

Notice: Use this form to request a **written response (on agency letterhead)** from the Department of Natural Resources (DNR) regarding technical assistance, a post-closure change to a site, a specialized agreement or liability clarification for Property with known or suspected environmental contamination. A fee will be required as is authorized by s. 292.55, Wis. Stats., and NR 749, Wis. Adm. Code., unless noted in the instructions below. Personal information collected will be used for administrative purposes and may be provided to requesters to the extent required by Wisconsin's Public Records law [ss. 19.31 - 19.39, Wis. Stats.].

Definitions

"Property" refers to the subject Property that is perceived to have been or has been impacted by the discharge of hazardous substances.

"Liability Clarification" refers to a written determination by the Department provided in response to a request made on this form. The response clarifies whether a person is or may become liable for the environmental contamination of a Property, as provided in s. 292.55, Wis. Stats.

"Technical Assistance" refers to the Department's assistance or comments on the planning and implementation of an environmental investigation or environmental cleanup on a Property in response to a request made on this form as provided in s. 292.55, Wis. Stats.

"Post-closure modification" refers to changes to Property boundaries and/or continuing obligations for Properties or sites that received closure letters for which continuing obligations have been applied or where contamination remains. Many, but not all, of these sites are included on the GIS Registry layer of RR Sites Map to provide public notice of residual contamination and continuing obligations.

Select the Correct Form

This form should be used to request the following from the DNR:

- Technical Assistance
- Liability Clarification
- Post-Closure Modifications
- Specialized Agreements (tax cancellation, negotiated agreements, etc.)

Do not use this form if one of the following applies:

- Request for an **off-site liability exemption or clarification** for Property that has been or is perceived to be contaminated by one or more hazardous substances that originated on another Property containing the source of the contamination. Use DNR's Off-Site Liability Exemption and Liability Clarification Application Form 4400-201.
- Submittal of an Environmental Assessment for the **Lender Liability Exemption**, s 292.21, Wis. Stats., **if no response or review by DNR is requested**. Use the Lender Liability Exemption Environmental Assessment Tracking Form 4400-196.
- Request for an **exemption to develop on a historic fill site** or licensed landfill. Use DNR's Form 4400-226 or 4400-226A.
- **Request for closure** for Property where the investigation and cleanup actions are completed. Use DNR's Case Closure - GIS Registry Form 4400-202.

All forms, publications and additional information are available on the internet at: dnr.wi.gov/topic/Brownfields/Pubs.html.

Instructions

1. Complete sections 1, 2, 6 and 7 for all requests. Be sure to provide adequate and complete information.
2. Select the type of assistance requested: Section 3 for technical assistance or post-closure modifications, Section 4 for a written determination or clarification of environmental liabilities; or Section 5 for a specialized agreement.
3. Include the fee payment that is listed in Section 3, 4, or 5, unless you are a "Voluntary Party" enrolled in the Voluntary Party Liability Exemption Program **and** the questions in Section 2 direct otherwise. Information on to whom and where to send the fee is found in Section 8 of this form.
4. Send the completed request, supporting materials and the fee to the appropriate DNR regional office where the Property is located. See the map on the last page of this form. A paper copy of the signed form and all reports and supporting materials shall be sent with an electronic copy of the form and supporting materials on a compact disk. For electronic document submittal requirements see: <http://dnr.wi.gov/files/PDF/pubs/rr/RR690.pdf>

The time required for DNR's determination varies depending on the complexity of the site, and the clarity and completeness of the request and supporting documentation.

Technical Assistance, Environmental Liability Clarification or Post-Closure Modification Request

Form 4400-237 (R 10/21)

Page 2 of 7

Section 1. Contact and Recipient Information

Requester Information

This is the person requesting technical assistance or a post-closure modification review, that his or her liability be clarified or a specialized agreement and is identified as the requester in Section 7. DNR will address its response letter to this person.

Last Name	First	MI	Organization/ Business Name
Schreiner	Evan		Wauleco, Inc.
Mailing Address			City
18 North Point Drive			Stevens Point
			State
			WI
			ZIP Code
			54481
Phone # (include area code)	Fax # (include area code)	Email	
(715) 346-8530	(715) 346-7842	Evan.Schreiner@Sentry.com	

The requester listed above: (select all that apply)

- Is currently the owner
 Is considering selling the Property
 Is renting or leasing the Property
 Is considering acquiring the Property
 Is a lender with a mortgagee interest in the Property
 Other. Explain the status of the Property with respect to the applicant:

Contact Information (to be contacted with questions about this request)

Select if same as requester

Contact Last Name	First	MI	Organization/ Business Name
Schreiner	Evan		Wauleco, Inc.
Mailing Address			City
18 North Point Drive			Stevens Point
			State
			WI
			ZIP Code
			54481
Phone # (include area code)	Fax # (include area code)	Email	
(715) 346-8530	(715) 346-7842	Evan.Schreiner@Sentry.com	

Environmental Consultant (if applicable)

Contact Last Name	First	MI	Organization/ Business Name
Iverson	Bruce	A	TRC
Mailing Address			City
708 Heartland Trail, Suite 3000			Madison
			State
			WI
			ZIP Code
			53717
Phone # (include area code)	Fax # (include area code)	Email	
(608) 826-3644	(608) 826-3941	biverson@trccompanies.com	

Section 2. Property Information

Property Name	FID No. (if known)
Wauleco, Inc.	737079310
BRRTS No. (if known)	Parcel Identification Number
02-37-000006	291-2907-354-0972
Street Address	City
125 Rosecrans Street	Wausau
County	State
Marathon	WI
Municipality where the Property is located	ZIP Code
<input checked="" type="radio"/> City <input type="radio"/> Town <input type="radio"/> Village of	54402
Property is composed of:	Property Size Acres
<input checked="" type="radio"/> Single tax parcel <input type="radio"/> Multiple tax parcels	8

Technical Assistance, Environmental Liability
Clarification or Post-Closure Modification Request

Form 4400-237 (R 10/21)

Page 3 of 7

1. Is a response needed by a specific date? (e.g., Property closing date) Note: Most requests are completed within 60 days. Please plan accordingly.

- No Yes

Date requested by: _____

Reason: _____

2. Is the "Requester" enrolled as a Voluntary Party in the Voluntary Party Liability Exemption (VPLE) program?

- No. **Include the fee that is required for your request in Section 3, 4 or 5.**
 Yes. **Do not include a separate fee.** This request will be billed separately through the VPLE Program.

Fill out the information in Section 3, 4 or 5 which corresponds with the type of request:
Section 3. Technical Assistance or Post-Closure Modifications;
Section 4. Liability Clarification; or Section 5. Specialized Agreement.

Section 3. Request for Technical Assistance or Post-Closure Modification

Select the type of technical assistance requested: [Numbers in brackets are for WI DNR Use]

- No Further Action Letter (NFA) (Immediate Actions) - NR 708.09, [183] - Include a fee of \$350. Use for a written response to an immediate action after a discharge of a hazardous substance occurs. Generally, these are for a one-time spill event.
- Review of Site Investigation Work Plan - NR 716.09, [135] - **Include a fee of \$700.**
- Review of Site Investigation Report - NR 716.15, [137] - **Include a fee of \$1050.**
- Approval of a Site-Specific Soil Cleanup Standard - NR 720.10 or 12, [67] - **Include a fee of \$1050.**
- Review of a Remedial Action Options Report - NR 722.13, [143] - **Include a fee of \$1050.**
- Review of a Remedial Action Design Report - NR 724.09, [148] - **Include a fee of \$1050.**
- Review of a Remedial Action Documentation Report - NR 724.15, [152] - **Include a fee of \$350**
- Review of a Long-term Monitoring Plan - NR 724.17, [25] - **Include a fee of \$425.**
- Review of an Operation and Maintenance Plan - NR 724.13, [192] - **Include a fee of \$425.**

Other Technical Assistance - s. 292.55, Wis. Stats. [97] (For request to build on an abandoned landfill use Form 4400-226)

- Schedule a Technical Assistance Meeting - **Include a fee of \$700.**
- Hazardous Waste Determination - **Include a fee of \$700.**
- Other Technical Assistance - **Include a fee of \$700.** Explain your request in an attachment.

Post-Closure Modifications - NR 727, [181]

- Post-Closure Modifications: Modification to Property boundaries and/or continuing obligations of a closed site or Property; sites may be on the GIS Registry. This also includes removal of a site or Property from the GIS Registry. **Include a fee of \$1050, and:**
 - Include a fee of \$300 for sites with residual soil contamination; and
 - Include a fee of \$350 for sites with residual groundwater contamination, monitoring wells or for vapor intrusion continuing obligations.

Attach a description of the changes you are proposing, and documentation as to why the changes are needed (if the change to a Property, site or continuing obligation will result in revised maps, maintenance plans or photographs, those documents may be submitted later in the approval process, on a case-by-case basis).

Section 4. Request for Liability Clarification

Select the type of liability clarification requested. Use the available space given or attach information, explanations, or specific questions that you need answered in DNR's reply. Complete Sections 6 and 7 of this form. [Numbers in brackets are for DNR Use]

Technical Assistance, Environmental Liability
Clarification or Post-Closure Modification Request

Form 4400-237 (R 10/21)

Page 4 of 7

"Lender" liability exemption clarification - s. 292.21, Wis. Stats. [686]

❖ **Include a fee of \$700.**

Provide the following documentation:

- (1) ownership status of the real Property, and/or the personal Property and fixtures;
- (2) an environmental assessment, in accordance with s. 292.21, Wis. Stats.;
- (3) the date the environmental assessment was conducted by the lender;
- (4) the date of the Property acquisition; for foreclosure actions, include a copy of the signed and dated court order confirming the sheriff's sale.
- (5) documentation showing how the Property was acquired and the steps followed under the appropriate state statutes.
- (6) a copy of the Property deed with the correct legal description; and,
- (7) the Lender Liability Exemption Environmental Assessment Tracking Form (Form 4400-196).
- (8) If no sampling was done, please provide reasoning as to why it was **not** conducted. Include this either in the accompanying environmental assessment or as an attachment to this form, and cite language in s. 292.21(1)(c)2., h.-i., Wis. Stats.:
 - h. The collection and analysis of representative samples of soil or other materials in the ground that are suspected of being contaminated based on observations made during a visual inspection of the real Property or based on aerial photographs, or other information available to the lender, including stained or discolored soil or other materials in the ground and including soil or materials in the ground in areas with dead or distressed vegetation. The collection and analysis shall identify contaminants in the soil or other materials in the ground and shall quantify concentrations.
 - i. The collection and analysis of representative samples of unknown wastes or potentially hazardous substances found on the real Property and the determination of concentrations of hazardous waste and hazardous substances found in tanks, drums or other containers or in piles or lagoons on the real Property.

"Representative" liability exemption clarification (e.g. trustees, receivers, etc.) - s. 292.21, Wis. Stats. [686]

❖ **Include a fee of \$700.**

Provide the following documentation:

- (1) ownership status of the Property;
- (2) the date of Property acquisition by the representative;
- (3) the means by which the Property was acquired;
- (4) documentation that the representative has no beneficial interest in any entity that owns, possesses, or controls the Property;
- (5) documentation that the representative has not caused any discharge of a hazardous substance on the Property; and
- (6) a copy of the Property deed with the correct legal description.

Clarification of local governmental unit (LGU) liability exemption at sites with: (select all that apply)

- hazardous substances spills - s. 292.11(9)(e), Wis. Stats. [649];
- Perceived environmental contamination - [649];
- hazardous waste - s. 292.24 (2), Wis. Stats. [649]; and/or
- solid waste - s. 292.23 (2), Wis. Stats. [649].

❖ **Include a fee of \$700, a summary of the environmental liability clarification being requested, and the following:**

- (1) clear supporting documentation showing the acquisition method used, and the steps followed under the appropriate state statute(s).
- (2) current and proposed ownership status of the Property;
- (3) date and means by which the Property was acquired by the LGU, where applicable;
- (4) a map and the ¼, ¼ section location of the Property;
- (5) summary of current uses of the Property;
- (6) intended or potential use(s) of the Property;
- (7) descriptions of other investigations that have taken place on the Property; and
- (8) (for solid waste clarifications) a summary of the license history of the facility.

Technical Assistance, Environmental Liability
Clarification or Post-Closure Modification Request

Form 4400-237 (R 10/21)

Page 5 of 7

Section 4. Request for Liability Clarification (cont.)

Lease liability clarification - s. 292.55, Wis. Stats. [646]

❖ **Include a fee of \$700 for a single Property, or \$1400 for multiple Properties and the information listed below:**

- (1) a copy of the proposed lease;
- (2) the name of the current owner of the Property and the person who will lease the Property;
- (3) a description of the lease holder's association with any persons who have possession, control, or caused a discharge of a hazardous substance on the Property;
- (4) map(s) showing the Property location and any suspected or known sources of contamination detected on the Property;
- (5) a description of the intended use of the Property by the lease holder, with reference to the maps to indicate which areas will be used. Explain how the use will not interfere with any future investigation or cleanup at the Property; and
- (6) all reports or investigations (e.g. Phase I and Phase II Environmental Assessments and/or Site Investigation Reports conducted under s. NR 716, Wis. Adm. Code) that identify areas of the Property where a discharge has occurred.

General or other environmental liability clarification - s. 292.55, Wis. Stats. [682] - Explain your request below.

❖ **Include a fee of \$700 and an adequate summary of relevant environmental work to date.**

No Action Required (NAR) - NR 716.05, [682]

❖ **Include a fee of \$700.**

Use where an environmental discharge has or has not occurred, and applicant wants a DNR determination that no further assessment or clean-up work is required. Usually this is requested after a Phase I and Phase II environmental assessment has been conducted; the assessment reports should be submitted with this form. This is not a closure letter.

Clarify the liability associated with a "closed" Property - s. 292.55, Wis. Stats. [682]

❖ **Include a fee of \$700.**

- Include a copy of any closure documents if a state agency other than DNR approved the closure.

Use this space or attach additional sheets to provide necessary information, explanations or specific questions to be answered by the DNR.

Section 5. Request for a Specialized Agreement

Select the type of agreement needed. Include the appropriate draft agreements and supporting materials. Complete Sections 6 and 7 of this form. More information and model draft agreements are available at: dnr.wi.gov/topic/Brownfields/lgu.html#tabx4.

Tax cancellation agreement - s. 75.105(2)(d), Wis. Stats. [654]

❖ **Include a fee of \$700, and the information listed below:**

- (1) Phase I and II Environmental Site Assessment Reports,
- (2) a copy of the Property deed with the correct legal description.

Agreement for assignment of tax foreclosure judgement - s.75.106, Wis. Stats. [666]

❖ **Include a fee of \$700, and the information listed below:**

- (1) Phase I and II Environmental Site Assessment Reports,
- (2) a copy of the Property deed with the correct legal description.

Negotiated agreement - Enforceable contract for non-emergency remediation - s. 292.11(7)(d) and (e), Wis. Stats. [630]

❖ **Include a fee of \$1400, and the information listed below:**

- (1) a draft schedule for remediation; and,
- (2) the name, mailing address, phone and email for each party to the agreement.

Technical Assistance, Environmental Liability
Clarification or Post-Closure Modification Request

Form 4400-237 (R 10/21)

Page 6 of 7

Section 6. Other Information Submitted

Identify all materials that are included with this request.

Send both a paper copy of the signed form and all reports and supporting materials, and an electronic copy of the form and all reports, including Environmental Site Assessment Reports, and supporting materials on a compact disk.

Include one copy of any document from any state agency files that you want the Department to review as part of this request. The person submitting this request is responsible for contacting other state agencies to obtain appropriate reports or information.

Phase I Environmental Site Assessment Report - Date: _____

Phase II Environmental Site Assessment Report - Date: _____

Legal Description of Property (required for all liability requests and specialized agreements)

Map of the Property (required for all liability requests and specialized agreements)

Analytical results of the following sampled media: Select all that apply and include date of collection.

Groundwater Soil Sediment Other medium - Describe: _____

Date of Collection: _____

A copy of the closure letter and submittal materials

Draft tax cancellation agreement

Draft agreement for assignment of tax foreclosure judgment

Other report(s) or information - Describe: Technical Memorandum Bio-Trap Study Results Regarding PCP Degradation

For Property with newly identified discharges of hazardous substances only: Has a notification of a discharge of a hazardous substance been sent to the DNR as required by s. NR 706.05(1)(b), Wis. Adm. Code?

Yes - Date (if known): _____

No

Note: The Notification for Hazardous Substance Discharge Form - Non-Emergency Only (Form 4400-225) is accessible through the RR Program Submittal Portal application. Directions for using the form and the Submittal Portal application are available on the [Submittal Portal web page](#).

Section 7. Certification by the Person who completed this form

I am the person submitting this request (requester)

I prepared this request for: _____

Requester Name

I certify that I am familiar with the information submitted on this request, and that the information on and included with this request is true, accurate and complete to the best of my knowledge. I also certify I have the legal authority and the applicant's permission to make this request.

Evan Schreiner

Signature

Jan 11, 2022

Date Signed

Treasurer

Title

(715) 346-8530

Telephone Number (include area code)

Technical Assistance, Environmental Liability Clarification or Post-Closure Modification Request

Form 4400-237 (R 10/21)

Page 7 of 7

Section 8. DNR Contacts and Addresses for Request Submittals

Send or deliver one paper copy and one electronic copy on a compact disk of the completed request, supporting materials, and fee to the region where the property is located to the address below. Contact a DNR regional brownfields specialist with any questions about this form or a specific situation involving a contaminated property. For electronic document submittal requirements see: <http://dnr.wi.gov/files/PDF/pubs/rr/RR690.pdf>.

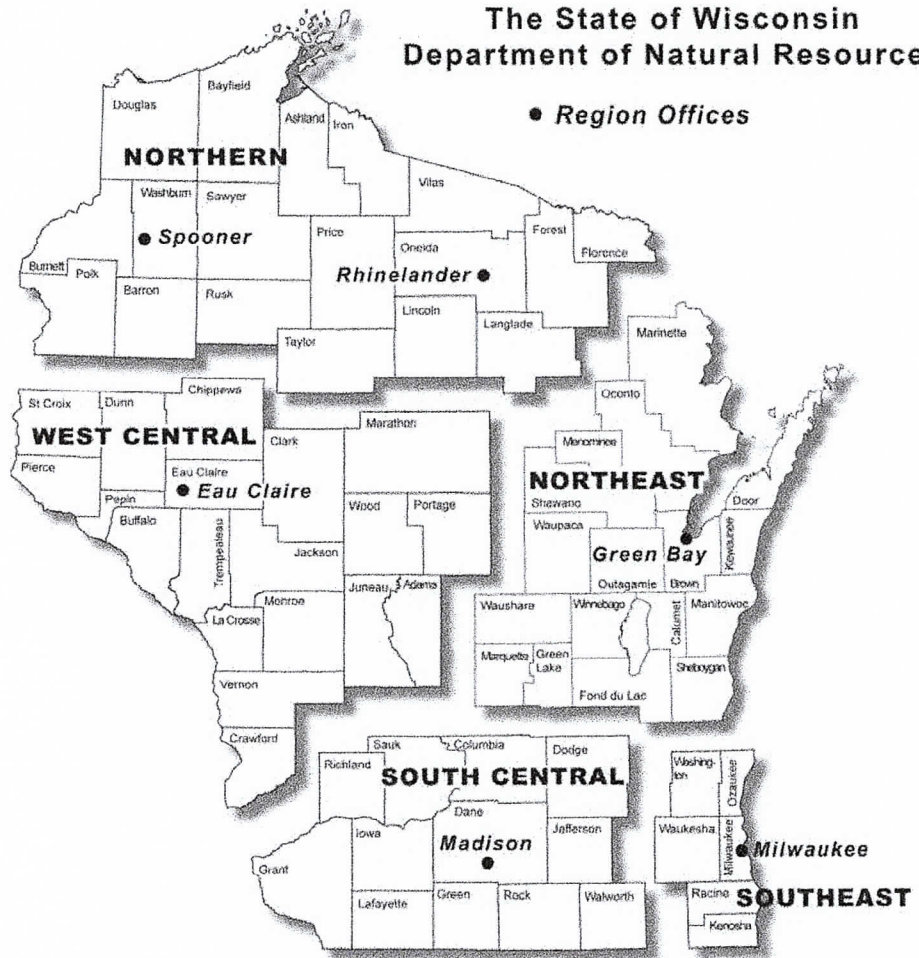
DNR NORTHERN REGION
 Attn: RR Program Assistant
 Department of Natural Resources
 223 E Steinfest Rd Antigo, WI 54409

DNR NORTHEAST REGION
 Attn: RR Program Assistant
 Department of Natural Resources
 2984 Shawano Avenue
 Green Bay WI 54313

DNR SOUTH CENTRAL REGION
 Attn: RR Program Assistant
 Department of Natural Resources
 3911 Fish Hatchery Road
 Fitchburg WI 53711

DNR SOUTHEAST REGION
 Attn: RR Program Assistant
 Milwaukee DNR Office
 1027 West St. Paul Ave
 Milwaukee WI 53233

DNR WEST CENTRAL REGION
 Attn: RR Program Assistant
 Department of Natural Resources
 1300 Clairemont Ave.
 Eau Claire WI 54702



Note: These are the Remediation and Redevelopment Program's designated regions. Other DNR program regional boundaries may be different.

DNR Use Only			
Date Received	Date Assigned	BRRTS Activity Code	BRRTS No. (if used)
DNR Reviewer		Comments	
Fee <input type="radio"/> Yes <input type="radio"/> No	Fee Amount \$	Date Additional Information Requested	Date Requested for DNR Response Letter
Date Approved	Final Determination		

Section 3. Request for Technical Assistance

Other Technical Assistance: Wauleco requests a technical meeting with the DNR to discuss the TRC document titled Technical Memorandum Bio-Trap Study Results Regarding PCP Degradation (Addendum to PCP Degradation Tech Memo, TRC 2020) dated January 2022.



Technical Memorandum Bio-Trap Study Results Regarding PCP Degradation

(Addendum to PCP Degradation Tech
Memo, TRC 2020)

Wauleco, Inc.
Wausau, Wisconsin

January 2022

BRRTS No. 02-37-000006

Prepared For:

Wauleco, Inc.

Prepared By:

TRC

708 Heartland Trail, Suite 3000

Madison, Wisconsin 53717



TABLE OF CONTENTS

1.0	EXECUTIVE SUMMARY	1
2.0	BACKGROUND	3
2.1	Introduction	3
2.2	Purpose and Background	3
2.3	Report Organization	5
3.0	BIO-TRAP STUDY RESULTS.....	6
3.1	Background and Methods	6
3.2	Summary of Results.....	7
3.3	Census Analysis.....	8
4.0	FINDINGS AND CONCLUSIONS	9
4.1	Findings.....	9
4.2	Conclusions.....	9
4.3	WDNR Review Request.....	10
5.0	REFERENCES.....	11

TABLES

Table 1: Bio-Trap Study Analytical Results

FIGURES

Figure 1: Site Location Map

Figure 2: Bio-Trap Sample Locations

Figure 3: PCP Isoconcentration Map (July 2021) and Bio-Trap Results

APPENDICES

Appendix A: Microbial Insights Bio-Trap – Stable Isotope Probing Protocol

Appendix B: Microbial Insights Bio-Trap Laboratory Reports

1.0 Executive Summary

This Technical Memorandum (Tech Memo) summarizes the results of a Bio-Trap Study performed at the Wauleco site located in Wausau, Wisconsin conducted pursuant to the Site Investigation Work Plan for a Bio-Trap Study Field Work (Bio-Trap Work Plan, TRC, December 2020) approved by the Department in a letter dated February 29, 2021 (WDNR 2021). The Tech Memo serves as an addendum to the August 2020 Technical Memorandum, Lines of Evidence of PCP Degradation (2020 PCP Degradation Tech Memo, TRC 2020), and relies, to some extent, on the data and presentations in the 2020 PCP Degradation Tech Memo. Results presented in this Tech Memo conclusively demonstrate that PCP is biodegrading in groundwater within the residual phase light non-aqueous phase liquid (LNAPL) footprint, through the central portion of the groundwater plume, and in downgradient groundwater.

U.S. EPA's Natural Attenuation guidance (EPA, 1998) described three lines of evidence to demonstrate whether contaminant degradation is occurring. The 2020 PCP Degradation Tech Memo (TRC, 2020) provided a detailed summary of the literature demonstrating that PCP naturally biodegrades and demonstrated satisfaction of EPA's lines of evidence #1 and #2¹.

U.S. EPA's guidance (EPA, 1998) line of evidence #3 is a field or microcosm study to directly demonstrate that biodegradation is occurring. WDNR's October 30, 2020 letter agreed that PCP can be naturally degraded and that PCP concentrations have decreased across much of the site. However, WDNR requested/stated that "additional field studies are necessary to support the conclusion that biodegradation is the primary natural attenuation mechanism for the central portion of the groundwater plume." Therefore, this Bio-Trap Study was completed to further evaluate whether biodegradation of PCP is occurring throughout the Wauleco Site, including through the central portion of the groundwater plume.

The Bio-Trap Study used PCP labeled with carbon 13 (¹³C), a stable carbon isotope found in nature at low concentrations, to demonstrate whether biodegradation was occurring. Bio-Traps baited with ¹³C labeled PCP were placed in select wells for 90 days after which the laboratory analyzed for ¹³C in bacterial biomass and dissolved inorganic carbon. The literature, included in the laboratory report by Microbial Insights (Appendix B), demonstrates that if the radio-labeled carbon present in the PCP is detected in the bacterial biomass or the dissolved inorganic carbon, then PCP is being biodegraded.

Analytical results (see Table 1) showed that:

- ¹³C was detected in all eight sample locations, both within the residual phase LNAPL footprint, through the central portion of the groundwater plume, and in downgradient wells (see Figure 3). Seven of the eight samples had high concentrations of ¹³C in the biomass, as those results are characterized by Microbial Insights.

¹ These lines of evidence are:

1. that a clear and meaningful trend of decreasing contaminant mass and/or concentration is occurring; and
2. that natural attenuation processes are active at the site at a rate which such processes will reduce contaminant concentrations.

- In addition, all eight samples showed the presence of ^{13}C in dissolved inorganic carbon, demonstrating that the PCP had been completely mineralized to carbon dioxide. Three were characterized as having high concentrations of ^{13}C , two moderate, and two low concentrations. Two of the three high concentration samples were from within the residual phase LNAPL footprint and one was in a downgradient location.

These Bio-Trap results conclusively demonstrate that biodegradation of PCP is occurring in groundwater throughout the Wauleco Site (i.e., within the residual phase LNAPL footprint, through the central portion of the groundwater plume, and in downgradient groundwater).

The moderate to high levels of biological degradation of PCP shown by the Bio-Trap Study, in combination with the concentration-distance analysis of biodegradation and dispersion in the 2020 PCP Degradation Tech Memo, demonstrates that this biodegradation is the primary natural attenuation mechanism occurring at the Site.

2.0 Background

2.1 Introduction

This Tech Memo is prepared as an addendum to the August 2020 Technical Memorandum, Lines of Evidence of PCP Degradation (2020 PCP Degradation Tech Memo, TRC 2020), and relies, to some extent, on the data and presentations in the 2020 PCP Degradation Tech Memo.

The Wauleco, Inc. (Wauleco), facility is located at 125 Rosecrans Street, Wausau, Wisconsin (Site; see Figure 1). The property is located in an area of mixed industrial and residential land use. The property is the location of a former window and patio door manufacturer from the early 1900s to the early 1990s. Manufacturing operations ceased in March 1991 and nearly all Site buildings were demolished by 1993.

As was common in the wood window manufacturing industry, surface coating on the exterior portions of wood windows manufactured at the site was performed using a wood preservative trade named Woodtox Preprime, manufactured by Kopper Chemical and Coating Company. Woodtox Preprime, commonly referred to as Penta, was a 5% solution of pentachlorophenol (PCP) dissolved in 85% mineral spirits, and 10% inerts. Penta was used at the Site from approximately 1944 until 1986.

2.2 Purpose and Background

The 2020 PCP Degradation Tech Memo presented information to demonstrate PCP biodegradation was occurring at the Wauleco Site, using the lines of evidence required by U.S. EPA's Natural Attenuation guidance (EPA, 1998). The data included:

- A comprehensive Conceptual Site Model (CSM) describing the Wauleco Site conditions, including the Light Non-Aqueous Phase Liquid (LNAPL) and groundwater.
- A comprehensive literature review demonstrating that PCP does biodegrade in both aerobic and anaerobic environments in soil and groundwater.
- A demonstration that PCP is biodegrading at the Wauleco Site using U.S. EPA's lines of evidence #1 and #2, as follows:
 - **Line of Evidence #1:** Data and analysis to satisfy EPA's first line of evidence for natural attenuation, by demonstrating a clear and meaningful trend of decreasing contaminant mass and/or concentration. This was demonstrated with both time-concentration and distance-concentration observations at Wauleco.
 - **Line of Evidence #2:** Data and analysis to satisfy U.S. EPA's second line of evidence for natural attenuation, through hydrogeologic and geochemical data that were used to demonstrate indirectly the types of natural attenuation processes active at the Site and the rate at which such processes will reduce contaminant concentrations.

U.S. EPA's guidance states that where lines of evidence 1 and 2 are inadequate or inconclusive then data from a field or microcosm study may be necessary.

A technical review of the 2020 PCP Degradation Tech Memo by the Wisconsin Department of Natural Resources (WDNR) was requested. The WDNR's technical review was presented in a WDNR letter dated October 30, 2020 (WDNR 2020) that stated in part:

- “Based on review of the technical memorandum, the Department agrees that PCP can be degraded naturally and that PCP concentrations in groundwater across much of site have decreased over time.”
- “The Department conceptually agrees with the findings and conclusions of this technical memorandum, however; additional field studies are necessary to support the conclusion that biodegradation is the primary natural attenuation mechanism for the central portion of the groundwater plume.”

To demonstrate whether PCP is degrading both outside and within the LNAPL footprint, TRC prepared the Site Investigation Work Plan for a Bio-Trap Study Field Work (Bio-Trap Work Plan, TRC, December 2020). As described in the Bio-Trap Work Plan, the purposes of this study were to:

- Respond to the WDNR's October 30, 2020 request for additional field studies.
- Determine whether bacteria capable of degrading PCP are present at the Wauleco site.
- Gather additional data to demonstrate that biodegradation of PCP is occurring within the central portion of the PCP groundwater plume. This data will be used to demonstrate whether PCP degradation is occurring throughout the PCP groundwater plume as well as beneath the area where residual phase LNAPL is present.
- Fulfill Wauleco's commitment in the 2020 PCP Degradation Tech Memo (refer to Section 1) to perform a study to assess the presence of necessary bacteria.

The Department approved of the Bio-Trap Work Plan sampling and analysis strategy in its letter dated February 29, 2021 (WDNR 2021).

As described in the Bio-Trap Study Work Plan, the study consisted of deploying bio-beads from Microbial Insights into eight wells across the residual phase LNAPL footprint and the downgradient PCP plume. The eight wells selected were presented in the Work Plan and approved by the Department. These bio-beads were spiked with PCP that contains the stable carbon isotope, carbon 13 (^{13}C). Following an approximate 90-day deployment period, the Bio-Traps were recovered to quantify whether ^{13}C contained in the PCP was incorporated into biomass and/or dissolved inorganic carbon (DIC).

When used as a carbon source, contaminant carbon is incorporated into biomolecules such as phospholipids, DNA, and proteins supporting growth of new cells (biomass). When used as an energy source, contaminant carbon is oxidized to carbon dioxide (CO_2) as part of cellular metabolism. Thus, detection of the ^{13}C “label” in the end products of biodegradation (bacterial biomass and CO_2) at the end of the study provides conclusive evidence of contaminant biodegradation. Detection of ^{13}C enriched dissolved inorganic carbon (DIC) which includes $^{13}\text{CO}_2$ conclusively demonstrates contaminant biodegradation and mineralization. Example peer reviewed publications describing these techniques, referred to as stable isotope probing, include Geyer, et. al., 2005, and Madsen, 2006, as well as numerous others. Analyses for ^{13}C in the

biomass and in DIC were conducted by Microbial Insights on the eight Bio-Traps deployed at Wauleco.

2.3 Report Organization

This report is organized as follows:

- **Bio-Trap Study Results** – Section 3
- **Findings and Conclusions** – Section 4
- **References** – Section 5

3.0 Bio-Trap Study Results

3.1 Background and Methods

The Bio-Trap study consists of:

1. A group of “bio-beads” that were amended by Microbial Insights with ^{13}C labeled PCP were delivered to the Wauleco Site. These bio-beads were then installed in the eight wells described in the Bio-Trap Work Plan (refer to Figure 2) on July 14, 2021 and recovered on October 12, 2021, for a deployment of approximately 90 days. Methods used for storage, deployment, recovery and shipping followed Microbial Insight’s Bio-Trap-Stable Isotope Probing Protocol (See Appendix A). During this time, groundwater flow through the bio-beads provides the conditions for bacterial growth on the beads, much like in the aquifer. The bacteria present in the aquifer seed the bio-beads, with subsequent growth in the bio-beads. The bio-beads are designed to retain the bacteria for subsequent analysis.
2. The bio-beads were then removed from the wells and analyzed for several properties. These properties, and a short description of their significance are presented below. Microbial Insights’ report provides much more detail on these properties and the literature references supporting the interpretation of these results. Analytical data were provided for:
 - **Total Biomass (cells/bead)** – This is a measure of the number of bacteria per bead and reflects the amount of biological activity in the aquifer. Microbial Insights has conducted hundreds of these studies and developed ranges of low, medium, and high biomass. These ranges are shown in the summary results on Table 1 as low (red), medium (yellow), and high (green), consistent with Microbial Insights’ report (see Appendix B).
 - **^{13}C enriched Biomass (cells/bead)** – This is a measure of the number of bacteria per bead that have incorporated ^{13}C originating from the ^{13}C labeled PCP present on the beads. As stated by Microbial Insights, the presence of ^{13}C within the biomass conclusively demonstrates whether degradation of PCP is occurring. Microbial Insights states that in their experience the ^{13}C presence is typically several orders of magnitude less than total biomass. Several orders of magnitude would be 0.1% of the total biomass containing ^{13}C . Therefore, over 1% of the total biomass is categorized by Microbial Insights as high, and are highlighted in green, on Table 1.
 - **Average and Maximum Enriched PLFA Delta (‰)** – These are measures of ^{13}C originating from the ^{13}C labeled PCP that ended up as a carbon molecule making up a phospholipid fatty acid (PLFA) in a bacterial body on the bio-beads. PLFA are a primary component of the bacterial cells, so that the PLFA Delta values greater than background conclusively demonstrates degradation of the ^{13}C labeled PCP. Microbial Insights’ experience shows ranges of high, medium, and low values for these analyses. These ranges are also color coded on Table 1.

- **Dissolved Inorganic Carbon (DIC) Delta (‰)** – This is a measure of the amount of ¹³C that has been mineralized (i.e., degradation of the PCP by the bacteria while using the PCP, or one of its degradation products, as a source of energy). DIC Delta (‰) naturally is in the range of -25‰ to -10‰. Microbial Insights states that ¹³C enriched DIC provides conclusive evidence of contaminant biodegradation even at low levels of DIC Delta values. Microbial Insights provides ranges of DIC Delta for low, moderate, and high amounts of biodegradation. These ranges are color coded on Table 1.

The Bio-Traps were deployed in eight locations across the area to satisfy the plan’s objectives of determining whether bacterial activity and PCP degradation are occurring at the Wauleco site, including within the central portion of the residual phase LNAPL footprint and throughout the PCP groundwater plume. As such, the locations for the eight Bio-Traps included:

Central Area of the Residual Phase LNAPL footprint:

- **W6R** – located within the residual phase LNAPL footprint, near the upgradient side, in a well that historically had mobile LNAPL;
- **W3A, W22, and W44** – Located in wells within the central portion of the residual phase LNAPL footprint. Downgradient of the former source area, but within the residual phase LNAPL footprint;
- **W10A** – Located in the furthest downgradient portion of the residual phase LNAPL footprint.

Northern Portion of the Residual Phase LNAPL footprint:

- **W2** – Located within the residual phase LNAPL footprint, on the north side, in a well that historically has had mobile phase LNAPL.

Southern Area of the Residual Phase LNAPL footprint:

- **W41** – Located adjacent to the southern portion of the residual phase LNAPL footprint, in a well that the WDNR has referred to as having a “recalcitrant” PCP concentration.
- **W11** – Located downgradient of the southern portion of the residual phase LNAPL footprint, in an area where PCP is shown to be degrading rapidly along the southeast concentration-distance profile (see discussion in the 2020 PCP Degradation Tech Memo).

3.2 Summary of Results

Microbial Insights laboratory report of the Bio-Trap results is included in Appendix B. Table 1 and Figure 3 summarize the results for the Bio-Trap analyses. Figure 3 illustrates the results for each Bio-Trap location, along with the extent of residual phase LNAPL.

As shown on Table 1 and Figure 3, all eight of the Bio-Trap locations conclusively demonstrated the degradation of PCP. In addition, all five Bio-Trap locations within the residual phase LNAPL footprint show high to medium levels of PCP degradation. The results show:

- Moderate levels of total biomass in all eight locations, indicating that there are moderate levels of bacterial activity present within the residual phase LNAPL footprint and in downgradient locations.
- All eight locations show the presence of enriched ^{13}C in the biomass and enriched in the PFLA. Microbial Insights and their list of peer reviewed journal articles state that this conclusively demonstrates that PCP is degrading within the residual phase LNAPL footprint and in downgradient locations.
- The presence of ^{13}C in mineralized forms of carbon (i.e., in dissolved inorganic carbon) conclusively demonstrates that PCP is being degraded while being used as an energy source for bacterial growth. As shown in Table 1, ^{13}C was detected at above background concentrations in all eight samples, and at moderate to high concentrations in five of the eight samples. Microbial Insights and their list of peer reviewed journal articles state that this conclusively demonstrates that PCP is degrading and is being mineralized to CO_2 within the residual phase LNAPL footprint and in downgradient locations.
- The ^{13}C was detected in dissolved inorganic carbon at high concentrations at locations W6R and W22 within the central portion of the residual phase LNAPL footprint and at location W11, downgradient of the southeast portion of the residual phase LNAPL footprint.
- Detection of ^{13}C in mineralized forms of carbon is a difficult form of ^{13}C to capture in the bio-beads because mineralized carbon occurs as gaseous carbon dioxide, which can be lost prior to being captured in the bio-beads or before analysis. Therefore, the amount of mineralization of PCP may be underestimated.

3.3 Census Analysis

Microbial Insights provided analysis to identify whether there were bacteria present that at least one research paper indicated as one set of bacteria that can biodegrade PCP. Analyses presented in Appendix B for the dechlorinating bacteria and the functional genes indicated low presence of these bacteria and genes. The conclusion from this analysis, in comparison with the Bio-Trap study is that the group of bacteria and genes targeted by these analyses are not the bacteria and genes that are the source of the conclusive demonstration of PCP biodegradation.

4.0 Findings and Conclusions

4.1 Findings

Key findings presented in this Technical Memorandum include the following:

- The Bio-Trap Study analytical results conclusively demonstrate that biodegradation of PCP is occurring in groundwater throughout the Wauleco Site (i.e., within the residual phase LNAPL footprint, through the central portion of the groundwater plume, and in downgradient groundwater).
- This demonstration that PCP is biodegrading is shown by the transfer of the stable isotope carbon 13 (^{13}C) that was built into the PCP that was amended to the bio-beads was transferred to biomass, as shown by ^{13}C being present in the biomass in all eight locations tested. In addition, the ^{13}C presence in dissolved inorganic carbon further demonstrates that the PCP, or its degradation products, are being used by the bacteria as an energy source.
- The comparison of the strength of the source of PCP to groundwater to the rate of PCP biodegradation qualitatively explains the changes in PCP in groundwater along each of the concentration-distance profiles. For example:
 - Where there is no overlying residual phase LNAPL, acting as a source of PCP to groundwater, the biodegradation rate results in decreasing PCP concentrations as groundwater migrates downgradient (e.g., near W11 along the Southeast Profile).
 - Where there is an overlying residual phase LNAPL, a strong source of PCP to groundwater can exceed the biodegradation rate, resulting in increases in PCP concentrations in groundwater, but a moderate to weak source of PCP to groundwater can be less than the biodegradation rate resulting in decreases in PCP concentrations in groundwater.
- The Centerline Profile has shown a change in the concentration profile from pre-2018 PCP trends (i.e., increasing PCP concentration between wells W03A and W10A) to progressively larger decreases in PCP concentration from 2019 to 2021.

4.2 Conclusions

The conclusions from this analysis are:

- Biodegradation of PCP is conclusively demonstrated throughout the Site through this Bio-Trap Study, particularly by detecting the radio-labeled ^{13}C in the PCP placed within the Bio-Traps showing up in the total biomass, the PLFA (i.e., bacterial biomass), and in some cases, within the dissolved inorganic carbon.
- This biodegradation of PCP was demonstrated throughout the Site, both within the residual phase LNAPL footprint and in downgradient areas.

This Bio-Trap Study responds to the WDNR's request in its October 30, 2020 review of the 2020 PCP Degradation Tech Memo, in particular, that PCP biodegradation not only can occur, but is conclusively demonstrated to be occurring throughout the Site and is the primary natural attenuation mechanism occurring that is resulting in the decline of PCP concentrations across much of the Site.

4.3 WDNR Review Request

Wauleco requests a technical meeting with WDNR to discuss the details of the Tech Memo.

5.0 References

- Geyer, R. et. al. 2005. *In Site Assessment of Biodegradation Potential Using Biotraps Amended with ¹³C-Labeled Benzene or Toluene*. Environmental Science and Technology, 2005, 39, 4983-4989.
- Madsen, E. 2006. *The Use of Stable Isotope Probing Techniques in Bioreactor and Field Studies on Bioremediation*. Current Opinion in Biotechnology. 2006, 17:92-97.
- TRC. 2020. Technical Memorandum Lines of Evidence of PCP Degradation. August 2020.
- TRC. 2019. Technical Memorandum Residual Phase LNAPL Investigation. December 2019.
- TRC. 2020. Bio-Trap Site Investigation Work Plan. December 2020.
- U.S. EPA. 1998. Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. EPA/600/R-98/128.
- WDNR. 2020. Department Review of Technical Memorandum Lines of Evidence of PCP Degradation Wauleco, Inc., 125 Rosecrans Street, Wausau, DNR BRRTS #02-37-000006. October 30, 2020.
- WDNR. 2021. Department Review of Bio-Trap Site Investigation Work Plan. February 29, 2021.

Table 1
Bio-Trap Study Analytical Results
Wauleco Project Site
125 Rosecrans Street
Wausau, Wisconsin

Sample Name	Well W2	Well W3A	Well W6R	Well W10A	Well W11	Well W22	Well W41	Well W44
Biomass & ¹³C Incorporation								
Total Biomass (Cells/bead)	3.91E+05	5.95E+05	2.29E+05	9.01E+05	6.37E+05	1.25E+06	7.31E+05	1.10E+06
¹³ C Enriched Biomass (Cells/bead)	1.12E+04	2.46E+04	1.76E+04	8.27E+03	7.26E+04	8.15E+04	1.09E+04	1.37E+04
¹³ C Enriched Biomass as Percent of Total Biomass	2.9%	4.1%	7.7%	0.9%	11.4%	6.5%	1.5%	1.2%
Average Enriched PLFA Delta (‰)	1240	1312	7048	72	11680	8630	405	515
Maximum Enriched PLFA Delta (‰)	4740	6906	10027	256	20627	28360	1291	4711
¹³C Mineralization								
DIC Delta (‰)	-2	-3	3456	-8	7474	6803	2	4

Community Structure (% total PLFA)

Firmicutes (TerBrSats)	0.00	5.96	0.00	2.29	6.07	3.58	12.11	1.67
Proteobacteria (Monos)	76.05	73.78	80.83	67.89	67.76	70.27	65.23	68.73
Anaerobic metal reducers (BrMonos)	0.00	0.00	0.00	5.49	6.45	1.70	0.00	0.55
Actinomycetes (MidBrSats)	0.00	0.00	0.00	0.00	2.88	4.96	0.00	0.00
General (Nsats)	23.95	20.28	19.17	23.11	16.84	18.45	20.60	22.10
Eukaryotes (Polyenoics)	0.00	0.00	0.00	1.21	0.00	1.03	2.06	6.94

Physiological Status (Proteobacteria only)

Slowed Growth	0.28	0.48	0.00	0.30	0.32	0.44	0.74	0.52
Decreased Permeability	0.00	0.13	0.00	0.36	0.00	0.06	0.00	0.05

Total Biomass Values (cells/bead)	Low	<1e5
	Moderate	1e5 to 9e6
	High	>1e7

Dissolved Inorganic Carbon (DIC) Delta (‰)	Background	-30 to -20
	Low	> -20 to 100
	Moderate	100-1000
	High	>1,000

Average and Maximum Enriched PLFA Delta (‰)	Low	0-100
	Moderate	100-1,000
	High	>1,000

¹³C is the stable isotope carbon 13 that was added to the biobeads as ¹³C-labeled PCP.

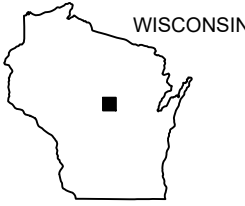
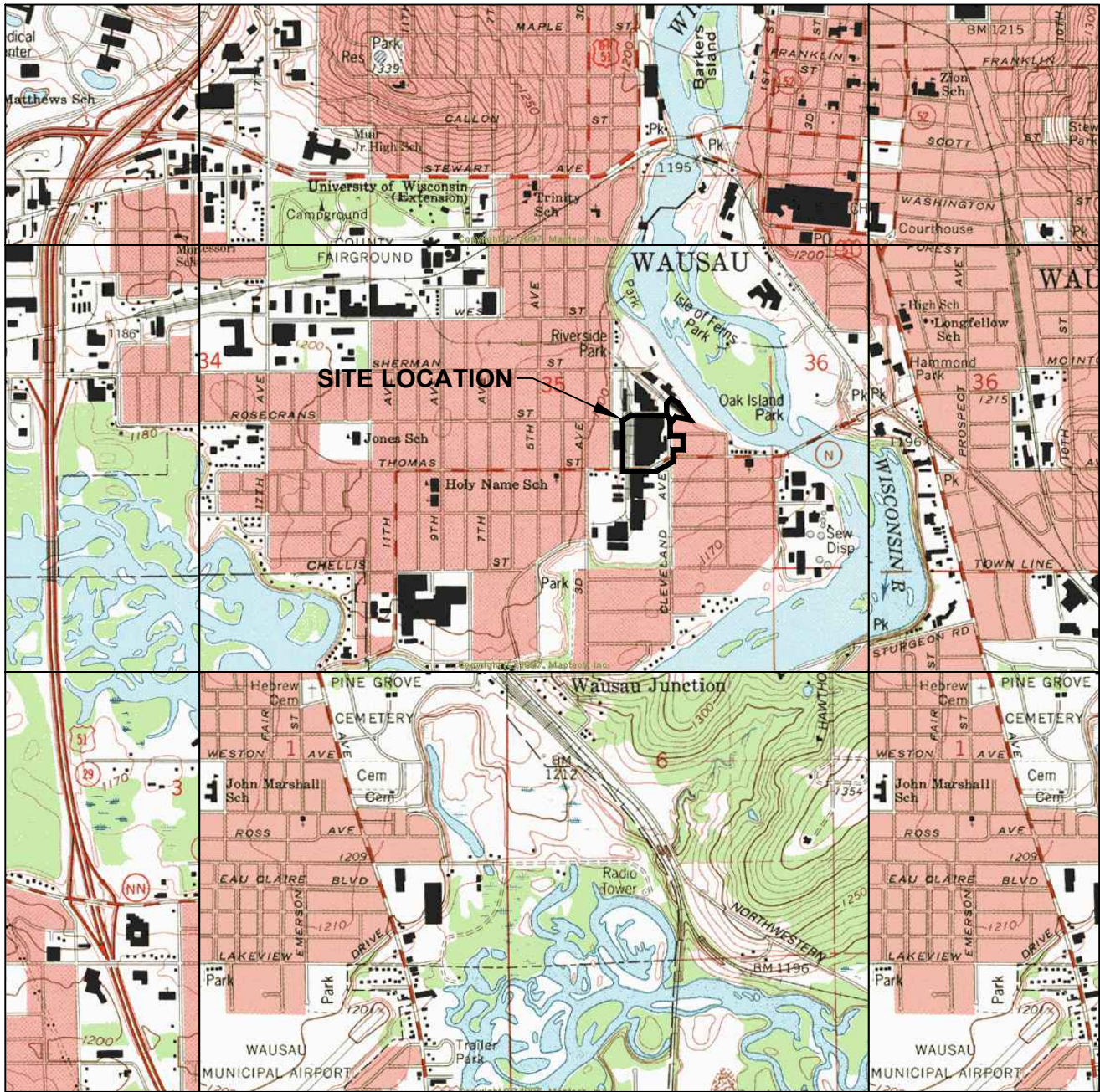
PLFA - are phospholipid fatty acids. PLFA are a primary component of the membrane of bacterial cells.

DIC - is dissolved inorganic carbon, and would consist of carbon dioxide, carbonate, and bicarbonate dissolved in groundwater.

Enriched PLFA Delta (‰) is a measure of the ratio of ¹³C to ¹²C (the typical stable carbon isotope). Increases in the Delta value indicate a source of ¹³C (i.e., from the ¹³C-labeled PCP).

Prepared by K. Quinn, 12/9/2021

Checked by S. Sellwood, 12/10/2021



NOTE
 BASE MAP DEVELOPED FROM THE WAUSAU WEST AND WAUSAU EAST, WISCONSIN 7.5 MINUTE U.S.G.S. TOPOGRAPHIC QUADRANGLE MAPS, DATED 1993. PART OF SECTION 35, T29N, R8E

QUADRANGLE LOCATION



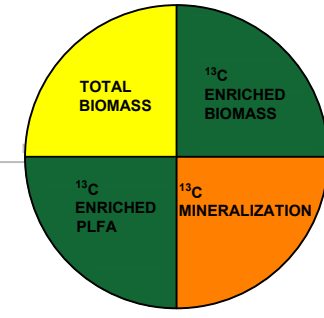
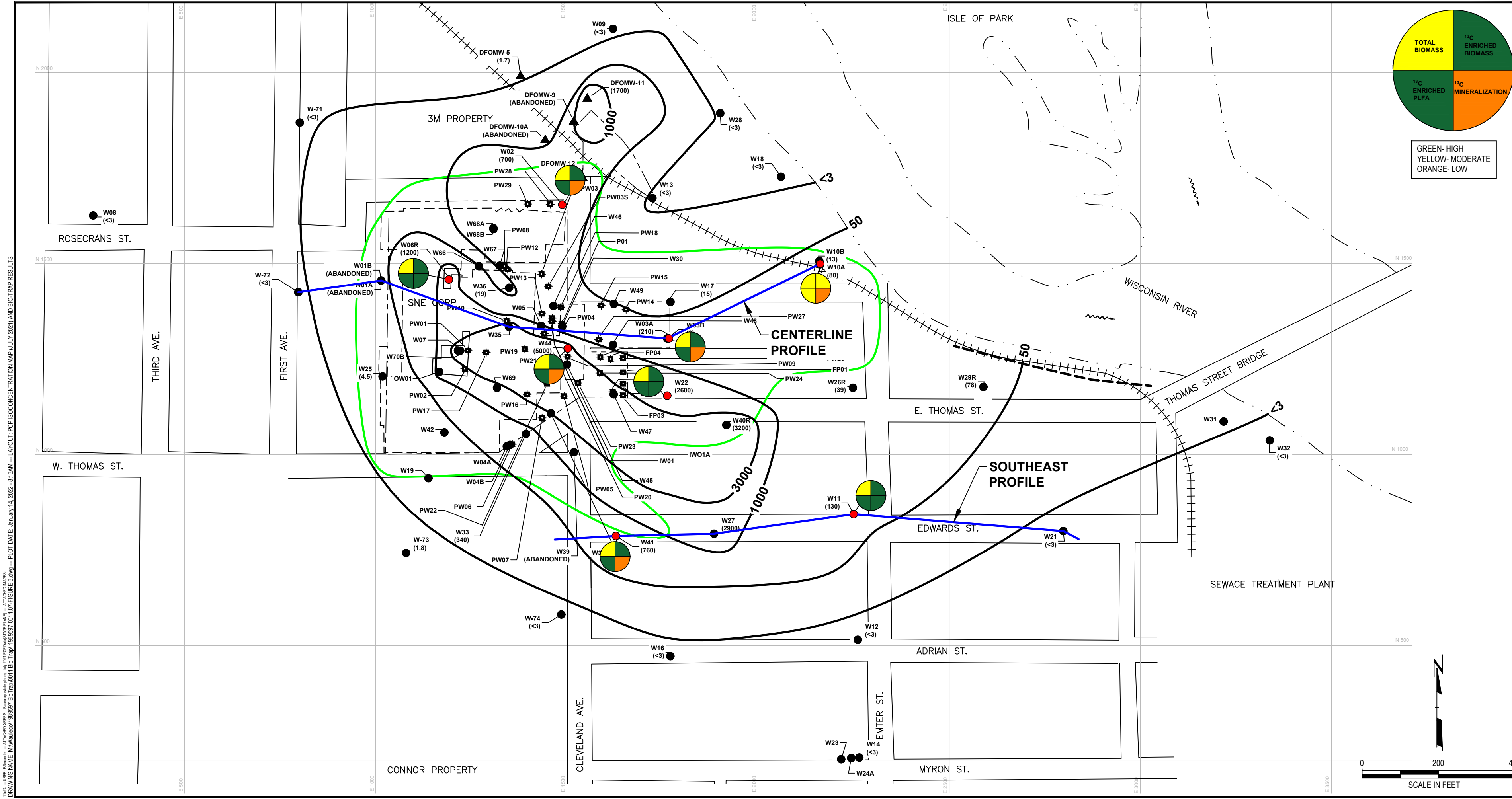
708 Heartland Trail
 Suite 3000
 Madison, WI 53717
 Phone: 608.826.3600

PROJECT:	WAULECO, INC. WAUSAU, WISCONSIN
TITLE:	SITE LOCATION MAP

DRAWN BY:	T.FIEBRANZ
CHECKED BY:	K. QUINN
APPROVED BY:	B. IVERSON
DATE:	JANUARY 2022
PROJ. NO.:	1989597.0011
FILE:	1989597.0011.01 SLM.dwg

FIGURE 1

8.541 -- USER: E:\awm\dwg -- ATTACHED XREFS: -- ATTACHED IMAGES: 06C, 06N, 06S, 06W, 06E, 06F, 06G, 06H, 06I, 06J, 06K, 06L, 06M, 06N, 06O, 06P, 06Q, 06R, 06S, 06T, 06U, 06V, 06W, 06X, 06Y, 06Z, 07A, 07B, 07C, 07D, 07E, 07F, 07G, 07H, 07I, 07J, 07K, 07L, 07M, 07N, 07O, 07P, 07Q, 07R, 07S, 07T, 07U, 07V, 07W, 07X, 07Y, 07Z, 08A, 08B, 08C, 08D, 08E, 08F, 08G, 08H, 08I, 08J, 08K, 08L, 08M, 08N, 08O, 08P, 08Q, 08R, 08S, 08T, 08U, 08V, 08W, 08X, 08Y, 08Z, 09A, 09B, 09C, 09D, 09E, 09F, 09G, 09H, 09I, 09J, 09K, 09L, 09M, 09N, 09O, 09P, 09Q, 09R, 09S, 09T, 09U, 09V, 09W, 09X, 09Y, 09Z, 10A, 10B, 10C, 10D, 10E, 10F, 10G, 10H, 10I, 10J, 10K, 10L, 10M, 10N, 10O, 10P, 10Q, 10R, 10S, 10T, 10U, 10V, 10W, 10X, 10Y, 10Z, 11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H, 11I, 11J, 11K, 11L, 11M, 11N, 11O, 11P, 11Q, 11R, 11S, 11T, 11U, 11V, 11W, 11X, 11Y, 11Z, 12A, 12B, 12C, 12D, 12E, 12F, 12G, 12H, 12I, 12J, 12K, 12L, 12M, 12N, 12O, 12P, 12Q, 12R, 12S, 12T, 12U, 12V, 12W, 12X, 12Y, 12Z, 13A, 13B, 13C, 13D, 13E, 13F, 13G, 13H, 13I, 13J, 13K, 13L, 13M, 13N, 13O, 13P, 13Q, 13R, 13S, 13T, 13U, 13V, 13W, 13X, 13Y, 13Z, 14A, 14B, 14C, 14D, 14E, 14F, 14G, 14H, 14I, 14J, 14K, 14L, 14M, 14N, 14O, 14P, 14Q, 14R, 14S, 14T, 14U, 14V, 14W, 14X, 14Y, 14Z, 15A, 15B, 15C, 15D, 15E, 15F, 15G, 15H, 15I, 15J, 15K, 15L, 15M, 15N, 15O, 15P, 15Q, 15R, 15S, 15T, 15U, 15V, 15W, 15X, 15Y, 15Z, 16A, 16B, 16C, 16D, 16E, 16F, 16G, 16H, 16I, 16J, 16K, 16L, 16M, 16N, 16O, 16P, 16Q, 16R, 16S, 16T, 16U, 16V, 16W, 16X, 16Y, 16Z, 17A, 17B, 17C, 17D, 17E, 17F, 17G, 17H, 17I, 17J, 17K, 17L, 17M, 17N, 17O, 17P, 17Q, 17R, 17S, 17T, 17U, 17V, 17W, 17X, 17Y, 17Z, 18A, 18B, 18C, 18D, 18E, 18F, 18G, 18H, 18I, 18J, 18K, 18L, 18M, 18N, 18O, 18P, 18Q, 18R, 18S, 18T, 18U, 18V, 18W, 18X, 18Y, 18Z, 19A, 19B, 19C, 19D, 19E, 19F, 19G, 19H, 19I, 19J, 19K, 19L, 19M, 19N, 19O, 19P, 19Q, 19R, 19S, 19T, 19U, 19V, 19W, 19X, 19Y, 19Z, 20A, 20B, 20C, 20D, 20E, 20F, 20G, 20H, 20I, 20J, 20K, 20L, 20M, 20N, 20O, 20P, 20Q, 20R, 20S, 20T, 20U, 20V, 20W, 20X, 20Y, 20Z, 21A, 21B, 21C, 21D, 21E, 21F, 21G, 21H, 21I, 21J, 21K, 21L, 21M, 21N, 21O, 21P, 21Q, 21R, 21S, 21T, 21U, 21V, 21W, 21X, 21Y, 21Z, 22A, 22B, 22C, 22D, 22E, 22F, 22G, 22H, 22I, 22J, 22K, 22L, 22M, 22N, 22O, 22P, 22Q, 22R, 22S, 22T, 22U, 22V, 22W, 22X, 22Y, 22Z, 23A, 23B, 23C, 23D, 23E, 23F, 23G, 23H, 23I, 23J, 23K, 23L, 23M, 23N, 23O, 23P, 23Q, 23R, 23S, 23T, 23U, 23V, 23W, 23X, 23Y, 23Z, 24A, 24B, 24C, 24D, 24E, 24F, 24G, 24H, 24I, 24J, 24K, 24L, 24M, 24N, 24O, 24P, 24Q, 24R, 24S, 24T, 24U, 24V, 24W, 24X, 24Y, 24Z, 25A, 25B, 25C, 25D, 25E, 25F, 25G, 25H, 25I, 25J, 25K, 25L, 25M, 25N, 25O, 25P, 25Q, 25R, 25S, 25T, 25U, 25V, 25W, 25X, 25Y, 25Z, 26A, 26B, 26C, 26D, 26E, 26F, 26G, 26H, 26I, 26J, 26K, 26L, 26M, 26N, 26O, 26P, 26Q, 26R, 26S, 26T, 26U, 26V, 26W, 26X, 26Y, 26Z, 27A, 27B, 27C, 27D, 27E, 27F, 27G, 27H, 27I, 27J, 27K, 27L, 27M, 27N, 27O, 27P, 27Q, 27R, 27S, 27T, 27U, 27V, 27W, 27X, 27Y, 27Z, 28A, 28B, 28C, 28D, 28E, 28F, 28G, 28H, 28I, 28J, 28K, 28L, 28M, 28N, 28O, 28P, 28Q, 28R, 28S, 28T, 28U, 28V, 28W, 28X, 28Y, 28Z, 29A, 29B, 29C, 29D, 29E, 29F, 29G, 29H, 29I, 29J, 29K, 29L, 29M, 29N, 29O, 29P, 29Q, 29R, 29S, 29T, 29U, 29V, 29W, 29X, 29Y, 29Z, 30A, 30B, 30C, 30D, 30E, 30F, 30G, 30H, 30I, 30J, 30K, 30L, 30M, 30N, 30O, 30P, 30Q, 30R, 30S, 30T, 30U, 30V, 30W, 30X, 30Y, 30Z, 31A, 31B, 31C, 31D, 31E, 31F, 31G, 31H, 31I, 31J, 31K, 31L, 31M, 31N, 31O, 31P, 31Q, 31R, 31S, 31T, 31U, 31V, 31W, 31X, 31Y, 31Z, 32A, 32B, 32C, 32D, 32E, 32F, 32G, 32H, 32I, 32J, 32K, 32L, 32M, 32N, 32O, 32P, 32Q, 32R, 32S, 32T, 32U, 32V, 32W, 32X, 32Y, 32Z, 33A, 33B, 33C, 33D, 33E, 33F, 33G, 33H, 33I, 33J, 33K, 33L, 33M, 33N, 33O, 33P, 33Q, 33R, 33S, 33T, 33U, 33V, 33W, 33X, 33Y, 33Z, 34A, 34B, 34C, 34D, 34E, 34F, 34G, 34H, 34I, 34J, 34K, 34L, 34M, 34N, 34O, 34P, 34Q, 34R, 34S, 34T, 34U, 34V, 34W, 34X, 34Y, 34Z, 35A, 35B, 35C, 35D, 35E, 35F, 35G, 35H, 35I, 35J, 35K, 35L, 35M, 35N, 35O, 35P, 35Q, 35R, 35S, 35T, 35U, 35V, 35W, 35X, 35Y, 35Z, 36A, 36B, 36C, 36D, 36E, 36F, 36G, 36H, 36I, 36J, 36K, 36L, 36M, 36N, 36O, 36P, 36Q, 36R, 36S, 36T, 36U, 36V, 36W, 36X, 36Y, 36Z, 37A, 37B, 37C, 37D, 37E, 37F, 37G, 37H, 37I, 37J, 37K, 37L, 37M, 37N, 37O, 37P, 37Q, 37R, 37S, 37T, 37U, 37V, 37W, 37X, 37Y, 37Z, 38A, 38B, 38C, 38D, 38E, 38F, 38G, 38H, 38I, 38J, 38K, 38L, 38M, 38N, 38O, 38P, 38Q, 38R, 38S, 38T, 38U, 38V, 38W, 38X, 38Y, 38Z, 39A, 39B, 39C, 39D, 39E, 39F, 39G, 39H, 39I, 39J, 39K, 39L, 39M, 39N, 39O, 39P, 39Q, 39R, 39S, 39T, 39U, 39V, 39W, 39X, 39Y, 39Z, 40A, 40B, 40C, 40D, 40E, 40F, 40G, 40H, 40I, 40J, 40K, 40L, 40M, 40N, 40O, 40P, 40Q, 40R, 40S, 40T, 40U, 40V, 40W, 40X, 40Y, 40Z, 41A, 41B, 41C, 41D, 41E, 41F, 41G, 41H, 41I, 41J, 41K, 41L, 41M, 41N, 41O, 41P, 41Q, 41R, 41S, 41T, 41U, 41V, 41W, 41X, 41Y, 41Z, 42A, 42B, 42C, 42D, 42E, 42F, 42G, 42H, 42I, 42J, 42K, 42L, 42M, 42N, 42O, 42P, 42Q, 42R, 42S, 42T, 42U, 42V, 42W, 42X, 42Y, 42Z, 43A, 43B, 43C, 43D, 43E, 43F, 43G, 43H, 43I, 43J, 43K, 43L, 43M, 43N, 43O, 43P, 43Q, 43R, 43S, 43T, 43U, 43V, 43W, 43X, 43Y, 43Z, 44A, 44B, 44C, 44D, 44E, 44F, 44G, 44H, 44I, 44J, 44K, 44L, 44M, 44N, 44O, 44P, 44Q, 44R, 44S, 44T, 44U, 44V, 44W, 44X, 44Y, 44Z, 45A, 45B, 45C, 45D, 45E, 45F, 45G, 45H, 45I, 45J, 45K, 45L, 45M, 45N, 45O, 45P, 45Q, 45R, 45S, 45T, 45U, 45V, 45W, 45X, 45Y, 45Z, 46A, 46B, 46C, 46D, 46E, 46F, 46G, 46H, 46I, 46J, 46K, 46L, 46M, 46N, 46O, 46P, 46Q, 46R, 46S, 46T, 46U, 46V, 46W, 46X, 46Y, 46Z, 47A, 47B, 47C, 47D, 47E, 47F, 47G, 47H, 47I, 47J, 47K, 47L, 47M, 47N, 47O, 47P, 47Q, 47R, 47S, 47T, 47U, 47V, 47W, 47X, 47Y, 47Z, 48A, 48B, 48C, 48D, 48E, 48F, 48G, 48H, 48I, 48J, 48K, 48L, 48M, 48N, 48O, 48P, 48Q, 48R, 48S, 48T, 48U, 48V, 48W, 48X, 48Y, 48Z, 49A, 49B, 49C, 49D, 49E, 49F, 49G, 49H, 49I, 49J, 49K, 49L, 49M, 49N, 49O, 49P, 49Q, 49R, 49S, 49T, 49U, 49V, 49W, 49X, 49Y, 49Z, 50A, 50B, 50C, 50D, 50E, 50F, 50G, 50H, 50I, 50J, 50K, 50L, 50M, 50N, 50O, 50P, 50Q, 50R, 50S, 50T, 50U, 50V, 50W, 50X, 50Y, 50Z, 51A, 51B, 51C, 51D, 51E, 51F, 51G, 51H, 51I, 51J, 51K, 51L, 51M, 51N, 51O, 51P, 51Q, 51R, 51S, 51T, 51U, 51V, 51W, 51X, 51Y, 51Z, 52A, 52B, 52C, 52D, 52E, 52F, 52G, 52H, 52I, 52J, 52K, 52L, 52M, 52N, 52O, 52P, 52Q, 52R, 52S, 52T, 52U, 52V, 52W, 52X, 52Y, 52Z, 53A, 53B, 53C, 53D, 53E, 53F, 53G, 53H, 53I, 53J, 53K, 53L, 53M, 53N, 53O, 53P, 53Q, 53R, 53S, 53T, 53U, 53V, 53W, 53X, 53Y, 53Z, 54A, 54B, 54C, 54D, 54E, 54F, 54G, 54H, 54I, 54J, 54K, 54L, 54M, 54N, 54O, 54P, 54Q, 54R, 54S, 54T, 54U, 54V, 54W, 54X, 54Y, 54Z, 55A, 55B, 55C, 55D, 55E, 55F, 55G, 55H, 55I, 55J, 55K, 55L, 55M, 55N, 55O, 55P, 55Q, 55R, 55S, 55T, 55U, 55V, 55W, 55X, 55Y, 55Z, 56A, 56B, 56C, 56D, 56E, 56F, 56G, 56H, 56I, 56J, 56K, 56L, 56M, 56N, 56O, 56P, 56Q, 56R, 56S, 56T, 56U, 56V, 56W, 56X, 56Y, 56Z, 57A, 57B, 57C, 57D, 57E, 57F, 57G, 57H, 57I, 57J, 57K, 57L, 57M, 57N, 57O, 57P, 57Q, 57R, 57S, 57T, 57U, 57V, 57W, 57X, 57Y, 57Z, 58A, 58B, 58C, 58D, 58E, 58F, 58G, 58H, 58I, 58J, 58K, 58L, 58M, 58N, 58O, 58P, 58Q, 58R, 58S, 58T, 58U, 58V, 58W, 58X, 58Y, 58Z, 59A, 59B, 59C, 59D, 59E, 59F, 59G, 59H, 59I, 59J, 59K, 59L, 59M, 59N, 59O, 59P, 59Q, 59R, 59S, 59T, 59U, 59V, 59W, 59X, 59Y, 59Z, 60A, 60B, 60C, 60D, 60E, 60F, 60G, 60H, 60I, 60J, 60K, 60L, 60M, 60N, 60O, 60P, 60Q, 60R, 60S, 60T, 60U, 60V, 60W, 60X, 60Y, 60Z, 61A, 61B, 61C, 61D, 61E, 61F, 61G, 61H, 61I, 61J, 61K, 61L, 61M, 61N, 61O, 61P, 61Q, 61R, 61S, 61T, 61U, 61V, 61W, 61X, 61Y, 61Z, 62A, 62B, 62C, 62D, 62E, 62F, 62G, 62H, 62I, 62J, 62K, 62L, 62M, 62N, 62O, 62P, 62Q, 62R, 62S, 62T, 62U, 62V, 62W, 62X, 62Y, 62Z, 63A, 63B, 63C, 63D, 63E, 63F, 63G, 63H, 63I, 63J, 63K, 63L, 63M, 63N, 63O, 63P, 63Q, 63R, 63S, 63T, 63U, 63V, 63W, 63X, 63Y, 63Z, 64A, 64B, 64C, 64D, 64E, 64F, 64G, 64H, 64I, 64J, 64K, 64L, 64M, 64N, 64O, 64P, 64Q, 64R, 64S, 64T, 64U, 64V, 64W, 64X, 64Y, 64Z, 65A, 65B, 65C, 65D, 65E, 65F, 65G, 65H, 65I, 65J, 65K, 65L, 65M, 65N, 65O, 65P, 65Q, 65R, 65S, 65T, 65U, 65V, 65W, 65X, 65Y, 65Z, 66A, 66B, 66C, 66D, 66E, 66F, 66G, 66H, 66I, 66J, 66K, 66L, 66M, 66N, 66O, 66P, 66Q, 66R, 66S, 66T, 66U, 66V, 66W, 66X, 66Y, 66Z, 67A, 67B, 67C, 67D, 67E, 67F, 67G, 67H, 67I, 67J, 67K, 67L, 67M, 67N, 67O, 67P, 67Q, 67R, 67S, 67T, 67U, 67V, 67W, 67X, 67Y, 67Z, 68A, 68B, 68C, 68D, 68E, 68F, 68G, 68H, 68I, 68J, 68K, 68L, 68M, 68N, 68O, 68P, 68Q, 68R, 68S, 68T, 68U, 68V, 68W, 68X, 68Y, 68Z, 69A, 69B, 69C, 69D, 69E, 69F, 69G, 69H, 69I, 69J, 69K, 69L, 69M, 69N, 69O, 69P, 69Q, 69R, 69S, 69T, 69U, 69V, 69W, 69X, 69Y, 69Z, 70A, 70B, 70C, 70D, 70E, 70F, 70G, 70H, 70I, 70J, 70K, 70L, 70M, 70N, 70O, 70P, 70Q, 70R, 70S, 70T, 70U, 70V, 70W, 70X, 70Y, 70Z, 71A, 71B, 71C, 71D, 71E, 71F, 71G, 71H, 71I, 71J, 71K, 71L, 71M, 71N, 71O, 71P, 71Q, 71R, 71S, 71T, 71U, 71V, 71W, 71X, 71Y, 71Z, 72A, 72B, 72C, 72D, 72E, 72F, 72G, 72H, 72I, 72J, 72K, 72L, 72M, 72N, 72O, 72P, 72Q, 72R, 72S, 72T, 72U, 72V, 72W, 72X, 72Y, 72Z, 73A, 73B, 73C, 73D, 73E, 73F, 73G, 73H, 73I, 73J, 73K, 73L, 73M, 73N, 73O, 73P, 73Q, 73R, 73S, 73T, 73U, 73V, 73W, 73X, 73Y, 73Z, 74A, 74B, 74C, 74D, 74E, 74F, 74G, 74H, 74I, 74J, 74K, 74L, 74M, 74N, 74O, 74P, 74Q, 74R, 74S, 74T, 74U, 74V, 74W, 74X, 74Y, 74Z, 75A, 75B, 75C, 75D, 75E, 75F, 75G, 75H, 75I, 75J, 75K, 75L, 75M, 75N, 75O, 75P, 75Q, 75R, 75S, 75T, 75U, 75V, 75W, 75X, 75Y, 75Z, 76A, 76B, 76C, 76D, 76E, 76F, 76G, 76H, 76I, 76J, 76K, 76L, 76M, 76N, 76O, 76P, 76Q, 76R, 76S, 76T, 76U, 76V, 76W, 76X, 76Y, 76Z, 77A, 77B, 77C, 77D, 77E, 77F, 77G, 77H, 77I, 77J, 77K, 77L, 77M, 77N, 77O, 77P, 77Q, 77R, 77S, 77T, 77U, 77V, 77W, 77X, 77Y, 77Z, 78A, 78B, 78C, 78D, 78E, 78F, 78G, 78H, 78I, 78J, 78K, 78L, 78M, 78N, 78O, 78P, 78Q, 78R, 78S, 78T, 78U, 78V, 78W, 78X, 78Y, 78Z, 79A, 79B, 79C, 79D, 79E, 79F, 79G, 79H, 79I, 79J, 79K, 79L, 79M, 79N, 79O, 79P, 79Q, 79R, 79S, 79T, 79U, 79V, 79W, 79X, 79Y, 79Z, 80A, 80B, 80C, 80D, 80E, 80F, 80G, 80H, 80I, 80J, 80K, 80L, 80M, 80N, 80O, 80P, 80Q, 80R, 80S, 80T, 80U, 80V, 80W, 80X, 80Y, 80Z, 81A, 81B, 81C, 81D, 81E, 81F, 81G, 81H, 81I, 81J, 81K, 81L, 81M, 81N, 81O, 81P, 81Q, 81R, 81S, 81T, 81U, 81V, 81W, 81X, 81Y, 81Z, 82A, 82B, 82C, 82D, 82E, 82F, 82G, 82H, 82I, 82J, 82K, 82L, 82M, 82N, 82O, 82P, 82Q, 82R, 82S, 82T, 82U, 82V, 82W, 82X, 82Y, 82Z, 83A, 83B, 83C, 83D, 83E, 83F, 83G, 83H, 83I, 83J, 83K, 83L, 83M, 83N, 83O, 83P, 83Q, 83R, 83S, 83T, 83U, 83V, 83W, 83X, 83Y, 83Z, 84A, 84B, 84C, 84D, 84E, 84F, 84G, 84H, 84I, 84J, 84K, 84L, 84M, 84N, 84O, 84P, 84Q, 84R, 84S, 84T, 84U, 84V, 84W, 84X, 84Y, 84Z, 85A, 85B, 85C, 85D, 85E, 85F, 85G, 85H, 85I, 85J, 85K, 85L, 85M, 85N, 85O, 85P, 85Q, 85R, 85S, 85T, 85U, 85V, 85W, 85X, 85Y, 85Z, 86A, 86B, 86C, 86D, 86E, 86F, 86G, 86H, 86I, 86J, 86K, 86L, 86M, 86N, 86O, 86P, 86Q, 86R, 86S, 86T, 86U, 86V, 86W, 86X, 86Y, 86Z, 87A, 87B, 87C, 87D, 87E, 87F, 87G, 87H, 87I, 87J, 87K, 87L, 87M, 87N, 87O, 87P, 87Q, 87R, 87S, 87T, 87U, 87V, 87W, 87X, 87Y, 87Z, 88A, 88B, 88C, 88D, 88E, 88F, 88G, 88H, 88I, 88J, 88K, 88L, 88M, 88N, 88O, 88P, 88Q, 88R, 88S, 88T, 88U, 88V, 88W, 88X, 88Y, 88Z, 89A, 89B, 89C, 89D, 89E, 89F, 89G, 89H, 89I, 89J, 89K, 89L, 89M, 89N, 89O, 89P, 89Q, 89R, 89S, 89T, 89U, 89V, 89W, 89X, 89Y, 89Z, 90A, 90B, 90C, 90D, 90E, 90F, 90G, 90H, 90I, 90J, 90K, 90L, 90M, 90N, 90O, 90P, 90Q, 90R, 90S, 90T, 90U, 90V, 90W, 90X, 90Y, 90Z, 91A, 91B, 91C, 91D, 91E, 91F, 91G, 91H, 91I, 91J, 91K, 91L, 91M, 91N, 91O, 91P, 91Q, 91R, 91S, 91T, 91U, 91V, 91W, 91X, 91Y, 91Z, 92A, 92B, 92C, 92D, 92E, 92F, 92G, 92H, 92I, 92J, 92K, 92L, 92M, 92N, 92O, 92P, 92Q, 92R, 92S, 92T, 92U, 92V, 92W, 92X, 92Y, 92Z, 93A, 93B, 93C, 93D, 93E, 93F, 93G, 93H, 93I, 93J, 93K, 93L, 93M, 93N, 93O, 93P, 93Q, 93R, 93S, 93T, 93U, 93V, 93W, 93X, 93Y, 93Z, 94A, 94B, 94C, 94D, 94E, 94F, 94G, 94H, 94



GREEN- HIGH
YELLOW- MODERATE
ORANGE- LOW

- LEGEND**
- W17 (15) ● MONITORING WELL LOCATION AND PCP CONCENTRATION (ug/L)
 - WELL WITH BIO-TRAP SAMPLER
 - PW12 ● EXTRACTION WELL LOCATION AND NUMBER
 - DFOMW-5 ▲ 3M GROUNDWATER MONITORING WELL
 - APPROXIMATE PROPERTY LINE
 - - - FORMER BUILDING OUTLINE
 - 50 PCP ISOCONCENTRATION CONTOUR INTERVAL VARIES
 - OUTLINE OF RESIDUAL PHASE LNAPL
 - - - APPROXIMATE LOCATION OF SHEET PILE WALL

- NOTES**
1. BASE MAP DEVELOPED FROM DRAWING A107250-1 OF THE SEPTEMBER 1992 SEMI-ANNUAL GROUNDWATER MONITORING REPORT BY KEYSTONE ENVIRONMENTAL, MWH DRAWING 2082658.302160101-B1, AND 3M WELLS LOCATION BASED ON 3M MAPS.
 2. GROUNDWATER SAMPLES OBTAINED BY TRC ON JULY 6, 7, 8, 12, 13, 14, 2021.
 3. ANALYTE CONCENTRATIONS OBTAINED FROM LABORATORY DATA BY CT LABORATORIES, INC.
 4. IN WELL CLUSTERS THE VALUE FROM THE SHALLOWEST WELL WAS USED TO DETERMINE ISOCONCENTRATIONS FOR THE ANALYTE.
 5. THE NR140 ENFORCEMENT STANDARD (ES) FOR PCP IS 1.0 ug/L. THE NR140 PREVENTIVE ACTION LIMIT (PAL) FOR PCP IS 0.10 ug/L.
 6. BIO-TRAP SAMPLERS WERE INSTALLED IN WELLS W02, W03A, W06R, W10A, W11, W22, W41, AND W44 FROM JULY 14 THROUGH OCTOBER 12, 2021.
 7. THE CITY OF WAUSAU INSTALLED A STEEL SHEET PILING WALL IN 2020 TO REPLACE A ROCK WALL ON THE WISCONSIN RIVER BANK LOCATED WEST OF THE THOMAS STREET BRIDGE.

PROJECT: **WAULECO, INC. WAUSAU, WISCONSIN**

TITLE: **PCP ISOCONCENTRATION MAP (JULY 2021) AND BIO-TRAP RESULTS**

DRAWN BY: T.FIEBRANZ	PROJ NO.: 1989597.0011
CHECKED BY: K. QUINN	
APPROVED BY: B. IVERSON	
DATE: JANUARY 2022	FIGURE 3

708 Heartland Trail
Suite 3000
Madison, WI 53717
Phone: 608.826.3600

FILE NO.: 1989597.0011.07-FIGURE 3.dwg

T:\04 - USER: E:\miller - ATTACHED: WARE - SHOWN: 3/14/2022 10:00 AM - JULY 2021 PCP DATA STATE PLAN - ATTACHED IMAGES:
 DRAWING NAME: M:\WAUSAU\1989597\Bio Trap\0011 Bio Trap\1989597.0011.07-FIGURE 3.dwg - PLOT DATE: January 14, 2022 9:13 AM - LAYOUT: PCP ISOCONCENTRATION MAP (JULY 2021) AND BIO-TRAP RESULTS
 Version: 2017-10-21

Appendix A: Microbial Insights Bio-Trap – Stable Isotope Probing Protocol

SAMPLING INSTRUCTIONS

Handling:

- Bio-Trap Samplers used for Stable Isotope Probing (SIP) are baited with ¹³C-labeled contaminant of interest (e.g. benzene, MTBE, chlorobenzene) adsorbed onto the powder activated carbon (PAC). Controlled laboratory conditions show only minimal loss of contaminant due to volatilization. However, special considerations must be taken into account when handling SIP Bio-Trap Samplers in order to reduce the risk of volatilization.
- SIP Bio-Trap Samplers are shipped out chilled, on blue ice, and it is essential that they should be kept cool (not frozen) until deployment.
- When retrieving the Bio-Trap Samplers that have been deployed in the field, they should immediately be placed on ice and shipped on ice for next day delivery. These steps will ensure the most accurate results.
- Although the contaminant is absorbed onto the beads, caution should be used in handling these Bio-Trap Samplers because the contaminant compounds are associated with possible health and safety risks.

Note: Clean latex gloves (or similar) should be used at all times when handling the Bio-Trap Samplers.

Storage:

It is important to minimize the amount of time that Bio-Trap Samplers are stored prior to being installed in the field. The physical properties of the Bio-Trap Samplers that make them an ideal medium for collecting microbes also increase the chances of microbial or chemical contamination. Bio-Trap Samplers need to remain sealed and refrigerated (not frozen) until they can be installed in the field.

Installation:

- Prior to installing Bio-Trap Sampler, the monitoring well may need to be purged if it has not been sampled in a while. If purging is necessary, MI recommends that three well volumes be removed to ensure contact with formation water and reduce well bore effect.
- Attach the Bio-Trap Sampler's nylon loop (provided) to a nylon line (not provided) and suspend Bio-Trap Sampler at a depth where significant contaminant concentrations exist. If no data are available on the vertical distribution of contaminants, then suspend the Bio-Trap Sampler in the middle of the saturated screened interval.
- If large fluctuations in the water level are anticipated during the period of incubation, the Bio-Trap Sampler should be suspended from a float (contact MI for further details). Be sure not to suspend the bio-trap in the NAPL zone.
- Once installed, incubation times can vary depending upon the scope of the project. A typical Stable Isotope Probing (SIP) study incubation period is 30 days but is project dependant. Please contact us if you have questions regarding the optimum deployment period for your samples.

Retrieval:

- Open the monitoring well and pull up the Bio-Trap Sampler. Cut and remove the braided nylon line used to suspend the Bio-Trap Sampler.
- Transfer the recovered Bio-Trap Sampler to labeled (well number and date) zippered bags, seal and then double bag in a larger (one-gallon) zippered bag, immediately place on blue ice in a cooler.
- Repeat above for all the Bio-Trap Samplers from the site.
- A chain of custody (COC) form must be included with each shipment of samples.
- In order to minimize the potential effect of these samplers on the monitoring well, MI recommends purging three well volumes from the test well following the retrieval of the SIP Bio-Trap Samplers.

Hold time for this analysis is 24-48 hours.

SHIPPING INSTRUCTIONS

Packaging Samples:

1. Samples should be shipped in a cooler with ice or blue ice for next day delivery. If regular ice is used, the ice should be double bagged.
2. A chain of custody form must be included with each shipment of samples. Access our chain of custody at www.microbe.com.

Shipment for Weekday Delivery:

Samples for weekday delivery should be shipped to:

Sample Custodian
Microbial Insights, Inc.
10515 Research Drive
Knoxville, TN 37932
(865) 573-8188

Shipment for Saturday Delivery:

Coolers to be delivered on Saturday **must be shipped via FedEx** to our **FedEx Drop Location** (FedEx will not accept shipments from any other carriers). To ensure proper handling the following steps must be taken:

1. FedEx shipping label should be marked under (6) Special Handling, check Hold Saturday.
2. The cooler must be taped with FedEx SATURDAY tape.
3. The shipping label must be filled out with the Drop Location address below. Our laboratory name must be on the address label.

4. You MUST **notify by email** customerservice@microbe.com with the tracking number of the package on Friday (prior to 4pm Eastern Time) to arrange for Saturday pickup. Please make sure you write "Saturday Delivery" in the subject line of the message. **Without proper labeling and the tracking number, there is no guarantee that the samples will be collected.**

Samples for **Saturday delivery** should be shipped to: Microbial Insights, Inc.
FedEx Drop Location
10601 Murdock Drive
Knoxville, TN 37932
(865) 573-8188

Notes:

- **Stable Isotope Probing (SIP) may preclude subsequent Compound Specific Isotope Analysis (CSIA) in the study well for a period of time. CSIA can be performed prior to SIP or at another location.**

Appendix B: Microbial Insights Bio-Trap Laboratory Reports

- Microbial Insights – SITE LOGIC Report Stable Isotope Probing (SIP)
- Microbial Insights – Census Analyses

**Microbial Insights
SITE LOGIC Report Stable Isotope Probing (SIP)**

SITE LOGIC Report

Stable Isotope Probing (SIP)

Contact: Ken Quinn **Phone:** 608-826-3653
Address: TRC Environmental Corporation **Email:** kquinn@trccompanies.com
708 Heartland Trail
Madison, WI, 53707

MI Identifier: 037SJ

Report Date: November 18, 2021

Project: Wauleco

Comments: Report revised 11/30/21 to include QAQC information. Report revised 12/3/2021 to expand the executive summary. Report revised on 12/17/2021 to expand the executive summary and add color shading for the result ranges.

NOTICE: This report is intended only for the addressee shown above and may contain confidential or privileged information. If the recipient of this material is not the intended recipient or if you have received this in error, please notify Microbial Insights, Inc. immediately. The data and other information in this report represent only the sample(s) analyzed and are rendered upon condition that it is not to be reproduced without approval from Microbial Insights, Inc. Thank you for your cooperation.

Executive Summary

A Stable Isotope Probing (SIP) study was performed to determine whether biodegradation of Pentachlorophenol (PCP) is occurring under existing site conditions. Bio-Trap® samplers baited with ^{13}C -labeled PCP were deployed in Wauleco W2, Wauleco W3A, Wauleco W6R, Wauleco W10A, Wauleco W11, Wauleco W22, Wauleco W41, Wauleco W44. Following a 90-day deployment period, the Bio-Traps were recovered to quantify ^{13}C incorporation into biomass and dissolved inorganic carbon (DIC). A complete summary of the SIP results is provided in Tables 1 and 2, and Figures 1 through 4. Following are the key observations from the results obtained for the monitoring wells.

Stable Isotope Probing (SIP) Results

- Quantification of ^{13}C -enriched PLFA conclusively demonstrated that PCP was metabolized under existing site conditions. The average PLFA $\delta^{13}\text{C}$ values for Wauleco W2, Wauleco W3A, Wauleco W6R, Wauleco W11, and Wauleco W22 were within the high range with values of 1240‰, 1312‰, 7048‰, 11680‰, and 8630‰, respectively. The average PLFA $\delta^{13}\text{C}$ values for Wauleco W41, and Wauleco W44 were within the moderate range with values of 405‰ and 515‰, respectively. The average PLFA $\delta^{13}\text{C}$ value for Wauleco W10A (72‰) fell within the low range.
- Quantification of ^{13}C -enriched DIC conclusively demonstrated that PCP was biologically degraded under existing site conditions. The average DIC $\delta^{13}\text{C}$ values for samples Wauleco W2, Wauleco W3A, Wauleco W10A, Wauleco W41, and Wauleco W44 were low with values of -2‰, -3‰, -8‰, 2‰ and 4‰, respectively. These results suggest that the mineralization of PCP was low at these locations.
- The average DIC $\delta^{13}\text{C}$ values for Wauleco W6R (3456‰), Wauleco W11 (7474‰), and Wauleco W22 (6803‰) fell within the high range. Detection of ^{13}C enriched DIC, which includes $^{13}\text{CO}_2$, conclusively indicates contaminant biodegradation and mineralization.
- Thus, detection of the ^{13}C “label” in the end products of biodegradation (bacterial biomass and CO_2) at the end of the SIP study provides conclusive evidence of contaminant biodegradation.
- The total PLFA biomass concentrations in all monitoring wells were on the order of 10^5 to 10^6 cells/bead, which was within the moderate range. PLFA analysis is one of the most reliable and accurate methods available for the determination of viable (live) biomass. Phospholipids break down rapidly upon cell death (1,2), so biomass calculations based on PLFA content do not include “fossil” lipids from dead cells.
- Community structure data is presented as a percentage of PLFA structural groups normalized to the total PLFA biomass. The relative proportions of the PLFA structural groups provide a “fingerprint” of the types of microbial groups (e.g., anaerobes, sulfate reducers, etc.) present, and therefore, offer insight into the dominant metabolic processes occurring at the sample location.
 - The PLFA community structure for sample Wauleco W2 was comprised of monoenoics (76.05%) followed by normal saturates (23.95%).
 - The PLFA community structure for sample Wauleco W3A was primarily comprised of monoenoics (73.78%) followed by normal saturates (20.28%) and indicators of firmicutes (5.96%).
 - Similar to Wauleco W2, the PLFA community structure for sample Wauleco W6R was comprised of monoenoics (80.83%) followed by normal saturates (19.17%).
 - The PLFA community structure for sample Wauleco W10A was primarily comprised of monoenoics (67.89%) followed by normal saturates (23.11%), anaerobic metal reducers (5.49%) and indicators of firmicutes (2.29%) and eukaryotes (1.21%).

- The PLFA community structure for sample Wauleco W11 was primarily comprised of monoenoics (67.76%) followed by normal saturates (16.84%), anaerobic metal reducers (6.45%), firmicutes (6.07%), and actinomycetes (2.88%).
- The PLFA community structure for sample Wauleco W22 was primarily comprised of monoenoics (70.27%) followed by normal saturates (18.45%), actinomycetes (4.96%), and firmicutes (3.58%). Less than 3% of the PLFA community structure was comprised of anaerobic metal reducers (1.70%), and eukaryotes (1.03%).
- The PLFA community structure for sample Wauleco W41 was primarily comprised of monoenoics (65.23%) followed by normal saturates (20.26%), firmicutes (12.11%) and indicators of eukaryotes (2.06%).
- The PLFA community structure for sample Wauleco W44 was primarily comprised of monoenoics (68.73%) followed by normal saturates (22.10%), and eukaryotes (6.94%). Less than 3% of the PLFA community structure was comprised of firmicutes (1.67%) and anaerobic metal reducers (0.55%).

Overview of Approach

Stable Isotope Probing (SIP)

Stable isotope probing (SIP) is an innovative approach to conclusively determine whether *in situ* biodegradation of a contaminant of concern is occurring.

With the SIP method, a Bio-Trap® is amended with a specially synthesized ^{13}C form of the contaminant of concern (e.g. ^{13}C -benzene). The ^{13}C essentially serves as a “label” to track biodegradation. For petroleum hydrocarbons and many other contaminants, biodegradation is a process whereby some microorganisms use the contaminant of concern as a carbon and energy source. When used as carbon source, contaminant carbon is incorporated into biomolecules such as phospholipids, DNA, and proteins supporting growth of new cells (biomass). When used as an energy source, contaminant carbon is oxidized to CO_2 as part of cellular metabolism. Thus, detection of the ^{13}C “label” in the end products of biodegradation (bacterial biomass and CO_2) at the end of the SIP study provides conclusive evidence of contaminant biodegradation.

To perform a SIP study, a Bio-Trap® is amended with the ^{13}C form of the contaminant of concern (e.g. ^{13}C -benzene) and deployed in an existing monitoring well for a period of 30 to 60 days. If present and active under the existing subsurface conditions, bacteria capable of utilizing the ^{13}C labeled contaminant of concern will colonize and grow in the Bio-Trap® over the course of the deployment period. Following recovery from the well, the Bio-Trap® is shipped to the laboratory and two approaches are used to conclusively evaluate contaminant biodegradation:

- Quantification of ^{13}C enriched phospholipid fatty acids (PLFA)
- Quantification of ^{13}C enriched dissolved inorganic carbon (DIC)

PLFA are a primary component of the membrane of bacterial cells and have long been used as a measure of microbial biomass. The detection of ^{13}C enriched PLFA during a SIP study indicates incorporation into microbial biomass and therefore conclusively demonstrates contaminant biodegradation.

Detection of ^{13}C enriched DIC which includes $^{13}\text{CO}_2$ conclusively indicates contaminant biodegradation and mineralization.

Results

Table 1. Summary of the stable isotope probing results obtained from the Bio-Trap® Units.

Sample Name	Wauleco W2	Wauleco W3A	Wauleco W6R	Wauleco W10A
Sample Date	10/12/2021	10/12/2021	10/12/2021	10/12/2021
MI ID	037SJ-1	037SJ-2	037SJ-3	037SJ-4

Biomass & ¹³C Incorporation

Total Biomass (Cells/bead)	3.91E+05	5.95E+05	2.29E+05	9.01E+05
¹³ C Enriched Biomass (Cells/bead)	1.12E+04	2.46E+04	1.76E+04	8.27E+03
¹³ C Enriched Biomass as Percent of Total Biomass	2.9%	4.1%	7.7%	0.9%
Average Enriched PLFA Delta (‰)	1240	1312	7048	72
Maximum Enriched PLFA Delta (‰)	4740	6906	10027	256

¹³C Mineralization

DIC Delta (‰)	-2	-3	3456	-8
---------------	----	----	------	----

Community Structure (% total PLFA)

Firmicutes (TerBrSats)	0.00	5.96	0.00	2.29
Proteobacteria (Monos)	76.05	73.78	80.83	67.89
Anaerobic metal reducers (BrMonos)	0.00	0.00	0.00	5.49
Actinomycetes (MidBrSats)	0.00	0.00	0.00	0.00
General (Nsats)	23.95	20.28	19.17	23.11
Eukaryotes (Polyenoics)	0.00	0.00	0.00	1.21

Physiological Status (Proteobacteria only)

Slowed Growth	0.28	0.48	0.00	0.30
Decreased Permeability	0.00	0.13	0.00	0.36

Legend:

NA = Not analyzed NS = Not sampled J = Estimated result below PQL but above LQL I = Inhibited ND = Result not detected

Total Biomass level (Cells/bd)	Low	E+03 to E+04
	Moderate	E+05 to E+06
	High	E+07 to E+08

Average and Maximum Enriched PLFA Delta (‰)	Low	0 to 100
	Moderate	100 to 1,000
	High	>1,000

Dissolved Inorganic Carbon (DIC) Delta (‰)	Background	(-25) to (-10)
	Low	> -10 to 100
	Moderate	100 to 1,000
	High	>1,000

Table 2. Summary of the stable isotope probing results obtained from the Bio-Trap® Units.

Sample Name	Wauleco W11	Wauleco W22	Wauleco W41	Wauleco W44
Sample Date	10/12/2021	10/12/2021	10/12/2021	10/12/2021
MI ID	037SJ-5	037SJ-6	037SJ-7	037SJ-8

Biomass & ¹³C Incorporation

Total Biomass (Cells/bead)	6.37E+05	1.25E+06	7.31E+05	1.10E+06
¹³ C Enriched Biomass (Cells/bead)	7.26E+04	8.15E+04	1.09E+04	1.37E+04
¹³ C Enriched Biomass as Percent of Total Biomass	11.4%	6.5%	1.5%	1.2%
Average Enriched PLFA Delta (‰)	11680	8630	405	515
Maximum Enriched PLFA Delta (‰)	20627	28360	1291	4711

¹³C Mineralization

DIC Delta (‰)	7474	6803	2	4
---------------	------	------	---	---

Community Structure (% total PLFA)

Firmicutes (TerBrSats)	6.07	3.58	12.11	1.67
Proteobacteria (Monos)	67.76	70.27	65.23	68.73
Anaerobic metal reducers (BrMonos)	6.45	1.70	0.00	0.55
Actinomycetes (MidBrSats)	2.88	4.96	0.00	0.00
General (Nsats)	16.84	18.45	20.60	22.10
Eukaryotes (Polyenoics)	0.00	1.03	2.06	6.94

Physiological Status (Proteobacteria only)

Slowed Growth	0.32	0.44	0.74	0.52
Decreased Permeability	0.00	0.06	0.00	0.05

Legend:

NA = Not analyzed NS = Not sampled J = Estimated result below PQL but above LQL I = Inhibited ND = Result not detected

Total Biomass level (Cells/bd)	Low	E+03 to E+04
	Moderate	E+05 to E+06
	High	E+07 to E+08

Average and Maximum Enriched PLFA Delta (‰)	Low	0 to 100
	Moderate	100 to 1,000
	High	>1,000

Dissolved Inorganic Carbon (DIC) Delta (‰)	Background	(-25) to (-10)
	Low	> -10 to 100
	Moderate	100 to 1,000
	High	>1,000

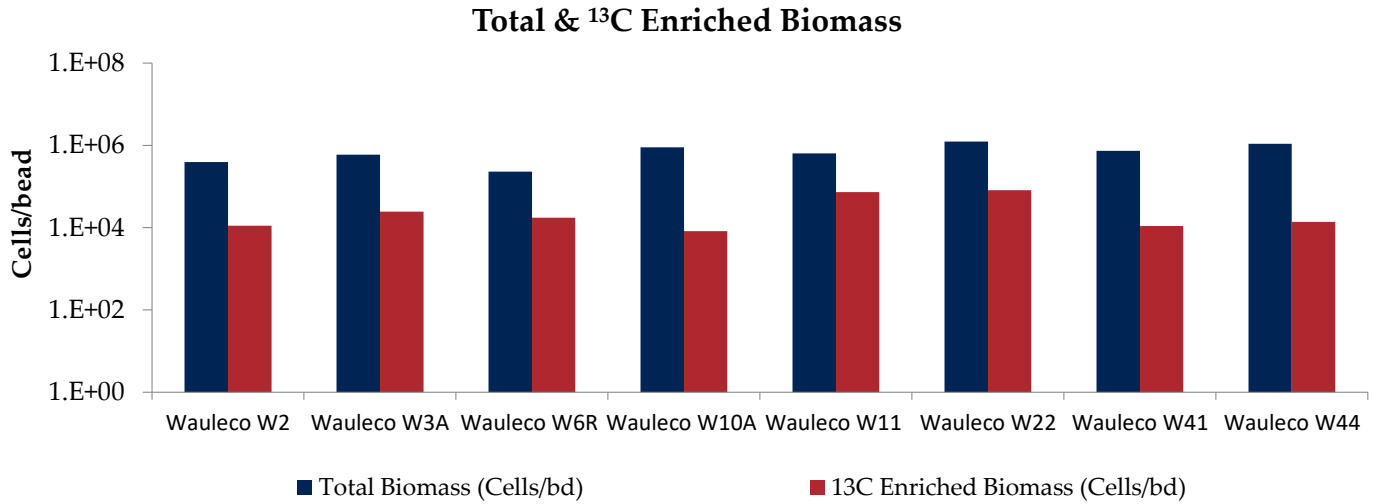


Figure 1. Biomass content is presented as a cell equivalent based on the total amount of phospholipid fatty acids (PLFA) extracted from a given sample. Total biomass is calculated based upon PLFA attributed to bacterial and eukaryotic biomass (associated with higher organisms).

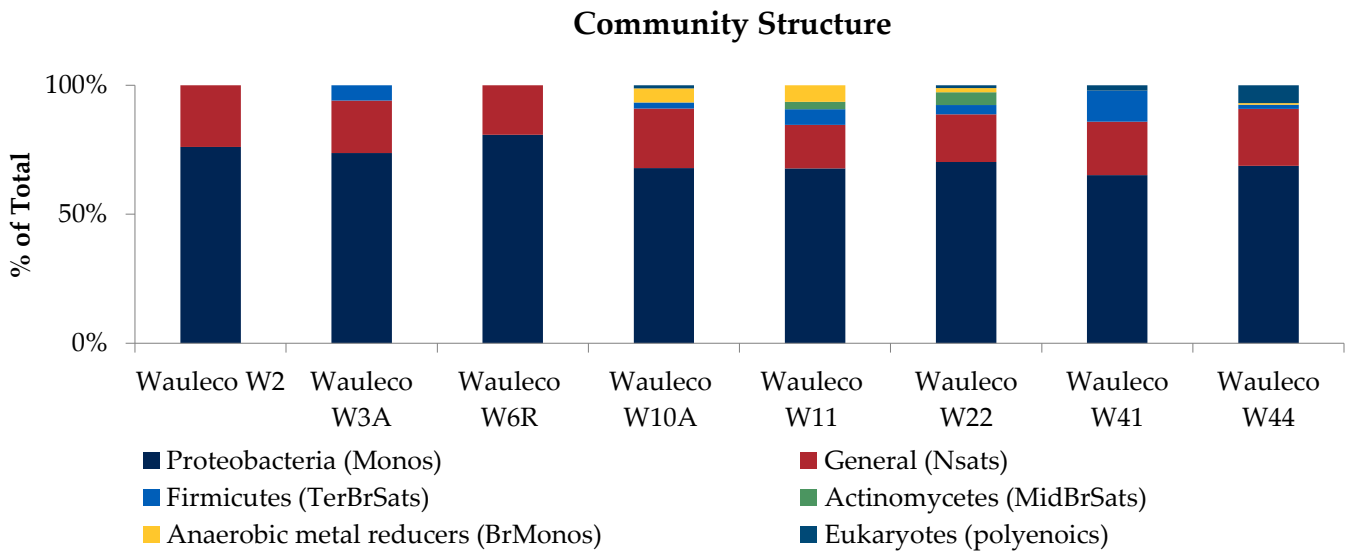


Figure 2. Relative percentages of total PLFA structural groups in the samples analyzed. Structural groups are assigned according to PLFA chemical structure, which is related to fatty acid biosynthesis. See the table in the interpretation section for detailed descriptions of the structural groups.

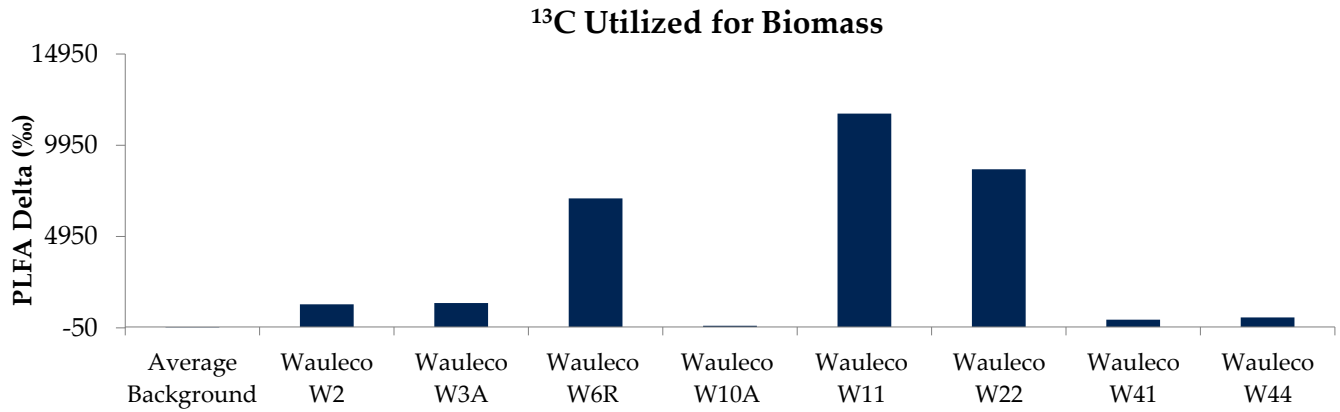


Figure 3. Comparison of the average Delta value obtained from PLFA biomarkers from each Bio-Trap[®] unit to the average background Delta observed in samples not exposed to ¹³C enriched compounds.

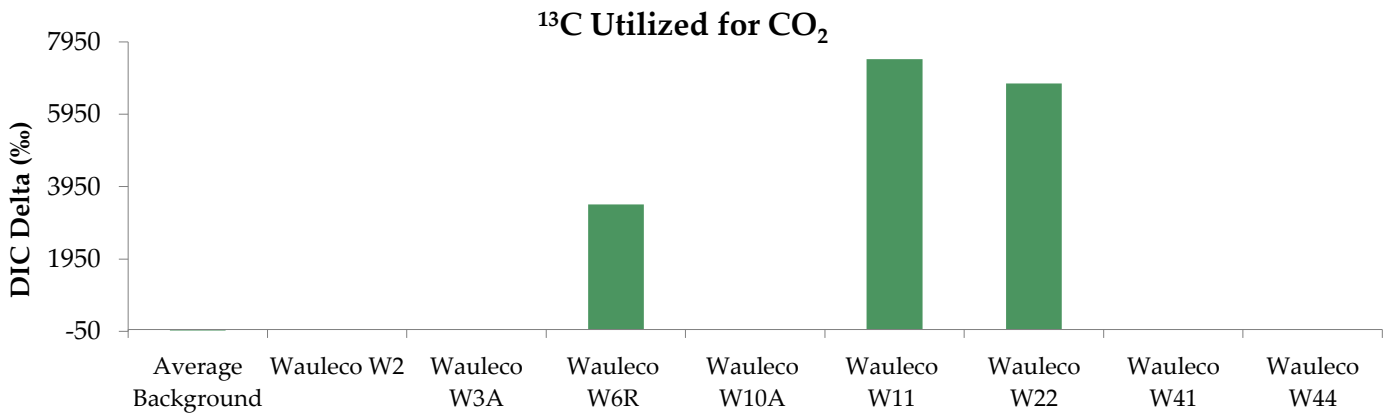


Figure 4. Comparison of the Delta value obtained from DIC from each Bio-Trap[®] unit to the average background Delta observed in samples not exposed to ¹³C enriched compounds.

Quality Assurance/Quality Control Data

Samples Received 10/13/2021

Component	Date Prepared	Date Analyzed	Arrival Temperature	Positive Control	Extraction Blank	Negative Control
PLFA	10/13/2021	10/29/2021	0 °C	112%	non-detect	non-detect
¹³ C-FAME	10/19/2021	11/1/2021	0 °C	Pass	not applicable	non-detect

Component	Date Prepared	Date Analyzed	Arrival Temperature	Mean Std. Deviation for Reference Material Replicates	Mean Absolute Accuracy for Calibrated Reference Materials
DIC	10/13/2021	11/15/2021	0 °C	±0.08 ‰	±0.15 ‰

Interpretation

Interpretation of the results of the SIP Bio-Trap® study must be performed with due consideration of site conditions, site activities, and the desired treatment mechanism. The following discussion describes interpretation of results in general terms and is meant to serve as a guide.

Biomass Concentrations: PLFA analysis is one of the most reliable and accurate methods available for the determination of viable (live) biomass. Phospholipids break down rapidly upon cell death (1,2), so biomass calculations based on PLFA content do not include “fossil” lipids from dead cells. Total biomass (cells/bead) is calculated from total PLFA using a conversion factor of 20,000 cells/pmole of PLFA. When making comparisons between wells, treatments, or over time, differences of one order of magnitude or more are considered significant.

Total Biomass		
Low	Moderate	High
10 ³ to 10 ⁴ cells	10 ⁵ to 10 ⁶ cells	10 ⁷ to 10 ⁸ cells

¹³C Enriched Biomass: For SIP studies, ¹³C enriched PLFA is determined to quantify ¹³C incorporation into biomass as a line of evidence. The detection of ¹³C enriched biomass provides conclusive evidence of contaminant biodegradation. However, biodegradation of a contaminant of concern is almost always performed by a small subset of the total microbial community. Therefore, the ¹³C enriched biomass is typically several orders of magnitude lower than total biomass.

Average and Maximum PLFA Delta ¹³C: Isotopic data is often reported as a delta value. The delta value is the difference between the isotopic ratio (¹³C/¹²C) of the sample (R_x) and a standard (R_{std}) normalized to the isotopic ratio of the standard (R_{std}) and multiplied by 1,000 (units are parts per thousand or “per mill” and denoted ‰). R_{std} is the international standard Vienna PeeDee Belemnite (VPDB) with an anomalously high ¹³C/¹²C ratio of 0.011237. Due to the high value of the R_{std}, computed delta ¹³C values for most natural compounds are negative on a per mill basis.

Under natural conditions, the background delta ¹³C value for PLFA is between -20 and -30‰. For a SIP Bio-Trap® study, biodegradation and incorporation of the ¹³C labeled compound into PLFA results in a larger ¹³C/¹²C ratio (R_x) and thus delta values greater than under natural conditions.

Typical PLFA delta values are provided below.

Enriched PLFA Delta (‰)		
Low	Moderate	High
0 to 100	100 to 1,000	>1,000

Dissolved Inorganic Carbon (DIC): Often, bacteria can utilize the ¹³C labeled compound as both a carbon and energy source. The ¹³C portion used as a carbon source for growth can be incorporated into PLFA as discussed above, while the ¹³C used for energy is oxidized to ¹³CO₂ (mineralized).

^{13}C enriched CO_2 data is often reported as a delta value as described above for PLFA. Under natural conditions, the delta ^{13}C value for CO_2 is typically in the range of -25‰ to -10‰ (3). For an SIP Bio-Trap[®] study, mineralization of the ^{13}C labeled contaminant of concern (increased $^{13}\text{CO}_2$ production) would lead to a greater value of R_x and thus a positive delta value.

The detection of even low levels of ^{13}C enriched DIC provides conclusive evidence of contaminant biodegradation. However, delta values between 0 and 100‰ are generally considered relatively low, values between 100 and $1,000\text{‰}$ are considered moderate, and values greater than $1,000\text{‰}$ are considered high.

Dissolved Inorganic Carbon (DIC) Delta and $\text{‰}^{13}\text{C}$		
Low	Moderate	High
0 to 100	100 to 1,000	>1,000

Community Structure (% total PLFA): Community structure data is presented as a percentage of PLFA structural groups normalized to the total PLFA biomass. The relative proportions of the PLFA structural groups provide a “fingerprint” of the types of microbial groups (e.g. anaerobes, sulfate reducers, etc.) present and therefore offer insight into the dominant metabolic processes occurring at the sample location. Thorough interpretation of the PLFA structural groups depends in part on an understanding of site conditions and the desired microbial biodegradation pathways. For example, an increase in mid chain branched saturated PLFA (MidBrSats), indicative of sulfate reducing bacteria (SRB) and Actinomycetes, may be desirable at a site where anaerobic BTEX biodegradation is the treatment mechanism, but would not be desirable for a corrective action promoting aerobic BTEX or MTBE biodegradation. The following table provides a brief summary of each PLFA structural group and its potential relevance to bioremediation.

Description of PLFA structural groups.

PLFA Structural Group	General classification	Potential Relevance to Bioremediation Studies
Monoenoic (Monos)	Abundant in Proteobacteria (Gram negative bacteria), typically fast growing, utilize many carbon sources, and adapt quickly to a variety of environments.	Proteobacteria is one of the largest groups of bacteria and represents a wide variety of both aerobes and anaerobes. The majority of Hydrocarbon utilizing bacteria fall within the Proteobacteria
Terminally Branched Saturated (TerBrSats)	Characteristic of Firmicutes (Low G+C Gram-positive bacteria), and also found in Bacteriodes, and some Gram-negative bacteria (especially anaerobes).	Firmicutes are indicative of presence of anaerobic fermenting bacteria (mainly <i>Clostridia/Bacteriodes</i> -like), which produce the H_2 necessary for reductive dechlorination
Branched Monoenoic (BrMonos)	Found in the cell membranes of micro-aerophiles and anaerobes, such as sulfate- or iron-reducing bacteria	In contaminated environments high proportions are often associated with anaerobic sulfate and iron reducing bacteria
Mid-Chain Branched Saturated (MidBrSats)	Common in sulfate reducing bacteria and also Actinobacteria (High G+C Gram-positive bacteria).	In contaminated environments high proportions are often associated with anaerobic sulfate and iron reducing bacteria
Normal Saturated (Nsats)	Found in all organisms.	High proportions often indicate less diverse populations.
Polyenoic	Found in higher plants, and animals.	Eukaryotic scavengers will often prey on contaminant utilizing bacteria.

Physiological Status (Proteobacteria): Some Proteobacteria modify specific PLFA as a strategy to adapt to stressful environmental conditions (4, 5). For example, *cis* monounsaturated fatty acids may be modified to cyclopropyl fatty acids during periods of slowed growth or modified to *trans* monounsaturated fatty acids to decrease membrane permeability in

response to environmental stress. The ratio of product to substrate fatty acid thus provides an index of their health and metabolic activity. In general, status ratios greater than 0.25 indicate a response to unfavorable environmental conditions.

Glossary

Delta (δ): A Delta value is the difference between the isotopic ratio ($^{13}\text{C}/^{12}\text{C}$) of the sample (R_x) and a standard (R_{std}) normalized to the isotopic ratio of the standard (R_{std}) and multiplied by 1,000 (units are parts per thousand denoted ‰).

$$\text{Delta} = (R_x - R_{\text{std}}) / R_{\text{std}} \times 1000$$

References

1. White, D.C., W.M. Davis, J.S. Nickels, J.D. King, and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40:51-62.
2. White, D.C. and D.B. Ringelberg. 1995. Utility of signature lipid biomarker analysis in determining in situ viable biomass. In P.S. Amy and D.L. Halderman (eds.) *The microbiology of the terrestrial surface*. CRC Press, Boca Raton.
3. Porowska, D. 2015. Determination of the origin of dissolved inorganic carbon in groundwater around a reclaimed landfill in Otwock using stable carbon isotopes. *Waste Management*. 39:216-225.
4. Guckert, J.B., M.A. Hood, and D.C. White. 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. *Applied and Environmental Microbiology*. 52:794-801.
5. Tsitko, I.V., G.M. Zaitsev, A.G. Lobanok, and M.S. Salkinoja-Salonen. 1999. Effect of aromatic compounds on cellular fatty acid composition of *Rhodococcus opacus*. *Applied and Environmental Microbiology*. 65:853-855.

Microbial Insights Census Analyses



10515 Research Drive
Knoxville, TN 37932
Phone: (865) 573-8188
Fax: (865) 573-8133



Client: Ken Quinn
TRC Environmental Corporation
708 Heartland Trail
Madison, WI 53707

Phone: 608-826-3653

Fax: 608-358-5193

Identifier: 037SJ

Date Rec: 10/13/2021

Report Date: 10/25/2021

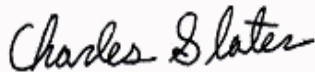
Client Project #:

Client Project Name: Wauleco

Purchase Order #:

Test results provided for: CENSUS

Reviewed By:



NOTICE: This report is intended only for the addressee shown above and may contain confidential or privileged information. If the recipient of this material is not the intended recipient or if you have received this in error, please notify Microbial Insights, Inc. immediately. The data and other information in this report represent only the sample(s) analyzed and are rendered upon condition that it is not to be reproduced without approval from Microbial Insights, Inc. Thank you for your cooperation.

Results relate only to the items tested and the sample(s) as received by the laboratory.

Client: TRC Environmental Corporation
Project: Wauleco

MI Project Number: 037SJ
Date Received: 10/13/2021

Sample Information

Client Sample ID:	Wauleco W2	Wauleco W3A	Wauleco W6R	Wauleco W10A	Wauleco W11
Sample Date:	10/12/2021	10/12/2021	10/12/2021	10/12/2021	10/12/2021
Units:	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Analyst/Reviewer:	HT/CS	HT/CS	HT/CS	HT/CS	HT/CS

Dechlorinating Bacteria

		Wauleco W2	Wauleco W3A	Wauleco W6R	Wauleco W10A	Wauleco W11
<i>Dehalococcoides</i>	DHC	<2.50E+01	<2.50E+01	<2.50E+01	<2.50E+01	<2.50E+01
<i>Desulfotobacterium spp.</i>	DSB	2.33E+02 (J)	5.83E+04	1.91E+01 (J)	5.35E+03	<2.50E+02

Functional Genes

		Wauleco W2	Wauleco W3A	Wauleco W6R	Wauleco W10A	Wauleco W11
PCP Regulator Gene	pcpR	<2.50E+02	<2.50E+02	6.92E+04	<2.50E+02	<2.50E+02
Maleylacetate Reductase	pcpE	<2.50E+02	<2.50E+02	<2.50E+02	3.84E+03	<2.50E+02
PCP-4-Monooxygenase	pcpB	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited
 < = Result not detected

Client: TRC Environmental Corporation
Project: Wauleco

MI Project Number: 037SJ
Date Received: 10/13/2021

Sample Information

Client Sample ID:	Wauleco W22	Wauleco W41	Wauleco W44
Sample Date:	10/12/2021	10/12/2021	10/12/2021
Units:	cells/bead	cells/bead	cells/bead
Analyst/Reviewer:	HT/CS	HT/CS	HT/CS

Dechlorinating Bacteria

<i>Dehalococcoides</i>	DHC	<2.50E+01	<2.50E+01	<2.50E+01
<i>Desulfitobacterium spp.</i>	DSB	<2.50E+02	<2.50E+02	<2.50E+02

Functional Genes

PCP Regulator Gene	pcpR	<2.50E+02	<2.50E+02	<2.50E+02
Maleylacetate Reductase	pcpE	<2.50E+02	<2.50E+02	<2.50E+02
PCP-4-Monooxygenase	pcpB	9.93E+04	<2.50E+02	<2.50E+02

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited
 < = Result not detected

Quality Assurance/Quality Control Data

Samples Received 10/13/2021

Component	Date Prepared	Date Analyzed	Arrival Temperature	Positive Control	Extraction Blank	Negative Control
DHC	10/13/2021	10/22/2021	0 °C	108%	non-detect	non-detect
pcpB	10/13/2021	10/22/2021	0 °C	100%	non-detect	non-detect
pcpR	10/13/2021	10/22/2021	0 °C	100%	non-detect	non-detect
pcpE	10/13/2021	10/22/2021	0 °C	81%	non-detect	non-detect
DSB	10/13/2021	10/22/2021	0 °C	99%	non-detect	non-detect