

An Altenews Interview with Mark Emalfarb, Founder and CEO of Dyadic (Symbol: DIL), and Dr. Glenn Nedwin, PhD, Dyadic's Chief Science Officer.

Dyadic (AMEX, Symbol: DIL) was founded by Mark Emalfarb as a company that supplied jeans makers with tools for stone-washing blue jeans. The company evolved from a clothing company into one of the premier bioscience corporations, specializing in the creation of enzymes, as stated in the company motto "From jeans to genes." Dyadic is now on the cutting edge of making enzymes for the production of cellulosic ethanol, and they also make enzymes from which a variety of fossil fuel-based products, such as chemicals and plastics, can be replaced with biomass-based substitutes. Mr. Emalfarb is assisted by his Chief Science Officer, Dr. Glenn Nedwin, who has a long history of working in the bioscience industry, and also holds a PhD in biochemistry from U.C. Riverside and an M.S. from MIT. I spoke with Mr. Emalfarb and Dr. Nedwin about the history of Dyadic, the development of cellulose-based ethanol, and how cellulosic ethanol can one day replace gasoline.

Altenews: Could you tell us about the future of cellulosic ethanol?

Mark: Look at the April presentation, on the Dyadic website (www.dyadic-group.com).

The goal of Dyadic is not just to make cellulosic ethanol, it is to create enzymes to produce glucose from biomass in order to replace not just ethanol, but all petroleum products. There are companies making methods to displace all petroleum products, with bacteria that eats glucose and makes polyesters and various plastics as well as most other petroleum-based products. Such companies include Iogen, Abendoa (which has a DOE grant), Cargill, Nature Works, and Metabolix (which is going public). The goal is to make every petroleum product from glucose, to use less petroleum and to take advantage of the future peak oil shortage of petroleum. It is not just about cellulosic ethanol, which is the tip of the iceberg. However, cellulosic ethanol will have a vital role to play in creating fuel in the future.

Altenews: I know that your company motto is "from jeans to genes." How did Dyadic get started, and how did Dyadic go from being a clothing-related company to being a bioscience company?

Mark: We started supplying Levis and other blue jean manufacturers with pumice, telling them how to stonewash better. We made \$4 million in 1987. Then Novozymes, the world's largest enzyme company, was able to displace pumice for stonewashing. That is like what we are doing with sugars to displace petrol, ethanol, and plastics. What happened was that we became a distributor for Novozymes. If a dinosaur was going to become extinct it might as well be us. We talked to Wrangler and others about how to wash jeans with enzymes, our market shrunk, there were more distributors, so we decided to make our own enzymes. We needed to know how to make enzymes when we were, as you say, a clothing company, so we looked around the world, and saw who were the experts in making enzymes. We landed at Moscow State University, after we had looked at Stanford and MIT. The Berlin wall was falling, all of a sudden Russian scientists

needed to be hired, so we hired these scientists to keep them on commercial products. The government never came up with the money they had promised us, so we put in our own money. We needed enzymes to break down cellulose, and methods to make those in large volumes at low cost, so we found a fungus, one growing on the ground in Russia. This one could be used to grow cellulose more efficiently, from cotton, wood and trees, and to get xylan. We developed this fungi, which could do the job making cellulase. It was a fungi scavenger on the floor, it secreted an enzyme to make the sugar that it lived on, that is how it metabolized. We needed to put it into overdrive, we couldn't make commercial quantities of enzymes using the natural fungus, so we took it to the U.S. from Russia, and we bred it, using mutagenesis. We used UV to kill off 99% of the spores, then we found the one to make what we wanted, and we bred a fungus that made more cellulase. Cellulase is the enzyme, cellulose is the polymer, it chops it into glucose. Xylanase breaks down xylans. Those are fermentable sugars. Cellulose and xylans are the two major ones from plants. There are microbes like yeast that make cellulose into ethanol. Dupont makes polymers like Sorona, and Cargill Nature Works engineered microbes that turn glucose to biodegradable plastics. We can make fermentable sugars cheaply from plant materials, through our enzymes that make it cost effective and efficient. We can make large volumes cheaply in fermenters. We have the tools to work with the fungus, to put genes in and take genes out, we know how it makes them, what levels it makes them at, and we can make it cheaper and more efficiently. We have made those enzyme mixtures for textiles, pulp and paper, animal feeds, cotton, wheat and barley. We have a broad based knowledge and expertise in breaking fiber down, we have the fungus organism that can be grown. We have been doing that since 1996. We have all the tools to put in and take out, to make better enzymes, to drive the price down. Every type of plant material has a different structure, and needs a different cocktail of enzymes.

Altenews: You have said that your enzyme will work on Douglas Fir trees. Will the product work on Poplar as well as Douglas Fir?

Mark: Each particular form of biomass requires a different pretreatment and a different enzyme, and produces a different kind of sugar. We can't develop an enzyme for every kind of tree, instead we develop enzymes for the kinds of biomass for which there is a demand. We develop a specific enzyme to go with a specific pretreatment to produce a specific glucose. We could develop an enzyme for any kind of biomass that can be fermented to produce sugars.

Altenews: Does Dyadic get government funding?

Mark: Yes, we are a participant in a DOE research grant.

Altenews: I know that you believe that Dyadic can expand not just by producing cellulosic ethanol, which you say is "just the tip of the iceberg," but by replacing all petroleum-based products with biomass-based products, and in your presentation you give a list of various petroleum-based chemical compounds that can be replaced with biomass-based chemicals. Aside from replacing gasoline, could you give a list of the major petroleum-based products that you hope to replace with biomass-based products?

Glenn: Look at the slideshow that goes with the presentation. There are hundreds of those. I can give you examples of four things happening today. DuPont's Sorona can be made into plastics and textiles. Using engineered micro-organisms, Cargill is making polylactic acid, and biodegradable plastics. ADM and Metabolix are making PHA, biodegradable plastics. When you unlock the sugar, glucose, that is locked up in polymer cellulose, which can be converted into sugar, you can make these things. When you unlock the sugar with enzymes, cellulases to break down the cellulose, once you have the sugars, you can train the micro-organisms to make chemicals, or ferment it into ethanol.

Altenews: Could you replace most petroleum products, including fuels and chemicals, with biomass-based replacements?

Glenn: Probably. The DOE looks into this. What it comes down to is the economics. We replace chemicals processes, making novel products, and a large extent of them can be replaced.

Altenews: I know that you produce many different enzymes, and that each enzyme goes with a specific pretreatment of a specific kind of biomass to produce a specific kind of sugar. Which kinds of biomass do you think have the best potential for creating cellulosic ethanol?

Glenn: There are many opportunities. Fiber and BBGs are now made in ethanol plants, there is sugarcane bagasse, wood pulp, and citrus peels. In the longer term: energy crops, switchgrass, which is high density, corn stover, leaf straw, rice straw, agricultural residue. In the short term you have to collect the biomass, in the long term there will be energy crops dedicated to it.

Altenews: Do you think that cellulosic ethanol will one day be able to compete with gasoline in terms of price?

Glenn: Yes. It is cheaper to make it now. The cost is \$1.20/gal to make cellulosic ethanol, and gas is \$3/gal.

Altenews: I know that Dyadic participates in a Department of Energy research grant. How important is it for the government to support renewable energy development?

Glenn: Huge, absolutely huge. There is some risk involved, so we need the government to continue to support it, for the economics. If you want to take this as a national security issue, 30% of the imported oil that we use can be replaced with carbohydrates and sugars, if you have the will to do it. They should mandate 10% ethanol in gas in all states, mandate that and you'll get there. There is enough biomass to do it.

Altenews: Will cellulosic ethanol one day replace corn-based ethanol in America?

Glenn: Absolutely, it will, no question. We will run out of corn, there will be competition between fuel and food and feed markets. Cellulosic ethanol will make farmers happy, anyone can grow those crops, the feedstock is everywhere, you can grow it anywhere you can grow a crop, not just where you can grow corn.

Altenews: Are you partnering with any other companies to help develop or manufacture your cellulosic ethanol?

Mark: We haven't disclosed that. There are some oil, agricultural and chemical companies that have needs, and we have tools to get the job done. We hope that we can get together to help solve the problem, because agricultural, chemical, petroleum, and ethanol companies all have same issues, they have to get beyond corn, get to cellulosic ethanol, and they need the enzymes. There are few people in the world who have these enzymes, it was a 10 year journey for us to get here, and we believe we are meeting that need. There are few people who could attempt to do that right now, it would take 5 to 10 years to get to where we are, with the evolution of the fungus. From the natural fungi, when they break down plants, those enzymes break down the plants into sugar, and it is inefficient, mother nature evolved that fungus over millions of years, to secrete enzymes that break down wood and fiber. They live off that sugar, the fungi that does this better than others. We found our C1 fungus on a forest floor, took it back to America, used mutagenesis, did the breeding, made it more productive, changed its structure. It was hard to culture and grow, too thick and viscous, we had to modify it to make it amenable to large scale production, able to make more and more protein. We changed the morphology of the structure, of that fungi, magnified it 100 times. The protein comes from the tips of it, so now we have 100 micro-hairs instead of one long hair, we can stir it up better, it has more tips, it produces more protein, the cells are smaller, we can put more tips in, make it more amenable to large scale production. We went from 1 quarter gram/liter of protein, to 80-100 grams/liter today. We can do that in 5 days in a tank, it is can be cultured, stirred, grown with low oxygen, agitated. We have an improved strain, improved fermentation process, a prolific fungus, a variety of enzymes, molecular tools. We can make more, put more copies in, isolate tools from the fungus, insert and delete DNA, get more of what we want, and less of what we don't want. We have spent over a decade developing this tool, manipulating the fungus, making different mixtures, modifying it, programming in ways to break down different biomass substrates, like corn stover, sugarcane bagasse, dried distillers grain. Everything needs a different mixture and cocktail of enzymes, and you have to create big volume at low cost, to improve the economics. To make fermentable sugars, you need a prolific fungus, one that is totally programmable.

In addition to the efforts for pulp and paper, cellulase, barley and wheat, we make therapeutic proteins, medicines. We are not just a cellulosic ethanol play, although we have been doing that for years, not just for food, pulp and paper, animal feedstock, and biofactories. We sequenced the genome of C1 to find new genes to make better enzymes at higher levels and more affordable. We can make antibodies and other things we want to make. We are working with the Scripps Research Institute, reprogramming the fungus to make antibodies and therapeutic drugs. We are 6 years into that journey, we can do it in 1/3 the time, grow C1 in 150,000 liters since 1996, we have a million cells grown, 10

times larger scale volume in 1/3 time, and nutrients to feed C1 are cheap. There are some challenges using this fungus, but we are overcoming those. Right now we are expressing genes for antibodies, and an enzyme protease for laundry detergent. You just need to chew up the protein. We have tools we put in, we can knock out the protease gene, and we recently reported being able to express antibodies at good levels. We are moving along the therapeutic path. The fungus can help reduce our dependence on foreign oil, we can make cheaper sugars from biomass, and help humankind by making these proteins in large volumes affordably. We will bring down the cost of healthcare, make better cheaper drugs, and we are also making enzymes for the pulp and paper industry, biorefining of pulp and paper. We get 20-60% energy savings, the ability to make stronger fibers, and when you put that into the manufacturing process, it saves trees and rainforests, we can use recycled fiber more efficiently and cost effectively, and the water is cleaner. We displace chemicals in the process. We can make more animal feed, help feed more of the world, use inedible grains for fuel rather than cattle feed, break down grains more efficiently. When you are converting polymers into sugars, think about barley and wheat, beta-glucanase breaks down into starch and xylose. You can feed the animals less, make more effective use of the grain. How much corn gets digested will change, if you digest it all you need to eat less, so you use less grain, and you feed more people.

Altenews: How important is the ability to make ethanol from cellulose in order to achieve energy independence?

Mark: It's obvious we're addicted to oil, and we need alternatives to oil. Near term as a source for transportation, the more ethanol that displaces oil the better. Replacing petroleum with sugars and glucose is critically important, both as a country and as a world. Everyone is growing plants and has waste, and we have methods to break plant materials down, cost effectively and abundantly, to displace as much petroleum as possible, and become energy independent, like Brazil. If Brazil can do this why can't America? We need political will, with every senator and governor on board. We need concerted, continual effort, and we have to drive that home.