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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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13/891,934

05/10/2013

Michael Scalzi

SCA-004

8286

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10/31/2014

EXAMINER

BERKE-SCHLESSEL, DAVID W

ART UNIT

PAPER NUMBER

1651

MAIL DATE

DELIVERY MODE

10/31/2014

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 13/891,934	Applicant(s) SCALZI ET AL.	
	Examiner David Berke-Schlessel	Art Unit 1651	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 5/10/13.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 1-19 is/are pending in the application.
5a) Of the above claim(s) ____ is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☐ Claim(s) ____ is/are rejected.
- 8) ☐ Claim(s) ____ is/are objected to.
- 9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date 11/27/13
- 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____.
- 4) ☐ Other: ____.

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Claim Objections

2. Claim 1 is objected to because of the following informalities: it appears that the preamble of claim 1 is missing the article "a." It seems that the preamble should read "A method for oxidizing volatile organic compounds and a biological attenuation process..." Appropriate correction is required.

Claim Rejections - 35 USC § 112

3. The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claim 4 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 4 is indefinite because it is unclear what type of metal is being introduced into the environmental medium. Based on the initial portion of the limitation, it would appear that the claim requires the administration of a trivalent (oxidized) metal;

however, the second portion of the limitation provides uncertainty with this interpretation, because it implies that a neutral metal is “previously introduced.”

Claim Rejections - 35 USC § 101/112

5. Claims 1-4 and 6-13 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a substantial asserted utility or a well-established utility.

The above claims include a method for oxidizing VOCs by the redox reaction between peroxide or persulfate and a trivalent metal. However, the independent claim provides no *actual* method steps wherein the reactants are actively introduced into the “environmental medium.” The independent claim only requires the “activation” of the peroxide or persulfate, which can happen naturally in the environment. Furthermore, the dependent claims (2-4 and 6-13) do not provide any positive method step limitations and do not require the exogenous administration of either the peroxide/persulfate or the trivalent metal, implying that both of these can be found naturally in the environment. Since the above claims are drawn to a method where no method steps exist, the invention has no real utility.

Claims 1-4 and 6-13 are also rejected under 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph. Specifically, because the claimed invention is not supported by either a substantial asserted utility or a well-established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale or otherwise available to the public before the effective filing date of the claimed invention.

7. Claims 1-19 are rejected under 35 U.S.C. 102(a)(1) as being anticipated by Block, et al (US Pat. 7,576,254). Block teaches the *in situ* and *ex situ* oxidation of volatile organic compounds by the administration of a peroxide or persulfate and a trivalent metal. See column 1, lines 9-14; column 2, lines 17-25; column 4, lines 26-34. While Block never explicitly describes a “biological attenuation process,” Block teaches every limitation of the claim, and would therefore inherently provide a “biological attenuation process.”

With respect to claim 1, Block teaches the *in situ* and *ex situ* oxidation of volatile organic compounds by the administration of a peroxide or persulfate and a trivalent metal. See column 1, lines 9-14; column 2, lines 17-25; column 4, lines 26-34. As such, the instant claim is anticipated.

With respect to claim 2, Block teaches soil and groundwater. See column 1, line 10. As such, the instant claim is anticipated.

With respect to claim 3, Block teaches sodium persulfate. See column 2, line 24. As such, the instant claim is anticipated.

With respect to claim 4, Block teaches the addition of Fe (III), which is a trivalent metal. See column 4, line 29. As such, the instant claim is anticipated.

With respect to claim 5, Block teaches the addition of Fe(II). See column 4, line 29. When Fe(II) is exposed to hydrogen peroxide (Fenton's reagent), it will oxidize to Fe(III). As such, the instant claim is anticipated.

With respect to claim 6, Block teaches that the trivalent metals are used as a catalyst with the peroxide/persulfate. See column 4, lines 26-30. Since a catalyst is interpreted to be the same as an "activator," the instant claim is anticipated.

With respect to claim 7, Block teaches Fe(III). See column 4, line 29. As such, the instant claim is anticipated.

With respect to claim 8, Block teaches Mn(II). See column 4, line 29. While Mn(II) is not the trivalent form (Mn(III) is the trivalent form), its exposure to an oxidizing agent, like a peroxide or persulfate, will cause at least some of the manganese to oxidize to Mn(III). Furthermore, since dependent claim 4 indicates that the metals do not have to be administered in their trivalent oxidation state, as long as the administration of Mn(II) results in the formation of some Mn(III), over the course of the method, the claim must inherently be anticipated.

With respect to claim 9, although Block does not say that the medium "returns to a reducing condition," Block teaches identical method steps. If Block teaches identical method steps, everything that follows must be inherent to the method. As such, the instant claim is anticipated.

With respect to claim 10, although Block does not mention a biological attenuation process, nor a mention of facultative biological reactions, Block teaches identical method steps. If Block teaches identical method steps, everything that follows must be inherent to the method. As such, the instant claim is anticipated.

With respect to claim 11, the instant claim is only describing the inherent redox reaction between the compounds already discussed by Block. Since Block teaches the same compounds, provided in the same method, the same redox reaction must also be present. As such, the instant claim is anticipated.

With respect to claim 12, although Block does not mention a biological attenuation process, Block teaches identical method steps. If Block teaches identical method steps, everything that follows must be inherent to the method. As such, the instant claim is anticipated.

With respect to claim 13, pyrite is FeS_2 . Since the reaction with Fe(III) and a persulfate will inherently produce some pyrite, and Block teaches the administration of Fe(III) and persulfate, Block must also inherently form pyrite. As such, the instant claim is anticipated.

With respect to claim 14, Block teaches wells. See column 3, line 47. Although Block does not mention if the wells are temporary or permanent, these are the only possible options. Therefore, the mere mention of using wells in the method of Block implies that both temporary and permanent wells were envisioned. As such, the instant claim is anticipated.

With respect to claim 15, Block teaches injection of the compounds. See column 3, line 29. Since injection requires some form of liquid pumping, the instant claim is anticipated.

With respect to claim 16, Block teaches injection rates of the compounds. See column 3, line 37. Since the control of injection rates requires some degree of pressure, the instant claim is anticipated.

With respect to claim 17, Block teaches wells. See column 3, line 47. Although Block does not mention if the wells are temporary or permanent, these are the only possible options. Therefore, the mere mention of using wells in the method of Block implies that both temporary and permanent wells were envisioned. As such, the instant claim is anticipated.

With respect to claim 18, Block teaches injection of the compounds. See column 3, line 29. Since injection requires some form of liquid pumping, the instant claim is anticipated.

With respect to claim 19, Block teaches injection rates of the compounds. See column 3, line 37. Since the control of injection rates requires some degree of pressure, the instant claim is anticipated.

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102 of this title, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole

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would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

9. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

10. This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

11. Claim 8 is rejected under 35 U.S.C. 103 as being unpatentable over Block, et al (US Pat. 7,576,254) in view of Lucas, et al (*Bioresource Technology*, 119, 174-180, 2012). While Block never explicitly teaches the administration of Mn(III), Block does suggest that Mn(II) could be used, wherein it would naturally oxidize to Mn(III) in the

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presence of an oxidizing agent like peroxide or persulfate. Regardless, Lucas teaches that the combination of Mn(III) (in the form of manganese (III) acetate), with peroxide, will effectively oxidize the highly recalcitrant lignin found in wood. See page 175, left column, second full paragraph. Additionally, Lucas posits that the reaction pathway may involve a Fenton-type reaction. See page 179, left column, fourth full paragraph. While Lucas never mentions that this composition would be useful for the oxidation of VOCs, Block notes that Fenton-type reactions are useful for the oxidation of VOCs. See Block, column 2, lines 17-25. Since Block suggests that Fenton-type reactions *could* be useful for the oxidation of VOCs, and the fact that Lucas teaches that the combination of Mn(III) and hydrogen peroxide undergo Fenton-type reaction, it would be obvious to use Lucas's method, like Block's, as a means of oxidizing VOCs. As such, the instant claim is rendered obvious.

Conclusion

12. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Chen, et al (*Journal of Hazardous Materials*, B87, 171-186, 2001); Gilmore, et al (US Pat. 6,726,406).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Berke-Schlessel whose telephone number is (571)270-3643. The examiner can normally be reached on Mon-Thu 7AM-4PM EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on (571)272-0926. The fax phone

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number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Blaine Lankford/
Primary Examiner, Art Unit 1651

/David Berke-Schlessel/
Examiner, Art Unit 1651

Notice of References Cited	Application/Control No. 13/891,934		Applicant(s)/Patent Under Reexamination SCALZI ET AL.	
	Examiner David Berke-Schlessel		Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-7,576,254	08-2009	Block et al.	588/320
*	B	US-6,726,406	04-2004	Gilmore et al.	405/128.5
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Lucas, Marcel; et al; "Evidence for room temperature delignification of wood using hydrogen peroxide and manganese acetate as a catalyst" Bioresource Technology, 119, 174-180, 2012
	V	Chen, Ge; et al; "The mechanism and applicability of in situ oxidation of trichloroethylene with Fenton's reagent" Journal of Hazardous Materials, B87, 171-186, 2001
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EAST Search History**EAST Search History (Prior Art)**

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2995	oxidiz\$6 same ((volatile adj organic adj compounds) or VOC)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 08:59
L2	640062	peroxide or persulfate	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 08:59
L3	172	l1 same l2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 08:59
L4	2177659	(trivalent near3 metal) or iron or manganese	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:01
L5	34	l3 same l4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:01
L6	1	l1 same (persulphate or persulfate) same ((trivalent near3 metal) or manganese)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:12
L7	2472	(persulphate or persulfate) same ((trivalent near3 metal) or manganese)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:15
L8	20	l1 and l7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:15
L9	475	(persulphate or persulfate) near5 ((trivalent near3 metal) or manganese)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:15
L10	5	l1 and l9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/10/28 09:15

EAST Search History (Interference)

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Evidence for room temperature delignification of wood using hydrogen peroxide and manganese acetate as a catalyst

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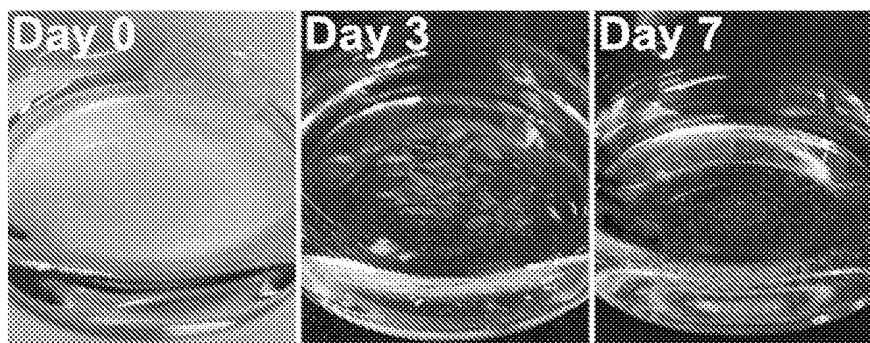
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HIGHLIGHTS

- ▶ $\text{Mn}(\text{OAc})_3$ catalyzes wood delignification with hydrogen peroxide at room temperature.
- ▶ Poplar sections turned into a fine powder within 4 days and without agitation.
- ▶ Raman microscopy showed the preferential oxidation of lignin-rich middle lamellae.
- ▶ Pretreatment for 2, 4, 7 days at room temperature led to improved glucose yields.
- ▶ This method could be a mild, inexpensive pretreatment of lignocellulosic biomass.

GRAPHICAL ABSTRACT



ARTICLE INFO

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ABSTRACT

Manganese acetate was found to catalyze the oxidative delignification of wood with hydrogen peroxide at room temperature. The delignification reaction was monitored by optical and Raman microscopy, and liquid chromatography/mass spectrometry. When exposed to H_2O_2 and $\text{Mn}(\text{OAc})_3$ in aqueous solution, poplar wood sections were converted into a fine powder-like material which consisted of individual wood cells within 4 days at room temperature and without agitation. Optical and Raman microscopy provided the spatial distribution of cellulose and lignin in the wood structure, and showed the preferential oxidation of lignin-rich middle lamellae. Raman spectra from the solid residue revealed a delignified and cellulose-rich material. Glucose yields following enzymatic hydrolysis were 20–40% higher in poplar sawdust pretreated with $\text{Mn}(\text{OAc})_3$ for 2, 4, and 7 days at room temperature than those in sawdust exposed to water only for identical durations, suggesting the viability of this mild, inexpensive method for pretreatment of lignocellulosic biomass.

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1. Introduction

Lignocellulosic biomass, including agricultural and forest wastes, is a potentially abundant and environmentally-friendly source of liquid transportation fuels and commodity chemicals (Sims et al., 2010). However, efforts to use lignocellulose for chem-

ical and fuel production have been hampered by the recalcitrant polymer lignin, which is resistant to chemical degradation. Multiple pretreatment strategies have been investigated to remove the lignin and improve the yield of fermentable sugars for the production of biofuels. However, most pretreatments are energy-intensive or generate toxic byproducts that affect their economic viability (Sims et al., 2010). One “green” pretreatment strategy has been to replicate and optimize the enzymatic activity of natural white rot fungi, such as *Phanerochaete chrysosporium*, that can degrade biomass completely, including the lignin. For instance, the

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reactivity of the Mn-dependent peroxidase enzyme produced from *P. chrysosporium* was studied with a series of lignin model compounds (Banci et al., 1999; Wariishi et al., 1989). The enzyme requires Mn(II) and H₂O₂ to degrade lignin, and the reaction is proposed to involve the oxidation of Mn(II) to Mn(III), a well known one-electron oxidant (Hofrichter, 2002; Khindaria et al., 1995). Consistent with this idea, stoichiometric Mn(OAc)₃ was shown to oxidize coniferyl alcohol (Önnerud et al., 2002), as well as guaiacyl and syringyl lignin models at a pH ranging from 2.5 to 4.1 at room temperature (Hammel et al., 1989; Henry, 2003; Kurek and Gaudard, 2000). Despite its promising stoichiometric reactivity, the use of Mn(OAc)₃ as a catalyst for the delignification of wood with H₂O₂ has not previously been reported.

A number of efforts have focused on using transition metal catalysts and H₂O₂ for the oxidative delignification of wood. The ability of several metalloporphyrins based on Fe, Co, Zn and Mn to oxidize lignin models was studied (Crestini et al., 2010; Zucca et al., 2007). Several other Mn and Fe complexes of synthetic macrocyclic ligands (Patt et al., 2001), including [(TACN)Mn(μ-O)₃Mn(TACN)](PF₆)₂ (Cui et al., 1999) (TACN = 1,4,7-trimethyl-1,4,7-triazacyclo-nonane) and polypyridyl ligands (Tzedakis et al., 2001), catalyzed the delignification of wood using H₂O₂. Methyltrioxorhenium catalyzed the oxidation of lignin models and lignin (Crestini et al., 2010). Although the activity of these catalysts was promising, their use has been limited by their complex and costly synthesis.

In contrast, using a simple transition metal salt catalyst could increase the economic viability of oxidative delignification. The addition of sulfuric acid or TiO₂ was found to improve the delignification of aspen and birch woods in an aqueous solution of H₂O₂ and acetic acid at 120–130 °C (Kuznetsov et al., 2011). A combination of Co(OAc)₂ and Mn(OAc)₂ was found to catalyze the aerobic auto-oxidation of lignin in acetic acid solution at 140–220 °C (Partenheimer, 2009). In ionic liquid solution, Mn(NO₃)₂ catalyzed the oxidation of various lignin and lignin models with air or O₂, but required elevated temperatures (100 °C) and high pressures (83 atm) (Stärk et al., 2010; Zakzeski et al., 2010). Herein, we report that Mn(OAc)₃ catalyzes the delignification of wood at room temperature in aqueous solution using H₂O₂. The reaction was monitored by optical and Raman microscopy, and the effect of the pretreatment on glucose yields from enzymatic cellulose hydrolysis was determined. The overall method is inexpensive, simple, and effective under ambient conditions.

2. Methods

2.1. Materials

All chemicals were obtained from Fisher Scientific and used as received. Unless specified otherwise, all manipulations were carried out under air at ambient temperature. Complexes Mn(pic)₃ (Hpic = picolinic acid), Mn(pic)(dipic)(H₂O) (Hdipic = dipicolinic acid), NMe₄[Mn(opba)(H₂O)₂] (opba = *o*-phenylenebis(oxamate)) and Mn(malt)₃ (malt = maltol) were prepared according to published procedures (see Supplementary data). Transverse sections of poplar wood (*Populus tremuloides*) of 50 μm thickness were prepared using a sliding microtome. The sections were dried in an oven for 4 h between glass slides at 60 °C to prevent curling.

2.2. Analysis

UV–Vis spectra were obtained on an Agilent UV–Vis spectrometer 8743. pH measurements were recorded using an IQ150 pH meter (IQ Scientific Instruments, Carlsbad, CA).

2.3. Oxidation of wood using transition metal salts

In a small glass vial, the appropriate metal salt (FeCl₂, CuCl, Cu(OAc)₂, Mn(OAc)₃, MnSO₄, NaVO₃, Fe(OAc)₂, or Co(OAc)₂, 0.10 mmol) was dissolved in deionized water (1.5 mL). Hydrogen peroxide (0.5 mL of a 30% aqueous solution, 4.4 mmol) was added dropwise, resulting in vigorous bubbling in some cases. As described below, monitoring the concentration of H₂O₂ in aqueous solutions (Chai et al., 2004) containing Fe(OAc)₂, Co(OAc)₂, or Cu(OAc)₂ and no substrate revealed that nearly all of the H₂O₂ was consumed within 24 h (see Fig. S1 in Supplementary data). A 5 × 8 mm² poplar section was then immersed in the vial, and the reaction was monitored by a time-lapse series of photographs collected over a period of 7 days. With Fe(OAc)₂, the wood microtome broke into two large fragments after 24 h, and no further fragmentation was observed over the next 6 days.

2.4. Determination of hydrogen peroxide concentration in transition metal salt solutions

Hydrogen peroxide concentrations were determined by spectrophotometric titration (Chai et al., 2004). A stock solution was prepared by dissolving (NH₄)₆Mo₇O₂₄·4H₂O (0.10 g, 0.081 mmol) in 250 mL of a 0.5 M solution of H₂SO₄. In a small glass vial, the appropriate metal acetate salt (Cu(OAc)₂, Mn(OAc)₃, Mn(OAc)₂, Fe(OAc)₂, or Co(OAc)₂, 0.10 mmol) was dissolved in deionized water (1.5 mL). Hydrogen peroxide (0.5 mL of a 30% aqueous solution, 4.4 mmol) was added dropwise. After 10–15 min, the initial concentration of H₂O₂ was measured by spectrophotometric titration after mixing 4 μL of the sample with 1 mL of a solution of (NH₄)₆Mo₇O₂₄·4H₂O in a quartz cuvette. The H₂O₂ concentration of the as-produced sample and the sample diluted ten times was provided by their absorbance at 350 nm. Calibration was performed with H₂O₂ solutions of concentrations 0%, 0.3%, 0.6%, 0.9% and 1.2%. The concentrations of H₂O₂ in each vial were determined again by titration once every 24 h for 7 days.

2.5. Oxidation of wood with Mn(OAc)₃

In a small glass vial, Mn(OAc)₃ (27 mg, 0.10 mmol) was suspended in deionized water (1.5 mL). A dark brown precipitate formed immediately. Upon dropwise addition of H₂O₂ (0.5 mL of a 30% aqueous solution, 4.4 mmol), slight bubbling was observed and the brown solid dissolved, forming a clear, colorless solution of pH 4.6. A 5 × 8 mm² poplar section was immersed in a vial containing 2 mL of this catalyst solution and a time-lapse series of photographs was collected over a period of 7 days. For high-resolution images, another 4 × 8 mm² section was kept immersed in 2 mL of catalyst solution contained in a polystyrene Petri dish for 7 days. The Petri dish was regularly filled with the catalyst solution to compensate for evaporation and keep the solution volume constant. Just before imaging, the Petri dish was partially drained to reduce foaming and keep the sample close to the low working-distance objective. Optical images were collected in transmission with a Zeiss Axiovert 200 M (Göttingen, Germany) inverted microscope using a 63× oil objective (NA 1.25). After the imaging session, the Petri dish was refilled with the catalyst solution.

2.6. Effect of Mn(OAc)₃/H₂O₂ pretreatment on glucose yield from cellulase treatment

In a glass vial, Mn(OAc)₃ (402 mg, 1.5 mmol) was suspended in deionized water (11.25 mL). Dropwise addition of H₂O₂ (3.75 mL of a 30% aqueous solution, 33.0 mmol) resulted in initial bubbling, forming a clear, colorless solution. To assess the efficiency of the pretreatment on the glucose yield after enzymatic hydrolysis,

500 mg of poplar sawdust (20–30 mesh) was placed in each of six glass test tubes. Three of the six samples were immersed for 2, 4, or 7 days in 5 mL of the $\text{Mn}(\text{OAc})_3/\text{H}_2\text{O}_2$ solution prepared as described above, while the other three were immersed in 5 mL of deionized water (control) for the same durations. After pretreatment, the supernatant solution was removed from the pretreated sample and the solid residue was immersed in 10 mL of water for 24 h to remove the remaining H_2O_2 and catalyst. Each sample was rinsed five times with 5 mL of deionized water before enzymatic hydrolysis. At each rinsing, excess water was extracted with a pipette. The rinsing was necessary since under otherwise equivalent conditions, the glucose yields of sawdust exposed only to H_2O_2 (2.2 M) were lower than those of sawdust exposed only to deionized water. These results suggested that the excess hydrogen peroxide and/or Mn inhibited the enzymatic activity involved in cellulose hydrolysis. Decomposition of H_2O_2 triggered by the addition of $(\text{NH}_4)_2\text{CO}_3$ reduced the enzyme inhibition, but the glucose yields measured in pretreated samples neutralized with $(\text{NH}_4)_2\text{CO}_3$ were still lower than those found in the controls for the same pretreatment time. In general, the presence of H_2O_2 and the Mn catalyst complicated the quantitative chemical analysis of the pretreatment products and the application of standard procedures that include HPLC and optical absorption measurements. Filtration and mass measurements were also complicated by the formation of precipitates following the neutralization with $(\text{NH}_4)_2\text{CO}_3$.

2.7. Enzymatic cellulose hydrolysis

Cellulases (Celluclast 1.5 L from *Trichoderma reesei*) and cellobiases (Novozyme 188 from *Aspergillus niger*) were obtained from Novozymes (Franklinton, NC). The weight ratios of Celluclast 1.5 L and Novozyme 188 to wood were 1% and 0.05%, respectively. The enzyme mixture of cellulases and cellobiases was prepared in 0.05 M sodium acetate buffer at pH 4.8. Five milliliters of enzyme solution was added to the six sawdust samples (500 mg each). The enzymatic hydrolysis took place in a water bath maintained at 55 °C for 24 h. After hydrolysis, the broth was filtered using 0.2 μm Spartan-3 filters with Teflon membranes (Schleicher & Schuell BioScience, Keene, NH) before liquid chromatography/mass spectrometry (LC/MS) analysis.

2.8. Raman microscopy

Raman spectra and images were collected with the 776 nm laser line of a tunable Ti:Sapphire laser (Mira 900-P, Coherent, Palo Alto, CA) coupled with a 532 nm solid state laser (Millenia V8s, Spectra-Physics, Mountain View, CA). The laser beam was focused to a line using a plano-convex BK 7 150 mm focal length cylindrical lens (CKX150AR.16, Newport Corp., Irvine, CA) and redirected to an inverted microscope (Carl Zeiss Axiovert 200, Göttingen, Germany) by a Raman edge dichroic (z785rdc, Chroma Technology, Rockingham, VT). At the sample, the laser line is about 1 μm wide and 100 μm long, with a total power of 80 mW at the sample. The sample was illuminated and the Raman signal was collected in a back-scattering geometry through the same C-apochromat 63 $\times$ water immersion objective (NA 1.2, Carl Zeiss). The Raman signal was focused onto the entrance of a Holospec f/2.2 spectrograph (Kaiser Optical Systems, Ann Arbor, MI) and filtered with a Holographic Super-Notch-Plus Filter (HSPF-785.0AR-2.0, Kaiser Optical Systems, Ann Arbor, MI). The signal was then dispersed with a holographic grating (HSG-785-LF, Kaiser) and imaged with a liquid nitrogen-cooled CCD camera (LN/CCD-1024E, Princeton Instruments, Trenton, NJ). The spectrometer was calibrated spectrally with 4-acetamidophenol, and spatially using a USAF-1951 resolution test target. The CCD images were corrected for CCD bias voltage, spherical aberrations, and the nonuniform power along the

focused laser line. Raman images were acquired line by line by moving the sample by 0.5 μm steps with an exposure time of 2 min for each line. Raman spectra were obtained by integrating CCD images spatially over the area of interest. Due to the weak signal-to-ratio for lignin, a linear background was subtracted from the integrated data. The exposure time for spectra is 30 s. Bright-field images were collected from an InfinityX-32 camera (Lumenera Corp., Ottawa, Canada) through the same objective.

2.9. Liquid chromatography/mass spectrometry analysis (LC/MS)

For quantitation of glucose, 400 μL aliquots of each sample were diluted with a 10 mM ($^{13}\text{C}_6$ 1,2,3,4,5,6- d_7 , M + 13) isotope enriched glucose standard solution in water such that the final concentration was 0.750 mM, 3 mM, and 6 mM in enriched glucose. A Thermo Scientific LTQ XL 2D linear quadrupole ion trap mass spectrometer interfaced with an electrospray ionization source and equipped with a Surveyor LC system was used. Separations were performed using a GlycoSep R HPLC column (4.6 mm $\times$ 150 mm) (ProZyme, California, USA). The injection volume was 10 μL . A 5 min linear gradient (acetonitrile 5–10%, 0.1% formic acid in water 88–83%, methanol 7%) was used at a flow rate of 500 $\mu\text{L}/\text{min}$. Retention times for both glucose and isotope enriched glucose were 2.9–3.4 min. After 5 min, the gradient changed to 83% acetonitrile to wash the column for 2 min, followed by a 3 min hold at original concentrations to re-equilibrate the column. Blank water runs were performed between each sample to ensure complete washing of the column. The mass spectrometer was operated in positive, single ion mode. The total ion count for the M + 1 peaks of glucose (m/z 181) and the isotope enriched glucose (m/z 194) were compared for each dilution concentration of each sample. The ratio of the two peaks, the known concentration of the isotope enriched glucose, and volume corrections were used to calculate the concentration of native glucose. The peak ratios were also compared using negative ion mode and found to be consistent across samples.

3. Results and discussion

To initially assess the ability of different Mn complexes to carry out the delignification of wood, multiple poplar wood sections of comparable sizes (50 μm thickness) were immersed in H_2O_2 solutions (2.2 M) containing one of the following complexes (50 mM): $\text{Mn}(\text{OAc})_3$, $\text{Mn}(\text{acac})_3$ (acac = acetylacetonate), MnO_2 , $\text{Mn}(\text{pic})_3$ (Hpic = picolinic acid), $\text{Mn}(\text{pic})(\text{dipic})(\text{H}_2\text{O})$ (Hdipic = dipicolinic acid), $\text{NMe}_4[\text{Mn}(\text{opba})(\text{H}_2\text{O})_2]$ (opba = o-phenylene-bis(oxamate)) and $\text{Mn}(\text{malt})_3$ (malt = maltol). Optical microscopy showed that only the solution containing $\text{Mn}(\text{OAc})_3$ caused visible degradation of the lignocellulosic matrix of poplar at room temperature within a few days (Figs. 1 and S3 in Supplementary data). For the other complexes, the poplar sections kept their integrity even after 7 days, and the samples remained unchanged microscopically. Control experiments under identical conditions with H_2O_2 only (2.2 M) and H_2O_2 (2.2 M) with an acetate buffer (150 mM) also showed no visible changes to the wood structure. No visible changes were observed using other transition metal salts under identical conditions, including FeCl_2 , CuCl , $\text{Cu}(\text{OAc})_2$, MnSO_4 , NaVO_3 , or $\text{Co}(\text{OAc})_2$ (see Supplementary data).

A microscopic study of the wood structure immersed in the $\text{Mn}(\text{OAc})_3/\text{H}_2\text{O}_2$ solution showed that the poplar section broke down into smaller fragments after 2 days at room temperature without any agitation. The fragments became visibly smaller, and after 5 days, the poplar section turned into a fine solid residue at the bottom of the vial. With only mild manual agitation, the poplar section became fragmented in <1 day. The pH of the $\text{Mn}(\text{OAc})_3$ solution was 4.6 before the introduction of the sample and remained so after 7 days.

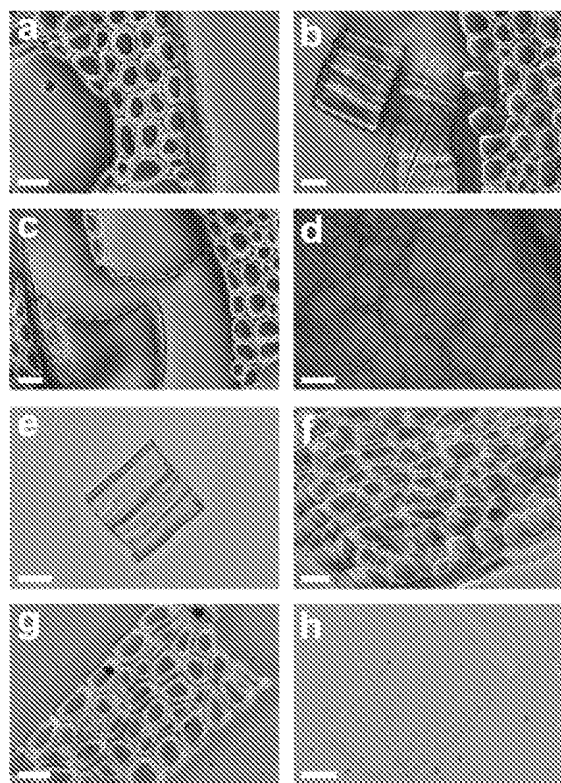


Fig. 1. Representative optical images of a poplar wood section after a treatment of (a–c) 1 day, (d) 2 days, (e, f) 3 days, (g) 5 days, and (h) 7 days with an aqueous solution of manganese(III) acetate and H_2O_2 at room temperature. The sample was imaged while immersed. The scale bar is 20 μm .

After a one-day treatment and mild manual agitation, the lignin-rich middle lamella between cells and the cell corners became darker and wider (Fig. 1a). The poplar section was cleaved along the ray parenchyma cells (Fig. 1a), the middle lamellae (Fig. 1b), and vessels (Fig. 1c). Rows of cells, as well as individual cells, became free from large fragments and laid on their side on the Petri dish (Fig. 1b). In some areas, loose material from the wood decomposition covered entirely the underlying wood structure (Fig. 1d). Some bubbling was also observed, particularly around cells damaged by mechanical action (cutting and manipulation during sample preparation), and along the ray parenchyma cells (see Videos in Supplementary data). After 3 days, only a few large fragments with more than several hundred cells remained (Fig. 1e–h). Isolated cells and short rows of cells were commonly seen (Fig. 1e). Although the middle lamellae between cells became looser and wider (Fig. 1f and g), the cellulose-rich cell walls remained relatively intact even after 5 days. However, after 7 days, even individual cells were visibly degraded (Fig. 1h). At this time, the sample was left to dry in air and microscopic images revealed a seemingly amorphous film covering the bottom of the Petri dish as shown in Fig. 1d.

To characterize the chemical degradation, Raman images were collected from the poplar section before and after a one-day catalytic treatment (Fig. 2). Two representative cells were selected from two different areas on the sample. The middle lamellae and cell corners in Fig. 2b, collected on a dried treated sample, appeared wider than those before treatment (Fig. 2a), and even more than those in Fig. 1a–c that were collected on a sample immersed in the catalyst solution. Raman images of cellulose and lignin were obtained by integrating over the wave number ranges that included the 1096, 1121, 1152 cm^{-1} bands from cellulose, and the 1602 and 1620 cm^{-1} bands from lignin (Lucas et al., 2010, 2011).

An overall decrease in the cellulose and lignin Raman signal was observed after the one-day treatment. This decrease is not attrib-

able to a thinning of the sample, since the laser penetration depth ($<6 \mu\text{m}$) was smaller than the sample thickness (50 μm) (Lucas et al., 2010). The catalytic degradation was most pronounced in the lignin-rich middle lamellae and cell corners. After treatment, the lignin signal was barely above noise level (Fig. 2f), with only traces remaining in the secondary cell wall layers closest to the lumen. The cellulose signal remained strong after treatment in the cellulose-rich secondary cell wall. In addition to the Raman images, Raman spectra were also collected on multiple dry poplar cells and the solid residues of treated wood (Fig. 3). The dry wood exhibited the cellulose and lignin bands imaged in Fig. 2, as well as bands located between 1270 and 1380 cm^{-1} , previously assigned to various stretching vibrations in cellulose and lignin (Lucas et al., 2010, 2011). Raman spectra of solid residues remaining upon evaporation of the pure catalyst solution exhibited a band around 940 cm^{-1} attributed to the acetate anion (Wang et al., 2005). After treatment, Raman spectra were collected from the remaining solid residues. The amorphous film, as shown in Fig. 1d, exhibited Raman bands from $\text{Mn}(\text{OAc})_3$ and cellulose. The dried treated poplar fragments (Fig. 1e and h) still exhibited strong cellulose bands at 1096 and 1121 cm^{-1} , while the lignin bands at 1602 and 1620 cm^{-1} and the bands located between 1270 and 1380 cm^{-1} became absent. Raman spectra and images confirmed the delignification and the loosening of the lignocellulosic matrix observed in Fig. 1 that eventually led to the fragmentation of the sample. Raman spectra of treated poplar showed that the delignified wood product became enriched in cellulose with little detectable lignin. The small cellulose signal decrease could indicate a loss of material or a loss in crystallinity (Schenzel et al., 2005).

To assess the pretreatment effect on biomass conversion, poplar sawdust pretreated with $\text{Mn}(\text{OAc})_3$ (50 mM) and H_2O_2 (2.2 M) for 2, 4 and 7 days was subjected to enzymatic hydrolysis for 24 h at 55 $^{\circ}\text{C}$. Poplar sawdust exposed for the same durations to water only were also prepared as controls. Before the enzymatic treatment of the wood, the excess solution containing H_2O_2 and Mn was removed and the solid residue was immersed in 10 mL of deionized water for 24 h. All samples, including controls, were rinsed five times with deionized water. The glucose concentration in the broth resulting from enzymatic hydrolysis was determined by LC/MS analysis. The glucose yield for the controls only exposed to water increased slightly with exposure time (Fig. 4). This can be partially explained by the overall expansion of wood cells in water, which can improve access of enzymes (Lucas et al., 2010, 2011). For the rinsed pretreated samples, the glucose yield was consistently higher than that of the controls, showing an improvement ranging from 20% to 40%. The improved glucose yield observed after a two-day pretreatment means that longer pretreatments of several days are unnecessary for this pretreatment to have a positive effect on biomass conversion. It is important to remark at this point that, for the pretreated samples, the aqueous solutions removed from the samples during rinsing contained an unknown amount of white insoluble solid residue, mostly constituted of individual delignified wood cells and amorphous cellulose, similar to those shown in Fig. 1d,e,h. In contrast, the solutions removed from the controls were clear. This material loss potentially reduced the amount of delignified wood and cellulose available for hydrolysis. The improved yields mentioned above therefore underestimate the pretreatment effect on biomass conversion.

Several different mechanisms are possible for the Mn-catalyzed reaction. Previous studies of the oxidation of arylglycerol β -aryl ether lignin dimers by Mn-peroxidase proposed both single electron transfer and hydrogen atom abstraction pathways (Henry, 2003; Kurek and Gaudard, 2000; Reddy et al., 2003; Srebotnik et al., 1997). Detailed studies suggest that hydride transfer, electron transfer, and hydrogen atom transfer pathways are all possible in the reactions of high-valent Mn-oxo dimers (Larsen et al.,

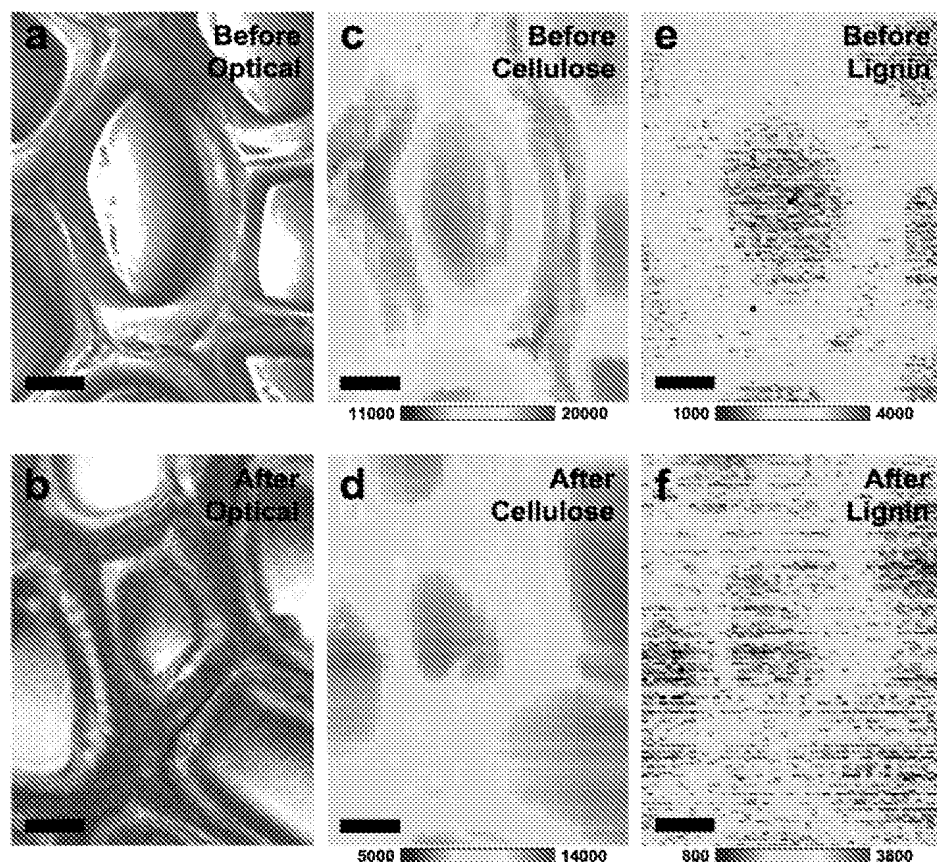


Fig. 2. Comparison between a poplar wood section before (top row) and after (bottom row) a one-day treatment with an aqueous solution of manganese(III) acetate and H_2O_2 at room temperature: (a, b) Optical microscopy images, Raman microscopy images of (c, d) cellulose and (e, f) lignin. The sample after treatment was left to dry overnight before imaging. The scale bar is 5 μm .

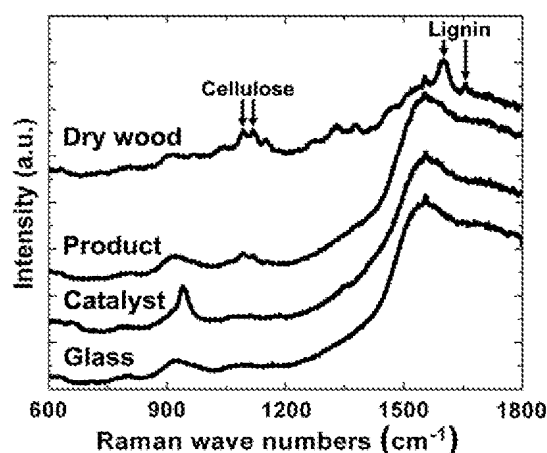


Fig. 3. Representative Raman spectra of a dry poplar section, a solid residue of treated wood (Product, as seen in Fig. 2e and h), a solid residue from an evaporated catalyst solution, and the glass substrate.

2002). Hydrogen atom abstraction was also proposed for oxidations of alkyl aromatics by Mn(IV) and Mn(V) oxo porphyrins (Arunkumar et al., 2009). A number of other mechanisms have been invoked for Mn-based oxidations, including oxo-transfer from a high-valent Mn complex, which is commonly proposed in olefin epoxidation and other oxidation reactions. Notably, Mn(II) salts were recently found to catalyze the epoxidation of olefins in acetonitrile/water solution using peracetic acid as the oxidant (Ho et al., 2008). Alternatively, a radical mechanism could be oper-

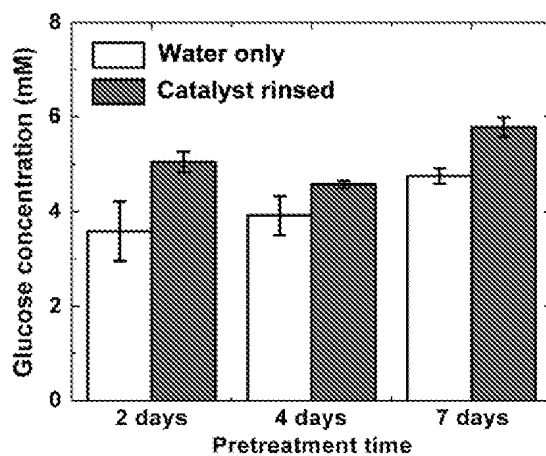


Fig. 4. Glucose concentration obtained after enzymatic hydrolysis as a function of pretreatment time with manganese(III) acetate. Each sample was split into three aliquots and the glucose concentrations are the average values from three repeat LC/MS analyses. The error bars represent standard deviations.

ative for the wood oxidation. Sawyer and others found that several Mn complexes, including Mn(salen) , catalyze oxygenated Fenton-type chemistry, activating O_2 in the presence of $t\text{BuOOH}$ to oxidize hydrocarbons via radical intermediates (Caudle et al., 1996; Sawyer et al., 1996). Concurrently, recent work involving treatment of spruce wood with several iron salts under acidic conditions showed that Fenton-type chemistry can degrade the lignocellulose matrix, producing fragments rich in cellulose (Arantes et al., 2009). However, a combination of MnCl_2 and 2,3-dihydroxy-

benzoic acid was found to be ineffective at inducing delignification under the same conditions (Arantes et al., 2009).

Due to the variety of pathways known for Mn-based oxidations, further experiments were carried out in attempt to gain insight into the reaction mechanisms. Upon dissolving $\text{Mn}(\text{OAc})_3$ in water, a dark brown precipitate formed immediately, presumably due to the disproportionation of $\text{Mn}(\text{OAc})_3$ in the absence of excess acetic acid (Davies, 1969; Önnnerud et al., 2002). Slow addition of H_2O_2 to the mixture resulted in initial bubbling accompanied by dissolution of the solid, forming a clear, colorless solution of pH 4.6. The absence of color suggests that $\text{Mn}(\text{II})$ is the dominant species in solution (Davies, 1969); both $\text{Mn}(\text{III})$ and $\text{Mn}(\text{IV})$ have been reported to be reduced to $\text{Mn}(\text{II})$ by H_2O_2 under acidic conditions (Davies et al., 1968; Wells and Mays, 1968). Consistent with this idea, a solution of $\text{Mn}(\text{OAc})_2$ (50 mM), HOAc (100 mM), and H_2O_2 (2.2 M) showed a catalytic activity similar to the original solution prepared from $\text{Mn}(\text{OAc})_3$ (50 mM) and H_2O_2 (2.2 M). Furthermore, identical UV–Vis spectra were obtained from an aqueous solution prepared from $\text{Mn}(\text{OAc})_3$ and H_2O_2 (2 eq.) and an aqueous solution prepared from $\text{Mn}(\text{OAc})_2$, HOAc (1 eq.), and H_2O_2 (1.5 eq.) (see Fig. S2 in Supplementary data).

The pH of the solution was critical for the catalytic activity. At higher pH, a solution of $\text{Mn}(\text{OAc})_2$ and H_2O_2 (unbuffered at pH ≈ 6.0), underwent rapid, vigorous bubbling due to catalytic disproportionation of the H_2O_2 . A similar reaction was observed upon raising the pH of the solution prepared from $\text{Mn}(\text{OAc})_3$ and H_2O_2 above pH ≈ 6 by addition of sodium carbonate or sodium phosphate buffer. This catalase-type reactivity of Mn is well known (Bodini and Sawyer, 1976) and resulted in complete consumption of the H_2O_2 within several hours at room temperature, preventing the delignification activity. Although vigorous disproportionation of H_2O_2 was observed, no reaction of the wood occurred when a microtome of wood was treated with a solution of $\text{Mn}(\text{OAc})_3$ at pH 6.5 (in Na_2HPO_4 buffer), suggesting that intermediates generated by the disproportionation of H_2O_2 are not responsible for the wood oxidation. In contrast, the H_2O_2 was consumed much more slowly in a solution of $\text{Mn}(\text{OAc})_3$ (50 mM) at pH 4.6, diminishing from an initial concentration of 2.2 M to approximately 1.1 M only after 7 days (see Fig. S1 in Supplementary data).

Detailed studies of the pH dependence of the catalytic reaction were complicated by the finding that the catalytic reaction was affected by the presence of other types of ions in solution. For instance, carrying out the reaction with added NaCl (0.2, 1, and 2 M) resulted in slower fragmentation of the wood. Likewise, increasing the concentration of acetate buffer (to 0.2, 0.5, 1, and 2 M) also resulted in a qualitative decrease in the observed fragmentation rate. No delignification was observed using $\text{Mn}(\text{OAc})_3$ and H_2O_2 in NaH_2PO_4 buffers (0.5 M, pH ~ 1.6 or 2.5). As discussed above, rapid disproportionation was observed upon addition of H_2O_2 to a solution of $\text{Mn}(\text{OAc})_3$ in Na_2HPO_4 buffer (0.5 M, pH ~ 6.5) or Na_2CO_3 buffer (0.5 M, pH ~ 9.9).

Overall, although the exact mechanism of the catalytic oxidation remains under investigation, both a radical Fenton-type pathway and a pathway involving oxo transfer from a high-valent Mn intermediate would be consistent with the observed delignification reaction. Given the complex heterogeneous nature of the wood substrate, the delivery of the catalytic reaction mixture inside the compact wood structure may also be a limiting factor in the delignification that needs to be investigated.

4. Conclusions

$\text{Mn}(\text{OAc})_3$ showed a remarkable activity for the delignification of wood at room temperature. Glucose yields following enzymatic hydrolysis were 20% to 40% higher in poplar sawdust pretreated

with $\text{Mn}(\text{OAc})_3$ for 2, 4, and 7 days at room temperature than those in sawdust exposed to water only for identical durations. The discovery of this mild, inexpensive pre-treatment offers the potential to facilitate biofuel production by efficient removal of lignin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2012.05.086>.

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The mechanism and applicability of in situ oxidation of trichloroethylene with Fenton's reagent

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Abstract

Fenton's reagent is the result of reaction between hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}), producing the hydroxyl radical ($\bullet\text{OH}$). The hydroxyl radical is a strong oxidant capable of oxidizing various organic compounds. The mechanism of oxidizing trichloroethylene (TCE) in groundwater and soil slurries with Fenton's reagent and the feasibility of injecting Fenton's reagent into a sandy aquifer were examined with bench-scale soil column and batch experiment studies. Under batch experimental conditions and low pH values (~ 3), Fenton's reagent was able to oxidize 93–100% (by weight) of dissolved TCE in groundwater and 98–102% (by weight) of TCE in soil slurries. Hydrogen peroxide decomposed rapidly in the test soil medium in both batch and column experiments. Due to competition between H_2O_2 and TCE for hydroxyl radicals in the aqueous solutions and soil slurries, the presence of TCE significantly decreased the degradation rate of H_2O_2 and was preferentially degraded by hydroxyl radicals. In the batch experiments, Fenton's reagent was able to completely dechlorinate the aqueous-phase TCE with and without the presence of soil and no VOC intermediates or by-products were found in the oxidation process. In the soil column experiments, it was found that application of high concentrations of H_2O_2 with addition of no Fe^{2+} generated large quantities of gas in a short period of time, sparging about 70% of the dissolved TCE into the gaseous phase with little or no detectable oxidation taking

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place. Fenton's reagent completely oxidized the dissolved phase TCE in the soil column experiment when TCE and Fenton's reagent were simultaneously fed into the column. The results of this study showed that the feasibility of injecting Fenton's reagent or H_2O_2 as a Fenton-type oxidant into the subsurface is highly dependent on the soil oxidant demand (SOD), presence of sufficient quantities of ferrous iron in the application area, and the proximity of the injection area to the zone of high aqueous concentration of the target contaminant. Also, it was found that in situ application of H_2O_2 could have a gas-sparging effect on the dissolved VOC in groundwater, requiring careful attention to the remedial system design. Published by Elsevier Science B.V.

Keywords: Fenton's reagent; In situ chemical oxidation; Groundwater; Soil; Trichloroethylene

1. Introduction

The in situ chemical oxidation technology uses chemical oxidants that directly destroy dissolved contaminants in a subsurface medium. This results in an increase in the concentration gradient and mass transfer processes between zones of high contaminant concentration and the groundwater containing the oxidant. Because the process is in situ, no surface treatment system for the contaminants is needed and there is no treated groundwater to be discharged. Trichloroethylene (TCE) is a dense non-aqueous phase liquid (DNAPL) with very low water solubility (~ 1100 mg/l at saturation) [1]. Residual TCE may exist in low permeable zones of the subsurface soil as blobs, globules and lenses. Slow dissolution of residual TCE in groundwater and the slow diffusion of TCE in groundwater trapped in soil micropores may be limiting factors in remediating TCE contaminated sites.

Among various oxidants explored for in situ oxidation of contaminants, Fenton's reagent (a mixture of hydrogen peroxide and ferrous salt) has gained a great amount of attention, due to its strong oxidation capabilities and low environmental impacts [2]. Fenton's oxidation technology has been applied in pilot-scale and full-scale removal of TCE from soil and groundwater at several contaminated sites in the past few years. Some of these studies have claimed to be successful [3,4], some of them found the TCE concentration to rebound a few months after the treatment, and some showed the limitations of this technology caused by subsurface heterogeneity [5]. Weeks et al. studied the use of Fenton's reagent for degradation of TCE in aqueous systems and soil slurries [6]. They used a constant aqueous concentration of 60 mg/l for TCE. However, they did not investigate Fenton's reaction in soil columns and the existing literature does not provide an in-depth understanding of the role of Fenton's reagent in the subsurface oxidation process.

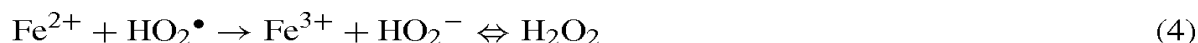
In this study, column experiments were designed to investigate: (1) the decomposition rate of H_2O_2 in a heterogeneous sandy soil under continuous flow conditions, (2) the possibility of oxidizing dissolved TCE with soil induced Fenton-type reaction, and (3) the oxidation of aqueous-phase TCE with Fenton's reagent in soil columns packed with undisturbed field samples. The soil induced Fenton-type reaction may be described as the reaction between H_2O_2 and Fe^{2+} ions naturally present in soil for the generation of hydroxyl radicals. In general, the redox potential (Eh) for deep groundwater is usually sufficiently low that, for a pH of less than about 8, iron is present as the soluble Fe^{2+} ion [7]. Therefore, presence of H_2O_2 in soils with sufficient iron content may produce a Fenton-type reaction. Batch

experiments were designed to study the competing capabilities of TCE and soil for Fenton's reagent or hydroxyl radicals.

2. Chemistry of Fenton's reagent

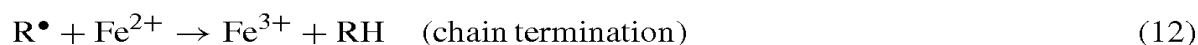
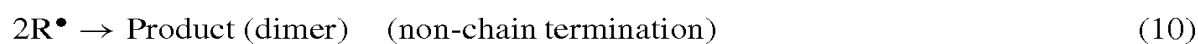
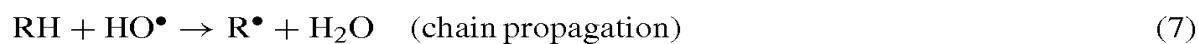
Fenton's reagent is a mixture of hydrogen peroxide (H_2O_2) and ferrous salt. It produces hydroxyl radicals that are strong oxidizers. Laboratory researchers have shown that Fenton's reagent is capable of oxidizing several chlorinated solvents in aqueous solutions [8,9] including TCE and tetrachloroethylene (PCE) in soil slurries or soil columns [10–12]. It is, therefore, reasonable to presume that Fenton's reagent may have a high potential for in situ applications.

Fenton's reagent is not stable. Once H_2O_2 and Fe^{2+} are mixed, several reactions take place simultaneously. These reactions produce hydroxyl radicals ($\text{HO}^\bullet$), hydroperoxyl radicals ($\text{HO}_2^\bullet$), Fe^{3+} , and O_2 (Eqs. (1)–(6)).



Although hydroxyl radicals, hydroperoxyl radicals, hydrogen peroxide, and oxygen are all oxidants, hydroxyl radicals have the strongest oxidation capability, and are considered responsible for oxidizing various organic compounds.

The mixture of organics and Fenton's reagent results in additional reactions, (Eqs. (7)–(12)) [13,14]. Hydroxyl radicals react strongly with the dissolved organic compounds, H_2O_2 , and Fe^{2+} ions in solution. They also react with other hydroxyl radicals themselves. The reaction rates of hydroxyl radicals are much faster than the generation of these radicals, which gives them a very short life span. Oxidation of the organics initially depends on the selectivity of the hydroxyl radicals for the target organic compounds and then on the selectivity and availability of these radicals to propagate the reaction.



Groundwater constituents and the chemistry of the soil surface may complicate Fenton's chemistry in the oxidation process. This effect was seen in the soil oxidant demand (SOD) when oxidation in DI water, groundwater, soil slurries, and soil columns were compared. During the in situ oxidation process, it is desirable to have little or no SOD, so that oxidants can effectively be distributed throughout the treatment zone. The soil-buffering capacity is an important factor to be considered. This is because where ferrous ions are present the optimal pH for Fenton's reagent for efficient reaction is in the range of 2–4. In order to achieve low pH conditions, soils may need to be acidified. Miller and Valentine conducted a study to investigate the oxidation behavior of quinoline and nitrobenzene in aqueous solutions in the presence of H_2O_2 and aquifer material (soil particle sizes of 0.18–0.84 mm) used as a filter media. They observed that reduction of pH in the filter media with acid-hydroxylamine reduced the manganese concentration by 80% and prolonged the lifetime of H_2O_2 . Manganese oxides have been shown to be effective catalysts for the decomposition of hydrogen peroxide [15].

3. Experimental procedure

Batch experiments were conducted in a zero-headspace reactor (Fig. 1). The reactor was a 100 ml airtight glass syringe (Hamilton part #: 86346) with a Teflon plunger to ensure that reactions were taking place under zero-headspace conditions. A Luer-lock valve (Hamilton part #: 35083) was connected to the syringe with a double-hub needle to facilitate the collection of samples.

Four different experiments in zero-headspace batch reactors were conducted (Table 1). Experiment FTG-B was conducted to study the oxidation of TCE in groundwater. Experiments FTS-B2, and FTS-B3, were conducted to study the impact of soil on the oxidation of TCE. Experiment FTS-B1 was conducted as a control with no addition of TCE. The initial pH levels of all the above experiments were controlled at 3 ± 0.05 with addition of appropriate quantities of 5 M sulfuric acid solution. Groundwater used for the experiments was taken from a site located in North Haven, Connecticut and was tested according to USEPA method-8260B for analysis of volatile organic compounds in soil and water samples, to ensure that it was free of chlorinated solvents or any of the other 60 target VOCs listed in that method [16].

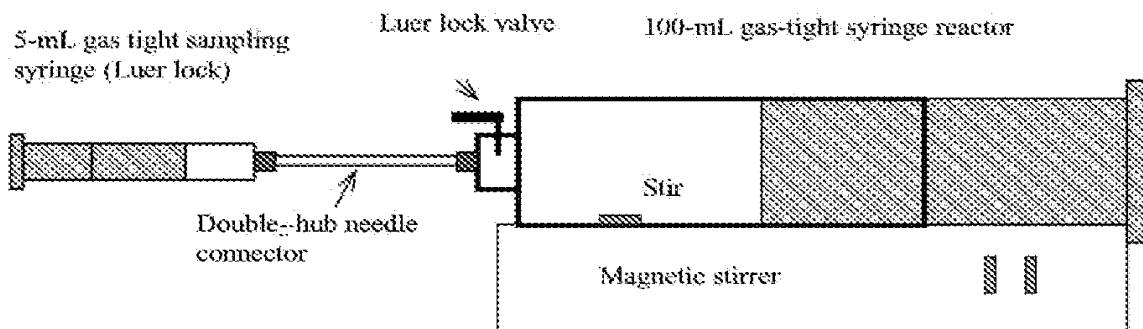


Fig. 1. Schematic of the zero-headspace batch reactor.

Table 1

Experimental conditions for Fenton's reagent oxidizing TCE in groundwater and soil slurries^a

Experiment (ml)	Groundwater (mg/l)	(H ₂ O ₂) ₀ (mg/l)	(Fe ²⁺) ₀ (mg/l)	(TCE) ₀ (mol:mol)	H ₂ O ₂ :Fe ²⁺ (g:g)	Solution:soil
FTG-B	~90	520	15	50	57.1:1	N.A.
FTS-B1 (control)	~90	1000	30	0	54.9:1	10:1
FTS-B2	~80	1000	30	98	54.9:1	10:1
FTS-B3	~45	1000	30	416	54.9:1	10:1

^a F: Fenton's reagent; T: TCE; G: groundwater; S: soil; N.A.: not applicable.

3.1. Column experiments

In order to assess the feasibility of Fenton's reagent application as an in situ oxidant for subsurface remediation, experiments were conducted in stainless steel columns with the dimensions of 76 mm (3 in.) long and an internal diameter of 43 mm (1.7 in.) (Fig. 2). Due to the short life span of H₂O₂, the column dimensions were chosen to allow the breakthrough of hydrogen peroxide solution in the columns. Undisturbed soil taken from soil cores was placed inside the experimental column. Duplication of column studies was not possible because natural undisturbed soil has a heterogeneous character and it was not possible to set-up two columns with identical soil conditions.

The chemical feed system consisted of a glass bottle for H₂O₂ stock solution, a Tedlar[®] bag containing TCE solution or TCE and Fe²⁺ stock solution, Teflon[®] tubing for the delivery of TCE and H₂O₂, and a peristaltic pump. All chemicals used were of reagent grade.

The soil column was prepared by inserting a 43 mm i.d. × 76 mm long stainless steel pipe into an aluminum core (minimizing soil disturbance) containing uncontaminated soil that was taken from a site located in North Haven, Connecticut (Table 2).

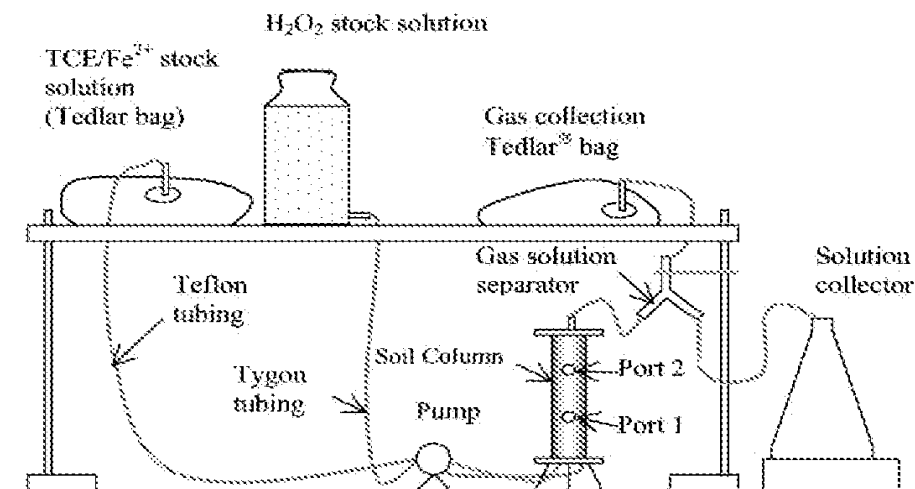


Fig. 2. Schematic of the 76 mm column set-up.

Table 2
Characteristics of the sandy soil used in the experiments

Parameter	Value
Moisture content ^a (saturated conditions) (%)	17–27
Porosity	~0.3
Organic carbon (%)	~0.02
Chromium (Cr) (μg/g)	24–40
Copper (Cu) (μg/g)	17–27
Iron (Fe) (μg/g)	11049–22111
Magnesium (Mg) (μg/g)	501–1603
Manganese (Mn) (μg/g)	281–541

^a Weight of moisture/weight of dry soil.

Soil was tested according to USEPA method-8260B for analysis of volatile organic compounds in soil and water samples to ensure that there was no trace of chlorinated solvents or any of the other 60 target VOCs listed in that method was present in the study soil [16]. The stainless steel column was then mounted on a tripod (Fig. 2). At each end of the soil column, a stainless steel screen and a thin layer of glass beads were added to retain soil in the column under flow conditions. Two small holes (ports 1 and 2) were drilled through the side of the column to facilitate the mounting of the sample collection fittings. Samples were collected periodically from the inlet, port 1, port 2, and the outlet using a 5 ml airtight syringe (Hamilton part # 81520).

The effluent collection system consisted of a Y-shaped connector serving as a water-gas separator, a piece of Teflon[®] tubing bent into a U-shape form to maintain the water level in the Y-shaped connector, a Tedlar[®] bag to collect the gas, and a volumetric flask to collect the effluent (Fig. 2).

Three sets of experiments were conducted with the column apparatus. The first experiment was designed to assess the soil oxidant demand under continuous flow conditions. The decomposition of H₂O₂ solutions was studied at concentrations of 0.1, 1, and 10 g/l. The pH level of influent groundwater without adjustment was measured to be 7 ± 0.05 . The groundwater used for the experiments had a good buffering capacity and no pH adjustment was needed to attain a pH of 7. For H₂O₂ concentration measurements, samples were taken from sampling ports 1, 2, and the outlet.

In the second experiment, the possibility of oxidizing TCE with soil-induced decomposition of H₂O₂ (a Fenton-type reaction) was evaluated. A TCE solution at 250 mg/l and an H₂O₂ solution at 10 g/l were simultaneously pumped into the column at a flow rate of 0.15 ml/min. In order to maintain the natural soil conditions, the pH levels of the two solutions were not lowered. The concentrations of TCE, H₂O₂, and Cl⁻ from two sampling ports and from the effluent were measured over time. The generated gas was collected in a Tedlar[®] bag, and TCE concentrations were measured at the end of the experiment.

The third experiment was designed to study the oxidation of TCE in a mixture of TCE-contaminated groundwater and Fenton's reagent in a soil column. Two solutions, one containing 30 g/l H₂O₂ and the other containing 900 mg/l Fe²⁺ in groundwater, were pumped continuously into the column at a flow rate of 0.15 ml/min. A 5 ml aqueous

solution saturated with TCE was then injected into the Fe^{2+} solution line within 10 mm of the column inlet port. The pH level of the solution was lowered to about 3 with addition of appropriate quantities of 5 M sulfuric acid solution. The concentrations of TCE, Cl^- , and H_2O_2 in the effluent were measured over time (Fig. 2).

4. Chemical analysis

4.1. Volatile organic compounds (VOCs)

A gas chromatograph (Model HP-5890, Series II Plus) connected to a mass selective detector (HP-MSD 5971A) was used for the analysis of VOCs in the aqueous solution. A HP-3365 Series II (Chem-Station software version A.02.12) was used for quantification of VOCs. The column used was a capillary column, 60 m-long (two interconnected 30 m columns), 0.319 mm i.d., 0.50 μm (film thickness) DB-1, J&W Scientific. The initial oven temperature was set at 35°C for 1 min and raised to 180°C at 8°C/min, then held at 180°C for 15.48 min. The total run time was 34.6 min. Samples were introduced into the GC/MSD through a Tekmar LSC-2016 connected to a Tekmar-LSC-2000 automated purge and trap system. Samples were purged with high purity grade helium for 12 min.

4.2. Chloride (Cl^-)

Chloride concentrations were determined by a DIONEX series 4000 ion chromatograph (HPIC) equipped with a DIONEX Ionpac AS12A 4 mm column, an anion micro membrane suppressor, and a conductivity detector. The eluent used was 2.1 mM sodium carbonate (Na_2CO_3)/0.8 mM sodium bicarbonate (NaHCO_3). The flow rate of the eluent was set at 1.5 ml/min, the typical pressure was between 12400 and 13800 kPa (1800 and 2000 psi), and the background conductivity was about 24 μS . The area integration method was used to quantify the concentration of Cl^- .

4.3. Hydrogen peroxide (H_2O_2)

The H_2O_2 solution was prepared from a 30% stock solution and standardized using a titration method with potassium permanganate [17]. The standardized H_2O_2 solution was used to produce a standard curve of H_2O_2 with a spectrophotometric method using cobalt (Co^{2+}) and bicarbonate [14]. The H_2O_2 concentrations were analyzed with the spectrophotometric method. The detection limit of the method was ± 0.01 mg/l of H_2O_2 . The concentration range measured was between 0 and 1 mg/l, at a wavelength of 260 nm. The spectrophotometer used was a Milton Roy Spectronic 601.

4.4. Ferrous Iron (Fe^{2+})

Ferrous iron was analyzed by the standard 3500-Fe D-Phenanthroline method, at a wavelength of 510 nm. The same Milton Roy Spectronic 601, spectrophotometer used for H_2O_2 analysis was used for the analysis of Fe^{2+} .

4.5. Quality control/quality assurance

All analytical instrumentations were calibrated prior to sample analysis based on the instrument's standard operating procedure used at the Environmental Research Institute. Calibration verification (CV), blanks, duplicates, and spikes were used during the system operation to control the precision of the instruments. A CV is a standard prepared from a source which is different than the source used for the initial calibration. The acceptance criterion for CV was 70 to 130% recovery and all CV recoveries were within the acceptable range [18].

5. Results and discussion

5.1. Batch experiments

During the batch experiments, Fenton's reagent was able to completely dechlorinate aqueous-phase TCE with and without the presence of soil. About 90–100% of TCE was degraded in 5 h (Fig. 3), and 93–102% of the stoichiometric releases of Cl^- were detected (Table 3). During each experimental run, the pH of the solutions decreased from 3 to as low as 2.43 (Table 3). Decreasing pH values may have been caused by the generation of CO_2 or H^+ . This is in accordance with the findings of Weeks et al. [5] and Gates et al. [19]. The H_2O_2 solutions are acidic, and if Fenton-type reactions occur, production of H^+ due to reduction of Fe^{3+} may result into lower pH values [19]. No VOC intermediates or by-products were found in the oxidation process. The only peak observed in the generated

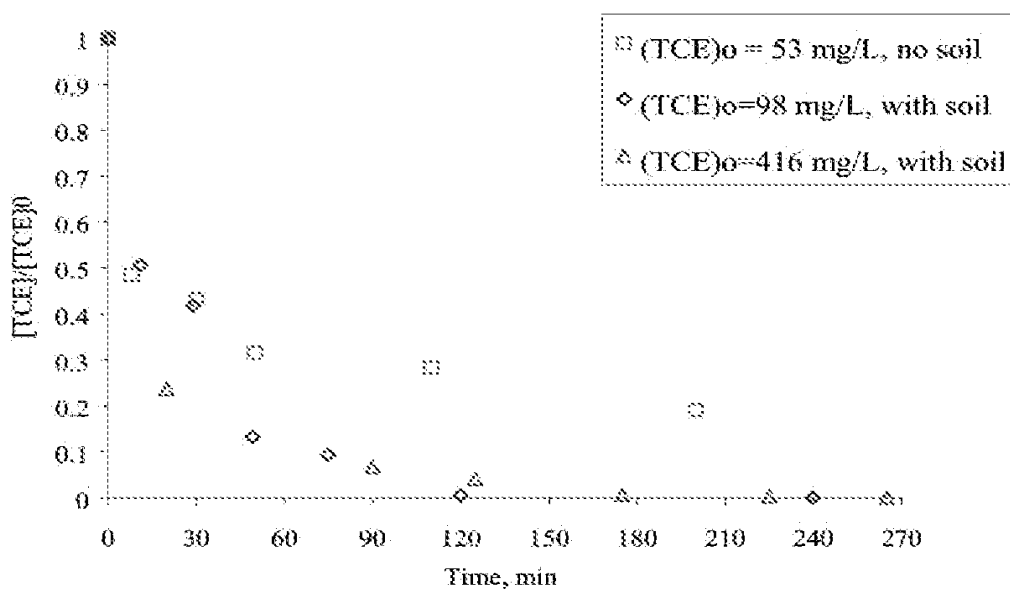


Fig. 3. TCE degradation in groundwater and soil slurries. Experimental conditions: zero-headspace reactors; $(\text{pH})_0 = 3$; for the experiment without soil, $(\text{H}_2\text{O}_2)_0 = 520 \text{ mg/l}$, $(\text{Fe}^{2+})_0 = 15 \text{ mg/l}$; for experiments with soil, $(\text{H}_2\text{O}_2)_0 = 1000 \text{ mg/l}$, $(\text{Fe}^{2+})_0 = 30 \text{ mg/l}$, soil:solution = 1:10 (g/g).

Table 3

Recovery rate of chloride and pH changes in the experiments on oxidation of TCE in groundwater and soil slurries^a

Experiment	Initial pH	Final pH	Cl ⁻ generated (mg/l)	Cl ⁻ predicted (mg/l)	Recovery rate (%)
FTG-B	3.01	2.77	44	43	102
FTS-B1	~7	6.5	N.A.	N.A.	N.A.
FTS-B2	3.0	2.71	73.7	79.3	93
FTS-B3	3.0	2.43	329	336.8	97.7

^a F: Fenton's reagent; T: TCE; G: groundwater; S: soil; B: batch experiment.

chromatograms of the GC/MSD chromatographic analysis was the TCE peak. Near the end of the first experiment (FTG-B), one sample was analyzed for total organic carbon (TOC). The concentration was lower than the detection limit, indicating no organic acid by-products were formed at high concentrations. Therefore, it is most likely that TCE was mineralized to CO₂, Cl⁻, and H⁺.

The soil iron content may have induced a Fenton-type reaction leading to the production of hydroxyl radicals. Therefore, in the presence of soil, TCE may have successfully competed for •OH against soil and other species (such as H₂O₂, Fe²⁺, and Fe³⁺) in the solution and was preferentially degraded.

In all of the three experiments, Fe²⁺ concentrations decreased to <0.1 mg/l within the first 30 min of the experiment. Oxidation of TCE continued afterward and was likely catalyzed by Fe³⁺ or regenerated Fe²⁺ (Eqs. (5) and (6)). The reaction rates were rapid in the first 100 min of the experiments and followed a slower trend afterwards. More than 56% of the TCE was destroyed within the first 30 min (Fig. 3). The observed overall degradation rate constant of TCE in soil slurries followed a pseudo first-order kinetic and ranged from 0.0283 to 0.033 min⁻¹ for aqueous TCE concentrations of 98 and 416 mg/l, respectively (Table 4).

Weeks et al. [6] found that the first-order model provided the best fit for change of concentrations of H₂O₂ and TCE as a function of time in their experimental studies. They also noted that the actual processes involved in the degradation of H₂O₂ and TCE are more complex than first-order model predictions. Degradation rate constants of TCE obtained in the batch experiments depended on the experimental conditions, such as the initial [H₂O₂]/[Fe²⁺] ratio and the initial H₂O₂ and Fe²⁺ concentrations. Reactions in the later stages may have been catalyzed by regenerated Fe²⁺ and possibly Fe³⁺. The degradation rate constants of H₂O₂ and TCE during the batch experiments are presented in Table 4. In

Table 4

Hydrogen peroxide and TCE degradation in aqueous and soil slurries

Experiment	Soil:water (w:w)	Initial H ₂ O ₂ (mg/l)	Initial Fe ²⁺ (mg/l)	Initial TCE (mg/l)	Initial pH	Final pH	KH ₂ O ₂ (min ⁻¹)	KTCE (min ⁻¹)
FTG-B	N.A.	520	15	53	3.010	2.77	N.A.	0.0057
FTS-B1 (control)	1:10	1000	30	0	3.000	2.72	0.0147	N.A.
FTS-B2	1:10	1000	30	98	3.000	2.71	0.0068	0.033
FTS-B3	1:10	1000	30	416	3.000	2.43	0.00378	0.0283

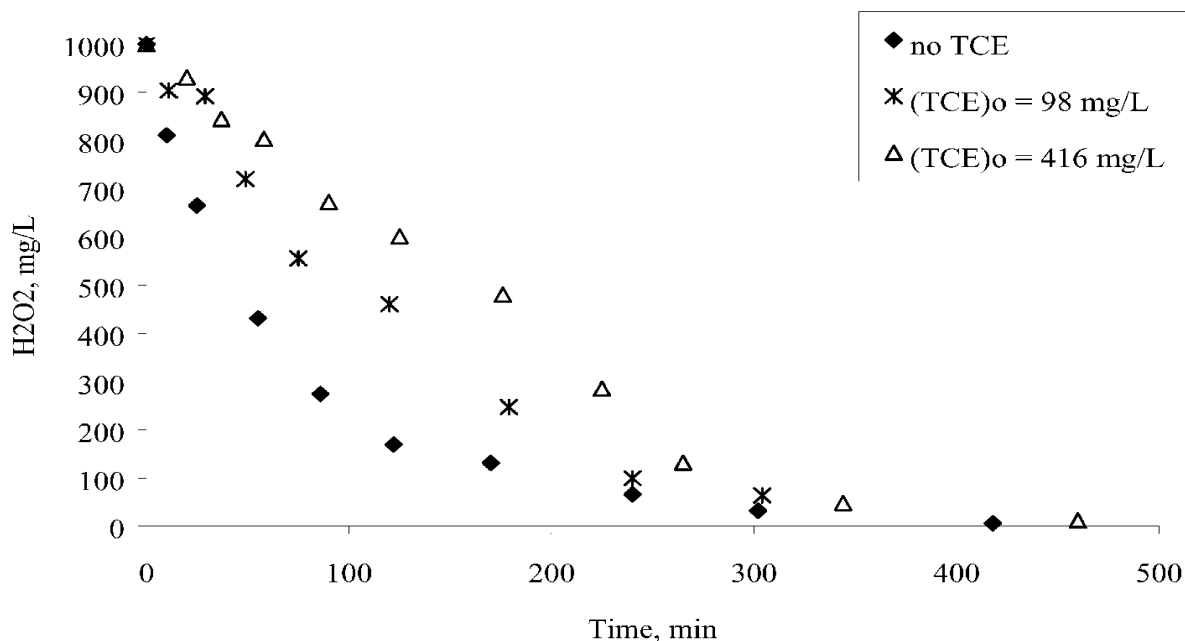


Fig. 4. The effect of TCE on H_2O_2 degradation in soil slurries. Experimental conditions: $(\text{H}_2\text{O}_2)_0 = 1000 \text{ mg/l}$; $(\text{Fe}^{2+})_0 = 30 \text{ mg/l}$; soil:solution = 1:10; total volume = 100 ml.

the experiments, oxidizing TCE in soil slurries (Fig. 4), higher initial TCE concentrations lowered the H_2O_2 degradation rates. At initial TCE concentrations of 0, 98, and 416 mg/l, the half-lives of H_2O_2 were 47, 102, and 165 min, respectively. In the experiment, oxidizing TCE in groundwater with no soil slurry (Fig. 5), the concentration of H_2O_2 only

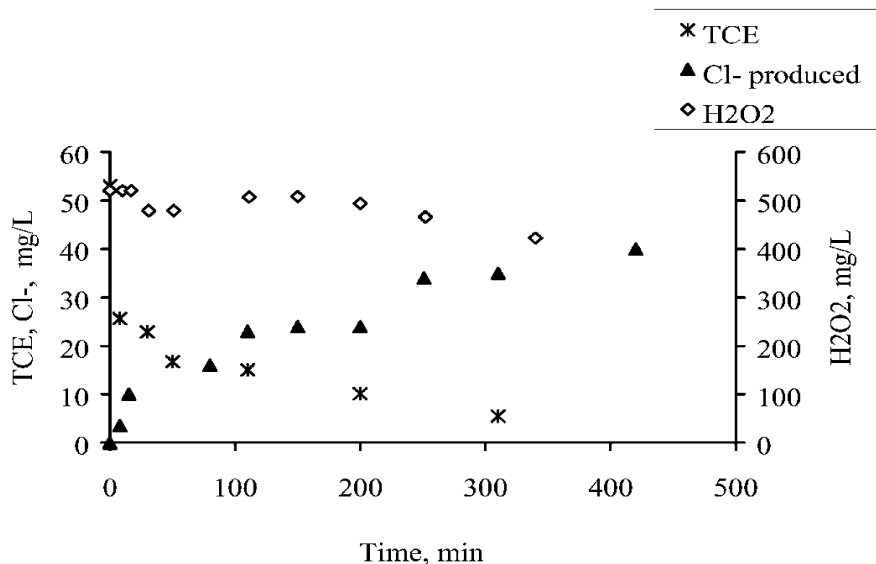
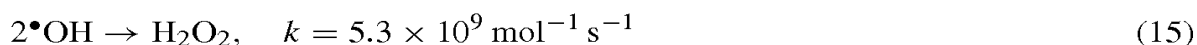
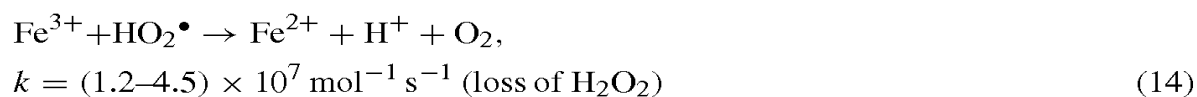
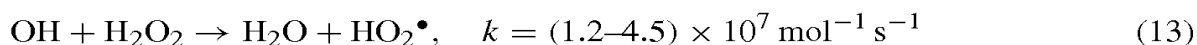


Fig. 5. Oxidation of TCE by Fenton's reagent in groundwater. Experimental conditions: $(\text{H}_2\text{O}_2)_0 = 520 \text{ mg/l}$; $(\text{Fe}^{2+})_0 = 15 \text{ mg/l}$; $(\text{TCE})_0 = 53 \text{ mg/l}$; total volume = 100 ml.

decreased by 10% throughout the experiment. Leung et al. [20] studied the oxidation of PCE, which has chemical properties very similar to those of TCE, with Fenton's reagent. They used the same method for H_2O_2 analysis; however, they did not run a control to compare the rates of H_2O_2 degradation with and without PCE. In the literature, many studies have mainly focused on the degradation of target compounds and have not provided data on H_2O_2 degradation. A control experiment (FTS-B1) was conducted to compare H_2O_2 degradation rates with and without the presence of TCE. The degradation rate constant of H_2O_2 decreased from 0.0147 (control-no TCE) to 0.00378 min^{-1} (initial TCE concentration = 416 mg/l).

The decomposition rate of H_2O_2 was slowed by TCE, and it appears that TCE competed better than other species in the mixture for $\bullet\text{OH}$ radicals. Walling [14] noted that various organic substrates could retard the decomposition of H_2O_2 by acting as $\bullet\text{OH}$ scavengers, so that less $\bullet\text{OH}$ reacts with H_2O_2 . The reaction rate constants [21], show that hydroxyl radicals prefer TCE to H_2O_2 ; therefore, when TCE is present, less H_2O_2 is consumed (Eqs. (13)–(16)).



This is an encouraging finding and implies that once Fenton's reagent is mixed with the TCE contaminated groundwater in the subsurface, H_2O_2 may persist until TCE is oxidized to a very low concentration. Scavenging of hydroxyl radicals by TCE is the first step of the oxidation process and it is possible that the intermediates will also scavenge the $\bullet\text{OH}$ radicals in the aqueous phase [6].

5.2. Column experiments

5.2.1. Soil oxidant demand

The results of H_2O_2 decomposition in a 76 mm column are given in Table 5. H_2O_2 had a very short life span in the sandy soil column. After 50 pore volumes, 10 g/l H_2O_2 groundwater solution could not break through 25 mm of sandy soil. Throughout the experiments, the H_2O_2 concentration was less than the detection limit (5 mg/l) at sampling ports 1, 2 and the effluent. The retention time in 25 mm of the soil column was 34 min (porosity = 0.3), implying that the life span of H_2O_2 was <34 min.

Iron and manganese contents of soil have been considered as potential causes of H_2O_2 decomposition [11,15,22]. For the soil studied, the iron and manganese contents were 12,950 and 306 mg/kg, respectively. High concentrations of iron and manganese in soil may explain the rapid H_2O_2 decomposition process.

Reducing the pH of the soil medium could decelerate the decomposition rate of H_2O_2 in the soil column. With influent H_2O_2 concentration of 15 g/l and a pH of 2.2, the effluent

Table 5
Decomposition of H_2O_2 in 76 mm soil column

Variables	Experiment		
	HC-1	HC-2	HC-3
Inlet H_2O_2 (mg/l)	100	1000	10000
Running time (day)	3.86	4.9	3
Effluent pH	7.4	7.4	7.4
Effluent Fe^{2+} ($\mu\text{g/l}$)	30	35	99
Retention time of port 1 (min)	34	34	34
H_2O_2 in port 1 (mg/l)	<5	<5	<5
H_2O_2 in port 2 (mg/l)	<5	<5	<5
H_2O_2 in effluent (mg/l)	<0.2	<0.2	<0.2

H_2O_2 concentration was about 150 mg/l after 10 days of steady flow through the column.

The sandy soil used in this experiment was a typical silica-based sandy soil found in Connecticut (Table 2). High oxidant demand was not expected for this type of soil, and the extremely rapid decomposition of H_2O_2 in this soil indicates that the decomposition of H_2O_2 in the subsurface could be a common problem for in situ application of Fenton's reagent.

The decomposition of H_2O_2 generates oxygen (O_2) [13]. The generation of gas was not observed when the inlet H_2O_2 concentration was 0.1 g/l, but gas started to form as the influent H_2O_2 concentration increased.

Overall, the fast decomposition of H_2O_2 in soils with high SOD causes two major concerns: first, a great portion of H_2O_2 may be consumed on the soil surface and the effectiveness of spreading H_2O_2 in the subsurface may require closer injection wells or higher injection H_2O_2 concentrations, and second, the gas generated in the subsurface may alter the flow path of groundwater or even the soil structure, especially when high H_2O_2 concentrations are applied. In a field study by Roberts and Bauer [5], 50% (by weight) H_2O_2 was injected under a pressure of 345 kpa (~ 50 psi) and caused soil fractures through the oxidation process. Therefore, relatively low H_2O_2 concentration and closer injection well may be a better choice for field applications.

5.2.2. Oxidation of TCE with soil-induced decomposition of H_2O_2

Although the decomposition of H_2O_2 was very rapid in the test soil, TCE was not degraded by the soil-induced decomposition of H_2O_2 . The total mass of TCE that eluted from the column was 103.85% of the total mass pumped into the column (Fig. 6), and throughout the experiment, the effluent and background Cl^- concentrations were the same. The mass balance of TCE shows that nearly 70% of the TCE was stripped into the gaseous phase (Table 6). Therefore, the decomposition of H_2O_2 induced by the test soil did not degrade TCE, and H_2O_2 degradation had a gas-sparging effect on the TCE-contaminated groundwater.

The gas-sparging effect of soil-induced decomposition of H_2O_2 on TCE can change the fundamental concept of in situ oxidation with Fenton's chemistry. The original purpose of

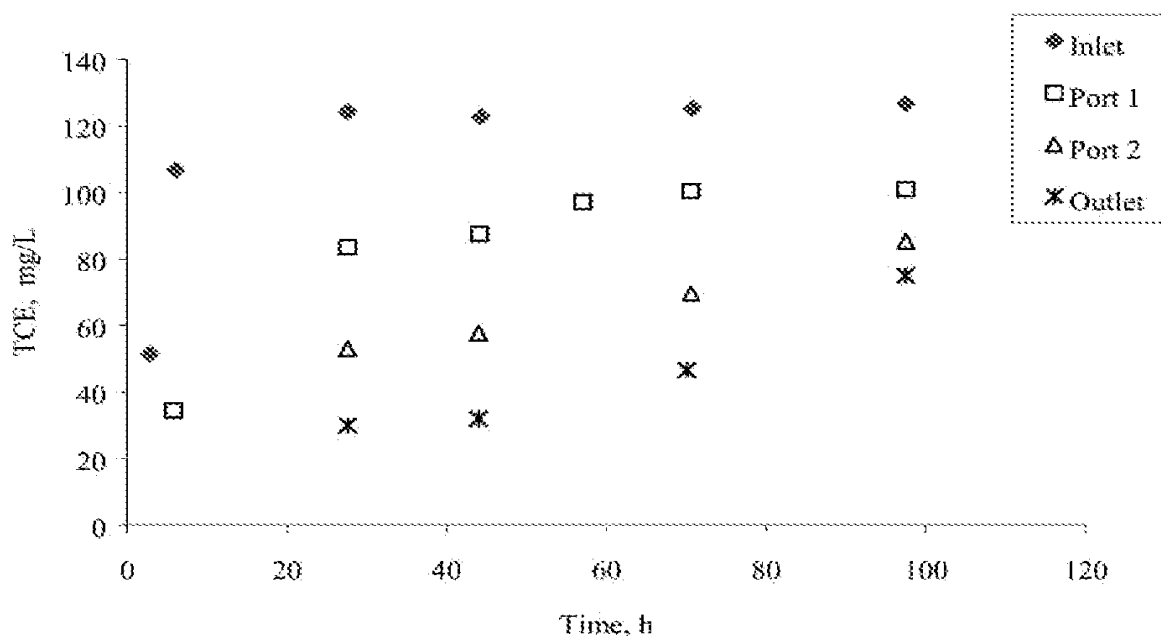


Fig. 6. Oxidation of TCE with H_2O_2 in 76 mm column. Experimental conditions: $(\text{H}_2\text{O}_2)_{\text{in}} = 10 \text{ g/l}$ at a flow rate of 0.15 ml/min , $(\text{TCE})_{\text{in}} = 250 \text{ mg/l}$ at a flow rate of 0.15 ml/min . The pH of both solutions was approximately 6.7.

using in situ Fenton's oxidation is to destroy the contaminants in the subsurface, instead it may act as a gas-sparging device for stripping of VOCs, especially in areas where ferrous ion does not reach the contaminated zone. Since, ferrous iron is not stable, after addition it will change to ferric iron relatively fast and may precipitate out of groundwater as $\text{Fe}(\text{OH})_3$ [5]. Therefore, the radius of influence of H_2O_2 from an injection well may not be the radius of influence of the Fenton's reagent. This gas-stripping effect on VOCs may also spread VOCs by vapor transport. Therefore, monitoring the possible pathway of vapor transport in the vadose zone may be necessary during the implementation of in situ oxidation technology with Fenton's reagent.

In some works related to pilot scale and full-scale in situ oxidation with Fenton's chemistry, an increase of VOC concentrations in monitoring wells was observed, but no calculations in regard to the total VOC mass in the vapor phase was presented and the radius of influences of ferrous iron was not reported either [23,5].

Table 6

TCE mass balance during the experiment "oxidation of TCE by H_2O_2 "

		Total TCE out (%)	Error (%)
TCE inflow to the column	207.35 mg		
TCE outflow in liquid phase	64.86 mg	30.12	
TCE outflow in gaseous phase	150.48 mg	69.88	
Total TCE out of column	215.34 mg		3.85

5.2.3. Oxidation of pulse-source TCE with Fenton's reagent

A solution of 30 g/l H_2O_2 at a flow rate of 0.15 ml/min and a solution of 900 mg/l Fe^{2+} ions at a flow rate of 0.15 ml/min were fed continuously and simultaneously into the 76 mm soil column from the two inlets located on the bottom of the column (the center of two inlets were 22 mm apart). The pH value of both influents was about 3. When the effluent concentration of H_2O_2 became stable, 5 ml of a TCE saturated aqueous solution (~ 1100 mg/l) was injected into the column through the Teflon[®] tubing at the ferrous iron solution line, (within ~ 10 mm of the column inlet) (Fig. 2). The effluent concentrations of TCE, Cl^- , and H_2O_2 were measured over time (Fig. 7). Results of oxidizing the pulse source TCE showed that TCE was completely oxidized in the column. The total Cl^- generated was 104.5% of the predicted amount. The difference could have resulted from the Cl^- analysis on the HPIC and in the measurement of the effluent volume. The results of this experiment indicated that TCE reacted with Fenton's reagent instantaneously as both solutions mixed in the sandy soil column. The generated gas was collected in a Tedlar[®] bag and analyzed for presence of any VOCs. Based on the GC-MS analysis of the gas, neither TCE nor the possible transformed TCE intermediates were stripped into the gaseous phase.

The results of this experiment indicated that Fenton's reagent was able to oxidize TCE even in high SOD soil. The lack of any TCE or other VOCs in the generated gas may point to the fact that Fenton's reagent was well mixed with the TCE-contaminated groundwater leading to a complete oxidation process. Pinpointing the location of TCE sources in the saturated zone can be an important factor in efficient application of Fenton's reagent because groundwater around the contamination source has the highest TCE concentration and delivering Fenton's reagent directly to the zone of highest contamination in high SOD soils can produce more efficient results.

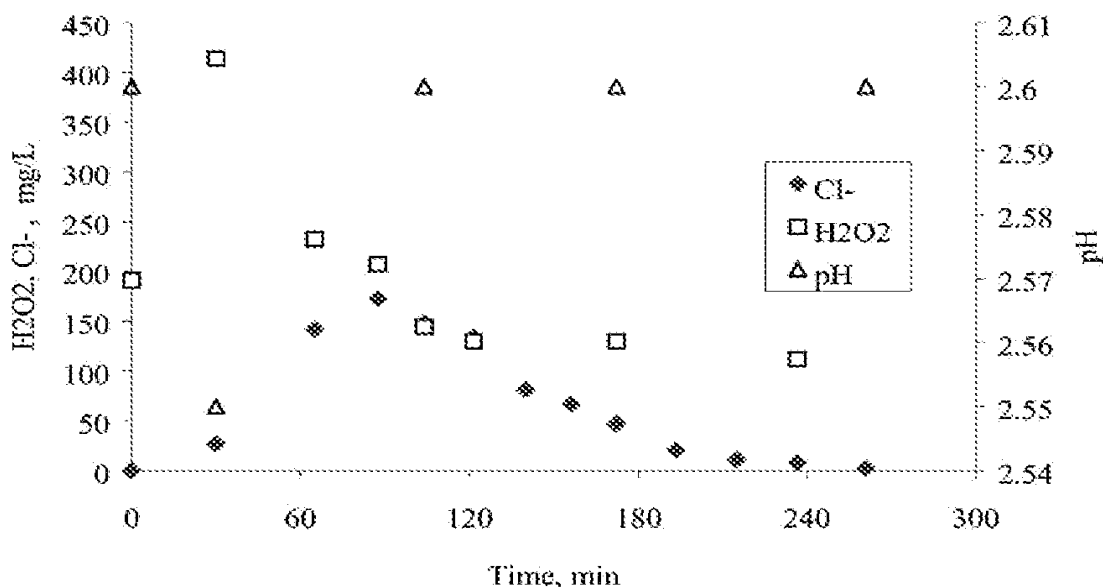


Fig. 7. Oxidation of a pulse source TCE in a 76 mm column. Experimental conditions: $(\text{H}_2\text{O}_2)_{\text{in}} = 30$ g/l at a flow rate of 0.15 ml/min; $(\text{Fe}^{2+})_{\text{in}} = 940$ mg/l at a flow rate of 0.15 ml/min. The pH level of both solutions was 3.

6. Conclusions

Rapid decomposition of H_2O_2 in some soils may limit the application of Fenton's reagent for in situ remediation of sites contaminated with chlorinated solvents. Prior to field application of Fenton's reagent, bench-scale column experiments may be performed to help in better understanding the decomposition patterns of H_2O_2 and the contaminants in the target soil. Determining the zone of influence of Fe^{2+} and H_2O_2 from the injection well is among the critical factors that need to be considered in real world field application of Fenton's reagent.

The soil-induced decomposition of H_2O_2 may not be capable of degrading VOCs, such as TCE. Delivering H_2O_2 and the ferrous salt solution to the zone of highest contaminant concentration and lowering the pH of the soil medium are important factors for successful in situ application of Fenton's reagent.

The use of high concentrations of H_2O_2 can generate a large amount of gas in a short period. In addition, Fenton's reagent produces an exothermic reaction that generates heat. The increased temperature of groundwater and the large amount of gas released from the reactions can strip VOCs into soil gas, creating new preferential pathways in the subsurface. Therefore, in field applications a relatively low H_2O_2 concentration and closer injection wells may produce better oxidation results than high concentration H_2O_2 and injection wells located further apart. Moreover, a careful chemical delivery and monitoring system should be established prior to the field application of Fenton's reagent.

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1	BLOCK, "Novel Activation Technologies for Sodium Persulfate In Situ Chemical Oxidation", Proceeding of the Fourth International Conference on the Remediation of Chlorinated and Recalcitrant Compounds, 2004.	☐
2	Innovative Environmental Technologies, Inc., "Zero Valent Iron Catalyzed Hydroxyl & Sulfate Free Radical Oxidation", PA, 2008.	☐
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L1 1404 OXIDIZ####(L)((VOLATILE (A) ORGANIC(A) COMPOUND) OR VOC)

=> s peroxide or persulfate or persulphate
L2 514366 PEROXIDE OR PERSULFATE OR PERSULPHATE

=> s (trivalent(3a)metal) or iron or ferric or manganese
L3 2234423 (TRIVALENT(3A) METAL) OR IRON OR FERRIC OR MANGANESE

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YOU HAVE REQUESTED DATA FROM 6 ANSWERS - CONTINUE? Y/(N):y

Appl. No. : 13/891,934 Confirmation No.: 8286
Applicant : Michael Scalzi
Filed : 05/10/2013
TC/A.U. : 1651
Examiner : BERKE-SCHLESSEL, DAVID W
Docket No. : SCA-004
Customer No. : 56184

Commissioner for Patents

P.O. Box 1450

Alexandria VA 22313-1450

REPLY UNDER 37 CFR § 1.111

Sir:

In response to the Office action of 10/31/2014, please amend the above-identified application as follows:

Amendments to the Claims are reflected in the listing of claims that begin on page 2 of this paper.

Remarks/Arguments begin on page 5 of this paper.

Listing of Claims:

1. (Currently Amended) A method for oxidizing ~~volatile organic compounds and~~
contaminants followed by a biological attenuation process in an environmental medium
containing one or more contaminants, the method comprising introducing into the
environmental medium the activation of peroxide or persulfate or a combination thereof
by the use of and one or more trivalent metals such that both oxidation of the one or more
contaminants occurs resulting in sulfate and metal to act as electron acceptors supporting
and biological attenuation of the one or more contaminants occurs.
2. (Original) The method of claim 1 wherein said environmental medium is soil or
groundwater.
3. (Original) The method of claim 1 wherein said persulfate is sodium, potassium, or
ammonium based.
4. (Currently Amended) The method of claim 1 wherein said environmental medium
contains source of said trivalent metals is indigenous or introduced as an oxidized
species from one or more previously introduced zero valent metals.
5. (Canceled)
6. (Currently Amended) The method of claim 1 wherein said one or more trivalent metals
act as activators for said persulfate so as to form sulfate free radicals.
7. (Currently Amended) The method of claim 1 wherein said one or more trivalent metals
are include Fe^{3+} .
8. (Currently Amended) The method of claim 1 wherein said one or more trivalent metals
are include Mn^{3+} .

9. (Original) The method of claim 1 wherein said biological attenuation process is accomplished as said environmental medium returns to a reducing condition.
10. (Original) The method of claim 9 wherein said biological attenuation process relies on facultative biological reactions.
11. (Currently amended) The method of claim 10 wherein said biological attenuation process utilizes evolved sulfate species and said one or more trivalent metals as terminal electron acceptors.
12. (Original) The method of claim 11 wherein said biological attenuation process results in resulting products including evolved sulfide and divalent metals.
13. (Original) The method of claim 12 wherein said resulting products further includes the formation of reactive geological minerals including pyrite.
14. (Currently Amended) The method of claim 1 wherein said one or more trivalent metals are introduced into said ~~targeted~~ environmental medium via temporary or permanent wells.
15. (Currently amended) The method of claim 1 wherein the ~~introduction~~ introducing into the environmental medium of said ~~peroxide or persulfate or combination thereof~~ is accomplished via gravity feeding, induced gas stream, a pump, or a combination thereof.
16. (Currently amended) The method of claim 1 wherein said one or more trivalent metals are delivered under pressure in either a gas or liquid stream.
17. (Currently amended) The method of claim ~~[[5]]~~ 1 wherein said one or more trivalent metals are introduced into said ~~targeted~~ environmental medium via temporary or permanent wells.
18. (Canceled)

19. (Canceled)

20. (New) A method for oxidizing contaminants in an environmental medium containing one or more contaminants, the method comprising introducing into said environmental medium a persulfate and one or more trivalent metals to cause oxidation of the one or more contaminants, which oxidation has resultant material, and wherein an amount of the persulfate and an amount of the one or more trivalent metals introduced into said environmental medium are designed to produce sufficient resultant material to optimize biological attenuation processes following the oxidation of said one or more contaminants.

21. (New) The method of claim 20, further comprising the steps of:

Testing the environmental medium to identify the one or more contaminants present and a level of each of the one or more contaminants;

Calculating an optimum dosage of the persulfate and the one or more trivalent metals to optimize biological attenuation processes following the oxidation of said one or more contaminants by the persulfate and the one or more trivalent metals;
and

Introducing said optimum dosage of the persulfate and the one or more trivalent metals into said environmental medium.

22. (New) The method of claim 21 wherein the persulfate and the one or more trivalent metals are combined before introduction into said environmental medium.

23. (New) The method of claim 21 wherein the persulfate and the one or more trivalent metals are introduced into said environmental medium sequentially.

REMARKS/ARGUMENTS

By this Response we have amended claims 1, 4, 6 – 8, 11, and 14 – 17 without prejudice or disclaimer. Claims 5, 18 and 19 are canceled without prejudice or disclaimer, and claims 20 – 23 are newly added. Claims 1 – 4, 6 – 17, and 20 – 23 (2 independent claims and 18 dependent claims) remain pending after entry of the amendments. Support for the amendments to the claims may be found in the specification as originally filed, for example in at least paragraph [0019] and [0022].

OBJECTIONS

Claim 1 is amended to correct the issue cited by the examiner in the preamble.

CLAIM REJECTIONS – 35 USC § 112

Claim 1 is amended to clarify that the environmental medium may contain one or more previously introduced zero valent metals.

CLAIM REJECTIONS – 35 USC § 101/112

The examiner rejected claims 1-4 and 6-13 as lacking utility. Claim 1 is amended to clarify the utility. Claim 1 is amended and to clarify the utility of introducing materials into an environmental medium containing one or more contaminants, and resulting in oxidation and attenuation of the contaminants.

Claims 2-4 and 6-13 depend from claim 1 and therefore overcome this rejection with the amendments to claim 1.

PRIOR ART CLAIM REJECTIONS – 35 USC § 102

With respect to claim 1, the examiner cites Block et al (US Pat. 7,576,254) and admits that Block does not teach a biological attenuation process. The applicant respectfully disagrees with the examiner that the biological attenuation process is inherent in the Block disclosure. Block states that “The goal is for the concentration of peroxygen compound in the injected solution to be just enough to result in the peroxygen compound reaction front traveling throughout the area of contamination requiring treatment in sufficient quantity to oxidize the contaminants present.” See Block Column 5 lines 43-47.

Claim 1 as amended includes a limitation which requires the treatment compound to have sufficient material to result in sulfate and metal to act as electron acceptors supporting biological attenuation of the one or more contaminants. Block does not teach this limitation, but teaches away from this limitation because Block teaches that the only goal of the compound is oxidation.

Block also teaches the use of chelated divalent metals as a catalyst. See Block column 4, lines 26-34. In the present application, in the BACKGROUND OF THE INVENTION, the applicant states that “The use of chelated divalent metal complexes prevents the second and critical step in the remedial process which is biologically mediated.” See paragraph [0002]. Block teaches a method that will prevent biological attenuation, therefore Block does not teach all the limitations of claim 1 as amended.

Claims 2-4 and 6-17 depend from claim 1 which is distinguished over the prior art for the reasons described above. As these claims depend from claim 1 they are also distinguished over the prior art.

PRIOR ART CLAIM REJECTIONS – 35 USC § 103

The applicant respectfully asserts that the examiner has failed to establish a *prima facie* case of obviousness. Even if the examiner established a *prima facie* case of obvious, the amendments to claim 1 clarify that Block teaches away from the invention as claimed. Even if the teachings of Block could be combined with the cited reference of Lucas (*Bioresource Technology*, 119, 174-180, 2012) the result would not teach all of the limitations of amended claim 1.

Claim 8 was rejected as obvious based on the combined teachings of Block and Lucas. Claim 8 includes the limitations of amended claim 1 and is therefore distinguishable over the prior art.

CONCLUSION

In this amendment the applicant has addressed all of the rejections and objections of the examiner as listed in the Office action dated 10/31/2014.

Applicant respectfully requests that a timely Notice of Allowance be issued in this case. The applicant further invites the examiner to contact the attorney of record to discuss this response, or to discuss any further action that appears to the examiner as necessary to advance the application towards a Notice of Allowance.

Respectfully Submitted,

By /Wayne Carroll/
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/891,934	05/10/2013	Michael Scalzi	SCA-004	8286

107047 7590 04/02/2015
IP Works, PLLC
P.O. Box 818
Doylestown, PA 18901

EXAMINER

BERKE-SCHLESSEL, DAVID W

ART UNIT	PAPER NUMBER
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1651

MAIL DATE	DELIVERY MODE
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04/02/2015

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 13/891,934	Applicant(s) SCALZI ET AL.	
	Examiner David Berke-Schlessel	Art Unit 1651	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 2/2/15.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

- 5) ☒ Claim(s) 1-23 is/are pending in the application.
5a) Of the above claim(s) ____ is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☒ Claim(s) 1-23 is/are rejected.
- 8) ☐ Claim(s) ____ is/are objected to.
- 9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some** c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) ☒ Notice of References Cited (PTO-892) | 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____. |
| 2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date ____. | 4) ☐ Other: ____. |

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Response to Arguments

Applicant's amendment, filed 2/2/15, with respect to the claim objections have been fully considered and are persuasive. The objection of claim 1 has been withdrawn.

Applicant's amendments, filed 2/2/15, with respect to 35 USC 101/112 rejections have been fully considered and are persuasive. The 35 USC 101/112 rejections of claims 1-4 and 6-13 have been withdrawn.

Applicant's arguments filed 2/2/15 have been fully considered but they are not persuasive. In response to the prior 35 USC 112 rejection of claim 4, the Applicant amended the claim; however there continues to be a degree of uncertainty with respect to the interpretation of the claim language. Currently the limitation reads "wherein said environmental medium contains one or more previously introduced zero valent metals," but when juxtaposed to the limitation found in the independent claim, "introducing... one or more trivalent metals," there continues to be indefiniteness. It is indefinite because the limitation of claim 4 suggests that zero valent metals were applied in an earlier step of the invention, but the independent claim explicitly claims that administration of trivalent metals, not zero valent metals. If the Applicant is suggesting that the zero valent metals are the "contaminants," this should be more clearly stated in claim 4. If the Applicant is suggesting that the zero valent metals are added in addition to the trivalent

metals, the Applicant is urged to amend to read upon this step. Additionally, it must be noted that zero valent metals rarely exist in nature, and most natural conditions will quickly oxidize zero valent metals, the noble metals are among the few exceptions.

On page 6 of the Applicant's Arguments, the Applicant argues that Block does not teach "biological attenuation." First, it must be noted that biological attenuation is an inherent process of soil-borne microbes. See Davis, et al (*Ground Water*, 32, 215-226, 1994). So, just the fact that Block does not specifically teach this is immaterial, because it will inherently and naturally occur whether Block's method is performed or not. The Applicant suggests that the new limitations get around Block, because the Applicant argues that Block provides "just enough" trivalent metal and persulfate to perform the oxidization, but not the biological attenuation. However, the Applicant has provided no evidence to suggest that Block's "just enough" will not enhance the inherent action of biological attenuation. Additionally, based upon the Applicant's balance equations, it is unclear how or why this is not inherently accomplished by Block's method, wherein it would be expected for sulfate and ferrous iron to form. See paragraphs [0023]-[0033] of the instant specification.

On page 6 of the Applicant's Arguments, the Applicant argues that Block "teaches away" from the claimed invention by providing "just enough" trivalent metal and persulfate to accomplish oxidization and not *necessarily* biological attenuation. As noted in the prior paragraph, biological attenuation is an inherent process of soil-borne microbes. Since Block never explicitly says "do not use this," or some other synonymous phrase, there is nothing in Block that would teach away from the claimed

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invention. Just because Block does not acknowledge the existence of an enhancement of biological attenuation, it does not mean that Block teaches away from this, it just means that Block never explicitly described it.

On page 6 of the Applicant's Arguments, the Applicant argues that since Block teaches chelators, and chelators can interfere with the enhancement of biological attenuation, then Block cannot anticipate the instant invention. While it is true that Block teaches the administration of metal chelates, Block indicates that this is one of several embodiments, wherein other embodiments do not use chelators. See column 4, lines 19-22 and claim 16. Additionally, there is no limitation indicating that chelators must be absent from the method.

The Applicant has amended claims 1, 4, 6-8, 11 and 15. However, the claim amendments still read upon, and appear to be anticipated by Block. Therefore, these rejections are maintained for the reasons stated above. The Applicant has added new claims 20-23. These will be discussed below. No claim is allowed.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly

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connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 21-23 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 21 and 23 have been added to the claim-set, but Applicant's Arguments do not suggest where in the specification the limitations of claim 21 can be found; additionally, a review of the instant specification indicates that none of the limitations of claim 21 are described. The claim requires several distinct steps: testing the environmental medium for contaminants, calculating an optimum dosage of persulfate and trivalent metal, and introducing the optimum dosage to the environmental medium. While the Examiner has no issue with the first and last steps of the claimed method, it is the calculating step the leads to significant Written Description issues. The instant specification has not clearly presented a method of calculating the optimum dosage, wherein the limitations of claim 20 are met. Since the instant specification does not provide a clear and concise method of calculating an optimum dose and since it is unclear if one skilled in the art would know how to make this *specific* calculation it is unclear if the Applicant was in possession of this embodiment at the time of filing.

Claims 22 and 23 are rejected insofar as they claim dependence on claim 21 and do not remedy the above stated issue. Additionally, since it is unclear how the Applicant

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has accomplished these calculations, there is no way to examiner these limitations on their merits, with respect to prior art.

2. The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claim 4 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 4 is indefinite because it is unclear what type of metal is being introduced into the environmental medium. Based on the initial portion of the limitation, it would appear that the claim requires the administration of a trivalent (oxidized) metal; however, the second portion of the limitation provides uncertainty with this interpretation, because it implies that a neutral metal is “previously introduced.”

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

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(a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale or otherwise available to the public before the effective filing date of the claimed invention.

5. Claims 1-4, 6-17 and 20 are rejected under 35 U.S.C. 102(a)(1) as being anticipated by Block, et al (US Pat. 7,576,254). Block teaches the *in situ* and *ex situ* oxidation of volatile organic compounds by the administration of a peroxide or persulfate and a trivalent metal. See column 1, lines 9-14; column 2, lines 17-25; column 4, lines 26-34. While Block never explicitly describes a “biological attenuation process,” Block teaches every limitation of the claim, and would therefore inherently provide a “biological attenuation process.”

With respect to claim 1, Block teaches the *in situ* and *ex situ* oxidation of volatile organic compounds by the administration of a peroxide or persulfate and a trivalent metal. See column 1, lines 9-14; column 2, lines 17-25; column 4, lines 26-34. As such, the instant claim is anticipated.

With respect to claim 2, Block teaches soil and groundwater. See column 1, line 10. As such, the instant claim is anticipated.

With respect to claim 3, Block teaches sodium persulfate. See column 2, line 24. As such, the instant claim is anticipated.

With respect to claim 4, Block teaches the addition of Fe (III), which is a trivalent metal. See column 4, line 29. As such, the instant claim is anticipated.

With respect to claim 6, Block teaches that the trivalent metals are used as a catalyst with the peroxide/persulfate. See column 4, lines 26-30. Since a catalyst is interpreted to be the same as an “activator,” the instant claim is anticipated.

With respect to claim 7, Block teaches Fe(III). See column 4, line 29. As such, the instant claim is anticipated.

With respect to claim 8, Block teaches Mn(II). See column 4, line 29. While Mn(II) is not the trivalent form (Mn(III) is the trivalent form), its exposure to an oxidizing agent, like a peroxide or persulfate, will cause at least some of the manganese to oxidize to Mn(III). Furthermore, since dependent claim 4 indicates that the metals do not have to be administered in their trivalent oxidation state, as long as the administration of Mn(II) results in the formation of some Mn(III), over the course of the method, the claim must inherently be anticipated.

With respect to claim 9, although Block does not say that the medium “returns to a reducing condition,” Block teaches identical method steps. If Block teaches identical method steps, everything that follows must be inherent to the method. As such, the instant claim is anticipated.

With respect to claim 10, although Block does not mention a biological attenuation process, nor a mention of facultative biological reactions, Block teaches identical method steps. If Block teaches identical method steps, everything that follows must be inherent to the method. As such, the instant claim is anticipated.

With respect to claim 11, the instant claim is only describing the inherent redox reaction between the compounds already discussed by Block. Since Block teaches the same compounds, provided in the same method, the same redox reaction must also be present. As such, the instant claim is anticipated.

With respect to claim 12, although Block does not mention a biological attenuation process, Block teaches identical method steps. If Block teaches identical method steps, everything that follows must be inherent to the method. As such, the instant claim is anticipated.

With respect to claim 13, pyrite is FeS_2 . Since the reaction with Fe(III) and a persulfate will inherently produce some pyrite, and Block teaches the administration of Fe(III) and persulfate, Block must also inherently form pyrite. As such, the instant claim is anticipated.

With respect to claim 14, Block teaches wells. See column 3, line 47. Although Block does not mention if the wells are temporary or permanent, these are the only possible options. Therefore, the mere mention of using wells in the method of Block implies that both temporary and permanent wells were envisioned. As such, the instant claim is anticipated.

With respect to claim 15, Block teaches injection of the compounds. See column 3, line 29. Since injection requires some form of liquid pumping, the instant claim is anticipated.

With respect to claim 16, Block teaches injection rates of the compounds. See column 3, line 37. Since the control of injection rates requires some degree of pressure, the instant claim is anticipated.

Claim 20 is rejected under 35 U.S.C. 102(a)(1) as being anticipated by Block, et al (US Pat. 7,576,254) as evidenced by Davis, et al (*Ground Water*, 32, 215-226, 1994). Davis indicates that biological attenuation is an inherent property of soil-borne microbes.

See page 215, "Abstract" section. While Block does not teach biological attenuation, Davis indicates that this occurs naturally. Furthermore, based upon the balanced equations presented in the instant specification, it appears that Block will provide amounts of trivalent metal and persulfates that will inherently optimize the biological attenuation process. As such, the instant claim is anticipated.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

6. Claim 8 is rejected under 35 U.S.C. 103 as being unpatentable over Block, et al (US Pat. 7,576,254) in view of Lucas, et al (*Bioresource Technology*, 119, 174-180, 2012). While Block never explicitly teaches the administration of Mn(III), Block does suggest that Mn(II) could be used, wherein it would naturally oxidize to Mn(III) in the presence of an oxidizing agent like peroxide or persulfate. Regardless, Lucas teaches that the combination of Mn(III) (in the form of manganese (III) acetate), with peroxide, will effectively oxidize the highly recalcitrant lignin found in wood. See page 175, left column, second full paragraph. Additionally, Lucas posits that the reaction pathway may involve a Fenton-type reaction. See page 179, left column, fourth full paragraph. While Lucas never mentions that this composition would be useful for the oxidation of VOCs, Block notes that Fenton-type reactions are useful for the oxidation of VOCs. See Block, column 2, lines 17-25. Since Block suggests that Fenton-type reactions *could* be useful for the oxidation of VOCs, and the fact that Lucas teaches that the combination of

Mn(III) and hydrogen peroxide undergo Fenton-type reaction, it would be obvious to use Lucas's method, like Block's, as a means of oxidizing VOCs. As such, the instant claim is rendered obvious.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Berke-Schlessel whose telephone number is (571)270-3643. The examiner can normally be reached on Mon-Thu 7AM-4PM EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Taeyoon Kim can be reached on (571)272-9041. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Blaine Lankford/
Primary Examiner, Art Unit 1651

/David Berke-Schlessel/
Examiner, Art Unit 1651

Notice of References Cited	Application/Control No. 13/891,934	Applicant(s)/Patent Under Reexamination SCALZI ET AL.	
	Examiner David Berke-Schlessel	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Davis, John W; et al; "Natural Biological Attenuation of Benzene in Ground Water Beneath a Manufacturing Facility" Ground Water, 32, 215-226, 1994
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes 	Application/Control No. 13891934	Applicant(s)/Patent Under Reexamination SCALZI ET AL.
	Examiner DAVID BERKE-SCHLESSEL	Art Unit 1651

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST search notes attached	10/28/14	DBS
STN search notes attached	10/28/14	DBS
Inventor search performed (8766030; 8776734)	10/28/14	DBS
EAST search notes attached	4/1/15	DBS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

/DAVID BERKE-SCHLESSEL/ Examiner.Art Unit 1651	
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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
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Natural Biological Attenuation of Benzene in Ground Water Beneath a Manufacturing Facility

by John W. Davis, Nancy J. Klier, and Constance L. Carpenter^a

Abstract

Subsurface unsaturated soils and ground water directly beneath a manufacturing site have been shown to contain benzene. The vadose zone appears to be the current source for benzene to the aquifer. Although the ground water contains several mg/l of contamination in the area immediately beneath the source, benzene was not detected in monitoring wells approximately 400 feet downgradient. Based upon the length of time benzene has been present in the ground water, as well as the permeability of the aquifer, physical processes alone such as adsorption and advection/dispersion are unlikely to account for the observed attenuation. Results from this investigation indicated the attenuation was primarily due to the natural biological processes occurring within the aquifer.

Evidence for the natural bioremediation of benzene from the ground water included: (1) analysis of ground-water chemistry, (2) laboratory studies demonstrating benzene biodegradation in aquifer samples, and (3) computer simulations examining contaminant transport. Laboratory studies indicated that under conditions similar to those encountered in the ground water, benzene was degraded to carbon dioxide by the naturally occurring microorganisms. The aerobic degradation of benzene in aquifer samples was quite rapid, with the time for 50% disappearance of the parent compound ranging from 4 to 14 days. In situ analyses of the ground water indicated significant levels of dissolved oxygen throughout the aquifer. Thus, the availability of oxygen should not limit the aerobic biodegradation of benzene. However, benzene was also shown to degrade under anaerobic conditions in microcosms prepared with aquifer material. Computer model simulations were also conducted to examine the processes influencing the transport of benzene in the aquifer. Results from the model simulations indicated biodegradation was the dominant process influencing attenuation of the contaminant.

Introduction

The facility subject to this investigation is located on the eastern seaboard of the U.S. During an investigation of the ground water at the site, benzene was detected in both the vadose zone and ground water. Benzene contamination in the unsaturated zone was thought to be confined to a small area north of the fire water lagoon (Figure 1). Chemical analysis of the soil from the unsaturated zone in this area indicated the highest concentration of benzene was detected in the region 20 to 35 feet below grade. This zone of contamination, localized in the unsaturated soil horizon, represents the most likely source of benzene contamination to the underlying aquifer.

Test drilling at the site revealed two major strata: 80 to 100 feet of glacial outwash deposits and bedrock. The unconsolidated deposits overlying the bedrock consisted of

sand, and clayey silt, with traces of gravel and grey brown gravel. The bedrock is a grey shale which is fractured in the upper few feet. Some of the fractures are filled with silt and clay. The water table begins at approximately 35 feet below grade and extends down to the bedrock (Figure 2). Horizontal ground-water flow is toward the east-southeast direction.

Analyses of ground water from monitoring wells (MW) installed at the site indicated that the highest concentrations of benzene were detected in MW 7, MW 13, and MW 14 (Figures 1 and 2). Benzene concentrations in these wells ranged from 3 mg/l to a high of 27 mg/l. The plume extended downgradient in an east-southeast direction towards MW 15 (Figure 1) where 0.30 to 0.91 mg/l of benzene were detected in ground-water samples. However, benzene levels at MW 15 were approximately 10- to 20-fold lower than the levels detected upgradient in the ground water underlying the source area. As the benzene plume moves through the aquifer, it appears to be under the influence of a downward vertical gradient since the highest concentration of benzene at MW 15 was localized 70 to 85 feet below grade, and not at MW 8 which is screened at 20 to 35 feet below grade. Benzene was not detected in the ground-

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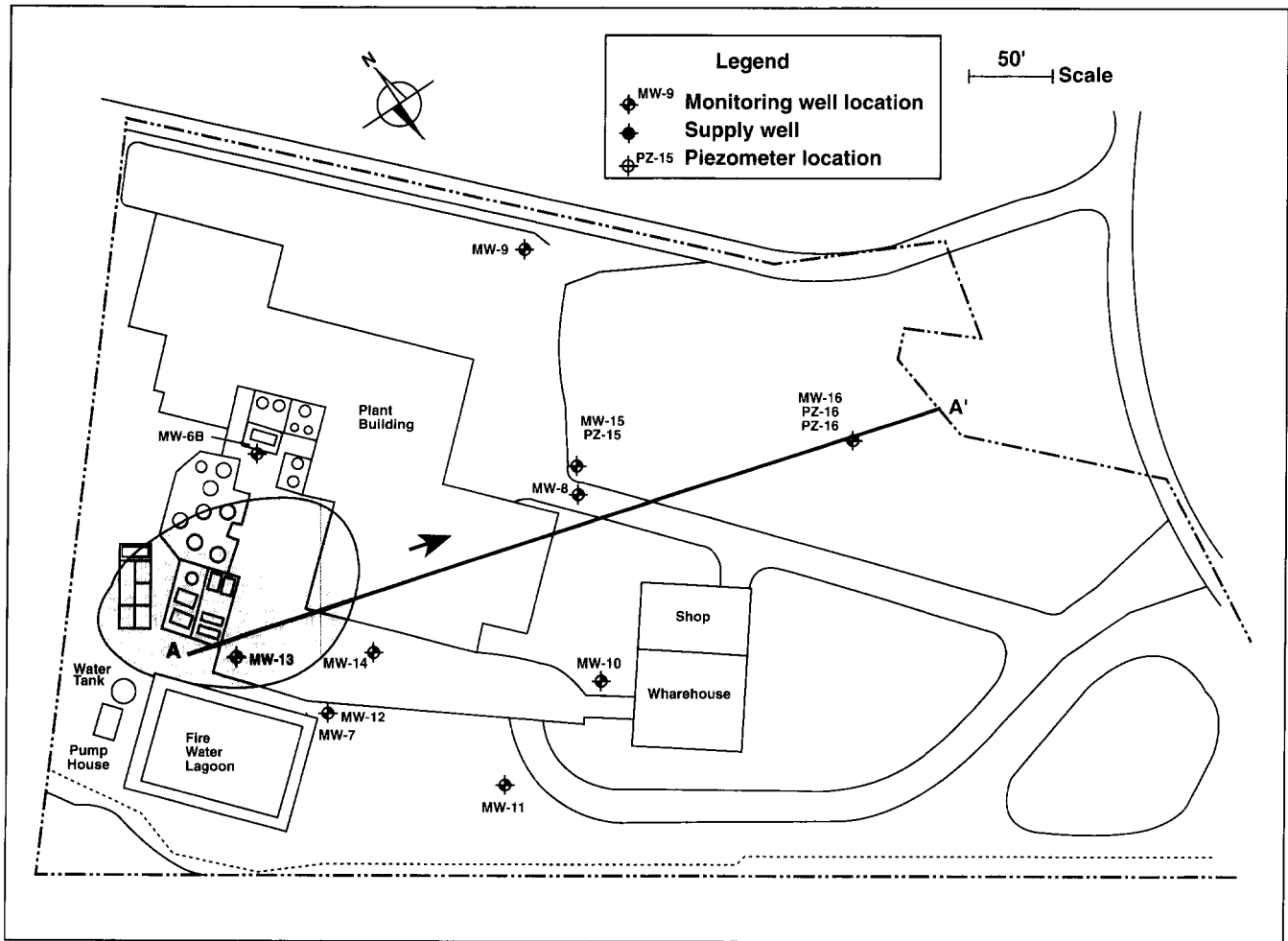


Fig. 1. Site map of the manufacturing site showing location of monitoring wells and source of benzene contamination. The shaded area represents the approximate zone of soil contamination. The general horizontal ground-water flow (see arrow) is in the east-southeast direction. For the BIOID model, movement of benzene through the aquifer was simulated along a transect A-A'.

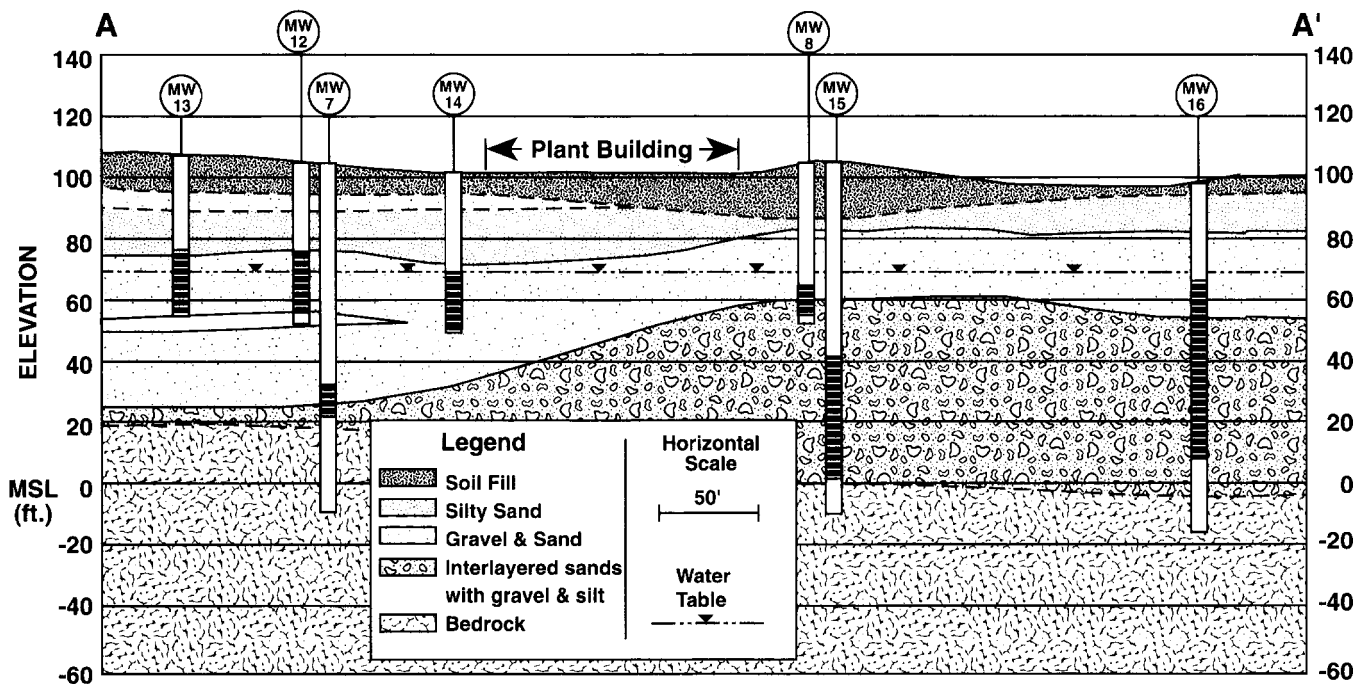


Fig. 2. Geological cross section: well location and elevation data. For each monitoring well, the shaded area represents the screened interval. Ground-water flow direction is from left to right along transect A-A'.

water samples taken downgradient at MW 16. However, it is unlikely that the benzene plume is sinking beneath the monitoring wells (e.g., MW 15 and MW 16) downgradient from the source. The screen bottoms in both MW 15 and MW 16 are set in bedrock. Since the bedrock permeability is significantly lower than the overlying sediments, it seems unlikely that a plume would be unaffected by this boundary and continue to sink. Ground-water flow would most likely follow a horizontal flow path along the bottom of the aquifer toward MW 16. Since benzene was not detected in ground-water samples downgradient from MW 15, it became apparent that attenuation of the plume was occurring during transport of the contaminant through the aquifer.

The objective of this study was to examine the importance of natural biological processes in the removal of benzene from the aquifer beneath the manufacturing site. Laboratory studies were conducted with aquifer soil and ground-water samples in order to develop site-specific information. The results from the laboratory studies were then incorporated into a solute transport model to further elucidate the processes occurring within the aquifer.

Methods

Test Chemicals

Radiolabeled benzene (ring- U - ^{14}C ; 69.2 or 19.3 mCi/mmol) was purchased from Sigma Chemical Company (St. Louis, MO). The purity of the radiolabeled benzene as determined in our laboratory by HPLC was $> 97\%$. Non-labeled, HPLC grade (99.9%) benzene was obtained from Aldrich Chemical Company, Milwaukee, WI. All other chemicals were of reagent grade.

Aquifer Material

Subsurface soil samples were collected in April 1991 from the aquifer beneath the manufacturing site. Samples were obtained from an area approximately 10 feet southeast of MW 15 (Figure 1) at depths ranging from 37 to 62 feet below the surface. Water-table measurements taken at the time of sampling placed the water table at approximately 36 feet beneath the surface.

In order to obtain aquifer samples for the laboratory study, a 35 foot borehole was advanced using rotary air drilling. Steel casing (10" ID) was inserted for the first 35 feet and the pipe was terminated one foot above the water table. The subsurface samples were obtained using a split spoon sampler. To prevent any cross contamination, the split spoon sampler was steam cleaned and rinsed with 70% ethanol prior to the initial sampling. For all subsequent samples, the split spoon was again rinsed with ethanol and allowed to air dry for a minimum of 0.5 h. The first subsurface soil sample, which was taken at the interface of the saturated zone, was discarded to minimize contamination of the aquifer soil with microorganisms from the vadose zone. Subsequent aquifer samples, which consisted of a mixture of soil and gravel, were taken from within the saturated zone. Once the test samples were removed from the boring, they were immediately transferred with a sterile spatula into

sterile glass containers, chilled, and then shipped by overnight express to The Dow Chemical Company laboratory in Midland, MI. In the laboratory, the soil samples were sieved through a sterile U.S. #6 standard sieve (to remove rocks and cobbles) composited, and stored at 4°C until used.

Ground-water samples were taken from MW 8 (Figure 2) using a clean polyethylene bailer. Prior to sampling, approximately two to three well volumes were removed from the formation and discarded. Water samples were collected in cleaned, sterile glass bottles and chilled on ice during transport.

Additional monitoring wells were sampled on a routine basis to further characterize the ground-water chemistry and extent of benzene contamination at the site. Ground-water temperature and dissolved oxygen measurements were performed in the field. Dissolved oxygen was determined by two separate methods. First, ground-water samples were obtained with a bladder pump under an N_2 -blanket. The pump-packer assembly was lowered to the bottom of the well and then raised approximately three to five feet. The packer units were inflated to 100 psi, a rubber hose was placed five to ten feet into the well, and the well casing was purged with nitrogen. This N_2 -blanket provided a boundary to the atmosphere. Several casing volumes of water were purged from the wells. After purging, the discharge line was attached to a closed chamber. The chamber allowed collection of field measurements in a flow-through system unaltered by surface conditions. Both dissolved oxygen and temperature were measured in this way. A "down-hole" measurement of dissolved oxygen was also performed by lowering an oxygen probe down into the well casing. These measurements did not differ substantially from the "chamber" method.

Biodegradation Studies

The biodegradation of benzene was examined in batch microcosms using the soil and ground-water samples obtained from the manufacturing site. Aerobic reaction mixtures were prepared by combining 25 g of soil with 30 ml of filter sterilized (0.45 μm) ground water in sterile 100 ml serum vials. All samples were amended with resazurin (0.0002%) as a redox indicator. The microcosms were then spiked with a stock solution of ^{14}C -benzene to achieve a final concentration in the reaction mixture of approximately 1.0 or 10.0 mg/kg. The level of the test substrate was chosen to represent the range of benzene concentrations typically observed in the ground water at the site. At the high concentration of the test chemical (10.0 mg/kg), reaction mixtures were prepared by first purging the microcosms with pure oxygen to ensure oxygen would not become limiting. After addition of the test substrate, the serum bottles were sealed with a Teflon®-faced butyl rubber septum and an aluminum crimp seal. Biologically inhibited controls were also included in the study to monitor for abiotic losses of the test substrate. Controls were prepared by first autoclaving (121°C @ 15 p.s.i.) the soil for 1 h on three consecutive days. The sterilized soils were then combined with filter sterilized ground water amended with mercuric chloride at an initial concentration of 1,000 mg/l.

To determine the fate of the contamination in the absence of oxygen, benzene biodegradation was also examined under anaerobic conditions. Ground water used in these studies was first purged with nitrogen gas for 0.5 to 1 h and transferred to an anaerobic glove box containing an atmosphere of 70% N₂, 28% CO₂, and 2% H₂. Reaction mixtures were prepared in the glove box by combining 10 g of soil with 5 ml of filter sterilized ground water in 20 ml sterile vials. All samples were amended with sodium sulfide (1 mM or 1.2 mg/microcosms) and resazurin (0.0002%) as a redox indicator. Since the soil and ground water were originally obtained under aerobic conditions, the microcosms were stored for one week in the glove box to equilibrate with the anaerobic atmosphere. Anaerobic conditions were achieved as indicated by the change in color of the redox dye from red to colorless. The resazurin remained colorless in the microcosms throughout the study indicating that anaerobic conditions were maintained in the test samples. After equilibration, the microcosms were amended with unlabeled benzene at a final concentration of approximately 1.0 mg/kg and sealed as described above. Selected microcosms were amended with either 1.5 mM sulfate or 5 mM nitrate in order to promote sulfate reducing or denitrifying conditions.

Aerobic microcosms were incubated on a rotary shaker (200 rpm) at the ambient ground-water temperature (12°C). Anaerobic samples were incubated in an inverted position and stored in sealed containers at 12°C under an N₂ atmosphere. The N₂ atmosphere in the containers was routinely replaced after the removal of a sample.

Analytical Methods

Aquifer microcosms were routinely monitored for the loss of the test substrate and the formation of degradation products. Aerobic reaction mixtures containing ¹⁴C-benzene were chilled on ice for approximately 30 minutes and then extracted with 10 ml of iso-octane on a reciprocating shaker. The distribution of ¹⁴C-benzene and any potential ¹⁴C-labeled metabolites was monitored using high performance liquid chromatograph (HPLC) connected to an on-line radioactivity monitor (RAM). Chromatography was performed using a ZORBAX ODS column (MAC-MOD Analytical, Inc., Chadds Ford, PA) with acetonitrile-water (50:50) as the mobile phase delivered at 1.0 ml/minute by a Waters model 6000 solvent delivery system. Radioactive compounds were detected using an on-line RAM unit (Berthold model 507A). The output signal was processed through a P. E. Nelson GC/LC Data System.

Total radioactivity in the aqueous fraction was determined by liquid scintillation counting (LSC). Triplicate samples of the iso-octane (50 µl) and aqueous (200 µl) fractions were counted in 10 ml of LSC cocktail (Aquasol®, NEN DuPont, Boston, MA). Recovery of ¹⁴C-benzene from the soil microcosms was determined to be greater than 90%.

The mineralization of ¹⁴C-benzene to ¹⁴C-carbon dioxide (¹⁴CO₂) in the reaction mixtures was also determined during the course of the study. ¹⁴C-carbon dioxide was collected by passing N₂ gas (> 500 ml/minute) through

the ground-water/soil mixtures which had been acidified with 200 µl of concentrated phosphoric acid. The purged gas was collected in a series of two traps, each containing 10 ml of 1N KOH solution. The traps were combined and 1 ml portions from the traps were analyzed by LSC. The production of ¹⁴CO₂ was confirmed by adding barium nitrate to the caustic solution, mixing for 30 minutes, and determining the radioactivity in solution after removal of the carbonate precipitate by filtration (Rapkin, 1962).

Benzene analysis in the anaerobic reaction mixtures was routinely performed using a Hewlett Packard 5710 gas chromatograph equipped with a Hewlett Packard 19359 head space analyzer, a flame ionization detector (FID), a split injector, and a 30 m × 0.53 mm (ID) GS-Q capillary column (J & W Scientific, Folsom, CA). The injector and FID were held at 200°C, and 250°C, respectively. The chromatograph was operated with a head pressure of 20 psi, split flow of 100 ml min⁻¹, with a split ratio of 10:1, and a septum purge of 5 ml min⁻¹. Nitrogen was used as the carrier gas, and samples were chromatographed using a temperature program with an initial temperature of 80°C which was immediately increased at a rate of 16°C/min to 230°C, and held at this temperature for 2 min. The head space analyzer was operated with the following conditions: bath temperature, 60°C; equilibration time, ≥ 10 h; valve-loop temperature, 65°C; loop volume, 1.0 ml; pressurization time, 0.25 min; and an injection time of 0.3 min. The concentration of benzene in the microcosms was determined using an aqueous external standard.

Analyses of the subsurface soil for organic and inorganic content and soil texture were performed by A & L Midwest Laboratories, Inc., Omaha, NE.

Computer Modeling

The migration of benzene in the aquifer was simulated using a one-dimensional transport code (BIO1D, GeoTrans, Inc., Herndon, VA). This model can be used to simulate the effects of advection and dispersion on the transport of a solute as well as the effects of adsorption and degradation (Srinivasan and Mercer, 1988). Hydrogeological parameters used in the model simulations were obtained from results of the field-site investigation.

The velocity (V) of the ground water was estimated using the following equation:

$$V = (K \cdot i) / \phi \quad (1)$$

where K is the hydraulic conductivity, i is the gradient, and ϕ is the porosity. The average hydraulic conductivity was estimated to be 32 ft/day based upon in situ slug testing performed at the site. The average porosity was estimated to be 0.35 and the water-table gradient was determined to be 0.0056 ft/ft. Based upon water-table measurements taken at the site over a seven month period, the gradient varied by 12% or less. The hydraulic gradient was consistently in the east-southeast direction (see Figure 3 for water-table elevation contours).

The dispersion coefficient (D) was calculated based upon the following equation (Freeze and Cherry, 1979):

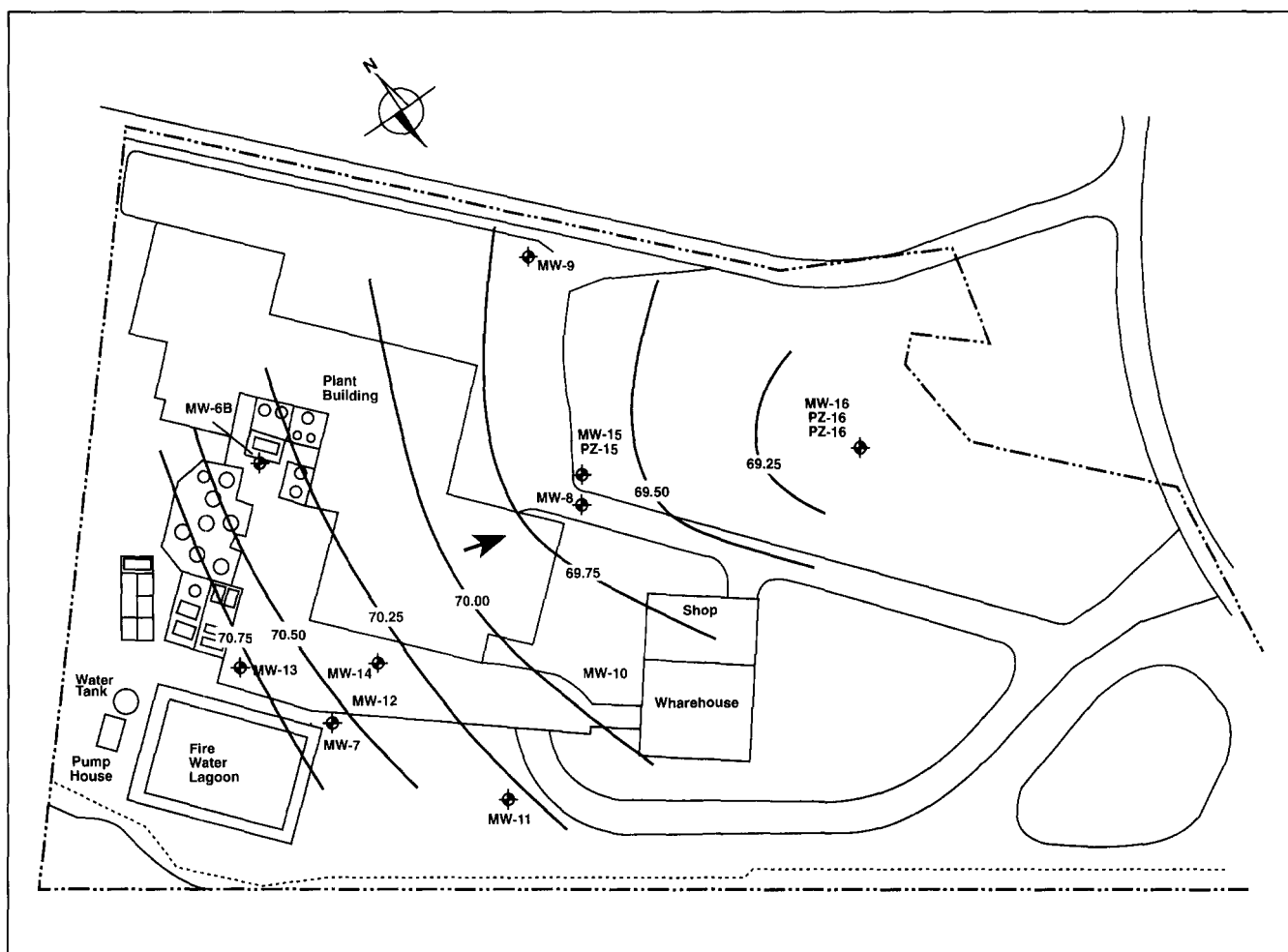


Fig. 3. Site map of the manufacturing site showing location of monitoring wells and water-table elevation contours. The general horizontal ground-water flow (see arrow) is in the east-southeast direction. The scale is identical to the scale included in Figure 1.

$$D = \alpha \cdot V \quad (2)$$

where α is the dispersivity of the soil (22.5 ft), and V is the velocity of the ground water [see equation (1)]. A range of dispersivity coefficients were estimated using the equations reported by Walton (1985) and Pickens and Grisak (1981). Using equation (2), a dispersion coefficient of $12 \text{ ft}^2/\text{day}$ was calculated. The dispersivity values were based upon a mean travel distance of approximately 300 feet which was the approximate distance between MW 13 and MW 15. The sorption of benzene onto the soil was assumed to be linear in the model simulations. A linear partition coefficient (K_p) for benzene was calculated according to the method of Karickhoff et al. (1979). Sorption constants, K_{oc} , were determined by normalizing the partition coefficient to the organic content (f_{oc}) of the soil. These K_{oc} values were determined based upon f_{oc} values for the soil of 0.009 and 0.014.

Biodegradation parameters for the model simulations were determined from the results obtained in the laboratory experiments. Biodegradation rates were assumed to be first order with respect to the concentration of benzene present. Biodegradation half-lives ($t_{1/2}$) were estimated from the results of the laboratory experiments based upon the time required for 50% disappearance of the parent compound.

Results

Aquifer Characteristics

After sieving the soil to remove the cobbles and rocks, a composite soil sample was analyzed for a series of physical and chemical properties including texture, organic carbon, and cation exchange capacity (Table 1). The soil was classi-

Table 1. Characterization of the Aquifer Solids at the Manufacturing Site

Parameters	
Soil type	Loamy sand
Texture	
—sand (%)	84
—silt (%)	8
—clay (%)	8
Organic carbon (%)	0.9
pH	6.9
Cation exchange capacity (meq/100 g)	3.9
Phosphorus (ppm)	
—weak Bray	15
—strong Bray	50
Nitrate (ppm)	3

Table 2. Benzene Concentration in Ground Water (Multiple Samples Were Taken Over Approximately a Four Year Time Period)

Parameter	Monitoring wells (benzene mg/l)						
	MW-8	MW-9	MW-11	MW-13	MW-14	MW-15	MW-16
Range (mg/l)	0.004 to 0.005	ND†	ND to 0.005*	1.9 to 27.0	2.1 to 15.0	0.058 to 0.910	ND to 0.0003
Mean	1.7			9.72	7.075	0.425	0.0003*
One standard deviation	1.9			10.629	5.706	0.305	
n = Samples	8	5	5	5	4	6	5

†None detected.

*Benzene detected in only one sample.

fied as a loamy sand based upon a relatively low silt and clay content. The cation exchange capacity was 3.9 meq/100 g and is consistent with the low percentage of clay present (8%). The f_{oc} of the soil was experimentally determined to be approximately 0.009.

Ground-water samples were collected to delineate the progression of the benzene plume with time as well as to further define the ground-water chemistry. Results of these analyses are summarized in Tables 2 and 3. The concentration of benzene in the monitoring wells represents the results of samples taken from October 1987 through July 1991. Benzene was routinely detected over a four year period in MW 8, 13, 14, and 15. The highest concentrations of benzene were recorded in MW 13 and 14 with the plume extending to MW 15. However, the mean downgradient concentration of benzene in MW 15 continued to be 10- to 20-fold less than the observed concentration in MW 13 and MW 14 (0.425 vs. 7.075 and 9.720 mg/l, respectively). Benzene levels fell to below the detection limit (< 0.001 mg/l) downgradient of MW 15. The contaminant was detected only once in MW 16 during the four year sampling period. Finally, MW 9 and MW 11 appear to be outside the influence of the benzene plume since benzene was not detected in MW 9 and detected only once during one sampling period (July 1991) in MW 11. The detection of benzene in MW 11 was probably an anomaly resulting from changes in ground-

water flow due to construction at the site. After construction was complete and ground-water flows returned to normal, benzene was not detected in MW 11.

Ground-water analyses for dissolved oxygen (DO) at the site indicated that the aquifer is essentially aerobic. A series of monitoring wells, MW 8, 9, 11, 13, 14, 15, and 16, were sampled on several occasions from December 1990 to July 1991. The remaining monitoring wells were not sampled. DO ranged from approximately 0.8 to 1.5 ppm throughout the monitoring wells indicating that the aquifer was aerobic (Table 3). However, there may be isolated zones within the aquifer where oxygen is limiting on a transient basis. If these anoxic zones are present they would most likely occur immediately downgradient from the source where the concentration of benzene in the ground water was the highest. These results suggest that oxygen would routinely be available for microbial activity occurring within the aquifer.

A wide range of values were reported for the inorganic carbon levels within the ground water. Low concentrations of inorganic carbon were observed in those monitoring wells (MW 9 and MW 11) which were relatively unaffected by the benzene plume. As summarized in Table 3, the inorganic carbon concentration ranged from approximately 2.5 to 5 mg/l in wells on the periphery of the benzene plume (MW 9 and MW 11). In contrast, the ground-water inorganic car-

Table 3. Ground-Water Characteristics

Parameter (mg/l)	MW-8	MW-9	MW-11	MW-13	MW-14	MW-15	MW-16
Dissolved oxygen	0.8		1.5	1.5	1.2	0.8	1.3
Ammonia, nitrogen	0.21	0.06	< 0.04	2.47	10.9	1.04	0.22
Nitrite, nitrogen	0.017	< 0.005	< 0.005	0.08	0.17	0.252	0.007
Nitrate, nitrogen	< 0.10	0.67	6.34	< 0.10	< 0.10	< 0.10	< 0.10
Kjeldahl, nitrogen	0.29	0.13	0.42	4.04	27.7	1.75	0.55
Phosphorus, ortho	< 0.15	< 0.15	< 0.15	< 0.15	1.77	0.69	< 0.15
TOC	0.57	0.89	1.37	5.94	19	4.04	3.67
Inorganic carbon	2.47	4.82	2.66	24.5	27	14.6	12.6
Well specification (ft above mean sea level)							
Ground elev. (ft)	105.7	155.6	97.2	103.7	102.3	105.3	98.6
Water elev. (ft)	69.57	69.69	70.26	70.81	70.36	69.5	69.14
Top screen (ft)	66.2	77.1	74.9	72.7	69.82	41.3	64.6
Bottom screen (ft)	56.2	57.1	54.9	52.7	49.8	1.3	4.6
Bedrock elev. (ft)	N.D.†	N.D.	N.D.	N.D.	N.D.	0.3	-1.4

*Anomalous dissolved oxygen measurement not included—measurement exceeded aqueous oxygen saturation.

†N.D.—No Data.

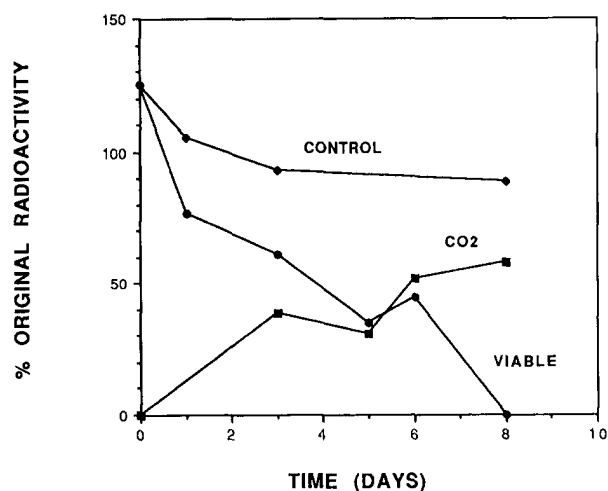


Fig. 4. Aerobic biodegradation of benzene in aquifer microcosms: nominal initial concentration of 1 mg/l.

bon levels were elevated in the vicinity of those wells which were directly impacted by the benzene plume. In MW 13 and MW 14, where benzene was routinely observed at concentrations approaching 10 mg/l, the inorganic carbon levels often ranged from five to tenfold higher (25 to 27 mg/l) than the basal levels observed outside the plume. The elevated concentrations of inorganic carbon observed may have resulted from high levels of oxidative microbial activity occurring in the ground water directly affected by the benzene plume.

Biological Activity in Aquifer Material

Laboratory studies were conducted to assess the impact of naturally occurring biological processes on the attenuation of benzene with the aquifer. Benzene biodegradation was examined in microcosms prepared from ground water and subsurface soil collected at the site. Initial studies, using ¹⁴C-benzene, were performed under aerobic conditions since field measurements demonstrated the presence of dissolved oxygen in the ground water indicating that the aquifer was aerobic. The levels of test chemical (nominal concentration of 1 and 10 mg/kg) used in the study were selected to represent those routinely observed in situ.

Under aerobic conditions, benzene was rapidly degraded in the laboratory microcosms (Figures 4 and 5). When the initial concentration of the test chemical was ~ 1 mg/kg, the time required for 50% disappearance of benzene was approximately four days (Figure 4). After eight days, ¹⁴C-benzene was no longer detected in biologically active microcosms. This degradation could be attributed to the naturally occurring biological activity within the microcosms since the loss of benzene in the biologically inhibited controls ranged from 2% to 18%. Furthermore, microbial adaptation was not required since a lag period preceding degradation was not observed in microcosms.

Somewhat different results were observed when the initial concentration of the test chemical was increased to ~ 10 mg/kg (Figure 5). A lag period of approximately 10

days preceded degradation, and the time required for 50% disappearance of benzene increased to 14 days. Benzene degradation was complete after 35 days and no ¹⁴C-benzene was detected in the microcosms.

The production of ¹⁴CO₂ in the biologically active microcosms further supports the conclusion that degradation was mediated by the naturally occurring microorganisms. Aerobic biodegradation resulted in the mineralization of benzene to carbon dioxide. At the completion of both of the aerobic degradations studies, approximately 40% to 60% of the initial test material was recovered as ¹⁴CO₂ (Figures 4 and 5). The remainder of the test material was presumably metabolized and incorporated into cell material. Accumulation of ¹⁴CO₂ rarely exceeded 1% in the biologically inhibited controls (not shown in Figure).

Ground-water analyses demonstrated that the aquifer was essentially aerobic. However, this does not preclude the possibility that anoxic conditions could slow down degradation on a transient basis. While benzene was rapidly degraded under aerobic conditions, the anaerobic transformation of benzene was somewhat slower. After 77 days, greater than 90% of the test material was degraded (Figure 6A). Most of this degradation could be attributed to biological processes, although approximately 30% of the benzene could not be recovered from the biologically inhibited controls. Note that in the biologically active microcosms methane was detected on a routine basis. The failure to recover all the benzene in the controls was most likely due to adsorption of the test material to the soil. In contrast, recovery efficiency of benzene from the microcosms in the aerobic studies was > 90% when the samples were extracted with iso-octane.

Similar results were observed in microcosms containing 2 mM sulfate (Figure 6B). Greater than 90% of the initial level of benzene disappeared after 77 days. The addition of sulfate to the microcosms likely promoted sulfate-reducing conditions as indicated by a change in the color of the soil from light brown to the characteristic greyish-black. In contrast, the presence of 5 mM nitrate did not promote the

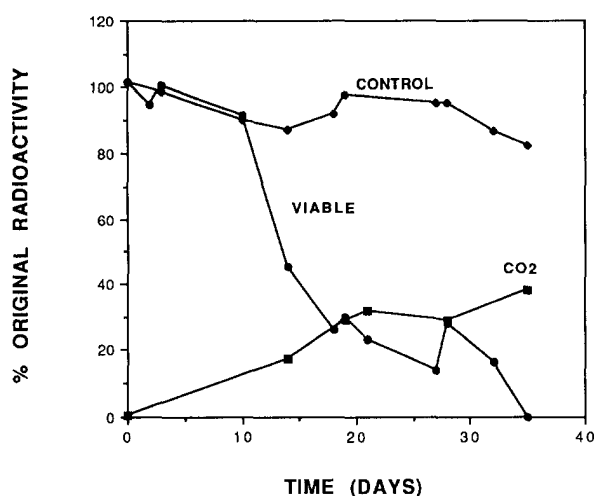


Fig. 5. Aerobic biodegradation of benzene in aquifer microcosms: nominal initial concentration of 10 mg/l.

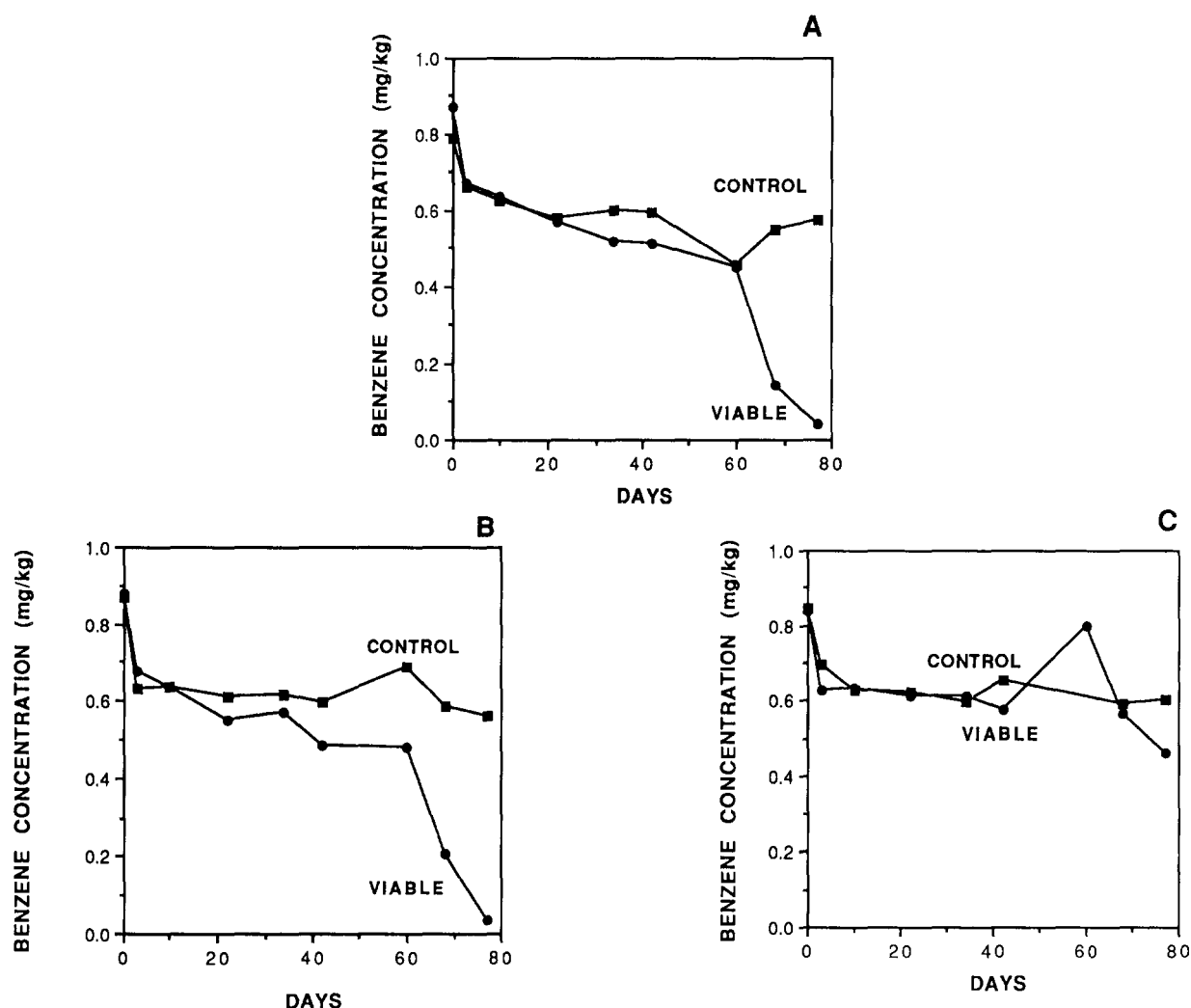


Fig. 6. Anaerobic biodegradation of benzene in aquifer microcosms: A) methanogenic, B) microcosms containing 2 mM sulfate, C) microcosms containing 5 mM nitrate.

anaerobic degradation of benzene (Figure 6C). After 77 days, most of the benzene loss in these microcosms could be attributed to abiotic processes since the level of benzene remained comparable in both the biologically active and inhibited control microcosms.

Computer Modeling

The importance of physical and biological processes on the attenuation of benzene in the aquifer was examined using a ground-water simulation model (BIO1D). This model is a one-dimensional, finite-difference code for simulating various aspects of solute transport. The model was used to simulate a flow system at the site with benzene as the reactive solute. The model included terms for both adsorption and biodegradation, as well as for advection/dispersion.

In the BIO1D model, movement of benzene through the aquifer was simulated along a transect A-A' (see Figure 1) parallel to the principal ground-water flow direction. The simulation predicted benzene transport for a distance of 500 feet downgradient from the source area over a period of approximately four years (1,400 d). This time interval was chosen based upon a minimum estimate of the length of

time in which benzene has been present in the aquifer. A constant benzene source of 15 mg/l was simulated at the upgradient model boundary. This concentration was chosen since benzene levels of 10 to 15 mg/l were routinely observed in monitoring wells (MW 13 and MW 14) lying directly below the source. The ground-water velocity was varied over a range from 0.25 to 0.50 ft/day. These values were chosen to bracket the velocity calculated from field measurements of hydraulic conductivity and gradient. Biodegradation was simulated using a laboratory derived half-life of five days. For selected simulations the biodegradation half-life was increased tenfold to 50 days.

The sensitivity of the model to variations in ground-water flow velocities are shown in Figures 7A and B. At higher flow velocity, 0.50 ft/day (Figure 7A), a minimal reduction in benzene levels was observed when only advection/dispersion were considered in the model simulations. After four years of simulated transport, the concentration of benzene at a distance 500 ft downgradient was approximately 13 to 14 mg/l (Figure 7A), which is essentially 87% to 93% of the concentration of benzene at the source. Decreasing the flow velocity by half (0.25 ft/day) resulted in an

approximate threefold reduction (from 13 to 4 mg/l) in the benzene concentrations at a distance of 500 ft downgradient (Figure 7A).

The sensitivity of the model to changes in adsorption was examined by varying the f_{oc} of the aquifer from 0.009 to 0.014. Increasing the f_{oc} resulted in a concomitant increase in the sorption constant (K_{oc}) for benzene from 0.75 to 1.16. Results from the sensitivity analysis demonstrated that benzene transport was highly influenced by adsorption. As seen in Figure 7A, at the higher flow velocity (0.50 ft/day), adsorption accounted for an approximate 33% to 57% reduction in the benzene levels within a distance of 200 feet (e.g., benzene reduced to levels of approximately 10.0 and 6.5 mg/l, respectively). The effects of adsorption were equally as important when the ground-water flow velocity was decreased. A reduction in the flow velocity by half (0.25 ft/day) resulted in a subsequent two to threefold decrease in the levels of benzene (approximately 70% to 85% reduction)

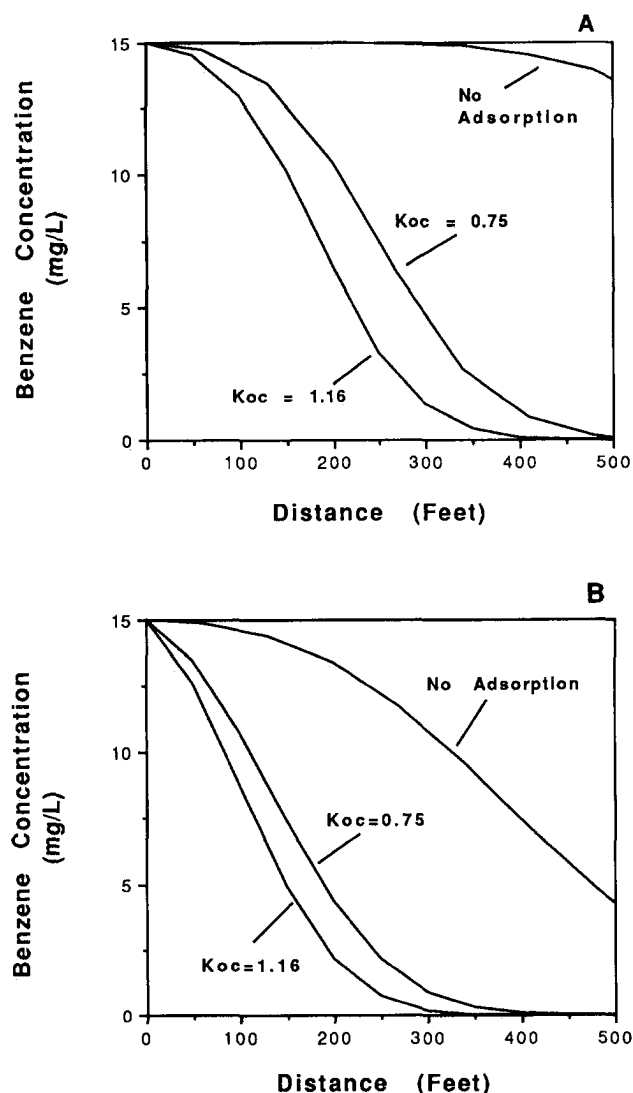


Fig. 7. Computer simulations demonstrating the effect of adsorption on the attenuation of benzene at two different ground-water flow velocities: A) 0.50 ft/day and B) 0.25 ft/day. Model simulations were conducted over a distance of 500 feet for approximately four years.

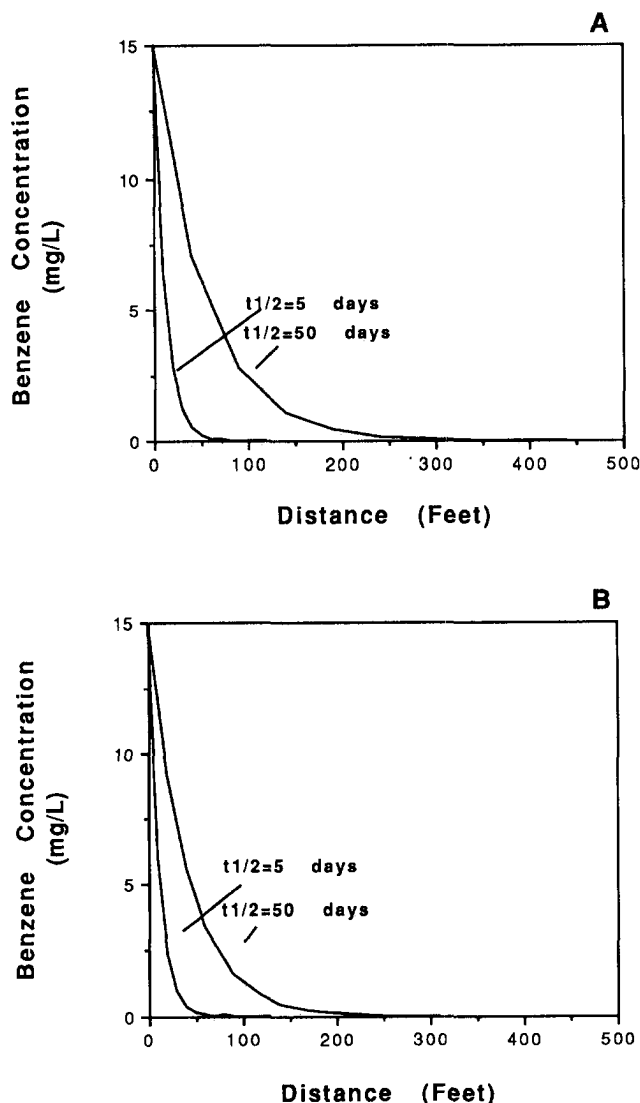


Fig. 8. Computer simulations demonstrating the effect of biodegradation on the attenuation of benzene at two different ground-water flow velocities: A) 0.50 ft/day and B) 0.25 ft/day. Model simulations were conducted over a distance of 500 feet for approximately four years.

within a distance of 200 feet (Figure 7B). Regardless of the f_{oc} , however, adsorption accounted for > 99% reduction in the benzene levels (less than 0.150 mg/l) at a distance of 500 feet at both flow velocities.

Computer simulations were also conducted to examine the effect of biodegradation on benzene attenuation in the aquifer (Figures 8A and B). Although previous simulations indicated that adsorption would retard benzene transport, significant concentrations of the contaminant would still be expected to occur 200 to 300 feet downgradient from the source. Results from the model simulations demonstrated that based on laboratory generated degradation rates, biological processes have a greater effect on benzene transport and attenuation than adsorption. When model simulations were conducted using a biodegradation half-life of five days, the benzene levels were reduced by > 99% (ground-water concentrations of less than 0.10 mg/l) within a distance of 60 feet, for both ground-water velocities examined (Figure 8).

Changes in the biodegradation half-life had the greatest influence on attenuation. For example, a tenfold decrease in the biodegradation half-life from 50 to 5 d resulted in a concomitant 500 to 1,000-fold decrease in the benzene concentration at a distance of 100 feet (Figures 8A and B). For both biodegradation half-lives examined, however, the levels of benzene were reduced by > 99% (ground-water concentrations of less than 0.05 mg/l) within a distance of 300 feet. Again, when the biological processes were considered, changes in flow velocities had a minimal influence on attenuation.

A sensitivity analyses was also performed to examine the combined influence of adsorption and biodegradation. The results of these simulations (data not shown) were nearly identical to those which examined the influence of biodegradation only. Taken together these results demonstrated that the biological processes are the dominant mechanisms influencing transport, and changes in the biodegradation rates can have significant impact on attenuation.

Discussion

Subsurface unsaturated soils and ground water directly beneath a manufacturing site have been shown to contain benzene. The vadose zone is the current source for the addition of benzene to aquifer. Although the ground water often contains up to 25 mg/l of benzene in the area immediately beneath the source (MW 13 and MW 14), the contamination was not detected in monitoring wells approximately 400 feet downgradient (MW 16). Based upon the length of time benzene has been in the ground water, as well as the permeability of the aquifer, physical processes alone such as advection/dispersion and adsorption are unlikely to account for this observed attenuation. Evidence to support the hypothesis that natural biological processes play an important role in attenuation of benzene in the aquifer include: (1) analysis of ground-water chemistry, (2) laboratory results demonstrating benzene biodegradation in aquifer samples, and (3) computer simulations examining benzene transport.

Measurements of the dissolved oxygen in the ground water at the manufacturing site indicated that the aquifer was essentially aerobic. In situ measurements conducted over an eight month period demonstrated DO concentrations ranged from 1 to 2 mg/l throughout the aquifer. Thus, DO levels in the ground water were sufficient for aerobic metabolism even in the immediate area beneath the benzene source (MW 14 and MW 13). Since oxygen was readily available in the ground water, benzene biodegradation was most likely an aerobic process and was not limited by the availability of an electron acceptor. However, biodegradation of benzene could substantially decrease if the demand for oxygen exceeded the availability.

The role of oxygen in the remediation of aromatic hydrocarbons in ground water has been well-documented (Chiang et al., 1989; Klecka et al., 1990; Wilson and Ward, 1987). Chiang et al. (1989) demonstrated a direct correlation between the availability of oxygen and the natural attenua-

tion of benzene, toluene, and xylene (BTX) in a sandy aquifer. They concluded BTX removal was directly related to DO concentration, and BTX was absent when the DO levels were greater than 0.9 mg/l. Hutchins and coworkers (1991a) also observed a correlation between oxygen availability and removal of benzene from contaminated ground water. Benzene levels were reduced from 0.760 to < 0.001 mg/l by recirculating oxygenated water through the contaminated zones.

Further evidence that natural biological attenuation is occurring in the aquifer examined in this investigation is supported by an increase in the inorganic carbon levels in the ground water. Laboratory evidence suggests that the naturally occurring microorganisms found in the aquifer were capable of mineralizing benzene to carbon dioxide. The inorganic carbon levels in ground water directly impacted by the benzene plume were five to tenfold higher than those in the area not affected by the plume (from approximately 2.0 - 5.0 mg/l to 25 - 27 mg/l). The increased inorganic carbon levels were primarily carbonates most likely originating from the aerobic biodegradation of benzene. Taken together, these results suggest high levels of microbial activity occurring in the vicinity of the benzene contamination.

Results from the laboratory studies provide additional evidence that the naturally occurring microorganisms are capable of degrading benzene under conditions similar to those occurring in the aquifer. Mineralization accounted for most of this loss with approximately 40% to 60% of the benzene routinely recovered as ¹⁴C-carbon dioxide. The biodegradation of benzene in subsurface environments has been reported by a number of groups (Delfino and Miles, 1985; Wilson et al., 1986a; Barker et al., 1987; Thomas et al., 1987; Major et al., 1988). Thomas and coworkers (1987) reported the biodegradation of high levels of benzene (800 ppb) by naturally occurring microorganisms present in soil, well water, and ground water. Barker et al. (1987) reported the natural attenuation of BTX (benzene, toluene, xylenes) compounds in a shallow sand aquifer. Field experiments were conducted where they added BTX to uncontaminated ground water and observed complete removal of the material after 434 days. The in situ removal rates of BTX from the ground water were similar to the biodegradation rates determined in laboratory experiments. Hadley and Armstrong (1991) reported on a state mandated survey that examined the presence of a broad range of contaminants in the wells serving water supplies throughout California. Contamination was found in 812 of the 7,167 wells examined and benzene was detected in only 10 wells, with benzene levels never exceeding 0.005 mg/l. In light of all the reported leaking underground gasoline tanks in the area, they concluded that the virtual absence of benzene from the ground water was due primarily to the natural biological processes occurring within the subsurface environment.

In the present study, an increase in the loss of benzene from the aerobic microcosms was observed when the initial concentration was increased from 1 to 10 mg/kg (an average loss of 6.7 vs. 13 $\mu\text{g day}^{-1}$ for 1 and 10 mg/kg, respectively). Benzene degradation in aquifer material has been shown to

be a function of substrate concentration and can be described by saturation kinetics. Alvarez and coworkers (1991) reported that the aerobic degradation of benzene in a sandy aquifer material was directly related to the initial concentration of the contaminant. An increase in the specific utilization rate was observed up to a benzene concentration of 100 mg/l, and no degradation was observed at 250 mg/l. This lack of degradation at the higher concentration was most likely due to the inhibition of the microbial populations.

In the current investigation, benzene biodegradation also occurred under anaerobic conditions. Benzene levels in laboratory microcosms were shown to be reduced by greater than 90% under anaerobic conditions. These results are consistent with those of others who have reported the anaerobic degradation of benzene in the laboratory as well as in situ (Van Beelen and Van Keulen, 1990; Edwards and Grbic-Galic, 1992). Grbic-Galic and Vogel (1987) demonstrated the anaerobic conversion of benzene to carbon dioxide by laboratory cultures of methanogenic bacteria. Wilson and coworkers (1986b) observed the anaerobic degradation of a series of aromatic hydrocarbons including benzene in aquifer material known to support methanogenesis. The anaerobic transformation of benzene has also been reported to occur in situ. Cozzarelli et al. (1990) observed the transformation of a variety of alkylbenzenes (including benzene) in anoxic ground water from a contaminated aquifer in Minnesota. They concluded that biological transformation of these contaminants was an important attenuation process occurring in anoxic ground-water environments.

Results from the current study suggest that the presence of nitrate did not promote the degradation of benzene under anaerobic conditions. The failure of benzene to degrade under denitrifying conditions has been reported by others. Hutchins et al. (1991b) and Kuhn et al. (1988) reported that benzene persisted in aquifer material under denitrifying conditions, although they observed the biotransformation of a variety of other substituted aromatic hydrocarbons. Benzene not only persisted, but was also reported to inhibit the denitrification process in the aquifer samples.

Somewhat different results were reported by Major and coworkers (1988). Under denitrifying conditions they observed the complete degradation of benzene, toluene, and xylenes. The discrepancy in the apparent fate of benzene under denitrifying conditions is most likely due to site-specific differences in the microbial populations.

Computer model simulations were conducted to evaluate the role of physical and biological processes in the attenuation of benzene. Physical and chemical parameters used in the model simulations were comparable to those observed beneath the manufacturing site. Computer analyses demonstrated that benzene transport was influenced by both ground-water velocity and adsorption. As expected, greater levels of attenuation were observed at a particular time and distance from the source with either a decrease in velocity or an increase in adsorption. Adsorption of non-ionic organic chemicals has been reported to be directly related to the soil organic content and involves the partitioning of the chemical into the soil organic matter (Karickhoff

et al., 1979; Chiou et al., 1979). In this investigation, benzene adsorption to the soil would be expected to occur given the level of organic content, and this is reflected in the model simulations. However, computer simulations also indicated that physical processes such as advection/dispersion or adsorption alone cannot account for the observed level of attenuation.

Model sensitivity analyses indicated that the transport and attenuation of benzene were influenced to a greater extent by biodegradation than variations in advection/dispersion or adsorption. When biological processes were incorporated into the model simulations, the concentration of benzene decreased rapidly over relatively short distances. These results suggest that the biological processes are the dominant mechanisms and may be responsible for the level of attenuation observed in the aquifer. In the absence of biodegradation, elevated concentrations of benzene would be expected to persist within the ground water.

The potential for benzene biodegradation has been demonstrated in the laboratory suggesting that the same biological processes may occur in situ. However, a wide range of physical, chemical, and environmental parameters are known to influence biodegradation rates (Klecka, 1985; Neilson et al., 1985), and benzene degradation occurring in situ may be slower than the rates observed in the laboratory. In order to account for this disparity, biodegradation rates used in the model simulations were decreased by a factor of 10 over the laboratory rates. Changes in the biodegradation rates had the greatest effect on attenuation and decreasing the biodegradation half-life from 50 d to 5 d resulted in a several hundredfold decrease in the level of benzene over the same distance. However, significant levels of attenuation (99%) were still observed over a distance of approximately 300 feet downgradient when the slower rates were used in the model simulations.

Evidence for the natural bioremediation of aromatic compounds in subsurface environments has been reported by others (Ehrlich et al., 1982; Klecka et al., 1990). Klecka and coworkers (1990) reported the natural bioremediation of low levels of phenolic and polycyclic compounds in the ground water below a Superfund site. They concluded that the natural biological processes occurring within the aquifer reduced the contaminants to near or below detection limit within a short distance downgradient of the source. Ehrlich et al. (1982) reported similar observations for contaminated ground water beneath a coal-tar distillation and wood treating plant. They identified the naturally occurring biological processes were responsible for removing phenolic compounds from the contaminated ground water.

The results from the present study demonstrate the importance of natural biological processes in the attenuation of benzene in subsurface environments. This investigation was conducted to support remedial action plans at the manufacturing site. This study supports the proposal that natural bioremediation is the predominant process responsible for the removal of benzene from the aquifer. Furthermore, as the source of benzene to the aquifer is removed from the unsaturated zone, natural bioremediation in the aquifer will eliminate benzene from the ground water.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/891,934	05/10/2013	Michael Scalzi	SCA-004	8286

38284 7590 06/04/2015
Ryder, Lu, Mazzeo and Konieczny, LLC
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Colmar, PA 18915

EXAMINER

BERKE-SCHLESSEL, DAVID W

ART UNIT	PAPER NUMBER
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1651

NOTIFICATION DATE	DELIVERY MODE
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06/04/2015

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

dryder@ryderlu.com

<i>Applicant-Initiated Interview Summary</i>	Application No. 13/891,934	Applicant(s) SCALZI ET AL.	
	Examiner David Berke-Schlessel	Art Unit 1651	

All participants (applicant, applicant's representative, PTO personnel):

(1) David Berke-Schlessel. (3) Michael Scalzi.

(2) Doug Ryder. (4) Antonis Karachalios.

Date of Interview: 01 June 2015.

Type: ☒ Telephonic ☐ Video Conference
 ☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
 If Yes, brief description: _____.

Issues Discussed ☐ 101 ☐ 112 ☒ 102 ☐ 103 ☐ Others
 (For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 1.

Identification of prior art discussed: _____.

Substance of Interview
 (For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Mr. Ryder supplied an agenda with potential claim amendments. Additionally, the inventors discussed the specific elements of the instant invention that were never described by the prior art; specifically, it was indicated that the ratio of trivalent metal and persulfate provides unexpected benefits that are not described by the prior art. Examiner Berke-Schlessel suggested including a ratio range in the independent claim as a means of suggesting that the claimed range imparts unexpected benefits over the prior art invention.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/David Berke-Schlessel/ Examiner, Art Unit 1651	
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Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews

Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael Scalzi	Examiner:	David W. Berke-Schlessel
Serial No.:	13/891,934	Group Art Unit:	1651
Filed:	May 10, 2013	Docket No.:	SCA-004
Title:	CHEMICAL OXIDATION NAD BIOLOGICAL ATTENUATION PROCESS FOR THE TREATMENT OF CONTAMINATED MEDIA		

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116

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P.O. Box 1450
Alexandria, VA 22313-1450

In response to the Final Office Action mailed April 2, 2015, the Applicant conducted an interview with the Examiner on June 1, 2015 in which the Applicant explained that the combination of persulfate and trivalent metals was such that after chemical oxidation residual material (sulfate and trivalent metal) remained that enables naturally occurring facultative cultures to utilize the residual material as terminal electron acceptors to promote the biological attenuation process of the one or more contaminants and prevents formation and accumulation of hydrogen sulfide which is a toxin to the facultative cultures. The Examiner requested that a ratio be included in the claims and the Applicants agreed to this.

Please amend the above-identified application as follows and consider the following arguments. As the Applicant believes that these amendments put the application in condition for allowance, the Applicant respectfully requests that these amendments be entered.

Please amend the specification as follows to correct a typographical error.

This technique utilizes the interaction between the occurring sulfate and ferric iron.

$$\text{Fe}^{2+} + 2\text{S}^{2-} \rightarrow \text{FeS}_2 \quad (21) \quad (14)$$

IN THE CLAIMS

Please amend the claims as follows.

1. (Currently Amended) A method for chemical oxidation ~~oxidizing contaminants~~ followed by a biological attenuation process ~~in~~ of an environmental medium containing one or more contaminants, the method comprising:

introducing ~~into the environmental medium~~ persulfate and one or more trivalent metals into the environmental medium, wherein the one or more trivalent metals activate the persulfate in order to chemically oxidize the one or more contaminants, wherein amount of the persulfate is selected to chemically oxidize the one or more contaminants and amount of the one or more trivalent metals is between approximately 17-30% of molecular weight of the persulfate so that at conclusion of the chemical ~~such that oxidation of the one or more contaminate occurs resulting in sufficient residual~~ sulfate and ~~metal~~ sufficient residual trivalent metals remain such that:

naturally occurring facultative cultures utilize the residual sulfate and the residual trivalent metal ~~to act~~ as terminal electron acceptors to promote the biological attenuation process of the one or more contaminants; and

the residual sulfate and the residual trivalent metal prevent formation and accumulation of hydrogen sulfide which is a toxin to the facultative cultures.

2. (Currently Amended) The method of claim 1, wherein the said ~~environmental~~ medium is soil or groundwater.

3. (Currently Amended) The method of claim 1, wherein ~~the said~~ persulfate is sodium, potassium, or ammonium based.

4. (Currently Amended) The method of claim 1, wherein the introducing one or more trivalent metals includes introducing ~~said environmental medium contains~~ one or more ~~previously introduced~~ zero valent metals and hydrogen peroxide to generate the one or more trivalent metals.

5. (Canceled)

6. (Currently Amended) The method of claim 1, wherein ~~said~~ the one or more trivalent metals activate the ~~act as activators for said~~ persulfate so as to form sulfate free radicals.

7. (Currently Amended) The method of claim 1, wherein ~~said~~ the one or more trivalent metals include Fe^{3+} .

8. (Currently Amended) The method of claim 1, wherein ~~said~~ the one or more trivalent metals include Mn^{3+} .

9. (Currently Amended) The method of claim 1, wherein ~~said~~ the biological attenuation process is accomplished as ~~said~~ the environmental medium returns to a reducing condition.

10-11. (Canceled)

12. (Currently Amended) The method of claim ~~[[11]]~~ 1, wherein the said biological attenuation process results in reduction of the residual sulfate and trivalent metals to resulting products including evolved sulfide and divalent metals.

13. (Currently Amended) The method of claim 12, wherein the sulfide and divalent metals combine and precipitate as said resulting products further includes the formation of reactive geological minerals including pyrite.

14. (Currently Amended) The method of claim 1, wherein the introducing one or more trivalent metals includes introducing the said one or more trivalent metals ~~are introduced into said environmental medium~~ via temporary or permanent wells.

15. (Currently Amended) The method of claim 1, wherein the introducing ~~into the environmental medium of said persulfate~~ includes introducing the persulfate ~~is accomplished~~ via gravity feeding, induced gas stream, a pump, or a combination thereof.

16. (Currently Amended) The method of claim 1, wherein the introducing one or more trivalent metals includes introducing the said one or more trivalent metals ~~are delivered~~ under pressure in either a gas or liquid stream.

17. (Currently Amended) The method of claim 1, wherein the introducing said one or more trivalent metals are introduced into said environmental medium via temporary or permanent wells includes introducing one or more divalent metals and hydrogen peroxide to generate the one or more trivalent metals.

18-19. (Canceled)

20. (Currently Amended) A method for oxidizing and biologically attenuating contaminants in an environmental medium containing one or more contaminants, the method comprising:

introducing ~~into said environmental medium~~ a persulfate and one or more trivalent metals into the environmental medium, wherein the one or more trivalent metals activate the persulfate in order to cause oxidation of the one or more contaminants, which wherein the oxidation of the one or more contaminants provides residual has resultant material, and wherein an amount of the persulfate is selected to chemically oxidize the one or more contaminants and an amount of the one or more trivalent metals is between approximately 17-30% of molecular weight of the persulfate so as introduced into said environmental medium are designed to produce sufficient residual resultant material such that:

naturally occurring facultative cultures utilize the residual material as terminal electron acceptors to optimize promote the biological attenuation process following the oxidation of the said one or more contaminants; and

the residual material prevents formation and accumulation of a toxin to the facultative culture.

21. (Canceled)

22. (Currently Amended) The method of claim [[21]] 20, wherein the persulfate and the one or more trivalent metals are combined before introduction into the said environmental medium.

23. (Currently Amended) The method of claim [[21]] 20, wherein the persulfate and the one or more trivalent metals are introduced into the said environmental medium sequentially.

24. (New) The method of claim 20, wherein the residual material includes sulfate and trivalent metal.

25. (New) The method of claim 24, wherein the biological attenuation process results in reduction of the residual sulfate and trivalent metals to sulfide and divalent metals.

26. (New) The method of claim 25, wherein the sulfide and divalent metals combine and precipitate as reactive geological minerals including pyrite.

27. (New) The method of claim 26, wherein the toxin includes sulfide and hydrogen sulfide and the pyrite results in the removal of potentially inhibitory levels of the sulfide and the hydrogen sulfide in the environmental medium.

28. (New) The method of claim 26, wherein the pyrite provides for abiotic surface oxidation of the environmental medium, providing additional attenuation of the one or more contaminants.

29. (New) The method of claim 20, wherein the introducing a persulfate and one or more trivalent metals into the environmental medium are done sequentially.

30. (New) The method of claim 20, wherein the introducing a persulfate and one or more trivalent metals into the environmental medium are done concurrently.

31. (New) The method of claim 1, wherein the introducing a persulfate and one or more trivalent metals into the environmental medium are done sequentially.

32. (New) The method of claim 1, wherein the introducing a persulfate and one or more trivalent metals into the environmental medium are done concurrently.

REMARKS

The Applicant has carefully reviewed and considered the Final Office Action mailed on April 2, 2015, and the references cited therewith.

Claims 1-4, 6-17, and 20-23 were pending in the Application. Claims 1-4, 6-9, 12-17, 20, 22 and 23 have been amended, claims 10, 11 and 21 have been canceled and claims 24-32 have been added. As a result of these amendments, claims 1-4, 6-9, 12-17, 20 and 22-32 are now pending in the Application with claims 1 and 20 being the independent claims.

Support for the claim amendments regarding the combination of persulfate and trivalent metals being such that after chemical oxidation residual material (sulfate and trivalent metal) remained that enables naturally occurring facultative cultures to utilize the residual material as terminal electron acceptors to promote the biological attenuation process of the one or more contaminants and prevents formation and accumulation of hydrogen sulfide which is a toxin to the facultative cultures can be found starting about half way down page 10 (attenuation process) until the end of the specification.

Support for the amendments that added the ratios can be found as notated below.

- 1) From the last paragraph on page 9 we know that sodium persulfate is activated through the presence of ferric iron (or other trivalent metals) in order to form sulfate radicals.
- 2) Equation (10) $S_2O_8^{2-} \rightarrow 2SO_4^{\cdot -}$ provides the equation for persulfate ($S_2O_8^{2-}$) forming sulfate ($SO_4^{\cdot -}$) radicals
- 3) Expanding equation 10 to sodium persulfate ($Na_2S_2O_8$) yields, $Na_2S_2O_8 \rightarrow 2Na^+ + S_2O_8^{2-} \rightarrow 2SO_4^{\cdot -}$
- 4) Substituting molecular weights for sodium persulfate (238 grams) and sulfate (96 grams) illustrates that 238g of sodium persulfate yields 192g (2×96) of sulfate

- 5) From equation 16 we know that trivalent iron or ferric iron (Fe^{3+}) reacts and becomes divalent or ferrous iron (Fe^{2+})
- 6) From the last paragraph of page 11 and equation 21, that was inadvertently labeled equation 14 in the specification (will correct in amendment), we know that sulfide (S^{2-}) and ferrous iron (Fe^{2+}) produce pyrite (FeS_2), $\text{Fe}^{2+} + 2\text{S}^{2-} \rightarrow \text{FeS}_2$
- 7) Expanding equation 21 to capture where the ferrous iron and sulfide come from results in, $2\text{Fe}_2\text{O}_3 + 8\text{SO}_4^- \rightarrow 4\text{FeS}_2 + 19\text{O}_2$
- 8) Substituting molecular weights for Fe_2O_3 , SO_4^- and FeS_2 illustrates that 320 grams ($2 * 160\text{g}$) of Fe_2O_3 and 768 grams ($8 * 96\text{g}$) of SO_4^- are required to produce 480 grams ($4 * 120\text{g}$) of FeS_2
- 9) Using molecular weights we can calculate that 224g of Fe^{3+} is required to produce the 320g of Fe_2O_3
- 10) Based on (4) above we can calculate that 952g of $\text{Na}_2\text{S}_2\text{O}_8$ is required to produce the 768g of SO_4^-
- 11) Accordingly 952g of $\text{Na}_2\text{S}_2\text{O}_8$ and 224g of Fe^{3+} are required to form FeS_2
- 12) Simplifying to 100g of $\text{Na}_2\text{S}_2\text{O}_8$, results in 100g of $\text{Na}_2\text{S}_2\text{O}_8$ and 23.53g of Fe^{3+} are required to form FeS_2 (amount of Fe^{3+} needed is 23.53% of the weight of $\text{Na}_2\text{S}_2\text{O}_8$)
- 13) Assuming a 25% range in that value, results in 17.65 – 29.41g of Fe^{3+} per 100g of $\text{Na}_2\text{S}_2\text{O}_8$ (approximately 17-30%) required to form FeS_2

35 USC § 112(a) or 35 USC § 112 (pre-AIA) 1st paragraph Rejection of the Claims

Claims 21-23 were rejected by the Examiner for failing to comply with the written description requirement since the Examiner believed that the specification did not clearly present the calculating step of claim 21. The Applicant has canceled claim 21 and submits that the

amendment to independent claim 20 is sufficiently supported in the specification as described above and claims 22 and 23 depend therefrom. The Applicant respectfully submits that the rejection has been overcome and requests that the rejection should accordingly be withdrawn.

35 USC § 112(b) or 35 USC § 112 (pre-AIA) 2nd paragraph Rejection of the Claims

Claim 4 was rejected as being indefinite for the noted reasons. The Applicant has amended claim 4 and it is submitted that the amended claim is definite. The Applicant respectfully submits that the rejection has been overcome and requests that the rejection should accordingly be withdrawn.

35 USC § 102(a)(1) and 35 USC § 103 Rejections of the Claims

Claims 1-4, 6-17 and 20 were rejected by the Examiner as being anticipated by *Block et al.* (US Patent 7,576,254). Claim 8 was rejected by the Examiner as being unpatentable over *Block et al.* in view of *Lucas et al.* (Bioresource Technology, 119, 174-180, 2012).

As noted above claim 1 has been amended to recite that combination of persulfate and trivalent metals (17-30% of molecular weight of persulfate) result in the chemical oxidation of one or more contaminants and residual sulfate and residual trivalent metal that enables naturally occurring facultative cultures to utilize the residual material as terminal electron acceptors to promote the biological attenuation process of the one or more contaminants and prevents formation and accumulation of hydrogen sulfide which is a toxin to the facultative cultures.

The Applicant submits that *Block et al.* do not disclose residual material at the end of chemical oxidation, let alone the residual material supporting the biological attenuation of the contaminants or prevention of formation and accumulation of hydrogen sulfide, as recited in the

claim 1. The Examiner contends that biological attenuation naturally occurs. While this may be true, for the biological attenuation to occur it needs something to activate or support it. The residual material acting as electron accepters accomplishes this. *Block et al.* clearly do not disclose residual material. In fact, as previously pointed out *Block et al.* specifically discloses using just enough persulfate to accomplish oxidation.

Furthermore, *Block et al.* do not disclose the ratio of trivalent metals to persulfate (trivalent metals are approximately 17-30% of molecular weight of persulfate).

The Examiner does not rely on *Lucas et al.* to disclose the combination of persulfate and trivalent metals resulting in residual sulfate and residual trivalent metal at the conclusion of chemical oxidation that enables naturally occurring facultative cultures to utilize the residual material as terminal electron acceptors to promote the biological attenuation process of the one or more contaminants and prevents formation and accumulation of hydrogen sulfide which is a toxin to the facultative cultures or the specific ratios with which to accomplish this (17-30% of molecular weight of persulfate in trivalent metals). The Applicant submits that *Lucas et al.* do not disclose this.

For at least the above reasons claim 1 is submitted to be patentable over the cited references. Claims 2-4, 6-9, 12-17, 31 and 32 depend from claim 1 and are submitted to be patentable for at least the reasons advanced above with respect to claim 1 and for the further features recited therein.

Independent claim 20 is submitted to be patentable over the cited references for at least reasons similar to those advanced above with respect to claim 1. Claims 22-30 depend from

claim 20 and are submitted to be patentable for at least the reasons advanced above with respect to claim 20 and for the further features recited therein.

CONCLUSION

The Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (215-997-0248) to facilitate prosecution of this application.

Respectfully submitted,

/DouglasJRyder-43073/

Douglas J. Ryder

Registration No. 43,073

Ryder, Lu, Mazzeo & Konieczny LLC

Customer No. 38284



UNITED STATES PATENT AND TRADEMARK OFFICE

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NOTICE OF ALLOWANCE AND FEE(S) DUE

38284 7590 06/19/2015
Ryder, Lu, Mazzeo and Konieczny, LLC
808 Bethlehem Pike
Suite 200
Colmar, PA 18915

EXAMINER

BERKE-SCHLESSEL, DAVID W

ART UNIT

PAPER NUMBER

1651

DATE MAILED: 06/19/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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13/891,934

05/10/2013

Michael Scalzi

SCA-004

8286

TITLE OF INVENTION: CHEMICAL OXIDATION AND BIOLOGICAL ATTENUATION PROCESS FOR THE TREATMENT OF CONTAMINATED MEDIA

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
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nonprovisional

SMALL

\$480

\$0

\$0

\$480

09/21/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** **Mail Stop ISSUE FEE**
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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

38284 7590 06/19/2015
Ryder, Lu, Mazzeo and Konieczny, LLC
808 Bethlehem Pike
Suite 200
Colmar, PA 18915

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/891,934	05/10/2013	Michael Scalzi	SCA-004	8286

TITLE OF INVENTION: CHEMICAL OXIDATION AND BIOLOGICAL ATTENUATION PROCESS FOR THE TREATMENT OF CONTAMINATED MEDIA

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0	\$0	\$480	09/21/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
BERKE-SCHLESSEL, DAVID W	1651	435-262500

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____
- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/891,934	05/10/2013	Michael Scalzi	SCA-004	8286

38284 7590 06/19/2015
Ryder, Lu, Mazzeo and Konieczny, LLC
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Suite 200
Colmar, PA 18915

EXAMINER

BERKE-SCHLESSEL, DAVID W

ART UNIT	PAPER NUMBER
----------	--------------

1651

DATE MAILED: 06/19/2015

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/891,934	Applicant(s) SCALZI ET AL.	
	Examiner David Berke-Schlessel	Art Unit 1651	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the Response After Final Action dated 6/4/15.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 1-4,6-9,12-17; 20 and 22-32. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|--|---|
| 1. ☐ Notice of References Cited (PTO-892) | 5. ☐ Examiner's Amendment/Comment |
| 2. ☐ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date ____ | 6. ☐ Examiner's Statement of Reasons for Allowance |
| 3. ☐ Examiner's Comment Regarding Requirement for Deposit
of Biological Material | 7. ☐ Other ____. |
| 4. ☐ Interview Summary (PTO-413),
Paper No./Mail Date ____. | |

/Blaine Lankford/
Primary Examiner, Art Unit 1651

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael Scalzi	Examiner:	David W. Berke-Schlessel
Serial No.:	13/891,934	Group Art Unit:	1651
Filed:	May 10, 2013	Docket No.:	SCA-004
Title:	CHEMICAL OXIDATION NAD BIOLOGICAL ATTENUATION PROCESS FOR THE TREATMENT OF CONTAMINATED MEDIA		

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

In response to the Final Office Action mailed April 2, 2015, the Applicant conducted an interview with the Examiner on June 1, 2015 in which the Applicant explained that the combination of persulfate and trivalent metals was such that after chemical oxidation residual material (sulfate and trivalent metal) remained that enables naturally occurring facultative cultures to utilize the residual material as terminal electron acceptors to promote the biological attenuation process of the one or more contaminants and prevents formation and accumulation of hydrogen sulfide which is a toxin to the facultative cultures. The Examiner requested that a ratio be included in the claims and the Applicants agreed to this.

Please amend the above-identified application as follows and consider the following arguments. As the Applicant believes that these amendments put the application in condition for allowance, the Applicant respectfully requests that these amendments be entered.



UNITED STATES PATENT AND TRADEMARK OFFICE

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BIB DATA SHEET

CONFIRMATION NO. 8286

SERIAL NUMBER 13/891,934	FILING or 371(c) DATE 05/10/2013 RULE	CLASS 435	GROUP ART UNIT 1651	ATTORNEY DOCKET NO. SCA-004	
APPLICANTS INNOVATIVE ENVIRONMENTAL TECHNOLOGIES, INC., Pipersville, PA; INVENTORS Michael Scalzi, Doylestown, PA; Antonis Karachalios, North Wales, PA; ** CONTINUING DATA ***** ** FOREIGN APPLICATIONS ***** ** IF REQUIRED, FOREIGN FILING LICENSE GRANTED *** SMALL ENTITY ** 06/08/2013					
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☒ No Verified and /DAVID W Acknowledged <u>BERKE-SCHLESSEL/</u> Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY PA	SHEETS DRAWINGS 0	TOTAL CLAIMS 19	INDEPENDENT CLAIMS 1
ADDRESS Ryder, Lu, Mazzeo and Konieczny, LLC 808 Bethlehem Pike Suite 200 Colmar, PA 18915 UNITED STATES					
TITLE CHEMICAL OXIDATION AND BIOLOGICAL ATTENUATION PROCESS FOR THE TREATMENT OF CONTAMINATED MEDIA					
FILING FEE RECEIVED 970	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit		

EAST Search History

EAST Search History (Prior Art)

<This search history is empty>

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1098	oxidiz\$6 same ((volatile adj organic adj compound) or voc)	USPAT; UPAD	OR	ON	2015/06/11 08:55
L2	203672	peroxide or persulfate	USPAT; UPAD	OR	ON	2015/06/11 08:56
L3	57	l1 same l2	USPAT; UPAD	OR	ON	2015/06/11 08:56
L4	203672	peroxide or persulfate or persulphate	USPAT; UPAD	OR	ON	2015/06/11 08:57
L5	482212	(trivalent near3 metal) or iron or manganese	USPAT; UPAD	OR	ON	2015/06/11 08:58
L6	14	l3 same l5	USPAT; UPAD	OR	ON	2015/06/11 08:58
L7	767	biological near5 attenuat\$6	USPAT; UPAD	OR	ON	2015/06/11 08:59
L8	0	l1 same l7	USPAT; UPAD	OR	ON	2015/06/11 08:59
L9	1	l1 and l7	USPAT; UPAD	OR	ON	2015/06/11 08:59

6/ 11/ 2015 9:00:08 AM
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1600x1200.wsp

Search Notes 	Application/Control No. 13891934	Applicant(s)/Patent Under Reexamination SCALZI ET AL.
	Examiner DAVID BERKE-SCHLESSEL	Art Unit 1651

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
EAST search notes attached	10/28/14	DBS
STN search notes attached	10/28/14	DBS
Inventor search performed (8766030; 8776734)	10/28/14	DBS
EAST search notes attached	4/1/15	DBS
Interference search notes attached	6/11/15	DBS

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
Y02W10	12	6/11/15	DBS
B09C1	10; 08	6/11/15	DBS
C02F2101	36; 306; 322; 327; 30; 34	6/11/15	DBS
C02F2103	06	6/11/15	DBS
C02F3	28; 006	6/11/15	DBS
C02F1	5245; 722	6/11/15	DBS

/DAVID BERKE-SCHLESSEL/ Examiner.Art Unit 1651	
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