

BIOCHEMICAL CONVERSION OF BIOMASS

Guest Lecturer: Stephen J. Clarke, Ph.D.

All right, as George said, I worked a long time in the sugar industry. I got involved in it when I lived in Jamaica and was teaching chemistry. And at the universe of the West Indies [INAUDIBLE] an interesting place to work. wonderful people, a bit of a chaotic country.

When I lived down there, it was a time of Michael Manley, I don't know whether you remember any of this. Michael Manley was the prime minister and he was a good friend with Fidel Castro. And a lot of my friends would say oh Jamaica is going to go communist, Jamaica will go communist. No, no, no, I never thought it would. Anarchy, now that's quite possible, chaos, quite possible but the organization required to become a communist country, that's not within the Jamaican character. They are just free and wonderful people.

Anyway, this is what I want to talk, overlaps quite a bit with what Dr. Philippides said, but it covers some of the same items but it's more of a personal perspective having been involved in a lot of these things for many years. After being in Jamaica, as you read your bio, I went to work as a research professor at the Audubon Sugar Institute, which is the premier research institute for sugar cane in the US, in Louisiana, in Baton Rouge. And then, I moved to work for private industry.

One of the reasons for the move, and George mentioned things like career choices, one of the reasons for the move was that when you are in academia, it is possible to do quite a lot of interesting experiments but it's possible to only do them at a small scale. So experiments and test tubes are easy. Experiments in buckets, not so bad. A million dollar pilot plan, not so easy.

The first task I had, when I went to Florida Crystals, was to establish the only, still the only, commercial scale membrane filtration system using the sugar industry and anywhere in the world. And within two years, we'd spent \$5 million. And that was my project. So it's a big, big difference with what you can do in the academic world.

We'll come back to various items as we go on career choices and so on. For the purpose of this talk, which is the biochemical conversion of biomass. The assumption is made that the basic science is adequate for the task, that the enzymes, the pretreatment, and so on, we know enough about it. It's not perfect, optimization will be required and improvements are inevitable as a technology matures. But it's sufficiently good to go on now. The focus here is on the development and implementation of practical technology at the commercial scale.

I'm doing the wrong way.

All right, energy or useful products, you're just used to a different way of saying what George said now. Energy, biomass as a source of reduced Carbon. That's a Carbon in a chemical form, which has got more hydrogen in it than CO₂ and it can be combusted directly to carbon dioxide. Completes the cycle because all of the biomass is generated by photosynthesis, which plants absorb CO₂ with

the energy from sunlight to produce these various compounds, primarily glucose based. And then when we burn things, we complete that cycle.

Biomass is a source of reduced Carbon that can be combusted directly to carbon dioxide or, preferably, after conversion to a more practical fuel, ideally a liquid fuel suitable for transportation applications. In other words, that's what we were talking about, because if you make ethanol, why do you make ethanol, you make ethical to burn it.

As George says, 72% of the energy in the biomass ends up in the ethanol. So we are burning that, it's a much better fuel. You couldn't run automobile if you had to put bagasse or bits of wood in the back of it. The alternative is useful products, conversion of carbohydrates, C5 and, or C6 and lignan to chemical intermediates suitable for reduction of finished materials that are functionally equivalent to materials currently produced from petroleum and other fuels.

We use a lot of plastics. And one of the goals is to be able to make plastics in a sustainable way. And I'm sure you're going to touch on this later, but they need not be the same material. You need not try and make polyethylene from fermentation. If you make ethanol and you can dehydrate the ethanol to ethylene, little bit of chemistry and then polymerize that ethylene. Doesn't really make too much sense. What we need to be able to do or make are functionally equivalent materials.

The ordinary user of a plastic bottle doesn't really care what it's made of. They care about its function. Whether you make plastic wrap for food out of polyethylene or polylactic acid, which is a typical material. Doesn't really matter. What the user is interested in, is it does it functionally equivalently. Because we have got used to materials that work every day in our lives, all the plastic bottles and all the rest of it. We need something which is functionally equivalent.

Now, just throw some numbers up, some energy perspective. On this, bagasse from a sugar cane plant 12,000 metric tons of cane a day. If we look at the bagasse from that, bagasse is roughly 25 to 28% percent on cane at a significant moisture content, usually 50%. The energy content, this is not talking about energy efficiency, or use of it, just the energy content is 2.9 times 10^4 gigajoules. That's 10^9 joules. No, 10^{12} joules. 10^{12} joules per day.

Ethanol from corn, at a 100 a plant, making a 100 million gallons a year, typical large plant, is 2.2 times 10^4 gigajoules a day. If you go to an oil refinery, and I chose 250,000 barrels of oil a day. A barrel is 42 gallons. The energy equivalent to that quantity of oil, at 250,000 barrels a day. It's a fairly typical size for an oil refinery. The largest in the United States, there's two of them, one in Louisiana and one in Texas. And these are running at 500,000, half a million barrels of oil a day. The largest in the world is in India, which produces 1.2 million, which processes 1.2 million barrels of oil a day.

And you can see there's a big difference, the amount of energy in the oil is much greater, in a typical plant than in a biomass related operation. And this gets back to another subject, which you need to give a little thought to, and that is, what we call energy density. If you think of density as weight per

unit volume, the energy density is quantity of energy that you can store in a unit volume. Something like gasoline has as a high energy density, ethanol has a somewhat lower energy density. If you go to something like wood, that's significantly lower again.

But some of the numbers, just to try and give you some perspective. The largest biomass fire power plants in the United States was at the sugar factory where I worked. And this has a total generating capacity, not an export capacity, because a lot of the power is used in the process of about 130 megawatts. A typical gas fired, combined cycle plant, and you could check on what combined cycle is at your leisure, is about 10 times greater. This is one unit. The new units being built for example, the one in West Palm Beach, is three of these so it's 3,750 megawatts capacity. Compare that with the largest biomass fire power plant.

So we need to think on a different scale when we talk of energy. Now, the other one that amused me, when I looked at it, if you take an 18 Wheeler, fully loaded, and you drive it from New York to Los Angeles, about 2,800 miles. And you use Biodiesel as a fuel, whether it's soy or canola, and you're getting eight miles a gallon. When I looked up the actual capacity, the fuel consumption of an 18 wheeler, the numbers were all over the place. Somebody claims 10, somebody claims four. It's all over the place. So being optimistic, I used eight. And 70 gallons per acre seems an average sort of number.

So you need five acres of soybeans to drive that one truck across the country. Now, you can grow a lot of food and stuff on five acres. So let's just try and put some of the energy issues in perspective. A little more, going back to sugar cane bagasse, sugar cane bagasse which is the residual fibrous material after the sugar juice after the sugar is squeezed out in the juice. It's traditionally used as a fuel for the operation. We sold a hundred year, more. Traditionally used inefficiently, a fuel for factory operations.

You didn't want to generate a large pile of bagasse, which would be a nuisance the rest of the year. So you were trying to burn it as you produced it. Typical operations require approximately 50% steam on cane, in other words, for every ton of cane you process, you need half a ton of steam. And under those conditions, negligible surplus for other applications, if you do the what George referred to earlier, the material energy balance, for a sugar factory and look at optimization of energy use in the process, you can get this below 20%. But you have to have a rather different energy platform in the factory.

Therefore allowing co-generation and sale of power, all of bagasse conversion to other things, it allows you the flexibility of using the bagasse for something else. 30 to 35% percent steam on cane is achieved in modern cane factories. And it's possible to push it down even lower but there are costs involved. It requires much tighter operational control. Typically a sugar factory will swing, the conditions will go up and down depending on the day of the week, whether the weather is bad or good and so on. And the system has got enough flex in it to allow this. If you want to be really efficient, you have to tighten up all your operations.

I don't know whether any of you are familiar with pinch technology. Pinch technology is a way of balancing energy availability and energy sources in an operation. And it gets into some quite complex modeling, like was just mentioned.

Significant capital costs, you need high pressure boilers rather than typical boilers which would run at 200 PSI in a traditional sugar factory. These will be running at 1,500, 1,200 PSI. So they're quite different engineering wise.

Biomass for fuel and energy. Energy is produced by combustion of the fuel to CO₂. Direct combustion, burn it as is, which is what a conventional sugar factory does with bagasse. After drying, size reduction, and pelletization you can make a superior fuel in terms of its handling capacity, storability, and so on. If you dry it, reduce it like into pellets which are typically, about the diameter of a pen, about an inch or so along.

You get into tarification at any stage?

No, we haven't.

You haven't mentioned that. Tarification is a thermal treatment after drying, which drives off the excess, it drives off some of the molecular water. Not the moisture, moisture is separate. Moisture is free water which just happens to be there on the material. If you go and tarify it, you reduce some of the molecularly attached water.

In other words, you dehydrate the molecules themselves. You pelletize this, it's much closer to coal. Now, it's a significant benefit to use as a fuel. There are significant, very significant costs in producing it because energy has to be put in to do the tarification.

After transformation by pyrolysis and cleanup, people made some various types of biocrudes. I'm not going to say much about that.

We'll cover those next week.

You cover those next week.

Conversion to syngas, of syngas to ethanol of all microorganisms, and this is being developed but has its teething troubles. In the industrial scale operations.

They know about the cyanide.

They know about the cyanide. And the Fischer-Tropsch synthesis of hydrocarbons. Again, you're taking these gasifications, carbon monoxide-hydrogen mixture with a catalyst and you can convert these two hydrocarbons. But the main focus here is the last one, hydrolysis to useful fermentable carbohydrates.

This is repeating a little bit of what George said. The inherent biochemical complexity of the structure biomass suggests that we should attempt to retain as much of this complexity as possible. When we talk about celluloids, it's a polymer of glucose. It contains the molecular complexity of glucose. So if we break down cellulose or hemi-cellulose into the individual free sugars, we still retain that chemical complexity and the biological usefulness of that material.

If you direct combustion and gasification, you destroy this complexity. So if we're going to go any biological route, we want to retain as much on the biological complexity inherent in the sugars as possible. Cellulose, hemi-cellulose, lignand should also mention that, lignand is a complex, oxygenated poly phenyl propane. Don't worry about that.

Hydrolysis of the insoluble polysaccharides by chemical treating, you can do it with acid, hydrolytic enzymes, cellulases and hemicellulases. A combination of the two. What George mentioned on the [INAUDIBLE] what was a combination of two.

Biomass hydrolysis, lignocellulose, primary products we've already dealt with that. The goal is to produce an intermediate product suitable for chemical or biochemical version to finished products, whether fuel or chemical feedstock.

For example, as George said, if you change the bug in the system, you convert the glucose, for example, to succinic acid or lactic acid or a whole variety of other things. Which then, have a usefulness as a chemical feed stock. And this is the Biorefinery concept.

Pros and cons of acid and enzyme hydrolysis. The early, early, early days of biomass conversion was done in Germany in the time of war because they wanted to make methanol and ethanol. So they would just use of sulfuric acid. The constraints of wartime economy change everything. So that was not something that was sustainable by any means.

Acid hydrolysis pros, rapid and inexpensive, George mentioned that. Pretreatment is not really necessary. And by pretreatment, I'll come to what is meant by treat treatment in a minute or what I mean by it. The cons, it's nonspecific and there are undesirable side products. With too much acid, you can make some degradation products from [INAUDIBLE] and from [INAUDIBLE] is the major component, which will inhibit fermentation, is not good news. So you do get a non-specificity.

As mentioned earlier, biological systems are very specific. You need to go into corrosion resistant materials for the equipment. The temperatures and pressures that were mentioned earlier also add to the corrosion issues. As an aside, in some of this development of corrosion resistant equipment, you need to use special alloys. Sometimes it's zirconium alloys, these are very, very, very expensive. And so you're going to add to the cost if you're going to do it this way.

Now, the short time helps a lot because you get more throughput in a small system when the residence time is low. In one case, where they were developing, and not too far from here, a acid

pre-treatment using fosric acid, in this case, they were having some problems with the design of the equipment. And my suggestion to the person doing the project was to build everything out of mild steel out of the cheapest material that you could machine as possible. Will it corrode? Surely it will corrode, who cares.

But if it runs for a week and it operates properly then we have the design right. If you have to design something in very, very expensive alloys and you design it wrong, you have other problems. So what, you have a piece of steel, you throw it in the scrap heap, it was cheap.

And so those issues. The last con of acid hydrolysis is neutralization and waste disposal, especially if your using something like sulfuric acid. If you have to neutralize the sulfuric acid with lime and calcium sulfite, then you could end up with a pile of calcium sulphate which is gypsum, which is Sheetrock. Not a nasty material but a lot of it, you could end up with more gypsum than you are producing ethanol. So who wants to build a waste plant? This is not a sustainable operation.

Enzyme hydrolysis, specific hydrolysis, pro-specific hydrolysis under mild conditions. Minimal unwanted side products because of the specificity of the enzyme. The long hydrolysis times, mentioned earlier, availability and cost of enzymes. The enzymes are not ideal, they are OK. That's what I mentioned right at the beginning, that they are adequate to the job, they are not there yet. There's recent work being done on high temperature and I'll mention that a little more later.

Pretreatment becomes essential. Pretreatment, lignin celluloids evolved to provide the structural integrity to biomass. If there wasn't any structure integrity to biomass all the trees would fall down. It has to be physically strong enough to have a matrix, strong enough to hold the trees up. It is, therefore, resistant to hydrolysis under natural conditions such as by enzymes.

Some of the enzymes found, initially, from fungi do cause tree rotting, But if you wanted to watch a tree rot in the forest, it will eventually rot but it takes a long, long time. The resistance is primarily due to the quasi crystalline structure of lignocellulose that prevents access of the polysaccharides, access to the polysaccharides by the hydroelectric enzymes mentioned earlier. The goal of pretreatment is to open this structure and convert the biomass to what we can call an enzyme ready condition. If you put untreated, unpretreated biomass in with an enzyme, you'll get a little reaction, very slowly. And That's not what you want, you want to push this reaction along as fast as you can.

A wide variety of approaches have been tested, Many work quite well in laboratory but have serious problems with scale up to commercial operations. And this is where some of my experience in the sugar industry gets-- it's critical. Because we want to have something which is scalable, something which can operate at a significant level. Doing something in a lab is fun, you can do anything you want in a lab, anything you want.

Some of them, some of the proposals, For example, to use-- I don't know whether you're familiar with organic liquid. Not organic liquid, ionic liquids. These are complex salts which are liquid. when

you think of salts, like sodium chloride or calcium sulfate or ammonium nitrate or any of these things, these are solids at normal temperatures.

Ionic liquids, I don't know whether you'll get into this. No, probably too exotic. These are very, very powerful solvents and some of them will dissolve cellulose and lignocellulose. However, they're very expensive and so you end up working using a solvent or a reagent, which is far more valuable than your finished product. And in order to make this work, you have to capture and recover and reuse all of this material. Not an easy thing to do.

And they also have significant and, as yet, improperly defined environmental impacts. When you look at the chemical structure, if you're a chemist, they said, I don't really want that stuff around. One of the earliest solvents for cellulose was based on cadmium. Nobody wants cadmium around, so we have to think of something for pretreatment which is environmentally benign.

So the evaluation of a pretreatment efficiencies by measuring the level of enzymatic, enzymatic hydrolysis using commercial enzymes. In all of the work that we did, we use commercial enzymes. We were not getting into production of enzymes. Whether you do this at a commercial scale plant would depend on local economics. You could make your own enzyme there or you could buy it that would be a straight commercial decision.

More on pretreatments, it's usually a combination of thermal, mechanical, and chemical treatments. Acid concentrated to a dilute, hydrochloric or sulfuric acid usually. There's been some more recent development using concentrated hydrochloric acid, which is a powerful solvent but quite a nasty material to handle. I have some burns on my arm, which a long time ago relate to that.

Also completely impractical things like hydrofluoric acid and Trifluoroacetic acid. These have all been proposed and it's fun to play in the lab, but you've got to take a completely different attitude when you get into commercial operations.

Problems of recovery, recycling, and disposal. If you are using one of these materials, like fluorides, and you are using an enormous amount of it, if your recovery is 99% that means 1% is going somewhere else. And that is just not good enough.

Alkali treatments, usually lime or sodium hydroxide due to the cost. Advantage, this is the approach that we use and I'll come to it in a minute, is the minimum hydrolysis of hemicellulose. Some lignin solubilization, part of the lignin solubilization occurs because you're opening up this structure. If you, probably have it in one of your references, a photo micrograph of lignocellulose where you see a crystalline structure which contains cellulose lignin and hemicellulose. Once this structure is broken up then, some of the, a minimum amount, but some of the lignin will be released.

There's been some work using ammonia, but ammonia is also not a very comfortable material to use a large scale. So the chemical industry does, so this is a more practical approach, for example, using ammonia then some of these other rather nasty materials.

Thermal treatments, the softening temperature for lignin is approximately 120 degrees. When I was at LSU, we did some work on bagasse but other materials as well. Well, we hot pressed it. Take the temperature up to about 130,140 degrees, just on bagasse, dry bagasse. Heat it so that the lignin would melt under pressure. We could try and make something like a board, a fiberboard, like a hard board, and it did work.

You could drill a hole in it, you could cut it with a table saw, do not get it wet because it collapsed. Anyway, so this is important, any pretreatment needs to get above this sort of temperature because it does help. The rate of pretreatment is much faster at high temperature increased production of undesirable materials, furfural and hydroxymethylfurfural. The higher the temperature you go to in the pretreatment, the more likelihood to produce some undesirable products.

Mechanical, disruption of the quasi crystalline structure using a mechanic approach is it produces high shear. And we need to learn here from the operating industries, which are large scale industries such as, the pulp and paper industry. These have a lot of technology for disruption of cellulosic material. It's an industry of a similar scale to the sugar industry, similar operating constraints. And so it's an industry that we should be looking at.

Mechanical systems used to produce paper and steam it, steam explosion is another method of doing this where you pressurize a material under temperature in a flash and release the pressure, it's like making popcorn. And if you take kernels of corn, you heat them up, and they will pop because the steam inside the kernels of corn will just explode. And this opens the structure. If you eat popcorn, you can digest a fair amount of popcorn when you eat it. If you ate the whole kernels, you wouldn't digest very much.

The advantage of mechanical systems is minimal waste production but higher energy costs. There's been some recent work from a sister institution. Where, they, at this place, suggested high shear alone. But some of the data is not adequate. And it's, I think very, very difficult to commercialize something like just a shear mechanical system using a ball mill. Are you familiar with that?

Experimental approach to pretreatment. I mentioned the top already ideas generate laboratory experimental work to confirm the idea. That's where you start. Almost anything is possible in the laboratory, you can do anything you want. Most important, next step is a practical evaluation. Define a process flow diagram even on the basis of very simple experiments at the laboratory. Define a process flow diagram. Have a look for process complexity.

Even think about, can the process be automated. Is novel equipment, special alloys required. Am I operating under conditions where I have to design radically new equipment or operate under severe corrosion conditions. Look at the recycle of water and reagents. Once you have an idea for a process, and you can sit down and do the thought experiments. A thought experiment is another version of what George was talking about.

Sit down with a blank piece of paper in a group of people with a whiteboard and just scribble. At that level, and then you rationalize your ideas this way. Waste disposal, any process is going to generate waste. Toxic reagent, process safety, et cetera. Then, do an estimate of the capital and process input. This is on the basis of the laboratory scale experiment.

Not easy to do, but you can get a ballpark number. If it's more than such a number, forget it, it's not going to work, go back to the laboratory. If it does give you numbers, which make some sort of sense, then proceed.

Now, the next few slides are the summary when George was at FIU and Florida Crystals Corporation collaborative project. This is a paraphrase of the title, bagasse pretreatment and enzymatic hydrolysis for the production of ethanol by fermentation. We later modified this to focus on the production of fermentable sugars. Ethanol production technology for such sugars is well established, perhaps less so for C5 sugars. This is still a work in progress.

The conditions we selected for pretreatment, pilot scale work, were treatment of whole bagasse with lime at 100 degrees C. Intense mixing with a higher shear pumping system, removal of lime along with organic acids, and solubilized lignin, not much by filtration, and PH adjustment for enzyme.

This treatment and the acid treatment are extreme pH, in the acid case, low pH, in this case, high pH . So there is pH adjustment for the enzyme. And enzymes have fairly narrow optimum pH range and optimum temperature range. So to make the enzymes work as efficiently as possible, if the enzymes were very fast and very stable, then you wouldn't be worried about these things . But they are the major cost to get the enzymes and the major time constraint on the process. So pH adjustment is necessary.

Process constraints, the other things we looked at. All equipment at the commercial scale to be off the shelf. We decided we're not going to start creating new pieces of equipment. No unique design and fabrication required. We didn't want to go out to an engineering company with an idea and say, build this piece of equipment but I'm not sure I got it right yet.

The goal was to have a custom process but not custom equipment. We would use conventional, available equipment in a nonstandard way. The other constraints we put on ourselves, no process conditions to be more severe and routine in the cane sugar industry, paper pulping industry. Especially for temperatures and pressures, we didn't want to get up to high temperatures and pressures.

Energy and water recovery, we built into the process. All process steps to be automatable more. I'm not sure automatable is a word, but it does convey the idea. Spell check told me I couldn't use it, but I thought it makes sense. Any process which you can't automate it, has no place in a modern industrial environment.

Safety is a paramount concern, and this goes back to some of the temperature pressure issues. Must be at the scale to be commercially viable. In the end, we need to be able to develop something which is, in the end, commercially viable.

To give you a little more detail-- we can go through this quickly-- mix on a high shear, bagasse 10 to 12% solids on a dry basis. With water at 100 degrees C and lime, 8 to 10% on bagasse for one hour. This is not optimized, some of the mixing issues we had made it take an hour, probably could be a lot less if you had a continuous operation rather than a batch.

Pump the slurry from one into the second stage and heat was recirculated to 120,140 degree C under pressure. We discharged to atmospheric pressure through a small orifice inducing flash and high shear. If you take something under the pressure, then release the pressure suddenly, especially if it's water, it will boil, it will flash. Heating by steam injection or scraped surface heat exchange, we looked at both of these and, in the end, I think we used steam injection.

The vapor condensed from the flash per process we reused in process. So if get these conditions right, we managed to recapture all the water that we added at this stage. Filtration of the slurry from item number two with the equivalent of a bell press, I don't know how much familiarity you have with industrial equipment, but this is a very routine piece of equipment used in many industries.

Enzymatic hydrolysis at the pilot scale. Treatment with enzyme cocktails from commercial vendors under the conditions specified by the vendor. I've not specified the suppliers because some of them were proprietary. If you're familiar with the industry, you know who the usual culprits are. But we were not into the business of developing enzymes. We were into business of using them. And so the constraints we have are, what is available commercially.

The ideal run time for us will be 48 hours, somewhat less than what George mentioned. This is a target value, we want to minimize this time. Typically would be 72 hours rather than 48 hours. We wanted to operate at high and solids content required to minimize concentration required for solubilized sugars.

If you use a very light slurry, if you use a 3% suspension of pretreated biomass in water, you're going to end up with something between 1% and 2% total sugar concentration. Far too low for any practical purpose. So the goal was to operate with as high a solids content as possible in the enzyme treatments stage. Mixing system that we developed was based on a sugar crystallizer. This is something that sugar factory was a mixture of molasses and sugar crystals, so capable of handling very viscous things. And we were able to do that.

Batch fed enzymes treatment proved superior. Batch fed is you don't add all the enzyme at once, you let the reaction go for a while, and add more enzyme and more substrate, more enzyme and more biomass. So this is a way of optimizing the use of the enzyme. Solubilized sugars recovered by filtration. The initial filtration of the pretreated materials to get rid of lime was very fast and very

easy because a lot of the fibrous structure from the cellulose remained in the product. And this makes filtration easy, that's why we use filter papers because filtration through paper is easy.

Once we solubilized the cellulose this filtration became a lot more difficult. It was sticky, slimy but I think we could figure it out. In fact, we did get this to work in the end using a completely different type of filter.

This is a picture, photo micrograph, of some incompletely pretreated bagasse to show you the contrast between the very fine fibers. This is-- that scale there is 100 microns so it tells you how fine it is. You can't read it. I can barely read it coming up to it close. That's what the picture of pretreated bagasse looked like, rather than the chunks that you would normally see.

Pilot plan results. Enzyme hydrolysis results depend on both pretreatment efficiency and the enzyme. It's difficult, not necessary, to separate the two stages. Because the enzyme is proof that the pretreatment worked, we did not have-- I'm not sure anybody has yet-- a pretreatment test. We could do it by filtration capacity but that's still difficult.

We observed that the thick slurry liquefies in 25 to 35% of the treatment time. If you looked at the production of free sugars with time, this would take, say, 60, 70 hours. Whereas, in 12 hours, or less sometimes. The viscosity of this material had gone down because you had broken down the high molecular weight material into lower molecular material. And then, this was still-- required enzymes to produce free sugars. But the lack of high molecular weight material meant that the viscosity went down.

So a batch fed approach attempts to maintain a high solids content, insoluble and soluble because what we're trying to do is to maintain a high solids content by solubilizing the lignocellulose into a soluble material within the viscosity limits of the slowly mixing system. If we had a better slowly mixing system, we may have been able to go to a higher density. The preferred enzyme dosage is 1 to 5% enzyme as protein and biomass solids. This process remains to be optimized.

This is some work that was done by our colleague with the enzyme dose, grams of enzyme per gram of dry solid. So it's anywhere from 1 to 5%. It gets close to the theoretical limit for glucose at this point. This maybe be a little optimistic, but that's the way the analytical data came out. As you increase the amount of enzyme, you get increased release of free sugars but you also increase your costs. You reduce your time but, there may, since the enzymes are the primary cost--

Project status, the basic process development is complete requires more elaborate and instrumented pilot plan for optimization. Based on the data we had, we did an estimation of the cost of producing mixed polysaccharides, that's free C5 and free C6. It's 12 to \$0.15 a pound. Current glucose prices is greater than \$0.20 a pound based on corn all costs included, energy costs, labor costs, enzyme costs, chemical costs, enzymes being the major issue.

This is where it stands, the problem of a lack of an established market for the product, and therefore, no support for going further at this time. So the project is in limbo, it's neither dead nor alive. Technologies develop, and this is typical of what happens in many, many of these projects. The justification of spending a lot of money on rebuilding a pilot plant larger and instrumented pilot plant for optimization. It is millions of dollars. And owners of private companies typically don't want to spend that money unless they can see that they can sell what they make.

And there's a problem of a lack of an established market. This is developing, so it's a watching brief at the moment.

Biomass conversion facilities are new and not yet proven technology. And they have high capital costs. Significantly higher than for established corn ethanol plants per gallon of production. But the benefits of being able to use something, which is not in the food chain, is very significant.

General comments on process development. I'll finish in about five minutes. Process robustness, including enzymes and microorganisms. If the process is so delicate that a little upset causes it not to work that's no good. It has to be robust. It has to take--

Let me tell a little story. When the pilot plant nearby, near here, was being started up, and it has a lot of high pressure equipment and very elaborate controls. I said to the guy in charge of it, when you have it running perfectly, go round the back and pull the power. Just stop all the power. You can't do that. It's going to happen, be prepared. How well does equipment respond.

It's obviously going to crash, especially if something is high temperature and the temperature drops. How much damage do you do, you don't really want to damage anything. How much damage you're going to do the process and how fast is your recovery time.

On a separate issue, when we developed the first membrane filtration system, and it was a part of an R&D project. And I have to be away for a few days, and so did my assistant. So we left it in the hands of one of the regular factory operators. Just run it, here's the instructions. We came back a few days later and the whole thing had crashed, bit of a mess.

It took my assistant and I two hours to have it up and running perfectly again. That is the sort of robustness that you want in the process. You don't want it to crash. But having being around industrial operations for awhile, it's always going to happen.

Minimize secondary side processes. This is a separate issue but if you looked at the process flow diagram, that George had earlier, then it's this going to this going to this going to this. If you are using some expensive reagent or solvent And you put in a solvent recovery system, water is easy, if you put in a separate solvent recovery system, then this may be more complex than the primary process stream. The secondary process has to be simple. Ideally not even that, except for water recovery.

So you don't want to make systems more complex, the simple straight through process is what you want. You don't want a lot of recycle, recovery loops in the process. They add complexity and uncertainty to the operation. Then, the-- I always like this one-- if the process is for fuel production, how should an overall energy balance be developed. Cradle-to-grave evaluations.

Take the extreme case, you build a plant for converting biomass to fuel. The plant is going to contain a lot of steel and a lot of concrete. I don't know if you aware of this, but concrete, cement production is one of the major sources of CO₂ production in the world.

Should you include, in the energy input into your plant, the cost of the energy going into the concrete, into the steel. Should you include the cost of energy used to produce the trucks that bring the material to the factory. It can get bigger and bigger and more and more stupid. But, bear in mind, your goal is energy production. If you're going to get energy out, your net energy is going to be somewhat reduced if you consider all of the other issues that go into it.

The other thing I learned was to think of the process from the operator's perspective. Your plant, any plant, is going to be run by operators. These are not your Ph.D. And master scientists. These are guys off the street. Smart very, very smart, most of them. But they have a very limited perspective. Give them something to work that works and they will make it work or they'll find a shortcut around it, which maybe you don't want to do.

On the membrane filtration system when we started it up, the operators very soon found out that if they diluted the string going into the system, it ran a lot faster. They just put more water in the system. But that wasn't producing anything. It was just producing a more dilute sugar solution. But they took a couple days to wonder, why are we running so well and we're making so little product. They were putting water into the system to do that.

Appreciate all the pieces of the puzzle at the start. Mind experiments, and this goes back to what I said at the start. Mind experiments, do think about, sit down with your colleagues around a whiteboard and argue about it. Nothing personal, just sort of, that'll never work, you missed that, you missed that, very useful.

And then, George mentioned the class of biomass production versus processing. All of what we've been talking about in my talk is processing operations. Biomass production is another issue. The great advantage of the cane industry is that the bagasse comes out in one place, in one factory. All of the dirty work of harvesting and collecting it is done for a totally separate justification, sugar production.

All right, some recent developments, the Department of Defense awarded to becoming operational in 2016 to three advanced biofuel companies to gasification, FischerTropsch, and one using wastelands. Brazil's Granbio, one of the big shot companies is commissioning a cellulosic ethanol plant so things are coming along. Kintex is another company based in Italy but developing cellulosic ethanol plants based on *Arundo donax* in Italy and USA.

This is an important one, probably as important as any, cellulase research identifies high temperature enzymes. Ideally, you want an enzyme which operates at a high temperature when nothing, no bugs will grow in the system. If you start looking at the enzyme profile, the preferred enzyme profile, especially for temperature, a lot of microorganism will grow under those conditions. You want to avoid that. If you look at the corn industry, the amylases used to solubilize starch all run at very high temperatures up to 100 degrees C, even hotter. So this will be a significant benefit, if efficient enzymes can be generated.

POET and DSM, these are big ethanol company in this country. DSM is an enzyme supplier. Joint venture to produce 25 million gallons per year of ethanol from crop waste, primarily corn [INAUDIBLE]. The 750 tons of corn waste a day, estimated capital costs \$250 million. That's a big chunk of change. C&E News, this is the American Chemical Society publication that everybody should read.

It will be sometime before making certain chemical from sugars is profitable. If the second quarter results posted by six bio-based companies are any indication. A lot of companies are struggling, the technology's good, . But they can't finance large scale operations necessary. For now, the best measure of success is progress towards operating smoothly at commercial scale. What does that mean?

Anyway, last slide, in many industries, there is a level of enthusiasm, you get your true believers and your more hard nosed realists. Best to be somewhere in the middle. There are people who believe passionately in biomass but, sometimes, they're not in touch with the real world.

Irrational exuberance is a term used by Alan Greenspan, former chairman of the FED, to describe the economic conditions in the late '90s. But Dilbert, if you don't read Dilbert, you should. We have incomplete-- this is the boss-- we have incomplete data so I'll use my intuition and experience to make a decision. To which the engineer replies, magical thinking fixes ignorance. So do not get carried away, do your hard thinking, no magical thinking. Magical thinking dooms you.

My question is, mainly, pertains to sugar cane.

All right.

Given all the constraint, to get more fuel out of the bagasse, wouldn't it be more economical to just take the juice out and used the bagasse for power instead of trying to get more fuel out of it?

Well, for example, there's is no sugar cane ethanol produced in the United States. And there is [INAUDIBLE] down in South Florida. It's probably one of the most efficient, energy wise, factories which has the large co generation facilities. So all the bagasse is burned. In fact, they burn some wood waste and other materials to be able to operate the power plant year round.

It is a local choice. In Brazil, a lot of the ethanol production is driven by government plants. It depends on local circumstances. If electric power is desirable, that's an easily sold material. Then, that is the preferred choice to make. Sugar makes more money.

There's another constraint on ethanol production from sugar cane, The same constraint as rum production, which is a better use, anyway, of sugar cane. and that is that, once you do the fermentation, you're left with all the non sugars in the waste stream. As I said earlier, you recover your ethanol by distillation. But you leave a lot of junk, very high BOD, dirty water behind.

This is handable in Brazil, not really handable in the United States at the moment. And certainly, not handled in the Everglades agricultural area. It's a tremendous amount of waste water. Very high BOD. So there's not a simple either or question, it's both and depending on where your circumstances are.

What's unhandalbe? Define handable.

You mean in the context? They can spray it on the fields in Brazil. The waste material is concentrated. Maybe in some cases, not in others, you can spray in on the fields. The waste contains a significant amount of potassium from the cane. It contains some phosphate, it contains a fair amount of nitrogen. It contains a lot of other junk which came from the soil, which is going back to the soil. But it does change the pH profile of the soil and all those other things.

So you need to have the right conditions and you need to have--

So are they relying on natural filtering to eliminate--

Well, it's natural-- with oxygen, with atmospheric oxygen, the BOD will reduce naturally. But it is-- eventually, but eventually, we're all dead.

Will it degrade?

It will degrade, but it is, to a certain extent, it's due to political and other issues, do the people accept it? They would not accept it in South Florida. There's a sugar factory in South Louisiana, which can go nameless. And we were discussing a project with them, which was similar to this, but would generate a lot of dirty, very dirty water. And the question was how to handle it. And the old Cajun, you know what Cajun is, South Louisiana person. The old man, traditional old Cajun, you scientists make things too complicated, when I have a lot of dirty water I get a big water truck with a leaky valve on it, find my dumbest driver and tell him to drive around South Louisiana every night. He comes back with a empty truck in the morning.

That is the solution to environmental. I don't think, but this is the attitude of some people. The environmental impact of many of these process has to be established. That's why it has to be clean and above board. Minimal use of nasty reagents, no heavy metals, no nothing.

No hiding your ingredients.

No hiding anything. Are you talking about the sugar industry? No, the sugar industry has a--

No, I'm not referring to the sugar industry.

As a--

But other issues in the everglades.

No, the Everglades, the Everglades is

I'm referring to the horizontal drilling and practices that are not fracking.

Not fracking. Well, semantics, semantics

Yeah, I know.

Political semantics.

Maybe. sugar cane.

Yeah, the sugar industry has, in the past, traditionally rode roughshod over all of this. It's changing now, they realize it's changing. But there's a legacy, which is the operating practices now, I think, would meet standards. But you still got a legacy of undesirable practises. Probably, the biggest one was the soil depletion. If you go to some part of Belle Glade near the experiment station, University of Florida experiment station, in the 1920s, a lot of the cane was being developed in that area.

They drove a big post into the ground market and marked it.

I've seen this.

You've seen it.

For the water level?

No, for the soil level.

[INAUDIBLE].

So the soil it-- remember the soil in South Florida is organic soil subject to atmospheric oxidation. Especially with the wrong practices. There are such things, when the soil gets dry, on a really hot,

sunny day. There are things called muck fires, which, remember that organic soil is almost like peat, you could, in fact-- we made the measurements on calorific value of the soil-- you could, in fact, put the soil in the boilers and burn it to generate power.

As a fuel.

As a fuel, but too high an ash, that will be the only problem. The only problem would be the height ash level. The calorific value is there so muck fires occur. And so if you go and look at this post over 50 years, 60 years, the level has dropped many feet. I think 10, 12 feet. The soil is down, way down. Some parts of the Everglades, the soil level on top, remember what the soil, what the Everglades basin--

The agricultural area is limestone with a layer of soil from the old lakes on top of it, The level of organics are. and this soil is being lost and it's down to only inches thick in certain areas. Still can struggle to grow cane on it. But this is been one of the legacies I'm talking about on the sugar industry. Practices now have changed, and it's flooding the fields, for example, during the summer. Growing rice on the fields, and flooding them during the summer minimizes this atmospheric oxidation in [INAUDIBLE].

I'm now here to defend or criticize [INAUDIBLE]. It's been a part of my life, and they are hardworking people. But sometimes they're stubborn, and they don't see a big picture.

They don't see the dirt plumes coming out in Naples on the other side of the state.

Huh?

They don't see the dirt plumes coming out in Naples on the other side of the state.

Yeah. That reminds me, have you ever heard of a-- completely separate story-- OK let me just out of sight, out of mind. It was an experiment done to test a language-- now probably told this punchline. The punchline's already-- anyway, it was a translation program. Could you translate something from English to Greek or English to French or something like that. The test was to translate it from English into the foreign language and get the program to translate it back into the original language. And what it came back was invisible idiot. From English to German back, out of sight, out of mind, invisibility. So you wonder about some of these wonderful apps for translation. You might want to-- where are you?

Anyway, any more questions? Any more issues?

Steve, I have a question. What do you see the trend in companies, large corporations with regard to sustainability. How seriously are they taking it and what do you see happening in the future as far as--

I think sustainability is a major issue. There is a sustainability committee, Florida Crystals Corporation, Gus Cepero is the chairman of it. And they are trying to walk a fine line between satisfying what the public perception of sustainability is and economics. Because economic costs are going to be there. But there's a balancing act and, in the end, if you don't maintain the sustainability of the soils, you lose everything.

And that is the way someone like Florida Crystals would look at it. But this is something, if we're going to stay in business, we have to operate sustainably. I think this is true of many companies. But, another example of where I think things go bizarre, is-- I don't see any over there-- yeah, there's one over there-- plastic bottles. These are Polyethylene terephthalate, PET, plastic bottles. These are made from polymerization of ethylene glycol, which is made from petroleum and Terephthalic acid, which is also made from petroleum.

And so the marketing guys at the big Coca-Cola and other places want to develop a bio-based equivalent. Well, the functionality of Polyethylene terephthalate is very, very good as the bottles get thinner and thinner as they improve their technology. So the goal was to try and make these bottles from sugar cane, for example. Because a lot of the Coke that goes in there contains sugar. So it'd be a nice marketing exercise. So make ethanol in Brazil, ship it to Taiwan, dehydrated it to ethylene in Taiwan, oxygenate it to make ethylene glycol. It's going to be a peroxide, usually a mercury catalyst, traditionally a mercury catalyst, but I'm not sure what they use now.

But you can convert that ethylene to polyethylene. Sorry, not ethylene to ethylene, glycol. Now, you take some sugar and put it into a catalytic reactor. This is Virent Technology. This is a company in the US which can take sugars, put the sugars in directly, and get out something like Paraxylene, which is a hydrocarbon. Which is then oxidized to Terephthalic acid, you combine with your ethylene glycol to make your Polyethylene terephthalate. So you make your plastic bottles from sugar. But it is insane. The costs of doing it make no sense. The marketing guys drive it. This is where the sustainability issues become crazy.

And the plastic bottles-- we've got, my wife uses them all the time and kids-- I don't, not so much. I don't drink tap water, I don't worry about getting the stuff in a little plastic bottle. But we are creating new necessities. Our grandfathers, when I was a kid, we never had these things., now, they're a necessity. No they're not. But the world is changing to create new necessities.

We all have one of these, I'm sure. This is an iPhone. and that's a new necessity. A small child, 10 years old without an iPhone, is not really a child anymore. everybody has to have one. One of Steve Jobs' comments was, we need to create new necessities. That's the marketing end. Is it really necessary? Is it?

Next time you go to buy some fruit at the supermarkets have a look at the little labels. When you're eating a pear that came from Chile or something that came from Australia, does this make sense. Not sustainable. This is one of my pet peeves. I grow my own veggies in my own garden, but the

sustainability issue is that we are developing technology, which is not sustainable. The food processing operation, not sustainable.

Go around-- that's one of the things you should do if you get a chance. Two things I would recommend you visit. One of them is an oil refinery, you all drive cars, I'm sure. How many of you have been in an oil refinery. You have, some of you, very good. So go and see what goes on in an oil refinery because you-- like small children, where does milk come from? Milk comes from Publix.

And they need to know. We don't pay enough attention to where things come from. Our Grandparents lived different lives.

Anyone? Anyone have questions?

Yeah.

I'm not from-- Well, I'm not from a science background so use that to understand my question. My question is, basically, all this enzymes and acids and everything that goes into the fermentation process and the breakdown of glucose and that. I don't know how these things are made. But with the future need of these things to achieve the end products, and renewable products. Are we also sure that these things are sustainable? I mean, how is acid gotten how are fermentation products gotten?

Well, you are, to me, you're asking two questions. I'll try and answer both. One of them is, the things like the acid and the chemical reagents. Those need to be minimized. They are, most of the time, fairly benign, can go back into the environment. We use lime for soil amendments. For example, in beet-sugar operations, you produce a lot of calcium carbonate.

That's a waste product of some cane refineries as well, cane sugar refineries. This can go back into soil amendments, rather than digging up fresh limestone for soil amendments, you can use this material. So that one is sustainable most the time.

Now the other one on the enzymes, the enzymes are produced by their own small fermentation system. Enzymes, you start with a microorganism, which you grow under whatever conditions appropriate for producing that enzyme. The enzymes are produced by microorganisms. So you have a parallel fermentation system. Whether you wish to have that at your location or somewhere else, is a decision to be made locally. If you're a very big operation, you may want to make your own enzymes.

So you will be diverting some of your sugar products from your process into your enzyme production facility. So, in that respect, that is sustainable. George any comment?

You need to decide whether you have the expertise. Or you want to bring the expertise for enzyme production inside your company, inside the core business. If it's not, you may not want to do it. You

can vertically integrate if you bring the enzyme production inside your operation. But you need to make a decision, because the enzyme companies have so much more expertise in that area than you do. But you may find it more beneficial to have them solve your enzyme related issues, than try to have to replicate what they do inside. It's going to be expensive and time consuming.

But the basics of enzyme production is sustainable fermentation, to produce the enzymes.

Yeah, yeah, it is sustainable.

[INAUDIBLE] problem without looking for enzymes?

No, the real challenge is finding the right enzyme. And what I refer to enzyme cocktails, because what we were looking at is a combination of cellulases, hemicellulases, and other supporting enzymes, like Beta-Glucosidase, it's a mixture.

Not work together.