Newsletter

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FROM THE TASK LEADER

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The opportunities for biofuels continue to increase. In late 2001, The European Commission proposed policies to significantly increase the use of alternative fuels in the EU countries (more detail included in the World Events/Information section). The proposals include mandates of minimum levels in alternative fuels in the transport fuel mix. The proposals also provide flexibility for European countries to adjust fuel taxation to accommodate this goal. Ethanol and biodiesel are expected to play a key role in this policy, particularly in the near-term.

This increased interest in biofuels is occurring throughout much of the world. The economic, environmental, and energy security benefits of bioenergy are increasingly appealing. As these benefits are being recognized by policy makers in many locations, the opportunities for biofuels continue to expand. Task 39 is dedicated to helping provide participating countries with the information and contact that will assist them in their deployment of biofuels.

EDITORS NOTES

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As I mentioned in the last newsletter, in an attempt to reduce the size of the newsletter and still cover the liquid biofuels area, I have decided to include just the headlines and links to the World Events/Information section.

I will be continuing to track down articles and sources from around the globe in the future. If you have any suggestions as to how you would like to see them organized or more importantly any links to share with the group please send them to me by email to the address at the top of this section.

TASK 39 WEBSITE

The Task 39 website is now officially online at the following address: http://www.forestry.ubc.ca/task39

Although the website is not complete we are hoping that it will give you a good idea of what we have in mind for both the website structure and informational content. Please feel free to send your comments or requests for added features of

content to me at the email address shown at the beginning of this section.

I am planning to continue to fill in more of the detail over the next couple of months. Please let me know if you feel there are particular links to other sites, reports, etc. that would be of interest to the general group, I am particularly interested in any links associated with any publically available work in your country or organization that would not generally be known to outside individuals.

WORLD EVENTS/INFORMATION

SPECIAL TOPIC - EUROPEAN COMMISSION PROPOSES TO ENCOURAGE ALTERNATIVE FUELS, STARTING WITH BIOFUELS

http://europa.eu.int/rapid/start/cgi/guestfr.ksh?p_action.gettxt=gt&doc=IP/01/1543|0|RAPID&lg=EN

Brussels, 7 November 2001

The European Commission has adopted an action plan and two proposals for Directives to foster the use of alternative fuels for transport, starting with the regulatory and fiscal promotion of biofuels. The Commission considers that the use of fuels (such as ethanol) derived from agricultural sources (i.e. biofuels) is the technology with the greatest potential in the short to medium term. The action plan outlines a strategy to achieve a 20% substitution of diesel and gasoline fuels by alternative fuels in the road transport sector by 2020. It concludes that only three options would have the potential to achieve individually more than 5% of total transport fuel consumption over the next 20 years: biofuels which are already available, natural gas in the medium term and hydrogen and fuel cells in the long term. One proposed Directive would establish a minimum level of biofuels as a proportion of fuels sold from 2005, starting with 2% and reaching 5.75% of fuels sold in 2010. The second proposed Directive would give Member States the option of applying a reduced rate of excise duty to pure or blended biofuels, when used either as heating or motor fuel.

The texts of the action plan Communication and the two proposals for Directives are available on the Europa website at:

http://europa.eu.int/comm/dgs/energy_transport/en/whats_new_en.html

http://europa.eu.int/comm/taxation_customs/whatsnew.htm

OTHER HEADLINES

Canadian Renewable Fuels Association (CRFA) begins extensive E-diesel testing
http://www.greenfuels.org/index.html

Canadian Renewable Fuels Association (CRFA) calls for Diesel Fuel Renewables Tax Policy
http://www.greenfuels.org/index.html

Canada's Iogen produces ethanol from wheat straw
http://www.bbiethanol.com/news/view.cgi?article=417

China begins work on ethanol plant
http://www.bbiethanol.com/news/view.cgi?article=437

Japan eyes ethanol to cut greenhouse gas emissions
http://www.bbiethanol.com/news/view.cgi?article=401

Australia takes plunge with ethanol
http://www.bbiethanol.com/news/view.cgi?article=398

Increased US ethanol use reduces greenhouse gases by 3.6 million tons in 2001
http://www.bbiethanol.com/news/view.cgi?article=434

U.S. DOE study concludes "No major infrastructure barriers exist" for a 5.1 billion gallon per year ethanol market
http://www.bbiethanol.com/news/view.cgi?article=435

Biodiesel distribution system coming to South Dakota
http://www.bbiethanol.com/news/view.cgi?article=439

Ethanol industry praises Bush comments
http://www.bbiethanol.com/news/view.cgi?article=451

US ethanol industry reaches all-time monthly production record to begin 2002
http://www.bbiethanol.com/news/veiw.cgi?article=454

Americans to get more ethanol in gas
http://www.bbiethanol.com/news/view.cgi?article=456

Oil patch and grain belt unite to promote renewables standard
http://www.bbiethanol.com/news/view.cgi?article=457

Gov. Davis' decision to delay MTBE ban a mistake
http://www.bbiethanol.com/news/view.cgi?article=461

California's delay in ban on rival to ethanol hits ADM
http://www.bbiethanol.com/news/view.cgi?article=462

U.S. Department of Energy (DOE) National Biofuels Program - Enzyme Sugar Platform Project Stage-Gate Meeting Presentations

- Introduction, Stage 2 Overview, and Technoeconomic Analysis
  http://www.ott.doe.gov/biofuels/pdfs/stage2_overview.pdf
- Stage 2 Technical Progress and Business Plan
  http://www.ott.doe.gov/biofuels/pdfs/stage2_details.pdf
- Detailed Stage 3 Plan
  http://www.ott.doe.gov/biofuels/pdfs/stage3_plan.pdf

Commentary and Contributions to Green Paper "Towards a European strategy for the security of energy supply"
http://europa.eu.int/comm/energy_transport/livrevert/default_en.htm

EU documents on Climate Change
http://europa.eu.int/comm/environment/climat/docs.htm#submissions

http://europa.eu.int/comm/environment/climat/home_en.htm

EU promotion of the use of biofuels for transport
http://europa.eu.int/comm/energy/library/comm2001-547-en.pdf

POLICY/REGULATORY ISSUES SUBTASK

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BRUSSELS MEETING

The Policy/Regulatory Working Group of IEA Bioenergy Task 39 met in Brussels, Belgium at the EU offices on October 18-19, 2001. This meeting was held to further define the scope of work for this Task period.

Eight of the nine participants including Austria, Denmark, Finland, Netherlands, Sweden, United Kingdom, United States, and European Commission were represented at the meeting. In addition, a representative from the PYNE pyrolysis network also attended the meeting as an observer.

WORKSHOP ON THE INFLUENCES OF POLICIES AND REGULATIONS ON THE IMPLEMENTATION OF BIOFUELS

The morning session of the Working Group meeting was organized to provide an overview of recent events in participating countries related to biofuels implementation. The workshop consisted of presentations by most of the meeting participants and general discussions. Several general conclusions were noted based on the presentations described above and the discussions by the group:

- There are many recent changes in policies relating to biofuels. Most notable is the proposal by the European Commission to encourage a significant level of biofuels in all transport fuels. This proposal is expected to increase the use of ethanol and biodiesel in the EU countries.
- There is substantial forward momentum toward much broader use of biofuels. The use of biofuels continues to expand in North America, and the policy changes in Europe set the stage for significant expansion in the EU countries.
- Many uncertainties still exist including availability of feedstock, choice of biofuel, and costs. However, market forces are expected to resolve these questions as the demad for biofuels increases.
- There is a general need for better information including standards, “agreed calculations”, and commonly based assumptions. Task 39 is assisting in these efforts.

INITIAL PROJECTS: PROVIDING INFORMATION ON CURRENT BIOFUELS IMPLEMENTATION

The Task 39 Policy and Regulatory Working Group outlined the following projects for 2002.

Update of Country Reports:

Task 39 will serve as a centralized source of timely, accurate information about biofuels on a country-by-country basis. Annual updates of the country reports will be prepared and will include information such as the amounts and types of biofuels used, the percentage of total motor fuel use, and updates of the mechanisms that support implementation.

Case Studies

Descriptive “case studies” will be prepared to provide general information about key biofuels. These descriptions willd be directed primarily toward larger-scale, commercial or near-commercial facilities that show biofuels successes. The case studies would be descriptive of representative or important facilities in a country but not comprehensive summaries on all such facilities. The format for the studies will be similar to that of related EC documents.

Ethanol and Biodiesel Standards

Appropriate standards for biofuels will be needed as international markets develop. The previous Task 27 examined standards briefly and found that while each country may have a standard for ethanol or biodiesel, no common standards exist. The lack of specific standards does not appear to hinder the use of ethanol, but standards were perceived to be more important for biodiesel where the composition can be variable. Updates of information on biofuels standards will be compiled.

Roadmaps and Strategies

Many countries have produced or are currently producing "Roadmaps" or other strategy plans concerning biofuels. While these important documents summarize the basis of national strategies for biofuels implementation, they are often un-noticed or difficult to obtain outside the country of origin. Task 39 will compile relevant documents to provide participants with a reference library.

Financial Instruments

Biofuels, at least in some cases, involve higher risk technologies that have difficulties attracting capital investments in conventional markets. Financial risk is also perceived differently in different countries based in part on previous successes (or the absence of experience) with biofuels. To assist participants, Task 39 will compile information describing existing or previous financial support mechanisms and their relative impact on biofuels implementation.

NEXT MEETINGS

The next meeting of the Task 39 Working Group on policy and regulatory issues was held at NOVEM in Utrecht, The Netherlands on March 13-14, 2002. This meeting will be reported in the next issue of the newsletter. Future meetings were suggested for late 2002 and for early 2003.

BIODIESEL SUBTASK

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The subtask on biodiesel is currently planning its projects for 2002 and 2003. More information will be available following the October 18-19 meeting in Brussels, described above.

ETHANOL SUBTASK

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We had a very successful meeting in Espoo, Finland last November. I would again like to congratulate Liisa Viikari on hosting and organizing this informative conference. You will find below the abstracts from the presentations included and if anyone is interested in a CD that contains the entire content of the presentations please contact David Gregg mailto:djgregg@interchange.ubc.ca.

The first organizational meeting of the ethanol subtask was also held at the Espoo meeting. Unfortunately as a result of the September 11th incident most of the North American participants were not available. Our next organizational meeting as well as a sponsored discussion on the progress in commercialization of biomass to ethanol technologies will be in Gatlinburg this coming April/May. These subtask organizational meetings will be used to review the objectives sent out in the Task Proposal, confirm future meeting dates and topics, discuss opportunities for exchange of personnel/students, determine interest in various technical roundrobins, discuss potential for exchange of technoeconomic modelling data, determine interest in site visits to PDU's/pilot plants/demonstration facilities, etc.

Hope to see everyone in Gatlinburg.

"RECENT TRENDS IN BIOETHANOL PRODUCTION" ESPOO, FINLAND NOVEMBER 12-13, 2001

This fuel ethanol technical meeting covered both policy, implementation and economics as well as process development and enhancement. The meeting was jointly sponsored by the Nordic Energy Research Programme, VTT Biotechnology and IEA Bioenergy Task 39.

A CD containing a full listing of all the abstracts and presentations has been created and is available for a nominal cost to cover production and mailing. If you interested in the CD please contact myself via email mailto:djgregg@interchange.ubc.ca.

POLICY, IMPLEMENTATION, ECONOMICS SESSION

NON-TECHNICAL BARRIERS TO FUEL ETHANOL GROWTH IN NORTH AMERICA

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An overview of the North American market for fuel ethanol is provided. The presentation reviews the history of ethanol market growth in the United States and Canada since 1980. The current extent of the industry in the two countries is provided.

An outlook for the fuel ethanol market in Canada and the United States is outlined, including the dominant market drivers in the two countries. The feedstocks, both starch and lignocellulosic materials, available to meet the projected market growth are presented.

The typical economics for a wheat to ethanol plant in Canada are developed. Some of the ethanol plant variables are examined to demonstrate the impact on ethanol production costs. The complex relationship between feedstock prices, oil prices and government tax incentives is highlighted for a Canadian plant.

The value of ethanol to a refiner is examined qualitatively. The critical success factors in developing an ethanol project are examined from the perspective of ethanol plant owners, potential lenders, and refiners and marketers.

FULL FUEL CYCLE ANALYSIS OF GREENHOUSE GAS EMISSIONS FROM FUEL ETHANOL IN CANADA

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Greenhouse and non-greenhouse gas emissions, such as carbon dioxide, nitrous oxide, methane, carbon monoxide, VOC's and particulate matter, have been shown to have a discernible impact on global climate and various aspects of human life on earth. The transportation sector is one of the main contributors to greenhouse emissions, especially in Canada and the United States. Alternative energy sources, such as ethanol derived from corn or agricultural residues, can play a major role in reducing such emissions by sequestering the atmospheric CO_2 during photosynthesis and providing feedstocks for the production of clean transportation fuels. In Canada, fuel-grade ethanol can be produced from corn in Ontario, from the large amounts of agricultural residues generated in all regions of the country and from the forestry waste available in Pacific and Atlantic Provinces. This strategy is an inherently 'clean' waste management scheme that can make significant contributions to mitigation of CO_2 and other air pollutants by replacing conventional practices such as field burning or wood incineration in beehive burners with the production of ethanol for the replacement of fossil fuels.

The greenhouse-gas emissions model developed by DeLucchi and recently (1999) modified by Levelton and (S&T)^2 calculates CO_2-equivalent emissions of carbon dioxide, methane, and nitrous oxide from all stages of the lifecycle of fuels and vehicles. A full fuel cycle includes production, processing, distribution, and utilization of the fuel. The model has been used to simulate the full cycle emissions for a variety of conventional and alternative fuels and for both light and heavy-duty vehicles in Canada. The Canadianized version of the model is capable of analyzing conventional and reformulated gasoline, natural gas, propane, ethanol from corn, wood and grasses, biodiesel from soybeans and canola, dimethyl ether from natural gas, and methanol and hydrogen from natural gas for fuel cell powered light-duty vehicles and buses.

This presentation discusses the full fuel cycle emission of primary greenhouse gases, i.e., carbon dioxide, methane and nitrous oxide, for ethanol fuel derived from corn, agricultural energy crops and agricultural residues in Ontario. Results for E10 and E85 for the year 2000 and the projected results for the year 2010 are calculated. The results are compared to the emissions from the production of gasoline in Ontario refineries. Consideration is given to the impact of ethanol on a refinery's octane pool. All cases studied result in a reduction of greenhouse gas emissions and a positive energy balance for the production and use of ethanol. The choice of feedstock has a significant impact on the magnitude of the reductions.

DANISH BIOFUELS-RTD FROM THE 1990'IES AND UP-TO-DATE

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This presentation briefly reviewed the history and current interest in biofuels from a Danish energy policy perspective. A number of driving forces for the past and current biofuel interest were discussed. One of the past by-products of interest in biofuels was the development of the

EMBIO LCA analysis programme. With the renewed interest in biofuels this analysis technique has been revived and was discussed in some detail to illustrate some points about the competitiveness of biofuels with petrol and other energy options under current Danish energy policy.

*LATEST INVESTMENTS IN BIOETHANOL PLANTS **IN EUROPE FOR FUEL PURPOSES***

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The use of bioethanol for fuel purposes has developed most rapidly in USA for the past five years. This development has also led to several investments in USA based nearly totally on the use of corn as raw material. Brazil has continued to use sugar cane based fuel ethanol in their gasoline but new investments have been rare.

In Europe, four countries, France, Spain, Sweden and the Netherlands, have been most active in developing the use of bioethanol in the fuels of vehicles. There have been new investments in fuel ethanol plants during the past five years. They all are based on agricultural raw materials.

In France one plant has been built in Artenay (250 000 l/d based on sugar beat juice) in 1995 and another one by Amylum in 1998 (120 000 l/d). In Spain, Cartagena, the first fuel ethanol plant was started up at the end of 1999. This plant is based on barley as raw material and has the capacity of 300 000 l/d of alcohol and 400 t/d of DDGS. In Sweden the first bioethanol plant for fuel purposes has been started up in spring 2001 in Norrköping. This plant is based on wheat, or alternatively barley, and has a capacity of 150 000 l/d.

In Spain, an other investment project based on grains has come in the construction phase recently. There are also some other European countries, where one could expect new investments in the next 5 year period. This, however, depends on the new directives expected from European Union in the near future and the taxation policy regarding the use of agricultural raw materials for ethanol production.

As to bioethanol production from cellulosic/hemicellulosic raw materials so far there have been new investments only for research and development purposes. Process research has taken place in laboratory and at bench scale level. There are also plans for demonstration plants and commercial plants, but so far no investment decisions have been made.

CLIMATE-NEUTRAL FUELS FOR A SUSTAINABLE DUTCH ENERGY SUPPLY SYSTEM

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Climate-neutral fuels - like bio(m)ethanol, Fischer Tropsch diesel, synthetic natural gas or hydrogen - can contribute to the sustaining of the energy sector and to the reduction of climate changing emissions. This was one of the conclusions of an inventory phase (1998-2000) in the Netherlands, commission by the Ministries of Environment and Economic Affairs. The ministries decided to continue with a Fuel Chain Demonstration Phase from 2001 to 2008. In order to accelerate the market introduction of these fuels the whole chain, from production of the resources up to the utilisation of the fuels in the transport sector is to be demonstrated.

The lay-out of the Fuel Chain Demonstration phase is to achieve in a three-staged process the introduction of climate neutral fuels. Those climate neutral fuels (i) should have a significant CO_2-reduction potential - both in market volume and as compared to the conventional alternative, (ii) should have low CO_2-reduction costs, (iii) should be able to be introduced in the current infrastructure, (iv) should be prepared for new technological developments, and last but not least, (v) should be supported by industrial parties. In 2001 parties are invited to form alliances. In 2002 support will be given to the development of a blueprint of a demonstration project. In 2003 the realisation of a demonstration project can start, followed by several years of environmental performance monitoring. By the year 2010 these fuels should have been introduced to the market.

The fuel chain demonstration phase puts strong emphasis on the organisational collaboration between all parties that are relevant for realising a whole chain project. The supporting programme, executed by Novem, under commission of the Ministries of Environment, Economic Affairs and Transport, is a good example of Transition Management.

October 2001, various applications for subsidy have been submitted. Over thirty industrial parties have been involved in these applications. The proposals concentrate on the formation of alliances and show that there is a strong interest of industrial parties to start the development of these new climate neutral substitutes for diesel, gasoline and natural for utilisation the transport and natural gas sector in the Netherlands. The Dutch programme is a good

example of Transition Management, involving all relevant stakeholders: industrial organisations, NGO's, local and regional governments, R&D institutes and public awareness builders.

SWEDISH FUEL ETHANOL DEVELOPMENT

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In The Green Paper ”Towards a European strategy for the security of Energy supply” the Commission discuss a goal to substitute 20 % of the fossil fuels in the transport sector by the year 2020. Furthermore the Commission have presented a directive to introduce 2 % biofuels in the transportsector to the year 2005.

The work presented in this paper has been focused on three parts

- The Swedish development of bioethanol in the transport sector and state of art
- case studies on production plants located in a biorefinery.
- preprojecting and building of a 13 Million EURO pilotplant for process development

In Sweden, as in many other countries, ethanol is the most widespread alternative to gasoline and diesel. Sweden has more then 400 buses running on neat ethanol, more than 400 US built FFV running on e85 and about 600 000 cars running on e5. In November 2001 the delivery of 3000 European Ford Focus FFV begins.

Feasibility studies with energy and material integration based on hydrolysis of agriculture and wood waste, show that the price of the ethanol is accounted for in the interval between 0,36-0,45 EURO /litre ethanol.

Ethanol production in a stand alone unit, based on cellulosic raw material is hard to motivate today due to high costs and low energy output. We have made about ten different feasibility studies in Sweden integrating ethanol production with municipal power plants, sawmills, pelletising units etc.

In such a bioenergy refinery, we can utilize up to 85% of the energy input in useful products such as bioethanol, electricity, lignin pellets and heat for dryers or househeating.

With energy- and CO_2 tax reduction on bioethanol, today ethanol fuels are competitive to gasoline in Sweden.

The next step in the development is to build a pilotplant based on two step dilute acid hydrolysis primarily for soft wood residues.

STATUS OF LIQUID BIO-FUELS IN THE UK, POLICIES AND REGULATIONS

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The UK has at last woken up to the idea of liquid bio-fuels. During 2001 the UK Government has announced a number of initiatives to encourage the production and use of liquid bio-fuels for transport. As well as again reducing duty on LPG and compressed natural gas, in March the Chancellor announced a 20p per litre cut in duty for bio-diesel to come into force in April 2002. At the same time bids were invited for the Green Fuels Challenge, for pilot projects to demonstrate benefits and explore production issues. Fuels to be considered are hydrogen, methanol, ethanol and bio-gas.

In addition to the above £15 million was made available for industrial research and pre-competitive development under the New and Renewable Energy Programme.

Throughout the year a UK energy review has been taking place to address the following issues; conflict of policy with environmental objectives, security and diversity of supply, potentially conflicting policy goals for energy prices.

PROCESS DEVELOPMENT & ENHANCEMENT SESSION

POTENTIAL OF OXIDOREDUCTASES IN LIGNOCELLULOSIC PROCESSING

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In Austria, among liquid biofuels production of biodiesel increased substantially in the last few years, which was also due to attractive tax regulations. In contrast, there is no tax exemption for ethanol based fuels. Nevertheless,

there are several industrial and academic research groups in Austria continuously contributing with their work to the biomass to ethanol process. While enzymatic hydrolysis of lignocellulosic materials has already been demonstrated in large scale more the 10 years ago, the focus of current research the development are new fermentation technologies and at TU-Graz, the development of more efficient enzymes systems. New bacterial oxidoreductases such as peroxidases and laccases are compared to the corresponding enzymes from lignocellulolytic fungi. While the latter are produced extracellularly in high amounts, bacterial enzymes are more stable at extreme conditions (pH, temperature) but low production yields are currently limiting the range of applications. Model substrates have been synthesized to study steric and redox effects during degradation of phenolics by these oxidoreductases. Degradation mechanisms of lignin model compounds in presence of redox-mediators are discussed.

PROGRESS REPORT ON IOGEN'S CELLULOSE-TO-ETHANOL PLANT

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Iogen Corporation of Ottawa, Canada is a manufacturer of cellulase and other industrial enzymes. Iogen has built and started up a 40 t/day demonstration plant that converts wheat straw to ethanol using an enzymatic conversion process. This presentation reports the progress to date and identifies some research areas that are important to improving the process.

OPTIMIZED BIOFUELS PRODUCTION FROM LIGNOCELLULOSIC BIOMASSES

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In Denmark it has been decided that bioethanol utilizing surplus straw will likely be required to meet their Kyoto commitment. A conceptual bioethanol process has been developed and is currently in the process of being developed and implemented that produces ethanol and methane. The process contains a wet oxidation pretreatment, SSF followed by a thermophilic fermentation for the production of ethanol and an anaerobic treatment of the bioethanol effluent combined with manure to produce methane. The extra step of producing the methane ensures optimal utilization of the biomass and a value addition of 34% or the ethanol price.

PENTOSE FERMENTATION BY YEASTS: ACHIEVEMENTS AND CHALLENGES

Merja Penttilä, Aristos Aristidou,
John Londesborough, Hannu Maaheimo,
Juha-Pekka Pitkänen, Peter Richard,
Laura Ruohonen, Laura Salusjärvi, and
Mervi Toivari
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The yeast *S.cerevisiae* is an efficient ethanol producer and tolerates the inhibitors present in lignocellulose hydrolysates. However, it cannot naturally utilise pentoses, and several groups have constructed recombinant strains which have been transformed with the *Pichia stipitis* genes encoding the NAD(P)H -linked xylose reductase (XR) which converts D-xylose to xylitol, and the NAD -linked xylitol dehydrogenase (XDH) which converts xylitol to D-xylulose, which is further phosphorylated by xylulokinase (XK) and enters the yeast pentose phosphate pathway (PPP). Overexpression of XK improves xylose utilisation. Although the recombinant *S.cerevisiae* yeasts can utilize xylose, ethanol production from xylose is still inefficient. Possible reasons for this are the lack of specific xylose uptake systems, inefficient PPP reactions, regulation of glycolytic activity and most importantly, the cofactor imbalances created in the XR and XDH reactions.

We have studied the limitations in xylose utilisation with various recombinant strains in batch and chemostat cultures and analysed the physiological consequences of pentose utilisation using e.g. NMR and genome-wide approaches. In particular the redox imbalances have been considered, and results on xylose fermentation by novel recombinant strains modified in their redox reactions will be discussed. We have recently also cloned all the genes involved in the fungal L-arabinose pathway and expressed these in *S.cerevisiae*. The redox cofactor imbalance is in this pathway even more severe than in the xylose pathaway and understanding the redox regulation and how to modify is thus of continuing importance.

POSSIBILITIES FOR PROTEIN ENGINEERING TO IMPROVE CELLULASES

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In nature the enzymatic breakdown of cellulose requires the coordinated action of several types of enzymes, which can broadly be divided into endoglucanases and exoglucanases (cellobiohydrolases). Often these enzymes have a two domain organization consisting of a catalytic domain and a substrate (cellulose) binding domain. It is interesting that a similar organization has evolved in bacteria and fungi independently. For a more efficient hydrolysis of cellulose for the purpose of for example fuel production it is desirable to increase the efficiency of enzymatic cellulose hydrolysis. The hydrolysis rate of cellulases is very slow, with a rate often as low as a few cuts per minute. It is not known what the rate limiting step is but it is unlikely that it is the actual hydrolysis event. More likely the rate limiting factor is the "pulling" out of the cellulose chain from its matrix, and the subsequent movement of the enzyme along the chain especially in the case of exoglucanases. Finding a productive site at the at the surface could also be rate limiting. For this reason we have studied the interaction between cellulases and cellulose from various points of view. In each of the domains several residues which interact with the substrate have been studied. The catalytic domain has in the case of exoglucaneses an extensive area of interaction along tunnels through which the cellulose chain is threaded, and the cellulose binding domains have surface residues which give specific properties to the interaction. We have also studied how the linking of the domains affect the overall properties of the enzyme. Through this work the posibilites for improvement of cellulases by protein engineering can be discussed.

ENGINEERING YEAST FOR IMPROVED RESISTANCE TO TOXIC COMPOUNDS IN LIGNOCELLULOSE HYDROLYSATES

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By-products formed in dilute-acid hydrolysis of lignocellulosic feedstocks have been shown to decrease the productivity and yield in ethanolic fermentations with the yeast *Saccharomyces cerevisiae*. Characterisation of fermentation inhibitors in dilute-acid hydrolysates of spruce and analysis of the chemical effects of detoxification methods suggest that phenolic compounds and furaldehydes play a prominent role among the inhibitory compounds. Specific removal of phenolic inhibitors from willow, spruce and sugarcane bagasse hydrolysates by the phenol oxidase laccase has shown the significance of this heterogeneous group of compounds. Heterologous expression of laccase from the wood-degrading fungus *Trametes versicolor* as well as overexpression of the *S. cerevisiae* gene encoding phenylacrylic acid decarboxylase have shown that genetic engineering is a possible strategy to increase the resistance of yeast strains to fermentation inhibitors. A comparison of the performance of industrial and laboratory yeast strains in the presence of a set of selected fermentation inhibitors indicated major differences with respect to resistance. In an effort to elucidate the molecular mechanisms behind the differences in resistance, DNA microarray analyses of *S. cerevisiae* cells exposed to inhibitors were employed. The results may lead to new possibilities to develop yeast strains suitable for fuel ethanol production from lignocellulose hydrolysates containing high amounts of inhibitors.

DEVELOPMENT OF ETHANOL PRODUCTION FROM SOFTWOOD USING A PROCESS DEVELOPMENT UNIT (PDU)

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The interest for production of fuel-grade ethanol from various lignocellulosic materials using enzymatic hydrolysis has been the subject of research for a long time. Much effort has been directed towards methods for improvement of the yields after some of the most important process steps, such as after pretreatment, after hydrolysis, or after fermentation. Progress has also been made in finding new and more efficient fermenting organisms, capable not only of a higher production rate but also having the ability to ferment sugars normally not fermented by conventional microorganisms.

However, there is a lack of reliable data stemming from larger-scale facilities. Most experiments to date have been performed in small-scale equipment. This often leads to poorly determined mass balances and an overall uncertainty about the effects of changes in experiments, such as a change of severity in the pretreatment step. To be able to avoid some of those difficulties a process development unit (PDU) has been constructed in Lund, Sweden. The PDU

consists of two steam pretreatment units, several fermenter vessels, enzymatic hydrolysis equipment, and down-stream processing units, such as an evaporation unit. The larger scale will make it easier to evaluate different process configurations with higher accuracy in the mass balances. The flexible PDU may also be rapidly reconfigured to mimic various process configurations, such as separate hydrolysis and fermentation, SSF or recycling of process streams. Results from some experiments performed in the PDU will be presented.

PILOT PLANT ACTIVITIES IN SWEDEN

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EnergyCenter North has made a process design of a pilotplant or Process Development Unit (PDU) with a capacity of about 400-500 liter ethanol/day from a feedstock input of 2 ton of dry substance/day. The plant is basically designed for development of the two step dilute acid hydrolysis process of softwood. It will be designed for a completion with enzymatic reactors when this technique is further developed for softwood. The second reactor is a countercurrent reactor which has a good potential to increase the yield and reduce the byproducts. (Simple drawings and P&I will be shown during the presentation)

The pilotplant will be open for cooperation with partners all over Europe and may be other countries. Location for the pilotplant will be in Ornskoldsvik northern part of Sweden, close to the existing sulfite pulp ethanol plant. Different feedstocks like hardwood and annual crops like straw and reed canary grass will also be tested in the pilotplant.

During the end of 2001 final discussions regarding finance are proceeding and the plant is supposed to be in operation in the first half of 2003. The pilotplant is linked to the four Universities in the region, The Univ. of Umeå, Mid Sweden Univ., The Technical Univ. of Luleå and The Univ. of Swedish Agriculture. The investment cost is about 12 million EURO and the annual running cost about 1.3-2.0 million EURO depending on the research program Sweden was carried out.

The plant is located together with an existing biobased municipality heat and powerplant in Northern part of Sweden. On the existing site there is also a pellettising plant with the annual capacity of about 75 000 ton dry pellets for household heating or delivery to MHP plants in the Stockholm area.

Process design for the production plant is basically the same as the pilotplant. Annual capacity of this plant will be about 75 000 000 liters of ethanol. The investment costs for the production plant will be about 130 million EURO. The plant can be in operation 2006-7 if the tests of the equipment and process development in the pilot plant follows the plans.

BIOETHANOL AS A ROAD TRANSPORT FUEL

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The UK Government announced the 'Green Fuel Challenge' on 25th July 2001, with the intention of encouraging pilot projects in hydrogen,bioethanol, biogas and methanol technologies. British Sugar submitted its proposal on the 28th September, titled ' Bioethanol as a Road Transport Fuel'. This paper outlines the content and aims of the BritishSugar proposal

POSTER SESSION

TWO-STEP STEAM PRETREATMENT OF SOFTWOOD WITH SO_2 IMPREGNATION FOR ETHANOL PRODUCTION

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Two-step steam pretreatment of softwood was investigated with the aim of improving the enzymatic digestibility for ethanol production. In the first step, softwood was impregnated with sulphur dioxide and steam pretreated at different severities. The first step was performed at low severity to hydrolyse the hemicellulose and release the sugars into the solution. The combination of time and temperature that yielded the highest amount of hemicellulosic sugars in the solution was determined. In the second step, the washed solid material from the optimised first step was impregnated once more with sulphur dioxide and steam pretreated under more severe conditions to enhance the enzymatic digestibility. The

investigated temperature range was between 180 and 220°C and the residence times 2, 5 and 10 minutes.

The effectiveness of pretreatment was assessed by both enzymatic hydrolysis of the solids and simultaneous saccharification and fermentation (SSF) of the whole slurry after the second pretreatment step, in the presence of antibiotics. For each pretreatment combination, the liquid fraction was fermented to determine any inhibiting effects. At low severity in the second pretreatment step a high conversion of cellulose was obtained in the enzymatic hydrolysis step while at a high severity a high conversion of cellulose was obtained in the second pretreatment step. This resulted in an overall yield of sugars that was nearly constant over a wide range of severity. Compared with the one-step steam pretreatment, the two-step steam pretreatment resulted in a higher sugar yield and in a slightly higher yield of ethanol. The overall sugar yield, when assessed by enzymatic hydrolysis, reached 80%. In the SSF configuration an overall ethanol yield of 69% was attained.

STRUCTURAL ANALYSIS OF WHEAT STRAW HEMICELLULOSE AFTER STEAM EXPLOSION AND WET OXIDATION

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In order to optimize the pretreatment, hydrolysis, and fermentation of lignocellulosic biomass, in this case wheat straw, analytical procedures were optimized for characterization of the hemicellulose fraction. The biomass was pre-treated by Wet Oxidation (WOX) or Steam Explosion (STEX), followed by isolation and purification of hemicellulose after the pretreatment. Analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Size-Exclusion Chromatogreaphy (SEC), were used for structural studies during the investigation.

WET OXIDATION PROCESS FOR IMPROVING ENZYMATIC HYDROLYSIS OF SOFTWOOD

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The wet oxidation process, a method involving oxygen and water at elevated temperatures and pressure, was previously shown to be an effective method for pretreating and fractionating wheat straw. In this study, the wet oxidation process of softwood (*Picea abies*) was studied with respect to reaction time, temperature and reaction pH. The factors affecting the enzymatic conversion were studied and a comparison to the commonly used steam pretreatment was made.

Wet oxidation of spruce chips was carried out in a loop-reactor constructed at Risø National Laboratory. Enzymatic hydrolysis of solid substrates was performed at 2 % w/w dry mater at 40°C using a mixture of Celluclast (30 FPU/g dry matter) and Novozym 188 (500 nkat of β-glucosidase /g dry matter).

The solid fraction after wet oxidation contained 58-64% cellulose, 2-16% hemicellulose and 24-30% lignin (w/w). The relative cellulose content increased due to the solubilization of hemicellulose, as well as part of the lignin fraction. The severity of the pretreatment affected the enzymatic hydrolysis, and the higher temperature clearly resulted in the better cellulose digestibility. However, in the extreme treatment conditions, the hemicellulose derived sugars were partly decomposed and converted to other products. The recovery of cellulose was high in all the tested conditions.

A high residual hemicellulose content seemed to have a negative effect on the hydrolysis. The optimum conditions for the pretreatment were 200°C for 10 minutes at neutral pH, when taking into account the enzymatic conversion and the dry matter yield. Also, without added chemicals (to adjust the pH), the process was more economical. The highest enzymatic conversion in 24 h hydrolysis was about 50 % w/w (using 30 FPU/g substrate). This corresponds to a sugar yield of 37g per 100g pretreated material. Similar hydrolysis of steam pretreated substrate yielded 41g sugars per 100 g pretreated material.

Our results suggest that wet oxidation offers an attractive means of pretreating softwood. The method and the enzyme preparation used in the hydrolysis would need, however, further optimization.

SURFACTANTS IN ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSE

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Addition of surfactants to enzymatic hydrolysis of lignocellulose increases the conversion of cellulose into soluble sugars. A number of surfactants were screened for their ability to improve enzymatic hydrolysis of steam pretreated spruce. Non-ionic surfactants were found to be the most effective. The conversion of cellulose after 96-h hydrolysis could be increased from 56 to 73% by addition of Tween 20 at 2.5 g/L. Experiments with different enzyme concentrations showed that enzyme loading in hydrolysis could be reduced with a factor of two when Tween 20 was included at 2.5 g/L. The mechanism is not known for the increase of lignocellulose hydrolysis by surfactant addition, and experiments were designed to explore mechanisms of surfactant effects. Studies of adsorption of the dominating cellulase of *Trichoderma reesei*, Cel7A (CBHI), during hydrolysis showed that the surfactant reduced enzyme adsorption to the lignocellulose substrate. Cel7A adsorption after 24-h hydrolysis was reduced from 88% to 64% with 5 g/L Tween 20, i.e. the surfactant increased the concentration of free enzyme in solution. Cellulase stability in presence of surfactants was studied by activity and fluorescence measurements. Surfactants were shown to have only weak effect on cellulase temperature stability. Our conclusions from studies of lignocellulose and delignified subtrates are that the improved conversion of lignocellulose with surfactant can be explained by the reduction of the unproductive enzyme adsorption to the lignin part of the substrate. This is due to the hydrophobic interaction of surfactant with lignin on the lignocellulose surface, which releases unspecifically bound enzyme. The presence of surfactant thus increases the amount of enzyme available for cellulose hydrolysis. A new approach with mixed charged and non-ionic surfactants has been introduced to further improve the positive effect of the surfactant addition.

HETEROLOGOUS EXPRESSION OF A FUNGAL LACCASE IN TRICHODERMA REESEI *AND* SACCHAROMYCES CEREVISIAE

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Ligninolytic enzymes of the basidiomyceteous white-rot fungus *Phlebia radiata* have been studied at VTT Biotechnology for more than a decade (1,2). *P. radiata* is known to produce one laccase (phenoloxidase) and three lignin peroxidases. Here we present the data of the heterologous expression and secretion of the *P. radiata* laccase. Our goal is to study the structure-function relationships of this laccase using both site-directed mutagenesis and directed evolution approaches. *P. radiata* laccase has been produced earlier in the soft-rot fungus *Trichoderma reesei* leading to the production level of 10- 20 mg / l of culture supernatant (3). In order to improve the heterologous laccase expression further in *T. reesei*, a gene fusion approach was chosen here. However, for directed evolution approaches a lower unicellular eukaryotic host is more suitable. Therefore *Saccharomyces cerevisiae* was chosen as the other host in our studies.

Heterologous Laccase Production in *Trichoderma reesei*

The amino terminal cellulose-binding domain (CBD) of *T.reesei* cellobiohydrolase Cel6A (CBHII) was fused in frame with the *P. radiata* laccase encoding cDNA. In addition, a proteolytic cleavage site for KEX2 (RDKR) was inserted between the regions encoding the CBD and the mature laccase. This cleavage site is recognised in the secretion pathway of *T. reesei.* The CBD-laccase fusion was expressed under the control of *cel7A* (*cbhI)* promoter and terminator sequences. This inducible promoter is one of the strongest in *T. reesei* resulting in production levels of tens of grams per litre of the native Cel7A (CBHI). The *cel7A* 5´and 3´flanking sequences were used to target the expression cassette in the *cel7A* locus in the fungal genome. In the most optimal case the wild type *cel7A* gene will be replaced with the CBD-laccase fusion cassette after homologous recombination.

T. reesei was cotransformed with the laccase-CBD fusion plasmid and a plasmid containing either the hygromycin resistance or the acetamidase selection marker. Transformants were screened by assaying the culture supernatants with ABTS (2,2´azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) as the substrate. The heterologously expressed laccase was demonstrated to have wild type enzyme like properties on the basis of the

enzymatic assay, mobility in SDS-PAGE gels and by immunoblotting. Furthermore it was shown that the CBD fusion partner was properly cleaved during the secretion of the enzyme. The fermentation cultivations are now under way to assay the production levels of the best producing clones.

Heterologous Laccase Production in *Saccharomyces cerevisiae*

Both a strong constitutive triose phosphate isomerase (TPI) promoter and an inducible galactokinase (GAL1) promoter were tested for the expression of the *P. radiata* laccase in yeast. The *S. cerevisiae* strain INVSc1 was chosen as a host and a 2m vector carrying the dominant blasticidin resistance marker was used in the transformations. Laccase producing transformants were assayed using ABTS as a substrate. In the case of the constitutive TPI promoter construction yeast transformants with the expression cassette did not survive the transformation. This suggests lethality of the enzyme when constitutively expressed. However, active *P. radiata* laccase could be expressed in yeast under the inducible GAL1 promoter. The heterologously expressed laccase was hyperglycosylated in *S. cerevisiae* when compared to the wild type laccase from *P. radiata*. After endo-H deglycosylation treatment the *S. cerevisiae* laccase had the same mobility as the native enzyme in SDS-PAGE gels. Immunoblotting of cell-lysate indicate that most of the produced laccase is secreted. Different cultivation conditions are now used to test the expression levels.

CELLULASE AND HEMICELLULASE PRODUCTION BY PENICILLIUM BRASILIANUM

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There is a growing demand for specific, efficient and cheap cellulases and hemicellulase for use in formulation of washing powders, in textile production, in pulp and paper industry and for degradation of lignocellulosic material in production of bio-ethanol. It is therefore of interest to gain more information about the production these enzymes of by the microorganisms and the regulation during growth on different carbon and nitrogen sources.

The production of these enzymes is normally measured by enzymatic assays, which is time consuming and often not specific for just one enzyme. Capillary electrophoresis is an analytical technique to separate and measure the proteins present in the cultivation broth. It is fast and offers automatic analysis of a large number of samples. Opposite to enzymatic assays, using capillary electrophoresis it is possible to measure several different enzymes with similar activity and this results in more detailed information about changes in the enzyme production pattern during growth on complex substrates.

In this study, the enzyme production in the filamentous fungi *Penicillium brasilianum* (IBT 20888) during growth on cellulose (Solka-floc FCC 200 or Sigmacell type 20), cellulose and oat spelt xylan, cellulose and corn steep liquor was investigated. The production of enzymes was measured using traditional enzymatic assays as well as by capillary electrophoresis.

A TECHNO-ECONOMIC EVALUATION OF DIFFERENT PROCESS CONFIGURATIONS FOR THE PRODUCTION OF FUEL ETHANOL FROM SOFTWOOD

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In this work technical and economic evaluations of different process configurations for production of fuel ethanol from softwood were performed. The focus was on the comparison between simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). In the evaluation the effect of the addition of fresh water on the overall ethanol cost was also investigated by running the processes with different concentrations of dry non-soluble solid material in the SSF and SHF. The results from the process simulations and the economic evaluation are also useful to determine the bottlenecks in the process and therefore act as a guide for future work.

The process simulations were carried out using the commercial flowsheeting program Aspen Plus. The process steps included were: steam-explosion, pretreatment, filtration, enzymatic hydrolysis, fermentation and incineration of solid residue. Downstream processing steps such as distillation and evaporation were also included. The models that were used for the various process steps were based on experimental data. The results from the simulations were used in the economic evaluation which were performed using the commercial program Icarus Process Evaluator.

The SSF cases give a lower ethanol cost than the SHF cases. This is mainly due to the higher overall ethanol yield and to the lower capital cost in the SSF compared

with SHF. The addition of fresh water has a very high impact on the ethanol production cost. If the process could be run with 8 % dry non-soluble solid material instead of 5 % dry non-soluble solid material with the same yield, the production cost would decrease by around 17 % for both SSF and SHF.

CHARACTERIZATION OF A SACCHAROMYCES CEREVISIAE *STRAIN ENGINEERED FOR XYLOSE UTILIZATION*

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Lignocellulose is a renewable and potentially cheap substrate for fuel ethanol production. After adequate hydrolysis of lignocellulose, its hemicellulose and cellulose fractions can generate hexoses (glucose, mannose, galactose) and pentoses (xylose, arabinose). These sugars can be converted to ethanol provided the proper biocatalyst for the fermentation step. Recombinant microbial platforms such as *S. cerevisiae, Zymomonas mobilis* and *Escherichia coli* are currently available for ethanol production from lignocellulose sugars (some of their features and requirements are summarized in Table 1). A xylose-utilizing of *S. cerevisiae* has been constructed and the ethanol production in a minimal medium containing a mix of sugars was evaluated.

THE FUNGAL L-ARABINOSE PATHWAY

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L-arabinose is a major constituent of plant material so that L-arabinose fermentation is of biotechnological interest. The bacterial pathway for Larabinose catabolism is well established, but very little is known about the fungal pathway. A fungal pathway for L-arabinose utilisation was described for the moulds *Penicillium chrysogenum* and *Aspergillus niger*. It is distinctly different to the bacterial pathway. It consists of an NADPH-linked reductase (aldoketo reductase) which converts L-arabinose to L-arabinitol, an NAD-linked dehydrogenase (L-arabinitol 4-dehydrogenase) which forms L-xylulose, an NADPH-linked reductase (L-xylulose reductase) which forms xylitol, an NADlinked dehydrogenase (xylitol dehydrogenase) which forms D-xylulose and a xylulokinase to make D-xylulose 5-phosphate. Although this pathway was described only for filamentous fungi, there are indications that it also exists in yeasts. Genes coding for the enzymes of this pathway are known except for the L-arabinitol 4-dehydrogenase (EC 1.1.1.12) and the L-xylulose reductase (EC 1.1.1.10). We have identified these genes from the mould *Trichoderma reesei* (*Hypocrea jecorina*) and functionally overexpressed in *S. cerevisiae*. We could demonstrate that the whole pathway is active in *S. cerevisiae*, in a strain where all the genes of this pathway are over-expressed, leading to growth on Larabinose v / nkat/mg.

FUTURE WORKSHOPS/SYMPOSIA

World Biofuels 2002
April 23-24, 2002
Seville, Spain
http://www.agra-net.com/NASApp/cs/ContentServer?pagename=agra/showEvent&id=20000003479

Power Crops for the Americas: The role of sugar cane, grains, and wood in the emerging biofuels economy of the Americas
May 6-7, 2002
The Inter-Continental Hotel
Miami, Florida, USA
http://www.agraeurope.com/NASApp/cs/ContentServer?pagename=agra/showEvent&id=20001004300

12th European Conference and Technology Exibition on Biomass for Energy, Industry and Climate Protection
June 17-21, 2002
Amsterdam, The Netherlands
http://www.etaflorence.it/events/amsterdam2002/conf-home.htm

10th Biennial Biomass Conference
Bioenergy 2002
Bioenergy for the Environment
September 22-26, 2002
Boise, Idaho, USA
http://www.uidaho.edu/bioenergy/

POLICY/REGULATORY ISSUES

Renewable Fuels Association
7th Annual National Ethanol Conference
Policy & Marketing: “A National Conversation about Energy and Economic Security"
February 27 - March 1, 2002
Loews Coronado Bay Resort
San Diego, California
http://www.ethanolrfa.org/nec.html

http://www.bbiethanol.com/nce/

IEA Bioenergy Task 39
March 13-14

NOVEM
Utrecht, The Netherlands
mailto:don.stevens@pnl.gov

IEA Bioenergy Task 39
September
Stockholm, Sweden
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BIODIESEL

US DOE - National Renewable Energy Lab (NREL)
Renewable Diesel Workshops:

March 8, 2002 – Albany, New York
http://www.bbiethanol.com/doe/conference.cgi?doeid=33

April 11, 2002 – Cincinnati, Ohio
http://www.bbiethanol.com/doe/conference.cgi?doeid=38

ETHANOL

IEA Bioenergy Task 39/American Chemical Society -
Division of Cellulose, Paper and Textile
Anselme Payen Award Session
"The Use of Enzymes for Modification of
Lignocellulosics"
April 7-11, 2002
Orlando, Florida, USA
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IEA Bioenergy Task 39
Current Bioethanol Commercialization Attempts
April 29, 2002
Gatlinburg, Tennessee, USA
Contacts:
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Jack Saddler mailto:saddler@interchange.ubc.ca

International Fuel Ethanol Workshop & Tradeshow
June 25th - 28th, 2002
Crown Plaza Springfield
Springfield, Illinois
http://www.bbiethanol.com/few/

World Ethanol 2002
November 6-7, 2002
London, UK
mailto:conferences@agra-net.com

IEA Bioenergy Task 39
Bioethanol Commercialization Progress
December 11-12, 2002
York, UK
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