigantea*, *Resinicium bicolor,* and two fungi isolated from Whakatane site soil referred to as B101 and B102. The latter strains were isolated from highly PCP-contaminated soil on either side of a former dip tank at the Whakatane site. As a baseline we also evaluated a U.S. strain of WRF, *Pleurotus ostreatus*. Fungal inoculum was prepared by cultivating pure cultures of each of the fungi on sterilized radiata pine and/or eucalyptus wood chips. The moisture contents of the chips were adjusted to 60% (wet weight basis) and then they were sterilized by autoclaving at 15 psi and 121˚C for 1 hour on two successive days. The chips were then be inoculated with mycelial slurry inocula produced from liquid cultures (2% glucose and 2% malt extract) of each fungal species. The inoculated chips were then incubated at 30˚C until they were thoroughly colonized by the fungi (about 2 weeks).

Experimental Units

Soil treatments for both studies were conducted in 272 ml canning jars with lids modified to allow adequate air exchange. Each jar contained approximately 30 g of the test soil (i.e. wet weight) and the appropriate amount of fungal inoculum and amendments. Three replicates were prepared for each treatment for each sample time-except for day 0. For day 0, samples were prepared on the side for each treatment from which 2 sub-samples were taken for analyses. The cultures were incubated at 30˚C (this would be the optimum biopile temperature) under high relative humidity to prevent moisture loss. Soil moisture

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contents were maintained as needed. Target compound concentrations were evaluated on the following days: 0, 14, 28 and 56.

Soil Analyses
Soil and soil inoculum mixtures from each experimental unit were air dried in plastic weigh boats and then ground to a fine powder using a commercial coffee grinder. The ground samples were stored dry in sealed glass containers.

To determine the concentrations of PCP, HpCDD, HpCDF, and OCDD 3 g subsamples from each sample were extracted with a 50:50 mixture of hexane and acetone with a Dionex Accelerated Solvent Extractor. Subsamples of the extracts were then analyzed using GC/ECD methods to determine extract concentrations of the analytes. PCP was analyzed as the trimethylsilyl derivative. PCP in extract subamples was derivitized using Sylon BTZ (Supelco Co.). GC/ECD analyses of derivatized extracts were performed on a Hewlett-Packard model 5890 gas chromatograph equipped with a 63Ni electron capture detector, a model 7673A autosampler, and a split-splitless capillary column injection port. Gas flows were: column flow 2 ml min^{-1}; total flow 60 ml min^{-1}. Operating temperatures were: 220˚C (injector) and 300˚C (detector); the carrier and makeup gas was nitrogen. The column was a DB-5 fused silica capillary column (30 m by 0.321mm; film thickness 0.25 um). The temperature program was as follows: initial 60˚C; hold for 1 min; split off for 0.5 min; ramp A, 10˚C min^{-1} for 9 min (60 to 150˚C); ramp B, 2˚C min^{-1} for 20 min (150 to 190˚C); hold at 190˚C for 5 min. GC/ECD analysis of extracts for HpCDD, HpCDF and OCDD were performed on the same instrument using the following conditions:

- Gas flows were - column flow 2 ml min^{-1}; total flow 30 ml min^{-1}.
- Operating temperatures were: 280˚C (injector) and 300˚C (detector); the carrier and makeup gas was nitrogen.
- The column was a DB-5 fused silica capillary column (30 m by 0.321mm; film thickness 0.25 um).
- The temperature program was as follows: initial 185˚C; hold for 2 min; split off for 0.5 min; ramp A, 8˚C min^{-1} for 8 min (85 to 285˚C); hold at 285˚C for 8 min.

Data analyses
Analyses of variance (ANOVA), using $\alpha = 0.05$, were performed on the percent difference between concentrations of the analytes on day 0 and day 56. The main effects included in the ANOVA were fungal treatment, inoculum application rate and surfactant addition.

RESULTS

Study One Whakatane soil

Initial concentrations after treatment applications are given in Table 1. There was significant variation in initial analyte concentrations among the treatments for all four analytes. This was an indication of the heterogeneity of the soil with respect to contaminant concentrations.

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Table 1
Initial concentrations of PCP (mg/kg), HpCDF (ug/kg), HpCDD (ug/kg), and OCDD (ug/kg) immediately after treatment application.

Treatment	PCP	HpCDF	HpCDD	OCDD
	(mg/kg)	----------(ug/kg)----------		
Control	83	313	135	472
P. ostreatus	92	262	189	508
"East side"	182	340	351	743
"West side"	115	378	331	1045
R. bicolor	154	323	307	644
P.gigantea	136	356	380	792

Fungal inoculation had a significant effect on the mean percent decreases of all four analytes among fungal inoculation treatments (Table 2). In all cases inoculation with any of the tested fungi resulted in a significantly greater decrease than no inoculation (i.e. control). Among the tested fungi, the greatest percent PCP decrease occurred in soils inoculated with B101. There were no significant differences among the fungal treatments in the degradation of HpCDF and HpCDD. Average percent decrease of these compounds was greater than 90% in all fungal treatments. Degradation of OCDD was greatest in soils inoculated with *P. gigantea* (Table 2). The percent OCDD decrease in all other fungal inoculated soils was less, significantly so, in soils inoculated with *R. bicolor*.

Table 2
Effect of fungal inoculum and control treatments on mean[1] percent decrease of PCP, HpCDF, HpCDD, and OCDD after 56 days of treatment.

Treatment	PCP	HpCDF	HpCDD	OCDD
Control	15.6c	5.4b	(33.3)b	(22.4)c
P. ostreatus	75.2b	98.5a	97.8a	82.1ab
B101	90.3a	95.7a	95.9a	69.3ab
B102	75.7b	97.0a	92.4a	81.0ab
R. bicolor	83.5ab	95.0a	91.6a	68.2b
P.gigantea	76.6ab	93.7a	91.5a	86.2a

[1] 'Means' within columns followed by the same letter are not significantly different.

Mean concentrations of all four analytes after 56 days of treatment were significantly less in fungal inoculated treatments compared to control treatments (Table 3). The lowest residual PCP concentration occurred in soils inoculated with B101. There were no significant differences among the fungal treatments in residual concentrations of HpCDF and OCDD. The lowest residual concentration of HpCDD occurred in soil inoculated with *P. ostreatus.* However, as with HpCDF and OCDD all the fungal treatments resulted in very extensive decreases in the concetration of HpCDD.

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Table 3
Mean[1] fungal inoculum treatment concentrations of PCP, HpCDF, HpCDD, OCDD after 56 days of treatment.

Treatment	PCP	HpCDF	HpCDD	OCDD
	(mg/kg)	----------	(ug/kg)----------	
Control	70c	263b	264c	557b
P. ostreatus	28b	4a	3a	98a
"East side"	13a	12a	12ab	210a
"West side"	28b	15a	30b	196a
R. bicolor	22ab	14a	24b	188a
P.gigantea	32b	21a	30b	95a

[1] 'Means' followed by the same letter are not significantly different.

The rate of fungal inoculation did not have a significant effect on the average percent decrease of any of the four analytes (Table 4). Application of EVO had no effect on the mean inoculum application rate percent decrease of PCP but significantly decreased the percent degradation of HpCDF, HpCDD and OCDD (Table 5).

Table 4
Effect of inoculum application rate on mean treatment percent decrease for inocuolum application rate of PCP, HpCDF, HpCDD, and OCDD after 56 days of treatment.

Inoculum application rate (wt inoc/wt soil)	PCP	HpCDF	HpCDD	OCDD
	----------		(%decrease)----------	
10%	83.7	96.1	92.6	78.9
20%	77.1	95.9	95	75.8

Table 5
Effect of EVO application rate on mean percent decrease of PCP, HpCDF, HpCDD, and OCDD after 56 days of surfactant treatment.

EVO addition rate	PCP	HpCDF	HpCDD	OCDD
	----------		(% decrease)----------	
0	71.8a	91.3a	82.9a	79.0a
3	76.8a	84.2b	81.6b	57.6b

The treatment combination that resulted in the greatest overall total percent decrease for the four analytes was inoculation with *P. ostreatus* (which is a US strain included for comparative purposes only) using an inoculum application rate of 10% and augmentation of the soil with 3% EVO. The second most effective was inoculation with the B101 at an inoculum application rate of 10% in the presence of 3% EVO (Table 6). Based on the degradation of PCDD/PCDFs only, the most effective treatments were inoculation with *P. ostreatus* at a rate of 10% with or without EVO and inoculation with the B102 isolate at a rate of 20% with or without EVO. Because similar results were obtained with or without EVO, it would not be necessary to use it in the field.

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Study Two Brookside and Kinleith soils

In the second study we only evaluated the relative abilities of B101 and B102 to decrease the concentrations of PCP HpCDF, HpCDD and OCDD in the two soils. Initial (Day 0) concentrations are given in Table 6.

Table 6
Initial concentrations of PCP, HpCDF, HpCDD and OCDD in the Brookside and Kinleith inoculated with either B101 or B102 or non-inoculated.

Treatment	PCP	HpCDF	HpCDD	OCDD
	(mg kg^{-1})			
	----------Kinleith soil----------			
Non-inoculated	710	1.3	16.1	51.4
B101	682	na[1]	na	na
B102	738	na	na	na
	----------Brookside soil----------			
Non-inoculated	473	1.3	9.6	18.3
B101	481	na	na	na
B102	464	na	na	na

[1] na= not assessed

After 56 days of treatment in the Brookside soil the concentration of PCP only decreased by about 34% whereas in soils inoculated with B101 or B102, the concentration of PCP decreased 98.7% and 98%, respectively. In the Kinleith soil there was a 33% decrease in the PCP concentration in the non-inoculated soil after 56 days. The PCP concentration in the soils inoculated with B101 and B102 decreased by 99% and 98.9%, respectively. Fungal inoculation in both soil, using either fungus resulted in rapid and extensive decreases in the concentration of PCP.

Table 7
Percentage decreases of OCDD, HpCDD and HpCDF in the Brookside and Kinleith soils inoculated with either B101 or B102 or non-inoculated, after 56 days.

Treatment	HpCDF	HpCDD	OCDD
	(%)		
	----------Kinleith soil----------		
Non-inoculated	10.0	3.0	(2.0)
B101	63.6	94.7	93.4
B102	53.3	93.5	91.6
	----------Brookside soil----------		
Noninoculated	11.3	9.1	(2.9)
B101	74.3	82.3	78.3
B102	68.0	80.3	75.15

There were only slight decreases in the concentrations of HpCDF, HpCDD and OCDD in noninoculated Kinleith and Brookside soils after the 56-day treatment period (Table 7). Inoculation with either B101 or B102 however, resulted in large decreases of all three

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analytes in both soils. Decreases were similar for both fungi. Decreases in the concentrations of HpCDD and OCDD were greater in the Kinleith soil than in the Brookside soil; whereas decreases in the concentration of HpCDF were greater in the Brookside than those observed in the Kinleith soil.

CONCLUSIONS

Inoculation of PCP/PCDD/PCDF-contaminated soils with selected isolates of WRF grown on locally available radiata pine or eucalyptus pulpwood chips resulted in rapid and extensive decreases in the concentrations of the contaminants. Minor decreases in the concentrations of the contaminants indicated that the decreases in the fungal inoculated soils were due to the pollutant-degrading activity of the inoculant fungi. In particular, treatment with either of two fungal species isolated from PCP/PCDD/PCDF-contaminated soil from around the former diptank at the Whakatane site effectively decreased the concentrations of the PCP, HpCDF, HpCDD, and OCDD. Based on these results the use of fungal-based remediation of the treatment of New Zealand soils contaminated with PCPs and associated PCDDs/PCDFs has excellent potential.

Work has been undertaken to demonstrate the effectiveness of fungal-based remediation, using isolate B101, at pilot-scale and further developmental work is underway to upscale this technology for application at a full scale and on a commercial basis.

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LARGE-SCALE PRODUCTION AND FIELD TESTING OF PELLETED FUNGAL INOCULA FOR USE IN FUNGAL-BASED REMEDIATION OF CONTAMINATED SOIL

Final Report

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LARGE-SCALE PRODUCTION AND FIELD TESTING OF PELLETED FUNGAL INOCULA FOR USE IN FUNGAL-BASED REMEDIATION OF CONTAMINATED SOIL

Summary

This report details the results a Phase II Small Business Innovation Research project conducted by EarthFax Development Corporation to evaluate large-scale production and field performance of pelleted fungal inoculum for use in bioremediation. Pellets were toll manufactured and production-scale equipment was identified and evaluated for coating and bagging the pellets. We initially intended for the field evaluation to be conducted on soil contaminated with polynuclear aromatic hydrocarbons (PAHs) that are contaminants at sites where creosote has been used in wood treating operations or at out-of-service coal gasification sites. Therefore, the field evaluation was originally planned for implementation at a creosote wood-treating facility in West Virginia owned by Appalachian Timber Services. However, during the first year of this Phase II project, the facility owners were granted permission by the US EPA to simply cap the site rather than pursue fungal-based or another active remediation alternative. We then evaluated an alternative location in Gainesville, FL that was the site of a former coal gasification plant. We obtained soil from this site and conducted a treatability study to evaluate the ability of three fungi to degrade PAHs in the soil: two white-rot fungi (i.e., *Pleurotus ostreatus* and *Irpex lacteus*), and another zygomycetous fungus that was isolated from pentachlorophenol-contaminated soil obtained from a former wood treating facility in Whakatane, New Zealand. The results of the treatability revealed that *I. lacteus* was the most effective fungus for degradation of PAHs in the Gainesville gas works soil and this fungus was selected for evaluation of the field evaluation of the pelleted inoculum. However, as the inoculum was being prepared for the field test during May and June of 2004, the city of Gainesville declined to have the field test conducted at their site. Since the inoculum had been prepared and was being stored under refrigeration, we attempted, unsuccessfully, to find an alternative site with soil contaminated with PAHs.

Although permission could not be obtained on short notice to conduct the field trial at an alternative PAH-contaminated site, we were able to obtain permission from both the site owner and the State of Montana to conduct the field portion of the project at an out-of-service wood treating facility located in Darby, Montana. The contaminants of concern at this facility were pentachlorophenol (PCP), polychlorinated dibenzo dioxins (PCDDs), and polychlorinated dibenzo furans (PCDFs). We also changed the focus of the field study, at the request of the granting agency (the U.S. Department of Agriculture) from a comparison of pelleted inoculum and site-produced to focus on the production and application of pelleted inoculum only, with replication of treatments. Approximately 5 tons of pelleted inoculum that was previously prepared at the L. F. Lambert Spawn Co. was delivered to the site in a refrigerated truck. The inoculum was thoroughly colonized at the time of receipt and did not exhibit contamination with any other microorganisms. To determine the shelf-life of the pelleted inoculum, several

bags of inoculum were retained under refrigeration at the Lambert facility and pellet samples were taken aseptically on a weekly basis (for two months), then every other week for another month, and sent to the EarthFax Development lab for testing inoculum potential using a fluorescein diacetate hydrolyzing activity (FDA) assay. This assay provides a measure of microbial biomass growth rate by measuring the activity of a number of enzymes (lipases, proteases, esterases) that are produced during fungal growth.

The field treatability study was initiated in August 2004 and run through May 2005. Five in-ground, forced-aeration biocells were established as follows:

- One control biocell (not inoculated),
- Two biocells inoculated at a rate of 5% (dry-weight basis) with pelleted inoculum, and
- Two biocells inoculated at a rate of 10% with pelleted inoculum.

Soil samples were taken at the time the study was established and at periodic intervals through the study period. Analysis of the samples for PCP and PCDDs/PCDFs revealed significant decreases in the concentration of PCP in the soils amended with 5% inoculum (70% decrease) and 10% inoculum (90% decrease) compared to a minimal decrease in the control soil (20% decrease). Decreases in the concentrations of PCDDs and PCDFS occurred in all treatments but tended to be greater in the fungal inoculated soils in all but one of the biocells. Decreases in toxicity, as normalized to the toxicity of 2,3,7,8-TCDD, were greater in fungal inoculated soil than in control soil. Treatment of PCDD/PCDFs may have been more effective if the soil had been amended with a surfactant to enhance the bioavailability (i.e. concentration in the soil solution) of these extremely hydrophobic compounds.

This project demonstrated that pelleted inoculum could be produced and delivered to a site for about $30 per ton of treated soil. This is a significant cost savings compared to producing the inoculum on-site. This project also demonstrated the technical effectiveness and ease of use of the pelleted fungal inoculum, which will increase both the effectiveness, and consistency of treatment by fungal-based remediation.

Task Overview and Results

Task I. Pellet production.

Status: Task Completed

Proposed Task:

Twelve tons of pellets for proposed investigations will be toll manufactured by Zeigler Bros., Inc. in Gardners, PA (717-677-6181). Pellets will consist percent by weight of 91% hardwood (e.g. oak, maple) sawdust, 6% soybean meal, 1% lime and 2% lignosulfonate. Ziegler Bros. will be responsible for obtaining the substrates, pelleting, packaging and delivering the pellets to the Isomedix Services facility located at 9 Apollo Drive, Whippany, NJ for gamma irradiation (to sterilize the pellets). The pellets will then be delivered to the L. F. Lambert Spawn Co. facility in Coatesville, PA where they will be stored.

Actual Task Results:

Twelve tons of pellets were produced by Zeigler Bros. and delivered to the L. F. Lambert Spawn Co. in 22.7 kg (50 lb.) Kraft paper bags. Lambert repackaged the Kraft paper bags in polypropylene overwrap bags and delivered the bagged pellets to CFC Logistics Irradiation Services in Quakertown, PA for gamma irradiation (Figure 1). This company was used instead of Isomedix because they were closer to Lambert and the cost of the irradiation was less. The overbags were used because they were less expensive and did not become brittle like the plastic used during the Phase I investigation and were easy to decontaminate for use in the clean room by spraying them with ethanol. Prior to gamma irradiation, testing was done on several bags loaded with pellets to determine the proper dose. The dosage used for irradiation was 16 kGr.

Task II. Test the Littleford Day 300 L KM mixer (i.e. KM mixer) for suitability for use in large-scale pellet coating.

Status: Task Completed

Proposed Task:

We have identified a mixer, the 300 L KM series continuous mixer from Littleford Day, Florence, KY (Mr. George Ely, 859-525-7600). This mixer is used for surface coating of dry products and has the capacity to process 142 L of material every two minutes. The KM series continuous mixers are filled through a port mounted on the topside of the charging end of the mixer. The typical working level of the product is

Figure 1. Kraft 22.7 kg bags of pellets in polypropylene overwrap bags.

nominally 50% of the total volume capacity. A series of plows on the interior of the mixer put the product into three-dimensional motion (a fluidized bed) that results in a superior mix achieved during a short retention time. Discharge of the product is through an adjustable weir valve at the bottom end opposite the charging end. Access doors are located in the front of the mixer for cleaning and maintenance. One liter of pellets weighs 820 g. Therefore, the 300L KM mixer can process 116 kg (dry weight) of pellets every two minutes. The mixer will be tested for its suitability for use in the pellet coating operation. This mixer was developed specifically for this type of application and there are many in commercial use. There is therefore, a high probability that it will be suitable for the pellet coating process. If the mixer provides acceptable coating without damage to the pellets, we will use it , along with conveying and bagging equipment (see task IV) to produce a line for the coating and bagging of sterilized pellets. To complete the proposed work we will lease ($900/wk) a KM Series 300 L continuous mixer from Littleford Day Inc.

Actual Task Results:

Littleford Day recommended that a new mixer, called the EasyClean Machine, be evaluated for coating the pellets (Figure 2). This new mixer is also a fluidized bed mixer and is designed for easy loading and cleaning. A pilot-scale machine was rented with a 450 L capacity. This capacity would accommodate commercial-scale production of the pelleted fungal inoculum. This machine provided excellent coating of the pellets with the alginate/fungal inoculum. Testing involved running the machine at different power settings (i.e. paddle speeds) and for varying lengths of time to provide the best pellet coating and minimization of pellet swelling and breakage. Visual assessments as well as assessing density, moisture content and overgrowth of the pellets were used to evaluate machining speeds and run times.

Figure 2. EasyClean fluidized bed mixer.

Task III. Design, have produced and test larger capacity grow-out/delivery bags.

Status: Task Completed

Proposed Task:

Work with representatives of the following polyethylene bag suppliers to develop bags that will hold up to 34 kg pellets for use in the grow-out phase and for delivery of the pellets: Tufpak, Ossipee, NH (603-539-4126) contact: Mr. Mike Wadlinger; Spa Flexible Packaging, Rouen, France (011 33 232 567 918), contact: Joe Phillips; Unicorn Imp and Mfg. Co., Commerce, TX (903-886-8282) contact: Lou Hsu. These companies manufacture, as part of their product lines, specialty packaging for the mushroom industry--which produce edible fruiting structures of WRF via solid substrate fermentation--and have experience in the production of grow-out bags. All three companies have expressed willingness to produce modified bags for testing in this project (communication from Dr. Christine Smith). Two of the manufacturers (i.e. Tufpak and Spa Flexible Packaging) supplied grow-out/delivery bags for the Phase 1 work. The bags are manufactured from polyethylene and are fitted with filters designed to allow a given amount of air exchange while minimizing moisture loss from the bag contents. Increasing or decreasing the size and/or number of perforations beneath the filter in the polyethylene or in the filter fabric itself varies aeration. The important factors in the design of the bags are the amount of aeration allowed by the filter(s) in relation to the volume of the bag, placement of the filters to provide even aeration throughout the bag, and the dimensions of the bag as they affect how deep the material, in this case, the coated pellets, lay in the bag. The bags tested during the Phase I work that gave the best and most even hyphal coat development had a pellet bed depth of about 10 cm. The ratio of pore size to bag volume used by the bag manufacturers is proprietary. Thus, we will have to rely on the manufacturers to produce larger bags for testing that have similar porosity to volume ratios along with proper placement of the filters. The bags will be tested for their ability to provide uniform hyphal coat development by filling them with various fill weights (these will depend on the sizes of the bags tested) of coated pellets. We will then follow the course of hyphal coat development visually. The goal will be to identify a bag that can provide acceptable hyphal coat development at a fill weight that is as close to 34 kg as possible.

Actual Task Results:

Results from the Phase I work demonstrated that breathability of the bags to provide adequate aeration throughout the pellets in the bag was important to get full colonization of the pellets by the fungus. To minimize handling, we also wanted to work with larger bags that fit within the racks (i.e. storage system) at Lambert. The bags used in Phase I work had the aeration strip oriented in the width-wise direction. This orientation did not provide adequate aeration to the ends of the larger bags. As a result, we worked with Tufpak to produce three types of bags that had the aeration strip, provided by perforated lanes in a herringbone pattern, oriented lengthwise over the entire length of the bags. The three bags were constructed as follows:

- Perforated lanes lengthwise down the center of the bag, upper side only, covered with a Tyvek filter,
- Perforated lanes lengthwise down the center of the bag, upper side only, with no filter, and
- Perforated lanes lengthwise, both top and bottom, with perforated lanes offset.

Fungal colonization of the pellets in the bags was assessed at several fill weights. These were 11.4, 13.6, 15.9 and 17.3 kg. Excellent colonization by *I. lacteus* was obtained with all bags at all fill weights. In the bags with the non-filtered perforations, there was a zone of no colonization caused by desiccation of the pellets directly under the perforation strip (Figure 3). This zone was small and may be acceptable given the extra cost of the bags that include a Tyvek filter over the perforations.

Figure 3. Bag with no filter above perforations with fully colonized pellets, showing area that is not colonized directly beneath perforations.

Task IV. Identify clean-in-place (CIP) equipment, or equipment that can be easily disassembled for sterilization by autoclaving, for conveying and bagging the pellets.

Task Status: Completed

Proposed Task:

Once we have identified a suitable grow-out/delivery bag (Task III) and worked with the KM mixer to determine the optimum throughput rate to obtain acceptable coating of the pellets (Task II), we will identify and test appropriate conveying and bagging equipment. To automate the process, the pellets will need to be conveyed from the KM mixer to an automatic bagger. An important factor to consider in equipment selection will be the ease with which the system can be cleaned and sanitized so that the sterility of the pellets can be maintained. There are several options for sanitation/sterilization. These include CIP systems that use either steam or dry heat (160-180 ^{o}C for 4-5 hours). In both cases the relevant components would be jacketed. Alternatively the system could be sanitized with a liquid like activated ClO_2. The other approach would be to use equipment that can be easily assembled/disassembled and sterilized by autoclaving. In all cases, the equipment would have to be thoroughly cleaned prior to sanitization/sterilization. Of equal importance is the ability of the equipment to handle and meter the coated pellets. Therefore, it will also be critical to find equipment that is capable of handling the density and viscosity of the coated pellets without degrading their quality. There are many manufacturers of conveying and bagging equipment who we can contact, based on their equipment fulfilling the requirements discussed above. We will ultimately select equipment to rent or purchase to develop a production line that will include the KM mixer, a conveyor and a bagger. The L. F. Lambert Spawn Co., where the pellet coating/bagging line will be engineered and where future production will take place, has several walk-in autoclaves at their facility that they could use to sterilize the equipment.

Actual Task Results:

All the coating equipment was located in a positive-pressure clean room with HEPA filtration of room air. The EasyClean mixer proved to be extremely easy to clean and sterilize. After use and wiping down the interior, the mixer was filled with quaternary cleaner dissolved in water and run for several minutes to dislodge residual pellet pieces and alginate. The mixer was then drained and filled with an activated ClO_2 solution and the mixer run again for several minutes to disinfect all interior surfaces. The mixer was then drained. Prior to using, the interior and the exterior of the mixer were swabbed with ethanol. The mixer emptied the coated pellets onto a 20-ft long 5-in auger contained in a 6-ft trough (Figure 4) that carried the coated pellets to the bagging station. The auger was attached to the mixer and cleaned by wiping off residual pellet particles and alginate followed by flushing with the quaternary cleaner, followed by a ClO_2 flush. For this exercise, the pellets were bagged by hand and the bags were closed by heat sealing. The bags were then rolled flat and place on a racking system

that was moved into a grow-out room. The racks were first placed in a room maintained at 22 ^{o}C. Once fungal growth started to produce enough metabolic heat to raise the temperature in the interior of the bags beyond 30 ^{o}C, the racks were moved to a room held at 18 ^{o}C and ventilated with fans to move the air and remove the metabolic heat.

Figure 4. Five inch auger connected directly to the mixer.

Task V. Bench-scale treatability study to determine the most effective pelleted inoculum application rate to use to decrease the concentration of PAH analytes in creosote-contaminated soil from the ATS site.

Task Status: Completed

Proposed Task:

The objective of Task VI is to conduct a bench-scale treatability study to determine the optimum application rate of fungal pelleted inoculum to use to decrease the concentrations of creosote-range PAHs in soil from the Appalachian Timber Services site. *Pleurotus ostreatus* will be used in this work because we know from prior work that the fungus very effectively degrades PAHs in soil from the ATS site (Lamar et al., 2002). The most effective pelleted inoculum application rate will then be used in the on-site study to compare the performance of the pelleted inoculum to on-site prepared inoculum applied at a rate of 10% (dry weight basis). The 10% application rate for the

on-site prepared inoculum was determined in a previous treatability study on the soil from the ATS site (Lamar et al., 2002).

Soil samples from the area of the ATS site from which soil will be excavated (approximately 120 y^3) to use in the on-site study will be taken and sent to the EarthFax Development laboratory. The samples will be pooled, well-mixed and sieved to pass a 2-mm screen. The soil will then be stored, dry, in a sealed container until use. The concentrations of the 16 PAH analytes (Table 1) will be determined on 10 randomly selected soil subsamples. For PAH analysis the soil subsamples will be loaded into a plastic weigh boat and allowed to air dry. Once dry the entire contents of each weigh boat will be ground. Approximately 3 g soil will be analyzed from each subsample in accordance with EPA Method 3545 (accelerated solvent extraction) using a Dionex ASE 200 and acetonitrile as extraction solvent. PAHs in the extracts will be analyzed using the following conditions: Column: Phenomenex Prodigy 5 ODS (3) reverse-phase (250 x 4.6 mm) proceeded by a Supelco SupelGuard LC-18 reverse-phase guard column. 15 ml samples will be injected and eluted with acetonitrile and water at a flow rate of 1.2 ml/min., with a 25 minute gradient from 40% to 100% acetonitrile. Column eluent will be monitored for absorbance at 254 nm.

The variability of the PAH analyte with the largest standard deviation will then be used to determine the number of samples required to detect a significant difference between the two inoculum treatments (as described below) during the on-site test.

The remaining soil will be used to conduct the treatability study. In this study the effect of pelleted inoculum application rate on the concentrations of the 16 priority pollutant PAHS will be assessed. *P. ostreatus* pelleted inoculum will be applied to the soil at 0 (control), 3, 5, and 10 %. The soil treatments will be conducted in 8 oz. canning jars with lids modified to allow adequate air exchange. Each jar will contain approximately 30 g of the test soil (i.e. wet weight) and the appropriate amount of *P. ostreatus* pelleted inoculum. Three replicates will be prepared for each treatment. The cultures will be incubated at 28ûC (this would be the optimum biopile temperature for this fungus) under high relative humidity to prevent moisture loss. Soil moisture content will be maintained by the addition of deionized water, as needed. PAH analyte concentrations will be evaluated on days: 0, 28 and 56.

When harvesting for PAH analysis, the treated soil in each replicate will be emptied into a plastic weigh boat and allowed to air dry. Once dry the entire contents of each weigh boat will be ground and approximately 3 g soil analyzed for PAH analyte concentrations as described above. The PAH analyte concentrations will be used to determine the percent decrease of each PAH analyte concentration using the following formula:

$$(([\text{PAH day 0}] - [\text{PAH analyte at day 28 or 56}]/[\text{PAH day 0}]) \times 100.$$

The data will be subjected to analysis of variance to test for significant differences in percent PAH analyte concentration among the tested inoculum application rates. The

most effective pelleted inoculum application rate will then be compared to the on-site prepared inoculum applied at a rate of 10% at large scale on site at ATS, as described below.

TABLE 1

Priority pollutant polynuclear aromatic hydrocarbon analytes and their industrial risk-based concentrations (from EPA, Region III).

Compound	Industrial Risk-Based Concentration (mg/kg)
acenaphthene	120,000
acenaphthylene	--
anthracene	610,000
benz[a]anthracene	7.8
benzo[b]fluoranthene	7.8
benzo[k]fluoranthene	78
benzo[a]pyrene	0.78
benzo[g,h,i]perylene	--
chrysene	780
dibenzo[a,h]anthracene	0.78
fluoranthene	82,000
fluorene	82,000
indeno[1,2,3-c,d]pyrene	7.8
naphthalene	41,000
phenanthrene	--
pyrene	61,000

Actual Task Results:

As indicated above, the Appalachian Timber site, where we originally intended to conduct the field investigation, became unavailable following a decision by the regulatory authority to allow capping of the site rather than active, on-site remediation. Treatability studies were then conducted on soil obtained from an alternative site located in Gainesville, FL. Site soil was contaminated with PAHs due to coal gasification activities of the former Gainesville Gas Company from the 1880s until the early 1950s. The abilities of three wood decay fungi, applied using pelleted inoculum, to degrade priority pollutant PAHs in soil from the site were evaluated in a bench-scale treatability study. The objective of the study was to determine the most effective combination of fungus, fungal pelleted inoculum application rate and the benefit of surfactant addition to use to decrease the concentrations of creosote-range PAHs in the soil. Three fungi: *Pleurotus ostreatus*, *Irpex lacteus* and an unidentified zygomycetous fungus isolated from PCP-contaminated soil in New Zealand, referred to as B101, were evaluated. Non-inoculated cultures were included as controls. The fungi were evaluated at 5% (i.e.

dry weight of inoculum to dry weight of soil) and 10% inoculum application rates. Finally, the ability of emulsified soybean oil (ESO), applied at 0, 1 and 3% (i.e. weight of ESO/ dry weight of soil) to enhance fungal degradation of the PAHs was evaluated.

The study was conducted on a highly-contaminated sample of soil collected from the Gainesville site. The sample was air-dried, well-mixed and sieved to pass a 2-mm screen. The soil was then stored dry, in a sealed container until use. The concentrations of 15 PAH analytes (Table 2) were determined on 10 three-g soil subsamples. Samples were extracted in accordance with EPA Method 3545 (accelerated solvent extraction) using a Dionex ASE 200 and acetonitrile as extraction solvent. PAHs in the extracts were analyzed using the following conditions: Column: Supelcosil LC-PAH 5 cm x 4.6 mm, 3 mm reverse-phase column preceded by a Supelco SupelGuard LC-18 reverse-phase guard column. The columns were located in a cabinet held at 35ûC. For HPLC analyses 50 mL of soil extract was diluted in 1 ml of acetonitrile. Ten ml samples were injected and eluted with acetonitrile and water at a flow rate of 3.0 ml/min., with a 6.2 minute gradient from 60% to 100% acetonitrile. Column eluent was monitored for absorbance at 254 nm.

The remaining soil was used to conduct the treatability study. *P. ostreatus*, *I. lacteus* and B101 pelleted inocula were applied to the soil at 0 (control), 5, and 10 % amended with ESO at 0, 1 and 3%. The soil treatments were placed in 20-ml scintillation vials topped with foam plugs, to allow adequate air exchange. Each vial contained approximately 5 g of the test soil (i.e. wet weight) and the appropriate amount of fungal pelleted inoculum. Three replicates were prepared for each treatment for each sample time. The cultures were incubated at 30 ^{o}C (i.e. the optimum biopile temperature for these fungi) under high relative humidity to prevent moisture loss. Soil moisture content was maintained by the addition of deionized water, as needed. PAH analyte concentrations were evaluated on days 0, 30 and 60. When harvesting for PAH analysis, the treated soil in each replicate was emptied into a plastic weigh boat and allowed to air dry. Once dry, the entire contents of each weigh boat were ground and approximately 3 g soil analyzed for PAH analyte concentrations as described above. The PAH analyte concentrations were then used to determine the percent decrease of each PAH analyte concentration as indicated above.

The initial concentration of total PAHs in the soil sample was 29,454 mg/kg (Table 2). At almost 3% by weight priority pollutant PAHs, this soil sample was considered highly contaminated. Concentration data for each analyte were subjected to an analysis of variance with time (days 0, 30, 60), inoculation treatment (control, *P. ostreatus*, *I. lacteus* and B101), inoculum application rate (0, 5, 10%) and ESO (0, 1% and 3%) as the main effects. The only factors that were significant were time and inoculum application rate. The data were then reanalyzed using these two main effects.

TABLE 2

Initial concentrations and percent decreases of 15 priority-pollutant PAHs after two months of bench-scale treatment in control soils and soil inoculated with *Pleurotus ostreatus*, *Irpex lacteus*, or B101.

Compound	Initial Conc. (mg/kg)	Percent Decrease			
		Control	*P. ostreatus*	*I. lacteus*	B101
acenaphthylene	1540	-0.45	19.01	74.85	17.85
acenaphthene	1795	22.75	39.17	78.46	34.59
fluorene	5936	-6.08	33.35	69.89	38.31
phenanthrene	1351	4.81	40.24	75.86	26.42
anthracene	805	-2.86	18.70	78.50	11.68
fluoranthene	4384	0.89	10.88	60.72	28.19
pyrene	6179	0.95	7.80	47.94	6.65
benz[a]anthracene	154	10.39	65.03	76.87	43.75
chrysene	636	-0.94	35.38	53.52	14.34
benzo[b]fluoranthene	903	-1.33	15.45	19.46	11.33
benzo[k]fluoranthene	762	-7.87	16.53	61.54	5.25
benzo[a]pyrene	684	-10.09	12.84	41.07	5.76
dibenzo[a,h]anthracene	2623	6.98	30.17	44.15	7.28
benzo[g,h,i]perylene	559	0.54	18.88	33.59	4.17
indeno[1,2,3-c,d]pyrene	1141	0.26	9.53	15.81	0.93
Total	29454	0.83	21.54	58.50	18.51

There was very little change in the concentrations of individual PAH analytes in non-inoculated (i.e. control) soils and only 0.83% change overall after two months (Table 2). Significant decreases in PAH analyte concentrations did, however, occur in fungal inoculated soils. PAH concentration decreases for individual analytes varied greatly but overall were modest and averaged 22% and 19% in soils inoculated with *P. ostreatus* and B101, respectively (Table 2). However, given the extremely high initial PAH concentrations and the presence of free tar in the soil, these percent decreases were considered acceptable. The greatest PAH concentration decreases occurred in soils inoculated with *I. lacteus*. Also, of the three fungi, this fungus appeared to colonize the contaminated soil most extensively. After two months of treatment the concentration of total PAHs had decreased an average of 59% and decreases for the individual PAHs ranged from 19% for benzo(b)fluoranthene to almost 79% for acenaphthene and anthracene. Given the extremely high initial PAH concentrations in this soil, this amount of degradation in two months time is impressive. Based on these results, *Irpex lacteus* was selected for use in the field trial to test pelleted inoculum performance.

Task VI. Large-scale production of pelleted inoculum for the field test.

Task Status: Completed

Proposed Task:

Four tons of sterile pellets will be coated, grown-out (for 7 days) and the resulting pelleted inoculum delivered to the site for mixing with the contaminated soil. Fungal biomass will be produced in our 15-L Applikon fermenter. The fungus will be incubated at 30ûC at a pH of 5.5. Approximately 36.8 g of *P. ostreatus* biomass are produced in a medium composed of 2% malt extract/2% glucose after 7 days of incubation. Therefore, a total of 442 g of biomass will be produced in the 12 L working volume. At the end of the incubation period, the fungal biomass will be aseptically fragmented by forcing the *P. ostreatus* biomass/liquid media through the Lobestar eductor into a sterile carboy. The fragmented biomass will then be transported to the L. F. Lambert Spawn facility to use in the pellet coating operation.

Actual Task Results:

Cultures of *Irpex lacteus* on 2% malt agar plates were sent from the EarthFax Development laboratory collection to the L. F. Lambert Spawn Company to be used in the production of pelleted inoculum. Originally, we intended to use liquid mycelial slurry inoculum to mix with an alginate solution to coat the pellets. However, we completed a series of experiments to determine if it was possible to replace the liquid inoculum with a solid substrate (i.e. vermiculite-based) inoculum. The liquid inoculum with and without fragmentation (i.e. in a blender) was compared to using the solid substrate inoculum for promoting colonization of the pellet surface by *I. lacteus*. We found the solid substrate inoculum to be superior. It produced more rapid colonization, which is important to minimize desiccation of the pellets during the overgrowth. The system described above, including the EasyClean Machine, the 5-in auger and manual bagging etc., were used to produce approximately 5 tons of pellets for the field study. Due to site changes, as discussed above, the inoculum was held in a cold room for about 1 month prior to shipping to Darby, MT while we were obtaining permission to use an alternative site. Six pallets of inoculum (4560 kg [10,032 lbs]) were shipped to the site in a refrigerated truck.

Task VIa. Determination of the shelf life of the overgrown pelleted inoculum.

Task Status: Completed

Proposed Task:

In the best-case scenario the pelleted fungal inocula will be prepared and delivered to the site ready for use on a specific date. However, delays of days to weeks that prevent the use of the pelleted inoculum on the day it was intended to be used,

could occur. One of the advantages of the pelleted fungal inoculum is that the alginate coat has a limited amount of water that is used by the fungus to produce the hyphal coat over the surface of the pellet. This water limitation was built in to the design of the pellets to prevent or minimize “flashing” or overheating of the fungal inoculum during transport due to the production and accumulation of metabolic heat and the consequent degradation of quality of the solid substrate inoculum.

Once the overgrowth process is complete, the pelleted inoculum in the growout bags would be palletized and loaded onto a refrigerated truck for delivery to the site or, in the event the pellets could not be used immediately upon delivery to the site, it would be stored under refrigeration (e.g. in a cold room) until immediate use could be assured. Storage could last from several days to several months.

Therefore, the effect of storage on the inoculum potential of the overgrown inoculum will be determined. In the past we have employed the fluorescein diacetate hydrolyzing activity (FDA) assay to assess the inoculum potential of pelleted inocula (Lestan et al. 1996). This assay provides a measure of microbial biomass growth rate by measuring the activity of a number of enzymes (lipases, proteases, esterases) that are produced during fungal growth (Soderstrom, 1977). The FDA assay is rapid, inexpensive and FDA activity of Phanerochaete chrysosporium-grown pellets, was shown to be highly correlated with mycelial dry weight (R2 = 0.89, P = 0.0001) and ergosterol content (R2 = 0.89, P = 0.0001) (a component of the fungal plasmalemma that is used as an indirect measure of fungal biomass (Davis and Lamar, 1992).)

Task VI originally involved the production of 4 tons of *P. ostreatus* pelleted inoculum to use in the inoculation of 60 yd3 of creosote-contaminated soil. This mixture would then be used to create one large biopile for assessment of the large-scale production and application of the pelleted inoculum as described in Task VIII of the original proposal. This task has been modified as described below to provide replication and to focus on the performance of the pelleted fungal inoculum. As a result of this modification we will produce a total of 6.35 tons of pelleted inoculum, 0.5 tons (approximately 7 bags) of which will be left in the cold room and samples of the pelleted inoculum taken periodically to assess the biological potential using the FDA assay. The remaining 5.85 tons will be used in the field evaluation of the bioremediation potential of the pelleted inoculum, as described below.

The 0.5 tons of bagged, overgrown pellets will be stored flat with aeration ports face up in a cold room (temp = 4ûC). Beginning with Day 0 three pellet samples will be taken aseptically from randomly selected locations representing the upper surface, the mid layer and the lower layer of the pellet mass from each bag every 3rd day and the FDA activity determined on the samples (a total of 21 samples each sample day).

The FDA activity will be determined as follows: 5 g of overgrown pellets will be disrupted with a glass rod in a glass tube containing 10 ml of 60 mM NaH_2PO_4, at pH 8. Hyphal and pellet substrate fragments will be separated from the buffer by filtration through a 45-mm nylon, low protein retention filter. Using this method, the FDA activity

of the particles that remain on the filter was less than 5.5% of that in the filtrate solution (Lestan et al. 1996). Ten ml of 10 mM FDA/DMS stock solution will then be added to 5 ml of the filtrate solution to achieve a final concentration of 20 mM. This reaction mixture will then be incubated for 30 min. at 24 ^{o}C and then assayed spectrophotometrically at 490 nm. The remaining filtrate solution will be used as a blank. FDA activity will be calculated from a linear calibration curve of fluorescein concentration vs absorbance, corrected for additional absorbance by DMS. The average biological potential of the pellets will be expressed as the mM of fluorescein released in 30 min. per g of dry pelleted substrate. An analysis of variance (ANOVA) will be performed to test for equality of mean biological potential response of the pellets taken from the different locations within the bags and over time.

Actual Task Results:

Two of the 17.3 kg bags of overgrown pellets were kept in the cold room at the L. F. Lambert facility. Pellet samples were taken from each bag from randomly selected locations on a weekly basis beginning the day the rest of the pelleted inoculum was shipped to Darby, MT. The samples were taken by placing the bags in a microbiological hood. Pelleted inoculum samples were taken by decontaminating the surface of the inoculum bag by swabbing it with ethanol, making a slit with a sterile blade and taking an approximately 100-g sample. These samples were then placed in pre-sterilized plastic bags, which were then placed on ice and sent to EarthFax Development via overnight shipping for determination of FDA activity. There were three pelleted inoculum samples from each bag for a total of six samples. The FDA activity of the samples was determined as described above. For each sample the absorbance at 490 nm was divided by the weight of the pelleted inoculum sample to standardize the FDA activity. A regression with time in days as the independent variable and the ratio of the standardized FDA activity as the dependent variable, was conducted. The analysis indicated that the slope of the regression line was not significantly different than zero. This indicates that after 51 days of storage in the cold room, that the inoculum potential, based on the FDA activity, did not change and that, based on results to date, the inoculum can stored for at least 2 months.

Task VIII. Field performance of the pelleted inoculum.

Task Status: Completed

Proposed Task:

The objectives of Task VIII were to assess the costs of using the pelleted inoculum and to evaluate the ability of the inoculum to promote fungal growth and pollutant degradation in a PCP/PCDD/PCDF-contaminated soil. The results of the first objective will be presented under Task IX. Fungal-based remediation is conducted by mixing the inoculum with the contaminated soil and treating the soil/inoculum mixture in a forced-aeration, covered biopile. Forced aeration is used to control the temperature of

the pile at approximately 30 ^{o}C by removing metabolic heat that is generated by fungal growth. The pile is covered to control the moisture content of the soil/inoculum mixture.

Actual Task Results:

Introduction

As indicated above, we were informed of the unavailability of the Gainesville site while the inoculum was being produced. Although we were unable on short notice to find a site with PAH contamination, we did find and secure permission to conduct the field evaluation at an out-of-service wood preserving facility in Darby, MT. The primary contaminants of concern at this site are pentachlorophenol (PCP) and associated polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The former S&W Sawmill is an inactive sawmill and wood treating facility, located about 0.5 miles north of Darby, MT, that operated from approximately 1964 to the 1990s. Facility operators used technical PCP mixed with diesel or similar carrier as a wood preservative. Leaks and spills of this material resulted in contamination of soil and groundwater at the site. The site is in a mixed residential/industrial area, about 0.25 mile west of the Bitterroot River. Residents in the vicinity use groundwater for drinking water supplies. Although this site does not contain the original project target contaminants (i.e. PAHs), PCP/PCDD/PCDF-contaminated soils represent a known market for treatment via fungal bioremediation and thus the Montana site was considered an appropriate location for this study.

Establishment of the Field Study at the S&W Sawmill Site

During the period of August 9 through 12, 2004, EarthFax Development Corporation constructed in-ground biocells (the owners of the site preferred that we use in-ground biocells versus aboveground biopiles to minimize the visual impact of the work), for a pilot-scale test of fungal-based soil remediation at the S&W Sawmill Site in Darby, Montana. The work at the site was conducted in general accordance with a work plan prepared by EarthFax (2004) and submitted to the Montana Department of Environmental Quality (MDEQ). The work plan was approved by MDEQ on August 3, 2004. Field work was conducted under an “Area of Contamination Authorization” issued by MDEQ on July 30, 2004.

EarthFax arrived on site on the afternoon of August 9, 2004. We met with Northwestern Energy regarding restoration of power to the site. We also staked out the 1,000 mg/kg PCP boundary in the vicinity of the support building as shown in Figure 5. Soil handling operations were conducted within this 1,000 mg/kg boundary.

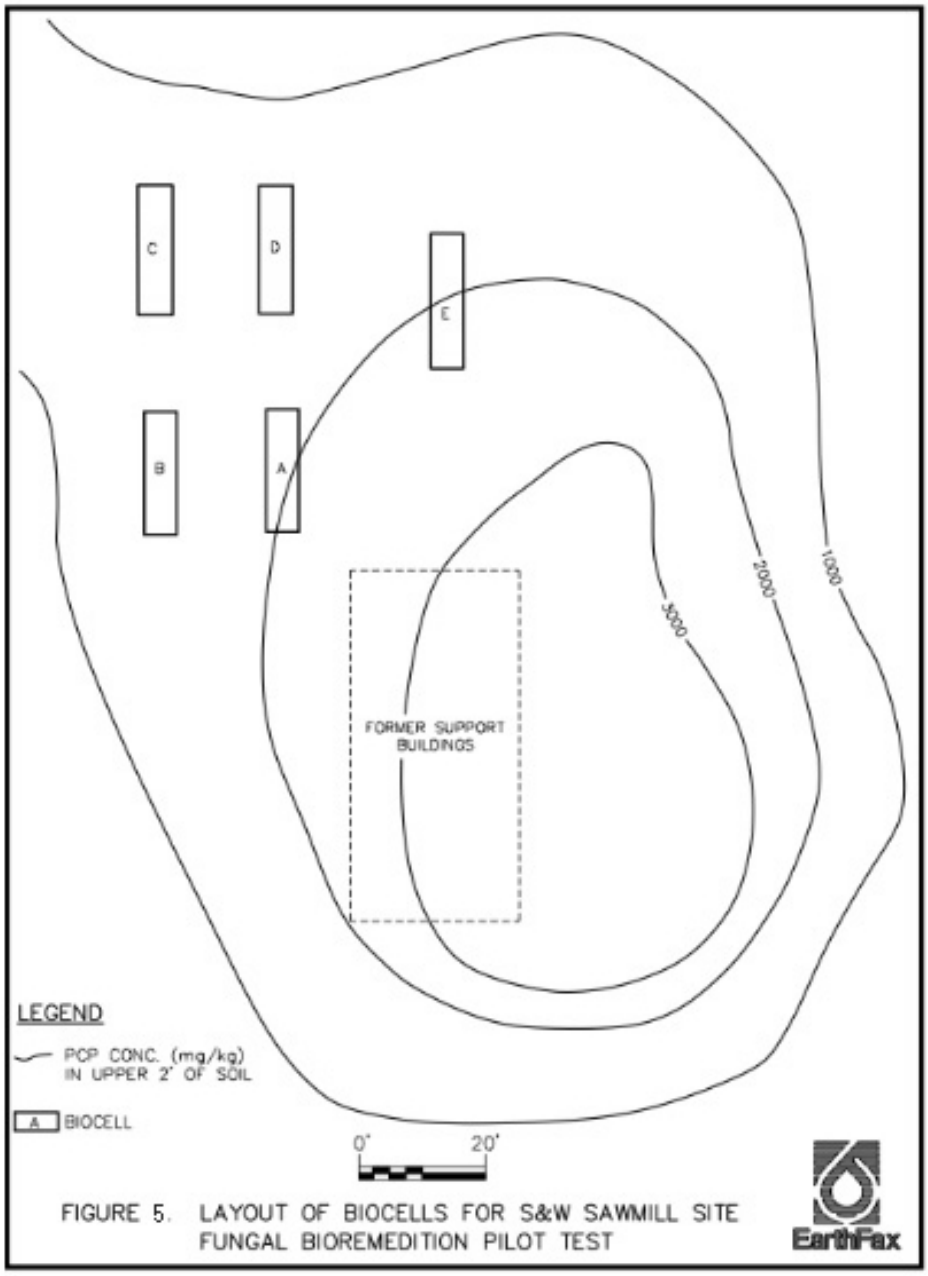

FIGURE 5. LAYOUT OF BIOCELLS FOR S&W SAWMILL SITE FUNGAL BIOREMEDITION PILOT TEST

We met on site with Bryan Douglas and Jim Royce (consultants to the owners of the site). Although the Draft Comprehensive RI Report estimated that PCP concentrations in soil might be higher on the east side of the support building, Mr. Douglas indicated that soil in this area is extremely cobbley. Hence, Mr. Douglas recommended that soil for the test be excavated from the north and northwest sides of the support building. Mr. Royce indicated that the pump house well provides water to the site fire hydrants. We used water from a nearby hydrant for dust suppression once

power was restored to the pump house. Two shallow trenches (approximately 2 to 3 feet deep, 2 to 3 feet wide, and 6 to 8 feet long) were dug approximately 10 to 12 feet from the north wall of the support building. Unlike the northwest trench, the northeast trench contained no visible or olfactory evidence of contamination. We decided to locate the biocells and obtain soil for the test from the area northwest of the building (Figure 6). The pelletized inoculum arrived on site and was off-loaded into a storage barn just south of the perimeter fence.

Figure 6. Excavating soil for use in the biopiles.

Biocell Construction

Five trenches, each approximately 5 feet wide, 5 feet deep, and 20 feet long, were excavated at the locations shown on Figure 5. The soil was screened using a vibrating screen with a 2-in grate that was set up within the 1,000 mg/kg boundary (Figure 5). Sholty Contracting of Missoula, Montana conducted excavation, screening, and other soil handling. The purpose of screening was to remove cobbles larger than about 2 inches in diameter from the soil. Cobbles screened from the soil were backfilled

into the bottom of the trenches, resulting in final excavation depths of 3.0 to 3.5 feet. The trenches were then lined with two layers of 10-mil polyethylene plastic.

Each of the biocells was outfitted with an aeration system that consisted of two 2-in diameter PVC pipes with 0.25-in diameter perforations, placed 6 inches apart, along the length of the pipe. One pipe was place in the bottom of the biocell, with perforations facing up, and covered with a 6-in layer of pea gravel to form a plenum (Figure 7). The other pipe was placed on top of the soil or soil inoculum mixture, with perforations facing down, after the biocell was filled with soil or soil/inoculum mixture, then covered with a 4-in layer of soil. The lower pipe was connected to a 4-in diameter return pipe and the upper pipe was connected to a 4-in diameter delivery pipe. The 4-in return pipe was connected to the inlet of a blower and the 4-in delivery pipe to the outlet on the blower (Figure 8). Thus, air was circulated through the soil or soil/inoculum mixture, continuously, by blowing air in from the top and pulling it through the soil from the bottom.

Figure 7. Pea gravel being loaded into the lined excavation.

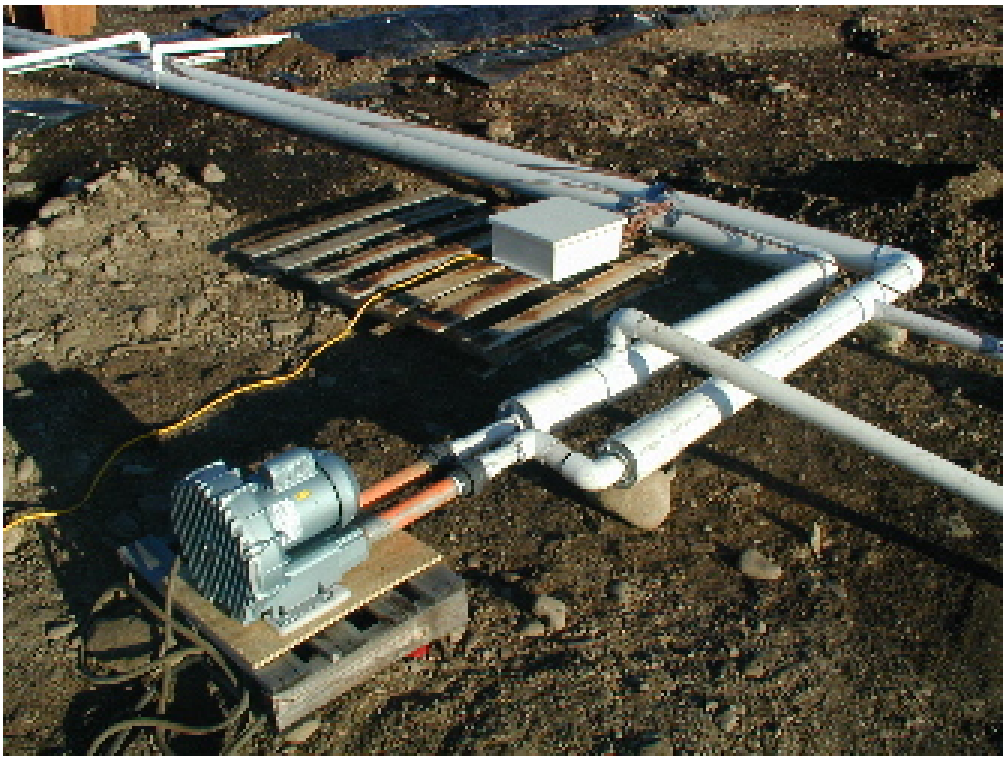

Figure 8. Aeration system consisting of blower and distribution piping.

A pile of approximately 60 yd^3 of screened soil was well mixed with front-end loaders. Five biocells (see Figure 5) were used for three treatments, consisting of a control (Biocell A, non-inoculated; 5% inoculum application rate (Biocells B and D), and 10% inoculum application rate (Biocells C and E). Each of the biocells required approximately 11 yd^3 of soil or soil/inoculum mixture. For the control treatment, soil from the screened pile was separated and moistened to 13% moisture content (dry weight basis) with city water from the fire hydrant. The soil was then loaded into the control biocell. The inoculated soil treatments were prepared by separating the appropriate volume of soil from the screened pile, moistening to 13% moisture content and spreading the soil out in a 1 foot deep layer. The appropriate amount of inoculum was then placed on top of this layer (i.e. by breaking open the appropriate amount of inoculum bags on top of the soil (Figure 9) and then mixing the soil and inoculum with the front-end loaders (Figure 10). The soil inoculum mixture was then loaded into the biocells (Figure 11).

Figure 9. Opening the bags of fungal pelleted inoculum on top of the soil to be mixed and treated.

Figure 10. Mixing soil and pelleted fungal inoculum.

The upper aeration pipe was then placed on top of the soil in the filled biocell, connected to the 4-in outlet pipe and covered with a 4-in layer of soil. Next, three temperature probes were inserted approximately 2 feet deep, 3 feet from either end and in the middle of the soil in each biocell. The probes were all connected to a data logger. One probe was also set up to measure ambient air temperature. The soil or soil/inoculum mixture in each of the biocells was then covered with a 10-mil thick polyethylene tarp and the edges of the tarp sealed with soil and rocks (Figure 12). One day after soil inoculation, the pelleted inoculum had already started to produce visible growth (Figure 13).

Figure 11. Loading inoculated soil into the biocell.

Figure 12. Biocells covered with polyethylene sheeting. Edges of sheeting were secured with soil.

Figure 13. Pelleted fungal inoculum one day after application to soil, showing new growth of *Irpex lacteus*.

Sample Collection and Maintenance

After mixing and moistening, and prior to loading the soil or soil/inoculum mixtures into the biocells, 10 random samples were collected from each biocell and placed into 170-ml glass jars. The samples were packed in ice and returned to the EarthFax Development laboratory in Logan, UT for analyses. EarthFax visited the site on August 28 and 29, 2004 to assess the condition of the biopiles. The piles were observed to be drier than desired. Drying of the soil/inoculum mixture sometimes occurs as pile moisture equilibrates during initial operation of the ventilation system. The covers were removed and additional water from the site fire hydrant was sprayed onto each biocell to raise moisture contents. The covers were then replaced.

EarthFax visited the site again on September 25, 2004 and collected additional samples from each pile for PCP analyses. Pile moistures were observed to be adequate during that visit, indicating that the efforts taken at the end of August were adequate. Soil samples were collected as outlined in the work plan, with the exception that samples were collected from the entire depth of the biocell at five randomly selected locations within each cell, rather than at two depths and three locations within each pile. Hence, five samples were collected from each pile rather than six. During the course of the project, we collected samples from the biocells on the following dates: September 11 and 26, 2004; October 23, 2004; December 15, 2004; March 26, 2005; and May 31, 2005. Five samples were collected from each biocell on each date, as described above.

Results

Results of PCP analyses performed by EarthFax on samples collected on each sample date are presented in Table 3. These samples were extracted using EPA Method 3545 and analyzed using EPA Method 8041. Initial PCP concentrations in the biopiles were generally in the 100 to 300 mg/kg range. Higher initial concentrations were anticipated since soil was excavated from areas where the surface PCP concentrations are typically in the 1,000 to 2,000 mg/kg range (see Figure 5). However, the rapid decrease in concentration with depth resulted in lower concentrations within the mixed soil.

Substantially greater decreases in the concentration of PCP in soil occurred in the inoculated biocells. While there was only a 20% decrease in the concentration of PCP in non-inoculated soil after 293 days of treatment, fungal inoculation resulted in an overall 63.1% decrease (to an average concentration of 72.9 mg/kg) in the soil treated at the 5% inoculation application rate and 89.3 % decrease (to an average concentration of 26.6 mg/kg) in the soils inoculated at the 10% inoculum application rate. As a point of comparison, the EPA Region III risk-based concentration of PCP acceptable under an industrial land-use scenario is 24.0 mg/kg. Temporal variations in PCP concentrations during the course of the study are given in Figure 14.

TABLE 3

Concentrations of PCP in each of the biocells on each sample date.[(a)]

Sample Date	Elapsed Time (days)	PCP Concentration (mg/kg)				
		A (Control)	B (5% inoc)	C (10% inoc)	D (5% inoc)	E (10% inoc)
8/11/04	0	121.2 (125.9)	169.0 (49.0)	224.1 (104.1)	318.4 (37.6)	284.1 (62.1)
9/11/04	31	89.5 (34.7)	157.3 (9.5)	130.7 (40.5)	145.8 (27.6)	174.2 (84.4)
9/26/04	46	105.9 (36.0)	121.4 (40.7)	140.6 (49.6)	211.1 (47.5)	135.8 (65.3)
10/23/04	73	101.7 (42.9)	152.3 (77.5)	120.8 (25.8)	201.4 (55.9)	120.7 (64.5)
12/15/04	126	101.1 (45.8)	137.8 (73.4)	118.9 (11.0)	135.1 (65.0)	106.6 (26.7)
3/26/05	227	94.0 (27.3)	115.4 (32.4)	88.6 (16.6)	74.4 (25.0)	64.9 (19.7)
5/31/05	293	96.9 (26.9)	101.2 (7.0)	28.2 (23.4)	44.6 (23.4)	25.0 (13.1)

[(a)] Concentrations on Day 0 are averages of 10 random samples taken prior to loading mixed soil into biocells. Concentrations on all other samples dates are averages of 5 samples taken as described in the text. Standard deviations given in parentheses.

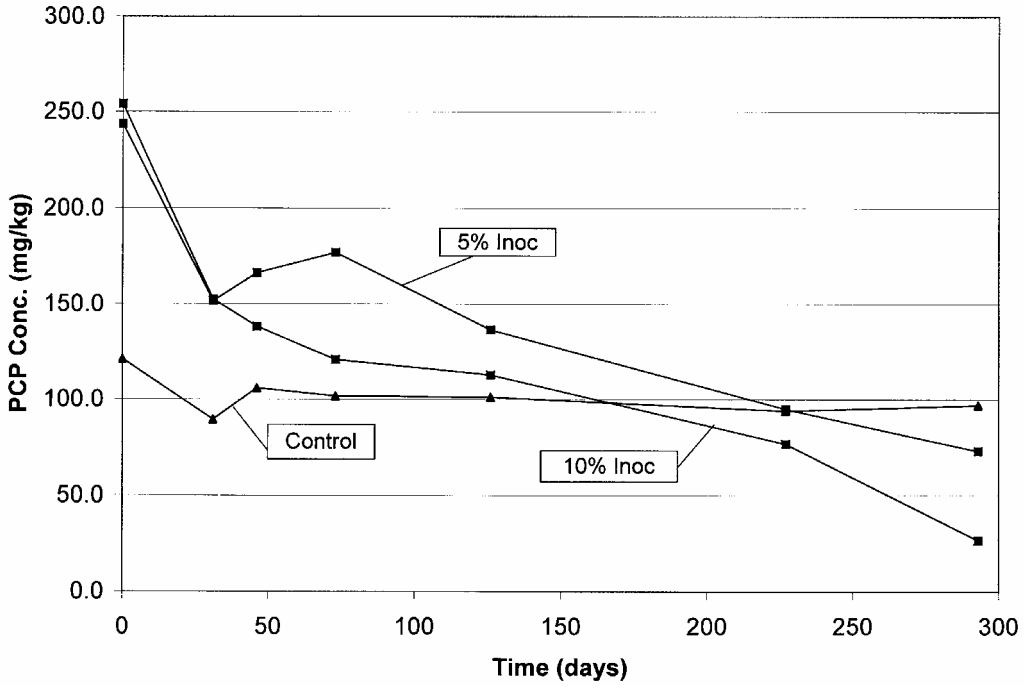

Figure 14. Concentrations of PCP with time.

As a quality-control measure, soil from each sampling round was split to Columbia Analytical Services (Kelso, Washington) for analyses of PCP in accordance with EPA Method 8151M. During the initial sampling event, composite samples (made up of a portion from each of the 10 individual samples) from biocells B and C were split to Columbia and compared against the average concentration of the 10 individual samples determined by EarthFax. During subsequent sampling events, individual samples were randomly selected and split, with one portion being analyzed by Columbia using EPA Method 8151M and one portion being analyzed by EarthFax using EPA Method 8041. Results of these split analyses are provided in Table 4. As indicated, relative percent differences were normally less than 25%. Furthermore, on average, the relative percent difference between analyses over the entire project is essentially zero. Hence, the analytical data from the EarthFax laboratory are considered valid.

TABLE 4

Relative percent differences between EarthFax and Columbia PCP analyses.

Date	Sample No.	PCP Conc. (mg/kg)		RPD (%)
		EarthFax	Columbia	
8/11/04	B-comp	169	160	5.5
	C-comp	224	180	21.8
10/23/04	C-5	131	220	-50.7
	A-5	94	76	21.2
12/15/04	E-1	140	180	-25.0
	D-1	77	72	6.7
3/26/05	C-2	65	69	-6.0
	E-5	88	60	37.8
5/31/05	A-3	74	72	2.7
	E-5	34	45	-27.8
			Average	-1.4

Composite samples were collected from each biopile on August 11, 2004 (day 0) and again on May 31, 2005 (day 293) for dioxin/furan analyses. Results of these analyses, as performed by Columbia Analytical using EPA Method 8290, are provided in Tables 5 and 6 for the two sample dates. As indicated, initial concentrations of 2,3,7,8-TCDD ranged from 5.09 to 9.29 ng/kg (parts per trillion), with total equivalent 2,3,7,8-TCDD (TEQ) concentrations ranging from 480 to 3,040 ng/kg. Final 2,3,7,8-TCDD and TEQ concentrations ranged from 2.51 to 11.55 ng/kg and 870 to 2,260 ng/kg, respectively.

TABLE 5

Initial (day 0) concentrations of PCDDs and PCDFs

Analyte	Concentration (ng/kg)				
	A (control)	B (5% inoc)	C (10% inoc)	D (5% inoc)	E (10% inoc)
PCDDs					
2,3,7,8-TCDD	6.28	6.42	6.74	5.09	9.29
1,2,3,7,8-PeCDD	129.15	36.10	168.64	261.41	138.15
1,2,3,4,7,8-HxCDD	357.68	109.25	405.48	572.87	408.63
1,2,3,6,7,8-HxCDD	3078.85	749.98	3914.81	5013.28	3412.67
1,2,3,7,8,9-HxCDD	1002.03	265.46	1438.75	1817.57	1005.25
1,2,3,4,6,7,8-HpCDD	73026.87	21625.70	102860.63	138506.93	90267.69
OCDDs	433029.08	137976.23	706698.01	1030082.30	733818.26
PCDFs					
2,3,7,8-TCDF	10.50	4.36	19.82	22.61	14.67
1,2,3,7,8-PeCDF	61.82	21.33	83.20	125.98	75.72
2.3.4.7.8-PeCDF	44.71	16.95	69.39	97.46	59.77
1,2,3,4,7,8-HxCDF	397.94	144.74	572.29	782.05	454.37
1,2,3,6,7,8-HxCDF	166.29	66.45	252.58	382.60	191.66
1,2,3,7,8,9-HxCDF	86.28	35.48	171.24	230.39	76.40
2,3,4,6,7,8-HxCDF	348.72	130.56	496.42	636.99	380.30
1,2,3,4,6,7,8-HpCDF	13557.52	4026.97	19604.51	25902.48	16503.27
1,2,3,4,7,8,9-HpCDF	<375.44	229.43	819.14	939.15	525.48
OCDFs	62913.69	20486.65	110322.44	157497.49	97426.18
Total TEQ[(a)]	1.62E+03	0.48E+03	2.26E+03	1.93E+03	3.04E+03

[(a)] Used toxic equivalency factors recommended by the World Health Organization taken from: Van den Berg, et al. 1998. Toxic Equivalency Factor (TEFs) for PCBs, PCDDs and PCDFs for Humans and Wildlife. Environmental Health Perspectives 106:775-792.

TABLE 6

Day 293 concentrations of PCDDs and PCDFs

Analyte	Concentration (ng/kg)				
	A (control)	B (5% inoc)	C (10% inoc)	D (5% inoc)	E (10% inoc)
PCDDs					
2,3,7,8-TCDD	2.51	3.30	3.25	5.59	11.55
1,2,3,7,8-PeCDD	72.23	86.14	86.05	145.92	217.86
1,2,3,4,7,8-HxCDD	207.63	219.00	263.86	462.72	676.56
1,2,3,6,7,8-HxCDD	1989.88	2193.12	1871.97	3521.90	3690.71
1,2,3,7,8,9-HxCDD	692.72	678.78	804.80	1125.71	1462.34
1,2,3,4,6,7,8-HpCDD	77991.79	23292.19	73818.80	118140.48	110391.64
OCDDs	642302.77	82109.61	577298.67	690734.00	577551.99
PCDFs					
2,3,7,8-TCDF	11.75	16.94	13.04	22.17	30.42
1,2,3,7,8-PeCDF	28.86	53.25	30.09	56.72	83.15
2.3.4.7.8-PeCDF	22.28	50.79	21.44	52.95	69.09
1,2,3,4,7,8-HxCDF	274.52	335.44	239.10	451.73	553.67
1,2,3,6,7,8-HxCDF	142.81	151.29	148.40	170.67	252.24

Analyte	Concentration (ng/kg)				
	A (control)	B (5% inoc)	C (10% inoc)	D (5% inoc)	E (10% inoc)
1,2,3,7,8,9-HxCDF	nd	8.55	nd	11.35	9.11
2,3,4,6,7,8-HxCDF	131.46	254.60	124.59	265.96	284.85
1,2,3,4,6,7,8-HpCDF	17663.93	11104.29	15905.18	19635.83	40282.47
1,2,3,4,7,8,9-HpCDF	1284.07	1051.85	1072.86	1057.05	956.95
OCDFs	124883.36	48582.14	104857.68	136455.42	110269.15
Total TEQ[(a)]	1.48E+03	0.87E+03	1.44E+03	2.18E+03	2.26E+03

[(a)] Used toxic equivalency factors recommended by the World Health Organization taken from: Van den Berg, et al. 1998. Toxic Equivalency Factor (TEFs) for PCBs, PCDDs and PCDFs for Humans and Wildlife. Environmental Health Perspectives 106:775-792.

The percent change in dioxin/furan concentrations during the 293 days of treatment is indicated in Table 7. Whereas the control pile exhibited an 8.6% decrease in TEQ concentrations, the 5%-inoculum pile exhibited an apparent increase and the 10%-inoculum pile exhibited an average TEQ concentration decrease of 31.0%. Apparent increases in individual concentrations in some dioxins/furans are undoubtedly a result of biopile heterogeneity.

TABLE 7

Percent decrease in PCDD and PCDF concentrations in 293 days.

Analyte	Concentration (ng/kg)				
	A (control)	B (5% inoc)	C (10% inoc)	D (5% inoc)	E (10% inoc)
PCDDs					
2,3,7,8-TCDD	60.0	48.6	51.8	-9.8	-24.3
1,2,3,7,8-PeCDD	44.0	-138.6	49.0	44.2	-57.7
1,2,3,4,7,8-HxCDD	41.95	-100.5	34.9	19.2	-65.6
1,2,3,6,7,8-HxCDD	35.37	-192.4	52.2	29.8	-8.2
1,2,3,7,8,9-HxCDD	30.87	-155.7	43.7	38.1	-45.5
1,2,3,4,6,7,8-HpCDD	-6.8	-7.7	28.2	14.7	-22.3
OCDDs	-48.3	40.5	18.3	32.9	21.3
PCDFs					
2,3,7,8-TCDF	-11.9	-288.5	34.2	2.0	-107.4
1,2,3,7,8-PeCDF	53.3	-149.6	63.8	55.0	-9.8
2.3.4.7.8-PeCDF	50.2	-199.6	69.1	45.7	-15.6
1,2,3,4,7,8-HxCDF	31.0	-131.8	58.2	42.2	-21.8
1,2,3,6,7,8-HxCDF	14.1	-127.7	41.2	55.4	-31.6
1,2,3,7,8,9-HxCDF	(a)	75.9	(a)	95.1	88.1
2,3,4,6,7,8-HxCDF	62.3	-95.0	74.9	58.2	25.1
1,2,3,4,6,7,8-HpCDF	-30.3	-175.8	18.9	24.2	-144.1
1,2,3,4,7,8,9-HpCDF	-584.0	-358.5	-31.0	-12.6	-82.1
OCDFs	-98.5	-137.1	5.0	13.4	-13.2
Total TEQ[(a)]	8.6	-81.2	36.3	-13.0	25.7

It should be re-emphasized that the laboratory treatability study for this project was conducted on PAH-contaminated soil from an abandoned gasworks site. Due to a last-minute change in site availability, and without the time to conduct a new laboratory investigation, it was necessary to apply the decisions from the initial laboratory study to the PCP/dioxin/furan-contaminated site in Montana. Although significant PCP degradation and noteworthy dioxin/furan degradation was achieved during the initial 293 days of the field investigation (particularly in the biocells inoculated at a rate of 10%), hindsight coupled with prior experience suggests that more extensive PCDD/PCDF degradation might have been achieved if a surfactant, for example emulsified soybean oil, had been added to enhance the bioavailability of hydrophobic pollutants like PCDDs/PCDFs.

Task IX. Perform a comparative economic analysis of pelleted and standard inoculum production and use.

Status: Completed

Task Description:

Cost data for production and application of pelleted and standard inoculum will be collected during the large-scale production of each for the field investigations and for inoculum application during set-up of the field investigation. For the standard inoculum, data will be collected on the costs of primary inoculum (which will be a liquid mycelial slurry inoculum produced in the EarthFax Development laboratory), materials (e.g., polyethylene tarps), equipment rental (e.g., front-end loader), and labor for both the inoculum preparation and mixing with contaminated soil. As explained above, ATS operated a railroad tie/mine timber production plant and produces hardwood splinters from the milling operation, which will be used as the substrate. We also have a steam generator at the site that will be used for pasteurization of the splinters prior to inoculation with primary substrate. Thus, there will be no costs for this test for inoculum substrate and pasteurization. However, we have solid data on costs for substrates and pasteurization from previous projects that will be used in the analysis. For the pelleted inoculum, data will be collected on the following costs: pellet substrates, packaging, labor and overhead, toll pelleting, pellet sterilization, delivery, equipment for mixing with contaminated soil. We already have solid costs for the alginate and fermentation media. The economic analysis will be expressed both in terms of cost/ton of pellets (for production and delivery) and cost/ton of contaminated soil. The latter will include both the production and application costs.

Actual Task Results:

The budget for production and delivery of pelleted inocula is based on a pelleted inoculum application rate of 5% (i.e. dry weight of pellets to dry weight of soil). Since the pellets are 3 times as dense as non-pelleted substrate, a 5% pellet inoculum application rate is equivalent to a 15% application rate using non-pelleted substrate.

The budget for production and delivery of the pelleted inocula consists of costs for: pellet substrates (used to make the pellets), alginate (the hydrogel used for coating the pellets), fermentation media (for producing liquid fungal mycelia used as the primary inoculum in coating the pellets), packaging (the bags used for grow-out and delivery of the pelleted inocula to the site), labor and overhead (cost of personnel, electricity, water, etc.), toll pelleting, pellet sterilization prior to coating, and delivery of the pellets from production facility to the contaminated site.

Budget explanation for pelleted inocula:

A. Costs associated with pellet substrates

Substrate	$/ton substrates	$/ton pellets	$/ton soil
Sawdust (75%)	36.51	26.63	1.33
Corn Glutan Meal (15%)	33.45	5.02	0.25
Starch (8%)	45.00	3.60	0.18
Lignosulfonate (2%)	16.10	0.32	0.02
Total		35.57	**1.78**

B. Alginate

Self-gelling alginate costs $4.00 per pound. The alginate is applied in a 1% solution. The moisture content of the pellets is 7%. Hence, for one ton of pellets there are 1860 lbs of dry pellets and 140 lbs of water. The final target moisture content of the pellets is 30%. Thus, the alginate is added in 657 lbs of water to a ton of pellets. Therefore a 1% alginate solution would contain 6.57 lbs of pellets, yielding the following costs:

$/ton pellets	$/ton soil
26.18	**1.31**

C. Fermentation Media

The costs for fermentation media are based on a typical production of 25 g of fungal biomass per liter of fermentation medium. Prior experience has indicated that an average of 1.24 g of fungal biomass is needed to provide adequate growth per ton of pellets. As a safety factor, this value was increased an order of magnitude to 12.4 g of fungal biomass per ton of pellets. Hence, a typical 8 L run would result in the production of 200 g of biomass. This would provide enough biomass for inoculation of 16.12 tons of pellets. Fermentation media costs for 8 L is $1.28. Therefore:

$/ton pellets	$/ton soil
0.08	**0.01**

D. Packaging

The pellets will be packaged in bags that cost $1.00 each and that hold approximately 40 lbs. Therefore packaging will cost:

$/ton pellets	$/ton soil
25.00	**1.25**

E. Labor and Overhead

Labor and overhead costs are based on production of mushroom logs, a similar process that also requires the application of liquid inoculum to lignocellulosic substrates. In the mushroom log production system, labor and overhead account for 40% of the costs, with overhead contributing $0.074 per lb of logs and labor contributing $0.149 per pound of logs. In this case, the labor force is made up of 24 hourly workers and 1 salaried worker. Production of pelleted inocula would require only 3 hourly workers. Therefore the labor figure of $0.149 per pound will be adjusted accordingly to $0.018 per pound. Combined, overhead and labor costs would run $0.092 per pound of pellets. This results in the following costs:

$/ton pellets	$/ton soil
184.00	**9.20**

F. Toll Pelleting

Pellets can be produced via toll manufacturing or by purchasing a pellet press and producing at the pellets at the inoculation facility. Initially, capital costs can be minimized by having the pellets toll manufactured. Based on experience obtained in this project, toll manufacture of pellets costs:

$/ton pellets	$/ton soil
128.00	**6.40**

G. Pellet sterilization

The cost of pellet sterilization is as follows:

$/ton pellets	$/ton soil
154.86	**7.74**

H. Delivery

Assuming a delivery range of 1500 miles, the cost of delivery is as follows, based on data provided by Lambert Spawn:

$/ton pellets	$/ton soil
60.00	**3.00**

Summarizing the above, the overall cost of producing pelleted inocula is:

	$/ton pellets	$/ton soil
Pellet substrates	35.57	1.78
Alginate	26.18	1.31
Fermentation media	0.08	0.01
Packaging	25.00	1.25
Labor and Overhead	184.00	9.20
Toll Pelleting	93.43	4.67
Pellet Sterilization	154.86	7.74
Delivery	60.00	3.00
	560.37	28.96

Budget explanation for solid secondary inocula produced on site:

Costs for the production of on-site secondary inocula were obtained from an actual commercial fungal-based remediation project that was conducted by EarthFax Development and its affiliate EarthFax Engineering at an out-of-service wood treating facility in North Carolina. The budget includes the cost and delivery of the substrate (in this case, cottonseed hulls), cost of producing the primary solid inoculum (a grain based spawn inoculum purchased from a commercial mushroom grower at a cost of $0.65/lb and applied at a rate of 15% dry weight of primary inoculum to secondary inoculum substrates), pasteurization (in this case, sterilization using methyl bromide), materials (plastic tarp to provide a base and cover during colonization of inoculum substrate by the fungus), equipment rental (mixing equipment, a tractor, and backhoe), and labor (self explanatory). These data indicate that the following costs were incurred for production of on-site inocula (based on a cost per ton of treated soil):

	$/ton of soil
Substrate (e.g. cotton seed hulls)	15.00
Primary inoculum	39.00
Pasteurization	2.00
Materials	0.50
Equipment rental	2.00
Labor	52.00
Total	110.50

Therefore, the cost of fungal inocula using on-site inoculum production vs. pelleted inocula is $110.50 per ton of treated soil compared to approximately $29.00 per ton of treated soil, respectively. This represents a savings of $81.50 per ton of treated soil. Coupled with the other costs associated with fungal bioremediation (biocell construction, mixing of soil and inocula, site monitoring, etc.), this savings in inoculum

production should reduce the cost of technology application by 30 to 50%, depending on the volume of soil being treated. Furthermore, a comparison of the results obtained from this investigation with prior experience, no loss in treatment effectiveness occurs through the use of pelleted inoculum vs. inoculum that has been prepared on site.

Conclusions

The following conclusions have been reached as a result of this investigation:

1. Pelleted inoculum was produced using a combination of toll manufacturing of the raw pellets, sterilization by gamma irradiation and coating and bagging using industrial-scale equipment. The pelleted inoculum was delivered from Coatesville, PA (located 40 miles west of Philadelphia) to Darby, MT (located in extreme western Montana) in a refrigerated truck in extremely good condition and supported a pure culture of *I. lacteus* with no signs of contamination by other microbes.
2. A solid vermiculite-based inoculum was found to be more effective in terms of rapidity of the pellet overgrowth process, than a mycelial slurry inoculum to use, along with the alginate, to coat the pellets.
3. A check of the inoculum potential of the inoculum, stored under refrigeration, over a 2 month period indicated that the viability of the inoculum was maintained without degradation.
4. A laboratory-scale treatability study designed to test the ability of three fungi to degrade PAHs in a highly contaminated soil from a gasworks site demonstrated the effectiveness of the pelleted inoculum to support good to excellent fungal growth in the soil. *Irpex lacteus* was identified as the most effective fungus to remove PAHs in this contaminated soil.
5. A field investigation to evaluate the large-scale use of the pelleted inoculum demonstrated that the inoculum was robust (i.e. did not break down when mixed with soil using front end loaders) and promoted good growth of and pollutant (i.e. PCP) degradation by *I. lacteus* in the soil at the 5% inoculum application rate level and excellent growth and pollutant degradation at the 10% rate.
6. Fungal inoculation resulted in the decreases in the concentrations of individual PCDD/PCDFs and in dioxin/furan TEQs in biocells inoculated at a rate of 10%. Better treatment of PCDDs/PCDFs may have been obtained through the use of a surfactant (e.g. emulsified soybean oil) to enhance the bioavailability of these extremely hydrophobic compounds.
7. An economic analysis indicated that a savings of about $82 per ton of treated soil could be achieved with pelleted inocula compared with on-site inoculum production. This will result in an estimated 30 to 50% savings in the cost of applying this technology, without a reduction in technology effectiveness.

A critical review of the application of white rot fungus to environmental pollution control

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REVIEW ARTICLE

A critical review of the application of white rot fungus to environmental pollution control

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Abstract

Research on white rot fungi for environmental biotechnology has been conducted for more than 20 years. In this article, we have reviewed processes for cell growth and enzyme production including the factors influencing enzyme productivity and the methods for enhancement of enzyme production. Significant progress has been achieved in molecular biology related to white rot fungi, especially related to the extraction of genetic material (RNA and DNA), gene cloning and the construction of genetically engineered microorganisms. The development of biotechnologies using white rot fungi for environmental pollution control has been implemented to treat various refractory wastes and to bioremediate contaminated soils. The current status and future research needs for fundamentals and application are addressed in this review.

Keywords: *White rot fungus; enzyme production; pollution control; molecular biology; bioremediation*

White rot fungus, which causes white rot on wood or trees, belongs to the basidiomycetes. The mycelia of the organisms can penetrate the cell cavity, and release ligninolytic enzymes to decompose xylon to a white sponge-like mass. Three types of extracellular enzymes are produced by the white rot fungus and these are nonselective yet effective in attacking lignin. These are often referred to as lignin modifying enzymes (LMEs), and they are lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase (Lac). Although, they effectively break down lignin, these fungi cannot utilize it as an energy source and it is assumed that they degrade it for access to the cellulose in the cell wall. The white rot fungus contains all three enzymes and is therefore able to degrade or mineralize several organic pollutants. The concept for the development of environmental technology using white rot fungus was proposed in 1980s (Bumpus and Aust, 1987; Tien, 1987; Aust, 1990).

Chemicals white rot fungi are able to degrade include many pesticides, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other halogenated aromatics (including dioxins), some dyes, 2,4,6-trinitrotoluene (TNT) and other nitro explosives, and other toxic chemicals such as cyanides, azide, carbon tetrachloride, and pentachlorophenol (PCP) (Cookson, 1995; Reddy and Mathew, 2001). To date, the fungi including *Phanerochaete chrysosporium, Trametes versicolor, Bjerkandere adusta, Pleurotus ostreatus*, have been studied for the biodegradation of xenobiotic organic pollutants and waste treatment. Some are summarized in Table 1. The typical white rot fungus—*P. chrysosporium*, has been investigated comprehensively, not only in microbiology, but also in engineering applications in the United States, China, and other countries.

Microbiology of white rot fungi

Recently, the research on the microbiology of white rot fungi has mainly focused on macro-aspect related engineering processes such as the characterization of cell growth and enzyme production, and on micro-aspects, that is, molecular biology.

Characteristics of cell growth and enzyme production

Characterization of cell growth and enzyme production is fundamentally important to develop environmental biotechnology for biodegradation of refractory organic pollutants and waste treatment. Research progress has been made in the areas of the characterization of white rot fungi including

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the incubation conditions of the mycelium formation, the formation of small extracellular molecular matters and cell mutation using laser irradiation.

Characteristics and conditions of cell growth

Factors including the medium composition, pH, concentration of spore suspensions, incubation duration and the mixing speed of the incubator, have a significant influence on the mycelium growth of the white rot fungus *Pe chrysosporium* (Li et al., 1999). A number of small extracellular molecular substances, which were separated using ultra filtration and molecular sieve chromatography, were found during white rot fungus growth (Yan et al., 2003). These substances were likely related to degradation of lignin. However, further identification of these substances is still needed. The optimum growth conditions for the formation of the mycelium pellets were found at the C/N ratio of 131, pH 4.5, a spore suspension of 10^6/mL with a mixing speed of 150 rpm, and the presence of Tween 80 (0.1%) to enhance the performance further (Li et al., 1999).

Growth conditions influenced the growth and activity of the fungus. A study on the impact of salinity on the growth of the white rot fungi *B. adusta, I. lacteus,* and *L. tigrinus* concluded that *I. lacteus* and *L. tigrinus* were less affected by salinity, but the growth of *B. adusta* was inhibited when the salt concentration reached 32‰ (Valentin et al., 2006). The growth of white rot fungus on the surface of a biofilm could be controlled by a pulsing flow and this could enhance the efficiency and stability of the decolorization of synthetic dyes (Moreira, Feijoo, and Lema, 2003). Kamitsuji et al. (2004) studied the incubation conditions of the white rot fungus and found that maximum growth was achieved after 8 days in peptone-glucose-yeast extract medium (PGY) and 30 days in glucose yeast extract medium (GY). Lang, Eller, and Zadrazil (1997) studied the growth of four different white rot fungi in the presence of soil microorganisms and found that the existence of Mn stimulated bacterial suppression of the white rot fungus.

Selection of the target strains

Much research has been carried out to select strains with high or specific biodegradation performance by various methods. The majority of this research was performed using laser and ultraviolet light (UV) treatments to obtain mutants. Guo, Xu, and Yang (2001) used a He-Ne laser at 632.5 nm to select a white rot fungus with a high lignin degradation rate. They found that the white rot fungus exposed to the laser with the power of 6mW and 7mW had enhanced lignin degradation by 33% and 39%, respectively compared with the untreated white rot fungus. With a higher power of 9 mW for 10 min of exposure, lignin degradation increased by 50%, but exposure for 20 min caused a lethality rate of white rot fungi L1 protoplasts near to 100%. The analysis of esterase isozyme indicated the presence of a stable mutant strain due to the laser treatment (Guo, Xu, and Yang, 2001). Using the UV-N^+ ion implantation, a genetically stable strain with high yield of polyphenol oxidase was obtained from the mutated white rot fungus. The activity of laccase with this strain was 15 times that of the original strain (Jia et al., 2002). Zhang et al. (2008) obtained a mutant strain of *P. chrysosporium* by using UV which had greatly enhanced activity for the decomposition of caprolactam with a high decomposing efficiency. The white rot fungus treated with UV had improved efficiency of the degradation of polyamide 6. However, treatment of laser or UV could also cause a mass-death rate of the fungus and could result in mutants with poor degradation performance (Guo, Xu, and Yang, 2001; Zhang et al., 2008). Therefore, more research remains to be carried out in this area.

Factors influencing enzyme production

Factors influencing enzyme production have been comprehensively investigated. Valaskova et al. (2006) studied enzyme production conditions of *P. ostreatus, T. versicolor* and *Piptoporus betulinus*. For *P. ostreatus*, after freeze-drying, the activities of MnP and Laccase were 60–65% less, and hydrolases were 30% less. *T. versicolor* and *P. betulinus* showed better stability of enzyme production, and most of the enzyme

Table 1. Research on biodegradation of organic pollutants by white rot fungi.

Organism	Pollutant or waste	Reference
Bjerkandera adusta	PAHs (dibenzothiophene, fluoranthene, pyrene and chrysene)	Valentin et al., 2007
	TNT	Eilers et al., 1999
	Daunomycin producing wastes	Kornillowicz-Kowalska et al., 2006
Irpex lacteus	PAHs (phenanthrene, anthracene, fluoranthene, and pyrene)	Baborova et al., 2006
	Synthetic dyes (Reactive Orange 16 and Remazol Brilliant Blue R.)	Svobodova et al., 2008
Lentinus tigrinus	Polycyclic aromatic hydrocarbons (PAHs)	Valentin et al., 2006
Trametes versicolor	Trichloroethylene (TCE)	Marco-Urrea et al., 2008
	Pentachlorophenol (PCP)	Ruttimann-Johnson et al., 1997
	Synthetic dyes (Amaranth)	Gavril and Hodson, 2007
	Polysaccharide	Zhu, Sun, and Cao, 2005
Phlebia radiata	TNT	Aken et al., 1999
Pleurotus ostreatus	PAHs	Lamar, White, and Ashley, 2002
Phanerochaete chrysosporium	PAHs (anthracene)	Mohammadi et al., 2009
	Molasses wastewater	Vahabzadeh, Mehranian, and Saatari, 2004
	Textile wastewater (Victoria Blue)	Gomaa, Linz, and Reddy, 2008

activities were maintained above 75%. Baldrian et al. (2005) studied the effect of metal ions on enzyme production and concluded that some microelements conferred a significant impact on enzyme activity. The peak of MnP activity was less affected in the presence of Mn, Cu, and Pb, although the average MnP activity in the presence of Mn was lower than that without Mn. Dekker et al. (2001) studied the impact of veratryl alcohol on enzyme production and concluded that laccase activity increased when veratryl alcohol existed in the culture medium, while the synthesis of amylase, pectinase, cellulase, and xylanase significantly decreased. Mougin, Kollmann, and Jolivalt (2002) found that laccase production by the white rot fungus *T. versicolor* was enhanced by several xenobiotics and their transformation products. The enzymatic activity in the culture medium increased 14-fold by adding 4-*n*-nonylphenol and 24-fold by adding aniline. Laccase activity was enhanced 10-fold by adding oxidized derivatives of the herbicide, 17-fold by adding *N*,*N*,-dimethyl-*N*-(5-chloro, 4-hydroxyphenyl) urea and 22-fold by adding 9-fluorenone. Lee (2007) studied the lignin peroxidase of *Phanerochaete* and demonstrated that in a cell-free culture medium with broth, the lignin peroxidase activity could reach 2800 U/L.

Immobilized techniques enhancing enzyme production

Immobilization of the white rot fungus *P chrysosporium* for biodegradation of TNT was reported by Rho et al. (2001). In recent years, immobilization techniques have been developed to enhance enzyme production. The researchers investigated different carriers (stainless steel net, polyamide fiber net, fiberglass net, and polyurethane foam) to immobilize *P. chrysosporium* for ligninolytic enzyme production (Liang, Zheng, and Gao, 2008b). As a result, *P. chrysosporium* immobilized on polyurethane foam showed much better performance in comparison with other carriers. The enzyme activity of *P. chrysosporium* immobilized on polyurethane foam was enhanced. The maximum manganese peroxidase (MnP) activity of the cultures immobilized on stainless steel net, polyamide fiber net and fiberglass net were 36.92, 428.27, and 62.76 U/L, respectively, while the activity of MnP immobilized with polyurethane foam was as high as 915.62 U/L. The activity of MnP of the immobilized culture with polyurethane foam was approximately 25, 2, and 15 times of that of immobilized culture with stainless steel net, polyamide fiber net and fiberglass net, respectively. The peak level of MnP of the immobilized culture on polyurethane foam, stainless steel net, polyamide fiber net and fiberglass net appeared on day 3, 6, 8, and 5, respectively. Using polyurethane foam, the enzymatic activity reached a peak level earlier than the others. In addition, the appearance of the peak was accordant to the time of complete consumption of total nitrogen. Zhu, Liu, and Hu (2007) studied laccase (Lac) production by the white rot fungus using three immobilized methods and indicated that the optimal immobilization was achieved by using glucose as carbon source, NH_4Cl as nitrogen source, pH 3.6 with calcium alginate-poval polymer as carrier.

Molecular biological research on white rot fungus

Molecular biology of white rot fungus has been investigated for years. In China, some researchers have utilized cell protoplasts and are studying the feasibility of cultivation of new strains with higher activity via protoplast fusion of the parent strains.

Methods for gene extraction

In molecular biology, a crucial step in the study is gene extraction. Developing successful extraction methods is essential for the molecular work. Qian et al. (2006) established methods for extraction of total RNA from white rot fungi using *Irpex lacteus* as target species. It took 1 h to extract total RNA from the mycelia. The total RNA detected by UV ion, A260/A280 was 1.9–2.0; gel electrophoresis analysis of 28S rRNA and 18S rRNA brightness ratio was about 2. The purity and integrity met the requirements of molecular biology. Shui et al. (2008) compared two methods (the STE law and the Trizol) to extract the total RNA from *Lentinula edodes, Ganoderma lucidum,* and *Polyporus* spp. and found that the STE method was more suitable than the Trizol method. The A260/A280 ratio of total RNA extracted from three mycelia by STE ranged from 1.80 to 2.10 and A260/A230 was more than 2.00. The RNA extracted by STE had clear bands, without tailing and hybrid. After the analysis of the RNA using an electrophoresis apparatus and a ND-1000 Spectrophotometer, the total quality RNA by STE was better than by the Trizol extraction method.

Gene cloning and the white rot fungus

Cloning technology has been investigated widely in order to understand the genetics and generate new strains or species with improved performance. For white rot fungi, gene cloning and gene construction has been investigated. Using genomic DNA from *Pycnoporus sanguineus* as template to enlarge fragments of the laccase gene through the full-length, the cDNA of the laccase gene was cloned by 5′/3′RACF, and encoding protein synthesis of amino acids, Zhao et al. (2004) obtained the amino acid residues with the highest homology of 96% with the laccase (Lcc3-2) from *Pychoporus sanguineus*. Guo et al. (2005) obtained the cDNA gene fragments from laccase Lcc1 using the RNA of the white rot fungus *Coriolous versicolor* as template through its methanol yeast expression plasmid pMETA-Lcc1 carrier, inserted this into *Pichia methbolica* pMAD16, and integrated this to the methanol *Pichia* chromosomes. Zhang et al. (2008) cloned the xylanase gene from the cotton Verticillium fungus, *Verticillium dahliae,* constructed a heterologous expression in Pichia, and then obtained the recombinant enzyme, which had activity within wide range of temperature and pH, and high degradation performance for beech wood xylan. In addition, the biological safety of genetically engineering bacteria using Pichia was constructed for a number of gene expressions in parallel. A new simple and efficient method, used to clone fungal laccase cDNA, was developed. With the gene intron of *Ganoderma lucidum*, Zhang et al. (2007) used an exon splicing PCR method to synthesize the cDNA of *Ganoderma lucidum* Laccase. Compared with the conventional methods,

the new method avoided not only manipulating the RNA, but also exploring enzyme expression conditions. If combined with genome walking PCR, this method could rapidly clone the laccase gene and cDNA. Rodriguez et al. (2008) cloned two new laccase genes, named pel3 and pel4, which originated from the white rot fungus *Pleurotus eryngii*. In addition, pel3 has been used for expression of *Aspergillus niger*. Moukha et al. (1999) cloned and sequenced the code of the white rot fungus *Pycnoporus cinnabarinus* fiber dehydrogenase. Lignin peroxidase is an important part of white rot fungi isozymes, and its coding gene relies mainly on the DNA probe cloned. Collins and Dobson (1995) cloned lip gene sequences from the four different kinds of white rot fungi. Kristiinan et al. (2006) described the genome and two new codes of the lignin peroxidase gene (Prlip1 and Pr-lip4) and the gene of lignin degradation was used in the gene expression of hardwood and softwood.

Environmental biotechnology with white rot fungus

In the past 20 years, white rot fungus has been investigated to develop biotechnology for the degradation of broad-spectrum, refractory organic pollutants in the environment based on their lignin degrading enzymes (such as Lip, MnP, and Lac). This research has been conducted for the degradation of many wastes and environmental pollutants, including dye wastewater, pesticides, PAHs, TNT, PCBs, chlorinated hydrocarbons, and other toxic organic compounds. White rot fungi can be applied in various environmental media (solid, liquid, and gaseous) for biodegradation.

Wastewater treatment

White rot fungi are known to degrade PAHs, chlorinated aromatic hydrocarbons (CAHs), polycyclic aromatics, PCBs, PCP, polychlorinated dibenzo(p)dioxins, the pesticides DDT and lindane, and some azo dyes (Aust, 1990; Cookson, 1995; Reddy and Mathew, 2001). In recent years, many researchers have indicated that white rot fungi are promising microorganisms in wastewater treatment. *P. chrysosporium* is the most investigated species. Fan et al. (2001) developed a process for dye wastewater which consisted of three parts-trifling electrolysis, biodegradation by white rot fungi and flocculation precipitation. COD_{Cr} removal reached 90%, and chroma declined from 12,800 to 80 and met the wastewater discharge standard in China. Li, Zhang, and Xu (2006) treated diazodinitrophenol-containing wastewater with white rot fungi containing peat and the discharged water reached the standards in China. Huang and Zhou (1999) used white rot fungi to treat the TNT wastewater and achieved degradation of TNT by more than 99%. Fang and Huang (2002) treated bleaching wastewater of paper pulp factory by a white rot fungus–coagulation process and CODcr and OD_{465} in the effluent were 185.1 mg/L and 0.0042 under optimal conditions. The removal of COD and OD_{465} was 99.4% and 86.5%, respectively. The authors used polyurethane foam as carriers to immobilize the white rot fungus *P. chrysosporium* for the biodecolorization of reactive dyes (Liang and Gao, 2008a). Results showed that stable decolorization was as high as 95% in an immobilized reactor system after incubating *P. chrysosporium* for only 2 days in comparison with 15% in a suspended culture for 5 days. The maximum activity of MnP was 915.62 U/L in the immobilized system, compared with 324.90 U/L in the suspended culture. Moreover, the consumption of the carbon and nitrogen substrate in the immobilized system was more rapid than that in the free suspended culture. The enhanced bio-decolorization in the immobilized culture of *P. chrysosporium* was due to the increased the activity of MnP related to the consumption of carbon and nitrogen substrates (Liang and Gao, 2008a).

Vahabzadeh, Mehranian, and Saatari (2004) studied the white rot fungus *P. chrysosporium* for treating molasses wastewater from an ethanol fermentation plant. In diluted wastewater, addition of spores caused a fading rate of up to 75% on the fifth day. The decolorization was found to be correlated to the activity of the ligninolytic enzyme system. The lignin peroxidase (LiP) activity was 185 U/L while manganese peroxidase (MnP) activity was 25 U/L. Gomaa, Linz, and Reddy (2008) studied the ability of the white rot fungus *P. chrysosporium* to decolorize Victoria Blue B (VB) in textile dyes. Inhibition of laccase production by adding various inhibitors to the shaken cultures had a great negative influence on decolorization. When sodium azide and aminotriazole were added to inhibit the activities of the endogenous catalase and cytochrome P-450 oxygenase, the decolorization rate decreased by 100% and 70% respectively. Addition of benzoate resulted in a decrease of 50%. Ergul et al. (2008) studied treatment of olive mill wastewater and the operational conditions of *T. versicolor* FPRL 28A IN. The results showed removal of phenolics by 78% in shake flasks and 39% under static condition. In continuously stirred tank reactors (CSTR), the removal of total phenolics reached 70%.

Bioremediation of contaminated soils

PAHs

Bioremediation of contaminated soils using white rot fungi has been investigated for many years (Aust, 1990). Andersson and Henrysson (1996) studied PAH degradation by five white rot fungi (*T. versicolor* PRL 572, *T. versicolor* MUCL 28407, *P. ostreatus* MUCL 29527, *Pleurotus sajor-caju* MUCL 29757 and *P. chrysosporium* DSM 1556) by adding anthracene, benz[a]anthracene and dibenz[a,h]anthracene to soil. The white rot fungi were cultivated in the soil contaminated with wheat straw and these pollutants. In a heterogeneous soil environment, the fungi displayed different degradation abilities. *Trametes* showed poor degradation performance while anthracene was completely transformed by *Phanerochaete* and *Pleurotus*. Marquez-Rocha, Hernandez-Rodriguez, and Vazquez-Duhalt (2000) studied the degradation of PAHs adsorbed by the white rot fungus, *P. ostreatus*. After 21 days, 50% of pyrene, 68% of anthracene, and 63% of phenanthrene were mineralized. The respective biodegradation percentage was increased to 75%, 80%, and 75% when 0.15% Tween 40 was added. Biodegradation of pyrene in the presence of a surfactant and H_2O_2 was 90%. Eggen and Sveum (1999)

investigated the effect of inoculation by the white rot fungus *P. ostreatus* for PAHs degradation in aged creosote contaminated soil. *P. ostreatus* had an overall positive effect on the degradation of PAHs, under the pre-conditions, the degradation increased with the increasing temperature. Chen et al. (2005) studied the degradation of PAHs by white rot fungi and found that temperature, medium composition, dissolved oxygen, and the moisture content in the soil influenced the degradation of PAHs.

TNT and other explosives

The explosives TNT, HMX, and RDX are integral components of many munitions. Degradation of TNT was investigated using four different strains of white-rot fungi (*Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Phlebia brevispara*, and *Cyathus stercoreus*) in liquid medium (Donnelly et al., 1997). The data indicated that within 21 days of incubation, all fungi were able to reduce the TNT concentration (from 90 mg/L) in the liquid medium to below detection limits. *P. sordida* showed a relatively high growth rate and the fastest rate of TNT degradation. Chemical analysis revealed that the major metabolites in the initial transformation of TNT were the monoamino-dinitrotoluenes, which were also degraded by select white-rot fungi. The study demonstrated that white-rot fungi are capable of metabolizing and detoxifying TNT under aerobic conditions in a nonligninolytic liquid medium. The degradation of TNT by white rot fungi involved two distinct steps, the first step was degradation to OHADNT and ADNT, the second step was to DANT (Aken et al., 1999). Axtell et al. (2000) reported that strains of *P. chrysosporium* and *P. ostreatus,* adapted to grow on high concentrations of TNT, were able to cause extensive degradation of TNT, HMX and RDX.

Chlorinated hydrocarbons

Much research has been conducted on the degradation of chlorinated hydrocarbons. Zou and Zhang (1998) investigated the degradation of chlorinated pesticides by white rot fungi and achieved degradation of chlorinated pesticide by over 90%. Ruiz-Aguilar (2002) reported that three white-rot fungi were used to degrade a mixture of PCBs at high initial concentrations from 600 to 3000 mg/L, in the presence of a nonionic surfactant (Tween 80). The PCBs were extracted from historically PCB-contaminated soil. Preliminary experiments showed that Tween 80 exhibited the highest emulsification index of the three surfactants tested (Tergitol NP-10, Triton X-100 and Tween 80). Tween 80 had no inhibitory effect on fungal radial growth, whereas the other surfactants inhibited the growth rate by 75–95%. PCB degradation ranged from 29% to 70%, 34% to 73%, and 0% to 33% for *T. versicolor*, *Phanerochaete chrysosporium,* and *Lentinus edodes*, respectively, in 10-day incubation tests. The highest PCB transformation (70%) was obtained with *T. versicolor* at an initial PCB concentration of 1800 mg/L, whereas *P. chrysosporium* could modify 73% at 600 mg/L. Zou and Zhang (1998) studied on the mechanism of degradation of chlorinated pesticides by white rot fungi by analyzing degradation products with a GC-MS. They found that the 1, 1-Cl on the atom was more?renovated? than the Cl atoms directly linked to the benzene ring during DDT degradation and that de-chlorination of the chloride atom on benzene was the rate-limiting step of the reaction. Kamei, Kogura, and Kondo (2006) studied the degradation of 4,4′-DCB by the white rot fungi *P. chrysosporium* and *Phanerochaete sp.* MZ142. The degradation ability of *Phanerochaete sp.* MZ142 was better than that of *Phanerochaete chrysosporium*. Hydroxylation of 4,4′-DCB by *Phanerochaete sp.* MZ142 was different from hydroxylation by *Phanerochaete chrysosporium*. The 4, 4′-DCB was initially hydroxylated to 2-OH-4, 4′-DCB and 3-OH-4, 4′-DCB by *Phanerochaete sp.* Although, 2-OH-4, 4′-DCB was not methylated, the metabolic pathway of 3-OH-4, 4′-DCB was branched to form 3-OCH_3-4,4′-DCB and to form 4-chlorobenzoic acid, 4-chlorobenzyl alcohol, and 4-chlorobenzaldehyde. Degradation of PCP by the fungus was reported previously (Ruttimann-Johnson et al., 1997; Ullah and Evans, 1999). Degradation of lindane by *P. ostreatus* was studied by several researchers in relation to degradation conditions (Rigas et al., 2005, 2007). The degradation of TCE by the white rot fungus *T. versicolor* produces 2,2,2-trichloroethanol and CO_2 with chloral as an intermediate (Marco-Urrea et al., 2008). This pathway is different from the aerobic metabolic degradation of TCE (Rittiman snd McCarty, 2001).

Limitations and technical challenges for application

A major limitation of the white-rot fungus is its sensitivity to biological process operations. The fungus does not grow well in a suspended cell system and enzyme induction is negatively affected by mixing actions and the ability of the fungus to effectively attach itself to a fixed medium is poor (Cookson, 1995). The majority of the research on fungal performance has been conducted on autoclaved soil or on synthetic media. Although, the results show that the white rot fungus efficiently and successfully degrades highly toxic complex organic pollutants under these conditions, the results may not be as significant when they are grown under natural environmental conditions with variable native soil organisms, temperature, moisture and pH (Reddy and Mathew, 2001; Hestbjerg et al., 2003). The application of white rot fungi under nonsterile conditions is a technical challenge, because native or contaminating bacteria grow faster than the fungi and compete for carbon sources and nutrients. Bacterial contamination usually causes deterioration in the degradation performance of the fungi. For example, a high and stable decolorization rate was seen under sterile conditions but the easily occurring bacterial contamination always led to a decline in dye decolorization efficiency (Heinfling et al., 1998).

Very few studies test biodegradation performance under field conditions. One problem with field application deals with the strict growth conditions required for most white rot fungi. For example, *P. chrysosporium* has a high temperature requirement (30–37°C) for growth and ligninolytic enzyme production (Hestbjerg et al., 2003) and like many white rot

fungi, it has low competitive capabilities in the environment. Only species of *Pleurotus* (major genera used for edible mushroom production) have a lower temperature requirement for growth and enzyme production and are less affected by native soil organisms than most other fungal species (Hestbjerg et al., 2003). Selection of fungal species with a competitive capability from nature and genetic engineering are needed for the future.

To date, there are limited reports of application of white fungi for large-scale waste treatment and soil remediation. *Phanerochaete chrysosporium*, a widely studied white rot fungus, has not shown positive results in any large-scale tests. A pilot-scale treatability study was performed at a former ordnance area of a USA Naval submarine base (Bangor, Washington) in the 1990s. The initial TNT concentration of 1,844 ppm was reduced 41%, however, the final concentration was well above the target level of 30 ppm. Concentrations of 1267 ppm and 1087 ppm were attained after 30 and 120 days of treatment, respectively. The test was considered a failure. No further testing was reported. Application of *P. ostreatus* has been reported to be successfully applied at both pilot and large-scale (Hestbjerg et al., 2003; Lamar, White, and Ashley, 2002). Field bioremediation studies using *P. ostreatus* were performed on site at the Yorktown Naval Weapons Station Yorktown (Yorktown, VA, USA) (Axtell, Johnston, and Bumpus, 2000). In two plots, 4.587 m^3 of soil, contaminated with TNT, HMX, and RDX, were blended with 2.294 m^3 of a substrate mixture containing nutrients that promoted the growth of fungi. In soil amended with growth substrate and *P. ostreatus*, concentrations of TNT, HMX and RDX were reduced from 194.0 ± 50, 61 ± 20, and 118.0 ± 30 mg/kg to 3 ± 4, 18 ± 7, and 5 ± 3 mg/kg, respectively, during a 62-day incubation period. However, in soil that was amended with this substrate mixture, but not with *P. ostreatus*, the concentrations of TNT, HMX, and RDX were also reduced substantially from 283 ± 100, 67 ± 20, and 144 ± 50 mg/kg to 10 ± 10, 34 ± 20, and 12 ± 10 mg/kg, respectively, during the same period. Thus, it was likely that addition of amendments that enhance the growth and activity of indigenous microorganisms was sufficient to promote the extensive degradation of these compounds in the soil. Although laboratory studies showed that *P. ostreatus* degraded TNT, the role of the fungus in this field test was not clear. Further application of fungal-based bioaugmentation was evaluated for the remediation of creosote-contaminated soil at a wood-preserving site in West Virginia (Lamar, White, and Ashley, 2002). Soil at the site contained creosote-range polycyclic aromatic hydrocarbons (PAHs). Two white-rot fungi (*P. ostreatus and Irpex lacteus*) were evaluated for remediation effectiveness in a two-month bench-scale treatability test. Both fungi produced similar results, with up to 67.3% degradation of total PAHs in 56 days. Pilot-scale testing was performed at the site using *P. ostreatus* grown on two local substrate mixtures. During the 276-day field trial, total PAHs were degraded by up to 93.2%. EarthFax Engineering, an US company tested fungal bioaugmentation using *P. ostreatus* with sawdust and cottonseed hull as a substrate. For dioxin degradation in soil at 2 m^3 test cell, the removal of 61–87% of different congeners were achieved after 282 days. For PCP degradation in a biocell containing 750 tons of soil with 51 ppm PCP and 21 ppm lindane, the average removal of PCP and lindane was 94% and 97%, respectively after two years.

Pilot- and full-scale applications are essential to evaluate the effectiveness and economy of the fungal technology. It is likely that many technical and engineering challenges remained in the application area. In order to apply white rot fungi for dye wastewater treatment, *P. chrysosporium* was incubated under nonsterile conditions and compared with sterile conditions. It was found that the selected immobilization method could maintain high decolorization performance under nonsterile conditions for the degradation of the reactive dye K-2BP (Gao et al., 2008).

Conclusions

The development of environmental technology using white rot fungi has been investigated for more than 20 years. Most fundamental work has been conducted with a variety of the fungi, especially the representative strain *Phanerochaete chrysosporium*.

Research progress has been achieved in the area of processes for cell growth and enzyme production, including the factors influencing enzyme productivity and the methods for the enhancement of enzyme production. Molecular biology related to white rot fungi, especially related to the extraction of genetic material (RNA and DNA), gene cloning and the construction of genetically engineered microorganisms is attractive.

The development of biotechnologies using white rot fungi has been implemented to treat various refractory wastes and to bioremediate contaminated soils. Degradation of many hazardous chemicals and wastes has been demonstrated on a laboratory-scale, especially under sterile conditions. The technical challenge remains for the application including bacterial contamination and for the scale-up of the process. The white rot fungus *P. ostreatus* has been applied for scaled-up bioremediation in the field. More research and development is still needed for cost-effective and sustainable application.

Declaration of interest

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Industrial and environmental applications of white-rot fungi

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Abstract

White-rot-fungi (WRF) are the only organisms able to degrade the whole wood components (i.e. lignin, cellulose and hemicellulose). This ability is due to the secretion of extracellular non-specific ligninolytic enzymes during their secondary metabolism usually triggered by nutrient exhaustion. The non-specificity of these enzymes enables them to transform a great variety of recalcitrant and hazardous pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides, fuels, alkanes, polychlorinated biphenyls (PCBs), explosives and synthetic dyes. In addition, their extracellular nature allows WRF to access non-polar and insoluble compounds. This makes WRF very appealing for their application to different industrial and biotechnological processes. Also, new potential commercial products and processes from the fungal treatment of lignocellulosic materials may arise. The implementation of such applications would contribute to the establishment of a more sustainable industry and the development of a circular economy.

Key words – biotechnology – degradation – lignin – ligninolytic enzymes – valorisation

Introduction

White-rot fungi (WRF), so-named because of the whitish colour of the delignified wood, are the only known organisms able to mineralise the recalcitrant and bulky heteropolymer lignin (Figure 1). This ability is due to the secretion of extracellular non-specific enzymatic complexes during their secondary metabolism (Wesenberg et al. 2003), usually under limited nutrient availability (C:N ratio) with nitrogen being the limiting nutrient for fungal growth in most wood and soils (Kirk & Farrell 1987). These enzymatic complexes mainly consist of lignin peroxidases (LiPs, EC 1.11.1.14), manganese-dependent peroxidases (MnPs, EC 1.11.1.13) and laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) together with accessory enzymes (Ruiz-Dueñas & Martínez 2009). The non-specificity of these enzymes enables them to transform a great variety of persistent chemicals with a structure similar to lignin (Mansur et al. 2003). Furthermore, their extracellular nature allows the fungi to access non-polar and insoluble compounds (Levin et al. 2003). This makes WRF very attractive for different industrial and biotechnological applications such as the production of biofuel from plant biomass, biopulping, biobleaching and the degradation of recalcitrant environmental pollutants.

WRF are ubiquitous in nature, particularly in hardwood forests as hardwood (e.g. birch and aspen) is more susceptible to the attack of WRF than softwood (e.g. spruce and pine) (Blanchette et al. 1990). WRF can degrade all wood components (i.e. cellulose, hemicellulose and lignin) or preferentially lignin. The former are named simultaneous or non-selective WRF and the latter

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selective WRF. The selective WRF are of special bioindustrial interest, since they remove lignin leaving the valuable cellulose intact (Dashtban et al. 2010). There are also WRF that cause both types of white-rot attack within one substrate (Blanchette 1984, Blanchette et al. 1985, Adaskaveg & Gilbertson 1986).

The mechanisms on how WRF degrade lignin are not fully understood but the fungal strain, the origin of lignocellulose and the culture conditions play a major role in the process (van Kuijk et al. 2015). Also, individual fungi can considerably vary their ability to degrade specific substrates under the same environmental conditions (Eriksson et al. 1990).

Potential applications of white-rot fungi

Bioremediation of environmental pollutants

One of the main environmental problems facing the world nowadays is the pollution of soil, water and air by toxic chemicals. Most of these chemicals are known to be carcinogenic and mutagenic posing a serious hazard to the ecosystem and human beings. Therefore such compounds have to be removed before entering into the environment. However, the in-use techniques for the treatment of these type of compounds are rather costly, time-consuming, mostly ineffective and sometimes generate hazardous sub-products (Grassi et al. 2011). Consequently, alternative methods to remove these hazardous recalcitrant compounds are needed. In this sense, the use of WRF represents a promising approach.

Due to the similarity between the chemical structure of lignin and those of many recalcitrant pollutants, such as polycyclic aromatic hydrocarbons (PAHs), pesticides, fuels, alkanes, polychlorinated biphenyls (PCBs), explosives and synthetic dyes (Figure 2), the use of WRF for the degradation of such pollutants has been considered (Paszczynski et al. 1991). This feature is the greatest advantage of using WRF in bioremediation, since a mixture of different pollutants is usually found in most polluted sites (Mester & Tien 2000). Also, WRF can bear a broad range of environmental conditions and, in addition to this, they can use lignocellulose for growth making them suitable for inoculation into polluted soils. Moreover, WRF can exert a positive effect on the growth of the indigenous micro-organisms facilitating the degradation of recalcitrant pollutants.

The first studies on pollutant degradation by WRF were performed with the white-rot fungus *Phanerochaete chrysosporium* (Figure 3A), which has become the model organism for lignin degradation studies (Bumpus et al. 1985). Since them, other species of WRF with promising ability to degrade recalcitrant pollutants have been described, including species belonging to the genera *Pleurotus*, *Bjerkandera*, *Coriolopsis*, *Phlebia* and *Trametes* (Rodríguez et al. 2004). In particular, the non-selective white-rot-fungus *Trametes versicolor* (Figure 3B) has been repeatedly used in assays as a WRF representative (Blanchette & Burnes 1988).

The biotransformation of pollutants by WRF entails different processes started either by the ligninolytic enzymes or the mycelial-bound redox system that produce free radicals, which can then perform either another enzyme-catalysed oxidation or non-enzymatic transformations *via* enzyme combustion. However until whole pollutant mineralisation, the use of different toxicity tests are needed to ensure the safety of the by-products formed (Jurado et al. 2011).

The ability of WRF to remove recalcitrant pollutants from wastewater has shown to be a good alternative to traditional wastewater treatment technologies. In addition, WRF have shown promising potential for the bioremediation of industrially-contaminated soils (Borràs et al. 2010; Anasonye et al. 2015). However, nowadays bioremediation on a commercial scale uses prokaryotes with comparatively few recent attempts to use WRF despite their clear advantages for bioremediation over bacteria (Table 1). In addition, WRF treatments would expand the substrate range of current treatments by degrading pollutants that prokaryotes cannot (Pointing 2001). Nevertheless, the use of WRF in bioremediation presents the following drawbacks: long growth cycle, requiring nitrogen limiting conditions, long hydraulic retention time (Banat et al. 1996, Saratale et al. 2009) and low pH for optimum enzyme activity (Doble & Kumar 2005) which make the maintenance of WRF in bioreactors problematic. Additionally, several operational problems,

such as formation of mycelia aggregates, electrode fouling and clogging, can made necessary the removal of fungal biomass from the bioreactors after short operation periods (Karthikeyan & Sahu 2014). Also, despite different authors have reported the potential of WRF to treat industrial wastewater, there are few studies at bioreactor scale operating in continuous mode and under non-sterilised conditions. Therefore, the application of WRF at industrial scale remains as a technical challenge.

Pulp and paper industry

During pulp and paper production, it is necessary to separate the cellulose fibres from lignin. This is performed by using mechanical or chemical methods. In chemical pulping, lignin is solubilised by chemicals resulting in a brown residual material that must be removed to produce white paper. For this, elemental chlorine has been used for a long time but currently delignification with oxygen and hydrogen peroxide is being used. However, they are less efficient in achieving a high degree of brightness than the chlorine reagents.

The treatment of wood chips with ligninolytic fungi prior to conventional pulping methods (mechanical, chemical or a combination of both) is named biopulping. WRF have been considered as potentially useful agents for biopulping because they reduce not only energy consumption but also chemicals, thus, being environmentally-friendly in contrast with the conventional pulping. In addition, biopulping not only removes lignin but also some of the wood extractives, thereby reducing the pitch content and effluent toxicity (Ali & Sreekrishnan 2001).

The biological delignification of wood by WRF was first considered by Lawson & Still (1957) at the West Virginia Pulp and Paper Company Research Laboratory (now Westvaco Corp.) (Akhtar et al. 1998). Since then, many researchers have studied the potential use of WRF in pulping processes and pilot mill trials have been started in the last decades (Farrell et al. 1997, Breen & Singleton 1999, Scott et al. 2002, Masarin et al. 2009). The efficiency of fungal pre-treatment utilising different lignocellulosic materials has been described and several patents have been published, the one based on the use of *Ceriporiopsis subvermispora* being the most optimised one (Gutiérrez et al. 2001). This species has also proven to be very competitive on both softwoods and hardwoods (Ferraz et al. 2007).

The pre-treatment of wood chips by WRF has shown to improve the effectiveness of kraft pulping and paper brightness (Fonseca et al. 2014). Therefore it can be considered as a possible alternative to chemical pulping since, in addition to this, it requires simpler equipment and produces an effluent with reduced BOD.

Valorization of lignocellulosic wastes

The accumulation of huge amounts of lignocellulosic wastes from human activity is considered a serious environmental problem (Dias et al. 2010). The major constituent of lignocellulosic materials is cellulose followed by hemicellulose and lignin (Figure 4). Cellulose and hemicellulose are macromolecules built from different sugars, whereas lignin is an aromatic polymer synthesised from phenylpropanoid precursors. The composition and proportions of these components vary between plants (Sánchez et al. 2009). Lignin degradation by using chemical and physical methods is a process neither environmentally-friendly nor economical. The use of WRF is being considered as an attractive alternative to transform these wastes into value-added products.

Production of relevant metabolites

Different lignocellulosic wastes have been used as support-substrates for the production of different metabolites of industrial or commercial interest by WRF, generally under solid-state fermentation (SSF) conditions. The use of such wastes not only reduces considerably de production costs but also helps to alleviate the economic and the environmental problems caused by their disposal. Although most of the produced metabolites are ligninolytic enzymes (Rodríguez-Couto & Sanromán 2005) other value-added products such as organic acids are also obtained (Table 2). In addition, recently the application of bioactive compounds produced by WRF to the food and

pharmaceutical industry has impelled the search for novel bioactive compounds of fungal origin (Wong et al. 2010). Moreover, their production has become an important field in modern biotechnology. Thus, the white-rot-fungus *Ganoderma lucidum* has been reported to produce several bioactive compounds with high therapeutic value (Paterson 2006). Also, recently the white-rot fungus *Cerrena unicolor* has shown to exhibit antiviral, immunomodulatory and anticancer activities (Mizerska-Dudka et al. 2015).

Bioethanol

Biofuel production from renewable sources has received increased interest in recent years as an alternative to the use of fossil fuels in many countries. Lignocellulosic biomass, mostly from agricultural and forestry wastes, is rich in carbohydrates and widely available, thus, providing attractive feedstocks for ethanol production. To maximise the use of carbohydrates from the biomass a pre-treatment process is required. The current in use technologies are costly hampering the commercialisation of bioethanol (Mosier et al. 2005). This has impelled the search for alternative processes such as those based on WRF. Thus, several WRF, such as *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor, Cyathus stercoreus* and *Ceriporiopsis subvermispora*, have been studied for the pretreatment of different lignocellulosic wastes (Wan & Li 2012; Knežević et al. 2013). However, despite that the use of WRF offers the following advantages over the current thermal or chemical pre-treament processes: simpler techniques, low energy requirements, no or reduced waste streams, reduced downstream processing costs and no or reduced inhibitors to ethanol fermentation (Keller et al. 2003; Nigam & Pandey 2009), substantial holocellulose (cellulose and hemicellulose) loss and long pre-treatment times are the main drawbacks of this process. So to ensure a cellulose-rich but highly delignified biomass for biofuel production, highly selective lignin degraders are preferred.

Ruminant feed

Cellulose and hemicellulose in most lignocellulosic wastes are highly linked to lignin which makes them difficult to digest by animals (Arora & Sharma 2009). This hampers the use of such wastes by rumen microbes and currently chemical and/or physical treatments are used to degrade lignin (Chaturvedi & Verma 2013). In search for alternative treatments to the use of chemicals or expensive physical treatments, the use of WRF is seen as a very attractive alternative. In particular to convert lignocellulosic wastes into ruminant feeding, selective lignin degraders are the preferred WRF since they left cellulose intact and, thereby, keep the energy value of such wastes. However, only small laboratory scale experiments involving singles substrates or fungal species have been conducted so far. Further optimisation is needed to develop an alternative treatment able to compete with the conventional treatments (van Kuijk et al. 2015).

Outlook

The practical use of WRF for biotechnological applications holds great potential. However before this can become a reality, progress related to process optimisation and cost reduction is needed. Searching for novel micro-organisms, taking advantage of the enormous microbial diversity existing in aquatic and terrestrial environments, is also required. The ocean is an enormous reservoir of untapped micro-organisms.

The advantages and disadvantages of using WRF or their enzymes in biotechnological applications should be evaluated before attempting industrial-scale operations. In comparison to fungal biomass, enzymes are still more expensive to produce at an industrial scale in spite of their potential for scaling up through gene technologies. However with the increasing advances in enzyme immobilisation technologies, efficiency in enzyme reusing both in amount and activity is probably to be greater than that of fungal biomass.

Table 1 Advantages of using white-rot-fungi (WRF) over bacteria for bioremediation (Maloney 2001).

(i) They use inexpensive and easily available lignocellulosic materials as a nutrient source
(ii) They can tolerate relatively high concentrations of pollutants due to their extracellular degradation system
(iii) They are able to survive in the presence of several xenobiotics that may be toxic to other microorganisms
(iv) They are able to degrade a mixture of chemicals thanks to their non-specific free-radical-based degradation mechanism
(v) They do not need pre-conditioning to a particular pollutant
(vi) They can tolerate a wide range of environmental conditions
(vii) The rate of degradation or biotransformation of a pollutant is proportional to its concentration and, so, the solubility of the pollutant is not important

Table 2 Examples of different value-added metabolites produced by white-rot fungi grown on lignocellulosic wastes under solid-state fermentation conditions.

White-rot fungus	Lignocellulosic waste	Product(s)	Reference
Fomes fomentarius	Granary waste (small grains, husks and straw, and straw of barley, oats, rye and wheat)	Crude protein	Hatakka & Pirhonen 1985
Nematoloma frowardii	Wheat straw	Manganese peroxidase, organic acids	Hofrichter et al. 1999
Phanerochaete chrysosporium, Phlebia radiata	Corncob	Protease	Cabaleiro et al. 2002
Dichomitus squalens, Phanerochaete sanguinea, Trametes ochracea, Trametes versicolor	Spruce wood chips	Oxalic acid	Mäkelä et al. 2002
Physisporinus rivulosus	Spruce wood chips	Manganese peroxidase, laccase, oxalic acid	Hakala et al. 2005
Ceriporiopsis subvermispora	*Pinus taeda* wood chips	Xylanase	Milagres et al. 2005
Trametes hirsuta	Grape seeds	Laccase	Rodríguez-Couto et al. 2006
Bjerkandera adusta, Pycnoporus sanguineus	Oak and cedar sawdust, rice husk, corn stubble, wheat straw, *Jatropha* seed husk	Cellulase, xylanase	Quiroz-Castañeda et al. 2011
Cerrena unicolor	Oat husks	Manganese peroxidase, laccase	Moilanen et al. 2015
Coriolopsis gallica	Sawdust waste	Laccases	Daâssi et al. 2016

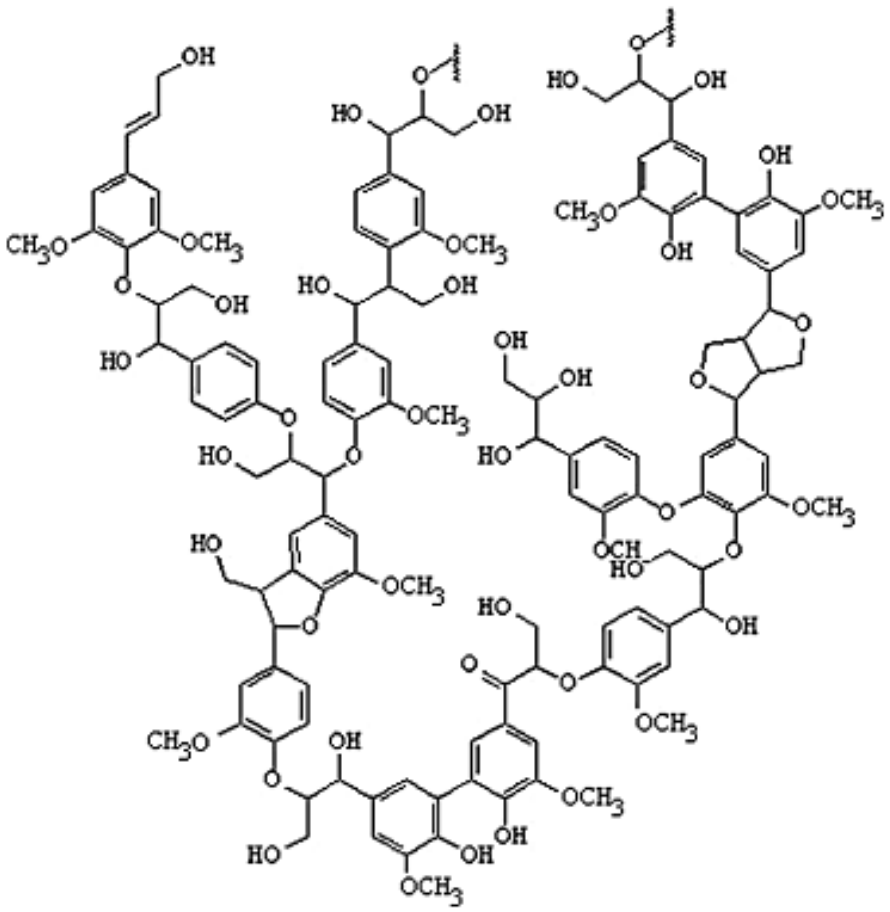
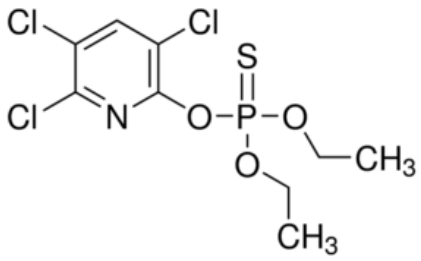
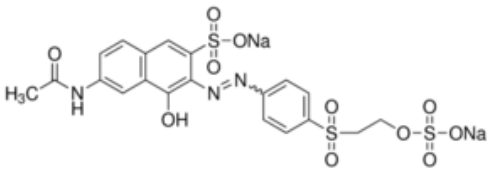


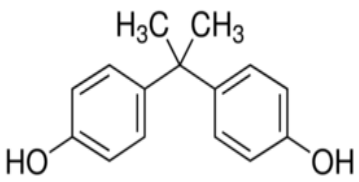
Fig. 1 – Schematic structure of a lignin molecule (source: www.research.uky.edu/.../green energy.html).



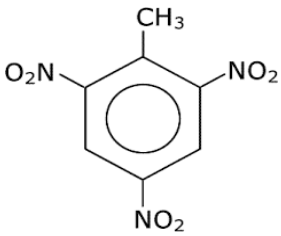
Chlorpyrifos



Reactive Orange 16



Bisphenol A



2, 4, 6-trinitrotoluene

Fig. 2 – Different environmental pollutants degraded by the white-rot-fungi.

A **B**

Fig. 3 – Pictures of the white-rot fungi *Phanerochaete chrysosporium* (A; source https://microbewiki.kenyon.edu/) and *Trametes versicolor* (B; source http://www.wisconsinmushrooms.com/) as grown in nature.

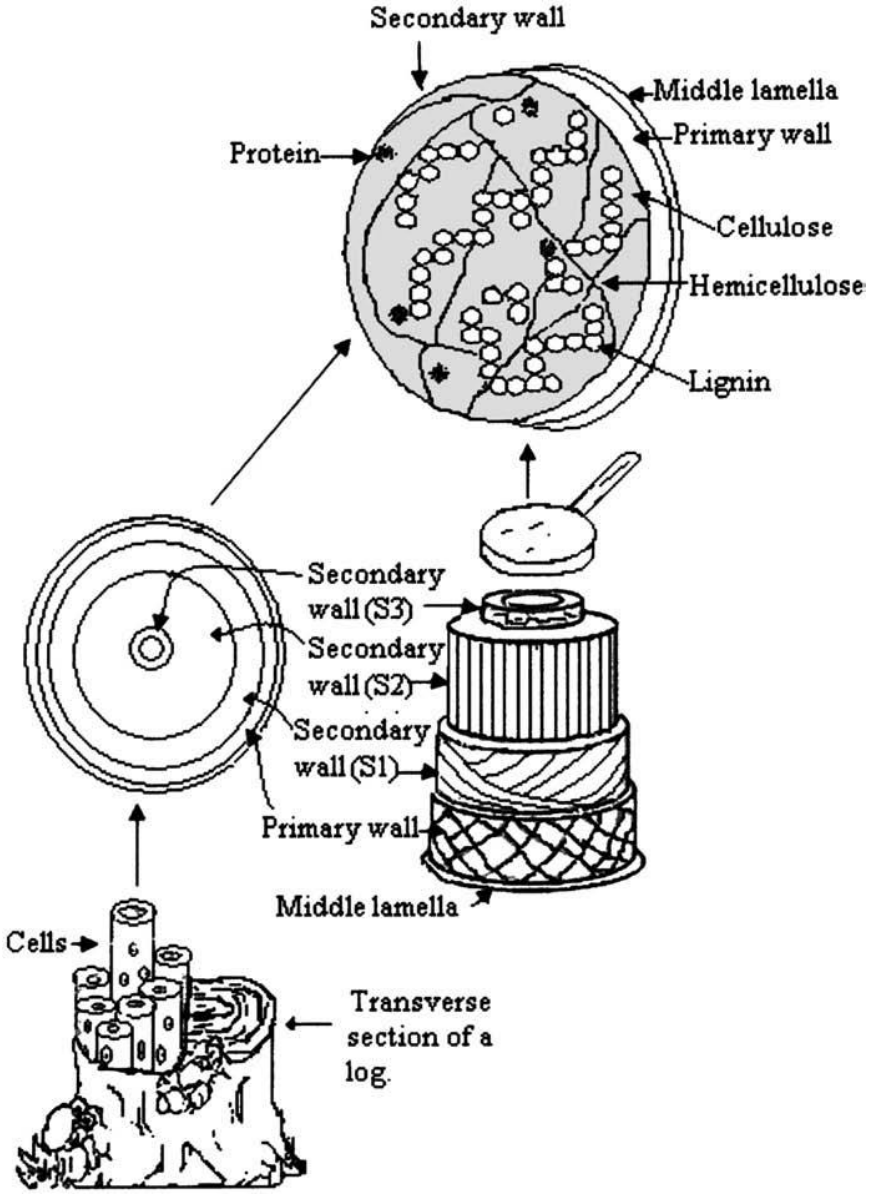


Fig. 4 – Major components of wood.

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Honors and Accolades

- Advisory Board Member, Journal of Pharmaceutical & Scientific Innovation, (2012)
- Cover Picture, Journal of Biotechnology, (2011)
- Member, Board of Editors, *World Journal of Pharmacology*, (Baishideng Publishing Group Co., Limited), (2011-2015)
- Member, Board of Editors, *Journal of Xenobiotics*, (Pagepress), (2011-current)
- Member, Board of Editors, *ISRN Chemical Engineering*, (Hindawi Publishing Corporation), (2011-current)
- Member, Board of Editors, *The Open Textile Journal*, (Bentham Science Publishing), (2010-current)
- Member, Board of Editors, *American Journal of Analytical Chemistry*, (Scientific Research Publishing), (2010-current)
- Member, Board of Editors, *International Journal of Current Trends in Science and Technology*, (Scientific Research Publishing), (2010-current)
- Managing Editor, *Frontiers in Bioscience*, (2010-current)
- I3 Professor, Recognition to an outstanding research activity, Spanish Ministry for Science and Innovation, (2008)
- Member, Board of Editors, *Food Technology and Biotechnology*, (2008)

- *Ramón y Cajal* Senior Fellow, Spanish Ministry for Science and Education, (2004-2008)
- *Isidro Parga Pondal* Senior Fellow, *Xunta de Galicia* (local government of Spain), (2004)
- Positive Evaluation by AQU (Catalan Agency for the Quality of the University System) for being an Associate Professor, (2006)
- Positive Evaluation by ANECA (Spanish Agency for the Evaluation of Quality and Accreditation) for being an Associate Professor, (2003)
- Extraordinary Doctoral Prize, University of Vigo, Spain, (2001)

Current Research

- Production of ligninolytic enzymes
- Design of bioreactors
- Immobilisation of microorganism and enzymes
- Biodegradation of recalcitrant compounds
- Bioprocesses for wastewater treatment
- Characterisation and purification of proteins

Biography

Susana Rodriguez-Couto was born in 1965 in Vigo, Spain. After graduating in Chemistry from the University of Santiago de Compostela, Spain, she obtained a Ph.D. from the University of Vigo, Spain. Immediately after the defense of her doctoral thesis, she joined the research group of Environmental Engineering and Bioprocesses at the department of Chemical Engineering of the University of Santiago de Compostela. Then, she joined the Department of Chemical Engineering of the University of Vigo as an Associate Professor, post that she kept until January 2004 in which she obtained a position as an *Isidro Parga Pondal* Senior Research Fellow (*Xunta de Galicia*, local government of Spain) at the same department. From December 2004 to December 2008, she hold a position as a *Ramón y Cajal* Senior Research Fellow (Spanish Ministry for Science and Education) at the Chemical Engineering Department of the Rovira i Virgili University in Tarragona, Spain. Since January 2009, she holds a permanent position as an IKERBASQUE (Basque Foundation for Science, local government of Spain) Research Professor at the Unit of Environmental Engineering of CEIT in Donostia-San Sebastian, Spain, where she is in charge of a new research line on Environmental Biotechnology.

Her main achievements are the development of methods for the production of enzymatic complexes by cultivation of different white-rot fungi under solid-state fermentation conditions and the efficient application of these enzymatic complexes to the decomposition of xenobiotic compounds, the design of different solid-state bioreactors and the design of enzymatic bioreactors. She has published about 95 international papers in outstanding journals (h-index 23) and 6 book chapters.

Further Information on Dr. Rodriguez-Couto and her research can be found on the ikerbasque website (http://www.ikerbasque.net/).

Selected Publications

Cost analysis in laccase production

Osma JF, Toca-Herrera JL, Rodriguez-Couto S
Journal of Environmental Management; Volume 92, Issue 11; Pages 2907-2912;
Published: 2011

Biodegradation of a simulated textile effluent by immobilised-coated laccase in laboratory-scale reactors

Osma JF, Toca-Herrera JL, Rodriguez-Couto S

Applied Catalysis A: General; Volume 373, Issues 1-2; Pages 147-153; Published: 2010

Decolouration of azo dyes by Phanerochaete chrysosporium immobilised into alginate beads

Enayatzamir K, Yakhchali B, Tabandeh F, Rodriguez-Couto S

Environmental Science and Pollution Research; Volume 17, Issue 1; Pages 145-153; Published: 2010

Industrial applications of laccases

Rodriguez-Couto S, Toca-Herrera JL

Biotechnology Advances; Volume 24, Issue 5; Pages 500-513; Published: 2006

Stainless steel sponge: a novel carrier for the immobilisation of the white-rot fungus *Trametes hirsuta* for decolourisation of textile dyes

Rodriguez-Couto S, Sanroman MA, Hofer, D, Gübitz GM

Bioresource Technology; Volume 95, Issue 1; Pages 67-72; Published: 2004

Memorandum

To:	Dave Bowers MTDEQ
From:	Colin McCoy, Tetra Tech Inc.
CC:	
Date:	11/20/2017
Re:	Dioxin Remediation Query

Dave,

As you requested, here is the summary of the internal email sent to our Remedial Strategies team inquiring as to whether anyone had experience in remediating dioxin in soils with methods alternative to (1) consolidation and capping, and (2) incineration. Two replies were received.

One email indicated the author was not aware of any other remedial alternatives for dioxin. It went on to discuss that depending on dioxin concentrations and hazardous waste determinations, incineration or landfilling was typically used.

The other email indicated the same experience, but discussed recently hearing of a project at the Vietnam Airport in Danang where thermal desorption is being used for destruction of dioxin in contaminated soils. The USAID website describing the project, listed the other technologies evaluated for dioxin remediation which included base-catalyzed decomposition, ball milling with active landfill, Geo-Melt™ Process (vitrification), passive landfill, active landfill, and incineration.

No other replies were received. Biodegradation of dioxin was not identified as an alternative.

In-Pile Thermal Desorption® (IPTD®) of Dioxin Contaminated Soil and Sediment

Ralph S. Baker, Ph.D., Gorm Heron, Ph.D., Jim Galligan, P.E., Steve McInerney and Stan Walker
(TerraTherm, Inc., Gardner, Massachusetts USA)
Niels Ploug
(Krüger A/S, Søborg, Denmark)

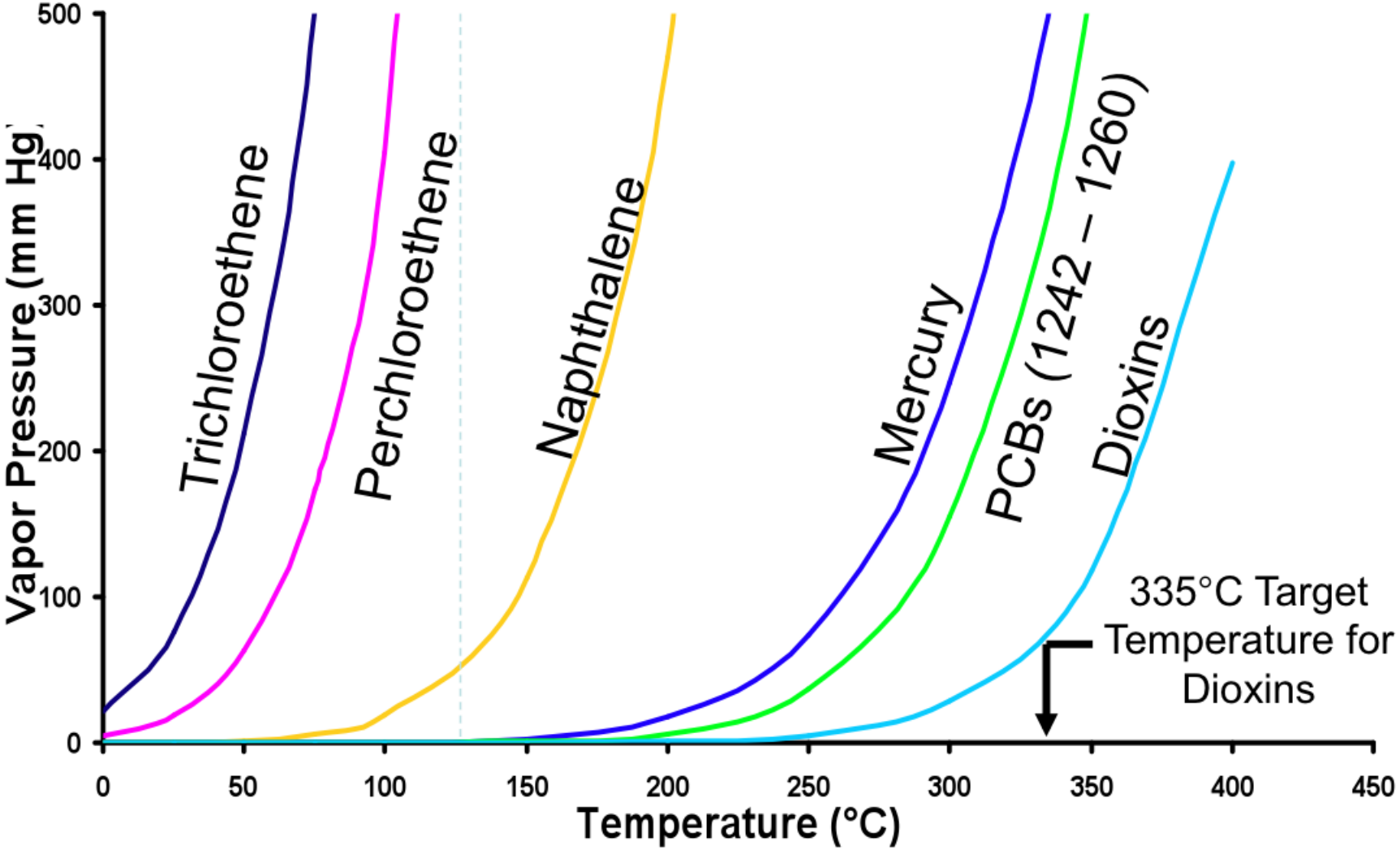
intersol'2014, Lille, France
20 March 2014

TERRATHERM

Overview

- Introduction to Thermal Conduction Heating and ISTD/IPTD® for Treatment of Dioxins
- MOE Japan IPTD® Demonstration
- USAID – Danang Airport, Vietnam IPTD® Project

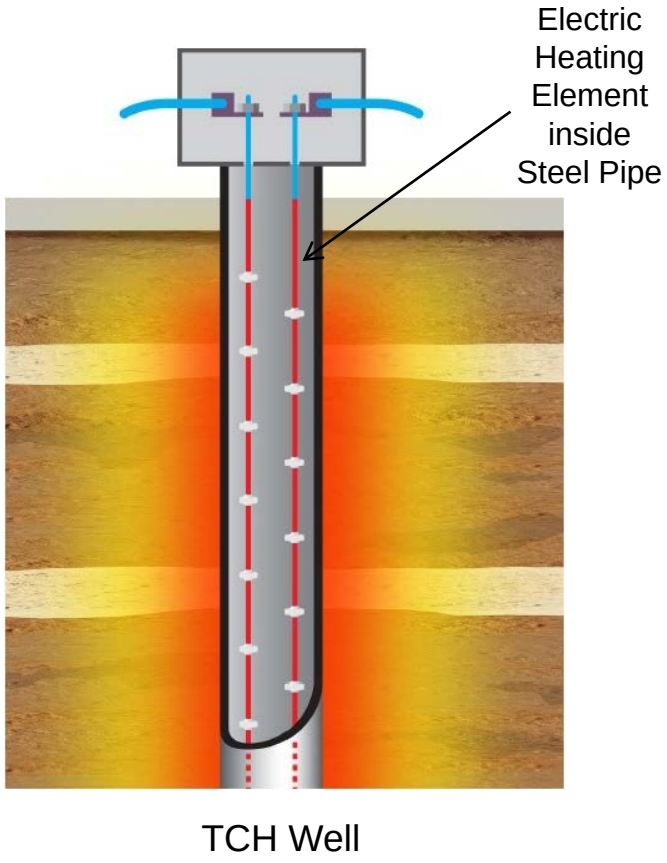
rbaker@terratherm.com



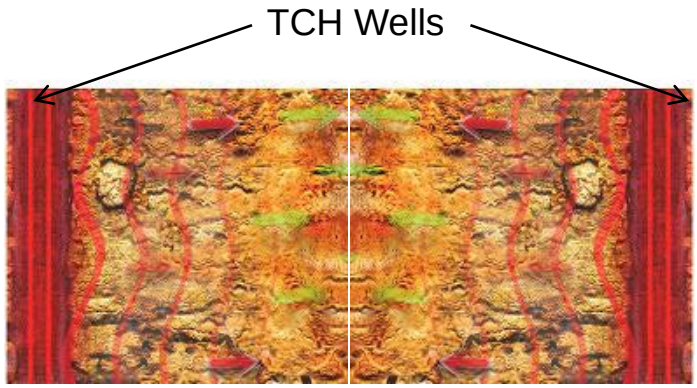
The vapor pressures of contaminants increase exponentially due to thermal conduction heating during the IPTD® process.

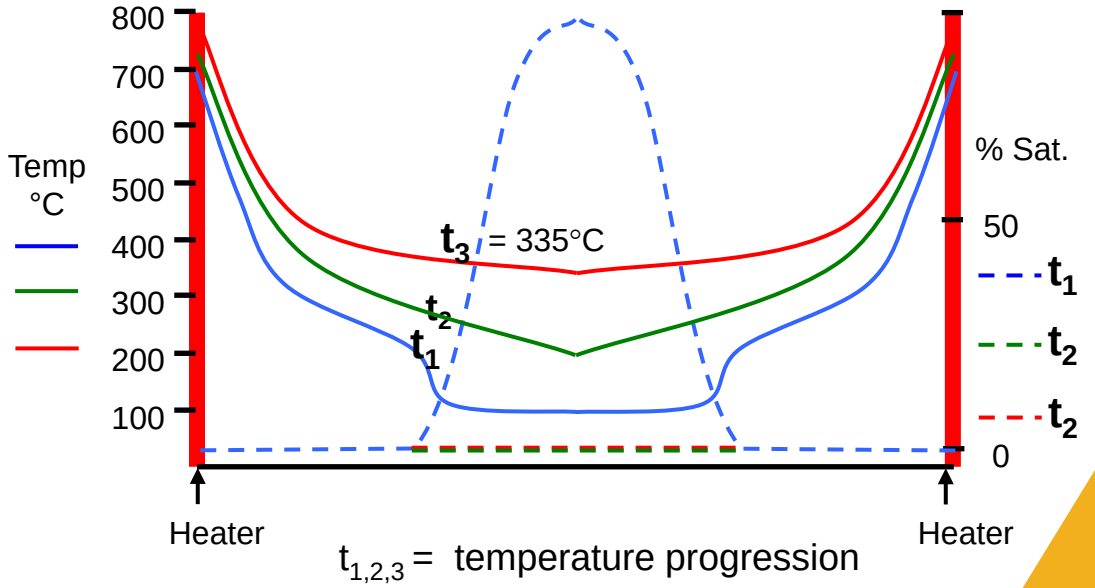
rbaker@terratherm.com

What is Thermal Conductive Heating (TCH)?



TCH Well





Heat spreads primarily by conduction → uniform heating

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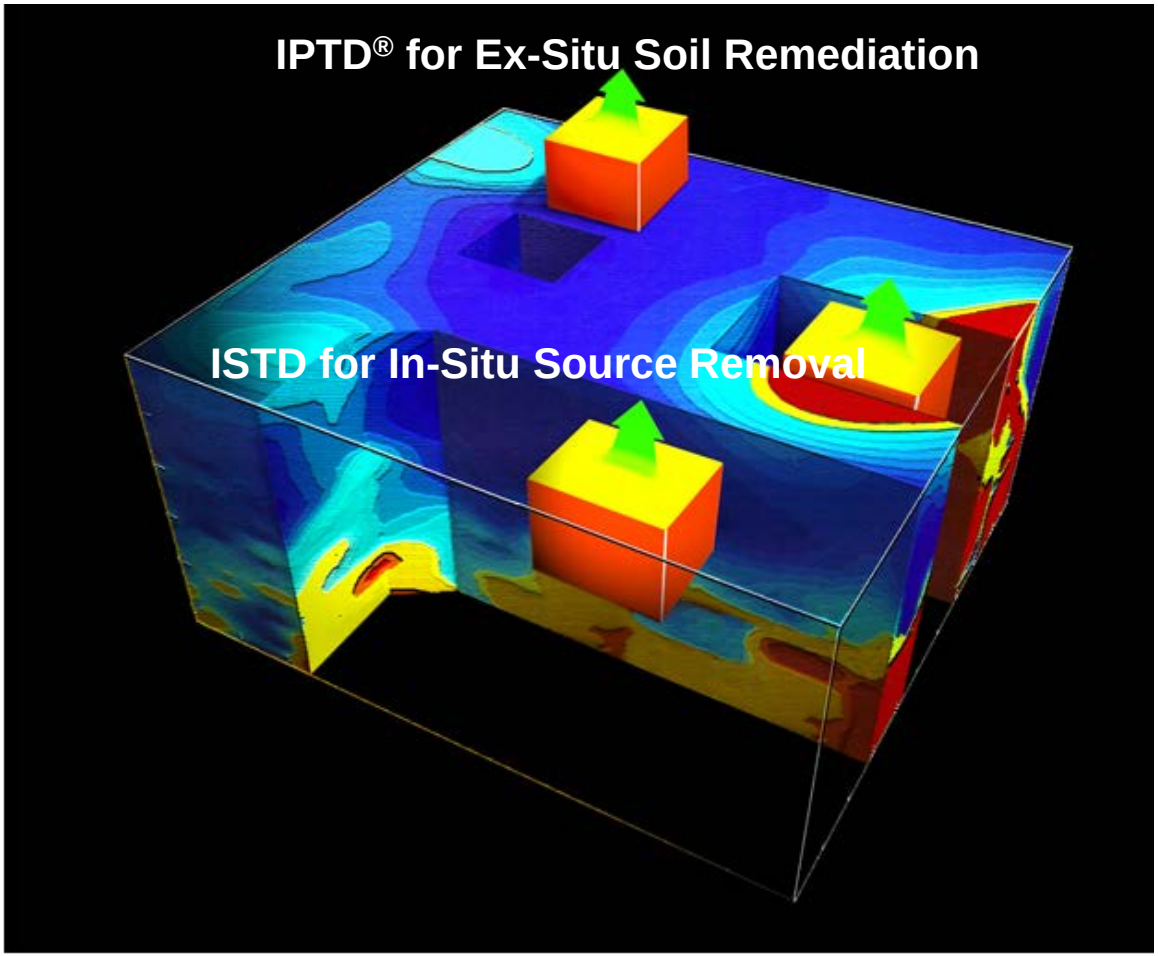
Two Ways to Apply TCH:
In Place (In-Situ) or Aboveground (Ex-Situ)



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Examples of ISTD and IPTD® Field Project Results for the Remediation of Dioxin in Soil and Sediment

Site	Treated Volume	Before treatment	After treatment	Source test
		Mean Soil Concentration	Mean Soil Concentration	Exhaust gas
	[m^3]	[pg-TEQ/g]	[pg-TEQ/g]	[ng-TEQ/Nm^3]
***Missouri Electric Works Superfund Site*, Cape Girardeau, Missouri USA**	5.7	6,500	3.2	0.0029
***Former US Naval Facility Centerville Beach*, Ferndale, California USA**	765	3,200	7.3	0.0055
***Southern California Edison* AOC-2, Alhambra, California USA**	12,615	18,000	110	0.0071
***Ministry of Environment*, Yamaguchi, Japan**	1.0	1,800	67.75	0.000018

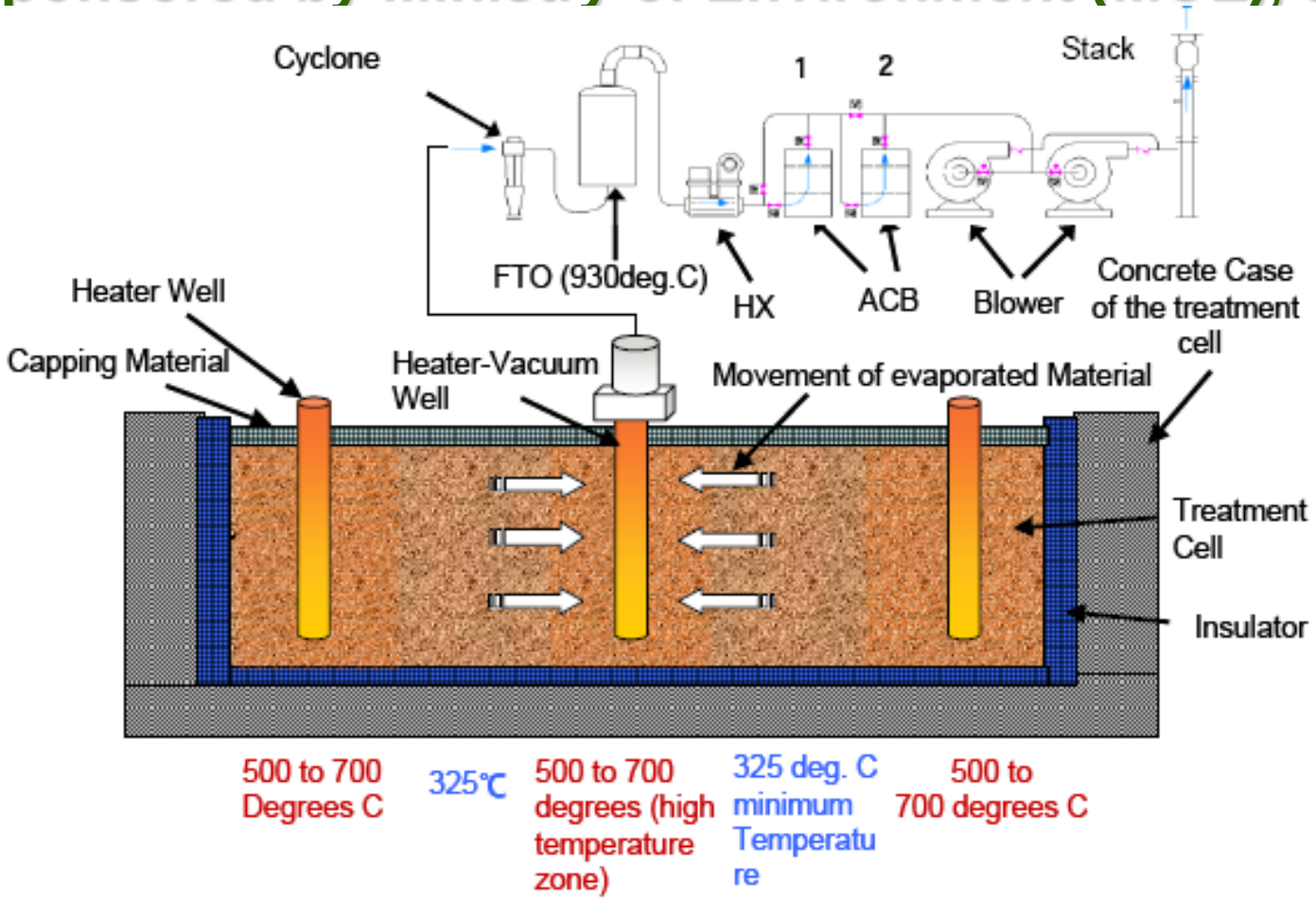
Presented at intersol'2007, Paris

TEQ = 2,3,7,8-Tetrachlorodibenzo-dioxin Equivalents (WHO)

(Heron et al. 2010; Baker et al. 2008; USEPA 1998; Conley and Lonie 2000)

IPTD® Demonstration 2009

Sponsored by Ministry of Environment (MOE), Japan



A joint project of TerraTherm, Inc. and SheGoTec Japan, Inc.

(Heron et al. 2010)

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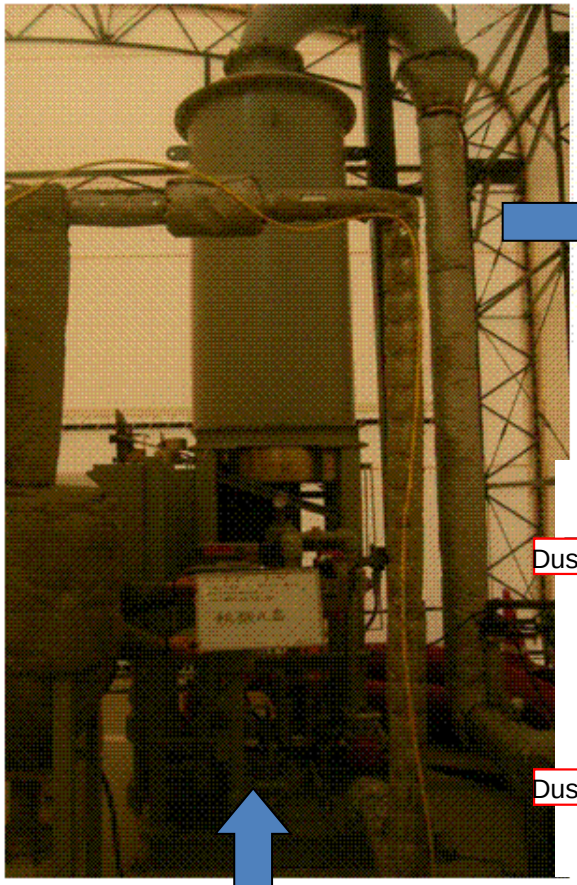

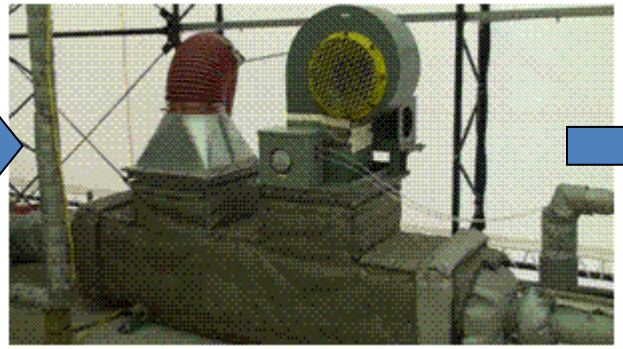

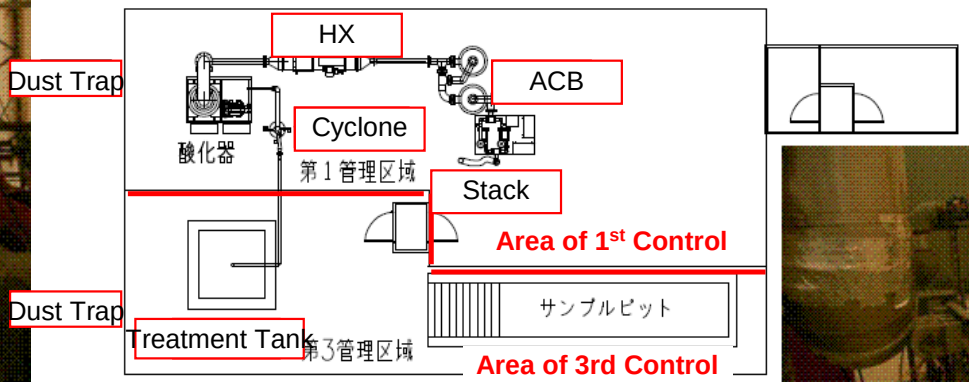

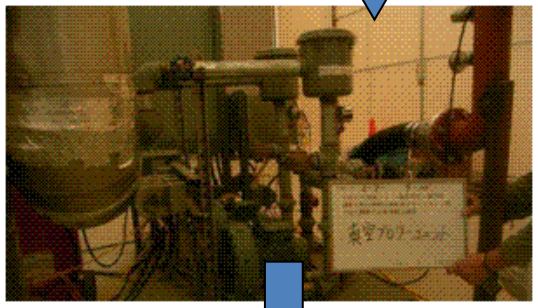

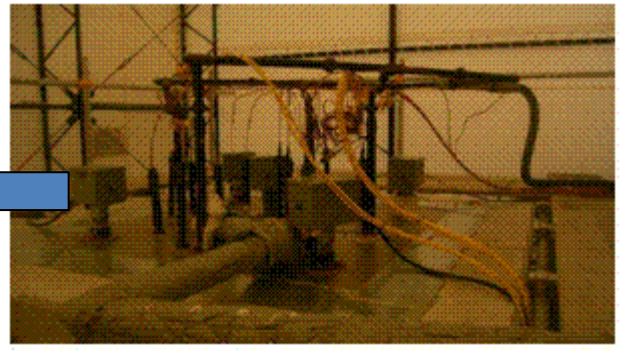

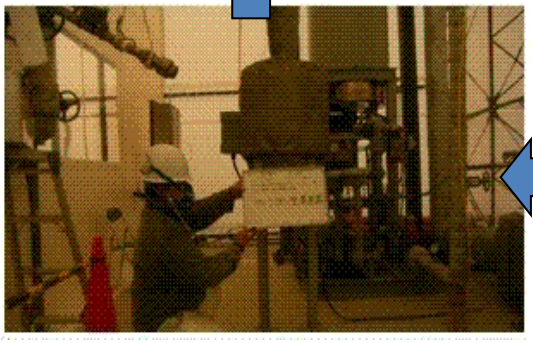

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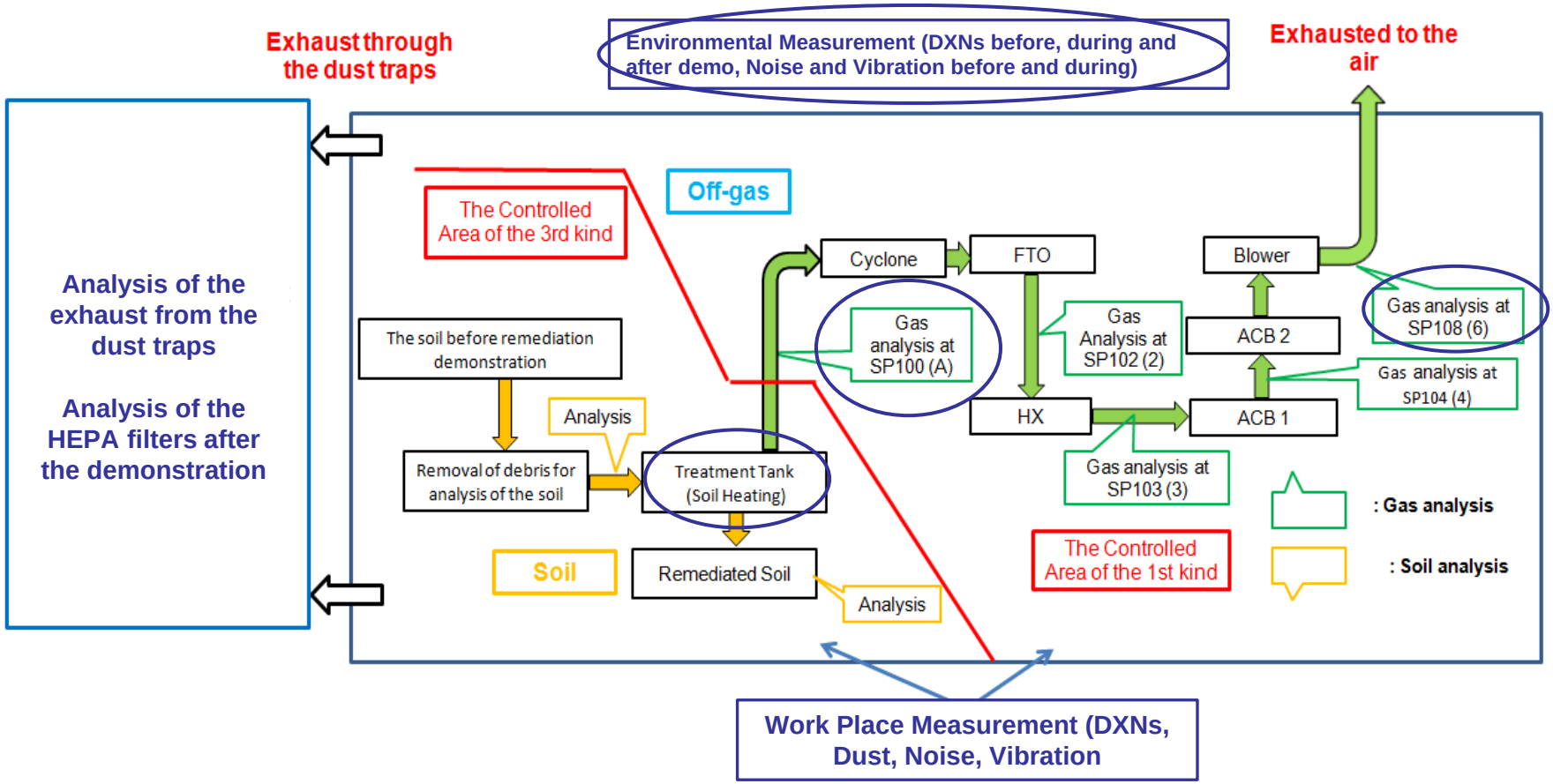
Monitoring Program



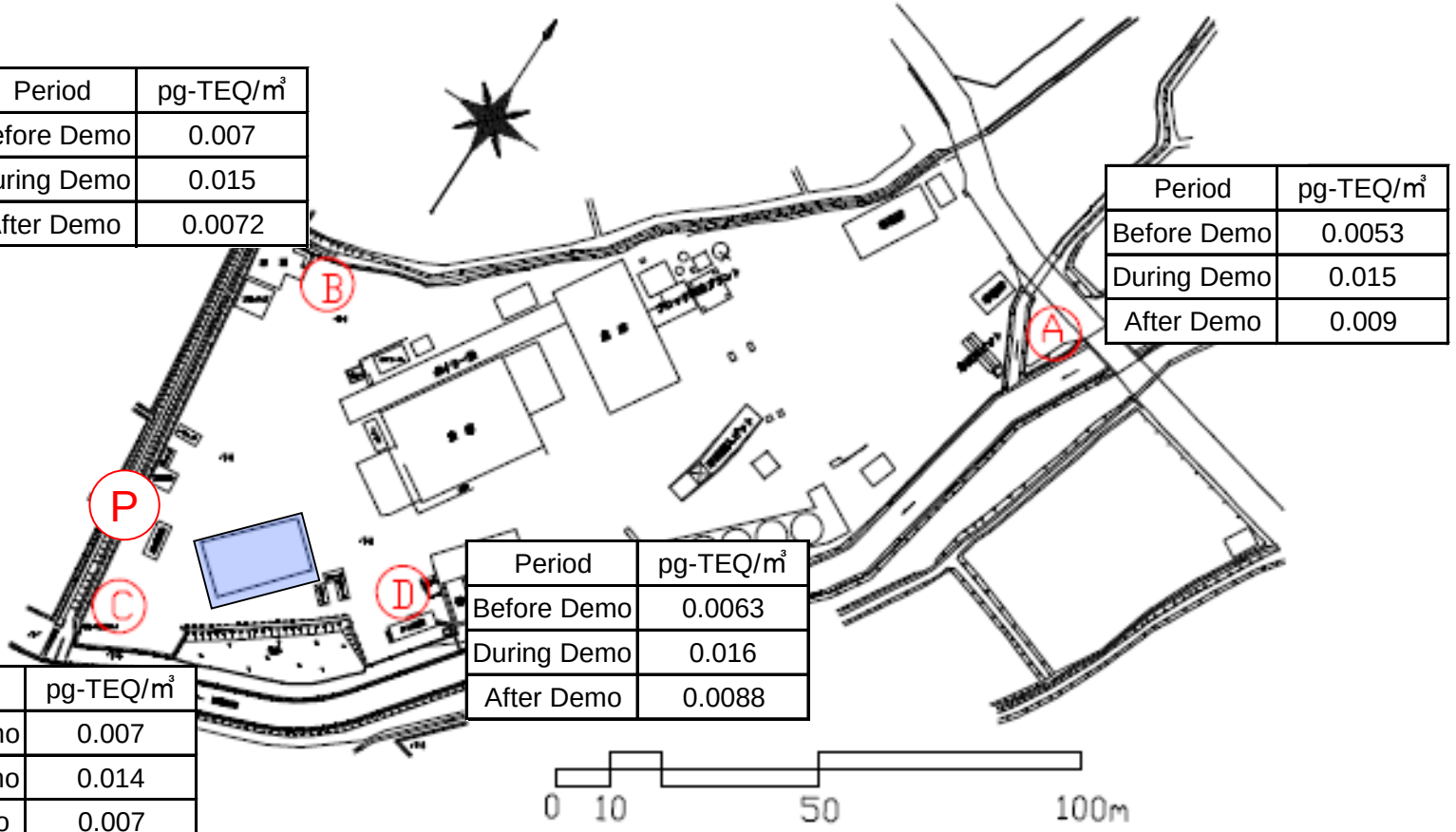
Monitoring the Surroundings

Period	pg-TEQ/m³
Before Demo	0.007
During Demo	0.015
After Demo	0.0072

Period	pg-TEQ/m³
Before Demo	0.0053
During Demo	0.015
After Demo	0.009

Period	pg-TEQ/m³
Before Demo	0.0063
During Demo	0.016
After Demo	0.0088

Period	pg-TEQ/m³
Before Demo	0.007
During Demo	0.014
After Demo	0.007



: Demonstration Tent

A to D : DXN monitoring Points

P : Noise/Vibration monitoring Points

Environmental Std.: 0.6pg-TEQ/m3

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Effectiveness of the IPTD® Technology

(Soil, before vs. after heating)

Removal Ratio	%	DXNs concentration before remediation (pg-TEQ/g)	DXNs concentration after remediation (pg-TEQ/g)
	96.24	1,800	67.75

67.75 pg-TEQ/g << Japan standard of 1,000 pg-TEQ/g

No changes in soil characteristics were observed

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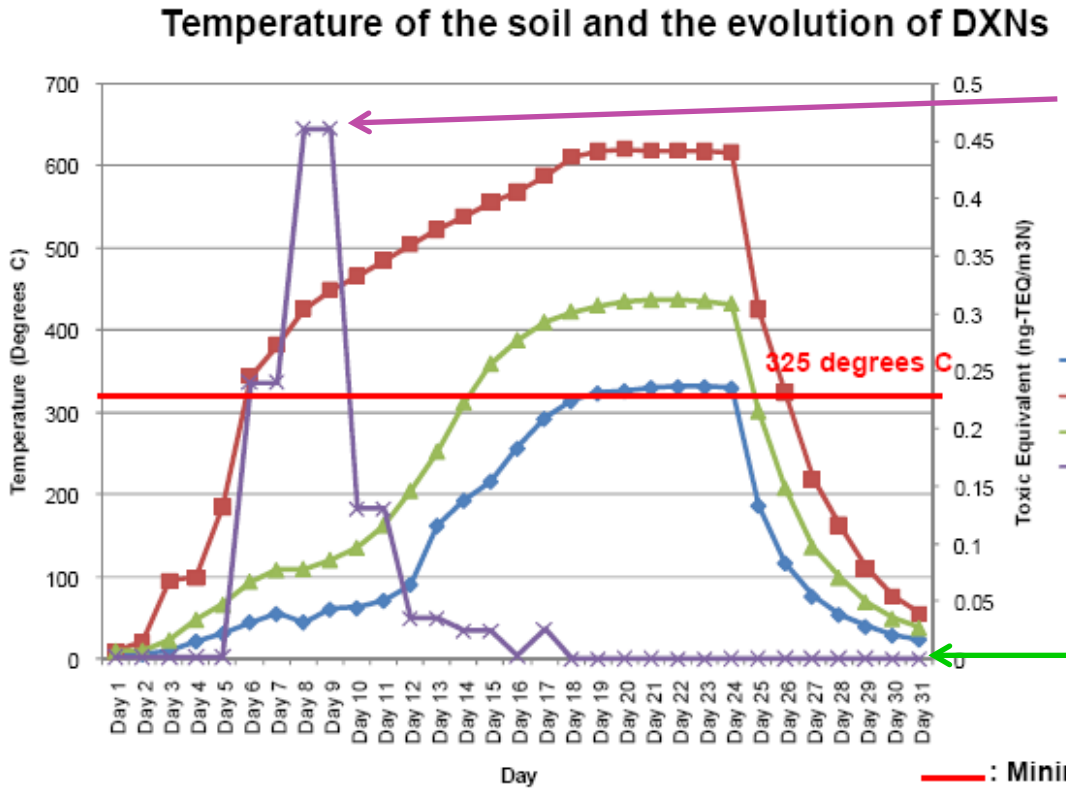
MOE IPTD® Demonstration Results



Peak concentration in gas evolved from soil (before AQC) = 0.46 ng-TEQ/m^3

Off-gas discharge = 0.00018 ng-TEQ/m^3 < 0.0006 ng-TEQ/m^3 environmental standard

⇒ IPTD® approved for treatment of dioxin-contaminated soil or sediment in Japan

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Examples of ISTD / IPTD® Projects for Treatment of Dioxins:

3. Danang, Vietnam

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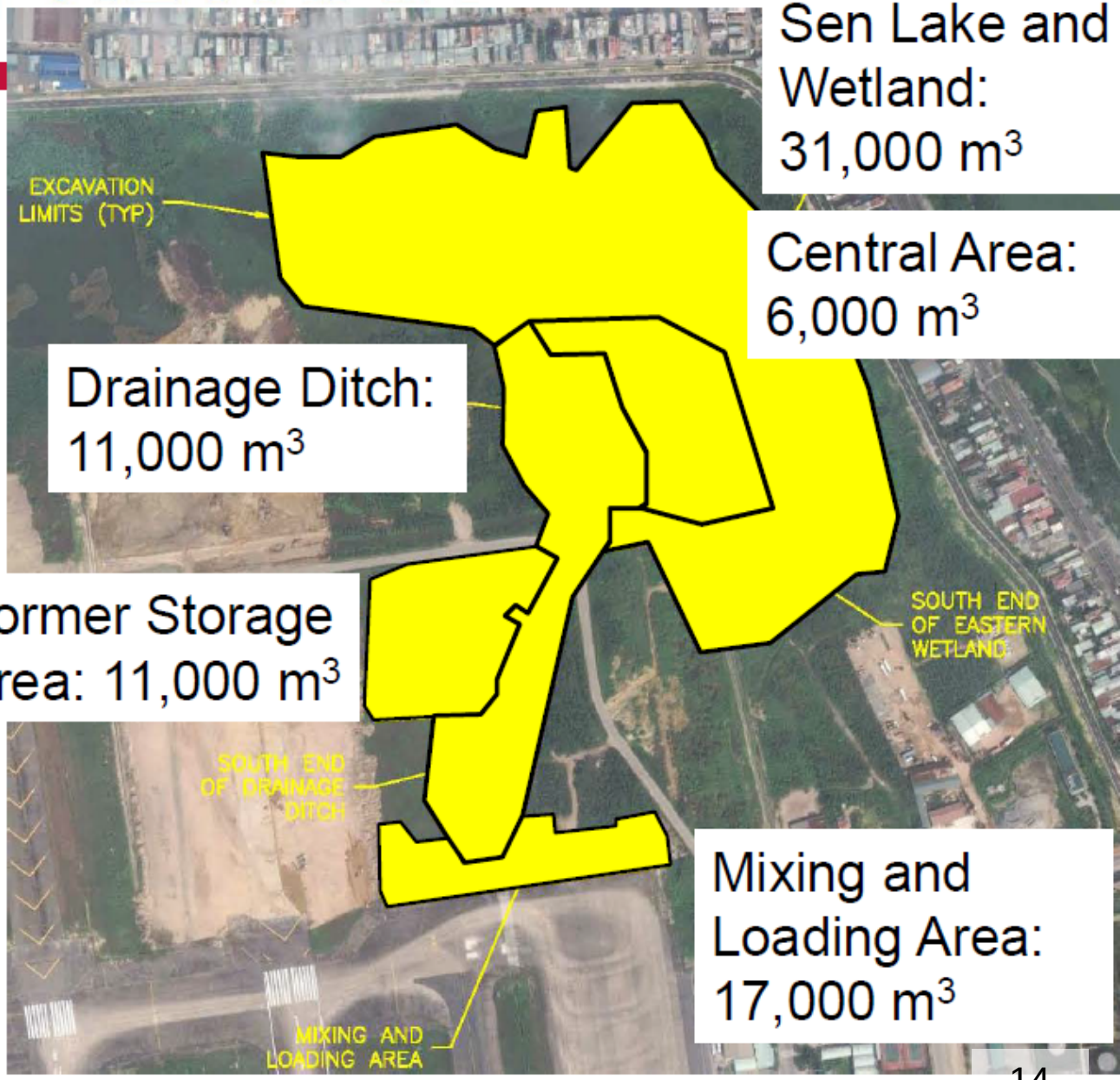
(Sorenson et al. 2011)

Areas Requiring Excavation

Total Area: approximately 190,000 m^2

Total Volume (with Pacer Ivy Storage Area to the south): 77,000 m^3

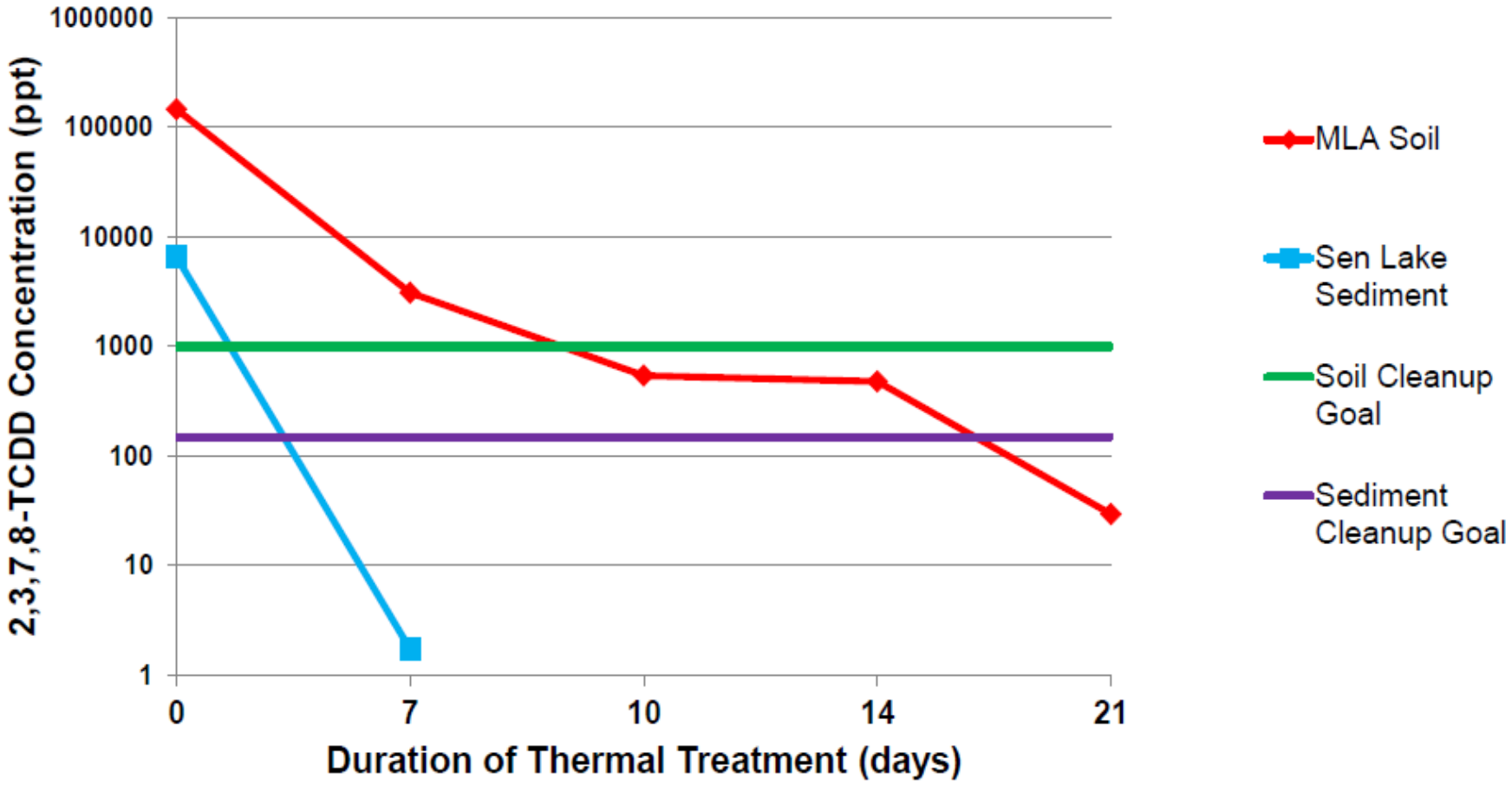


(Sorenson et al. 2011)

DETAILED EVALUATION: SUMMARY OF EA FINDINGS

Alternative	Final Remedy to Meet Clean Up Goals	Implementable	Potential Environmental Impact	Estimated Cost
No Action	No	Yes	Highest	Externalized
Active Landfill	Uncertain	Yes with challenges	Second highest	$31M
Passive Landfill	No	Yes with challenges	Third highest	$36M
ISTD/IPTD	Yes	Yes with challenges	Lowest	$34M

Treatability Testing Results (Sorenson et al. 2011)



3-D Simulation of IPTD® System Construction and Operation

(Courtesy of USAID and CDM Smith)

Under Construction

Pile containment structure (Photo: TetraTech)

Installing heaters into pile structure (Photo: CDM Smith)

http://www.usaid.gov/vietnam/environmental-remediation

USAID
FROM THE AMERICAN PEOPLE

Vietnam: Environmental Remediation of Dioxin Contamination at Danang Airport

Progress Report: January 1, 2014 to January 31, 2014

Construction Nearing Completion

Containment structure and liquid/vapor treatment system (Photo: Tetra Tech)

http://www.usaid.gov/vietnam/environmental-remediation

Current Status

- Construction nearly complete
- Commission: March-April 2014
- Phase 1 Heating: April – August, 2014
- Phase 2: 2016.

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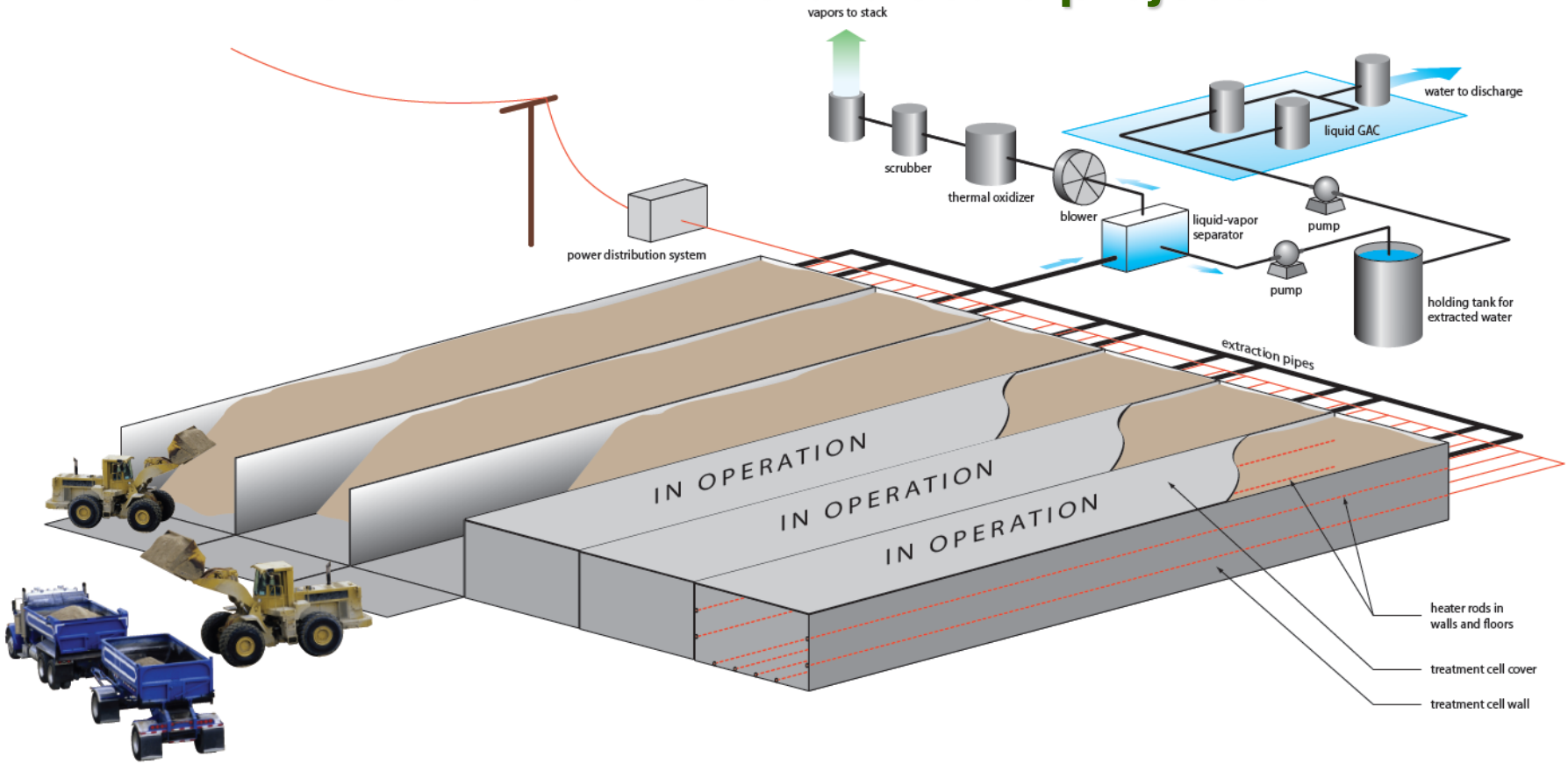
IPTD® Adapted for Mobile or Fixed Ops

for small- or moderate-scale projects



Drive in / Drive out capability. Load / unload with no obstructions!

U.S. Patents 8,348,551 and 8,562,252 issued in 2013.

International patents pending.

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IPTD® is More Sustainable than Off-Site Thermal Desorption or Landfilling

IPTD®:

- Eliminates transportation back and forth to a treatment facility:
  - saves fuel and resources
  - Reduces neighborhood impacts and risk of spreading of hazardous dust/particulates.

- Eliminates long-term management at landfills where the soil/sediment is stored.

- Requires less energy than kiln-style treatment, for which the heat losses are higher.

Questions?

References

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Conley, D.M., and C.M. Lonie. 2000. "Field Scale Implementation of In Situ Thermal Desorption Thermal Well Technology." pp. 175-182. In: G.D. Wickramanayake and A.R. Gavaskar (eds.) *Physical and Thermal Technologies: Remediation of Chlorinated and Recalcitrant Compounds*. Battelle Press, Columbus, OH.

Heron, G., R.S. Baker, J. Galligan, T. Mahoney, G. Anderson, K. Tawara, and H. Braatz. 2010. "In-Pile Thermal Desorption for Treatment of Dioxin-Contaminated Soil in Japan." Paper E-008, in K.A. Fields and G.B. Wickramanayake (Chairs), *Remediation of Chlorinated and Recalcitrant Compounds—2010*. Seventh International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2010). Battelle Memorial Institute, Columbus, OH.

Sorenson, K.S., R.E. Chichakli, P.M. Chenevey, J.G. Montera, T.M. Diep, P.J. McNamee, T.G. Boivin, R.S. Baker, F. Donovan and H. Handler. 2011. "Technology Selection and Conceptual Design for Cleanup of Dioxin Contamination at the Da Nang Airport Hot Spot, Viet Nam." In*: Proceedings of the 31st International Symposium on Halogenated Persistent Organic Pollutants* (Dioxin 2011), Brussels, Belgium, August 21-25, 2011.

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