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 and Butamax, I respectfully submit this nomination for the 2012 Presidential Green Chemistry Award Challenge Program. An electronic copy of this nomination entitled "2012 Presidential Green Chemistry Challenge Award Nomination DuPont Isobutanol.pdf" was emailed to greenchemistry@epa.gov on December 28th, 2011. The main contact information for this nomination is included below:

Production of Isobutanol from the Synergy of Metabolic and Process Engineering

Nomination Date:	December 28th, 2011
Primary Sponsor:	DuPont Company 1007 Market Street Wilmington, DE 19898
Contact Person:	William D. Provine, Ph. D. Science Director – BCS&E - BioFuels DuPont CR&D, Ex. Sta. 328/409 P. O. Box 80328, Wilmington, DE 19880-0328 phone: (302) 695-2201; fax: (302) 355-2041 email: william.d.provine@usa.dupont.com
Contributing Organization:	Butamax™ Advanced Biofuels, LLC. Route 141 & Henry Clay, P.O. Box 80356 Experimental Station – Bldg 356 Wilmington, DE 19880-0356

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: Production of Isobutanol from the Synergy of Metabolic and Process Engineering

Recent Milestone: DuPont in collaboration with Butamax™ Advanced BioFuels has developed an integrated fermentation and process engineering technology using a yeast biocatalyst to convert biomass to isobutanol at best-in-world rate, yield and titer at laboratory-scale. Our commercialization partner Butamax™ Advanced BioFuels has begun operation in 2011 of a demonstration plant for the isobutanol process technology located in Kingston upon Hull in the UK. We have also developed a foundational and sizeable patent portfolio including the issuance of key enabling patents (including US 7,851,188 and US 7,993,889, Fermentative Production of Four Carbon Alcohols, in 2010 and 2011, respectively).

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

US Component: The development efforts to create the isobutanologen strains with the integrated process technologies were undertaken at DuPont's R&D labs in Wilmington, Delaware.

Abstract: DuPont was the first to create and develop from renewable resources the integrated biological and process technologies for the commercially viable microbial production of isobutanol, an advanced biofuel with significant technical and commercial advantages over ethanol. Our strategy to accelerate to market and maximize impact from the technology in the United States is based upon retrofitting existing ethanol plants for isobutanol production using feedstocks currently employed by the ethanol industry, such as corn grain and sugarcane, as well as other next generation feedstocks such as lignocellulosic biomass and macroalgae (seaweed). The sugars from these feedstocks are fermented to isobutanol using a proprietary yeast strain which has been engineered with a novel biosynthetic pathway capable of converting glucose derived carbon from pyruvate to isobutanol. This yeast-based isobutanologen was selected because it is a drop-in biocatalyst suitable for retrofitted ethanol production plants. Key pathway enzymes were selected based on isobutanol-specific characteristics and cofactor requirements, and competing by-product reactions were eliminated to maintain flux through to isobutanol production. A major challenge in the commercialization of microbially produced isobutanol for fuel is related to the intolerance of the biocatalyst to commercially relevant aqueous titers of isobutanol. The nominated technology uses *in-situ* product removal methods to provide an efficient integrated process capable of minimizing production cost and environmental footprint. The yeast strain performance (rate, titer and yield) is significantly superior to that typically associated with traditional acetone-butanol-ethanol (ABE) fermentation and has met technical milestones demonstrating cost effective biological production of isobutanol for a variety of end-uses including chemical and fuel markets. Our technology facilitates the displacement of incumbent petroleum based routes of isobutanol synthesis and the direct replacement of refined gasoline from crude oil with a greener biotechnological route. Furthermore, it does so with multiple advantages over incumbent technologies by providing a significant reduction in greenhouse gas emissions, economic benefits at the local and national levels with the preservation and creation of new jobs, and increased national security through domestic drop-in fuel supply.

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 advanced biofuels such as cellulosic ethanol and biobutanol (www.DuPont.com/BioFuels). Our cellulosic ethanol program enables the cost effective conversion of non-food feedstocks to sugar then onwards to fuel and the biobutanol program converts sugar to an improved fuel molecule – isobutanol. DuPont and our joint venture (with BP Biofuels) Butamax™ Advanced Biofuels LLC (www.butamax.com) were the very first to identify and demonstrate biobutanol production via synthetic biochemical pathways including the description of a novel biochemical pathway to convert the C6-sugar glucose to isobutanol (2005).

During the seven year development period, we have established a foundational and sizeable patent portfolio with applications directed to technologies such as metabolic pathways, tolerance, protein engineering, and process engineering which will enable the low-cost practice of renewable isobutanol production[1-8]. Issued patents for key technologies include claims directed to microorganisms and methods employing the most viable metabolic pathway for the formation of isobutanol. DuPont's accomplishments are a significant step towards commercializing a green process that is intended to ultimately replace the current petrochemical-based butanol production process. To this end, DuPont formed with BP Biofuels the Butamax™ joint venture in 2009 to commercialize the technology and steward the commercial enterprise to market.

Biocatalyst Development

Our strategy for biocatalyst development uses an established commercial yeast fermentation host, the most appropriate drop-in biocatalyst for retrofitting current ethanol production facilities. The dry-mill industry currently exclusively uses yeast and yeast biology facilitates supply logistics. Using synthetic biology and metabolic engineering, we have created a novel isobutanol production pathway and a microbe that can ferment corn grain glucose (from starch) into isobutanol at a high rate, yield and titer. The enzymes required for the biochemical conversion of glucose to pyruvate to isobutanol in yeast are designated collectively as the isobutanol pathway (Figure 1).

This pathway is efficient with respect to the number of enzyme reaction steps and may be used with any feedstock that can provide carbon through glycolysis. Natural diversity provides enzymes with quantitative differences in their kinetic constants (typically exemplified *in vitro* as k_{cat}, K_M, K_I, etc.), and a key consideration is the choice of enzyme for each of the steps in the pathway. Our design criteria for selecting a combination of pathway enzymes included balancing expression and flux through the reaction steps to deliver a high isobutanol production rate and eliminating inhibitory intermediate metabolites. Key enzyme choices for the selected pathway include a feedback resistant acetolactate synthase (ALS), a highly active ketol-acid reductoisomerase (KARI), a dihydroxyacid dehydratase (DHAD) that is active in the cytosol, an α-ketoisovalerate-specific decarboxylase (KIVD), and an isobutyraldehyde-specific and feedback resistant branched chain alcohol dehydrogenase (ADH).

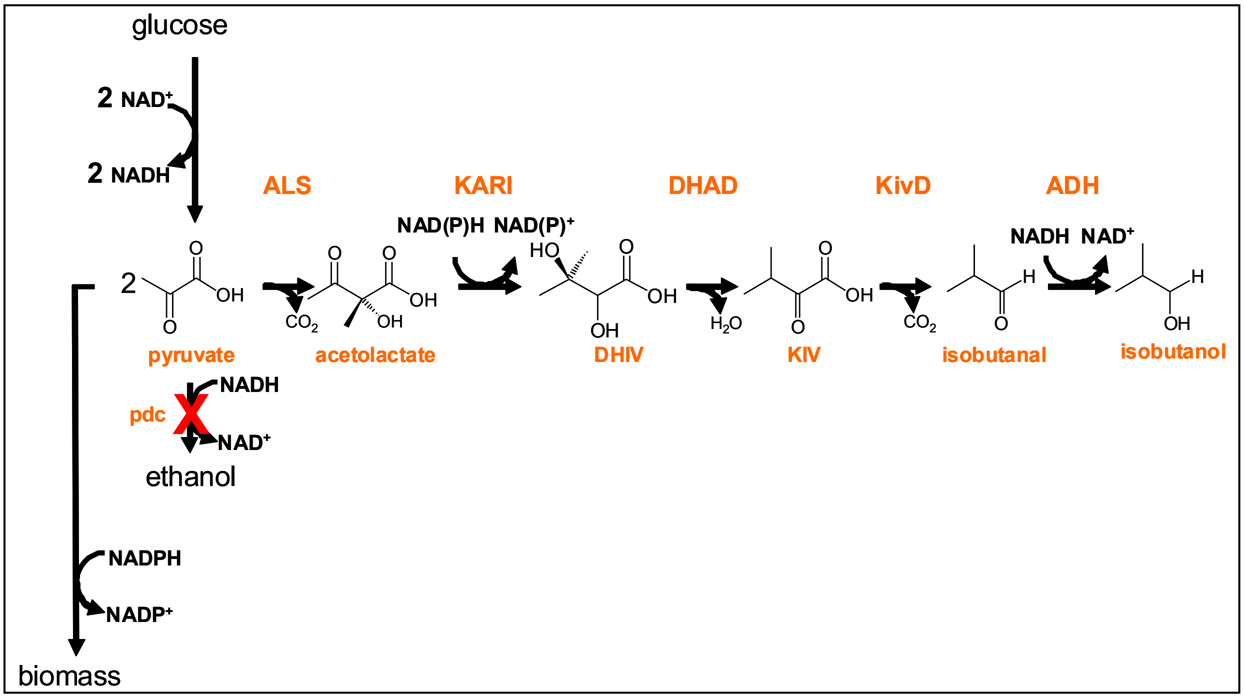

Figure 1: Isobutanol Pathway. Abbreviations: ALS, acetolactate synthase; KARI. ketol-acid reductoisomerase, DHAD, dihydroxyacid dehydratase; KivD, α-ketoisovalerate decarboxylase; ADH, alcohol dehydrogenase; DHIV, 2, 3-dihydroxyisovalerate; KIV, 2-ketoisovalerate; NAD+, nicotinamide adenine dinucleotide (oxidized); NADP+, nicotinamide adenine dinucleotide phosphate (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); NADPH, nicotinamide adenine dinucleotide phosphate (reduced).

A challenge to developing a high yield isobutanol production process was the need for a biocatalytic pathway from glucose to isobutanol that is redox-balanced under anaerobic fermentation conditions, as for ethanol fermentation. Glycolysis through the Embden-Meyerhoff pathway supplies two NADH reductant molecules, while the isobutanol pathway consumes two NADH (one in the conversion of acetolactate to 2, 3-dihydroxyisovalerate by KARI and the second in the terminal aldehyde to alcohol conversion by ADH). Natural diversity provided an effective NADH-dependent ADH, and the nucleotide cofactor preference of a KARI enzyme was changed from NADPH to NADH in order to establish a redox balanced pathway to achieve the yield goal. Also, DHAD requires loading of the enzyme with Fe-S cluster for proper activity. In yeast, Fe-S cluster biosynthesis is localized in the mitochondria. Thus, in order to maximize flux through a cytosolic engineered pathway in a eukaryotic host, we identified a bacterial enzyme that has high DHAD activity in the yeast cytosol.

Product Removal

A final challenge that had to be met was achieving a fermentative volumetric production of isobutanol comparable to the equivalent fermentative volumetric production of ethanol. This in turn translates into achieving effective concentrations of isobutanol in fermentation far in excess of the yeast half maximal inhibitory concentration (IC_{50}). We have met this challenge and the nominated technology maintains an aqueous concentration of isobutanol below a certain level while maintaining a high level of isobutanol specific productivity. In comparison to other existing technologies producing a dilute concentration of butanol and by-products (such as the ABE process), our technology achieves high effective isobutanol concentrations while eliminating the need for energy and material intensive separation and purification steps and allowing fermentation to be carried out at ambient temperature and pressure. *In-situ* product removal methods have been developed to address product inhibition by a range of chemicals produced by fermentation. Our technology optimizes strain performance and product removal, ultimately providing an efficient and integrated process for the production of isobutanol, in which the process energy is minimized. This solution can be fully integrated with any existing ethanol plant retrofitted for the production of isobutanol.

Human Health and Environmental Benefits

As an advanced biofuel, isobutanol can offer significant advantages over fossil-based fuels and ethanol, most notably including the ability to reduce Greenhouse Gases (GHG) compared to gasoline or traditional ABE fermentations. Life Cycle Assessment (LCA) is used to quantify the Well-to-Wheel (WTW) GHG emissions and fossil fuel energy consumption throughout the supply chain for producing and using isobutanol. The term WTW is used to describe impacts from the entire life cycle of a fuel, from extracting materials from the ground to combustion of the fuel in the vehicle. For biofuels, this includes everything from fertilizer production and tractor fuel to biorefinery process energy use, transportation of the fuel through distribution, and finally combustion emissions from the vehicle.

To benchmark isobutanol with existing technologies, GHG results are quantitatively compared to conventional gasoline[9] and traditional biological production of n-butanol using ABE technology. To fairly compare, inputs for the different fuel options need to be aligned in the LCA models, meaning that the same life cycle inventory data are used for all of the cases being compared. For the ABE and isobutanol models, the same data for corn, transportation, natural gas and electricity inputs were used.

As shown in Figure 2, process designs for producing renewable isobutanol are superior as compared to incumbent conventional gasoline and *n*-butanol from ABE in terms of WTW GHG emissions. The results compare relative GHG emissions based on emissions per energy content of the fuel. The isobutanol results shown are for a base case process which produces dried distillers grains as a byproduct and uses a natural gas fired boiler. The GHG emission results for isobutanol may be decreased further depending on the selection of process improvement options. Two ABE process options are shown in Figure 2. The “Current” result shows GHG emissions for a typical ABE process currently operating. The “ANL” result shows the GHG emissions for a future ABE process based on an Argonne National Laboratory[10] report detailing potential improvements to the ABE process. The nominated isobutanol technology reduces GHG impact by 40% to 75% as compared to gasoline and current ABE butanol production respectively. Due to its inherently lower vapor pressure, isobutanol also has a lower fugitive emission potential when compared to other existing fuels such as gasoline or ethanol.

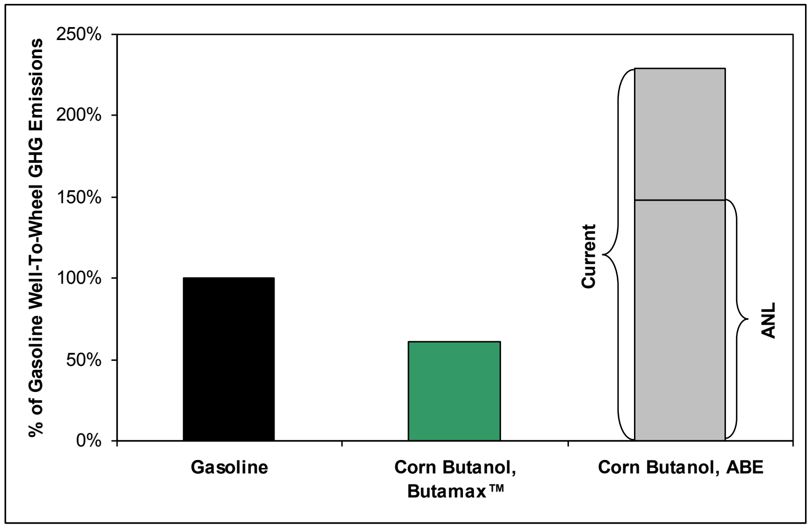

Figure 2: Corn Grain Biofuels GHG Emissions per Unit of Energy Content

Applicability and Impact

The world needs better biofuels. Specifically, biofuels that reduce greenhouse emissions, decrease reliance of foreign oil, benefit the economy, work as drop-in fuels in the current infrastructure in order to delay or bypass ethanol blend wall limits. They must perform on par with incumbent fuels and be available at large volumes at a price that is competitive with petroleum-based fuels. The nominated technology can produce such a biofuel, isobutanol, that can be blended for use in the current fleet at high levels, is able to be integrated into the existing petroleum and first generation biofuel infrastructure, and has a carbon footprint that will meet or exceed DOE's goal for significant reduction in GHG emissions. Butamax™ has closely examined the goals set by the 2007 Energy Independence Security Act (EISA) and has determined that isobutanol is a fuel that can achieve these goals and can support well over 10 billion gallons per year of production in the United States alone.

Isobutanol based biofuels are also expected to be economically viable based on superior attributes which include 1) higher energy density, 2) high octane number, 3) favorable distillation qualities, 4) low vapor pressure, 5) high compliance value in fuels, 6) materials compatibility because of reduced corrosivity, 7) low toxicity, 8) ease of transportation due to the ability to blend at the refinery, 9) lower hygroscopicity, and 10) greater compatibility with existing fuel infrastructure and engines.

Additionally, due to its lower oxygen content, isobutanol can be blended to a 16% volume versus the 10% limit for ethanol in gasoline under current oxygen limits. Moreover, Butamax sees potential to blend isobutanol at even much higher blend levels. The Renewable Fuel Standard (RFS) requires increasing the volume of renewable fuel in gasoline blends through 2022[11] and isobutanol will be a critical enabler to meet these national objectives.

The US currently imports approximately two-thirds of its petroleum from foreign sources, and the economic impact of importing this oil is enormous. Isobutanol production has the potential to utilize the starch from 4.2 billion bushels of corn grain while leaving behind a high value animal feed product, as well as the 1.3 billion tons of sustainable cellulosic biomass (USDA/DOE, 2005)[12] available annually, to provide domestically produced liquid transportation fuel. A 40 million gal/yr corn to isobutanol plant has the potential to displace 730,000 barrels of crude oil. A sugarcane butanol process will also produce excess electricity for the grid, and accounting for the excess electricity a 40 million gal/yr cane to isobutanol plant would displace 870,000 barrels of crude oil. An additional economic benefit is the creation of local farm community employment[13, 14].

Virtually all of the world's butanol supply is currently produced via petrochemical routes. The incumbent butanol production process, known as oxo-synthesis or hydroformylation, involves combining propylene (by-product of petroleum refining) with synthesis gas (CO and H_2) and a cobalt or rhodium catalyst at moderate temperatures and pressures (less than 100°C and less than 20 bar g), to produce a mixture of *n*-butanol and isobutanol isomers (Figure 3).

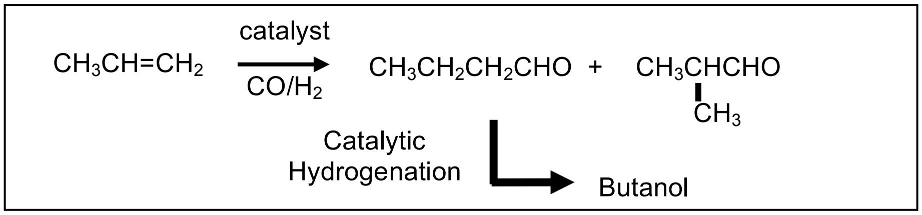

Figure 3. Butanol production process from petroleum.

The nominated process, a unique synergy generated as a result of DuPont's combined metabolic and process engineering approach, can produce isobutanol in a significantly more sustainable and environmentally friendly manner (ambient temperature and pressures) than the current petroleum-based technologies. The nominated technology for isobutanol production is also vastly superior to the *n*-butanol ABE process, which is no longer considered a commercially viable technology due to its high cost of manufacturing and undesired by-product of acetone. Our analysis indicates that the acetone generated from a single reasonably sized ABE process biofuel production facility would provide much of the needed global volume of acetone.

Butamax™ has constructed and is operating an isobutanol technology demonstration plant in Hull, UK (see inset, at right) to support scale-up and optimization of the commercial production design package. In addition, the nominated isobutanol process can be retrofitted to any existing ethanol plant, minimizing the capital investment required. On December 1st 2011, Butamax™ announced a major step forward toward first commercial production. Butamax™ has agreed commercialization principles with Highwater Ethanol (a leading ethanol producer located in Lamberton Minnesota) and intends to provide its technology for retrofit of Highwater Ethanol's 50 million gallon per year plant. Butamax™ has also announced its intentions to broadly license and make the technology available to the US market in an expedited manner through formation of an Early Adopters Club with Highwater Ethanol as its first member.

Another difference between our isobutanol effort and other drop-in fuel efforts is the fact that DuPont has parallel programs to develop processes that enable the conversion of cellulosic biomass as well as macroalgae (seaweed) to sugar and/or other intermediates and the conversion of those components into final products. The first product from such approaches is envisioned to be ethanol. The rationale for an ethanol first approach is to avoid taking on too many technical risks in any one program and to accelerate low-cost fuel products to market, but isobutanol from these other feedstocks is a clear goal and is enabled by the nominated isobutanol technology herein. Assuming these developments are successful, macroalgae aquafarming in our oceans has the potential to produce a feedstock at a cost in the same range as terrestrial-based substrates, while cellulosic feedstocks enable the use of non-food feedstocks for conversion to isobutanol. Macroalgal aquafarming may also be helpful in remediating coastal dead zones.

An additional difference between the DuPont effort and other firms who are in this space is our focus on fuels. Our focus on fuels mandates that the cost of manufacture becomes a critical consideration in all aspects of development. While we believe that biologically-derived chemicals are a crucial step forward to a more sustainable future, it will be the development of economically competitive biofuels that will drive significant environmental change.

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