Presidential Green Chemistry Challenge
US Environmental Protection Agency
c/o Dr. Carol Farris
EPA East, Room 5133
1201 Constitution Avenue, NW
Washington, DC 20004

December 28th, 2011

Dear Dr. Farris,

On behalf of DuPont, I respectfully submit this nomination for your consideration under the 2012 Presidential Green Chemistry Challenge Award Program. An electronic copy of this nomination under the file name "2012 Presidential Green Chemistry Challenge Award Nomination DuPont Cellulosic Ethanol.pdf" was emailed to greenchemistry@epa.gov on December 28th, 2011. The main contact information for this nomination is included below:

Development of a Commercially Viable Integrated Cellulosic Ethanol Production Process

Nomination Date: December 28th, 20111

Primary Sponsor: DuPont Company
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Wilmington, DE 19898

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We look forward to your response to this nomination and please don't hesitate to call or contact me if you need additional information in support of this nomination.

Sincerely yours,

William D. Provine, Ph.D.
Science Director – Central Research & Development
DuPont Company

Project Title: Development of a Commercially Viable Integrated Cellulosic Ethanol Production Process

Recent Milestone: DuPont started up in 2009 and has successfully operated since then a 250,000 gallon per year cellulosic ethanol demonstration facility in Vonore, TN. At this demonstration facility, we are validating and optimizing all components of a novel cellulosic ethanol technology at a pre-commercial scale and developing design data for the first commercial facility (nameplate capacity of 27.5 million gallons per year of cellulosic ethanol) that is planned to be constructed in Nevada, IA where DuPont purchased land in 2011 in anticipation of a 2014 plant start-up.

Focus Area: Focus Area 1 – Technology that uses Greener Synthetic Pathways.

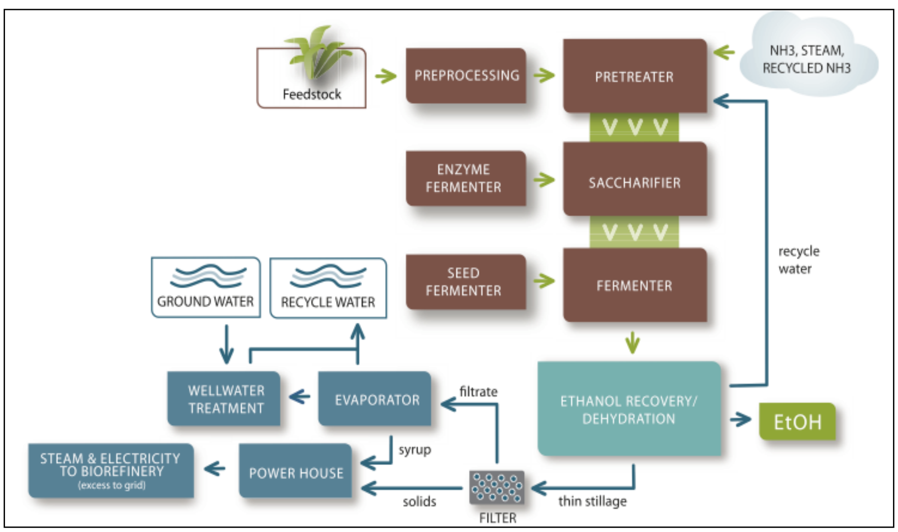
US Component: The key component technologies in our nominated technology were developed in R&D labs at DuPont Experimental Station, located in Wilmington, Delaware, and at DuPont's Genencor facilities in Palo Alto, California, partly funded by grants from the US Department of Energy. Furthermore, the technology is currently being piloted at our demonstration facility in Vonore, TN and the first plant is anticipated to be in Nevada, IA. The technology will be extensively licensed to other parties throughout the United States thereafter in order to maximize commercial impact.

Abstract: DuPont has developed and scaled up an integrated biochemical process for the production of ethanol from non-food lignocellulosic biomass, such as corn stover. Our process is a novel integrated pathway, with three major technology components, for the production of ethanol at sufficiently high yields and titers to achieve commercially viable economics. To optimize the process it was necessary to consider and innovate all three conversion steps holistically. First, a novel dilute ammonia biomass pretreatment process decouples the carbohydrate polymers from the lignin matrix with minimal formation of compounds which inhibit subsequent fermentation, thus eliminating the need for costly "detoxification" steps which are common in other cellulosic ethanol technologies. Next, an enzymatic hydrolysis step uses a novel suite of high performance enzymes to depolymerize and hydrolyze both cellulose and hemicellulose to high titers of fermentable sugars in a single sugar stream. Thirdly, we integrated and optimized the metabolic pathways of a recombinant bacterium, *Zymomonas mobilis*, to simultaneously metabolize both 6-carbon (glucose) and 5-carbon (xylose) sugars to efficiently produce ethanol at high yields and titers from the "raw" hydrolysate. This unique integration of three technology components enables a very efficient, "clean" flowsheet with minimal steps, a reduced environmental footprint, and reduced cost and capital versus other known cellulosic ethanol processes. We have achieved commercially viable ethanol yields of > 80 gallons/US ton of biomass and ethanol titers in excess of 80 g/L consistently at the semi-works (200 L) scale. In our 250,000 gallon per year demonstration facility in Vonore, TN yields of > 70 gallons/US ton of biomass and ethanol titers in excess of 70 g/L have been demonstrated. The performance achieved by this unit has enabled the announcement of plans to construct the first commercial facility in Nevada, IA using corn stover biomass for commercialization in 2014. Comprehensive "Well-to-Wheel" Life Cycle Analyses shows that our combined process has the potential to achieve more than a 100% reduction in greenhouse gas (GHG) emissions compared to gasoline, which is substantially better than current grain based ethanol GHG performance.

Science and Innovation

DuPont has made a major commitment to the development of renewable, sustainably sourced fuels, chemicals and materials. In biofuels, two parallel programs are underway to commercialize cellulosic ethanol and biobutanol (www.DuPont.com/BioFuels). This nomination describes the integration of chemistry, biology and process engineering to develop a commercially viable, scalable technology platform for the production of cellulosic sugar and its conversion to ethanol. The work required the integration of a novel pretreatment process, the development of improved enzymes for hydrolysis, and the genetic engineering of a novel, highly efficient fermentation host (www.DuPont.com/Cellulosic_Ethanol). The result is a high yield, scalable, commercially viable process for the production of fuel ethanol from lignocellulosic biomass, such as corn stover and other non-food materials. Ethanol produced using this novel technology, when used to replace gasoline, represents a net 'sink' in greenhouse gas emissions (greater than 100% reduction) per unit of energy, which is a major step forward in the transition to a sustainable, renewable economy. This technology has been validated at a demonstration plant in Vonore, TN with the first commercial scale deployment in Nevada, IA in 2014.

The production of cellulosic ethanol is considerably more complex than the well established process to produce ethanol from starches, such as corn grain. There are three main components to the linocellulosic biomass - cellulose, hemicellulose and lignin. Converting these to ethanol requires disrupting the lignin to provide access to the carbohydrates, removing organic acids linked onto the hemicellulose, depolymerizing the cellulose and hemicellulose to fermentable monomer sugars, and fermenting both the 5-carbon and 6-carbon sugars to ethanol. The nominated technology represents the development of a unique integrated pathway for this conversion, which incorporates a relatively mild alkaline pretreatment to disrupt the lignin and de-esterify the hemicellulose, followed by enzymatic hydrolysis of both the hemicellulose and cellulose and finally fermentation of glucose, xylose and arabinose to ethanol in a single step with a single organism. The integrated process is illustrated schematically in Figure 1.



Scalable, economical ethanol production requires accomplishing these steps at high yields and concentrations in a low capital process, with minimal non-renewable energy requirements. Our integrated process capitalizes on the inherent advantages of each step. The process steps are described in detail below.

Figure 1: DuPont Cellulosic Ethanol Process

Pretreatment

Pretreatment is used to increase the reactivity of the carbohydrate polymers in the plant cell wall to the action of hydrolytic enzymes. In particular, hemicellulose is a mixed sugar polymer that is extensively acylated and also linked to the non-carbohydrate polymer, lignin through ester bonds.

The chosen pretreatment for this process uses ammonia and steam at moderate temperatures[1,2] to remove side chains such as acetic, ferrulic and coumeric esters, and to disrupt the lignin matrix structure to provide better enzymatic access for the subsequent hydrolysis step, while leaving the carbohydrate polymer intact, as shown in Figure 2.

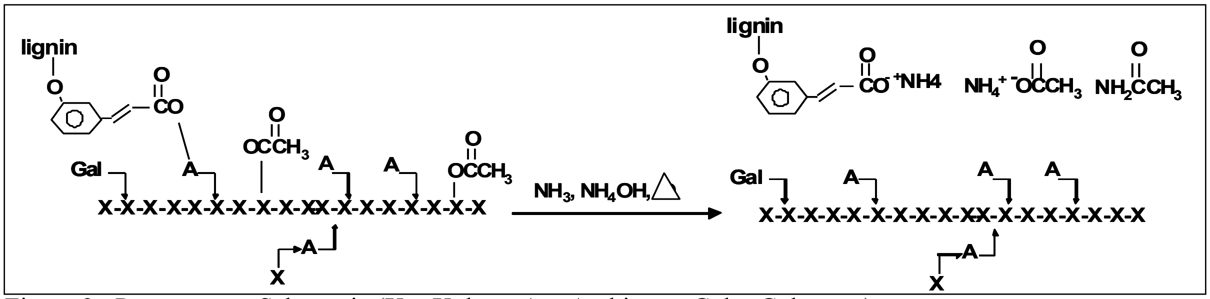


Figure 2: Pretreatment Schematic (X = Xylose, A = Arabinose, Gal = Galactose)

The process can be run at high solids (up to 70% biomass), typically requiring less than 10% ammonia by weight per unit of biomass, and the unreacted ammonia is readily recycled via a simple flash. Acetic acid is an unwanted by-product of pretreatment which is inhibitory to subsequent fermentation. The incorporation of ammonia results in a fraction of the acetyl groups being converted instead to acetamide, which is non-inhibitory, and reduces the amount of acetic acid produced by roughly 50%. By allowing us to operate at high solids and reducing or eliminating the production of inhibitory by-products, the dilute ammonia pretreatment of the nominated technology results in a significant reduction of the energy consumption, cost and capital requirements compared to competitive pretreatment processes using dilute acid.

Enzymatic Hydrolysis

The ammonia pretreatment process leaves most of the cellulose and hemicelluloses intact. Efficient utilization of both cellulose and hemicellulose requires a unique stoichiometry and combination of enzymes that do not exist together either in nature or in any single commercial product. Development of such a mixture required optimization of the cellulose and hemicellulose degrading components of our process enzymes.

As illustrated in Figure 3, there are at least six different classes of activities required for efficient degradation of cellulose and hemicellulose. They are the depolymerizing endoglucanases, two types of exoglucanases that act on the ends of the cellulose polymer, the beta-glucosidases that produce glucose monomers from oligosaccharides, and a variety of molecules whose synergistic contributions to cellulose degradation are not completely understood. Hemicellulose is a hetero-polymer composed of at least three different carbohydrate monomers - xylose, glucose, and arabinose - making its degradation even more complex than cellulose. There are many classes of hemicellulose degrading enzymes and a variety of activities within those classes, including endo and exo enzymes, beta xylosidase activities and L arabinofuranosidase. Selecting the most efficient of each enzyme type and optimizing their ratios is critical to achieving the necessary high sugar yields at low enzyme dose.

We chose *H. jecorina* (Hj) as the starting point for developing an enzyme suite for hydrolysis of ammonia pretreated biomass, because it produces one of the most efficient cellulase systems known[3]. Our first task was to further increase the specific performance of the cellulase mixture. This was accomplished by determining which activities were limiting the mixtures performance and then using several approaches to mitigate those limitations. Using this kind of reasoning we chose several enzymes to be engineered for better performance and searched genomes for cellulases that would enhance the performance of the *H. jecorina* mixture.

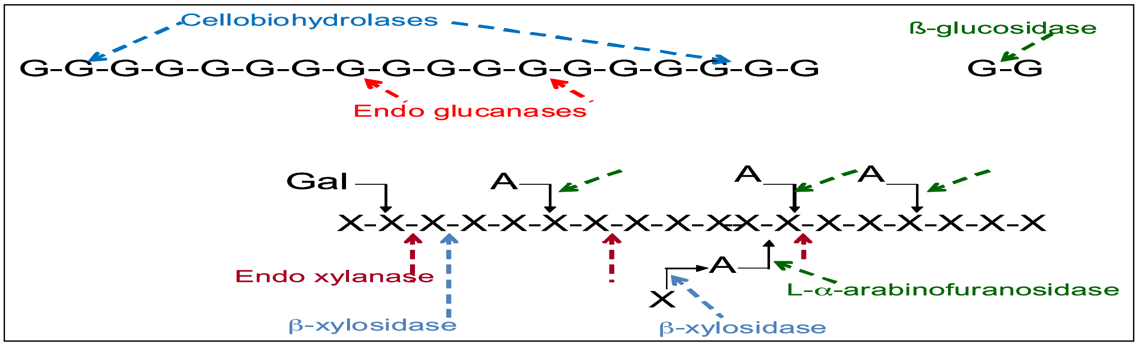


Figure 3: Key Enzyme Activities Required to Hydrolyze Cellulose and Hemicellulose (G = Glucose, X = Xylose, A = Arabinose, Gal = Galactose)

H. jecorina's genome is known to be deficient in hemicellulases. Therefore our next challenge was to increase the hemicellulose degrading capability of the Hj cellulase. Towards this end we screened hemicellulases from a variety of genomes, searching for enzymes that work together with the Hj cellulases to specifically degrade the hemicelluloses from ammonia pretreated biomass. Incorporating these enzymes resulted in a near doubling of the amount of fermentable xylose released in biomass hydrolysis.

The result of this work is a mixture of enzymes with a combined specific performance on cellulose and hemicelluloses that has never been achieved before at industrial production scale. We have essentially doubled our ability to release xylose and significantly increased glucose release, while at the same time roughly halving the enzyme dose requirements; thereby cost effectively producing a mixed sugar stream which can be fermented to produce cellulosic ethanol.

Fermentation

To capitalize on the high titer mixed sugar stream produced by the pretreatment and enzymatic hydrolysis processes, the final component of the nominated technology is the development of a single ethanologen strain capable of simultaneously fermenting glucose and xylose at high yield and titer. This was accomplished starting with an organism, *Zymomonas mobilis*, known to efficiently ferment glucose to ethanol and to have high tolerance to ethanol, and adding the pathway for xylose, followed by metabolic engineering to optimize the xylose pathway. In summary, *Zymomonas mobilis* uses the Entner-Duoderoff pathway to convert glucose to pyruvate and then to ethanol, as shown in Figure 4. This pathway diverts a minimal amount of the carbon to biomass production (cell growth) and results in a high metabolic yield of ethanol.

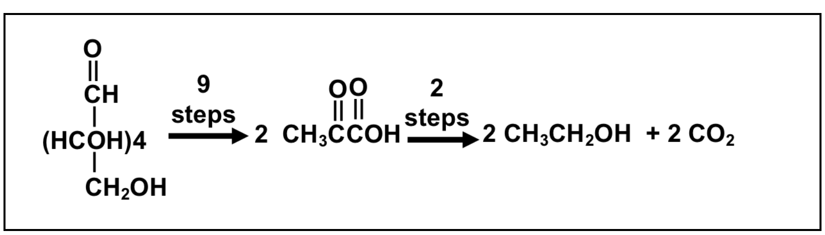


Figure 4: Conversion of Glucose to Ethanol via the Entner-Duoderoff pathway

As shown in Figure 5, a xylose-fermenting *Zymomonas mobilis* was then constructed using technology developed by the National Renewable Energy Laboratory (NREL) by integrating two operons, P_{gap}*xylAB* and P_{gap}*taltkt*, into the genome via sequential transposition events.

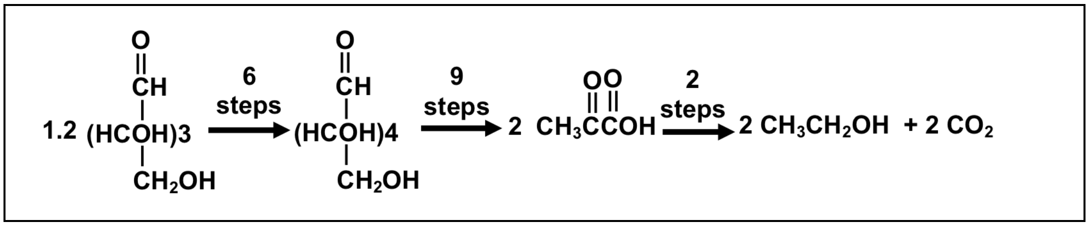


Figure 5: Conversion of Xylose to Ethanol in Recombinant Z*ymomonas mobilis*

While capable of using xylose, this first generation strain did not meet the ethanol production rate or titer required for the overall process. Knock out of the gene responsible for the formation of xylitol, an unwanted and inhibitory by-product, improved performance. The xylose conversion rate was further improved by sequentially increasing activity at two steps in the pentose metabolism pathway, leading to an organism in which the newly added xylose pathway functions at almost 50% of the endogenous rate of glucose conversion to ethanol. Adaptation of the strain for growth in the presence of inhibitors found in the hydrolysate from dilute ammonia pretreated corn cob also improved ethanol yield and titer. Patents on control of xylitol formation and on the use of a higher efficiency xylose isomerase gene have been issued, and patents have been filed on the effective, novel adaptation to ethanol production in process hydrolysate [4,5,6,7].

The recombinant *Zymomonas mobilis* strains derived from the metabolic pathway engineering and adaptation described above have demonstrated the ability to directly ferment process relevant hydrolysate in a single step, consuming over 98% of the starting monomer glucose, over 95% of the starting monomer xylose and producing ethanol at a rate of over 1.4 g/L/hr, achieving final ethanol titers in excess of 80 g/L at the 200L semi-works scale using corn cobs as a feedstock[8]. Most fermentation organisms are either incapable of co-fermenting glucose and xylose, requiring two separate fermentation operations, or do so inefficiently, leaving a significant fraction of the xylose unconverted to ethanol. By successfully integrating efficient metabolism of both C5 and C6 sugars, our technology significantly improves the yield, and therefore reduces the cost, of cellulosic ethanol compared to other fermentative approaches.

Human Health and Environmental Benefits

This technology enables the production of greater than 10 billion gallons per year of transportation fuel in the US from a renewable source and furthermore allows the use of non-food resources such as corn stover and switchgrass. In addition, because the biorefinery co-products can be used as fuels on and off-site and can thereby displace fossil fuels, this lignocellulosic technology creates a greenhouse gas (GHG) 'sink' (a net negative value for Well-to-Wheel (WTW) GHG emissions) compared to either gasoline or current grain ethanol technology.

Life Cycle Assessment (LCA) was integrated with process development early in DuPont's biofuel research and development programs. The LCA methodologies used are in accordance with the series 14040 ISO standards for LCA[9]. We have completed comprehensive WTW LCA studies, including the impact of producing the biomass, transportation of the biomass to the conversion facility, the conversion process, and transportation from the conversion facility. In partnership with Michigan State University, a rigorous LCA of the corn grain and stover production and harvest system was completed and the results are published in the International Journal of Life Cycle Assessment[10].

The GHG footprint of gasoline has been well studied, and the US EPA reports WTW GHG emissions of about 93 g CO_2 eq/MJ[11]. Ethanol derived from corn grain is another major incumbent fuel in the US. There is a wide range of WTW GHG emission results reported by various sources for dry grind corn grain ethanol, with literature values ranging from 30 to 98 g CO_2 eq/MJ[12,13]. By contrast, the DuPont integrated cellulosic ethanol conversion technology has been modeled to produce ethanol with WTW GHG emissions of -17 g CO_2 eq/MJ[14]. The net negative GHG emissions are a result of using the biorefinery co-products as fuels. The syrup co-product is used in the onsite boiler to produce the steam needed to run the conversion process. The nominated technology can operate at moderate temperatures and produces ethanol at high titers, reducing the energy required for product recovery and purification. The steam requirements to produce ethanol are less than the energy available from burning the residual syrup co-product. This excess steam can be exported to displace steam based on a fossil fuel such as natural gas. In addition, in commercial production the solid co-product from the biorefinery, lignin filter cake, will also be used as fuel for power production, on-site or externally, thereby displacing the fossil fuel coal.

Figure 6 compares the GHG footprint of the new cellulosic ethanol technology to those of gasoline and dry grind ethanol. In the cellulosic ethanol case, the credits for the co-products are shown in the stacked bar chart below the 0 line. These displacement credits from off-setting the use of fossil fuels represent a significant reduction in overall GHG emissions. On a MJ basis, more lignin filter cake is made in the cellulosic ethanol process than excess steam. Additionally, on a MJ basis, displacing coal will have more GHG benefits than displacing natural gas. This cellulosic ethanol technology shows the potential to be significantly better than both gasoline and corn grain ethanol, even when compared to the optimistic GHG emission value of 30 g CO_2 eq/MJ for a dry grind corn ethanol process

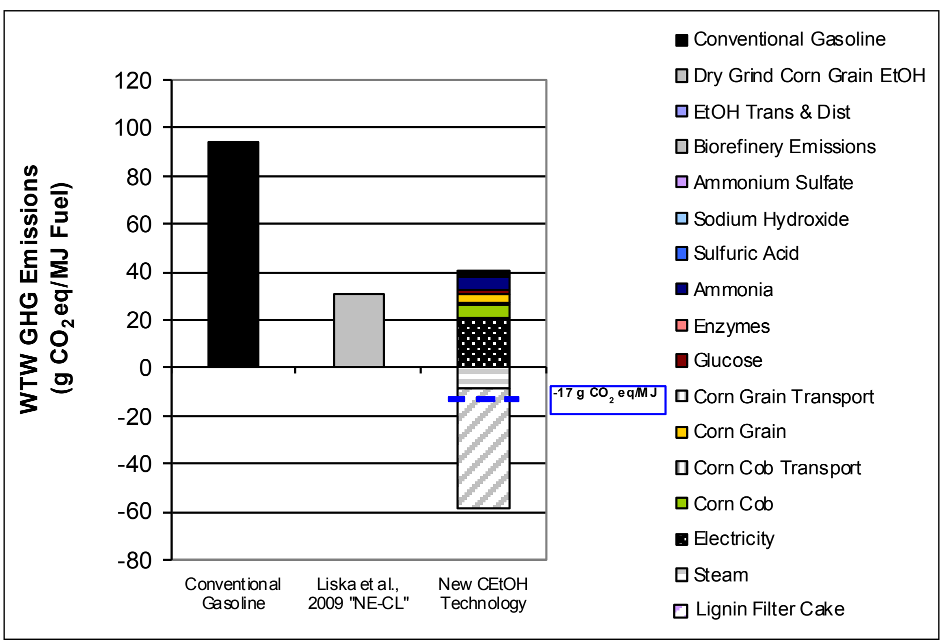


Figure 6: DuPont Cellulosic Ethanol Technology WTW GHG Emissions Compared to Gasoline and Dry Grind Corn Grain Ethanol

Applicability and Impact

Developing sustainable solutions to our energy needs is a major priority for the United States and the world. The ability to produce liquid transportation fuels from renewable resources such as lignocellulosic biomass is one key component of a sustainable future. Under the Renewable Fuels Standard (RFS), the US is targeting the use of 21 billion gallons of advanced biofuels by 2022, of which 16 billion gallons is anticipated to be cellulosic biofuels.

The nominated technology combines advances in three technology elements (pretreatment, hydrolysis and fermentation) and the synergy obtained from the integration enables the economic, scalable production of ethanol from biomass at high yields in a commercially relevant process. While the progress and achievements in all three aspects of this technology are in themselves significant, it is only by developing these in parallel in an integrated, holistic approach that a commercially viable solution can be brought to fruition. We have demonstrated the ability to reproducibly produce ethanol from corn cobs at a yield in excess of 80 gallons/dry US ton of biomass, leading to an expected cost of the ethanol produced of less than $2/gallon based on corn cob feedstock at $50/dry US ton. This cost basis will enable the US to meet its aspiration of 16 billion gallons per year production of cellulosic ethanol in US through DuPont owned or licensed production facilities.

This achievement provides a demonstration of the power of the creative combination of chemistry, biochemistry and metabolic engineering to deliver unique, valued solutions. This technology is soon to be extended to produce other biofuels, such as isobutanol, and other value added chemicals, from non-food feedstocks. Broad deployment of this breakthrough technology offers significant promise for sustainably supporting our energy needs for transportation while reducing the production of greenhouse gases.

References:

1. US Patent 7,781,191, "Treatment of Biomass to Obtain Target Chemical"
2. US Patent 7,819,976, "Biomass Treatment Method"
3. Lantz et al, Hypocrea jecorina CEL6A protein engineering. *Biotechnology for Biofuels* 2010, 3:20
4. US Patent 7,629,156 "Ethanol Production in Fermentation of Mixed Sugars Containing Xylose"
5. US Patent 7,741,084 "Ethanol Production using xylitol synthesis mutant of xylose-utilizing Xylose"
6. US Patent 7,741,119, "Xylitol synthesis mutant of xylose-utilizing Zymomonas for ethanol production"
7. US Patent 7,803,623, "Zymomonas with improved ethanol production in medium containing concentrated sugars and acetate"
8. Viitanen, P., "A xylose utilizing Zymomonas mobilis strain for ethanol production from high concentrations of mixed sugars", presented at the 31st Symposium on Biotechnology for Fuels & Chemicals, San Francisco, 2008
9. ISO 14040 92006), Environmental Management – Life Cycle Assessment – Principles & Framework, 2nd Edition 2006-07-01.
10. Kim, S., B.dale, and R. Jenkins, 2009. Life Cycle Assessment of Corn Grain and Corn Stover in the United States. The International Journal of Life Cycle Assessment 14: 160-174. Published on-line January 20, 2009, at http://www.springerlink.com/content/c220515747622673/fulltxt.pdf, assessed November 2009.
11. US Environmental Protection Agency (EPA), Renewable Fuel Standard Program (RFS2) Regulatory Impact Analysis, February 2010
12. CARB Staff Report: Proposed Regulation to Implementation the Low-Carbon Fuel Standard for California (LCFS) – Initial Statement of Reasons. Volume 1: Released March 5, 2009. Volume 2: Released March 5, 2009.
13. Liska et al., "Improvements in Life Cycle Energy Efficiency and Greenhouse Gas Emissions of Corn Ethanol" Journal of Industrial Ecology, Volume 13, Number 1, 2009.
14. Jenkins, R., Alles, C., Johnas, C., "Sustainable Solutions from Field to Fuel for Advanced Biofuel Production", Presented at the Sustainable Feedstocks for Advanced Biofuels, Soil and Water Conservation Society Workshop, September 28 – 30, 2010, Atlanta, GA.