

<u>환경에너지공학</u>

바이오 연료 생산의 도약 및 발전

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- [00:00] So I was told that you are dealing with bio energy in this portion of the class.
- **[00:01]** Let's go. Okay, so this is going to deal more with some of the technical issues of cellulose to liquid fuels.
- **[00:17]** And it is strongly still from the center but little straights with all of these centers are doing worldwide.
- [00:24] It's the same and the barriers are the same.
- [00:26] So the opportunities are non edible plants, parts of plants and part of that is this fuel verses food debate.
- **[00:45]** Use of the most abundant organic material on earth and largely that organic matters are made up of sugar polymers based on glucose.
- **[01:00]** And that glucose can be converted to ethanol or other chemicals by reforming or by microbial fermentation processes.
- **[01:06]** So there are basically two approaches.
- **[01:08]** One is superior chemical [?00:13]caddis approach, the other is microbial approach.





- [01:14] So far the microbial approach has been the most feasible being lower cost in producing one product or perhaps if you have other products but welldefined products.
- **[01:32]** So usually microbial [?01:36]incimedic processes their feature specificity.
- [01:39] Their catalyst is very specific.
- **[01:44]** For chemical catalysts are not specific.
- **[01:47]** So you get more of a mixture and often less efficiency.
- **[01:54]** But the challenge is the sugars are trapped in insoluble fibers, so here are cellulose microfibers and here is lignin wrapped around all of these things.
- **[02:15]** So it's not just pure cellulose it's got lignin wrapped all around it.
- [02:20] That's what gives plant the structure why trees can stand so tall in the windstorm because of that physical structure of that matrix being wrapped together.
- **[02:32]** So the challenge is how do you get those apart and get the cellulose free so you can get the sugars.
- **[02:45]** The fibers are also held together by hemicelluloses and lignin, so lignin is the major component that's a phenolic polymer.
- **[02:59]** Not all plant cell walls are the same.
- [03:05] They have different components, different types of cellulose, different hemicelluloses, different lignin contents, lignin itself is often a, it's not a specific enzyme that causes a specific reaction.
- **[03:29]** It causes a radical reaction so the lignin can cross-link in various ways.
- **[03:30]** So it's not consistent product.
- [03:36] So the point here is because not all plant cell walls are the same then if you try to use these processes with this different kind of cellulose that not are going to work so well.
- **[03:51]** So some people like the idea of growing one crop, they would be genetically engineered crop, optimized for the conversion.





- **[04:05]** But others say that it's certainly not reasonable.
- [04:10] There is, going to be different environmental condition for reasons of different biomass to be grown and therefore the process should take all kinds of biomass.
- **[04:22]** But then that makes conversion process more challenging.
- [04:30] So I said there are three of these biology centers, the largest is called Great Lakes Bioenergy Research Center, and it has researchers at these locations in the Midwest, and the few others indicated there.
- [04:51] There's also one headquartered in Tennessee right here, the three researchers are in this locations, and also one headquarter in Berkley, California with most researchers out there.
- [05:05] So rather big effort though, compared to the problem not that much of a huge effort.
- [05:21] So this is some of the productivity, of the research centers in terms of publications, invention disclosures, patent applications, and licenses for operations.
- **[05:39]** So our center has this kind of mission.
- [05:46] Center-wide expertise and breadth to develop integrated solutions, sustainable crops with desirable biofuel traits, and energy-efficient production of fuels and chemicals that are currently derived from petroleum.
- [06:05] So this will be the stages, cropping systems, pretreated biomass, the hydrolysate and the biofuel production.
- **[06:22]** with these being a sort of the technical components that are important for scientific the discovery but the point is that it should all be integrated.
- [06:32] So that one component is not working with the not knowledge of the other.
- **[06:37]** So for example I work in a sustainability component where [?06:41] we've worked a lot with the plant development people.
- **[06:44]** Because they need to know what is important from environmental point of view in their plant development work.
- **[06:57]** So corn stover is an initial biomass feedstock that we are using.





- **[07:03]** There is a lot of supply and it's very similar to other grasses and crop residues.
- **[07:11]** So the base pretreatment preserves the 5 and 6 carbon sugars for conversion to fuel and provides a lignin fraction for downstream use.
- [07:23] And we use yeast and bacteria and chemical conversion platforms.
- **[07:30]** And we use for the microbes, yeast and E.coli., and also chemical processes with chemical catalyst.
- **[07:41]** These are the kind of products ethanol, levulinic acid, gamma-valerolactone, fatty acids and hydrocarbons.
- **[07:54]** So it's more than one product.
- [07:56] The sustainability I have talked a little bit, I've talked about it a lot already so I will not say a lot here.
- [08:03] So the goals are to design a sustainable biofuel system, model alternative biofuel systems at field for the real regional scales, economic, environmental and social objectives.
- **[08:19]** Economic objectives in profitability and the basis for farmer, refiner and policy makers about what to plant, where and when.
- ♥ [08:34] When I talked about the 8 model systems before, so some of the issues I need time to pay back carbon debt associated with moving to new biofuel crops.
- [08:55] So when you move to a new biofuel crop then you stimulate more carbon loss, so you need to pay that back, at sometime in the overall carbon economy.
- **[09:08]** Carbon and nitrogen ecosystem benefits of perennial crops I've talked about that before, I didn't talk about the beneficial insects.
- **[09:15]** So one of the researchers has done a study on these beneficial insects.
- [09:20] It need to versifying cropping systems that maintain beneficial insects like these right here that control [?09:32] eithuds which are problematic to soybeans.
- **[09:35]** And that beneficial cost was 240 million dollars per year of chemical pest control.





- [09:44] Another words by having this more divertive ecosystem with insects that are for predators of [?09:50]eithuds and the farmer didn't need to use insecticides saving overall the region 240 million dollars.
- **[10:00]** So that would be an example for ecosystem service, by having this different type of landscape that provided that the predators to control the [?10:05] eithuds.
- ▲ [10:25] And so this is a landscape simplification another words these are the high corn producing areas, so the darker it gets its landscape simplification because everything is corn and soybeans.
- **[10:40]** It's the high productive areas through here and then with that comes the relatively high insecticide use.
- [10:52] So there will be some marginal lands of this areas, if they grew biofuels and they supported the biodiversity of other insects, like predatory insects.
- **[11:04]** Then it would reduce those kinds of cost in the... on the productive lands.
- **[11:21]** Okay, that's sustainability.
- **[11:23]** Now what's going on with the plants?
- **[11:28]** So the plants that I showed before in that gradient were existing plants.
- **[11:34]** Now I'm talking about bio engineer plants for biofuel production.
- **[11:43]** So can we develop productive energy crops that can be easily processed in fuels?
- [11:48] So the big problem is how to get the cellulose out so can one alter the structure of that cellulose, hemicellulose and lignin to make it more easily digestible.
- [12:02] So alter lignin for reduced recalcitrance to processing, increase the energy density of biofuel crops, i.e. producing oils and manipulate hemicelluloses for improved processing.
- [12:20] Hemicelluloses are five carbon sugars that are not easily readily fermented.
- **[12:22]** So when once the make those also fermented to ethanol.





- [12:31] So [?12:31]non oil or the cellulose but the hemicelluloses is converted to ethanol and that can be officially converted the lignin lag behind, the lignin can be burnt.
- **[12:41]** For example, okay, so one of the strategies okay.
- [12:49] So lignin altering the structure, oils, increasing the plant energy density others produce more oils and the hemicelluloses altering type, improve conversion.
- **[13:00]** So the approach has advent gene discovery in modern crops.
- **[13:08]** So these are different crops that have plants done [?13:15[just like crops that have particularly unique features relative to this components.
- [13:23] So to understand the genetics controlling the production of those components in those plants so those gene components then can be put in to switch grass, for example.
- **13:40** So that's the general approach of this center.
- **[13:44]** Take information that nature's already provided with other plants about import pathways and move it to crop of interest.
- [13:56] Ok. so, zip-lignin, so the modified lignin, softening the lignin called ziplignin for lower energy lignocellulose processing release sugars and aromatics for subsequent processing in bioconversion.
- **[14:16]** Ok, so, making having the plant with modification of lignin bond here so that is more easily broken apart.
- [14:26] Without altering the plants ability to stand up, or its acceptability to insects
- [14:38] Accumulate oils and hydrocarbons in vegetative tissue to produce drop in fuels and increase energy yield per ton of harvested biomass.
- **[14:47]** Ok, so this energy yield, the hydrocarbons are the most energy rich material and if you think about it, ethanol, has rich on it.
- **[15:02]** It's already oxidized.
- **15:05**] Alkane does not.
- **[15:06]** So it's more energy rich.





- **[15:08]** So that's oils.
- **[15:13]** So you want more of that, you get more energy.
- **[15:17]** But you [?15:18] wanted in vegetative tissue right now some plants produce a lot of it in seeds but the seed isn't a big part of the plant.
- **[15:26]** [?15:27] So you can't use produce in vegetative materials.
- **[15:29]** And then all of the C6 which is easily converted and C5 poorly converted to hemicellulose sugar composition to improve fuel production.
- **[15:43]** So you want to go from C5 five to C6.
- **[15:47]** So those of three things.
- **[15:49]** So again, this particular plant, I don't know what it is, is the source of this Acetyl-TAG gene, which is what is used for this biofuels, this drop in biofuels.
- **[16:05]** Ok. So, that's altering the plant but that's got to be done in conjunction with enzyme system for the conversion.
- **[16:18]** We can't make the plant produce something if you can't convert it.
- **[16:23]** So, it's got to be a package
- **[16:26]** The enzyme sweet and plant genes have to work together.
- **16:32** So, optimize mixtures to reduce cost of sugar release.
- **[16:37]** So one of the cost now of this kind of conversion is the enzymes.
- **[16:43]** That is the major driver in the cost of this process
- [16:48] So you want the enzymes to be produced cheaply and you want to reuse them and you want them last as long as possible and you want [?16:57] as efficient as possible
- **[17:01]** So the bio-prospecting identifies thousands of candidate enzymes.
- [17:05] There is a lot of efforts going on a lot of place to come over better enzymes.
- **[17:09]** Here from this centers for example, one of the investigator who is specialist in the microbiology of leaf cutter ants in the tropics





- **[17:18]** So you see these leaf cutter ants, they are very efficient [?17:23] had converting the ligno-cellulose in those leaves for their growth
- **[17:28]** So he is doing total genome sequencing of the microbes in the gut.
- **[17:36]** In the gut and in the homes of this leaf cutter insects to find the genes producing the enzymes that are efficient in the ligno-cellulose conversion.
- [17:48] So that's one example again of looking at nature for this kind of enzymes.
- **[17:54]** And other centers are doing the other things like this.
- **[18:00]** All kinds of different insects that seem to be good at this job.
- **[18:09]** So this is an example of an eleven component mixtures of enzymes.
- [18:13] These are different kinds of enzymes here, and different combinations that give a particular yield with glucose and xylose.
- [18:25] Xylose would be hemicellulose.
- [18:29] So, identifies thousands of candidate enzymes, in vitro expression of proteins with this system, and with the carbohydrate binding modules, CBM modules, of how these enzymes bind to the carbohydrates which is the first step in their efficient attack of carbohydrates
- **[18:50]** So it's combinatorial analysis of bacteria and fungi enzymes mixtures.
- **[18:57]** In other words you have all these candidate different enzymes.
- **[19:00]** How do you mix them together in efficient way to come up with optimum set of enzymes for the most efficient conversion
- **[19:13]** Screen for limiting and missing enzymes like xylose, fucose release, optimize substrates and pretreatment products.
- **[19:23]** I'll talk a little more about that
- **[19:29]** 5-enzyme, a C. thermocellum, this is a bit important one to study, uses two times less protein than current mixes.
- [19:42] And rapidly leads for microbial expression in other words improve the expression of these in microbes





- **[19:50]** So a lot of biochemistry and genetic engineering.
- **[19:59]** So this is the basic pathways that we are dealing with here.
- **[20:04]** So at the top, the ligno-cellulosic biomass so you can use catalyst to produce these kinds of products.
- **[20:15]** Chemical catalyst, or you can pre-treat the biomass release this C5 and C6 carbon sugars.
- **[20:27]** And these are the standard pathways of metabolism in different organisms, and you can manipulate them to produce different products.
- **[20:37]** So, here, for example, is ethanol, here is one butanol, iso-propanol other butanol, pentanol, prenoids and so forth.
- **[20:50]** So all of these have some potential value for particular products beyond fuels.
- **[21:05]** But the pathways at least in the key organisms are very well known a lot of genes controlling the pathway are known.
- **[21:14]** So it's the matter of manipulating those genes to get the desirable product.
- [21:20] So the goals is tap microbial diversity to optimize the flow of carbon and electrons to precursors and fuels.
- [21:29] Produce co-products from "waste" (acids, aromatics, CO2) and of course increase biofuel's sustainability.
- [21:36] So this is the key central pathway here.
- **[21:46]** Now, going back to altering lignin to decrease recalcitrance.
- [21:54] Lignin today the weakest bonds cleave at a 170 degree in alkali or 190 degree in acid.
- **[22:01]** So, the future, the weakest bonds cleave at less than 100 degree replace backbone ethers with esters.
- **[22:11]** So, this becomes esters rather than ether.
- [22:15] So, esters are easily hydrolyzed.





- [22:21] Ethers are stronger.
- [22:23] So that will be a goal to make that.
- **[22:30]** So the plant produces that kind of lignin, it may not be a stronger lignin but [?22:42] for a biomass crop that may not be important.
- **[22:43]** If it is a tree, then that's a problem.
- **[22:46]** If it is not strong but for bio mass crop, switch grass might be okay.
- **[22:54]** Ok, so again, this is coniferyl ferulate, is the key to zip-lignin approach.
- **[23:08]** So, that particular bond, this is component lignin here.
- [23:14] That particular bond make it an esters rather than an ether.
- **[23:18]** Well, this is ester right here.
- [23:26] And as I said, part of this goal has been to use plant natural products to find those kinds of genes.
- [23:35] And this particular plant has this kind of gene.
- **[23:40]** So it has this monolignols and it has ferulate rather coumarate, and that makes it more digestible.
- **[23:59]** So using that example, one can take that gene and then move it to another plant, or the biofuel plant and make its lignin more digestible.
- [24:17] Ok. So, identified genes to produce "designer" lignin varieties, express those in plants.
- **[24:29]** And field trials are now under way with poplar which is a fast growing tree to assess lignin to assess that affect on lignin.
- [24:40] Now, the next thing was to increase biomass energy density to accumulate oils in vegetative non-seed tissues.
- **[24:47]** And this is that plant that you saw before.
- **[24:51]** It produces oils and its vegetative tissues and it produces this acetyl-TAG which is indicated here, compare to other fuels.
- **[25:06]** And that's better from an energy point of view.







- **[25:11]** So this is the oil production by an ethanol production by different plants.
- **[25:19]** So canola is an oil producing plant.
- [25:23] Corn, of course, ethanol.
- [25:25] Perennial grass potentially more ethanol, but grass that with 5% oil, 10% oil, 20% oil.
- **[25:34]** These being possible by moving some of these genes from that plant that produce oils in the vegetative material into something like switch grass.
- [25:43] So now, switch grass produces not only ethanol, but oil, this more dense fuel.
- [25:53] So 20% oil one would predict two times increase in energy density per ton of biomass
- **[26:08]** So "designer" plant.
- [26:12] But if you think about it, I mean, you may think that this uh... is a fictitious also, you know, something hard to imagine.
- [26:24] But if you think, you know hybrid seed corn began to be popular right after the World War II.
- ♥ [26:35] And the tremendous increase in productivity and another value of corn over that period of time by research, this doesn't seem impossible to me.
- **[26:47]** Because one has already done it, not quite in the same way with especially corn and soy bean.
- **[27:01]** So this is that Acetyl-TAG as drop-in biodiesel
- ♥ [27:10] So who is the [?27:11]아르고 expert here, biodiesel 아고 expert not here.
- **[27:18]** So this stands for Acetyl-triacetylglycerols, often called ac-TAGs.
- [27:27] It's a modified triglycerides partially acetylated without glycerol byproduct.
- [27:34] So the endosperm, so this is the plant, Burning bush seed, that's why



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it [?27:40] has three heads.

- **[27:42]** It's an ornamental, burning bush, thread.
- **[27:45]** Its endosperm has 95 percent of this product.
- [27:53] Embryo, 70 percent, so ESTs are expressions of sequence TAGs, so that is the mRNA produced by this seed, at the component that's being synthesized.
- [28:16] So those are all sequenced and then one can interpret what genes are important for that production.
- [28:25] So the idea of then again, is to use this then in a biofuel crop.
- [28:32] And that would be a drop-in biofuel, so here's what I showed you before and it is very similar for Diesel number 4.
- [28:43] Or the soy bean oil is out of range for using as a biodiesel.
- **[28:50]** It's got to be further processed, that's why it's called drop-in.
- **[28:55]** It's all ready to go.
- [28:58] The plant produces large amount of that, all you need to do extract it and ready for the diesel engine, drop-in fuel.
- [29:10] And I guess experimentally now they've been able to get transgenic Arabidopsis and this, whatever this plant is, to produce 60 percent of this kind of material.
- [29:28] At least in seeds and 5 percent in vegetative tissues of model plants.
- [29:35] So the goal would be some larger portion, this will be 5 percent here, done experimentally so far and one could potentially increase that.
- [29:51] So deconstruction, that's plant engineering to come up with a more desired traits of the plant.
- [29:59] Just as we've done for food production, more desired plants, traits for food production, this is more desired traits for energy production.





- [30:11] So the deconstruction is goal to improve release increase of monomers or short changed oligomers from the lignocellulose.
- **[30:20]** So [?30:21] for a long time has developed [?30:26] afa x process, it's the pressurized ammonia treatment process for expanding the lignin.
- [30:32] So improved alkaline pretreatments to open the matrix of plant cell walls, that's the kind of thing that our center does.
- **[30:39]** And improve the enzyme cocktails capable of releasing the useful intermediates from these polymers, what I mentioned before.
- **[30:47]** So it goes ended with how do you blow it apart, then do you have the right enzyme makes to go with that.
- [30:57] So here's the alkaline pretreatment, so this will be a model of the plant biomass.
- [31:04] And then this ammonia and pressure and temperature then blows it apart like this.
- [31:15] And then the cellulose and sugars come out and you can do the conversion.
- [31:20] And what remains behind is the lignin and you can do something else for that like burning.
- [31:29] And this is the enzyme cocktail here that goes into this stage so those sugars are converted to product.
- [31:42] So this would be the lignin product from the existing pretreatment and this is what it looks like from this [?31:54] afax process, this ammonia oxylithed process, so you can actually pour it out.
- **[32:00]** Then the conversion, okay so...
- [32:07] Modified plants, deconstructing the plants wall and then the conversion.
- [32:13] Okay so improve the efficiency of biomass-to-ethanol, determine the effects of diverse feedstocks on the microbes and engineer routes to next generation biofuels.





- **[32:24]** So again bioengineering becomes important.
- **[32:27]** So this is the Genomics-Enabled Yeast Improvement, so highthroughput screening nearly 600 wild strains on 6 different hydrolysates from that deconstruction process.
- **[32:42]** So who grows the best at the appropriate conditions, genome sequencing are large number of these yeast.
- **[32:53]** So [?32:56] little bit genetic blueprint is available for the modification when one wants to make of that.
- **[33:00]** Then develop that yeast strain and one of the goals is new genes to improve xylose metabolism, that's one of the hemicelluloses.
- [33:12] So this was the... what was produced under aerobic and anaerobic conditions without the improvement.
- [33:22] And this is in with the genetic modifications, so you can see much more xylose consumed with the genetic modification.
- [33:34] Another thing is stress tolerance because they are producing alcohols and that's a stress product.
- [33:42] So can we get them to tolerate more ethanol or other components in this hydrolyslate?
- **[33:50]** And so here would be the two mutant strains that are much more tolerant than the parent strain indicated there.
- **[34:03]** Okay so the central intermediates are important, so start with lignocelluloses, this is the microbial synthesis, this is the chemical catalysis.
- **[34:21]** C5 and 6, sugars to pyruvate and Acetyl-CoA, this can go onto ethanol, this can go to fatty acids in the biodiesel or to alkanes.
- [34:29] This would be chemical catalysis producing these kind of products that also have value as fuels.
- [34:44] So what started off to be just the goal is ethanol production now everybody realizes that there're better solutions, more feasible, more economic





solutions than just ethanol.

- [35:05] Though its primarily liquid fuels is the desirable product and if there're co products with that are higher value, of course.
- [35:14] Okay and we talked about this before and in closing then who does this work?
- **[35:22]** So this is the number of people at they belong to the center that were at the all center retreat last year.
- [35:33] So there are about 400 scientists, students, and staff, faculty, graduate students, post docs, other scientists, technicians.
- **[35:42]** So if you want a good research opportunity in the future when you get your Ph.D, you can come to one of these biofuel centers and be part of the team.
- [35:58] Because great things can be done and I think important solutions will happen.
- **[36:04]** Okay, so I said in the beginning that there were three of this centers, so this is our center, the one in Berkeley and the one in Tennessee.
- ♥ [36:21] Okay, so to sum up, the overall issues are how to make the plant better for biofuel product, how can deconstruct plant cell wall.
- [36:40] And then what microbial systems to produce what microbial products in an efficient way.
- **[36:45]** Those are the basic research challenges anywhere in the world that people are dealing with this problem.
- [36:56] Okay, so any questions?
- **[37:05]** Are you going to make it, are we going to get it done, any doubters?
- **1** [37:19] I can't tell.
- **[37:24]** I can't tell whether you are optimistic or not.
- **[37:27]** Who's optimistic?





- **[37:30]** Show me if you're optimistic if we can make... two?
- [37:36] Okay, who thinks it will fail?
- **[37:45]**Nobody, then what about the rest of you?

