Matt: Take it away, Ralph.

Ralph: *cringes at the sound of my voice*

Hazen: I did my Ph.D at Wake Forest University but I was actually doing my research at the Savannah River site. So this site is one of DOE's big nuclear sites, where they produced material for both nuclear weapons and for civil purposes, like a strange type of nucleides that would be put into satellites and things like that as power sources. So while I was there, my doctorate work was actually looking at thermal effluence from nuclear reactors and how they are affecting bacteria – they would affect fish and caused fish disease and alligator disease. If you go to my website, that's why you see a picture of me holding an alligator

Matt: You know, that was actually my last question, you went ahead and took care of it (laughter)

Hazen: And he is dead…

Matt: Oh. God bless him.

Hazen: The BBC had done a special for NOVA, and they found out that I was one of the only people that would dive in this reservoir where these alligators were. So, they asked me to pretend that I was an alligator and pull this float around because we had radio transmitters on these alligators. And then, supposedly, they tried to catch the alligator, you see the guys in the boat being jerked around – that was all me underwater. But they didn't even acknowledge me in that movie. What were they gonna say? Especially NOVA, the epitome of science documentaries? "Thanks to Terry Hazen, for playing the alligator."

Matt: That would have looked good on your CV at the time.

Hazen: And of course, they did all of the splicing and everything, it looked like they dragged the alligator on shore when actually they had already killed that alligator. And that is the alligator that is on my website.

Matt: That's crazy.

Hazen: Anyway, I got familiar with a lot of the containment issues and problems that the Department of Energy had. And I was also working on pollution problems related to () And I had a grant from the North Carolina Board of Science and Technology and later on from the Water Resources Research Institute while I was a graduate student. At one time I had two master students and a postdoc working for me as a graduate student for my Ph.D. At (Albemarle Sound) we were looking at the effects of a nitrogen fertilizer factory and a pulp mill, their effluents on (Albemarle Sound) So it's the Chowan River, there on upriver. So actually because of that work, we showed that it would increase the densities of these bacteria that would cause these fish diseases, an epizootic of these fish diseases, and in large-mouthed bass in particular, which got all the locals excited. Because of those papers and reports that we published, the state of North Carolina declared (Albemarle Sound) as a nutrient sensitive area. The nitrogen

fertilizer factory had to close down, and the warehouse plant pulp mill had to put a bunch of scrubbers in it. After finishing my Ph.D. and paying myself as a postdoc for one year, I went down to the University of Puerto Rico as an assistant professor, quickly rose up through the ranks and started studying coral reefs and effects of the Bacardi effluent on those and a tuna fish factory effluent, and in general, what we call non-point sources – so, human fecal contamination. And the problem is, fecal coliform, which are the standards for measuring recent human fecal contamination don't work in the tropics because they can survive for a very long time. In fact, I could find E. Coli in the tops of trees in bromeliads, so these epiphytes in the trees. CNN did a special science report and actually filmed me and my students taking samples. But we also worked on oil spills. There were some oil spills down in Puerto Rico and I did some work on those too. But I looked at a variety of contaminants. But I was mainly just trying to see what effect the bacteria had on that, and if there was an increased risk for humans for getting diseases and things like that.

Matt: So you've spent a lot of time looking at the genomic population of bacteria and what not?

Hazen: No – this was way back before we did genomes. And the only thing we could do was we did isolations and that sort of thing.

Matt: Wow.

Hazen: So this was way before you were born. (laughter) I was there for eight years. I was professor and chairmen of the department by the time I left, and they were trying to make me dean, and I didn't want to do that because chairmen of the department was bad enough – 45 faculty members, so it was a big department and it was taking up too much of my research time. Anyway. Then, the Savannah River plant made me an offer. And this time, it wasn't through … I was working through the Savannah River ecology laboratory as part of the University of Georgia, at the Savannah River site – so this time, it was the Savannah River – what became the Savannah River National Laboratory. They actually made me an offer I couldn't refuse. SO I became the head of the environmental biotechnology section there, and we were specifically looking at microbes related to chlorinated solvents and deep subsurface and how deep that went, and were there unique organisms that degrade things that we could utilize. And so we had a gigantic culture collection and we started to do some pilofane analysis and some really basic genomic analysis back then. SO that was in – oh I started that in 1988.

Matt: And so this was part of the emerging biotechnology field, at this point.
Hazen: Right. Then I did a bunch of reviews for the department of energy, relating to that. And in the meantime, at Savannah River, I've got five patents up there that are all related to bioremediation. And those are all patented by Westing House. And Westing House ran the Savannah River National Laboratory for the Department of Energy.

Matt: Are your patents genetic patents or ...?
Hazen: They're process patents. So gaseous nutrient injection and use of triethylphosphate, which is made by Kodak – or originally was – and basically we can inject that as a gas and I've

developed some techniques for doing that. And also using methane. I demonstrated that methanotrophs will degrade over a thousand different compounds with methane monooxygenase that they produce. So that patent was actually licensed by, as far as I can tell, probably about fifty multinational companies. Whenever they used it at a government site, they didn't have to pay a royalty. But they did if they used it at a non-governmental site. But DOE didn't charge them very much because – technically DOE is the signee on my patent – and so, DOE wasn't out to make money, they were just trying to make sure that no one sold it back to them.

[continued]

To clean up sites. But I showed that you could use it in creosote oil, trichloroethylene, tetrachloroethylene, dichloroethylene, vinyl chloride, that sort of thing. And I developed some systems for vinyl chloride if we're looking at a fiber glass factory in South Carolina how they could remove that with a bioreactor system that basically ran the gas through that, so a variety of things like that. So I've been doing this for a long time.

Matt: That's amazing. The utilization of methane is a very interesting topic.

Hazen: I've used it for creosote, for a variety of things. I've never been into bioaugmentation, which is use of a microbe because normally, everything I've found in the literature and all the studies I've done is that usually, the organisms that you've grown up become lab rats. When you put them out into the environment, they immediately die because they are not well adapted to those environments which are much more stressful and extreme. Then they become good nutrients for the indigenous microbes. It looks like you're seeing some effect by doing a bioaugmentation when actually you're not. It would be much cheaper just to add nutrients. The other possibility, if you go and read my chapters on biostimulation, you can see the different things that can be used, such as intrinsic bioremediation. That needs to be the goal all the time because a lot of the time, there's enough nutrients in the environment that they can take care of the contaminants. Just wait. But there may be some reason that you can't wait very long because of risk. Then you may need to add nutrients, do some engineering controls.

Matt: That's definitely one of our questions is that, given our platform, we're kind of leaning in two different directions: There's bioremediation, but there's also profits on the upgrading of this toxic waste.

Hazen: Then I went to Berkeley National Laboratory. I started looking at radioactive and metal contaminated sites, looking at if we could reduce particular contaminants and get them demobilized so they didn't move, either radionuclides or chromium. In the case of chromium, I showed that you could add lactate, and you could use a polylactate compound and inject that into the subsurface. Basically, it would set up a zone where, as the water moved through that zone with the chromium, it would immediately form a salt and precipitate out. It wouldn't move any further past that sort of microbial wall that was created. I demonstrated that full

scale at the Hanford site, in eastern Washington State. That was a full scale demonstration and that was actually used by DOE. We tried it at Oak Ridge and at a site at Y12 and I started working on that when I was at Berkeley a few years ago. And so I did technical assistance request on all of the DOE sites and making recommendations on what they should do. In some cases, the best thing to do is nothing at all, but that's very difficult for some people to understand. You can introduce more problems if you add a lot of nutrients or nonindigenous organisms.

Ralph: You mentioned a book that you had written, what is the title of that work again?

Hazen: It's *Hydrocarbons*. If you go to my website, look under publications, go back to 2010, you'll see biostimulation, groundwater bioremediation. Of course I've been doing oil all along, and when I was at Berkeley, in 2010 when Deepwater Horizon happened, I was already working indirectly for BP, through a bioenergy center at UC Berkeley. BP had a contract with the university and they subcontracted me at the national lab, so that way there wouldn't be a conflict of interest. Everything I do is public. I was given about $2 million a year to do enhanced microbial hydrocarbon production. Basically looking at abandoned oil wells and creating some changes in oil composition so that you could either get more oil out or transform it into methane or hydrogen. We had been working on that when Deepwater Horizon happened. Immediately they indirectly asked us to go down there and be part of the response phase.

Matt: Just to clarify, in your hydrocarbon production, is oil your substrate going in?

Hazen: Yes, we're trying to modify the oil. In some cases, it becomes so viscous that you can't get it out. If you can get the bugs to produce biosurfactants, or a combination of dispersants and bacterial biodegradation, you could sweeten the oil too. You then make it better as product and basically have in situ refining occurring. There's still a lot of interest in that. But because of Deepwater Horizon, I had the whole group working on that and I could immediately get out there. We were out there continuously on two different ships from May 25th until October 20th, so we have the longest continuous set of data: 10,000 samples. I published about 25 or 30 papers related to that. Probably published around 25 or 30 more as time goes by. Even though it's been 7 years.

Matt: Just with all those samples, you keep on finding …?

Hazen: We find out new things, new techniques that we can use. We've got them all stored in -80's. I'm also looking at uranium contaminated sites and extreme sites out at Y12 that are very low in pH, very high in nitrates. In fact, the world's highest nitrate well concentrations in the well are at Oak Ridge, where we find 11,000 parts per million in the water, which is just … no place anywhere has that level. But we do still find organisms in that, but they've had 60 years to develop.

So that brings us up to the time where they made me an offer here. I just turned 60 in 2011, and I could technically retire from the University of California system. They have a very nice

retirement program. So I said, what the heck, I'm going to come here and try to do more stuff at Oak Ridge but also be back at the University. I started out at the University and I needed to come back so I could teach you all some of the things I learned. So I have a lot of graduate students, a lot undergraduates. I usually have about 10 undergraduates working with me and about 10 graduates and 3 postdocs. I'm only required to do research. What's the next question?

Matt: Would it be more pertinent for us to, instead of trying to extract our toxins from out of the soil, could we not also just take the BTX from the factory, convert in the lab, and take that as our own bioremediation? Does that still qualify as bioremediation, even if you're not necessarily taking it out of the field itself?

Hazen: Well, you're using the BTX as a feedstock for a particular product, and that's fine. And there may be process where you could use the BTX, but it would be pretty expensive to try to pull it out of contaminated soil. You could do that by aeration. You can use in situ air stripping to get a lot of stuff out. Instead of using a bioreactor to degrade that BTX compound – so the process we used at Savannah River, we had a thermal system that basically would heat the air up enough to completely degrade the chlorinated solvents as they came back up. But in this case you could put a bioreactor on there that would be converting this into a product. And so you would be cleaning up the environment and making a product. That might be very cost effective, it might be advantageous for you to do. But you're using a physical process tied to a biotransformation process. There's one person that does quite a bit of that here, and that's Tom Zawodzinski.

Matt: Interesting. Thank you. In regards to BTX, we made one platform last year; this year we're looking at expanding the enzymatic library, but with the current enzymes we have right now, they are sterically hindered such that they can't actually degrade benzene and o-xylene. Toluene and m and p xylene are fine because their carbon arms are farther away. Does that necessarily disqualify our efforts if we are only getting rid of 3 of 5 BTX compounds?

Hazen: Yeah, probably. You'll have to look at the standards for each one of those. Benzene can be pretty toxic, and xylene too. But that's why with BTX compounds you'll probably have to use a combinatorial process. But what I found with gaseous nutrient injection, even though you're injecting gas and methane is a low percentage of the air, it is technically an aerobic system. However, there are anaerobic microniches that are created in the soil where you get aerobic degradation of tetrachloroethylene. Aerobic degradation of tetrachloroethylene is damn near impossible, but the two are opposite of each other. Vinyl chloride is degraded very fast under aerobic conditions, but very slow under anaerobic conditions. Just the opposite of tetrachloroethylene. Tetrachloroethylene is degraded practically not at all under aerobic conditions but under anaerobic conditions, it goes very fast. If you combined the two, basically a bulk – and I showed this in some of papers – we showed this in soil columns, is that you have enough anaerobic niches in an environment that even though there is tetrachloroethylene present, it's degraded too. Because it's being automatically degraded down to trichloroethylene

and then aerobically, the bugs are very fast at degrading trichloroethylene and any other toxic daughter products, dichloroethylene and vinyl chloride that might be created under anaerobic reductive dechlorination.

Matt: How imperative is it to implement kill switch machinery in bioaugmented organisms if you are going to release them into the environment for remediating purposes?

Hazen: There's a particular source for getting permits to do that. Gary Sayler had to go through that process for a NAH7 gene that he put into a naphthalene degrader. And he put onto that NAH7 a reporter gene that would basically give off light. He set up a complete system out at Oak Ridge with these large optics that you could see for particular strains under particular conditions how much light was being generated. It sounds bizarre, but it did work.

Matt: You mentioned that strains have a susceptibility to becoming lab rats. Is directed evolution for organic solvent tolerance and outdoor conditions a viable option before putting your bug out into the environment.

Hazen: You can try to do that but you'll spend a lot of money doing that, so it might not be worth your while. However, it technically is possible to create genes that you potentially could put onto either phages or plasmids and those could be injected very easily and give them new capabilities for degrading the contaminants. That is technically possible. You would have to go through the same permitting procedures. I have some lectures on that, that I've done – you can go online and see those. Just look up my name. It may even be on YouTube. A lot of the YouTube stuff you'll see is on Deepwater Horizon, but I've done some other stuff too that's on there.

Matt: I've really enjoyed this talk, I found it very interesting. With that being said, I just want to thank you again for all that you've done.

Hazen: Sure. You're welcome.