ENOIL BIOENERGY SA

Research laboratories
Biofuels & Bioenergy development

2015

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An elite research centre dedicated to the development of renewable and sustainable biofuels.

Everyday, millions of tons of greenhouse gas are emitted into our atmosphere from our consumption of fuel refined from crude oil. Scientists have forecasted our temperature to rise by 3 deg C in the next 50 years. This in turn will have devastating effects such as melting of polar caps, increased sea level, heatwaves, droughts and severe winters. Biofuels are organic fuels made from plants and vegetables such as soya bean, corn, palm, sugar cane. As they take in carbon dioxide from our air in making these biofuels, they do not contribute to greenhouse gases and global warming.

Biofuels

Biofuels are any liquid, solid or gaseous fuels produced from organic matter. The extensive range of organic materials used for biofuel production includes starch and sugary plants such as corn, wheat or sugar cane; oily plants such as rape seed, soya beans or jatropha; vegetable oils and animal fats; wood and straw; algae and organic waste and others. Biofuels are commonly referred to as first generation, mainly bioethanol and biodiesel, or second generation, which cover a variety of technologies currently in the pipeline.

In the wake of environmental concerns and volatility of oil supply the development of alternative fuels is crucial to the future of the transport sector. This need has been widely recognized by policy makers and resulted in the introduction of European Biofuel Directive and other relevant Directives in the EU and Worldwide.

Liquid fuels derived from oil supply 96 percent of the nation's transportation sector. This reliance, together with growing global demand for oil, the concentration of oil reserves in the Middle East and greenhouse gas emissions from oil production and consumption, raise significant geopolitical and environmental concerns about oil dependence.

Conversion of renewable sources of energy to liquid fuels provides an avenue to address these concerns. However, today's technology for converting biomass to liquid fuels is not scalable to amounts sufficient to displace a large fraction of oil consumption. Currently, ethanol made from corn is a renewable resource used to produce liquid fuels in the United States, but this new market and the associated increases in demand for corn have already significantly affected food prices and agricultural practices.

Producing alternatives to gasoline from other types of biomass, alternative crops, switchgrass and agricultural waste, for example, would eliminate "food vs. fuel" concerns. However, economic, scalable and sustainable production of ethanol from cellulose is difficult and expensive. It requires focused, multidisciplinary research involving biochemistry, genetics, enzymes and catalysts to identify the best plant candidates, enhance plant growth, improve biomass fermentation and optimize biofuels processing. As with other renewables, the dynamics of the natural resource base, including the effects of droughts, floods, and infestations, must be taken into account to ensure that biofuels represent robust, sustainable, affordable and environmentally benign supplies of energy. Land use, water use and biodiversity are examples of important issues for biofuels production at a scale that materially reduces petroleum use and carbon dioxide emissions.

Not a new technology

Biofuels are not a recent invention and have a long history in the motor industry, stretching right back to the development of the internal combustion engines of the 1800s. Rudolf Diesel designed his compression engine to run on peanut oil, while Nicolaus Otto's pioneering spark-ignition engine was developed to run on ethanol.

The case for bioethanol was in particular championed by Henry Ford who had a vision to "*build a vehicle affordable to the working family and powered by a fuel that would boost the rural farm economy*". He himself also owned an ethanol plant and his famous mass produced Model T Ford first ran on bioethanol.

First generation biofuels

The two most common 1st generation biofuels are bioethanol from starch or sugar crops and biodiesel from oil-rich plants. As these fuels are primarily derived from crops which may also be used as food for animals and humans, these type of fuels have been criticised for diverting food away from the human food chain to the engine. Biofuels' impact on the environment and food prices and sustainability of production are often scrutinized in mass media.

Research in the field suggests however, that whilst still depending on method of production, the overall green house gas emission savings are positive and in some cases zero carbon production is possible as carbon emitted during the burning of biofuels is compensated by the carbon absorbed by the plants as they grow.

Impact on biodiversity may be negative, however, in many cases the cultivation of biofuels may enhance biodiversity, especially if underutilized agricultural land is used for production. Biofuels will only negatively impact food markets if they compete for land with the agricultural sector.

Second generation biofuels

Second generation biofuels are broadly speaking the biofuel technologies still in the pipeline. It is these sustainable biofuels that will provide the source for the future. Some of the new technologies focus on increasing yields from plant-derived fuels; others look at the application of microbiological research to improve energy efficiency and range of renewable feedstocks for biofuel production.

The Biofuel Research Centre focuses on 2nd Generation biofuels produced by microbial fermentation of non-food crops. These carbon-rich lignocellulosic materials are renewable and widely available. Sources include agricultural waste such as corn stover, straw and bagasse ; industrial waste such as sawdust and paper pulp; woody biomass from forestry; municipal solid waste including household food and garden waste and paper products; and specific non-food energy crops such as switchgrass. Conversion of lignocellulosic biomass is attractive and even more so if biomass which is otherwise regarded as waste can be used as the substrate. In this case, the overall energy yield and carbon footprint will be improved compared to first generation biofuels as there is no need to cultivate and harvest the crops. How we best harness this energy stored within lignocelluloses may provide the solution for biofuels in the future.

Microbial biofuels

Microorganisms convert biomassintochemicals that can be used astransport biofuels.Production of fuel by microbesis not new - mankind has been fermenting and distilling alcohol for millennia; the only difference is that the alcohol is destined for use incombustion engines rather than human consumption.Microbial production of methane, ethanol and butanol have been around for many years, while biohydrogen and oil from algaeremain for future development.

- Methane is produced by anaerobic digestion of waste organic materials. Its use as a fuel is limited with mostused inCombined Heat and Power (CHP) systems.

- Biohydrogenis used in hydrogen fuel cells to generate electricity. Currentproduction is limited by thecost of reactorsrequired forphotochemical synthesis and the low yields from anaerobic fermentation.

- Oil from microalgae is a new technology. Over 40% of the mass of some microalgaeis oil which can be extracted and used as biodiesel. The biggest challenge to oilgae is to reduce the cost of cultivation and improve the biology of oil production.

- Bioethanol is currently produced by yeast (*Saccharomyces cerevisiae*) fermentation of sugar and starch crops. Any move away from food crops is limited by the sugars that can be used by yeast. Glucose is the preferred substrate and conversion of othercarbohydrates such as those in lignocellulose into ethanol requires genetic engineering of novel yeastand bacterial strains.

- Biobutanol is now recognised as a superior biofuel to ethanol. In addition to the beneficial fuel properties, the solventogenic clostridia used inthefermentation alsohave an inherent advantage. Clostridiacan utilise a wide variety of carbohydrates, allowing all the carbohydrates, in addition to glucose, inbiomassto be converted to fuel.

Biomass

One of the main costs in biofuel production, in terms of both economics and environment, is thebiomass feedstock. The choice offeedstock iscentral to the controversy surrounding biofuels today, withcurrenttechnologies associated with the useof food as fuel and largescale changes in land usage.For biofuels to have any meaningful impact on energy, biomass feedstocks must be widely available atlow costand without negative environmental impact.Lignocellulose - the non-food componentof plants, fits this description.Future technologies are based on harnessing the energy locked within lignocellulose, converting the carbohydratecomponents to biofuel.

Lignocellulose biomass includes:

- Agricultural waste such as straw, corn stover and bagasse.

- Industrial waste such as sawdust and paper pulp.

- Woody biomass from forestry.

- Municipal solid waste including food and garden waste and paper products.

- Specific non-food energy crops such as switchgrass.

Conversion to biofuel first requires hydrolysis of the biomass to yield a fermentable susbtrate. This step often involvesa combinationofphysical, chemical and enzymatic treatments.Complete hydrolysis of the polysaccharide polymers yields glucose from cellulose and a complex mixture of sugars from hemicellulose including pentoses (xylose and arabinose) and hexoses (glucose, mannose, galactose and rhamnose).The second step isto ferment the hydrolysate to biofuel.In order for a lignocellulose process to be efficient, it is desirable for the microbes to utilise all of thecarbohydrates. It is here that solventogenic clostridia havethe advantage,enabling complete conversion of lignocellulosic sugarsto butanol and co-products.

Enoil BioEnergy Research Laboratories

Biofuels research is one of the most dynamic areas within biotechnology. As the well-established “First generation biofuels” compete with food production it is necessary to search for alternative feedstocks and new techniques.

Biofuel production often requires instruments capable of operating at or beyond the limits of traditional bioprocesses. This may include high temperatures, anaerobic conditions, and/or multi-step procedures. Conversion of complex structures typically requires a pre-treatment and enzyme-catalyzed degradation to simple sugars suitable as substrates for fermentation.

Scientists at the Enoil BioEnergy Research Laboratories (EBERL) conduct research in a variety of areas in bioenergy development. The EBERL program focuses on a thorough, systems-level understanding of bacterial and yeast metabolism, gene regulation, and stress response for elucidating principles to help rationally engineer bacteria and yeasts for improved biofuel production.

In order to accomplish their goals and objectives, researchers in the EBERL program will implement automated processes, including the use of an integrated parallel bioreactor system and automated bioreactor sampling system, to conduct experiments for optimizing yeast strain characterization and selection. Incorporating tools such as parallel bioreactor systems and automated bioreactor sampling technologies can significantly reduce project timelines and increase the efficiency of the microbial strain characterization and selection process.

Coupling Parallel Bioreactors and Seg-Flow automated on-line sampling technologies enabled our research staff to implement remote-controlled, automated process trigger sampling as an integral part of its yeast strain characterization activities.

By integrating this functionality into their high-throughput screening and selection process, EBERL research scientists are better able to rapidly characterize process events, parameters and stress responses that impact yeast strain gene regulation and, ultimately, biofuel productivity.

In Enoil BioEnergy Research Laboratories (EBERL) our researchers are working to better overcome the physical, chemical, and biological barriers to liberating sugars from energy crops and converting them into such biofuels as ethanol, biodiesel, or hydrogen.

Ongoing Research and Research Programs at the Enoil BioEnergy Research Laboratories (EBERL)

- H_2O-CO_2 Pretreatment
- Enzyme discovery and characterization
- Fermentation
- Systems Biology
- Composting and Molecular Ecology
- Engineer bacterias
- Nanoscale enzyme imaging
- Biomass characterization and analytical systems
- Industrial Ecology

The ultimate goal for the Enoil BioEnergy Research Laboratories (EBERL) is to provide the fundamental science to underpin a cost-effective, advanced biofuels industry. Using systems biology approaches, the Enoil BioEnergy Research Laboratories (EBERL) are focusing on new strategies to reduce the impact of key cost-driving processes in the overall production of cost competitive biofuels from biomass. For these biofuels to be adopted on a large scale, they must represent environmentally sustainable and economically competitive alternatives to existing fuel systems. New strategies and findings emanating from the centers' fundamental research are addressing challenges for cost-effective advanced biofuels production:

- Designing synthetic biological systems to produce renewable chemicals and fuels
- Combustion; advanced energy conversion systems; environmental emissions control; algae-based biofuels; conventional biofuels
- Biochemical, molecular and ecological analysis of phototosynthetic algae. Emphasis on chloroplast evolution, photosynthetic processes, and harmful algal bloom formation, population diversity, lipid biogenesis and biofuel production.
- Combustion Characteristics of Bio-Derived Fuels for Aerospace, Power Generation, and other Transport Applications
- Fractionation of lignocellulosic biomass and bioconversion natural products of into biofuels(bioethanol and biodiesel) and biochemicals (sugar alcohols, glycols).
- Microreactors for biomasss to biofuel conversion; Nanoporous materials for energy application; large-scale energy storage
- Advanced Biofuels and Co-Products from Mixed Feedstocks
- Characterization of novel microbial strains for biofuel production, development of optimized biomass, and the use of symbiotic microorganisms for enhanced plant growth in a sustainable manner.
- Develop next-generation bioenergy crops by unraveling the biology of plant development.
- Discover and design enzymes and microbes with novel biomass-degrading capabilities.
- Develop transformational microbe-mediated strategies for advanced biofuels production.

Patents, innovation and applications

We're engaged in fundamental scientific research to develop alternative, renewable fuels and bio-chemicals from biomass to reduce fossil fuel dependence and preserve the environment. Enoil BioEnergy Research Laboratories provides a unique intellectual and physical framework where investigators are addressing several key renewable energy and raw materials challenges while addressing an obligation to train tomorrow's biorenewable workforce.

- Enoil BioEnergy Research Laboratories brings each research project to a point where an entrusted innovator – entrepreneurs and corporations – with savvy and resources can transform discoveries into products to address economic and environmental concerns.

- Enoil BioEnergy Research Laboratories investigators with access to more than 2,000 m^2 of laboratory space have access to state-of-the art, in many cases customized, equipment supported by powerful computing capabilities to collect, analyze and manage data.

- Enoil BioEnergy Research Laboratories investigators are well-recognized experts in a broad range of disciplines.

- Enoil BioEnergy Research Laboratories investigators collaborate along an intellectual continuum that reflects the multifaceted challenges faced in biorenewable fuels and bio-chemicals research.

- Enoil BioEnergy Research Laboratories is vertically integrated to focus on the major challenges of biorenewable research: biomass feedstock development; biological and chemical conversion process development; and economic and environmental impacts.

Patents pending and licensing

Bringing biofuels and other bioproducts to market requires expertise in licensing, marketing, derisking, logistics and supply chain management, as well as process scale-up. Enoil BioEnergy Research Laboratories works with entrepreneurs and established business to successfully navigate the commercialization process.

Innovations

Innovative reactor set to harvest alternative fuel is a reactor that will be able to produce the next generation of alternative fuels.

A new biofuel production process created by Enoil BioEnergy Research Laboratories investigators produces more than 20 times the energy of existing methods. A novel way to use microbes to produce biofuel and hydrogen, all while consuming agricultural wastes.

Compared with petroleum, biomass raw materials are inexpensive. Much of the raw materials are now considered waste -- stems and stalks left over after plants are harvested. But the processing costs for turning plant materials into ethanol, biodiesel and other biochemicals traditionally have been much higher than the costs for turning crude oil into gasoline and diesel fuel. This has been a longstanding roadblock for widespread acceptance of biofuels. When gasoline was cheap and plentiful, not many people wanted to pay more for ethanol, despite its many environmental benefits. Today through an Enoil BioEnergy Research Laboratories innovative production process will be possible to obtain with competitive costs biofuels, biodiesel, biojet fuels and other biochemicals.

A new production process created by Enoil BioEnergy Research Laboratories investigators allow to produce biochemicals using multy waste sources.

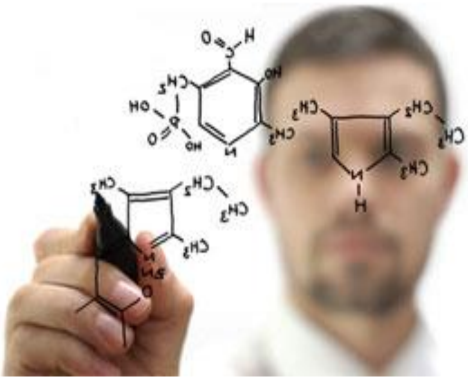
Engineering the biofuels of the future. One aspect of our research includes cold flow properties. A problem with traditional biodiesel fuels is their behavior at low temperatures: they tend to gel and turn solid. This means that traditional biodiesel fuels cannot be used in many countries during a large portion of the year. Today through an Enoil BioEnergy Research Laboratories innovative production process will be possible to obtain with competitive costs stable biofuels, stable biodiesel, and biojet fuels and other biochemicals which could be used to low temperatures too.

Another project uses computer simulations to predict vapor pressures and densities of various biocompounds. Vapor pressure and density are important at engine startup. We are going to develop a method to understand how to predict both of these factors in any given compound.

Enoil BioEnergy Research Laboratories investigates how the algae used to treat waste water can be converted into viable biofuels. Algae could be an ideal source of biofuel. It grows in water and thus does not displace food crops or increase the cost of food. Little energy is needed to grow these microscopic plants and minimal infrastructure would be needed to harvest them. Also, algae contains less cellulose than terrestrial plants, which means that less energy would be needed to break down the biomass to produce fuel.

Enoil BioEnergy Research Laboratories is working to grow algal species that are easy to harvest and that store higher levels of oil in their cells. This “ecological engineering” can tailor algal fuel sources that are maximally productive for different climates and nutrient levels.

Enoil BioEnergy Research Laboratories investigators are collaborating to develop techniques to improve the production of liquid transportation fuels using microalgae. The researchers are identifying and developing microalgal strains that can be harvested economically and processed into finished transportation fuels. This basic research will answer fundamental algal biology questions regarding oil production that could lead to the development of cost-effective, algal-based jet fuel. Researchers are combining a high-throughput, robotics-based platform with two advanced chemical characterization techniques to characterize lipid production by algal species. The goal is to provide spectroscopic data for analysis.



Patents: Right of First Refusal Agreement

ENOIL BIOENERGY SA (Enoil BioEnergy Research Laboratories) has a Right of First Refusal with ENOIL GROUP SA which generally licence the patents to ENOIL REFINERIES which the Group is developing worldwide through several JVs and Partnership Agreements.

Patent Licensing

Patent licensing may come about in different ways, and patent licenses can be classified as exclusive or non-exclusive.

Patent Licenses

A patent grants its owner the right to exclude others from practicing the patented invention, and it does not give the patent owner the right to practice the patented invention. Licenses should be understood in this context.

- Exclusive license: Under an exclusive license, a patent owner transfers all indicia of ownership to the licensee only retaining the title to the patent. From the point of view of the patent owner, he surrenders all rights under the patent (including the right to sue for infringement and the right to license) to the licensee. In essence, the licensee steps into the shoes of the patent owner and acquires the right to sub-license the patent and sue for patent infringement. However, the exclusivity can be limited by a field of use. That means that the licensee gets a promise from the patent owner that the patent will not be licensed to anyone else in a stipulated field of use.

- Non-exclusive license: By granting a non-exclusive license, the patent owner essentially promises not to sue the licensee for patent infringement. Some people think that by acquiring a non-exclusive license the licensee acquires the freedom to operate in the space protected by the licensed patent, but this may or may not be the case. It depends on whether or not the licensee's products infringe other patents.

Advance in royalty fees: For exclusive license agreements, it is very common that the licensee would pay an advance toward the first year's royalty.

Yearly minimum royalty or performance requirements: This is either a yearly minimum payment or a yearly unit volume that the licensee must hit to retain exclusivity. The amount usually gets larger every year to allow for the licensee to ramp-up and distribute your product. Also, it provides the inventor with assurance that the licensee will seek to develop your invention and not just let it sit dormant.

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