Pacific Northwest
NATIONAL LABORATORY

PNNL-29786

Biotic Degradation of Acetonitrile

Limitations, Controls, and Conversion Rates

April 2020

Christopher E. Bagwell
Matthew Asmussen

U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

DISCLAIMER

PACIFIC NORTHWEST NATIONAL LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC05-76RL01830

Printed in the United States of America

Available to DOE and DOE contractors from the
Office of Scientific and Technical Information,
P.O. Box 62, Oak Ridge, TN 37831-0062;
ph: (865) 576-8401
fax: (865) 576-5728
email: reports@adonis.osti.gov

Available to the public from the National Technical Information Service
5301 Shawnee Rd., Alexandria, VA 22312
ph: (800) 553-NTIS (6847)
email: orders@ntis.gov <https://www.ntis.gov/about>
Online ordering: http://www.ntis.gov

PNNL-29786

Biotic Degradation of Acetonitrile

Limitations, Controls, and Conversion Rates

April 2020

Christopher E. Bagwell
Matthew Asmussen

Prepared for
the U.S. Department of Energy
under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory
Richland, Washington 99354

Summary

This document has been prepared to support Washington River Protection Solutions (WRPS) in their evaluation of technologies to remediate organic compounds in the Liquid Effluent Retention Facility (LERF)/Effluent Treatment Facility (ETF) during direct feed low activity waste (DFLAW) operations at Hanford. This report summarizes the results of a review of the available literature describing aerobic and anaerobic biological treatment of acetonitrile and proposes a test matrix to evaluate microbial degradation rates of acetonitrile. Numerous studies describe acetonitrile degradation by pure culture strains of bacteria, fungi, and yeast; however, the purpose of this review was to provide estimations of degradation under conditions that more closely approximate the Liquid Effluent Retention Facility (LERF) basins at the Hanford Site. To this end, documented limitations, controls, and conversion rates are summarized for mixed and natural microbial communities being maintained or operated as a biological treatment for wastewater processing.

Based on the literature evaluation, the inherent capacity and rates of microbial degradation of acetonitrile are sufficiently high that if applied in the LERF basins, acetonitrile removal could be achieved. To be clear, though, published studies have not been conducted under conditions that approximate or that could be directly related to the LERF basins. Thus, a focused test matrix the encompasses LERF relevant conditions to specifically measure microbial degradation of acetonitrile is proposed and the results would provide the strong technical justification for use of this low-cost efficient biological process at LERF during DFLAW operations and beyond.

Key highlights of this review include the following:

- Acetonitrile can be readily degraded by many microorganisms as a sole source of carbon and nitrogen under oxic conditions at parts-per-million levels. The primary limitations on aerobic degradation activity and rate include acetonitrile toxicity at high concentrations (g/L, far above the expected mg/L concentrations projected at LERF) and the efficient supply of O_2 to bacteria catalyzing acetonitrile conversion to acetic acid and ammonium.
- The published literature generally regards anaerobic degradation of acetonitrile as a negligible process, though there are a few published studies that demonstrate anaerobic degradation of acetonitrile does occur and at an acceptable rate for effective removal. The potential for anaerobic degradation of acetonitrile (that is under low to no O_2 conditions) should be systematically investigated for conditions that are specifically related to the LERF wastewater. Additionally, since NO_3 is a likely product from aerobic degradation of acetonitrile, the potential for anaerobic degradation pathway(s) to contribute to the overall removal of acetonitrile from the LERF is justified.
- Alkaline pH of the process streams directed to LERF/EFT should not present a legitimate challenge for aerobic or anaerobic degradation of acetonitrile. Published laboratory studies demonstrate degradation processes under circum-neutral pH buffered conditions. In a field deployment scenario, microorganisms residing in the LERF storage basins will become naturally adapted to the alkaline pH, organic and inorganic composition of the LERF wastewater. A previous investigation has already demonstrated the presence of a robust native microbial community in the LERF basins.
- Acetonitrile degradation rates will be influenced by the other organic constituents present in LERF/ETF waste streams. Nearly all of the documented organic compounds present in LERF/EFT waste streams can be used by microorganisms as a viable carbon and energy source for growth. The ready supply of organic carbon in LERF/EFT waste streams will be expected to support a robust microbial community and sustain degradation activity in the LERF basins. However, certain constituents (e.g., phenol, cresol) may be toxic to microbial activity and could potentially interfere with acetonitrile degradation. Specific

laboratory testing will be necessary to measure the rate and extent of acetonitrile degradation that can be expected or achieved in the LERF/ETF waste streams.

- Finally, a comprehensive experimental plan is proposed for quantifying aerobic and anaerobic degradation of acetonitrile in conditions that approximate the LERF basins. These evaluations will be made using simulated LERF/EFT waste streams and a typical wastewater treatment microbial community in order to estimate the rate and extent to which natural processes can degrade acetonitrile during interim storage in the LERF basins.

Acknowledgments

We acknowledge the support and contributions of Rodney Skeen (WRPS), and PNNL staff members David Peeler (project management), James Szecsody (technical review) and Matt Wilburn (technical editing).

Acronyms and Abbreviations

DRE	Destruction and removal efficiency
EMF	Effluent Management Facility
ETF	Effluent Treatment Facility
LERF	Liquid Effluent Retention Facility
UV	Ultraviolet
WRPS	Washington River Protection Solutions
WTP	Waste Treatment and Immobilization Plant

Contents

Figures

Tables

1.0 Introduction

The current waste feed profile for the condensate liquid stream from the Waste Treatment and Immobilization Plant (WTP) Effluent Management Facility (EMF) on the Hanford Site contains several organic species, including acetonitrile at a concentration of approximately 46 mg/L (RPP-RPT-60974). Table 1 is a reproduction of the organic composition of the 2018 EMF condensate waste profile provided in the *ETF New Waste Stream Acceptance Package for WTP Effluent Management Facility* (RPP-RPP-60974). This effluent stream will be sent to the Liquid Effluent Retention Facility (LERF) and subsequently processed in the Effluent Treatment Facility (ETF). The delisting petition for the ETF set the allowable maximum acetonitrile concentration to 1.2 mg/L (40 CFR 261, Appendix IX, Table 2). This limit equates to the need for an ETF system destruction and removal efficiency (DRE) for acetonitrile of approximately 97.4%.[1] The current ETF system does not meet the acetonitrile DRE requirement and the operating contractor is designing a steam-stripping system for this purpose (RPP-RPT-61923).

Table 1. EMF condensate waste profile from RPP-RPT-60974.

Chemical Constituent	EMF Effluent Concentration (mg/L)	Chemical Constituent	EMF Effluent Concentration (mg/L)
1,4-Dioxane	3.11E-04	bis (2-Ethylhexyl)phthalate	1.00E+01
2-Butanone	1.46E+00	Butanal	7.66E-02
2-Butoxyethanol	1.21E+00	Dichloromethane	1.44E-02
2-Hexanone	1.41E-04	Diethyl phthalate	5.53E+00
2-Nitrophenol	6.29E+00	Di-n-octylphthalate	5.44E-01
2-Propanone	4.24E+00	Formate	3.64E+00
4,6-Dinitro-o-cresol	2.17E+01	Glycolate	1.30E+00
Acetate	8.59E-01	m-Cresol	3.12E+00
Acetonitrile	4.64E+01	Naphthalene	1.78E+00
Acetophenone	1.37E-03	1-Butanol	1.93E-02
Acrylonitrile	8.80E-01	Nitrobenzene	9.51E-01
Aroclors (Total)	9.24E-04	N-Nitrosomorpholine	8.36E+00
Benzene	1.29E-05	N-Nitroso-N,N-dimethylamine	1.78E-02
o-Cresol	4.21E+00	Propionitrile	8.78E-04
Phenol	4.27E+00	Tributyl phosphate	1.17E+00
p-Nitrophenol	3.84E-02	---	---

It is important to note, however, that the handling of the EMF waste stream may alter the concentration of organics prior to its introduction to the EMF. Specifically, there is an opportunity for microbial activity to reduce the concentration of acetonitrile. This opportunity is created by the fact that EMF liquids will not be directly fed to the ETF. Rather, the EMF effluents will first be routed to an outdoor retention basin located in the LERF. Figure 1 provides an aerial view of the LERF. The LERF is located south of the ETF and is currently composed of three 7.8-Mgal, covered, double-lined basins designed to store aqueous wastewaters. The basin liners are made of high-density polyethylene (HDPE) and the floating cover is composed of very low-density, non-transparent polyethylene (WHC-SD-W105-SAR-001). The floating liner does not always form a perfect seal with the basin liquid, and the air space between floating liner and the liquid is connected to atmospheric air through 21 breather vents that are equipped with carbon absorbers to remove volatile organic compounds that may evaporate from the basin waters.

[1] The DRE assumes an acetonitrile concentration of 46.4 mg/L at the inlet and 1.2 mg/L in the effluent stream.

EMF condensate wastewater will accumulate in a designated LERF basin for up to 1 year before it is processed. During accumulation there is no active agitation within the basin. The presence of microbial activity within the LERF basins has been observed in past studies focused on the source of particulate matter responsible for fouling the ETF inlet filters (RPP-21533, Rev 0.; RPP-RPT-22879, Rev. 0; WRPS-1804803). In these works, a large quantity of gelatinous material was reported in the ETF inlet filter units and it was speculated that biological growth was the primary source since the feed material (242-A evaporator condensate) was filtered prior to its introduction to the LERF basin. In addition, a study conducted in 1995 characterized the types and populations of microorganisms in the LERF basins storing 242-A evaporator process condensate (see Appendix A of RPP-21533). Aerobic heterotrophic bacteria along with denitrifying and fermenting bacteria were among those observed to be active in the basins' alkaline (pH=10) wastewater. It is anticipated that EMF effluent wastewater (having a pH between 9 and 10) will have a similar ability to maintain microbial activity.

Figure 1. Liquid Effluent Retention Facility storage basins.

2.0 Degradation Pathways

2.1 Chemical Decomposition

A number of chemical processes have been described for acetonitrile decomposition, including combustion; alkaline hydrolysis (Gilomen et al. 1995); the Radziszewski reaction using H_2O_2 (RPP-RPT-61697) and advanced oxidation processes using Fenton reagent, ozone, and TiO_2 catalyst (Augugliaro et al. 2004; Micaroni et al. 2004). The most efficient chemical decomposition process, though, was shown to be photodegradation and photocatalysis. Each of these processes would require the addition and permitting of a new unit of operation to the Hanford flowsheet.

Aqueous solutions of up to 20% acetonitrile can be rapidly decomposed by exposure to ultraviolet (UV) light and natural sunlight (Micaroni et al. 2004). Thermal decomposition did not contribute significantly to the loss of acetonitrile mass (measured as dissolved organic carbon) in these studies. Photolysis of acetonitrile has been shown to be positively correlated with sunlight intensity. UV light (365 nm) at 1.1 to 2.0 mW/cm decreased the aqueous concentration of acetonitrile (84% in water) by 52% in 4 hours and 70% in 6 hours, and complete destruction was measured by 27 hours. Since dissolved organic carbon was used as a proxy for acetonitrile in these investigations, the production of volatile or toxic intermediates was not evaluated. Several studies, though, have documented that photocatalytic oxidation of acetonitrile in gas and liquid phases can result in the production of cyanogen (C_2N_2), cyanate (HNCO), cyano radicals (•CN), and possibly free cyanide, all of which have high toxicity (Augugliaro et al. 1997, 1999a,b; Lichtin and Avudaithai 1996). Hypochlorite (ClO-) has been shown to inhibit the photodegradation of acetonitrile.

Experimental rates for the chemical decomposition of acetonitrile describe ideal conditions and likely near maximum reaction kinetics. As such, published rate values cannot be reliably extrapolated to predict photodegradation of acetonitrile in industrial wastewaters, though more realistic values are available for other organonitriles. The extent to which photochemical decomposition would occur in a natural setting would be majorly impacted by the LERF/ETF waste streams (water quality, dissolved organic matter, acetonitrile loading) and configuration of the LERF basin (depth, surface area, floating liner, UV exposure). There is a dearth of information available on the environmental stability of acetonitrile, or the potential for interacting and coincidental degradation pathways, both chemical and microbiological.

2.2 Biological Degradation

Because many different categories of microorganisms (bacteria, fungi, and yeast) are capable of degrading acetonitrile, mixed community biological treatments have been devised for cost effective and efficient treatment of acetonitrile containing wastewaters (Dhillon and Shivaraman 1999; Dias et al. 2000; Egelkamp et al. 2017; Håkansson and Mattiasson 2002; Li et al. 2007, 2008; Linardi et al. 1996; Manolov et al. 2005; Muñoz et al. 2005; Nagle et al. 1995; Nawaz and Chapatwala 1990; Nawaz et al. 1989; Van der Walt et al. 1993). It is expected that such a process could be utilized in the LERF basins without major capital facility investments and with minimal permitting. Acetonitrile can be degraded aerobically and anaerobically by microbes as a sole source of carbon and nitrogen, though nearly all of the published studies describe the aerobic pathway, with corresponding reaction kinetics and conversion efficiencies. Aerobic, and presumably anaerobic, degradation of acetonitrile is catalyzed by two different enzymatic processes (Figure 2). The first is a two-step enzymatic process that converts acetonitrile to the end products acetate and ammonia (Ahmed and Farooqui 1982; Asano et al. 1980; Lou et al. 2001; Thompson et al. 1988). In the second, microorganisms are able to degrade amides and nitriles to carboxylic acids and ammonia in a single-step enzymatic reaction.

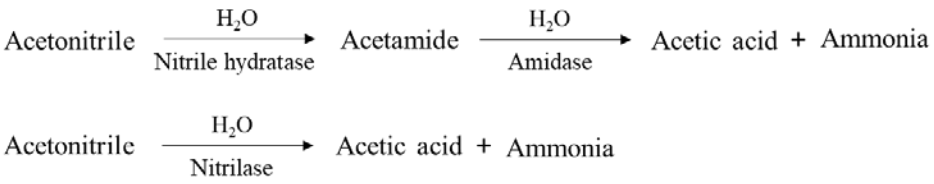

Figure 2. Two pathways for enzymatic hydrolysis of acetonitrile.

Numerous studies have described the degradation of acetonitrile by a variety of pure culture strains (Chapatwala et al. 1990; Egelkamp et al. 2017; Li et al. 2013; Linardi et al. 1996; Nawaz et al. 1989, 1990; Sorokin et al. 2007). These publications do provide evidence for effective microbial degradation of acetonitrile and in certain cases at extremes in environmental conditions (e.g., pH, salinity, °C). However, degradation rate values from these types of studies are not directly transferrable for potential treatment of waste streams in the LERF basins. Because this information cannot be meaningfully extrapolated for the intended purposes of this review, discussion of the literature on pure culture strains will not be considered further.

Specific characteristics of mixed community bioreactors catalyzing the aerobic degradation of acetonitrile are summarized in Table 2. In general, published literature describes aerobic degradation of acetonitrile as the sole carbon and energy source in reactor systems inoculated with activated sludge from wastewater treatment facilities. Limited information is available for anaerobic degradation of acetonitrile; more discussion on this topic is provided below. In these continuous-fed bioreactor systems, the key regulating controls on acetonitrile degradation capacity are biological toxicity at mass loadings much higher than the projected concentrations in the LERF basins (> 7 g / L: Li et al. 2007; Muñoz et al. 2005), adequate supply of O_2 (Manolov et al. 2005), and the buildup of ammonia in unbuffered systems (Li et al. 2008; Manolov et al. 2005). Utilization of mixed organic constituents in LERF/ETF waste streams will ultimately determine the rate and extent to which acetonitrile is degraded in the LERF basins.

Numerous studies emphasize the development of biofilm reactors to achieve acceptable conversion efficiencies at high mass loading of acetonitrile (Li et al. 2008; Parkin and Speece 1983). Again, the inherent limitation to bacterial degradation is acetonitrile toxicity at high concentrations (~g/L with mg/L levels expected in the LERF basins). Li et al. (2013) demonstrated a strong positive correlation between acclimation of a mixed community bioreactor and degradation of acetonitrile to the activity of biofilm-forming bacteria. When these biofilm-forming bacteria were tested individually, not a mixed community, acetonitrile was not degraded. These results demonstrate the important influence of synergistic associations that accelerate bacterial growth and degradation activity. Furthermore, the establishment of a biofilm community provides enhanced resistance to acetonitrile toxicity and permits efficient wastewater treatment. Biofilm-forming bacteria have been documented in the LERF basins, though acetonitrile toxicity is not expected to be an impediment to degradation activity at the projected concentrations expected at LERF, as shown in Table 1.

Aerobic degradation of acetonitrile can result in the accumulation of acetate, NH_4^+, and/or NO_3^-, which could, if particularly high concentrations are achieved (~mg/L), affect the rate or sustainability of acetonitrile conversion depending on how quickly these resources are used. The production of acetate and ammonium would provide a reliable supply of organic carbon and biologically available nitrogen. Rapid uptake and efficient use of these products would be expected given the establishment of a mixed microbial community in the LERF (e.g., Håkansson and Mattiasson 2002), though specific rates will depend on the organic composition of the waste streams directed to LERF. The production of nitrate would provide an alternative terminal electron acceptor that could support anaerobic degradation of acetonitrile. The combined activity of aerobic and anaerobic heterotrophic bacteria and fermenters in the LERF basin would

be expected to prevent the accumulation of these products (acetate, NH_3, NO_3), and collectively contribute the removal of acetonitrile from LERF wastewaters.

Acetonitrile is a potentially toxic and highly volatile pollutant of many industrial wastewaters. Numerous studies express caution regarding the possibility that conventional pond aeration and mixing could promote acetonitrile losses due to increased flux to the atmosphere; however, few studies have actually quantified a change in concentration that could be attributed to volatilization. In fact, short-term (hours) laboratory studies measured negligible evaporative losses of acetonitrile during active aeration of bioprocess reactors (Håkansson and Mattiasson 2002). It remains unclear how significant these potential losses could be in natural systems.

The published literature generally regards anaerobic degradation of acetonitrile as a negligible process, though there is limited evidence which suggests that anaerobic pathways can contribute to the degradation of acetonitrile (Li et al., 2007). Given the paucity of data for this pathway, the potential for acetonitrile degradation in the presence of alternative terminal electron acceptors should be systematically investigated. Since there will be no active mixing of the LERF basin, aerobic degradation of acetonitrile will likely be constrained by oxygen diffusion at the air-liquid interface. Thus, degradation pathways under low oxygen conditions would be particularly relevant in the LERF basins. Furthermore, since NO_3 is a likely product from aerobic degradation, denitrification conditions could become established in the LERF and contribute to the removal of acetonitrile from the LERF. Anaerobic degradation processes should be evaluated in simulated wastewater and conditions that are directly related to those expected for the LERF basins.

In short, the published studies cited herein and those summarized in Table 2 clearly demonstrate efficient and complete aerobic degradation of acetonitrile at concentrations that far exceed those expected at the LERF (46 mg/L). As such, we would certainly expect acetonitrile degradation to occur in the LERF basin, though technical justification is needed to establish degradation rates under relevant conditions for the LERF.

Table 2. Mixed microbial systems for aerobic degradation of acetonitrile.

Source	Loading (g/L/d)	Degradation Rate (g/L/d)	Efficiency (%)	Products	Reference
Activated sludge from wastewater. Oxygenated, stirred biofilm reactors	1 - 1.58	0.77 - 1.2	53, single reactor 100, dual reactors	Acetate, NH_3 – single reactor NH_3 – dual reactors	Håkansson and Mattiasson 2002
Activated sludge, membrane aerated biofilm reactor. Surface area = 84.5 m^2/m^3	11.29 ± 0.32 $g/m^2/d$	1.05	96.7 ± 3.14	NH_3, 34.2 - 52.7 mg/L	Li et al. 2008
Aerated packed-bed bioreactor	a) 0.43 b) 0.77 c) 0.99 d) 1.56 e) 2.76 g) 3.0	a) 0.43 b) 0.77 c) 0.81 d) 1.04 e) 1.03 f) 0.79	a) 100 b) 99.5 c) 81.6 d) 66.7 e) 37.2 f) 26.2	NH_3	Manolov et al. 2005
Algal – mixed community stirred tank photobioreactor	2.5	2.3	100	NH_3, low levels	Muñoz et al. 2005
Mixed community moving-bed-biofilm reactor R1: 3 g/L activated sludge R2: 2.4 g/L activated sludge R3: 1.8 g/L + 0.6g/L pure strains	R1: 0.8 R2: 0.25 R3: 0.25	Not detected	R1: 95 R2: 97 R3: 100	R1: Acetamide, Acetic acid R2: None R3: None	Li et al. 2013

3.0 Experimental Plan

With the very promising literature evidence for microbial degradation of acetonitrile, testing in relevant LERF conditions to measure acetonitrile degradation rates is highly prudent. The experimental and testing approach to measure degradation rates of acetonitrile under LERF basin conditions are provided here. Experimental batch reactors will be prepared, in duplicate, to measure microbiological degradation of acetonitrile from simulated LERF/ETF waste streams as defined by the LERF/ETF waste feed profile under aerobic and anaerobic conditions (NO_3, SO_4, and Fe^{3+}). A summary of the experimental matrix, treatments, and sampling is provided in Table 3.

Briefly, simulated LERF wastewater will be pH adjusted (pH 8, 9, and 10) to approximate the anticipated pH range of the LERF basin. Effluent (100 mL) will be aliquoted into 125-mL serum bottles, leaving 25 mL of headspace to permit adequate O_2 exchange for aerobic degradation and gas phase sampling for quantitative measurement of acetonitrile volatilization. The starting concentration of acetonitrile will be set at 46 mg/L based on the EMF condensate waste profile (RPP-RPT-60974), though higher concentrations (< 100 mg/L) will also be examined to ensure degradation capacity should actual concentrations exceed (if only temporarily or occasionally) the current projections. Microbiological degradation testing will use an inoculum obtained from effluent fluid from the City of Richland wastewater treatment plant aeration basin. Sterile control tests (heat killed biomass and without biomass) will quantify abiotic losses of acetonitrile.

Microbiological batch reactor treatments will measure acetonitrile degradation under aerobic (20% O_2 headspace composition) and anaerobic conditions. For the latter, the simulated LERF basin solution composition will be supplemented with NO_3, SO_4, or Fe^{3+} (concentrations will be set by waste feed profile for the condensate liquid stream from the WTP/EMF) and sparged with a N_2 stream to remove bulk O_2. Serum bottles will be sealed with PTFE/silicone septa and aluminum crimp caps. The bioreactors will be incubated at room temperature (25 °C) on a rotary shaker (180 rpm). Batch reactors will be sampled at a minimum on a weekly basis for up to 30 days.

For chemical analyses, aqueous sub-samples will first be centrifuged (10,000*g* at 4 °C for 20 min) or filtered (0.2-µm pore size) to remove particulates. Measurements will include pH, total anions (by ion chromatography), as well as the contents of acetonitrile, acetamide, acetic acid (by gas chromatography-mass spectrometry, GC-MS), and ammonia (using a HACH kit). Microbial respiratory activity will be assayed by measuring concentrations of electron acceptors, specifically NO_3, NO_2, SO_4 and O_2. Total microbial biomass will be measured by spectrophotometry (630 nm) or by whole cell protein content using the Lowry assay.

Table 3. Laboratory testing (n=2) to measure biotic (aerobic and anaerobic) and abiotic degradation of acetonitrile (46 – 100 mg/L) at a range of pH values (pH 8-10) relevant to LERF basin wastewater.

Test	Biomass	Kill	ACN	NO_3	SO_4	Fe^{3+}	O_2	Purpose
1	X	X	X	X	X	X	X	Heat killed control
2			X	X	X	X	X	Biotic control
3	X			X	X	X	X	No substrate control
4			X	X	X	X	X	Abiotic control
5	X		X				X	Aerobic degradation
6	X		X	X				Anaerobic denitrification
7	X		X			X		Anaerobic iron reduction
7	X		X		X			Anaerobic sulfate reduction
8	X		X	X	X	X	X	Aerobic-anaerobic degradation

4.0 Summary and Recommendation

From the literature evaluation, microbial degradation rates of acetonitrile are sufficiently high that if applied to the LERF basins, acetonitrile removal could be achieved. However, these studies were conducted in controlled laboratory setting and do not directly translate to the LERF basins. Thus, a focused test matrix in LERF relevant conditions to measure microbial acetonitrile degradation rates has been proposed and would provide a strong technical justification for the implementation of this low-cost process at LERF during DFLAW operations and beyond. Key highlights include the following:

- Overwhelming evidence demonstrates efficient degradation of acetonitrile by aerobic and anaerobic bacteria, fungi, and yeast. While experimental results from pure cultures strains of microorganisms do not directly relate to relevant conditions at the LERF, these studies support a technical basis for acetonitrile degradation in the LERF basin once the natural microbial community establishes.

- Oxic/aerobic microbial activity is known to occur at LERF in a mixed microbial community. Acetonitrile can be readily degraded by many microorganisms as a sole source of carbon and nitrogen under oxic conditions at part per million (ppm) levels. The primary limitations on aerobic degradation activity and rate include the acetonitrile toxicity at high concentrations (g/L, far above the expected mg/L concentrations projected at LERF) and efficient supply of O_2 to bacteria catalyzing acetonitrile conversion to acetic acid and ammonium. It is likely that testing under oxic conditions in simulated LERF wastewaters will demonstrate successful acetonitrile degradation.

- Due to the static nature of the LERF basins, the diffusion of oxygen through the depth of the basin may be restricted and anaerobic conditions will likely develop. The potential for anaerobic pathways to contribute to acetonitrile degradation has been largely ignored in the published literature. Still, of the few studies available, anaerobic degradation is a promising option for acetonitrile removal though the rates may be slower than aerobic pathways. The potential for acetonitrile degradation in the presence of alternative terminal electron acceptors (e.g., NO_3) should be systematically investigated as it is a scenario directly relevant to the LERF basins.

- The alkaline pH of the streams directed to LERF/ETF should not present a legitimate challenge for aerobic or anaerobic degradation of acetonitrile. Published lab studies all demonstrate the process under buffered conditions at circum-neutral pH. In a field deployment the microorganisms present in the LERF basins will become naturally adapted to the alkaline pH of the system. A previous investigation has already demonstrated the presence of a robust native microbial community in the LERF basins.

- Acetonitrile degradation rate will be influenced by the other organic constituents present in LERF/ETF waste streams. Nearly all of the documented organic compounds present in the LERF/ETF waste streams can be utilized by microorganisms as a viable carbon and energy source for growth. The ready supply of organic carbon in the LERF/ETF waste streams will be expected to support a robust microbial community and sustain degradation activity in the LERF basins. However, certain constituents (phenol, cresols) may be toxic to microbial activity and could potentially interfere with acetonitrile degradation. Specific lab testing will be necessary to measure the rate and extent of acetonitrile degradation that can be expected or achieved in the LERF/ETF waste streams.

- Finally, a focused, yet comprehensive, experimental plan is proposed for quantifying aerobic and anaerobic degradation of acetonitrile in conditions that approximate the LERF basins. These evaluations

will be made using simulated LERF/ETF waste streams and a typical wastewater treatment microbial community to estimate the rate and extent to which naturally occurring biological processes can degrade acetonitrile during interim storage in the LERF basin. The proposed experimental plan would generate data that could be immediately used to build a strong technical case for the implementation of microbiological degradation of acetonitrile at LERF.

5.0 References

40 CFR 261, "Identification and Listing of Hazardous Waste," Appendix IX, Wastes Excluded Under §§ 260.20 and 260.22, Table 2. *Code of Federal Regulations.*

Ahmed AE and MYH Farooqui. 1982. "Comparative toxicities of aliphatic nitriles." *Toxicol. Let.* 12:157-163.

Asano Y, Y Tani, and H Yamada. 1980. "A new enzyme 'nitrile hydratase' which degrades acetonitrile in combination with amidase." *Agric. Biol. Chem.* 44:2251-2252.

Augugliaro V, AB Prevot, JC Vázquez, E García-López, A Irico, V Loddo, S Malato Rodríguez, G Marcì, L Palmisano, and E Pramauro. 2004. "Photocatalytic oxidation of acetonitrile in aqueous suspension of titanium dioxide irradiated by sunlight." *Adv. Environ. Res*. 8(3-4):329-35.

Augugliaro V, EG López, V Loddo, G Marcì, and L Palmisano. 1999a. "Degradation kinetics of iron(III) cyanocomplexes in irradiated systems." *Adv. Environ. Res*. 3:179-188.

Augugliaro V, JB Gálvez, JC Vázquez, EG López, V Loddo, MJ López Muñoz, SM Rodríguez, G Marcì, L Palmisano, M Schiavello, and JS Ruiz. 1999b. "Photocatalytic oxidation of cyanide in aqueous TiO_2 suspensions irradiated by sunlight in mild and strong oxidant conditions." *Catal. Today* 54:245-253.

Augugliaro V, V Loddo, MJ López Muñoz, G Marcì, and L Palmisano. 1997. "Photocatalytic oxidation of cyanides in aqueous titanium dioxide suspensions." *J. Catal.* 166:272-283.

Chapatwala KD, MS Nawaz, JD Richardson, and JH Wolfram. 1990. "Isolation and characterization of acetonitrile utilizing bacteria." *J. Ind. Microbiol.* 5:65-69.

Dhillon JK and N Shivaraman. 1999. "Biodegradation of cyanide compounds by a Pseudomonas species (S1)." *Can. J. Microbiol.* 45:201-208.

Dias JCT, RP Rezende, and VR Linardi. 2000. "Biodegradation of acetonitrile by cells of Candida Guilliermondii UFMG-Y65 immobilized in alginate, k-carrageenan and citric pectin." *Braz. J. Microbiol.* 31:61-66.

Egelkamp R, D Schneider, R Hertel, and R Daniel. 2017. "Nitrile-degrading bacteria isolated from compost." *Front. Environ. Sci.* 12:56. https://doi.org/10.3389/fenvs.2017.00056

Gilomen K, HP Stauffer, and VR Meyer. 1995. "Detoxification of acetonitrile — water wastes from liquid chromatography." *Chromatographia* 41:488-491.

Håkansson K and B Mattiasson. 2002. "Microbial degradation of acetonitrile using a suspended-carrier biofilm process." *Biotechnol. Lett.* 24:287-291.

Li C, Y Li, X Cheng, L Feng, C Xi, and Y Zhang. 2013. "Immobilization of *Rhodococcus rhodochrous* BX2 (an acetonitrile-degrading bacterium) with biofilm-forming bacteria for wastewater treatment." *Bioresour. Technol.* 131:390-396. https://doi.org/10.1016/j.biortech.2012.12.140

Li T, J Liu, R Bai, and F-S Wong 2008. "Membrane-aerated biofilm reactor for the treatment of acetonitrile wastewater." *Environ. Sci. Technol.* 42:2099-2104.

Li T, J Liu, R Bai, D-G Ohandja, and F-S Wong. 2007. “Biodegradation of organonitriles by adapted activated sludge consortium with acetonitrile-degrading microorganisms.” *Water Res.* 41:3465-3473.

Linardi VR, JCT Dias, and CA Rosa. 1996. “Utilization of acetonitrile and other aliphatic nitriles by a *Candida famata* strain.” *FEMS Microbiol. Lett.* 144:67-71.

Litchin NN and M Avudaithai. 1996. “TiO_2-photocatalyzed oxidative degradation of CH_3CN, CH_3OH, C_2HCl_3, and CH_2Cl_2 supplied as vapors and in aqueous solution under similar conditions.” *Environ. Sci. Technol.* 30(6):2014-2020.

Lou WY, MH Zong, and SL Liu. 2001. “Research progress on enzymatic hydrolysis of nitrile.” *Microbiol.* 28:76-81.

Manolov T, K Håkansson, and B Guieysse. 2005. “Continuous acetonitrile degradation in a packed-bed bioreactor.” *Appl. Environ. Biotechnol.* 66:567-574

Micaroni RC, da CM, MIMS Bueno, and W de F Jardim. 2004. “Degradation of acetonitrile residues using oxidation processes.” *J. Braz. Chem. Soc.* 15(4):509-513.

Muñoz R, M Jacinto, B Guieysse, and B Mattiasson. 2005. “Combined carbon and nitrogen removal from acetonitrile using algal-bacterial bioreactors.” *Appl. Microbiol. Biotech.* 67:699-707.

Nagle NJ, CJ Rivard, A Mohagheghi, and G Philippidis. 1995. “Bioconversion of cyanide and acetonitrile by a municipal-sewage-derived anaerobic consortium.” In: Hinchee RE (ed) *Bioremediation of inorganic*s. Battelle, Columbus, pp. 71-79.

Nawaz MS and KD Chapatwala. 1990. “Simultaneous degradation of acetonitrile and biphenyl by Pseudomonas aeruginosa.” *Can. J. Microbiol.* 37:411-418.

Nawaz MS, KD Chapatwala, and JH Wolfram. 1989. “Degradation of acetonitrile by *Pseudomonas putida.*” *Appl. Environ. Microbiol.* 55:2267-2274.

Parkin GF and Speece RE. 1983. “Attached versus suspended growth reactors: response to toxic substances.” *Water Sci. Technol.* 15:261-289.

RPP-21533. 2004. *Test Plan for the Biocide Treatment of LERF Basin 42 Feed Using Glutaraldehyde*. Rev. 0, CH2M Hill Hanford Group, Inc., Richland, Washington.

RPP-RPT-22879. 2004. *Liquid Effluent Retention Facility Basin 42 Studies*. Rev. 0, CH2M Hill Hanford Group, Inc., Richland Washington.

RPP-RPT-60974. 2019. *ETF New Waste Stream Acceptance Package for WTP Effluent Management Facility*. Rev. 0, Washington River Protection Solutions, LLC, Richland, Washington.

RPP-RPT-61697. 2019. *222-S Laboratory Study of the Reaction of Acetonitrile with Alkaline Hydrogen Peroxide for the Effluent Treatment Facility.* Washington River Protection Solutions, Richland, WA.

RPP-RPT-61923. 2019. *Effluent Treatment Facility Assessment of Flowsheet Impacts from the Hanford Tank Waste Treatment and Immobilization Plant Effluent Management Facility Waste Profile*. Rev. 0, Washington River Protection Solutions, LLC, Richland, Washington.

Sorokin DY, S van Pelt, TP Tourova, S Takaichi, and G Muyzer. 2007. “Acetonitrile degradation under haloalkaline conditions by *Natronocella acetinitrilica* gen. nov., sp. nov.” *Microbiol.* 153:1157-1164.

Thompson LA, CJ Knowles, EA Linton, and JM Wyatt. 1988. “Microbial biotranformations of nitriles.” *Chem. Brit.* 24:900-902.

Van der Walt JP, EA Brewis, and BA Prior. 1993. “A note on the utilization of aliphatic nitriles by yeasts.” *System Appl. Microbial.* 16:330-332.

WHC-SD-W105-SAR-001. 1991. *Final Safety Analysis Report: 242 A Evaporator Liquid Effluent Retention Facility*. Rev. 0, Westinghouse Hanford Company, Richland, Washington.

WRPS-1804803. 2019. “Liquid Effluent Retention Facility Basin Particle Size Results - October 2018.” Interoffice Memorandum from JA Kadinger to MJ Linberg, dated January 10, 2019. Washington River Protection Solutions, Richland, Washington.

PNNL-29786

Pacific Northwest National Laboratory

902 Battelle Boulevard
P.O. Box 999
Richland, WA 99354
1-888-375-PNNL (7665)

www.pnnl.gov