

2022 Spring WRRRC Seminar Series

Biodegradation of Petroleum Hydrocarbons Controls Their Fate and Transport in Subsurface Environments

Dr. Tao Yan

Time	Speaker	Dialogue
0:12	Keri Kodama (host)	<p>I'm Keri Kodama, I'm a postdoc for the WRRRC and I'm the seminar co-coordinator with Aurora Kagawa-Viviani, who I haven't seen come in yet. But she's been hard at work with the Water Commission and the Red Hill task force at UH. So she has been very busy in these last couple of months. I'll let her introduce herself if she makes it in later.</p> <p>I'd also like to introduce the Director of WRRRC, Tom Giambelluca, who I'm sure many of you know. But Tom, if you want to stay—say a few opening words, before we turn it over.</p>
0:45	Tom Giambelluca (director)	<p>Thanks, Keri. Yeah, and thank you and Aurora for all the hard work you do to coordinate this seminar series. And thank you everyone who's tuned in for the first seminar of this semester. And I'm sure Keri's going to mention this, that we intend to focus the semester's seminars on the Red Hill topic, not exclusively, but you know, we'll focus as much as we can on this issue this semester. And so please be on the lookout for future seminar announcements as as they come in. Thank you. I'll turn it back to Keri. Thanks Keri.</p>
1:26	Keri Kodama	<p>Yes, Tom said we're having a general theme of Red Hill water crisis. And so we're trying to bring in various experts on impacts and mitigation efforts involved. So today, we're starting off with one of our faculty members at WRRRC, Dr. Tao Yan, who will be talking about the biodegradation of petroleum hydrocarbons.</p> <p>(1:46) So, Dr. Tao Yan is a professor at the Department of Civil and Environmental Engineering and Water Resources Research Center at the University of Hawai'i. His research interests are in the areas of environmental health microbiology, and has recently focused on the microbiol—biological quality of Hawai'i's coastal water, wastewater infrastructure for further human health protection, including pandemic detection, bacterial antimicrobial resistance in natural and built environments, and biodegrade—degradation of recalcitrant organic compounds in the environment. He is a strong advocate and practitioner of the transdisciplinary education of the next generation of environmental engineers and has mentored numerous MS and PhD students and postdoctoral research fellows.</p> <p>(2:31) Before I turn it over just some quick logistical notes. The presentations in general will be between 45 and 50 minutes with time for questions at the end. So please hold all your questions until that time, or feel free to leave them in the chat, so I can call</p>

		<p>on you later to either read your question or you can—or I can relay it to the speaker for you if you prefer. And you'll also see a periodic reminder in the chat to fill out an attendance form. Please do so as this will help us keep track of our audience engagement. And with that, I'd like to turn it over to Dr. Yan.</p>
3:02	Tao Yan (speaker)	<p>All right. Thanks Keri for that introduction.</p> <p>So today I'm going to talk about biodegradation of petroleum hydrocarbon. And my title here actually is a little bit more than what I'm going to discuss because as I was preparing this, I found that the material in this topic is tremendous. So I will today spend the time focused on the biodegradation part and talk a little bit about some case studies in terms of how biodegradation controls feed and transport in subsurface environment.</p> <p>(3:41) Okay, so I want to give a quick introduction about my own experience with petroleum hydrocarbon and biodegradation. And I did a, you know, that was way back, 2000. I did my master—master's degree in environmental engineering back in China. And I worked on this numeric modeling of groundwater flow and hydraulic containment of petroleum contaminants in this city called Zibo City, in Shangdong Province, China. And that's where the, you know, China has a bunch of—several large government owned petrol—petrochemical companies, and this Sinopec is one of the biggest. So, and this caught—this company, called the Qilu Petrochemica Company, operates in Zibo City and if they're petrochemicals, petroleum, hydrocarbons contaminated groundwater. So my task then was to do some numerical modeling.</p> <p>(4:39) And then I came to the United States due to my PhD and biodegradation, and at that time the project was on polychlorinated biphenyls (PCBs) in sediments so my focus was on how microorganisms under anaerobic conditions can remove chlorines from these polychlorinated compounds. So think about this, you know, 20—almost 20 years later and then in Hawai'i, we are also running into problems of petroleum contamination. And also, you know, as biodegradation is one of the most important factors controlling the fate and transport of these pollutants in the environment.</p> <p>(5:24) You know, my—my experience 20—16 years ago, seems will come back to be of useful. So I want to first talk about forever chemicals because I worked on PCBs and back then PCBs at the beginning in 1960s, 70s, was considered to be a fair—forever chemicals. And of course, now we know that PCBs can be degraded, but when you look at the chemical structures of PCB, which is a biphenyl ring, and with hydrogen substituted by chlorine, so you can—this substitution can happen at any of these locations that will give you a very complex mixture of PCBs, you can have up to 209 congeners. And, you know, there was a very interesting story about how our conception of the biodegradability of PCBs have changed.</p>

	Tao Yan—continued	<p>(6:30) At the beginning, ever—everybody believed that it's going to be in the environment forever. But as we started to look at natural environments, then people started to realize that the chromatograph of PCBs in river sediments, in particularly Hudson River where General Electric dumped a lot of these PCBs into Hudson River, the chromatograph actually shifted from the more chlorinated compounds towards the less chlorinated compounds, which indicates that there's dechlorination going on, that really started the process.</p> <p>(7:07) So, I spent four years on my PhD looking at how we can enhance the PCB anaerobic dechlorination. So, this is one of the figures that I got in my PhD dissertation. I just wanted to look at the x-axis, which is time. So you know, from the point I set up the reactor, it took, you know, 200 days for me to observe something happening in the system. So in this graph shows that the more chlorinated—more highly chlorinated biphenyl, which is 2,3,4,5 - biphenyl, will gradually lose chlorines and we will start have this and less chlorinated compounds.</p> <p>(7:53) And, you know, my PhD dissertation led to this one paper that I'm pretty proud of, which is that we were the first group that identified that this microorganism responsible for PCB anaerobic dechlorination, and this group of organisms is called the <i>Dehalococcoides</i>. And they are a group of obligatory dehalorespirers, meaning that they can only use chlorinated organics as their terminal electron acceptors for their respiration. So that was a paper that we published and in which, you know, later on, of course, there's more study that showed that, corroborated our observation there.</p> <p>(8:45) So, by talking about PCBs being forever, but then we know that there are going to be degraded, I also want to mention that recently, we were all probably heard of this, another group of persistent organic compounds called PFAS. And this is also considered to be forever, several years back, but now there's also evidence showing that microorganisms can degrade those chemicals as well.</p> <p>(9:16) So, you know, when you think about these organic compounds, and even those highly chlorinated—highly fluorinated compounds can be biodegraded then for petroleum hydrocarbons. So there are not going to be forever when they enter into our environment.</p> <p>(9:37) And this is to, you know, if you just think about how many oil spills that has occurred, both in water and in—on land. For example, I have this couple of major events of oil spill in ocean water. Start from 1969, Santa Barbara oil spill to the most recent 2010, Deepwater Horizon spill. So a huge amount of oil gets spilled into the ocean water, which is here on this NASA photo here, you can see this sheen of oil on the ocean water surface. So those petroleum hydrocarbons, some of them will end up in the</p>
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	Tao Yan—continued	<p>marine sediments, but a lot of them will be dispersed into the ocean water and eventually be degraded.</p> <p>(10:34) Another common occurrence of petroleum hydrocarbon contamination is oil spills on land. And this is most frequently linked to this—these underground storage tanks (USTs), so this, you know, since the establishment of UST program in 1988, the law actually was passed in 1984. And there has been documented more than 500,000 releases by these USTs. And even with all this stringent regulation, inspection monitoring, today, you can still expect about 5,000 new releases every year, and the most intimate example to us is this recent Red Hill spills.</p> <p>(11:28) So I want to also before I go further into biodegradation, I want to lay a little bit more foundation on what are petroleum hydrocarbons, which I'm sure a lot of you are very familiar with. And we look at petroleum hydrocarbon, it's really a very, very complex mixtures, just like the PCBs that I talked about, you can have 209 different congeners. When it comes to petroleum hydrocarbon, you can have numerous— a large number of different types of hydrocarbon compounds, they can be—some of them, a very small fraction can be polar, most of them are nonpolar, and among the nonpermanent hydrocarbons. And we're very familiar with the alkanes, the cyclicals, alkenes, and aromatics.</p> <p>(12:18) So I'm going to highlight you this monocyclic aromatic hydrocarbons (MAH), because this group is the main risk drivers when it comes to petroleum hydrocarbon contamination. And this group includes benzene, toluene, ethylbenzene, so those are BTEs. And also the three isomers of xylenes: ortho-xylene, meta-xylene, and para-xylene. And these compounds, like I said, they are the main risk driver, because they have—one of them, benzene is a lone human carcinogen, and ethylbenzene is a probable— possible human carcinogen, and when you look at the EPS drinking water standards, and benzene has the smallest maximum contamination level, which is 0.005 ppm.</p> <p>(13:18) So when, you know, later on we're also going to talk about the solubility of these different petroleum hydrocarbon compounds. And you will see that these aromatic compounds have the best water solubility so when you combine water solubility to their toxicity, then it's easy to understand why most of the study today on the fate, transport, and toxicity health impacts focus on BTEX compounds and some, you know, some study also include this MN to the end methylnaphthalenes and naphthalene to it because their solubility and toxicity are also high. So petroleum hydrocarbon can be degraded.</p> <p>(14:06) So, you know, they're not forever chemicals. And when you look at how petroleum hydrocarbon are degraded in the environment, so, this is a general chemical reaction that outlines the components here involved. So, you know, I use this $C_xH_yO_z$ to indicate hydrocarbon. So here, you know, of course, we shouldn't</p>
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	Tao Yan—continued	<p>have that <i>O</i> there because these are hydrocarbons. And so, this hydrocarbon, they function as electron donors and in a redox process mediated by microorganisms, you should have electron acceptors. So this can be oxygen or can be non-oxygen, electron acceptors, like nitrate, sulfate, ferric iron, magnesium, so on and so forth.</p> <p>(14:58) And then this process is mediated by microorganisms—microorganism, microbial biomass function as the catalyst in this reaction. And in that process, the micro—microorganisms benefit, and they grow their biomass or they maintain their biomass and the carbon that—petroleum hydrocarbon gets oxidized in carbon dioxide and in the water.</p> <p>(15:27) So when you think about this degradation process from this general chemical reactions, you can say that this is really a tripod, their reaction needs 3 footings. The first one is that you need to have these microorganisms, these microorganisms need to have the metabolic capabilities to carry out those biodegradation. So they can either be anabolism or catabolism. So either they just degrade the petroleum hydrocarbon for energy catabolism or they grab—use the carbon for—to building their biomass anabolism. And that is also how these microorganisms build a biomass, so you need to have enough biomass to achieve fast enough degradation rates.</p> <p>(16:24) The second key components, second group of key components is the hydrocarbon themselves—the petroleum hydrocarbons. They're not always bioavailable, and the solubi—the different compounds can have different solubilities, but in general, they're not very soluble, they're generally hydrophobic. Therefore, there—no solubility can restrain their availability to microorganisms for biodegradation, and, and even also among these different petroleum, hydrocarbon compounds, some are more likely to be degraded, and some are less likely to be degraded. For example, when you look at alkane versus BTEXMN compounds, that alkanes tends to be more easily degraded by microorganisms, whereas BTEXMN compounds tends to be more resistant or recalcitrant.</p> <p>(17:29) The third group of components is environmental conditions. So here we're looking at a biologic system where the microorganisms needs to grow. I talked about, you know, there are microorganisms that will use oxygen as their terminal electron acceptors for their respiration, so those are aerobic microorganisms. There are also microorganisms that don't or can't use oxygen as their electron acceptor. And there are other use some other non-oxygen terminal electron acceptors or TEAs, such as nitrate, sulfate, ferric iron. And those will also determine how fast, whether or not bio- degradation will occur and how fast they occur, if they do. Some other environmental conditions like nutrients availability, like nitrogen and phosphorus will also—can also affect the biodegradation process.</p>
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	Tao Yan—continued	<p>(18:35) So first I want to talk about microorganisms that are capable of degrading petroleum hydrocarbon. So I want to start with this Supreme Court case where I found this to be quite interesting. And back in 1980, there was this landmark supreme—US Supreme Court case decision. Diamond versus Chakrabarty and Chakrabarti was working for General Electric. And he filed the first US patent for genetically modified organism so he called it "multiplasmid hydrocarbon-degrading <i>Pseudomonas</i>."</p> <p>(19:17) So hydrocarbon here is specifically for oil petroleum hydrocarbon. So, government—the US government rejected that. So they had an eco-fight and the case eventually ended up at the US Supreme Court who determine—which determined that in these life—human made a microorganism is patentable subject matter. So, basically, it says that if you create an organism, then you can patent that and make money out of it. So this was the first ever US patent on a living organism. And it is really the start of the US biotech industry.</p> <p>(19:58) So today when you look at all this biotech companies, seed companies, monoclonal antibody, so on and so forth, and it's really this landmark Supre—Supreme Court case that started the whole thing.</p> <p>(20:14) But, you know, what is <i>Pseudomonas</i> and this organism itself is actually very commonly found in soil bacteria, which also speaks to other bacteria that can degrade petroleum hydrocarbon, which is also very commonly found in environment and very diverse. So, <i>Pseudomonas</i> is a group of common soil bacteria and now we know that many <i>Pseudomonas</i>, not just <i>Pseudomonas putida</i>, which is the organism that Supreme Court case was about, many <i>Pseudomonas</i> can degrade diverse organic compounds, including petroleum hydrocarbons.</p> <p>(20:54) And this super <i>Pseudomonas putida</i>, this bacterium itself has a very interesting story. So, it was first isolated in Japan in 1960s. So, this is the first organism, it was easy to isolate. So, nowadays, if you go out try to isolate <i>Pseudomonas</i> from soil near—on campus, you will be able to do that. And so, this organism was isolated and then was shared widely with researchers around the world. And in 1973, people found that this strain, <i>Pseudomonas putida</i> mt-2 was able to degrade aromatic—hydrocarbon aromatic compounds, hydrocarbons, from oil. And then 1974, researchers identified that degradation capability was linked to this plasmid. Plasmid is a piece of DNA in the bacterial cell that can be moved around from one species to another, to one cell to another. So, this was really the first catabolic plasmid that was identified, at the time most of our understanding about plasmid was an antibiotic resistance plasmid. Then 1976, this plasmid was isolated, and then 1981, this first patent on a bacteria GMO was was filed. So, there was a—the original researcher who discovered for—isolated <i>Pseudomonas putida</i> actually wrote a really nice story about how this one microorganism from Japan</p>
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	Tao Yan—continued	<p>traveled around the world and reshaped the landscape of microbial genetics.</p> <p>(22:51) So, what the, the contribution from this—from Professor Chakrabarti is that he created a superbug. So, what he did is that, you know, before then there are other researchers who isolated these two groups independently identified this plasmid, it's called TOL plasmid pWWO. And these researchers found that this plasmid encodes metabolic capabilities that use toluene and xylene, which are two of the BTEX compounds that we're going to talk about later, as the sole carbon source for growth.</p> <p>(23:35) And they also found that this plasmid can be transferred horizontally from one bacteria to another, that also has implication later on. Because if you have plasmid that can degrade the petroleum hydro compound, that can transfer from one species—one species to another, then you would expect this capability to be widespread in the environment. Some other researchers, so, you're here, this is a biochemical pathway that shows how this tall plasmid in <i>Pseudomonas putida</i> were able to degrade totally through these different pathways.</p> <p>(24:14) So at around that time, several other research groups have also identified other plasmids that are able to degrade other petroleum hydrocarbons, not only toluene and xylene. So what Chakrabarty did was he took those different plasmids, he put them into <i>Pseudomonas putida</i>, and then he fixed them in those cells with UV irradiation. So, that led to this he called <i>Pseudomonas putida</i> superbug, which demonstrated very high efficiency integrating oil spills that any of the other individual bacterial species, <i>Pseudomonas</i> species. He didn't really make a lot of money out of this, this case, because later on people found that a lot of—they can just go out to the environment and isolate different types of bacteria with the capability—capability of degrading petroleum hydrocarbons.</p> <p>(25:19) So, today, some reviews have indicated that there's more than 79 different bacteria genera that can degrade petroleum hydrocarbon. So, I copied a small sub list of bacteria genera that are commonly found in the soil environment that can degrade petroleum hydrocarbon.</p> <p>(25:43) So, the ubiquitous distribution of these bacteria means a couple of things. So, first of all, petroleum hydrocarbon in the environment will not stay there forever. So they will be, eventually be degraded, which also means that if you go out to the environment and you try to detect for petroleum degrading bacteria, that detecting itself doesn't necessarily mean that significant occurrence of by—significant occurrence of biodegradation, because you will always detect something out in the environment.</p> <p>(26:21) And what is really important here is how much can you detect because the biomass of those microorganisms will</p>
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	Tao Yan—continued	<p>determine the reaction kinetics, which will determine the extent or rate of biodegradation of petroleum hydrocarbon. So, I just want to give a quick review of some basic biodegradation kinetics. So, we look at how microorganisms degrade substrates in the environment, if the substrate is the rate limiting substrate.</p> <p>(27:00) So, in this case BTEX or other petroleum hydro compound and its degradation rates can be expressed by this differential equation. So, S stands for substrate. So, how fast the substrate is changing is a function of k, which is the maximum specific substrate utilization rate; S, which is the substrate concentration; X here stands for the biomass divided by this K_S, which is half saturation coefficient plus substrate concentration itself.</p> <p>(27:38) So, we look at how fast a substrate can degrade in the environment based on Monod kinetics. Here we assume that bacteria is not growing, so this X here this is the biomass, is not going to change for that short period of time. How fast the petroleum hydrocarbon can degrade depends on the types of microorganisms you have in the system, because that will determine K_S, sorry, not u_{max}, K, that's the maximum specific substrate utilization rate as well as K_S. So, that is the half saturation coefficients, which also indicates the affinity of that bacteria towards the substrate. And also the biomass of the microbes that degrades those petroleum hydrocarbon is also important. So, that's this part and also the limiting substrate concentration S. So, this equation really speaks to that three components of biodegradation of petroleum hydrocarbon in the environment.</p> <p>(28:49) You know, of course, Monod kinetics is not the easiest model that people like to use. And later on, we're going to introduce some of the kinetic observation in the laboratory and also in the—laboratory and also in field observations, and people tends to use first order reaction kinetics to describe it. And so, this—if you take this Monod kinetics and you—focus that on this region where the substrate concentration is way less than the half-saturating coefficients K_S here, then this Monod kinetics can be simplified into this form and then we can simplify this $k X$ divided by K_S as k_1. So, that gives you a first order reaction kinetics approximation of Monod kinetics.</p> <p>(29:57) So, another—so, talk about those, you know, might—we have microorganisms that can degrade petroleum hydrocarbon and another component is the hydrocarbon themselves, different hydrocarbons have different properties that can affect by degradation. So, solubility is one of the most important parameter that affects the bioavailability and the degradation of those compounds by microorganisms. This is because microorganisms have to uptake those compounds into their body or at least on their membrane, on their cell surface, before they—their enzymes can attack these compounds and degrade them.</p>
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	Tao Yan—continued	<p>(30:44) So we look at the solubility of the different petroleum hydrocarbons, you can say that they're, you know, based on whether you're talking about aliphatics or aromatics, you're looking at two different types of behavior. Generally, for the alkanes and alkenes, they have very low solubility, and when the carbon number increase, then the solubility decrease, drops precipitously. Aromatics on the other hand, have higher solubility, so aromatics, these are the blue circle here, have higher solubility than the aliphatics. Note that this is a log scale. So, you know, their solubility actually are a lot more higher than aliphatics. And also follow the similar trends, more carbon—effective carbon number in those compounds, you will have less and less solubility.</p> <p>(31:48) So, one thing to notice is, you know, when you look at it, there are aromatic compounds the BTEX compounds, we said that BTEX compounds drives the health risks of petroleum hydrocarbon pollution in the environment, benzene has the highest solubility, 1800 milligram per liter; toluene, 530; xylene, those three isomers have 160 to 190 milligram per liter. So, these are a lot higher than aliphatic compounds.</p> <p>(32:22) So, another factor that comes into play is that when you think about petroleum hydrocarbon mixture in the environment, it's a mixture and how each of those compounds to dissolve in water, actually has to fight between partition in the fuel mixture itself and to dissolve in water. So that partitioning can be calculated or can be indicated by this concept called effective solubility. So, this is really, you know, when you look at NAPL mixture, non-aqueous phase liquid, you know, its solubility is equal to its pure solubility, solubility of pure compounds times its mono fraction within that NAPL mixture. So, that gives you this S_i^e, effective solubility.</p> <p>(33:19) Another factor that has a lot to do with bacteria for petroleum hydrocarbon is pseudo-solubility, and this actually can have implications in in transport behavior of these compounds. So, it turns out that, you know, there's a lot of microorganisms that can degrade petroleum hydrocarbon, this has phobic compounds, but this hydrophobic compounds in particular alkenes have very low solubility in water. So in order for those microorganisms to degrade them they—they want to get these alkenes into water and the way they do that is by producing, secrete biosurfactants into the environment, and those biosurfactants will then go attach onto the oil droplets then they were created this emulsification and typically, these are small oil droplets between 0.1 to 1.0 micrometer size and that's about the same size as microorganisms here. And then, you know, that can significantly increase the surface area of the wa—the oil in the environment, and allow bacteria to grow on those—on those alkenes specifically.</p> <p>(34:46) So this is a picture from a study from this group and they showed that, you know, when they add bioemulsifiers, emulsifiers produced by bacteria into this oil water mixture and they're able to</p>
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	Tao Yan—continued	<p>create this water in oil emulsion on top as well as this oil in water that is water in oil that nowhere is oil in water emulsion. So basically, it creates a lot of—significant more surface area for the microorganisms to approach. Whereas when they don't have bioemulsifiers that don't really see that phenomena.</p> <p>(35:29) So, I also mentioned that, you know, different for the petroleum hydrocarbon this mixture, different components, different type of petroleum hydrocarbon can have different biodegrade affinity and typically this general trend of degradability is observed. So, linear alkanes tends to be mostly—most biodegradable, then it's branched alkanes. Then it's monoaromatics, BTEX compounds, then cyclic alkanes, and the polycyclic aromatic hydrocarbon (PAH) compounds like naphthalene, like methylnaphthalene, pyrene, those are very, very resistant recalcitrant in the environment. You're—they're very resistant to biodegradation.</p> <p>(36:22) So, how fast can these compounds be degraded? So I looked up some review papers, this review paper reported this first order rate coefficients here and this they found that petroleum hydrocarbon under aerobic condition has a first order rate coefficient of 0.445 day^{-1} whereas we look at BTEX, B—benzene, ethylbenzene, toluene, and xylene, their rates are much much smaller. This is almost 1000—1000 times smaller than petroleum hydrocarbon. You know, of course, these numbers, this rate coefficients are good for inter-study, intro-study comparison, like within the same study, because remember that we talked about this first order rate coefficients. So this k_1 it is a function of this k, that's the maximum substitution utilization rate, this K_S, which K_S, which is the half coefficients, but it also depends on the biomass concentrations for different biomass in a study then you can have different rate coefficients.</p> <p>(37:44) So, the third group of environmental factors that affect biodegradation of petroleum hydrocarbon is the environmental conditions. The most important environmental condition here is oxygen or non-oxygen. So, we have—studies have shown that when petroleum hydrocarbon are present in the environment, with good availability of oxygen, then they aerobic biodegradation will occur, which is typically much faster than anaerobic biodegradation and this—which is also why most of the laboratory and field observation experiments focused on aerobic biodegradation. But petroleum hydrocarbon will not only stay in aerobic condition.</p> <p>(38:35) For example, when you look at the oil spill in the ocean, most of the oil eventually will end up at the ocean sediments. We look at the land, you know, fuel spill on land by US TEAs and these oil, they may end up in the vadose zone, in the soil in the vadose zone, which is supposed to be have air present, but the fuel themselves will occupy the space and then start biodegradation, aerobic biodegradation consume those limited oxygen available in</p>
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	Tao Yan—continued	<p>the vadose zone and create anaerobic environments. So, when you look at, you know, the biodegradation of petroleum hydrocarbon in—under anaerobic conditions, then researchers have found that many other non-oxygen terminal electron acceptors such as sulfate, nitrate, carbon dioxide, so, these are methanogenesis, ferric iron, manganese. These alternative electron acceptors can also support anaerobic biodegradation petroleum hydrocarbon, but when you compare that the rate of degradation of anaerobic degradation to aerobic degradation, anaerobic degradation is much much slower.</p> <p>(40:03) So another factor that's important here is of course temperature. And we all know that you know for biological processes that you need to have optimal temperature to facilitate the growth of microorganisms to gain biomass, so that they can function as catalysts to carry out the biochemical reactions. So, here is a study done by Atlas et al. back in 1975 and they showed that at 10°C and 20°C you—they observed different rates of biodegradation, in terms of evolution of carbon dioxide from their petroleum hydrocarbon.</p> <p>(40:45) The third group of environmental conditions that's important is nutrients and nitrogen, in particular nitrogen and phosphorus. So, this is because when you think about microbial biomass, there are catalysts and they—but they need to grow themselves from very small amount to larger amount and we look at the microbial biomass, they need all of these elements and among them nitrogen and phosphorus are typically limited in petroleum hydrocarbon contaminated environments. Some studies showed that to degrade one kilogram of petroleum hydrocarbon you need at least you need around 150 grams of nitrogen and 30 grams of phosphorus. And this was also supported by studies in marine environments where when they add nitrogen phosphorus to marine environments, they significantly increase their biodegradation rates or in soil environments like in groundwater aquifer by adding nitrogen here, nitrogen is more limited in terrestrial systems, nitrogen amendments increased biodegradation rates.</p> <p>(41:56) So those are some of the, you know, the three major areas of requirements or three major groups of components for biodegradation of petroleum hydrocarbon in the environment. And I'm going to quickly go over some of—one of the most studied petroleum hydrocarbon sites, and just talk about some of the quick— some of the observations that these scientists have made.</p> <p>(42:24) So this is at a, Bemidji in Minnesota. So that's the spill happened back in 1979. So, this, about 1.7 million liters of crude oil was spilled at the site, some a good portion of them have been recovered through various means, but, you know, a significant amount has—was left in the at—the site. So, this was the most studied site by USGS, you know, hundreds of papers have been published, maybe not hundreds of paper, a lot of paper have been</p>
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	Tao Yan—continued	<p>published. So, what they have observed is that with all those efforts, you know, they did five years of pumping scheme and then 30 years of natural attenuation back in 2009, they still observed significant amount of oil at the subsurface.</p> <p>(43:25) Another group of observation is that when others, the spill happened in 1979, methanogenic degradation began by 1987, observable methanogenic degradation of petroleum hydrocarbon was observed by 1987. And they also observed that substantial degradation of n-alkanes, those aliphatic—saturated aliphatic compounds were degraded under methanogenic conditions, which is shown by—which was observed by looking at these chromatographs.</p> <p>(44:07) So, they also found that iron reduction was very important. They extracted iron—ferric iron in sediments near the oil plume and which they found to be 30% less than the background, which means that the petroleum hydrocarbon has supported anaerobic iron degradation and here the ferric iron was used as the terminal electron acceptor whereas, the petroleum hydrocarbon was used as the electron donor.</p> <p>(44:42) And they also observed organic acids are the main metabolic intermediates of biodegradation and in the biodegradation process is structurally dependent. And this is basically they found that the persistent compounds benzene and ethylbenzene decreased slowly in the anoxic zone whereas the more biodegradable compounds, toluene and xylene, decreased very quickly.</p> <p>(45:14) And what's very most interesting to me was that, you know, they observed that anaerobic iron reduction limited plume growth. So, when the, you know, this was a—when they compared how fast the front of the BTEX was moving over time and also they observed—they monitored the different redox reactions from iron-reduction to methanogenesis. They found that this iron-reduction was controlling how fast the plume was growing. And also, you know, due to some further estimation of degradation rates, so, you know, for BTEX, 98, 98—95% of the BTEX mixture was benzene, which, you know, some—corroborates the fact that benzene and ethylbenzene degrades really slowly out of the site. And their estimation of the kinetics is $3 \times 10^{-3} \text{ day}^{-1}$, so that's a first order reaction kinetics. To transform that into half-life, so, that is equivalent to 231 days.</p> <p>(46:37) So, that's a quick summary of, you know, what are the major components of biodegradation and some of the papers that I was able to—have been able to read on at this well studied site, Bemidji.</p> <p>(46:56) And now, you know, I'd like to open the floor up for questions, comments, and hopefully, we can have a discussion on this topic.</p>
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47:03	Keri Kodama	Thank you so much for the excellent presentation. So yeah, we're gonna open the floor for questions. We have two in the chat, one from Don Thomas, and one from Paul Eyre. So I'll let Don go ahead first.
47:18	Donald Thomas	Okay. Actually, I got many questions, but I guess the first one is, is there a way to optimize the nutrient content that is required for your biomass to accelerate the breakdown of these products? And of course, where I'm going with this is, you know, okay, we've got a spill site, would this be an opportunity for us to actually do some research on that and look at ways of accelerating that breakdown process? Thanks.
48:04	Tao Yan	Yeah. So that is theoretically possible and actually, at this Bemidji site and they actually, while their study they look at other which location most biodegradation occurs, and they have found this site where the rainwater recharge occurs most and that's where most of the biodegradation occurred and they, their explanation was that because the rainwater carries in additional nitrogen and mostly nitrogen or and phosphorus and that has narrowed to the observed enhancement of biodegradation at the site. So, it is theoretically possible to introduce nitrogen compounds to enhance the biodegradation, but you also need to be careful about the consequence, you know, the inadvertent consequence of introducing nitrogen to groundwater aquifer, because, you know we also know that nitrogen compounds, especially nitrate in the groundwater can also be important pollutants.
49:18	Keri Kodama	Okay, Paul, go ahead.
49:20	Paul Eyre	Thank you, and thank you Dr. Tao Yan. I've been promoting, proposing that an oxidizing solution be injected into the subsurface beneath the tanks to break down the contamination there, which is a source of the contamination down in the aquifer. But we have to be careful not to, that this process doesn't generate its own set of contaminants. So I wonder if there's a similar concern with your bacteria that you don't generate, I mean, is there a chance you could create a mutated toxic bacteria? Or are there other forms of contamination that you have to be careful with? With your process?
50:14	Tao Yan	Yeah, good question. So, you know, for biodegradation, the when, when you look at the biodegradation process itself, it's mostly natural process, so you don't really do anything, they call it monitored natural attenuation. And if you want to implement bioremediation, so, you want to intervene, then the option would be one, to provide nutrients like Don just mentioned, and that the likelihood of that to create some, some more harmful bacteria down there is not very high. So, you know, of course, you will, you will have to conduct monitoring, and this can be done relatively easily, you know, because if you're doing a bioremediation out at the site, then you will have monitoring wells,

	Tao Yan—continued	and then you can look, at nowadays we have the capability to look at, you know, what are the microorganisms in the sample, and how they are changing over time. And then you will, can easily delineate, you know, whether your action of promoting bioremediation out at the site has led to the development of some hazardous or more toxic bacteria in the water.
51:36	Paul Eyre	Thank you.
51:39	Keri Kodama	Okay, we have time for at least one more question.
51:43	Tom Giambelluca	If I could just ask a very simple general question, Tao. First of all, thank you very much for that great talk. I learned a lot. But can you kind of outline how? And yeah, and what the limits would be for implementing enhancing biodegradation at Red Hill, specifically, you know, how we go about it? And, what are the limits? And what are the, what are the cautions that we should take in trying to enhance biodegradation?
52:21	Tao Yan	<p>Yeah, I think, you know, first of all, it's going to be costly. So that's, you know, no matter what you're trying to do, so we mentioned you there are different process, there's aerobic process, which is a lot faster. And there's an anaerobic process, which you depends on, you know, what's what type of alternative terminal electron acceptors, you have there like ferric iron and manganese, or you pump additional non-oxygen electron acceptors, like sulfate nitrate in there.</p> <p>(52:53) And all those processes are going to be costly. So that, that's number one. And number two is that you also need to be careful about the inadvertent consequences, you know, if you're, will that process actually lead to the mobilization of, of these pollutants, because, you know, when you look at the petroleum hydrocarbon or JP-5 in this case, and they are not very water soluble. So, you know, the, the most water soluble ones are BTEX compounds, and it seems like you're, they're not very high in this particular blend.</p> <p>(53:36) So when you promote bioremediation, then you know, you could because by remediation, biodegradation products are mostly polar. So they're soluble bacteria is going to eat the food and they're going to excrete some waste that some intermediates, so these compounds can travel even further and, you know, mingle with the water much easily and move much faster. So that's another potential factor that we need to consider.</p>
54:14	Tom Giambelluca	Thank you.
54:16	Paul Eyre	How will you introduce your bacteria to the contaminated system? It's all deep underground.
54:25	Tao Yan	Oh, so you don't need to introduce anything. So, you know, when you look at the diversity of microorganisms capable of degrading petroleum hydrocarbon and, you know, you can assume that they

	Tao Yan—continued	<p>are there in the environment. And the limiting factor is how to grow them to a critical mass that can carry out biodegradation at a rate that's appreciate, appreciable, that can be observed or that's relevant to our lifespan. So, it's really how fast those microorganisms can, can grow up. So that's why I talked about these environmental conditions, nutrients, nitrogen, phosphorus, because nitrogen and phosphorus are typically limited under those type of conditions.</p> <p>(55:19) Temperature is another factor. You know, if it's too cold, you know, not here, but if you are in Minnesota then, you know, temperature can be really cold, really low in winter. So those factors if you meet them, and then, you know, the biodegradation can speed up, you know, of course, there's also the fact that some of the compounds itself is toxic to microorganisms.</p> <p>(55:49) So, you know, at the center of the plume, when you have high concentration of all those compounds, then the activity or the growth of microorganisms can also be limited, then that will limit, limit your reactive zone to the outer area sphere, where the concentration and other environmental conditions are suitable for microbial activities.</p>
56:16	Keri Kodama	<p>Okay, we had one question from Gina, she had Okay, we had one question from Gina, she had you Tao's contact information, and you can follow up with him later on. Gina, go ahead.</p>
56:36	Gina Hara	<p>My question is, Have you considered how Japanese farming, Korean natural farming harvest the indigenous microbes, and they can quickly make a whole large amount, it just takes a little time and not much money by using, by harvesting them, then, like you said, with the nutrients they can make a large field of them and can make even a whole tank of them we're doing.</p> <p>(57:07) So I wanted to ask you, they even have the core sample from under the Red Hill tank of the soaked cores, if is it possible to do a test where you attenuate the indigenous microbe with the JP-8 eating microbes and see what the byproduct is. Because the 17 holes that originally were made, I asked the scientists that came to the second meeting for Red Hill in 2016, if they think it would work to make a slurry and pour it down the holes, as like a to chase them. But a risk maybe, I don't know what the byproduct risk would be. But a risk would be the soil becomes so alive, that it will become soft, and the tank may sink down. But, they said that's a good idea. But I wasn't supposed to talk about it there because the Navy said don't talk about it, a Navy PR person said don't talk about it. But I want to ask your opinion.</p>
58:17	Tao Yan	<p>About injecting some bacteria, slurry of bacteria that you farm outside into, underground into the subsurface system is that?</p>
58:28	Gina Hara	<p>Korea natural farming and Japanese natural farming is not afraid of aerobic situation to make fertile soil and the microbe will use the fuel or components as their, that's what they are hungry for, right,</p>

	Gina Hara— continued	those microbes. And if they are friends with the indigenous microbes, then it will contact where, it'll, it won't fight with each other when it goes down into the, into that environment.
58:58	Tao Yan	Yes. So the, you know, the sounds like you're talking about bioaugmentation, introducing some microbes from other sources into a system, environmental sources. And, you know, the chance of the success of that really depends on the consortium, the microbial consortium that you develop, you know, going back to the <i>Pseudomonas putida</i> that I talked about in discussion, and that microorganism was so popular because when you grow them in the lab and put them into the environment, they can still survive there. And a lot of other microorganisms can't do that. So I don't know. (59:42) You know, a lot of other microorganisms can do really well in the laboratory conditions, but once you put them into a different environment conditions and they die off rather quickly. So I don't know, you know, when you talk about augmenting the microbial system, whether those microbes will survive and thrive under those conditions and needs to be investigated.
1:00:04	Gina Hara	I understand what you're saying. I mean to say attenuate, which means to introduce the local microbes in the soil with the microbe that eats that fuel and make them friends so that they do survive with each other. The, you know, otherwise they fight and they disappear.
1:00:26	Tao Yan	Yeah, that that needs to be, I guess you can try to investigate that in the laboratories to see if they can survive in optimal laboratory conditions, and then try it out in an actual system.
1:00:41	Gina Hara	I don't want to take up all the time. Thank you.
1:00:47	Keri Kodama	Okay, Tom requested we get in one more question, so sorry to keep people a bit longer. But Barry, would you like to go ahead and ask your question?
1:01:00	Barry Usagawa	The one I put in chat, is that what you're talking about?
1:01:06	Keri Kodama	Yeah.
1:01:08	Barry Usagawa	Can the microbial degradation be spatially modeled if we knew where sufficient monitor wells to define where the plume is?
1:01:19	Tao Yan	Hey Barry, can you repeat that question? Sorry.
1:01:25	Barry Usagawa	I'm wondering if you could spatially model the degradation rates if we knew where the plume is?
1:01:32	Tao Yan	Yeah, if you knew, if we knew the size of the plume, that's, that's one part. And another part is that we need to know the biomass concentration, you know, within that plume? And we also need to know what are the environmental conditions like, you know, whether it's aerobic, anaerobic? Does it have not enough nutrients, nitrogen, phosphorus, to support those degradation? And so that,

	Tao Yan—continued	you know, then we can try to model that process. And of course, that has to be corroborated or calibrated by field observation, like in the groundwater, you know, what do you observe there?
1:02:26	Barry Usagawa	Thank you.
1:02:28	Keri Kodama	All right. I think that will wrap it up for today's seminar. Thank you, everyone, for joining us. And if you want to follow up with Dr. Yan, get his contact information, please feel free to contact me. I'll leave my email in the chat. And I'll put you in contact. Our next seminar will be two weeks from now on March 4, and that will be given by Dr. Han Tseng, and she'll be talking about her dissertation research on cloudwater interception. But yeah, thank you so much Tao for giving that talk and taking the time to discuss with us today. Thank you everyone for being here. And, Tom, if you want to give any closing remarks.
1:03:26	Tom Giambelluca	Now just to reiterate, thanks to our speaker, Tao. Great job, and to you Keri and Aurora, and behind the scenes April and Pat, for facilitating the seminar, and everybody tuned in. Please keep in touch and look for our future seminars, including the one that Keri just mentioned on March 4 with Han Tseng. Thank you, everybody. Aloha.