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EXPENSIVE PETROLEUM GENERATES INCREASED OPPORTUNITIES FOR AGRICULTURAL COMMODITIES

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The rapid, sustained rise in petroleum prices creates opportunities for processors to replace petrochemicals with functional equivalents derived from agricultural commodities. This opportunity began with development of fuel replacement molecules such as ethanol and fatty acid methyl esters, which has created a new base price for commodities related to their energy value. It is continuing with development of new derivatives of fats and carbohydrates that replace petrochemicals directly or replace their functionality with different molecules.

Vegetable oils naturally resemble hydrocarbons. They can be modified further into hydrocarbons or into derivatives with myriad applications from replacements for detergents (soap) to heterobranched derivatives that make superior lubricants. However, high and increasing demand for vegetable oil for use in food likely will limit the quantity of vegetable oil available.

Carbohydrates, in the form of starch derived glucose and sucrose from sugar cane, are bulk feedstocks available in high purity. They are converted readily by chemical or biological means into a variety of platform molecules. Today, fermentations produce ethanol and lactic acid, for example, in large quantity and recent announcements of demonstration facilities to produce succinic acid, isobutanol and 1,4-butanediol are in the press. Advances in genetic engineering and synthetic biology hold the promise of new transformations developing. As with ethanol fermentation, production cost will be a prime driver in making these new fermentations commercial successes; innovative ways of minimizing the cost of fermentation will be required.
Chemical catalysis is an effective way of reducing the functionality of carbohydrates to yield more stable molecules comparable with petrochemicals. Dehydration and hydrogenation can remove oxygen and decrease functionality. Molecules such as isosorbide, hydroxymethylfurfural, furfural and their derivatives can function in many roles now served by petrochemicals, and, in many cases, produce products superior to those they replace.

An untapped source of carbohydrate feedstock is lignocellulose. It is a problematic resource to the developer. Unlike starch and sucrose, it is a mixed and somewhat recalcitrant feedstock that is not resolved readily into pure components. An alternative use of this feedstock being explored by ADM is in animal food, displacing corn from the diet. In this application, treated crop residue is mixed with a protein source and fed to the animal in place of the normal corn diet. Initial feeding trials with cattle suggest the replacement food is an adequate diet and may allow greater weight gain per pound of food consumed than the more common corn diet. This replacement makes the corn starch formally fed to cattle available for processing. An additional advantage of this approach is the starch displaced from animal food is replaced in the animal diet with currently unused crop residue grown on the same land.
IMPROVING WATER USE EFFICIENCY IN BIOFUELS PRODUCTION: ENERGY SUPPLY, RELIABILITY AND ENVIRONMENTAL CONSIDERATIONS

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Transportation fuels currently used in the US are derived primarily from petroleum, of which 58% is imported. To reduce dependence on imported oil, the US has developed initiatives to assess and develop alternative transportation fuels from domestic supplies such as biomass, oil shale, coal and natural gas. The scale up of the alternative fuels production needed to keep oil and petroleum imports constant is projected to require up to 8 million bbl/day of alternative fuels by 2035. Much of this fuels alternative fuels development is water use intensive and much of the production will be focused in certain regions of the country, making water consumption for refining and processing of transportation fuel alternatives a potentially significant regional issue, especially in regions with already limited water supplies.

These concerns have led many researchers and energy and water policy and management administrators to identify improving water use efficiency in alternative fuel processing and refining as a major need and challenge. We will provide an overview of current water issues and challenges in biofuels production, as well as the science and technology research and development efforts needed to improve fresh water use and consumption efficiency in biofuels production while improving biofuel supply reliability, energy security and environmental performance.

BACKGROUND

Alternative transportation fuels include renewable biofuels, fuels refined from nonrenewable crude oils produced from oil shale and oil/tar sands, and nonrenewable synthetic liquid fuels derived from coal and natural gas. Also of longer term interest are hydrogen from fossil and biomass sources, from electrolysis of water using wind or solar power or from nuclear power. Biogas (primarily methane) produced from the anaerobic digestion of agricultural wastes and the organic fraction of municipal solid wastes is also a form of biofuel that can be used for generation of electrical power and heat. Refining of conventional gasoline and diesel fuels consumes 1.5 gallons of water for every gallon
of fuel produced (Gleick 1994, CH2MHill 2003). The US currently refines about 880 million gallons of petroleum products per day, resulting in water consumption for fuel refining on the order of 1 billion gal/day, which represents a baseline reference to compare water use estimates for future alternative fuels production.

In Figure 1 is an illustration of most of the major energy feedstock and processing pathways being used or investigated for production of alternative fuels (EIA 2006, Huber et al 2006). Included are both thermochemical pathways used for most feedstocks and biochemical pathways used for ethanol and oil based biodiesel. Processing pathway details and technology approaches generally are documented and the subject of ongoing research (DOE 2005, Huber et al 2006, NREL 2007).

Figure 1. Feedstocks and pathways for several transportation fuel alternatives (Adapted from: EIA 2006, Huber, et al 2006).
Efforts are underway nationally to advance biofuel production from various forms of biomass. Research and development is continuing on conversion technologies and processes to improve biofuel production efficiencies and reduce costs. Biofuels production currently is experiencing the most rapid growth and interest relative to all alternative fuel options, with starch grain (primarily corn based) ethanol production exceeding the Renewable Fuels Standard mandate of 7.5 billion gal/yr by 2012 set by the Energy Policy Act of 2005. Biofuels based on conversion of cellulosic biomass will be required to enable reaching the current national biofuel goals of 35 billion gal/yr by 2017 and 60 billion gal/yr (nearly 4 million bbl/day) by 2030 (DOE 2006a, DOE 2006b). Successful development and commercialization of emerging lignocellulosic conversion processes and technologies is of growing interest for building a broader biorefining capacity to convert biomass into biofuels and numerous other products (DOE 2005, Ragauskas et al 2006). The authors of the joint DOE-USDA “billion ton” study (ORNL, 2005) concluded the US could produce over one billion dry tons of biomass each year from various agricultural, forestry and municipal sources, enough to generate the 60 billion gallons of fuel ethanol.

The Joint Research Agenda Roadmap on Breaking the Biological Barriers to Cellulosic Ethanol (DOE 2006a), the Biofuels Initiative Needs Requirements Document (DOE 2006b), the 30x30 Workshop and Draft Strategic Plan (DOE 2006c), the Federal Biofuels Posture Plan Workshop documents (DOE 2006d), the Thermochemical Roadmap Workshop (DOE 2007) and the National Biofuels Action Plan Workshop Summary Report and the Algal Biofuels Roadmap are examples of recent technical roadmap and programmatic guidance and direction for priority research and development for biofuels development. The research scope addressed by the above efforts includes biofuel feedstock production, biochemical conversion technologies and processes, thermochemical conversion technologies and processes, and technology integration and deployment.

Water is used directly and consumed within various input feedstock and intermediary product development, conditioning, conversion, separation and cooling processes associated with all alternative transportation fuel alternatives highlighted in Figure 1, as well as with oil shale refining, which is not included, but essentially is thermal heating of oil shale, either in situ or ex situ, to liberate trapped oils and light hydrocarbons for downstream recovery using traditional refining methods. Water related issues are beginning to be noted in many of these efforts but currently with limited emphasis and technical detail or specificity. Only recently has attention begun to be placed on the relationship between biofuels and water quality and on the broader water quantity implications of biofuels production (NAS 2007, Paul 2006).
An overview of water use and water consumption for several alternative approaches considered to supplement domestic fuel supplies is provided in Table 1. Estimated ranges of water use are given both in terms of the relative per unit energy content of the fuel and in terms of gallons of water consumed per gallon of liquid fuel produced (DOE 2006).

Table 1. Alternative transportation fuel production water use and consumption.

<table>
<thead>
<tr>
<th>Fuel Type and Process</th>
<th>Relationship to Water Quantity</th>
<th>Relationship to Water Quality</th>
<th>Water Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional Oil &amp; Gas</td>
<td>Water needed to extract and refine; Water produced from extraction</td>
<td>Produced water generated from extraction; Wastewater generated from processing;</td>
<td>7 - 20 gal/MMBTU</td>
</tr>
<tr>
<td>- Oil Refining</td>
<td></td>
<td></td>
<td>2 - 3 gal/MMBTU</td>
</tr>
<tr>
<td>- NG extraction/Processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biofuels</td>
<td>Water needed for growing feedstock and for fuel processing;</td>
<td>Wastewater generated from processing; Agricultural irrigation runoff and infiltration contaminated with fertilizer, herbicide, and pesticide compounds</td>
<td>12 - 160 gal/MMBTU</td>
</tr>
<tr>
<td>- Gran Ethanol Processing</td>
<td></td>
<td></td>
<td>2000 - 31600 gal/MMBTU</td>
</tr>
<tr>
<td>- Corn irrigation for EOH</td>
<td></td>
<td></td>
<td>4 - 5 gal/MMBTU</td>
</tr>
<tr>
<td>- Biodiesel Processing</td>
<td></td>
<td></td>
<td>13000 - 60000 gal/MMBTU</td>
</tr>
<tr>
<td>- Soy Irrigation for Biodiesel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lignocellulosic Ethanol and other synthesized Biomass to Liquid (BIL) fuels</td>
<td>Water for processing; Energy crop impacts on hydrologic flows</td>
<td>Wastewater generated; Water quality benefits of perennial energy crops</td>
<td>24 - 150 gal/MMBTU (ethanol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14 - 90 gal/MMBTU (diesel)</td>
</tr>
<tr>
<td>OilShale</td>
<td>Water needed to Extract / Refine</td>
<td>Wastewater generated; In-situ impact uncertain; Surface leachate runoff</td>
<td>1 - 9 gal/MMBTU</td>
</tr>
<tr>
<td>- In situ retort</td>
<td></td>
<td></td>
<td>15 - 40 gal/MMBTU</td>
</tr>
<tr>
<td>- Ex situ retort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OilSands</td>
<td>Water needed to Extract / Refine</td>
<td>Wastewater generated; Leachate runoff</td>
<td>20 - 50 gal/MMBTU</td>
</tr>
<tr>
<td>Synthetic Fuels</td>
<td>Water needed for synthesis and/or steam reforming of natural gas (NG)</td>
<td>Wastewater generated from coal mining and CTL processing</td>
<td>35 - 70 gal/MMBTU</td>
</tr>
<tr>
<td>- Coal to Liquid (CTL)</td>
<td></td>
<td></td>
<td>20 - 24 gal/MMBTU</td>
</tr>
<tr>
<td>- Hydrogen RE Electrolysis</td>
<td></td>
<td></td>
<td>10 - 50 gal/MMBTU</td>
</tr>
<tr>
<td>- Hydrogen (NG Reforming)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Ranges of water use per unit energy largely based on data taken from the Energy-Water Report to Congress (DOE, 2007)
2 Estimates based on unvalidated projections for commercial processing. 1 Assuming rainfed biomass feedstock production

Liquid fuel alternatives derived from biofuels, coal, natural gas, oil shale and oil/tar sands are expected to be in the range of 3 to 6 times more water use intensive than conventional petroleum based fuels (Table 1). Water use for biofuels development can be even greater, depending on whether irrigation is used for the biomass feedstock production.

Water quantity and quality issues are tied closely to both energy feedstock production and the conversion processing associated with each of the alternative fuels considered. Future alternative fuels development and production, based on current and
projected approaches and processes, likely will increase demand on fresh water resources by at least a factor of 3 relative to current water consumption for traditional petroleum fuels refining. The regional production and refining nature of many of these alternative fuels, especially biofuels, is suggestive these increased water demands will have larger impacts in some regions and could pose relevant competition for available regional water supply resources in regions with already limited water supply availability.

SUGGESTED HIGH IMPACT RESEARCH AND DEVELOPMENT DIRECTIONS

In the sections, tables and discussions below, we provide a summary of the science and technology needs and research and development priorities identified to address the emerging water quality and quantity issues associated with large scale biofuels production from a series of regional Energy Water Needs Workshops coordinated by Sandia National Laboratories for the Department of Energy from 2005 to 2006 (Pate et al 2007). More than 500 representatives from water management, energy management, energy and water utilities, energy and water providers, economic developers, environmental groups and tribal groups attended these workshops and provided suggestions on regional issues and needs. Surprisingly, identified needs were similar, with only minor regional differences and priorities, across the western, central and eastern regional workshops conducted.

Major national water related research needs identified for large scale biofuels production included:
1) reducing fresh water and overall water use in biofuels process cooling,
2) reducing fresh water use for biomass feedstock production and
3) reducing fresh water consumption and water use in biofuels and biomass processing.

One of the largest demands and consumption of water for most alternative fuel processing technologies is associated with cooling. Technical needs and approaches for reducing water use for process cooling in fuel production are similar to those associated with cooling applications for thermoelectric power production. Cooling water demand can be reduced not only with better cooling technologies, but also through improvements in integrated fuel processing system design and implementation that optimize thermal energy management to minimize the amount of waste heat generated from processing. Another potentially large water demand for biofuels is irrigation for biomass feedstock production. While there are efforts to move to rain fed biomass feedstock, irrigation of even a small percentage of these crops to improve fuel supply reliability could dwarf
most other biofuel water demands. Finally, there are opportunities for reducing water use and consumption during feedstock pretreatment, processing and conversion.

**REDUCE WATER DEMAND FOR BIOFUEL PROCESS COOLING**

Alternative fuels production is an emerging industry where infrastructure build up is in the early phases. With the exception of the relatively rapid growth underway with grain based ethanol production and vegetable/animal oil based biodiesel production, other alternative fuels are in research and development or at a limited pilot scale test phase in the US. This provides the opportunity to develop, implement and evaluate the performance of water saving cooling technologies, as well as optimizing energy utilization and management to reduce waste heat cooling demands and overall water use, while in the early stages of infrastructure build up. The impact can be an earlier realization of improved water efficiency and greater cumulative reduction in water demand as emerging alternative fuel production industries develop. An associated area for reducing water demand is in the design and integration of alternative fuel processing systems and facilities to better optimize energy management, heat utilization and reduce the total net cooling load and associated water demand for the processing facility.

Table 2. Reducing water demand for biofuel process cooling.

<table>
<thead>
<tr>
<th>Research Needs</th>
<th>Research Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved fuel process cooling to reduce net water demand and use</td>
<td>• Assess alternative cooling approaches, modified to appropriately scale and match biofuel processing needs</td>
</tr>
<tr>
<td></td>
<td>• Conduct cost/benefit performance tradeoffs of water efficient cooling</td>
</tr>
<tr>
<td></td>
<td>• Assess cooling approaches compatible with available nontraditional water resources</td>
</tr>
<tr>
<td>Improved integrated thermal management to reduce net cooling demand in fuel processing</td>
<td>• Improve integrated systems and facilities design, analysis and implementation for more productive transfer, utilization and management of heat and water utilization among processes and systems</td>
</tr>
<tr>
<td></td>
<td>• Maximize utilization of available heat and reduce waste heat cooling demand through integration and use of combined heat and power (CHP)</td>
</tr>
</tbody>
</table>
Improve alternative fuel process cooling

As in thermoelectric power plants, dissipation of waste heat from nonconventional fuel feedstock pretreatment and fuel processing typically uses and consumes relatively large quantities of water for cooling. Improved materials and systems that can reduce net water demand and consumption for cooling can bring about water savings in alternative fuel production. Cooling system research needs highlighted in Hightower (2010) represents directions that can address reduction of water use for cooling in processing of most alternative biofuels including research to evaluate novel cooling technologies such as dry and hybrid cooling, the use of nonfresh waters for cooling etc.

Integrated energy use to reduce cooling requirements in processing

Reducing the amount of waste heat to be dissipated in alternative fuel processing will reduce the demand for cooling in the overall fuels production enterprise. This involves making maximum productive use of heat energy generated in exothermic processes to supply heat needed for other endothermic processes and by using what would otherwise be waste heat for use in generation of combined process heat and electric power (CHP) for auxiliary processes such as moisture reduction pretreatment (drying) of biomass gasification input feedstock and water treatment and reuse throughout the fuel processing facility. Additional study and improvements are needed to reduce cooling requirements and increase net water use efficiency and reduce makeup water demand in integrated alternative fuel processing systems. Integrated system design, implementation and pilot scale up demonstrations are needed to validate performance, assess cost/benefit tradeoffs and lower technical risks for commercialization.

ASSESS AND MITIGATE WATER ISSUES FOR BIOMASS FEEDSTOCK PRODUCTION

Water use for growing biomass feedstock can range from no direct withdrawals, represented by entirely rain fed crop and forest lands, to the withdrawal and application of up to several acre feet (1 acre foot = 325,850 gal)/acre/yr for irrigated crops (USDA 2004). When irrigation is used for biomass production, water demand and consumption become large, resulting in thousands of gallons of water consumed per gallon of fuel produced from the biomass (Table 1). Biofuel production based on commodity crops in the US today is a combination of irrigated and rain fed crop acreage. This likely will continue as long as market conditions and policies are favorable, even with the expected eventual commercial viability of biofuel production from cellulosic biomass from crop
residues, forest wastes and rain fed energy crops. Net increases in irrigation used for
grain based ethanol production and crop based biodiesel production could occur under
several possible scenarios. Market conditions and policies will determine what producers
do. Increased demand for biofuels, along with favorable economic conditions (eg, high
oil prices, biofuels production tax incentives and demand for increased energy crop yield
or reliability) could drive increased use of irrigation for biofuels. Future use of irrigation
for perennial lignocellulosic energy crops in some regions during dryer conditions is
possible as a means of ensuring higher and more reliable crop productivity.

Table 3. Assess and mitigate water issues in biomass feedstock production.

<table>
<thead>
<tr>
<th>Research Needs</th>
<th>Research Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assess and quantify impacts of increased irrigation of energy crops and resulting biofuel cost/benefit tradeoffs</td>
<td></td>
</tr>
</tbody>
</table>
- Review/improve estimates of future expansion capacity/likelihood  
- Assess potential increased demand and impact on water supplies  
- Assess potential increased impacts on water quality  
- Assess tradeoffs of increased feedstock production yield and reliability with irrigation vs increased variability and uncertainty without |
| Assess impacts on hydrologic flows of regional expansion of perennial energy crop production |  
- Review/improve estimates of future energy crop expansion/location  
- Develop modeling approaches to assess hydrologic, soil and crop production impacts at regional and local scales and validate with field experiment studies  
- Review/assess water use impacts and risks for perennial crops  
- Assess risk of potential hydrologic flow impacts on water supply quantity vs mitigation approaches  
- Develop best management practices and metrics to reduce or avoid adverse impacts and improve cost/benefit tradeoffs on water supplies |
| Assess regional climate impacts on future irrigation needs for resource reliability |  
- Develop regional climate modeling capabilities to assess regional climate variability impacts on long term droughts and biomass reliability to identify future crop and irrigation needs |
| Research biofuel production from nontraditional biomass feedstock |  
- Explore algal based and other nontraditional biofuel feedstock production that can use nontraditional waters, waste water and reduce impacts on fresh water resources |
The application of irrigation for growing additional grain, sugar, and oil commodity crops to meet growing demand for biofuels, or to increase biomass feedstock yields and better insure the reliability of supplies in specific regions, would be the largest single source of additional water demand to meet expanded biofuel production needs in the future. This could translate into an additional several billion gal/day of water demand for biofuels production, depending on the volume of net irrigation increase that would occur. This is expected to be less of an issue in using agricultural crop residues, other agricultural and forestry wastes, and in moving toward the future use of largely rain fed cellulosic energy crops. However, large scale production of perennial energy crops involving tens of millions of acres, even when rain fed, could have water resource impacts and unintended local consequences due to alterations of hydrologic flows (Hall 2003). Potential impacts include soil moisture deficits, reduced subsurface aquifer recharge and reduced surface inflows to adjacent lakes, streams and rivers.

Key elements of this will be better understanding and characterization of issues and tradeoffs associated with increased use of irrigation for biomass energy crops; issues, risks, tradeoffs and approaches to mitigate possible hydrologic flow impacts that could result from massive national expansion of perennial energy crop production; and the exploration of alternative biomass feedstock, like oil producing microalgae, that could offer the potential for relatively high biofuel productivity using nontraditional waters and land not suitable for agriculture.

Assess water impacts of increased irrigation for biofuel feedstock

There are cost/benefit tradeoffs associated with the use of water and high value agricultural land to produce irrigated crops for biofuels vs other competing products and markets. The use of commodity starch and oil crops for ethanol and biodiesel is supporting the current rapid increase in US biofuels production but is expected to reach a total production limit of 15 to 20 billion gal/yr due to competing market needs and constraints. The expected limit still represents a several fold increase over historic biofuels production, which can represent a significant additional water demand if the agricultural sector responds in a way that creates a net increase in water use for irrigation to supply the growing demand for biofuel feedstock.

As of 2002, total US irrigated farmland was 55 million acres of 442 million acres of total cropland or 13% of total acreage (USDA 2006). The proportion of irrigated vs rain fed crops currently used for ethanol and biodiesel production is not known and is not tracked; however, but it would not be unreasonable to expect the percentage of total corn and soy produced with irrigation (18 and 10%, respectively) would be consistent
with the percentage of corn and soy used for biofuel production, which would increase considerably if more grain based biofuels are developed. The issues, tradeoffs, risks and impacts of increased water demand for biofuel feedstock irrigation is an area where additional critical analysis and quantitative assessment is needed from an energy water and transportation fuel supply reliability perspective, especially as it pertains to fuel supply reliability in drought conditions and under future climate change considerations.

Assess hydrologic flow impacts from energy crops

Projections for potential future growth of perennial cellulosic biomass energy crops vary over a range of 40 to 60 million acres or more in support of biorefinery based production of biofuels and other biomass based coproducts. This is in addition to the production and use of agricultural commodity crops and other agricultural and forestry waste biomass (ORNL 2005, Perlack 2006). Wide scale planting of perennial energy crops (herbaceous grasses and fast rotation woody crops) could alter hydrologic flows due to their deep, extensive root systems and dense canopies. Depending on local hydrologic conditions, this would contribute to local water deficit impacts during dryer periods (Hall 2003). Relative water use efficiency of biomass production with herbaceous and woody perennial energy crops is higher than for many other agricultural commodity crops but absolute water consumption also is relatively large (McLaughlin et al 1998). As with other biofuels, cellulosic energy crops could see additional use of irrigation, depending on energy markets, production incentives and concerns about ensuring reliability and productivity of energy feedstock supplies from drier regions or during what could be abnormally dry periods in wetter regions.

Also, water quality impacts likely will be associated with any additional agricultural run off involving the use of pesticide, fertilizer and herbicide inputs for increased biofuels production and will have to be assessed. A combination of modeling and field experimental work is needed to identify, understand and mitigate risks of potential adverse water quality impacts from large scale energy crop production.

Past and current work on annual and perennial energy crops by the USDA, in partnership with DOE, should be leveraged and extended to more thoroughly model and assess hydrologic flow impact issues and mitigation strategies and water quality issues from efforts to optimize feedstock availability. The goal would be to develop and establish guidelines for feedstock production plantations, metrics for monitoring and fertilizing, and best management practices to avoid or to minimize problems that could arise in certain locations or regional watersheds under either high production conditions or drier conditions where increased irrigation would be required for energy crop and fuel supply reliability needs.
Model climate change impacts on biofuel reliability and irrigation needs

Development and production of biofuels have regional issues and concerns with respect to their relationships with potential climate change impacts and precipitation rates. Resource reliability considerations include reliability and supply risks for biofuel production due to weather variability, extreme weather events and potential climate change for different regions of the country expected to produce and supply biomass feedstock.

Key research needs include development of climate models that can capture and predict regional precipitation and weather conditions and effects of changing climate and short and long term variability of weather patterns on available water supply quantity, quality and timing, both in terms of precipitation and irrigation water availability. Fundamental questions to be answered include what the interrelated water, short/long term weather variations and climate change impacts will be on reliability, quality, timing and volume of biomass production, and related biofuel production, supply availability, price and reliability over time. Also of interest is whether future climate trends will require additional irrigation to support long term biomass feedstock reliability.

Low water use alternative biomass feedstock research

Oil producing microalgae represent one type of promising renewable feedstock for biofuels which can be grown without need for higher quality arable land, while using nontraditional waters and CO₂ waste streams as a nutrient source. Large scale algal growth places minimal stress on the supply and demand cycle for agricultural products utilized for human and animal consumption and can be scaled to allow for higher overall production levels on a per acre basis when compared to conventional terrestrial biomass plants, particularly those that produce oils. The DOE funded Aquatic Species Program (ASP) that operated during the period 1978-1996 (DOE 1998), along with work by various others since that time, including the recent algal biofuels research roadmap, provides a solid foundation from which to launch a focused effort to advance technologies and processes needed to produce nontraditional biofuels.

Algal oil based biofuels can have higher energy density and potentially are more fungible within existing transportation fuel infrastructure than ethanol. The projected high uptake of CO₂ from concentrated waste stream sources provides additional greenhouse gas abatement benefits. Considerable research and development is needed to overcome several existing impediments to the cost effective production and scale up of these nontraditional biofuel resources.
Reduce water consumption in biofuels processing and biomass conversion

Water use, consumption and impacts in biofuels production predominantly are associated with biomass feedstock production, and with biorefinery utility functions that provide steam, process heat and process cooling. All other processing and conversion water used in current biofuel processing plants and in future biorefinery system designs appear to be moving toward achieving “zero discharge” with the intent of maximizing capture, treatment and reuse of water within the processing and conversion system. This is driven largely by the need to ensure compliance with environmental regulations, but has the added benefit of improving water use efficiency.

Since many processes and technologies are in development, major emphasis has been, and continues to be, placed on making the needed and critical advances in the efficiency and cost performance of the technologies and processes, with water impacts being of lesser concern. As technologies and processes mature, and given the emerging issues associated with water demand and availability in the context of the expected major biofuels production volume increase, incremental improvements can provide cumulative water savings.

There are several opportunities in biofuels processing to improve water use efficiency. In the future, ethanol and other biofuels are expected to be produced from a range of biochemical and thermochemical pathway processes. The biochemical pathway typically consists of the sequential use of mechanical preprocessing, thermochemical pretreatment, hydrolysis and enzymatic saccharification to break apart the material into its component sugar molecules that can be converted to ethanol through fermentation and distillation. The nonconvertible lignin component is a solid residue byproduct that can be burned to generate CHP, or can be converted through thermochemical means to biofuel or other products.

Alternatively, the thermochemical pathway typically uses either gasification or pyrolysis to convert the biomass material into a mixture of char and synthesis gas or pyrolysis oil that can be further synthesized or refined into a variety of fuels and other products. Biomass gasification requires removal of tars and other contaminants from the syngas (gas scrubbing/conditioning) prior to downstream biofuel synthesis, typically involving significant water use. For thermochemical processing, biomass feedstock must be put through mechanical preprocessing to break the biomass material into smaller and more uniform pieces for more efficient handling and processing. The material also must be dried to reduce moisture content for more efficient processing.
Table 4. Reducing fresh water use in biofuels and biomass processing.

<table>
<thead>
<tr>
<th>Research Needs</th>
<th>Research Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduce water use in biomass feedstock pretreatment for cellulosic deconstruction</td>
<td>• Deconstruction treatments effective with higher concentration (less diluted) mixtures and less water use/consumption</td>
</tr>
<tr>
<td>for biochemical conversion and to minimize drying for feedstock gasification</td>
<td>• Treatments capable of more efficient and economical water reuse</td>
</tr>
<tr>
<td></td>
<td>• Improved drying/dewatering using excess/waste process heat</td>
</tr>
<tr>
<td>Reduce water use in biochemical conversion processes including, hydrolysis/fermentation, distillation /separation and anaerobic digestion</td>
<td>• Hydrolysis and fermentation with higher solids/biofuel concentrations</td>
</tr>
<tr>
<td></td>
<td>• Lower energy, higher efficiency distillation and/or membrane water/biofuel/residuals separation</td>
</tr>
<tr>
<td></td>
<td>• Higher efficiency anaerobic digester with high solids ratio (≥15%)</td>
</tr>
<tr>
<td>Reduce water use in thermochemical gasification and synthesis</td>
<td>• Syngas conditioning/scrubbing with minimal water use/consumption</td>
</tr>
<tr>
<td>Identify water quality requirements for biofuels processing and opportunities for use of nontraditional water resources</td>
<td>• Identify water quality requirements for biofuel processing and assess applicability of using nontraditional waters to replace fresh water consumption</td>
</tr>
</tbody>
</table>

The biofuel production processes described above offer areas of opportunity for reduction of water use and consumption during feedstock processing and conversion. Research topics identified in Table 4 could yield improvements in water use efficiency by emphasizing reducing water use in emerging biochemical and thermochemical processes.

**Improve biomass feedstock pretreatment**

Pretreatments being explored and developed to deconstruct cellulosic biomass for biochemical conversion to biofuels include mechanical crushing and exposure to strong and weak aqueous acid solutions, hot water, steam and enzymes. Water is used and consumed to varying degrees, depending on the process and solids concentrations. Research that puts additional emphasis on the potential reduction in water use and consumption, or that provides for more cost effective water treatment and reuse, is recommended. Water use efficiency improvements must be complementary to primary concerns for increasing processing efficiency and cost effectiveness for biofuel production. Drying biomass feedstock to low moisture content is a critical element in
biomass pretreatment for efficient thermochemical conversion using gasification. Through research, implementation and testing of improved integrated systems approaches to put waste heat from the gasifier to productive use in feedstock drying, net water use efficiency can be improved by reducing overall system cooling load. Testing and demonstration is needed to validate performance and assess cost/benefit tradeoffs.

**Develop improved biochemical conversion processes**

Research to enable hydrolysis and fermentation processes to work efficiently and effectively with higher concentrations of solids and ethanol content can reduce water use and consumption as well as the volumes of water needing to be treated for reuse. Again, water use efficiency improvements will need to be complementary to primary concerns for increasing processing efficiency and cost effectiveness for biofuel production.

Research and development that can enable anaerobic digester systems to operate efficiently and cost effectively with higher solid content (≥15%) waste streams can increase biogas productivity with a wider range of waste streams and reduce water demand and use through the ability to process less dilute mixtures.

Research and development of improved, high efficiency, lower energy separation technologies using advanced materials and membrane designs can improve water use efficiency by displacing distillation and its associated cooling demands, and by providing more cost effective water treatment and reuse. This overlaps to some extent with the broader discussion of water treatment in other sections but would involve specific approaches and materials for integration within biofuel production processes.

**Improved thermochemical conversion processes**

Research and development of technologies and processes that can reduce fresh water use in syngas scrubbing and conditioning to remove tars and other unwanted contaminants could provide incremental improvement to overall system water use efficiency.

**Evaluate use of nontraditional waters in alternative fuels production**

Demands on freshwater resources for biofuel production can be reduced by expanding use of nontraditional waters. Such waters can be used in both biomass feedstock production and in biofuel processing to displace or augment fresh water use and consumption. Major issues and considerations involve determining regional and local availability and matching nontraditional water source quality with biofuel development and processing requirements.
SUMMARY AND CONCLUSIONS

A large reduction in water demand for alternative biofuels processing can be achieved through research, development and implementation of more water efficient cooling technologies and through improved fuel processing systems integration, energy management and heat utilization to bring about a net reduction in overall heat dissipation load, thereby lowering cooling requirements and cooling water demand. Also, with application of low water use cooling approaches, such as dry or hybrid cooling, these improvements could reduce biofuel process water consumption by 25 to 50%.

In addition to water demand for process cooling, another major water impact with biofuels production is associated with the potential use of irrigation for higher reliability biomass feedstock production. Reducing water demand impacts for irrigation can be achieved through successful transition from commodity crop based biofuels, where irrigation is used more frequently, to expansion based predominantly on rain fed cellulosic biomass, much of which is expected to be in the form of perennial energy crops. Still, irrigation water needed to ensure biofuel supply reliability must be considered, especially as climate change variations on additional irrigation needs are considered. A 10% reduction in irrigation needs would be a significant improvement in fresh water consumption.

Wide spread production of rain fed perennial energy crops can have undesirable impacts on water supplies in local areas and regional watersheds due to alterations in hydrologic flows in drier growing regions and during drier periods. These effects need to be better understood and management practices developed to avoid or mitigate adverse impacts. Another unique opportunity is in the research and development of biomass feedstock, like algae, that could have major productivity advantages over more conventional biomass while making effective use of nontraditional waters and reducing biofuel production demand on both fresh water resources and arable land. Finally, improvements to reduce water use in biomass conversion and processing research to enable use of nontraditional or waste waters for alternative biofuel processing could also reduce future fresh water consumption.
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HIGH THROUGHPUT AUTOMATED MOLECULAR BIOLOGY PLATFORM FOR PRODUCTION OF FUEL ETHANOL YEAST CAPABLE OF EXPRESSING HIGH VALUE HETEROLOGOUS PROTEINS

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The majority of fuel ethanol currently produced in the United States is made from corn starch. Projections indicate that corn supplies will not be able to meet the increasing demand for biofuels. Lignocellulosic biomass, an abundant and renewable carbon source, has the potential to supplement starch feedstocks for the production of fuel ethanol (Perlack et al 2005) but current technology is constrained by production costs. The profitability of ethanol production from lignocellulosic biomass will be improved if high value coproducts also are generated.

Current processes for fuel ethanol production from starch yield substantial amounts of corn oil as a byproduct. Corn oil triacylglycerides are converted to fatty acid ethyl esters (biodiesel) and glycerol by transesterification with ethanol. One method of catalyzing this transesterification reaction is with lipase enzymes (Akoh et al 2007). An integrated biorefinery combining starch ethanol and cellulosic ethanol facilities may become cost effective if biodiesel is produced as a coproduct using lipase catalyzed, single step column transesterification (Nielsen et al 2008) with low cost lipases expressed in large quantities in a recombinant yeast strain capable of cellulosic and corn ethanol production. We have engineered such a recombinant ethanologenic yeast strain to express a lipase that could be used for biodiesel production from the corn oil byproduct of the starch ethanol process in an integrated biorefinery.

Use of lipases as biocatalysts to accomplish transesterification would help to decrease costs associated with the traditional method of biodiesel production as well as to
overcome some of the technical drawbacks (Akoh et al. 2007). The biocatalytic process not only represents a simplification over the traditional biodiesel production processes that utilize homogeneous alkaline catalysts but also produces a high quality product that satisfies ASTM specifications (ASTM 2009). Although the cost of the enzymatic catalyst remains a hurdle compared to the less expensive chemical catalysts, the use of recombinant DNA technology to produce large quantities of lipases and the use of immobilized lipases may lower the cost of biodiesel production, while reducing downstream processing problems (Akoh et al., Torres et al. 2003, Villeneuve et al. 2000).

Optimization of lipases and development of strains to express large quantities of these biocatalysts are necessary for inexpensive production of biodiesel. The scripting of automated protocols and scheduling of PCR assembly steps on a robotic platform developed in our laboratory have the potential to be used in an iterative fashion for rapid production of any gene open reading frame (ORF) and are demonstrated in this work for production of a lipase gene. Also, it is possible to immobilize lipases on specialty resins to establish a continuous single step column process for biodiesel production. Sepabeads® EC-EP resins are polymethacrylate beads with epoxy functional groups on the surface that can anchor lipase catalysts through covalent linkages to a polyhistidine tag on the lipase (Mateo et al. 2002).

The lipase gene construct that we assembled was based on the Candida antarctica lipase B (CALB) sequence registered in GenBank as Z30645, which consists of 1029 nucleotides. An overall stepwise PCR assembly method was implemented using 38 overlapping oligonucleotide segments, 36 with 50 nucleotides, 1 with 40 nucleotides and 1 with 15 nucleotides to construct the complete CALB ORF. Segments were added sequentially. Each added oligonucleotide segment overlapped at the 5' end with 25 nucleotides of the previous oligonucleotide and overlapped at the 3' end with 25 nucleotides of the next oligonucleotide. The assembled CALB sequence consisted of 1030 nucleotides. The PCR mixture containing the CALB product was separated using electrophoresis on a 1% (w/v) agarose gel. The desired band was cut out of the gel and purified using a GENECLEAN® II kit. The sequence of lycotoxin-1 variant C3 (Hughes et al. 2006 and 2008, Yan and Adams 1998), an amphipathic peptide potentially intended to facilitate movement of the expressed enzyme to the surface of the yeast cell, also was assembled and placed in frame with the CALB ORF sequence using the integrated robotic platform to give the CALB Lyt-1 C3 ORF.

The full length purified assembled CALB or CALB Lyt-1 C3 ORFs were TOPO cloned into pENTR D TOPO® vector; the resulting reactions were transformed into TOP10 chemically competent Escherichia coli cells as described previously (Hughes et al. 2006). The resulting colonies were subjected to a plasmid preparation procedure using
QIAprep 96 Turbo Kit protocol as described previously (Hughes et al 2007). Presence of the assembled ORF inserts was verified using restriction enzyme digestion and separation by agarose gel electrophoresis. Inserts were sequenced using the procedure described previously (Hughes et al 2006).

The CALB and CALB-Lyt-1 C3 inserts were moved from pENTR D TOPO® into respective pYES2 DEST 52 expression vectors using LR Clonase™ II reaction. The pYES2-DEST 52 plasmids containing CALB or CALB Lyt-1 C3 were extracted using the QIAprep 96 Turbo Kit protocol as described previously (Hughes et al 2007) and used to transform the diploid strain PJ69-4 (S. Fields, Washington University, Seattle, WA) to determine if the enzymes were heterologously expressed as CALB 6xHis and CALB Lyt-1 C3 6xHis and if they possessed lipase activity. The INVSc1 yeast strain also was transformed with the inserts.

Yeast strains were grown in CM 2% glucose minus URA selective medium and then spotted on plates containing either galactose or glucose and 5% (v/v) corn oil (Lincolnland Agri-Energy, Palestine, IL) with 0.1% (v/v) Triton X-100 and incubated for 5 days. A sample of corn oil containing the yeast cells was taken from the surface of the galactose plate and used for SEM analysis (Bang and Pazirandeh 1999).

On CM 2% glucose minus URA, the control strain PJ69-4 did not grow (Figure 1A right) because it did not contain the pYES2 DEST 52 plasmid with ura3. Strain PJ69-4 with pYES2 DEST 52 CALB and PJ69-4 with pYES2 DEST 52 CALB Lyt-1 C3 showed growth (Figure 1A, right) on the selective medium indicating presence of the pYES2 DEST 52 plasmids. The results for the three yeast strains grown in CM 2% galactose medium and spotted on plates containing corn oil and Triton X-100 are presented in Figure 1A (left). In galactose, the GAL1 promoter drives expression of CALB His and CALB Lyt-1 His. The region corresponding to CALB His was only slightly larger than the control and the enzyme did not appear to be available to interact with the corn oil. The region corresponding to CALB Lyt-1 His was larger than the region for CALB His and had spread across the corn oil on the plate, suggesting the expressed enzyme with the amphipathic Lyt-I sequence was available at the surface of the yeast cell and capable of interacting with corn oil.

In the scanning electron micrographs (Figure 1B) of cells taken from the galactose plate, it also appeared that the PJ69-4 yeast cells expressing CALB Lyt-1 His were on top of the oil mixture (Figure 1B, left) also indicating the lipase was interacting with the oil. The PJ69-4 yeast cells expressing CALB His were immersed in the corn oil similar to the PJ69-4 control.
Figure 1. Transformation of PJ69-4 with lipase constructs (A, top) and scanning electron micrographs of yeast colonies from CM 2% galactose plate (B, bottom).

Fermentations were performed in a Fedbatch-pro fermentation system maintained at 30°C with stirring (150 rpm). Yeast cells were incubated in glucose medium for 2 days to achieve a high density culture for initiating fermentation in the reactor. Fermentation in the reactor with glucose as the carbon source was incubated at 30°C for
48 hr, followed by growth on galactose at 30°C for 72 hr with cell growth, ethanol production and glucose or galactose consumption measured over time for the control strain and all transformed strains.

Results for all yeast strains in glucose medium are shown in Figure 2A (left). After 48 hr fermentation, the culture was shifted to galactose medium. Results for all yeast strains in galactose are shown in Figure 2A (right). With glucose as the carbon source, the maximum production of ethanol for PJ69-4 CALB His and PJ69-4 CALB Lyt-1 His strains was 9.3 g/L (Figure 2A, 48 hr). This level was as high as control PJ69-4 strain indicating that presence of plasmid did not affect ethanol production. Cell growth for all strains was similar and all strains completely consumed the glucose substrate. With galactose as the carbon source, cell growth was greater for the PJ69-4 CALB His and PJ69-4 CALB Lyt-1 His strains than for control, possibly related to expression of lipase. All strains, control and transformed, completely consumed the galactose substrate after 72 hr on YPG medium (Figure 2A, 120 hr). Maximum ethanol production with galactose for control and transformed strains was similar at 7.2 g/L (Figure 2A, 120 hr).

It is possible that a single step fermentation strategy, in which the lipase enzyme is expressed simultaneously with production of ethanol, would save time and material costs. Protein could be isolated from the fermentation supernatant prior to distillation of the ethanol product. The sequential strategy proposed here makes use of the same fermentation reactor for the second stage (lipase expression) after the ethanol has been removed as in the basic dry grind process. Enzyme would be isolated for biodiesel production in the same way using either fermentation strategy.

Cells taken from the galactose culture were pelleted; the supernatant and resuspended intact cell pellets were assayed for biodiesel production. Evaluation of the expressed CALB enzymes in a biodiesel assay showed the lipases were active in catalyzing the formation of ethyl esters (biodiesel) from ethanol and soybean oil (Figure 2B). The amount of ethyl ester production was the highest for the supernatant from intact PJ69-4 yeast cells transformed with CALB Lyt-1 His (890 µg/gram oil), followed in decreasing order by the pelleted intact cells of INVSc1 with CALB His (660 µg/gram oil), of PJ69-4 with CALB Lyt-1 His (650 µg/gram oil), and of PJ69-4 with CALB His (540 µg/gram oil). Lower levels of ethyl esters were produced by the supernatant from intact cells of PJ69-4 with CALB His (230 µg/gram oil) and INVSc1 with CALB His (98 µg/gram oil). The presence of the amphipathic Lyt-1 sequence appeared to favor production of ethyl esters from the supernatant, suggesting the enzyme was present at the surface of the cells and so was found in the supernatant to a much greater extent than CALB without Lyt-1.
In all cases, ethyl esters produced with intact cells or supernatant contained predominantly C16 fatty acid chains (87 to 89%) rather than C18 chains. The percent of C16 (89%) vs C18 (11%) is the exact reverse of the ratio found in the soybean oil used as substrate (89% C18 vs 11% C16). The 89% C18 fatty acids found in soybean oil are
85% unsaturated (23% 18:1, 54% 18:2 and 8% 18:3) and 4% saturated. The 11% C16 in soybean oil is from a saturated fatty acid (palmitic). This soybean oil fatty acid profile is similar to that of corn oil, which is also 89% C18 and 11% C16 (palmitic), but the 89% C18 are 87% unsaturated (28% 18:1, 58% 18:2 and 1% 18:3) and 2% saturated.

The properties of biodiesel are dependent on fatty acid ester composition, and modifying the fatty acid profile has been suggested as a way to improve the low temperature properties or oxidative stability of biodiesel (Knothe 2009). An important property specified in biodiesel standards is the cetane number (CN), which is related to ignition delay time. The higher the CN, the shorter the ignition time will be. The CN of fatty acid esters increases with increasing saturation and increasing chain length. Favoring C16 fatty acid ester production from soybean oil increases the saturation of the fatty acid esters produced and increases the CN. Increasing the saturation also increases the oxidative stability of the biodiesel (Knothe 2009). Conversion to ethyl esters and ethanol production was monitored by gas chromatography.

CALB Lyt-1 His enzyme from cultures of PJ69-4 yeast cells were prepared, Ni bead purified and immobilized on Sepabeads® resin to evaluate their activity in the production of ethyl esters (biodiesel) compared to that of nonimmobilized recombinant CALB Lyt-1 His. The immobilization procedure was adapted from Resindion (Binasco, Italy) [Silvia et al]. The specific activity of the enzyme was calculated by dividing µg ethyl esters produced/L by mg lipase/L. The highest specific activity (167 µg ethyl esters/mg lipase) was obtained using the nonimmobilized CALB Lyt-1 His enzyme from intact cells, indicating a large quantity of active enzyme was expressed and secreted from the intact cells. The specific activity of CALB Lyt-1 His enzyme from lysed cells was 4 fold lower (43 µg ethyl esters/mg lipase), indicating possible inhibition by lipid cell debris. Purified CALB Lyt-1 His enzyme immobilized on EC-EP resin gave a specific activity of 82.3 µg ethyl esters/mg lipase, about half that of the nonimmobilized enzyme.

Recombinant lipases produced in this work are designed to be bound to a resin for use in single column production of biodiesel from ethanol and corn oil in a combined cellulosic and starch ethanol biorefinery. Lipases expressed in a cellulosic ethanol yeast strain would provide low cost biocatalysts for production of both bioethanol and biodiesel from cellulosic biomass. Use of this yeast strain could increase the profitability of an integrated biorefinery that combines starch ethanol and cellulosic ethanol facilities by catalyzing conversion of corn oil (triacylglycerides) byproduct to fatty acid ethyl esters (biodiesel) and glycerol in a single step column transesterification with ethanol produced in the facility.
ACKNOWLEDGEMENT

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LITERATURE CITED


PHENOLIC ACIDS IN CEREAL GRAINS AND THEIR INHIBITORY EFFECT ON STARCH LIQUEFACTION AND SACCHARIFICATION

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BACKGROUND

Numerous authors point to the inhibitory effect of phenolic compounds on enzymatic starch hydrolysis by $\alpha$-amylase and amyloglucosidase, an effect which generally is attributed to the ability of polyphenols to decrease enzyme activity by binding to enzymes/proteins (Thompson and Yoon 1984, de Jong et al 1987, Rohn et al 2002, Funke and Melzig 2005, Chethan et al 2008, Shobana et al 2009, Sreerama et al 2010). However, phenolic acids with their carboxyl and hydroxyl groups also are capable of binding with starch and other polysaccharides through hydrogen bonds, chelation or covalent bonds, forming substitutions or bridges/cross links (Gibson and Strauss 1992). Few investigators have reported the contribution of the reaction of phenolic acids with starch on the inhibition of starch amylolysis, even though the interaction of tannic acid and catechin with starches (Deshpande and Salunkhe 1982) as well as the interference of gallic and chlorogenic acids with the starch-iodine reaction (Sharma et al 1992) have been reported.

OBJECTIVES

This present study was designed to investigate the effects of phenolic acids either alone or in combination on the hydrolysis of starch from triticale, wheat, barley and corn sources. Also, we assessed the nature of those effects and whether they were influenced by heating at temperatures similar or close to that of used during liquefaction/jet cooking.
RESULTS AND DISCUSSION

The presence of phenolic acids in cereal grain is thought to influence starch amylolysis during liquefaction and saccharification of whole grain flours. As a basis for remodeling starch amylolysis systems and for understanding inhibition mechanisms, phenolic acid compositions and concentrations in whole grain flours of triticale, wheat, barley and corn were analyzed by HPLC. Total phenolic acid contents (sum of 11 phenolic acids) in the 4 grains were 1171, 1732, 1599 and 2331 µg/g, respectively, with more than 76% found in the bound form. Ferulic acid, coumaric acid and protocatechuic acid were the major phenolic acids in triticale and wheat. Gallic acid also was rich in triticale. Ferulic acid, coumaric acid, hydroxybenzoic acid, gallic acid and catechinhydrate were predominant in barley. In corn, ferulic acid, coumaric acid, gallic acid, catechinhydrate, naringin and syringic acid were abundant.

Based on these profiles, commercially obtained pure phenolic acids were added individually and collectively to isolated starches at amounts either equivalent to or 3x those in whole grains. The degree of starch hydrolysis with α-amylase (liquefaction) and amyloglucosidase (saccharification) decreased up to 8% when individual phenolic acids were added. Decreases were more pronounced when phenolic acids were added collectively (4 to 5% with α-amylase and 9 to 13% with sequential α-amylase and amyloglucosidase). For a phenolic acid-starch-enzyme model system, a phenolic acid-enzyme interaction was the dominant contributor to the interference, but a phenolic acid-starch/dextrin interaction also was important. Heating augmented interactions among phenolic acids and enzymes and starch/dextrin. Phenolic acids thus contribute to the resistance of starch to enzymatic hydrolysis and/or the loss of enzyme activity during starch amylolysis.

BENEFITS

The finding on the interaction of phenolic acid with starch/dextrin under boiling is new. It indicates the high temperature liquefaction step is not only costly but can trigger interactions of phenolic compounds with dextrins, thus negatively influencing starch bioconversion efficiency. Therefore, we are providing useful information to cereal crop breeders in the selection of grain varieties low in phenolic compounds, and to bioethanol processors for optimization of primary grain processing operations such as milling and pearling that remove outer most grain tissues (ie bran layers) enriched in phytochemicals, such as phenolic compounds.
ACKNOWLEDGEMENTS

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LITERATURE CITED

ENZYMATIC PROCESS FOR DP4 PRODUCTION

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ABSTRACT

Maltotetraose producing enzyme, a genetically modified alpha-amylase from Pseudomonas saccharophilia expressed in Bacillus licheniformis was evaluated to produce DP4 syrup under optimized conditions (pH 5.5 and 60°C) using 32% DS liquefied starch (10 DE) for 48 hr. The resulting syrup was found to contain DP4 greater than 45% with only 3.3% DP1 and 8% DP2. A maltotetraose producing amylase can be used to produce high DP4 syrup in the wet milling industry.

INTRODUCTION

Maltotetraose (G4 or DP4) syrup is one of many commercially important products derived from enzymatic treatment of starch. Conversion of vegetable starches, especially cornstarch, to maltotetraose and lower sugars, such as glucose or maltose, is a rapidly expanding industry.

The current process consists of two sequential enzyme catalyzed steps that result in glucose or maltose production. The first enzyme catalyzed step is starch liquefaction. Typically, starch suspension is gelatinized by rapid heating to 85°C or more. Alpha-amylases (EC 3.2.1.1) are used to degrade the viscous liquefact to maltodextrins. Alpha-amylases are endohydrolases that catalyze the random cleavage of internal α-1,4-D-glucosidic bonds. As alpha-amylases break down starch, viscosity decreases. Because liquefaction typically is conducted at high temperatures, thermostable alpha-amylases, such as an alpha-amylase from Bacillus sp., are preferred for this step.

A second enzyme catalyzed saccharification step is required to break down the maltodextrins. Glucoamylases and/or maltogenic alpha-amylases commonly are used to catalyze hydrolysis of nonreducing ends of maltodextrins formed after liquefaction, releasing D-glucose, maltose and isomaltose. Debranching enzymes, such as
pullulanases, can be used to aid saccharification. Saccharification typically takes place under acidic conditions at elevated temperatures, e.g., 60°C, pH 4.3.

G4 (also referred to as DP4) syrup has a number of advantageous properties compared to sucrose syrups. For example, partially replacing sucrose with G4 syrup in a food reduces the sweetness of foods without affecting taste or flavor. G4 syrup has high moisture retention in foods and exhibits less deleterious Maillard reaction products because of its lower glucose and maltose content. G4 syrup also has higher viscosity than sucrose, thus improving food texture.

G4 syrup depresses the freezing point of water less than sucrose or high fructose syrup, so G4 syrup can better control freezing points of frozen foods. After ingestion, G4 syrup also affects osmotic pressure less than sucrose. Together, these characteristics make G4 syrup ideally suited as an ingredient in foods and medical products. Also, G4 syrup imparts gloss and can be used advantageously as a paper sizer. In this study, SAS3, a genetically modified G4 producing amylase from *Pseudomonas saccharophilia* expressed in *Bacillus licheniformis*, was evaluated to produce maltotetraose syrup from liquefied starch.

**MATERIALS**

- SPEZYME® Fred liquefied starch (34% ds, 10 DE)
- G4-forming amylase (SAS3, 40.8 BMK/g)

**METHODS**

Starch liquefied was adjusted to 5.5 pH with NaOH; each 100 g of liquefied starch was incubated at 60°C for DP4 saccharification by dosing SAS3 at 0.04 BMK/g ds. The reaction was carried out up to 39 hr in a temperature controlled water bath. To track DP4 production during incubation, samples were withdrawn periodically and the reaction stopped by heating in boiling water. Enzyme inactivated samples were analyzed by HPLC using an oligosaccharide Ag²⁺ column (Phenomenex, Torrence, CA) at 80°C. Mobile phase was DI water.
RESULTS AND DISCUSSION

SAS3 was able to produce DP4 concentrations of >45% within 24 hr with 0.04 BK/g ds from liquefied starch as a substrate. SAS3 reached maximum DP4 concentrations in 24 hr but extended incubation resulted in loss of DP4 yield (Figure 1). DP4 saccharification time will need to be considered to maximize DP4 production.

Figure 1. Maltotetraose production using SAS3 enzyme.

LITERATURE CITED

MEMBRANE GLUCOSE DEMUDDING BY A DECANTER-MEMBRANE SYNERGY PROCESS: CHALLENGES AND BENEFITS

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Dextrose syrups are produced from starch by liquefaction combined with saccharification. In the conventional process, rotary vacuum filters (RVFs) with a diatomaceous earth (kieselguhr) coating as filter aid often are used for removal of the so called mud phase after liquefaction and saccharification. This mud phase consists of insoluble impurities and suspended solids such as proteins, fats and enzymes. The use of these RVFs commonly is associated with high investment and operating costs, eg, high costs for kieselguhr and for the disposal of kieselguhr combined with sugar losses in the kieselguhr. Furthermore, RVFs introduce a high complexity to the process combined with safety issues for operators.

Alternatively, closed systems with microfiltration (MF) and ultrafiltration (UF) have been proposed either as stand alone units or in combination with high speed separators (HSS) or decanters for concentration of the mud fraction and purification of sweeteners (Lancrenon et al 1994, Singh and Cheryan 1997, Singh and Cheryan 1998). Apart from replacing the RVFs, these approaches eliminate the need for filter aid. Furthermore, downstream processing costs are reduced since the membrane processes are removing more color and turbidity from the dextrose syrups. The state of the art concepts in the industry are either: 1) stand alone units with polymeric or inorganic membranes as open channel modules, tubular or plate and frame or 2) a process combination of HSS with polymeric spiral wound modules or decanters with inorganic tubular modules.

Despite their inherited advantages over RVFs, the electrical power consumption of these concepts with either open channel membrane modules or with HSS as an initial step is higher than for RVFs alone. To overcome this, a novel concept has been developed combining a decanter with spiral wound membranes. The key to this concept is the optimization of the decanter performance to provide a feed stream which can be handled by a spiral wound module and thus provide a more robust and cost reduced alternative to the HSS concept (Figure 1).
Figure 1. Membrane-decanter concept for starch demudding.

The initial test and development of this concept started independently on the Alfa Laval decanter and membrane sites. The first membrane tests for demudding were carried out by Alfa Laval Business Centre Membranes, previously DDS Filtration/Danish Separation Systems (DSS) during the 1990s. In these tests, flat sheet GR40PP membranes (polysulphone, molecular weight cut off (MWCO) 100 kDalton) and FS40PP membranes (fluoro polymer, MWCO 100 kDalton) were tested in an M39 open channel plate and frame module. It was possible to use the GR40PP and FS40PP membranes in the M39 module for demudding of wheat based and corn based syrups. However, the use of open channel plate and frame modules was associated with high investment and operating costs.

Independent of the membrane development, the Alfa Laval Product Centre Decanter from 2001 and onwards tested decanters for demudding of wheat-starch based syrups. A two phase decanter could remove up to 99% of the mud but the colour removal was inferior compared to the RVFs. On this background, the idea to use the combination of a two phase decanter with spiral wound modules emerged. In 2008, the first combined test of a two phase decanter with a spiral wound membrane unit was conducted at a producer of wheat starch based syrup. Tests were conducted on a high maltose syrup and glucose DE45 using a full scale STNX438 decanter and pilot scale membrane unit with a 4 in spiral wound module. The decanter could remove up to 99% of the mud and achieve a relatively dry mud phase with low sugar loss, while the membrane unit polished the syrups exceeding the quality achieved by the RVFs on site (Figure 2). Furthermore, it was confirmed the spiral wound module could handle the feed from the decanter and could be cleaned with a simple alkaline cleaning.
The first two lines based on this new demudding concept have now been operating on an industrial scale since the beginning of 2011 at a producer of corn based syrup in Europe. The first of these lines combined an STNX 938 decanter with an Alfa Laval Membrane-UF 10 membrane unit with sequential cleaning and is demudding 25 to 35 m³/hr of glucose DE95. The second line consisted of an STNX 438 decanter with an Alfa Laval Membrane-UF 10 membrane unit with sequential cleaning and is demudding 15 to 20 m³/hr of glucose DE40. Both lines are working as expected from the results obtained during concept development. We will show the successful development of the new concept for demudding of starch based sweeteners from initial tests to the first successful installation of the concept on an industrial scale.
LITERATURE CITED


ZEIN PURIFICATION:
THE PROCESS, THE PRODUCT, MARKET POTENTIAL

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ABSTRACT

We will present an overview of a zein purification, decolorization and deodorization process, methodologies to assess those properties and applications of the purified product. The process involves column filtration of commercial zein solutions through a combination of molecular sieve and activated carbon packed columns to produce a white, odorless, purified product. Diagnostic tools to assess that product involve spectroscopic methodologies to monitor the removal of odor and color. Purity of product is determined by proximate analyses. Ready markets for a purified, odorless, colorless zein product are its usage in gluten free baked products and for producing biodegradable gum bases, chewing gums, edible packaging films, coatings, and encapsulants for drugs, flavors and sweeteners. Growing areas where purified zein holds promise are in a variety of in vivo applications involving soft tissue augmentation, grafts, wound healing, scaffolding for artificial organs and as a replacement for skin replacements.

INTRODUCTION

Biofuels are generated from the biomass of renewable resources as opposed to petroleum based transportation fuels where reserves are becoming limited and fossil fuel prices are increasing. According to a recent world agricultural supply and demand estimate, 43% of the domestic corn crop is projected for ethanol production in 2010-2011. Biofuels will grow fast in the next decade in the automotive fuel market as mandated by the Energy Independence and Security Act (EISA) of 2007 where the production of 36 billion gallons of biofuels is to be produced by 2022. In that act, corn ethanol is to be capped at 15 billion gallons per year. Use of the corn ethanol industry coproducts is essential to make ethanol production economically feasible without
subsidies. Within those coproducts, whether production is from corn gluten meal (wet milling) or distillers dried grains (dry grind), is a “golden nugget” termed zein. Zein is a prolamin that has a multitude of potential uses in the food, medical, pharmaceutical and cosmetic industries, particularly if the zein is processed further to purify it and free it of yellow color and off odor. The objectives are three fold: 1) the process for zein purification that generates a colorless, odorless product, 2) analyses of the product to define consistency for a commercial operation and 3) new market potentials for the purified zein product.

THE PROCESS

Our zein purification process for this investigation (Sessa 2008, Sessa and Palmquist 2009) uses a combined column filtration with a molecular sieve and an activated carbon to produce a white, odorless, purified zein. Materials used for this process include Freeman zein (FZ) purchased from FloChemical Corp., Ashburnham, MA. Data are from three different lots, namely F40008064C, F40009051C1 and F40009101C13. Zeolite 5A molecular sieve, as 8 to 12 mesh beads was obtained from Sigma-Aldrich Corp., St. Louis, MO; Norit Rox 0.8, extruded plant material, was purchased from Norit America Inc., Marshall, TX.

Two columns, one with dimensions 120 cm x 28 mm (id) and the second with dimensions 60 cm x 28 mm (id) were hooked in series. The first column was packed with 70% ethanol, washed and equilibrated Zeolite 5A; the second column was packed with 70% ethanol washed, and equilibrated Norit Rox 0.8 such that the ratio of zeolite to activated carbon was 3:1 (w/w). Zein samples, 300 mL of 8% solids dissolved in 70% ethanol were passed through the columns at 20 to 30 mL/hr. Column eluents, 10 mL collected in test tubes, were monitored for protein elution by UV absorbance at 280 nm (Figure 1).

Test tubes with UV 280 nm absorbances were pooled and dialyzed against deionized water for 2 days. Contents of the dialysis casings were freeze dried. One passage through the combined columns resulted in some visible evidence of yellow color. To effect a more complete decolorization, column eluents were recycled through the 2 columns until visible absorbance at 448 nm was at a desired level of removal required for a particular usage. The column eluent, collected as one batch, was dialyzed and freeze dried. Column filtration designations were defined as CF1 for 1 passage through the 2 columns vs CF followed by the number of recycles through those columns. Column resins can be regenerated. Zeolite 5A can be regenerated by elution with 0.2 M HCl in 70% ethanol; whereas, activated carbon can be regenerated with 0.2 M NaOH in 70%
ethanol. Activated carbon can be reactivated for further usage. Zeolite, an aluminosilicate (clay), and activated carbon that become “spent” are both “green” and can be discarded. Zeolite 5A at $51.76/kg (Sigma) and Norit Rox 0.8 at $19.10/lb, sold in 44 lb bags, are relatively inexpensive. Lesser priced activated carbons that are suitable in the column filtration process are commercially available (Sessa and Palmquist 2009).

![Figure 1. Protein elution pattern for FZ through two columns hooked in tandem. Column 1 was packed with Zeolite 5A and column 2 with Norit Rox 0.8 in ratio 3:1, by wt.; elution with 70% ethanol; 10 ml/test tube.](image)

**THE PRODUCT**

Proximate analyses of zein before and after column filtrations were performed at the Agricultural Experiment Station Laboratories, University of Missouri-Columbia, Columbia, MO. Data represent means of one replicate from each of the three different lots of FZ. Sulfur content was measured by inductive coupled plasma optical emission spectroscopy. In addition to the proximate analyses, given as dry basis (db) in Table 1, a ratio 280:325 nm of zein before and after column filtrations was recorded. That ratio was used to assess the removal of diferuloylputrescine, a contributor to the off odor of zein,
Table 1. Proximate analysis (db) and ratio 280 nm:325 nm of zein before and after column filtration (CF).

<table>
<thead>
<tr>
<th>%</th>
<th>FZ</th>
<th>FZ-CF1</th>
<th>FZ-CF24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein(^a)</td>
<td>88.5(1.2)</td>
<td>99.7(1.3)</td>
<td>100.1(0.5)</td>
</tr>
<tr>
<td>Crude fat</td>
<td>1.86(0.18)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ash</td>
<td>2.48(0.02)</td>
<td>0.11(0.00)</td>
<td>0.02(0.00)</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.68(0.03)</td>
<td>0.61(0.01)</td>
<td>0.55(0.03)</td>
</tr>
<tr>
<td>280 nm:325 nm(^b)</td>
<td>2.48(0.02)</td>
<td>7.59(1.71)</td>
<td>15.49(2.06)</td>
</tr>
</tbody>
</table>

Data represent the mean (± 1 sd) of one replicate from three lots of Freeman zein (FZ) where CF1 is one cycle and CF24 is 24 cycles through tandem columns.

\(^a\)Dumas N x 6.25
\(^b\)Ratio 280 nm:325 nm is indicative of removal of diferuloylputrescine, the major contributor of odor, where a higher number is indicative of removal.

As depicted in Table 1, one passage of zein through the tandem columns yielded a purified zein protein product (based on Dumas N x 6.25), removed residual crude fat, substantially removed ash and diminished sulfur content. Diminished sulfur content may be attributed to the removal of a β-zein component during the purification process (Sessa and Woods 2011). The 280:325 nm ratio was higher than the starting ratio. Even with these improvements, some yellow color was visible in product solutions. To effect further removal of yellow color, FZ was subjected to recycling of the column eluents through the tandem columns. FZ-CF24, recycled 24 times, gave products with diminished ash contents and sulfur contents and increased the 208:325 nm ratio to yield a “near colorless” zein product. Mean recoveries of purified zein product with one cycle, FZ-CF1, were 55.6±10.4% and for FZ-CF24 recoveries were 51.0±7.9%.

The yellow color of commercial zeins is attributed to the xanthophylls, lutein and zeazanthin. We devised a simple colorimetric assay to demonstrate degree of color removal (Sessa and Woods 2011). A series of dilutions of zein, dissolved in glacial acetic acid, were assessed at 448 nm with glacial acetic acid as blank. Plots of three replicates each for FZ, FZ-CF1 and FZ-CF24, with their respective natural log of absorbances vs natural log of dilutions were prepared (Figure 2).
Figure 2. Visible spectra of FZ at λ448 nm before and after column filtration (CF) where number following CF indicates number of cycles through tandem columns packed with Zeolite 5A and Norit Rox 0.8.

FZ had the most color, FZ-CF1 possessed lesser color than the original; whereas, FZ-CF24 had the least color. This method can be used as a comparative, qualitative, diagnostic tool to demonstrate degree of color removal in a commercial operation. Complete removal of all yellow color from zein may be impossible without denaturing protein. A proposed model for zein, based on molecular dynamic simulations, suggests that three lutein molecules fit into the core of the three triad helical segments that stabilizes zein configuration (Momany et al 2006). Based on this model, complete removal of all the yellow color would require conformational changes resulting in diminished helical content. However, Sessa and Woods (2011) reported that purification of commercial zein products via the column filtrations through Zeolite 5A and an activated carbon yielded a “near colorless”, purified zein product with enhanced helical character. Those findings were based on FTIR and CD analyses. Only a portion of those xanthophylls entrenched within the helices could be solubilized and adsorbed onto the column media.
MARKET POTENTIAL

Utilization of zein for food, medical and pharmaceutical applications has been limited by the lack of availability of a high purity zein that is decolorized and deodorized. Gluten free baked products are an area of interest because 1% of Americans are gluten intolerant which results in celiac disease and cannot eat baked goods from wheat and other gluten containing flours (Busken 2011). Corn, sorghum, potato, quinoa, soy, tapioca and rice are sources that can be used as replacements for wheat flour. The challenge is to mimic the absorption, structural, elastic and baking characteristics of wheat. Such is the case with the use of corn zein together with starch, hydroxypropyl methylcellulose, sugar, salt, yeast and water to form a “wheat-like cohesive, extensible viscoelastic dough” (Schober et al 2008, Schober et al 2010). To achieve a specific volume, zein was defatted. Our patent pending process (Sessa 2008) uses column filtration rather than organic solvent extraction, not only to defat, but also to decolorize and deodorize zein. Elimination of the off odor and off taste should yield an improved baked product.

Another area of interest for a decolorized, deodorized zein product is directed to producing biodegradable gum bases, chewing gums, edible packaging films, coatings, encapsulants for drugs, flavors and sweeteners (Liu and Lee 2004). Again, off odor and yellow color are deterrents for zein usage in those products.

Purified zein holds promise in a variety of _in vivo_ applications involving soft tissue augmentation, grafts, wound healing, scaffolding for artificial organs and as a substrate for skin replacements. Zein, in the form of microspheres, can be used _in-vivo_ to release biologically active agents for specifically targeted drug delivery when administered to humans or animals (Bernstein et al 1997). Prolamin polar lipid composites can be used as a protective film for wound healing and wound protection as well as a “cosmetic agent” for improving skin appearance, topical gels, patches or films to deliver active agents (Fotinos 2002). Biodegradable porous scaffolds, composed of zein, poly(e-caprolactone) interdispersed with hydroxyapatite particles are foamed with supercritical carbon dioxide for bone tissue engineering (Salerno et al 2010a,b,c). Electrospun structures from protein based biomaterials possess unique properties preferred for tissue engineering and other medical applications. Electrospun zein fibers, cross linked with citric acid to enhance water stability and cytocompatibility, can be used for tissue engineering and other medical applications (Jiang et al 2010). Electrospun protein fibers as a scaffold may surmount the major problem in tissue engineering to create a vascular network to feed the growing tissue. Yet to be investigated is the use of high purity zein to construct bioartificial organs from a biomimetic, 3 dimensional scaffold (Geltser 2005). Currently, tissue engineers can grow simple body tissue, such as
knee cartilage, by shaping a porous scaffold made of a biodegradable plastic. This scaffold is seeded by immersing the scaffold into a patient’s cell followed by immersion in a nutrient solution. The cells multiply and clump with the scaffold, the scaffold dissolves to yield cartilage ready for transplantation. Many applications for usage of highly purified zein products in the food, medical and pharmaceutical demonstrate their excellent market potential.

LITERATURE CITED


ZEIN RECOVERY FROM COPRODUCTS OF A RAW STARCH FERMENTATION BIOETHANOL PROCESS

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INTRODUCTION

Zein was first identified by Gorham in 1821 but did not appear in the marketplace until the 1930s. For the next 20 yr, zein found uses in many different fields, such as coatings, adhesives, fiber, binders and plastics (Lawton 2002, Shukla and Cheryan 2001). With the introduction of petroleum products, the need for zein diminished. Today, zein is known as a specialty product that is used mostly as pharmaceutical and food coatings. Interest and demand for zein is growing due to consumer demand for biobased products.

Traditional zein has been extracted from corn gluten meal (CGM) (Lawton 2002). Zein initially was extracted from corn and corn meal, but was switched to CGM. Commercial recoveries were greater for CGM because of its higher protein content. Today, most of the zein available commercially is obtained from CGM. A relatively new high protein corn coproduct, distillers dried grains, has become available in the last 20 yr. Distillers dried grains did not exist in large quantities in the 1930s, when the original zein extraction and recovery research was done. Annual production of distillers dried grains with solubles (DDGS) is estimated to be 16 million tons (Weiss et al 2007). At an average protein content of 30%, distillers grains would be a good starting material for zein extraction.

A number of advantages make extraction of distillers grains an attractive choice for zein production by alcohol producers. They have the solvent needed for zein extraction and the distillation equipment for the recovery of the solvent. In addition, distillers grains do not need to be dried prior to extraction if extracted at an ethanol facility. In older literature, distillers grains were not a good choice for zein extraction because of low zein yields (Wolf and Lawton 1997). As ethanol production increased, more emphasis has been placed on nutritional quality of distillers grains. This emphasis has resulted in improved recovery of zein. A number of published reports and patents
have been issued recently on the extraction and recovery of zein obtained from bioethanol coproducts (Cookman and Glatz 2009, Xu et al 2007).

MATERIALS AND METHODS

High protein distillers dried grains (HP), beer and 200 proof ethanol were obtained from POET Research Center, Scotland, SD. Distillers dried grains (DDG) and beer were obtained from POET Biorefining, Chancellor, SD. Hydrochloric acid, sodium hydroxide (50% w/w), 2-mercaptoethanol, sodium metabisulfite, trifluoroacetic acid (TFA) and acetonitrile were purchased from Sigma-Aldrich. CGM was purchased from Customer’s Supply Distribution.

ZEIN EXTRACTION AND RECOVERY

Samples of HP, DDG and CGM were extracted with 70% (w/w) aqueous ethanol with and without NaOH. Extractions were done in duplicate at 70°C and for 30 min using a solvent to solids ratio of 5:1. Two hundred fifty g of 70% aqueous ethanol was mixed with 50 g (db) of sample. If NaOH was used, 2.3 ml of 50% (w/w) aqueous NaOH was added to the mixture. Extraction took place in a 1000 ml, 3 neck round bottom flask stirred at 300 rpm. Temperature was controlled with a J-KEM Model 150 controller; to minimize ethanol loss, the flask was equipped with a condenser. After extraction, the mixture was centrifuged at 4500 rpm using an IEC-HN-SII centrifuge. Solids were air dried and saved for analysis. The liquid fraction (zein solution) was pH adjusted to about 7 using 6 N HCl. Zein was recovered from the solution by pouring it into 2500 ml of cold (~0°C) water.

Precipitated zein was recovered by centrifugation and air dried. Zein was extracted from beer using a similar extraction as described previously except the solids were recovered from the liquid portion of the beer prior to extraction. Beer was centrifuged at 4500 rpm using a Beckman Model J-6B centrifuge. The liquid fraction was discarded and solids were used for zein extraction. The dry matter of the solids was obtained by drying a sample at 100°C for 4 hr. The percent dry matter was used to calculate how much 200 proof ethanol needed to be added to achieve a 70% aqueous ethanol extraction medium. For the extraction some 70% aqueous ethanol was added to achieve a 9:1 solvent to dry solids ratio. Other than the change in solvent to solids ratio, the extraction of beer was the same as the extraction of the dry samples.
HP STEEPING WITH SODIUM METABISULFITE

One hundred fifty g HP was mixed with 750 g of 1% aqueous metabisulfite. The mixture was steeped for 4 days at room temperature. After steeping, the mixture was centrifuged at 4500 rpm. The liquid fraction was oven dried and retained for analysis. Solids were washed twice with water and oven dried. Dried solids were extracted as described previously.

RP-HPLC

HPLC separations were performed using a Dionex UltiMate 3000 HPLC system with UV detection at 214 nm using a Jupiter 3 µm C18 300 Å column (Phenomenex) maintained at 50°C and a 0.5 mL/min flow rate under gradient conditions starting at 72% of mobile phase A (0.1% TFA) and 28% of mobile phase B (0.1% TFA in acetonitrile) at 0 min and progressing linearly to 39.5% mobile phase A and 60.5% mobile phase B at 50 min with a 10 min hold.

RESULTS AND DISCUSSION

HP and DDG used in this study were from two different POET biorefineries. Both biorefineries use a raw starch hydrolysis during the fermentation process, where corn was not cooked prior to fermentation. HP was from a POET biorefinery that fractionates the corn prior to fermentation; therefore, only the endosperm was subjected to fermentation. There was a difference in protein content of the coproducts from the two biorefineries; potential zein in each sample was estimated based on its protein content. Recovery efficiency was calculated from the recovered zein. This was done by dividing the recovered zein by the potential zein available in the sample. Because protein content of the recovered material varied between extractions, only the protein portion of the recovered material was used in the recovery efficiency calculation. Yields of zein extracted from HP and DDG depended on whether NaOH was used in the extraction solvent (Table 1). There was little difference in yields of zein recovered from CGM whether or not NaOH was present in the solvent during extraction (Table 1).
Table 1. Zein recoveries from extracting DDG, HP and CGM.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein starting material (%)</th>
<th>Potential zein available (g)</th>
<th>Zein recovered Yield (g)</th>
<th>Efficiency of recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDG1 (no NaOH)</td>
<td>29.0</td>
<td>5.8</td>
<td>2.4</td>
<td>41.7</td>
</tr>
<tr>
<td>DDG1 (NaOH)</td>
<td></td>
<td></td>
<td>4.7</td>
<td>81.5</td>
</tr>
<tr>
<td>HP2 (no NaOH)</td>
<td>43.4</td>
<td>10.8</td>
<td>4.5</td>
<td>41.7</td>
</tr>
<tr>
<td>HP2 (NaOH)</td>
<td></td>
<td></td>
<td>9.1</td>
<td>87.2</td>
</tr>
<tr>
<td>CGM2 (no NaOH)</td>
<td>69.8</td>
<td>17.5</td>
<td>13.6</td>
<td>77.7</td>
</tr>
<tr>
<td>CGM2 (NaOH)</td>
<td></td>
<td></td>
<td>14.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

*DDG (distillers dried grains), HP (high protein distillers dried grains) and CGM (corn gluten meal)
**Denotes whether NaOH was added to 70% aqueous ethanol during extraction.
140% of the available protein assumed to be zein for whole corn DDG.
250% of the available protein assumed to be zein for HP and CGM.

On an actual yield basis, greater amounts of zein can be obtained from CGM than from HP or DDG (Table 1). However, recovery efficiency for zein extracted HP and DDG compare favorably with zein extracted from CGM as long as NaOH is present during the extraction. What is curious is how inefficient the recovery of zein is from HP and DDG when NaOH is not present during the extraction. Heat is known to denature proteins. In a traditional bioethanol facility, DDG proteins are subjected to elevated temperatures during 3 processing steps, cooking, distillation and drying. HP and DDG used in this study were from a raw starch hydrolysis fermentation; therefore, corn was not subjected to a cooking step. To eliminate the 2 other heating points, zein was extracted from beer solids. Beer solids were obtained by recovering the solids from the beer prior to distillation.

The recovery of zein from beer solids shows the same trend as recovery from HP and DDG, regardless of whether the solids were from a whole corn plant or a fractionation plant (Table 2). If NaOH was present during extraction, recovery efficiency was 80%. If no NaOH was used during extraction, recovery efficiency was less than 40%. Heat the proteins experience during ethanol production does not seem to be the reason for poor zein extraction efficiency for DDG, HP or beer solids.
Table 2. Extraction of zein from beer solids.

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Protein starting material (%)</th>
<th>Potential zein available (g)</th>
<th>Zein recovered Yield (g)</th>
<th>Efficiency of recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole corn(^1) (no NaOH)</td>
<td>32.9</td>
<td>6.58</td>
<td>2.4</td>
<td>36.5</td>
</tr>
<tr>
<td>Whole corn(^1) (NaOH)</td>
<td></td>
<td></td>
<td>5.2</td>
<td>79.0</td>
</tr>
<tr>
<td>Frac(^2) corn(^3) (no NaOH)</td>
<td>39.8</td>
<td>9.95</td>
<td>3.6</td>
<td>36.2</td>
</tr>
<tr>
<td>Frac corn(^2) (NaOH)</td>
<td></td>
<td></td>
<td>7.4</td>
<td>74.4</td>
</tr>
</tbody>
</table>

*Denotes whether NaOH was added to 70% aqueous ethanol during extraction.
\(^1\)40% of the available protein assumed to be zein for whole corn DDG (distillers dried grains).
\(^2\)50% of the available protein assumed to be zein for HP (high protein distillers dried grains) and CGM (corn gluten meal).
\(^3\)Frac is fractionated corn; only the endosperm goes to fermentation.

RP-HPLC analysis of the recovered zein extracted with and without NaOH showed differences in the type of zein extracted. Using our RP-HPLC procedure, \(\alpha\)-zein came out starting at 40 min and \(\beta\)-zein and \(\gamma\)-zein came out at 25 min. For zein extracted from HP without NaOH, only \(\alpha\)-zein was obtained (Figure 1A). For zein extracted from HP with NaOH, not only \(\alpha\)-zein but also \(\beta\)-zein and \(\gamma\)-zein were recovered (Figure 1B). Similar trends (data not shown) were detected for zein extracted from DDG and beer solids. If NaOH was used during the extraction, \(\beta\)-and \(\gamma\)-zein were recovered along with \(\alpha\)-zein. If no NaOH was used during extraction, only \(\alpha\)-zein was obtained.

The increased amount of zein recovered was not just additional \(\beta\)-zein and \(\gamma\)-zein; also, there was an increase in the amount of \(\alpha\)-zein recovered. Area under the curve was used to estimate the amount of \(\beta\)-zein and \(\gamma\)-zein extracted from HP. \(\beta\)-zein and \(\gamma\)-zein account for 25% (Figure 1B) of the recovered zein or 2.3 g according to Table 1 (9.1*0.25). If the amount of \(\beta\)-zein and \(\gamma\)-zein (2.3 g) is removed from the total yield (9.1 g), an estimated yield of \(\alpha\)-zein extracted using NaOH is 6.8 g. Comparing the yield of zein recovered when NaOH was not used during extraction with the estimated amount of \(\alpha\)-zein recovered when NaOH was used during extraction, over 2 g of additional \(\alpha\)-zein was obtained when using NaOH during the extraction. Similar results were seen when NaOH was used to extract DDG and beer solids. Not only were \(\beta\)-zein and \(\gamma\)-zein obtained when NaOH was used during extraction but there also was a greater amount of \(\alpha\)-zein obtained.
RP-HPLC analysis of recovered zein obtained by extracting CGM with and without NaOH was indicative that using NaOH makes little difference on zein obtained (Figure 2). When CGM was extracted without NaOH, some β-zein and γ-zein were seen in the zein. When ethanol coproducts were extracted without NaOH, no β-zein and γ-zein were seen in the RP-HPLC results. When NaOH was used during extraction of CGM, only slight increases in β-zein and γ-zein were seen. Estimates from the area under the curves for β-zein and γ-zein (Figure 2) for zein obtained without and with NaOH present during extraction were 2.3 and 2.9%, respectively.

One difference between CGM and ethanol fermentation coproducts is that no sulfur dioxide steeping takes place during ethanol fermentation. Steeping is done to break disulfide bonds in the protein matrix to free the trapped starch granules (Johnson and May 2003). β-zein and γ-zein contain many disulfide bonds; these proteins are on the periphery of the protein bodies (Lending and Larkins 1989). By steeping corn with sulfur dioxide, β-zein and γ-zein on the periphery of the protein bodies are disrupted. This disruption of the protein body allows for good extraction efficiency when zein is extracted from corn gluten meal regardless of whether NaOH is used during extraction.
Figure 2. RP-HPLC of zein extracted from CGM: A extracted without NaOH; B extracted with NaOH.

To obtain good extraction efficiencies when extracting zein from HP, it was necessary to use NaOH during extraction. It was hypothesized the protein bodies were not altered and remained intact during fermentation. To test this hypothesis, HP was steeped for 4 days in a 1% aqueous sodium metabisulfite solution to emulate steeping in a corn wet milling facility. Zein was extracted from the steeped HP using 70% aqueous ethanol and 70% aqueous ethanol containing NaOH. Zein extraction efficiency was improved when extracting metabisulfite steeped HP (Table 3). There was no increase in extraction efficiency when using NaOH during the extraction of metabisulfite steeped HP.
Table 3. Zein extracted from metabisulfite steeped HP.

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Protein starting material (%)</th>
<th>Potential zein available (g)</th>
<th>Zein recovered, Yield (g)</th>
<th>Efficiency of recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steeped HP (no NaOH)</td>
<td></td>
<td></td>
<td>7.2</td>
<td>63.7</td>
</tr>
<tr>
<td>Steeped HP (NaOH)</td>
<td>45.1</td>
<td>11.3</td>
<td>8.9</td>
<td>78.8</td>
</tr>
</tbody>
</table>

*Denotes whether NaOH was added to 70% aqueous ethanol during extraction

Figure 3. RP-HPLC of zein extracted from sodium metabisulfite steeped HP.

RP-HPLC results of zein extracted from metabisulfite steeped HP looked similar to the RP-HPLC results obtained for zein extracted from corn gluten meal. Some β-zein and γ-zein were obtained even when no NaOH was used during extraction (Figure 3).
Even if NaOH was in the 70% aqueous ethanol during extraction, little or no additional β-zein and γ-zein was obtained, compared to steeped HP extracted without NaOH. One reason for the decrease in the amount of β-zein and γ-zein seen in the RP-HPLC results was that γ-zein is water soluble when reduced (Paulis and Wall 1977). Water soluble γ-zein would be lost in the steep liquor. This proved to be the case when the steeped liquor was analyzed by and RP-HPLC (Figure 4).

![Figure 4. RP-HPLC of γ-zein obtained from sodium metabisulfite steep liquor.](image)

**CONCLUSIONS**

Zein was extracted and recovered from coproducts of a raw starch fermentation process. Yields of zein obtained from extracting these coproducts can be improved if NaOH was used along with 70% aqueous ethanol during extraction. Protein bodies of corn are thought to remain intact during ethanol production. Because protein bodies remain intact during ethanol production, NaOH is needed to break disulfide bonds along the periphery and disrupt the protein bodies. Zein recovery from HP also can be increased by steeping HP with metabisulfite prior to extraction. Steeping is thought to act like sulfur dioxide steeping in corn wet mill facilities and break disulfide bonds and disrupt the protein bodies. Steeping breaks the disulfide bonds in γ-zein, rendering it water soluble; γ-zein can be found in the steep liquor.
LITERATURE CITED


BACTERIAL CONTAMINATION OF COMMERCIAL STARCH BASED ETHANOL PRODUCTION

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Bacterial contamination is a continual problem in commercial fermentation cultures, particularly in fuel ethanol fermentations, which are not performed under sterile, pure culture conditions (Connolly 1999). Chronic bacterial contamination poses a constant drain on the sugar available for conversion to ethanol and the bacteria scavenge essential micronutrients required for optimal yeast growth and ethanol production. Acute infections occur unpredictably; bacterial byproducts such as acetic and lactic acids inhibit yeast growth and may result in “stuck” fermentations that require costly shut downs of facilities for cleaning (Makanjuola et al 1992, Nareanrathan et al 1997). Despite efforts to prevent contamination with extensive cleaning and disinfecting procedures, saccharification tanks, continuous yeast propagation systems and notoriously drug resistant biofilms can act as reservoirs for bacteria which continually reintroduce contaminants (Skinner and Leathers 2004, Skinner-Nemec et al 2007).

Although it is believed that lactic acid bacteria are the primary bacterial contaminants of fuel ethanol fermentations, a variety of gram positive and gram negative bacteria, including species of Lactobacillus, Lactococcus, Pediococcus, Enterococcus, Acetobacter, Gluconobacter and Clostridium (Connolly 1999; Skinner and Leathers 2004; Lushia and Heist 2005), have been isolated from fuel ethanol fermentations. We conducted a quantitative study on the natural occurrence of bacteria in commercial corn based fuel ethanol production. Samples were collected from one wet mill and two dry grind fuel ethanol facilities at strategic time points and locations along production lines.

Lactobacillus species were the most abundant isolates from all 3 plants, averaging 51, 38 and 77% of total isolates from the wet mill and the first and second dry grind facilities, respectively (Table 1). Although populations varied over time, individual facilities tended to exhibit characteristic bacterial profiles, suggesting the occurrence of persistent endemic infections. Contamination in the wet mill facility consistently reached $10^6$ colony forming units/ml (CFU/ml), while titers from dry grind facilities were more variable but often reached $10^8$ CFU/ml. Antibiotics were not used in the wet mill operation. One dry grind facility added antibiotic to the yeast propagation tank only,
while the second facility dosed the fermentation with antibiotic every 4 hr. Neither
dosing procedure appeared to reduce reliably overall contamination, although the second
facility showed less diversity among contaminants.

Table 1. Bacterial genera found in surveys of fuel ethanol plants.\(^a\)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Wet mill</th>
<th>Dry Grind #1</th>
<th>Dry Grind #2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium</em></td>
<td>0 to 20</td>
<td>1 to 2</td>
<td>0</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>0 to 9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>44 to 60</td>
<td>37 to 39</td>
<td>69 to 87</td>
</tr>
<tr>
<td><em>Lactococcus</em></td>
<td>0 to 4</td>
<td>0 to 6</td>
<td>0</td>
</tr>
<tr>
<td><em>Leuconostoc</em></td>
<td>0 to 6</td>
<td>1 to 8</td>
<td>0 to 8</td>
</tr>
<tr>
<td><em>Pediococcus</em></td>
<td>0 to 6</td>
<td>19 to 24</td>
<td>0 to 4</td>
</tr>
<tr>
<td><em>Weisella</em></td>
<td>0 to 2</td>
<td>18 to 24</td>
<td>0 to 6</td>
</tr>
</tbody>
</table>

\(^a\)Data are derived from Skinner and Leathers (2004). Values represent a range over multiple samplings.

In addition to planktonic growth in the fermentor, bacteria may exist in biofilms throughout the commercial production environment. A great deal of literature is available on biofilm formation, but little is relevant to fuel ethanol production. In a preliminary study, we found for the first time that bacterial contaminants were capable of forming biofilms under laboratory conditions (Skinner-Nemec et al 2007). Fermentor samples from a commercial fuel ethanol production facility were used to inoculate a biofilm reactor and purified bacterial isolates were identified. Biofilms were composed of many of the same species, with lactic acid bacteria predominating, present in production samples. More recently, we developed methods to evaluate rapidly the antibiotic susceptibility of fuel ethanol contaminant biofilms (Rich et al 2011).

Pure cultures of *Saccharomyces cerevisiae* are stressed by added acetic and lactic acids (Narendranath et al 2001, Thomas et al 2002, Bayrock and Ingledew 2004) but the literature is contradictory regarding the effects of contaminating microorganisms. Narendranath et al (1997) reported ethanol reductions of up to 7.6% when \(10^5\) to \(10^9\) CFU of various *Lactobacillus* species were introduced into wheat mash fermentations. In contrast, Chin and Ingledew (1994) found that wheat mashes artificially infected with *L. fermentum* or *L. delbrueckii* at \(10^8\) CFU/ml were not impaired seriously in ethanol productivity. Thomas and coworkers (2001) found that various species of *Lactobacillus* inoculated at \(10^7\) CFU/ml did not affect fermentation rates or yeast viability in corn
mashes. However, if bacteria were precultured in mash for 24 hr to a density of \( >10^9 \) CFU/ml, ethanol production was reduced up to 22%.

Recently, we developed a model system to measure quantitatively the effects of bacterial contamination on fuel ethanol production (Bischoff et al 2009). The effects on corn mash fermentations experimentally infected with lactic acid bacteria were species and dose dependent (Table 2, Figure 1). Thus, the deleterious effects of contamination may depend on the specific contaminants present and culture conditions employed.

### Table 2. Challenge of *S. cerevisiae* fermentations with lactic acid bacteria.

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>Δ Ethanol (g/l)</th>
<th>Δ Glucose (g/l)</th>
<th>Δ Lactic (g/l)</th>
<th>Δ Acetic (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. fermentum</em> 0315-1</td>
<td>7</td>
<td>-15.8</td>
<td>31.4</td>
<td>2.88</td>
<td>1.52</td>
</tr>
<tr>
<td><em>L. fermentum</em> 0315-25</td>
<td>3</td>
<td>-16.1</td>
<td>28.7</td>
<td>2.52</td>
<td>1.27</td>
</tr>
<tr>
<td><em>L. brevis</em> 84</td>
<td>3</td>
<td>-29.6</td>
<td>54.1</td>
<td>4.90</td>
<td>2.17</td>
</tr>
<tr>
<td><em>L. amylovorus</em> 0315-7B</td>
<td>3</td>
<td>-5.00*</td>
<td>ND*</td>
<td>3.94</td>
<td>-0.025*</td>
</tr>
<tr>
<td><em>L. delbrueckii</em> ATCC 4797</td>
<td>3</td>
<td>-0.458*</td>
<td>ND*</td>
<td>2.30</td>
<td>0.0889</td>
</tr>
<tr>
<td><em>W. confusa</em> 0216-2</td>
<td>10</td>
<td>-3.3</td>
<td>7.87</td>
<td>1.96</td>
<td>0.515</td>
</tr>
</tbody>
</table>

*aCultures used corn mash as feedstock and were inoculated with approximately \( 10^7 \) CFU/ml *S. cerevisiae*. Cultures were challenged with the indicated strains at a density of \( 10^7 \) CFU/ml. Data are reported as the change in mean values of the indicated parameter between challenged cultures and unchallenged controls. N indicates the number of replicate cultures. ND indicates that residual glucose concentrations were not detectable. *Mean values between challenged and unchallenged controls were not different (\( P > 0.05 \)). Data are from Bischoff et al (2009).
Figure 1. Dose response on ethanol fermentation by varying inoculum of \textit{L. fermentum} 0315-1. Cultures of \textit{S. cerevisiae} grown on corn mash feedstock were challenged with the indicated inoculum of \textit{L. fermentum} 0315-1. The following products were measured after 72 hr incubation: ethanol (■), residual glucose (□), lactic acid (▲) and acetic acid (♦). Data are reported as mean values ± standard deviation of triplicate cultures. Final concentrations for the control culture (no bacterial challenge) were as follows: ethanol = 108 ± 3 g/l, glucose = 4.8 ± 3.6 g/l, lactic acid = 0.63 ± 0.01 g/l and acetic acid = 0.31 ± 0.04 g/l. Data are from Bischoff et al (2009).

A variety of antimicrobial agents have been described to control bacterial contamination in ethanol fermentations. Many are antibiotics, including virginiamycin, penicillin, tetracycline and monensin (Stroppa 2000, Bayrock et al 2003, Hynes et al 1997), also used in clinical and veterinary medicine or in food animal production. Other nonantibiotic agents include urea hydrogen peroxide and hop acids (Narendranath et al 2000, Ruckle 2006). Chemical disinfectants like chlorine dioxide also are marketed for use in fuel ethanol production. In industrial fermentations in the US, however, the most common commercially available products used to control contamination are based on the antibiotics virginiamycin or penicillin (Connolly 1997, Lushia and Heist 2005).
The emergence of drug resistant strains may limit the effectiveness of these agents. Decreased susceptibility to virginiamycin has been observed in *Lactobacillus* species isolated from dry grind ethanol plants that use virginiamycin; the emergence of isolates with multidrug resistance to both virginiamycin and penicillin also has been reported (Bischoff et al 2007, Lushia and Heist 2005). Thirty-eight percent of *Lactobacillus* isolates from a dry grind ethanol plant that routinely used virginiamycin were found to possess the virginiamycin resistance gene vatE (Bischoff et al 2007). New antibacterial agents and new drug management methods may need to be developed to mitigate the emergence of resistant strains. Current efforts include the development of antibacterial proteins and bacteriophage as replacements for antibiotics.

Other issues regarding the use of antibiotics to control contamination involve the potential for drug residues to enter the food chain via animal feed produced from distillers grains. It is believed the instability of penicillin at the fermentation pH and the heat lability of virginiamycin during the drying process effectively degrade these drugs (Shurson et al 2003). However, depending on the proximity of feed lots, the distillers grains may not be dried and other classes of drugs, such as macrolides, may be used in commercial ethanol fermentations. Data on the stability of these other drugs in distillers grains is lacking; currently there is no regulatory monitoring for drug residues in distillers grains.

Since treatment for contamination often is prophylactic, which necessitates the addition of antibiotics to each fermentor, control and treatment of bacterial contamination may be a critical control point for decreasing costs and improving efficiency of both starch and cellulosic ethanol production. Drug resistance and regulatory constraints may limit future use and effectiveness of antibiotics. Biotechnology can supplement or replace antibiotics for effective contamination control in commercial fuel ethanol production.

**LITERATURE CITED**


SOLVING PROBLEMS IN FUEL ETHANOL PRODUCTION

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INTRODUCTION

Even the best run plant encounters many operational problems and less well run ones encounter many more. Some of these problems can cause unsafe conditions but more often they hurt only profitability. Inefficiency, down time, excessive maintenance, environmental penalties and off spec products are the most common results of Murphy’s Law in ethanol plants.

We will not present a long introduction, because most readers will be familiar with the process, at least in general terms. We will discuss some examples of problems, both common and unusual, and the methods used to overcome them, that have occurred in dry grind fuel ethanol plants.

DEPOSITS

Deposits inside process equipment are common problems. They decrease flow and increase backpressure, impede heat transfer and provide a hideout for bacterial infections. The first step is to identify the material. About half the deposits have been primarily organic, composed of proteinaceous material and/or mash. While caustic clean in place (CIP) works reasonably well for these deposits, there are many instances where large areas do not get clean. The deposit becomes an “anchor” for future deposits, but even worse, a breeding ground for bacteria and a significant source of infection. This is especially true with plate and frame heat exchangers where even a short period of poor flow and distribution can lead to material stuck in crevices.

One organic deposit that caused widespread problems appeared in many ethanol plants during a recent winter. A sticky brown material with a consistency between
chewing gum and soft rubber was plugging filters in load out areas. It was suggested by some equipment engineers, who were not chemists, that its origin was the corrosion inhibitor added to the ethanol. However, the rubbery material was insoluble in the corrosion inhibitor or in ethanol. It was, however, quite soluble in gasoline. It also had a small and variable amount of inorganic material (1 to 8%), which appeared to be entrained solids rather than inherent to the deposit.

An infrared spectrum of the material showed an excellent match to certain butyl polymers. Knowing the identity of a deposit allows one to make informed hypotheses, rather than hasty accusations. The polymer was similar to those commonly used as gasoline additives. In this case, the polymer was added to the gasoline to make it flow through the pipeline with less turbulence (ie, less drag.) When this gasoline was used as denaturant for the ethanol, the polymer found itself in a solvent where it was insoluble and gummed up filters were the result. Denaturant suppliers, while not admitting they had anything to do with the problem, generally have agreed to avoid the use of such polymers in gasoline to be used for denaturing ethanol.

**Inorganic Deposits**

Inorganic material presents a greater diversity of deposits than the organics. Depending on temperature, concentration, pH and other parameters, different compounds will precipitate in different places. Typically, these are harder than organic material and are not removed by caustic CIP. They usually can be removed with acid CIP but this often is slow. The formation of inorganic deposits can often be reduced or prevented by altering conditions or adding inhibitors.

Inorganic compounds characteristically are soluble in water to an extent defined as the solubility constant, $K_{sp}$. $K_{sp}$ for an ionic compound is equal to the concentration of the positive ion ($M^+$) times the concentration of the negative ion ($X^-$).

$$K_{sp} = [M^+] * [X^-]$$

If $[M^+] * [X^-] > K_{sp}$, the solution is supersaturated and the compound can precipitate. If $[M^+] * [X^-] < K_{sp}$, it is undersaturated and can dissolve.

As any chemistry student will tell you, it is never that easy. The solubility constant is not truly a constant. It changes, often significantly, with temperature. Most compounds become more soluble as the temperature increases but some do just the opposite and precipitate when heated. This causes the “lime” deposits on the bottom of a coffee pot and similar deposits in boilers and heat exchangers.
Furthermore, the negative ion is often a weak acid or base so only a fraction of the total is actually in the form that can react with M$^+$ to form a precipitate. This fraction depends on pH of the water. For example, bicarbonate ion, HCO$_3^-$, can become carbonic acid (H$_2$CO$_3$) at low pH, or it can become carbonate ion (CO$_3^{2-}$) at high pH. At neutral pH, about 0.1% of the total is present as CO$_3^{2-}$, while at pH 9, roughly 10% is CO$_3^{2-}$. Since the formation of calcium carbonate depends on CO$_3^{2-}$, not either of the other two forms, CaCO$_3$ is 100 times more likely to precipitate at pH 9 than at pH 7. By the same logic, going to low pH reduces the fraction in the CO$_3^{2-}$ form so much that [M$^+$] * [X$^-$] is now less than K$_{sp}$ and the deposit dissolves. This is how acid cleaning works.

But wait! It still is not that easy! These considerations are based on thermodynamics, which tells us what will happen after an infinite amount of time. Precipitation is also a kinetic process and we can use this to our advantage. We do not have to stop precipitation, we just have to make sure it does not happen where we do not want it. There are inhibitors which slow down precipitation so much that it occurs after water has left the process. (This is one of the major problems with zero liquid discharge, ZLD, because the water never leaves.) There also are dispersants which allow the precipitation to occur, but keep the solid from sticking to surfaces. Now let us look at 3 compounds of particular interest: calcium oxalate, magnesium phosphate and silica.

**Calcium Oxalate**

Calcium oxalate, CaC$_2$O$_4$.xH$_2$O, is well known as “beerstone.” It is a hard, tenacious material that dissolves slowly, even in 5% acid solutions. Oxalate is produced by most plants, apparently as part of the cell wall but for reasons that are not well understood. Also, there are numerous reports of fungi related to yeast that produce large amounts of oxalate during fermentation; therefore, it is possible some oxalate is formed this way. Corn is low in calcium content but water used for the process can have low, medium or high levels of calcium, depending on the source of the water.

Calcium oxalate is more soluble at high temperature. The K$_{sp}$ is low, so that at low temperature, only a few ppm oxalate can remain in solution. At high temperature, this increases (Table 1).
Table 1. Oxalate solubility.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Maximum oxalate, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ppm Ca, 75°C</td>
<td>11.5</td>
</tr>
<tr>
<td>250 ppm Ca, 75°C</td>
<td>2.3</td>
</tr>
<tr>
<td>250 ppm Ca, 55°C</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Although calcium oxalate dissolves slowly, the longer the stillage/syrup remain hot and the higher the temperature, the more oxalate will dissolve. In evaporators, the concentration increases as the water is removed. (If half the water is removed, the dissolved material stays behind, and is at double the concentration.) The solution also cools during evaporation, making things even worse in the case of calcium oxalate. This explains why virtually all the calcium oxalate deposits we have seen in ethanol plants have been in the evaporators. One particularly severe case involved a plant that tried to recycle RO reject water, which had high levels of calcium, into their process. The increase in calcium in the evaporators led to heavy deposits of calcium oxalate. Hydroblasting extended a shutdown by more than a day.

Other than keeping calcium levels low, there are options for preventing beerstone. Reducing levels in whole stillage and thin stillage tanks decreases the time available for the oxalate to dissolve. Keeping pH below 4.5 also will help in theory, but this can be problematic. We found inhibitors that extended the time between cleanings by a factor of 2 to 3.

**Magnesium Phosphate**

The form of magnesium phosphate commonly found in ethanol plants is magnesium monohydrogen phosphate, MgHPO₄.xH₂O. It is soluble at moderate pH; therefor, it forms mainly in evaporators where the concentration in multiplied over that in fermentors. Struvite, ammonium magnesium phosphate, NH₄MgPO₄.6H₂O, also has been reported but mainly at the front end of the plant where ammonia is added. Both magnesium and phosphate come from corn. Typical concentrations are given in Table 2.
Table 2. Inorganic concentrations and pH in corn.

<table>
<thead>
<tr>
<th></th>
<th>Plant A</th>
<th>Plant B</th>
<th>Plant C</th>
<th>Plant D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syrup</td>
<td>TS</td>
<td>Syrup</td>
<td>TS</td>
</tr>
<tr>
<td>SO₄</td>
<td>4950</td>
<td>1130</td>
<td>3495</td>
<td>600</td>
</tr>
<tr>
<td>PO₄</td>
<td>18900</td>
<td>3200</td>
<td>20500</td>
<td>4140</td>
</tr>
<tr>
<td>Ca as Ca</td>
<td>124</td>
<td>39</td>
<td>149</td>
<td>34</td>
</tr>
<tr>
<td>Mg as Mg</td>
<td>3250</td>
<td>580</td>
<td>2680</td>
<td>570</td>
</tr>
<tr>
<td>K</td>
<td>10250</td>
<td>1790</td>
<td>11000</td>
<td>2290</td>
</tr>
<tr>
<td>SiO₂</td>
<td>250</td>
<td>45</td>
<td>218</td>
<td>47</td>
</tr>
<tr>
<td>pH</td>
<td>5.12</td>
<td>5.35</td>
<td>4.64</td>
<td>4.65</td>
</tr>
</tbody>
</table>

To better understand conditions that allow precipitation, laboratory studies were performed. By combining various volumes of concentrated solutions of magnesium chloride, potassium monohydrogen phosphate (adjusted to the desired pH) and ionic strength adjuster, a series of experiments was conducted that spanned the range of expected concentrations. Samples were stored in sealed vials at 20, 50 or 70°C. Only the most concentrated at the highest pH showed precipitation in less than a few hours. For those which did not precipitate over night, a small crystal from another vial was placed in the vial and crushed to provide seed crystals. Once all precipitation had stabilized, and solutions presumably were near equilibrium, pH and PO₄ were measured. The concentration of magnesium was calculated using the difference between the initial and measured phosphate, determining the amount of Mg theoretically consumed by the lost PO₄, and subtracting that from the initial Mg. A graph of (ppm Mg) * (ppm PO₄) is shown in Figure 1.

MgHPO₄ is less soluble at higher pH. This is because the fraction of total phosphate that is in the HPO₄⁻² form increases over this pH range. When the concentration of HPO₄⁻² is calculated using known pKa values, a nearly constant value of [Mg²⁺][HPO₄⁻²] is found. This value, 5*10⁻⁵ M², is consistent with previously reported values. Samples stored at 70°C had slightly lower solubility; those at 30°C had slightly higher but differences were small. Ionic strength in the range 0.6 to 0.8 also had a small effect, with higher I resulting in higher solubility (as determined by analytical concentrations rather than ionic activities). When I was kept constant but sulfate increased in the solution, solubility increased 15%. This was attributed to the formation of a soluble MgSO₄ ion pair.
Figure 1. Magnesium and phosphate concentrations.

Preventing magnesium phosphate scale would require adding acid to lower pH but this can be expensive and increases sulfur present in DDGS. Certain polymeric compounds have been found that can inhibit precipitation and reduce deposits.

Silica

Silica is an occasional problem deposit. Silica solubility is less affected by pH but more affected by temperature. At 100C, solubility is 400 ppm, while at 50C, it is only 180 ppm. Silica can come from the water, but deposits are more common where sorghum is used as the source of starch. Sorghum has more silica than corn. The longer stillage remains at high temperature, the more silica will dissolve. As this concentrates and cools in the evaporators, silica will supersaturate. Hard deposits can result, which are unaffected by strong acid and only slightly attacked by strong caustic. This has been a nuisance where it has had to be removed mechanically from syrup lines. However, it is probable that when grasses or stover will be used for fermentation, it will be a much worse problem, because these contain substantial amounts of silica. No silica inhibitor yet available is approved for applications where it ends up in animal foodstuffs.
Environmental Compliance/Product Quality

The fermentation process produces large volumes of carbon dioxide, along with the ethanol, and smaller amounts of numerous organic compounds. One of the most important from an environmental compliance aspect is acetaldehyde. It is regulated as a hazardous air pollutant (HAP).

During the fermentation process, a great deal of carbon dioxide is given off and vented to the atmosphere. This carries away a significant amount of ethanol in the vapor form. To recover this valuable product, vented gas usually is washed with water in a countercurrent packed bed chimney called a “scrubber”.

Also among the vapors produced is acetaldehyde. This is considered a HAP and most plants need to remove it from the gas which is vented to the atmosphere. This usually is done by dosing the water in the scrubber with bisulfite ions (HSO$_3^-$). Bisulfite reacts with acetaldehyde to form hydroxyethanesulfonic acid. This is not volatile and remains in the aqueous phase. Certain catalyzed forms of bisulfite react more rapidly and completely with acetaldehyde, allowing lower feed rates while also decreasing unreacted bisulfate, which can become a yeast stressor, in scrubber bottoms.

It is important to minimize the amount of water sent to the process, and to minimize the amount of bisulfite. However, it is essential to remember that “minimize” and “optimize” are not the same! If the water flow is too low, excessive amounts of bisulfite are needed and ethanol vapor also escapes.

The plant must pay for expensive testing to show that it meets air pollution permit limits. Once it has shown that it passes at a certain flow rate of water and a certain dose of bisulfite, it cannot operate at a lower flow or lower dose (without retesting). These parameters are set during the initial testing, but over time scrubber effectiveness may become impaired through buildup of deposits, usually microbial slime layers but sometimes iron based as well. Slime diverts water from the off gas; therefore, there is incomplete mixing. Demisters often become fouled as well, causing increased backpressure in the fermenters. We developed a regimen of quarterly cleaning with an acidic oxidizing agent which has biodispersant capability. This removes biological and inorganic deposits and restores the scrubber to original capacity.

Experience is vital in determining problem causes. At one plant, the amount of pollutants, expressed in tons/yr emitted through the scrubber, was determined to be over permit limits by the testing laboratory. In reviewing the data, in terms of ppm, results were normal. The testing laboratory had mismeasured the diameter of the scrubber at 34
instead of 23 inches; therefore and had calculated the amount of gas to be twice what it actually was!

Another case illustrates one of the more unusual air pollutants that can appear. A plant implemented a program to reduce acid consumption. This plant had a continuous emissions monitoring system on their thermal oxidizer; when acid was cut back, NO\textsubscript{x} increased. This made no sense but when acid was restored, NO\textsubscript{x} decreased; when acid was again cut back, NO\textsubscript{x} again went up. Realizing the only reasonable source of nitrogen was ammonia vapor, it was determined there was an ammonia leak, which excess acid feed had neutralized to the nonvolatile ammonium form. When acid was decreased, NH\textsubscript{3} was no longer neutralized and escaped through the vapor system to the TO, where it was oxidized to NO\textsubscript{x}. When this was fixed, both acid and ammonia consumption decreased.

**Corrosion Inhibitor**

One final example of unexpected observations involved the final product. Because ethanol is more corrosive than gasoline, standard practice is to add 30 to 100 ppm corrosion inhibitor. This generally was done by running a pump at a low rate during transfer, or by running a pump at higher rate for part of the time the transfer was being done. Since the amount of inhibitor added to each transfer was small, and the compounds in the inhibitors were difficult to analyze, it was difficult to know precisely how much was added to each transfer. Over a longer period, it was simple to determine that since, say, 60 gallons of inhibitor were used, and 1,000,000 gallons of ethanol were produced, the average concentration was 60 ppm during that time. With a traced inhibitor, it becomes possible to determine accurately the amount in each transfer, by simply measuring the tracer. Since some plants feed inhibitor directly into the transport vessel (rail car or tank truck) rather than a storage tank, this is particularly important. At one plant, it was discovered the valves on the inhibitor feed lines were controlled incorrectly, so that rail cars received extra inhibitor, while tank trucks received too little. Once this was corrected and the feed rate properly adjusted, each load had the correct amount of inhibitor.

In conclusion, there are innumerable opportunities for improvement in any process, especially so with a relatively young industry such as fuel ethanol. Logic and methodical hard work complement intuition and guesswork. Experience must be humbled properly by the astonishing ingenuity of Mother Nature, who keeps coming up with new ways to surprise us.
The author would like to thank the US Water Services Ethanol Process Technologies team, particularly Roy Johnson, Monty McCoy, Todd Emslander, Charlie Kroeger and John McInnis, and Duane Weber and Nicole Johnson of US Water Services analytical department.

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IMPACT OF PRETREATMENT AND DOWNSTREAM PROCESSING TECHNOLOGIES ON ECONOMICS, ENERGY AND WATER USE IN CELLULOSIC ETHANOL PRODUCTION

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While advantages of biofuels have been reported (Wyman et al 2005, Mosier et al 2005), investigators also highlight challenges in large scale production of biofuels (Pate et al 2007). In particular, challenges in energy and water use in production of biofuels need to be addressed in a holistic manner for establishing biofuels as long term sustainable alternatives to fossil fuels. In this context, cellulosic ethanol is proposed as a more sustainable alternative to fossil fuels. Water and energy use in cellulosic ethanol plants is dependent on technologies used for conversion of feedstock. Choice of pretreatment and downstream processing technologies is critical in this context. Tradeoffs among capital cost, production cost, energy and water use exist and must be examined to obtain site and feedstock specific technologies for deployment of appropriate conversion technologies.

A comprehensive technoeconomic analysis was performed for conversion of cellulosic feedstocks to ethanol using some of the most common pretreatment technologies (Ballesteros et al 2004, Bjerre et al 1996, Laser et al 2002, Lloyd and Wyman 2005, Mosier et al 2005, Sun and Cheng 2005, Wyman et al 2005). Detailed process models incorporating feedstock handling, pretreatment, simultaneous saccharification and cofermentation, ethanol recovery, coproduct utilization to produce process steam and electricity, waste water handling were developed using SuperPro Designer (Intelligen, Inc., Scotch Plains, NJ). The process model was developed for an ethanol plant with a processing capacity of 250,000 metric ton biomass/yr.

The ethanol production process was divided into four sections: front end operations (eg, cleaning and size reduction of biomass), pretreatment of biomass, simultaneous saccharification and cofermentation (SSCoF) and downstream processing (Figure 1). Downstream processing consisted of pure ethanol recovery using distillation and molecular sieves, coproduct (lignin) recovery and utilization to generate steam and electricity and waste water treatment. Alternative scenarios were modeled by developing models for four pretreatment technologies, dilute acid (DA), dilute alkali (AL), hot water
(HW) and steam explosion (SE), and two alternative technologies (anaerobic digestion and multiple effect evaporators) for handling process streams. Detailed chemical oxygen demand (COD) and energy calculations were performed for various waste streams to determine methane production during anaerobic digestion and steam and electricity production from combustion of waste streams.

Equipment costs were calculated based on built in cost models in SuperPro designer. For individual pieces of equipment, especially in bioethanol plant (pretreatment reactor, pneumapress filter, shredder, fermenters), cost models were used based on earlier models for cellulosic (Aden et al 2002, Laser et al 2009) and corn ethanol (McAlloon et al 2000, Kwiatkowski et al 2006) plants. Models were developed to analyze the performance of alternative scenarios on a consistent basis.

![Diagram](image)

Figure 1. Generic cellulosic ethanol production process.

Tall fescue (*Festuca arundinacea Schreb*) containing 31% cellulose, 20.2% hemicellulose, 14.4% lignin, 19.4% extractives, 5% protein and 10% ash (Kumar and Murthy, 2011) was used as a model feedstock. Tall fescue grass was chosen as a model feedstock as it comprises the largest fraction of the grass straw residues available for

Capital costs for an ethanol plant processing 250,000 tons feedstock/yr were $1.92, 1.73, 1.72 and 1.72 for processes using DA, AL, HW and SE pretreatment technologies, respectively. Projected ethanol yields were 252.62 (67.80), 255.80 (67.58), 255.27 (67.44) and 230.23 L/dry ton (60.83 gal/dry ton) for conversion processes using DA, AL, HW and SE pretreatment technologies, respectively. The standard case for analysis of different pretreatment technologies consisted of 25% water recycle to anaerobic digester.

Unit costs of ethanol were $0.84, 0.89, 0.81 and 0.86/L ethanol for processes using DA, AL, HW and SE pretreatment technologies, respectively. Energy uses were 0.811, 0.800, 0.825 and 0.576 MJ/L ethanol for processes using DA, AL, HW and SE pretreatment technologies, respectively. Water uses were 5.96, 6.07, 5.84 and 4.36 kg/L ethanol for processes using DA, AL, HW and SE pretreatment technologies, respectively, for the standard case.

Table 1. Overall economics of an ethanol production plant with 25 MT biomass processing for various pretreatments.

<table>
<thead>
<tr>
<th></th>
<th>Dilute Acid (DA)</th>
<th>Dilute Alkali (AL)</th>
<th>Hot Water (HW)</th>
<th>Steam Explosion (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Investment (MM$)</td>
<td>114.63</td>
<td>102.77</td>
<td>101.89</td>
<td>91.36</td>
</tr>
<tr>
<td>Operating Cost (MM$/yr)</td>
<td>50.06</td>
<td>52.70</td>
<td>48.10</td>
<td>45.83</td>
</tr>
<tr>
<td>Ethanol Revenue (MM$/yr)</td>
<td>65.41</td>
<td>65.21</td>
<td>65.07</td>
<td>58.64</td>
</tr>
<tr>
<td>Ethanol (MM gal/yr)</td>
<td>15.76</td>
<td>15.71</td>
<td>15.68</td>
<td>14.14</td>
</tr>
<tr>
<td>Ethanol Unit Cost ($/gal)</td>
<td>3.18</td>
<td>3.35</td>
<td>3.07</td>
<td>3.24</td>
</tr>
</tbody>
</table>

*DA: dilute acid; AL: dilute alkali; HW: hot water; SE: steam explosion.

Raw material costs were 45.1, 51.3, 47.3 and 48.5% of total ethanol production cost (Table 2). Major contributions (80 to 96%) to raw material costs were from feedstock and enzyme price for all pretreatments. Hence, potential reduction in ethanol production cost could be achieved by reducing feedstock or enzyme price.

A challenge in cellulosic ethanol production is fermentation of pentose sugars, which are a significant part of biomass. Efficiency of xylose utilization is low for many microorganisms (Bertilsson et al 2008, Chandrakant and Bisaria 2000). Pentose fermentation efficiency of 70% was assumed for model simulations in the standard case analysis for all pretreatments. Sensitivity of pentose fermentation on ethanol price was
investigated by varying the efficiency from 10 to 90 % for dilute acid and steam explosion pretreatment processes (Table 3).

Table 2. Economics of cellulosic ethanol plants using different pretreatments processes.

<table>
<thead>
<tr>
<th>250,000 tons/year capacity</th>
<th>DA*</th>
<th>AL</th>
<th>HW</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Investment ($)</td>
<td>114,626,000</td>
<td>102,766,000</td>
<td>101,889,000</td>
<td>91,359,000</td>
</tr>
<tr>
<td>Operating Cost ($/yr)</td>
<td>50,063,000</td>
<td>52,699,000</td>
<td>48,102,000</td>
<td>45,831,000</td>
</tr>
<tr>
<td>Ethanol Revenue ($/yr)</td>
<td>65,414,000</td>
<td>65,205,000</td>
<td>65,071,000</td>
<td>58,642,000</td>
</tr>
<tr>
<td>Ethanol (gal/yr)</td>
<td>15,763,325</td>
<td>15,712,997</td>
<td>15,680,495</td>
<td>14,141,994</td>
</tr>
<tr>
<td>Ethanol Unit Cost ($/L($/gal))</td>
<td>0.84 (3.18)</td>
<td>0.89(3.35)</td>
<td>0.81(3.07)</td>
<td>0.86(3.24)</td>
</tr>
<tr>
<td>Direct Fixed Capital ($)</td>
<td>106,376,000</td>
<td>94,680,000</td>
<td>94,236,000</td>
<td>84,259,000</td>
</tr>
<tr>
<td>Equipment Cost ($/yr)</td>
<td>45,373,000</td>
<td>39,515,000</td>
<td>39,170,000</td>
<td>34,963,000</td>
</tr>
<tr>
<td>Installation ($/yr)</td>
<td>19,182,000</td>
<td>18,247,000</td>
<td>18,376,000</td>
<td>16,512,000</td>
</tr>
<tr>
<td>Facility Dependent Cost ($/yr)</td>
<td>17,824,000</td>
<td>15,831,000</td>
<td>15,745,000</td>
<td>14,078,000</td>
</tr>
<tr>
<td>Raw Material Cost ($/yr)</td>
<td>22,562,000</td>
<td>27,052,000</td>
<td>22,731,000</td>
<td>22,233,000</td>
</tr>
<tr>
<td>Others Operating($)</td>
<td>9,677,000</td>
<td>9,816,000</td>
<td>9,626,000</td>
<td>9,520,000</td>
</tr>
<tr>
<td>Others Capital($)</td>
<td>50,071,000</td>
<td>45,004,000</td>
<td>44,343,000</td>
<td>39,884,000</td>
</tr>
<tr>
<td>Effluent Water</td>
<td>207,368,654</td>
<td>208,634,536</td>
<td>206,764,293</td>
<td>128,427,707</td>
</tr>
</tbody>
</table>

*DA: dilute acid; AL: dilute alkali; HW: hot water; SE: steam explosion.

Table 3. Sensitivity of ethanol price to pentose fermentation efficiency.

<table>
<thead>
<tr>
<th>Pentose fermentation efficiency (%)</th>
<th>Ethanol Price ($/L)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilute Acid (DA)</td>
<td>Steam Explosion (SE)</td>
</tr>
<tr>
<td>10</td>
<td>1.201</td>
<td>1.248</td>
</tr>
<tr>
<td>20</td>
<td>1.121</td>
<td>1.161</td>
</tr>
<tr>
<td>30</td>
<td>1.050</td>
<td>1.084</td>
</tr>
<tr>
<td>40</td>
<td>0.987</td>
<td>1.016</td>
</tr>
<tr>
<td>50</td>
<td>0.931</td>
<td>0.955</td>
</tr>
<tr>
<td>60</td>
<td>0.881</td>
<td>0.902</td>
</tr>
<tr>
<td>70</td>
<td>0.839</td>
<td>0.855</td>
</tr>
<tr>
<td>80</td>
<td>0.797</td>
<td>0.807</td>
</tr>
<tr>
<td>90</td>
<td>0.763</td>
<td>0.765</td>
</tr>
</tbody>
</table>

*DA: dilute acid; SE: steam explosion.
Biomass price was observed as a major contributor in total material cost. The impact of biomass price on production cost of ethanol was investigated for dilute acid and steam explosion pretreatment models (Figure 2). Biomass price of $50/ton was assumed for development of actual models, which resulted in biomass costs of $0.2095 and 0.2335/L ethanol for dilute acid and steam explosion pretreatments, respectively. Sensitivity of ethanol cost to biomass price was studied by changing grass straw price from $25 to 100/ton (Figure 2).

Interdependence of water and energy use in the ethanol production process was investigated for DA pretreatment by varying the percentage of water diverted to anaerobic digestion process and multiple effect evaporators. While multiple effect evaporators allow reuse of water they also lead to additional energy use. Anaerobic digestion process can be used to treat process water at relatively low energy cost without reusing the effluent water. Hence the percentage of process water diverted to multiple effect evaporators and anaerobic digester represents a tradeoff in energy and water use.

For DA process, water and energy use showed a 48.6% increase (5.96 to 8.86 kg/L ethanol) and 9.91% decrease (37.87 to 34.12 MJ/L ethanol) as the liquid stream from filter press to anaerobic digester increased from 25 to 50% (Figure 3). Correspondingly, unit price of ethanol and unit capital cost increased from $0.84 to 0.85/L ethanol and $1.92 to $1.96/L ethanol. Since electricity was produced from excess steam not used in the process, electricity production decreased with increasing energy use.
(or decreasing water use) due to increasing use of steam for process needs. Electricity production decreased from 1.13 to 0.77 kWh/L ethanol as the liquid stream from filter press to anaerobic digester decreased from 50 to 25%; correspondingly, process energy use increased from 36.61 to 40.90 MJ/L ethanol. We must address tradeoffs in capital costs, pretreatment and downstream processing technologies in addressing energy and water use in cellulosic ethanol plants.

Figure 3. Impact of process water treatment on capital cost, energy and water use.

**CONCLUSIONS**

Four process models for an ethanol production plant with 250,000 metric ton/yr grass straw processing capacity using different pretreatment technologies were developed using Super Pro Designer. Capital cost of the ethanol production plant ranged from 91.36 MM$ for steam explosion pretreatment to 114.63 MM$ for dilute acid pretreatment for this plant. Unit costs of ethanol were $0.84, 0.89, 0.81 and 0.86/L ethanol for processes using DA, AL, HW and SE pretreatment technologies, respectively. Steam energy use were 0.214, 0.211, 0.218 and 0.152 MJ/MJ ethanol for processes using DA, AL, HW and SE pretreatment technologies, respectively. Water uses were 5.96, 6.07, 5.84 and 4.36 kg/L ethanol for processes using DA, AL, HW and SE pretreatment technologies, respectively, for the standard case.
Biomass and enzymes were major contributors in total raw material cost. Cost of ethanol production was sensitive to pentose fermentation efficiency. Energy from lignin residue was sufficient to supply total steam required for ethanol production plant for all pretreatment processes. Potential for ethanol cost reductions existed in increasing pentose fermentation efficiency and reducing biomass and enzyme costs. Depending on the type of downstream technologies used for water treatment, energy and water use varied from 41.83 to 29.51 MJ/L ethanol and 3.07 to 14.67 kg water/L ethanol, respectively. We demonstrated the importance of considering the energy and water dependence in designing cellulosic ethanol plants.

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INFLUENCE OF STENOCARPella MAYDIS INFECTED CORn ON THE DRY GRIND ETHANOL PROCESS

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Introduction

Stenocarpella ear rot (formerly Diplodia ear rot) is resurfacing as a concern in the central United States corn belt. There are reports of some fields containing more than 50% mummified ears. Ears infected within two weeks of silking may be completely mummified with white to grayish brown mycelium covering light weight, shriveled, and lusterless light brown kernels. The severity of ear rotting decreases sharply with later infection dates during kernel development. Ears infected later in the growing season may appear normal with disease symptoms seen as discolored kernel embryos after the ear is broken in the middle. It is notable that the feeding of Stenocarpella corn to animals is not considered a risk. The influence of S. maydis infected grain on corn ethanol production is unknown. This is of significant concern because the U.S. corn ethanol processes 40% of domestic corn and generates 12 billion gallons of fuel ethanol per year.

For this study, Stenocarpella infected corn was manually harvested from a central Illinois farm in 2010 and the corn graded into five categories; an uninfected control was also included. Corn ears were graded based upon visual criteria designed to fractionate the corn from early to late infection after planting. The visual selection criteria were validated by measuring ergosterol. This fungal associated sterol is not produced by plants and is often used as an indicator of fungal infection in grain. Ergosterol concentrations were observed to increase with severity of infection (Figure 1). No ergosterol was detected in the uninfected control. The mildest infected corn is often referred to as “hidden” Stenocarpella because the infection is only noticeable as a

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1Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.
discoloration of the kernel tip ends. It is notable that this sample was determined to have significant ergosterol content as its detection would be unlikely by corn processors.

To our knowledge there are no published data on the effect of ear rot infection on corn ethanol production. In the dry grind process, the majority of corn is converted into ethanol. For this study, the fate of corn of was modeled from arriving at the facility gate through the production of ethanol and distillers dried grains with solubles (DDGS). When corn first arrives, it is analyzed for bushel test weight, moisture and chemical composition. The latter is measured using near infrared spectroscopy (NIR). The corn is processed into ethanol using one of two schemes. Corn starch can be either liquefied and simultaneously saccharified and fermented (SSF) into ethanol using a combination of alpha and gluco-amylase enzymes or it can be converted directly using the newer granular starch hydrolyzing amylases. Following fermentation, the ethanol is removed from the beer and the spent grains are dried and marketed as DDGS. DDGS is used as animal feed, especially for beef and dairy production. For this study, the set of six corn samples described above were compared for bulk and chemical properties. Chemical compositions were independently determined by wet chemistry and NIR methods. The corn was subsequently converted to ethanol using both processes and evaluated for ethanol productivities and yields. Finally, the spent grains, generated by the traditional method, were processed to DDGS, which was analyzed for composition.

Figure 1. Ergosterol present in infected corn. Corn class 1 was an uninfected control.
Bulk and Chemical Properties

The corn samples were evaluated for bushel test weights, mean kernel sizes (measured by displaced air method), and moistures. Test weights declined progressively from 63.5 to 36.4 lb/bu (Figure 2), which means that all but the least infected corn was lower than standard weight. Moisture contents were 9.1 to 17.2% (w/w) and followed no noticeable pattern (data not shown). Decline in kernel size mirrored that of test weights (Figure 2). Therefore, the more severe infections lead to a costly decrease in productivity and impeded seed development.

![Graph showing test weights and kernel sizes for infected corn samples.](image)

Figure 2. Test weights and kernel sizes for infected corn samples. Corn class 1 was an uninfected control.

Each sample was evaluated for starch, protein, fiber and oil (Table 1). Starch content is the most important trait because it determines the theoretical ethanol yield. In this case, relative starch content was constant for all but the most infected sample and the mean value (73.7% w/w, db) is within the expected range for dent corn. Crude protein and fiber contents were on average 1% higher than observed for the control. These increases might be associated with fungal protein and “fibrous” cell wall material. Unexpectedly, corn oil decreased with severity of infection and was lowest for the most severely infected corn sample. This decline in oil has broad implications. In some types
of processes (i.e., corn wet and dry milling) the germ is recovered and the oil extracted. When corn is fed either directly or as DDGS, a decline in oil can be expected to lower the energy value of the feed; oil has a high energy density. Therefore infection directly impacted kernel size, bushel weight, and oil contents. Specific analysis of the corn oil also revealed another concern. Only 0.8% of the oil extracted from the control sample was in the form of free fatty acids. In contrast, oils extracted from the infected samples contained 6.8 to 28% free fatty acids. Higher free fatty acid contents of infected samples suggest that the fungus produces an extra-cellular lipase activity. In summary, the fungus affected oil content and quality but not starch nor the theoretical ethanol yield on a mass basis.

**Table 1. Grain composition for control and infected grains**

<table>
<thead>
<tr>
<th>Corn Class</th>
<th>Starch</th>
<th>Protein</th>
<th>Fiber</th>
<th>Oil/Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73.4±0.4</td>
<td>8.6±0.0</td>
<td>8.6±0.4</td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>2</td>
<td>74.6±0.2</td>
<td>9.5±0.1</td>
<td>9.5±0.6</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td>3</td>
<td>74.7±0.3</td>
<td>9.6±0.2</td>
<td>9.6±0.8</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>4</td>
<td>74.4±0.5</td>
<td>9.7±0.1</td>
<td>9.7±0.4</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>5</td>
<td>73.2±0.7</td>
<td>10.1±0.1</td>
<td>10.1±0.2</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>6</td>
<td>71.9±1.2</td>
<td>9.6±0.1</td>
<td>9.6±0.5</td>
<td>1.5±0.1</td>
</tr>
</tbody>
</table>

Sample compositions also were analyzed by NIR measurement. NIR is able to quickly measure corn composition on whole corn kernels. However, the basis of the measurement is a reference or calibration set of corn. Measurement of type of corn not included in this reference set, and possibly including ear rot corn, might give erroneous results. As corn is analyzed at the facility gate by NIR measurement, any possible inaccuracy becomes a concern. So, the same six samples were also analyzed for starch, protein, and oils/lipids by NIR and these results compared to wet chemistry results cited earlier. Maximum differences between the NIR and wet chemistry results for any one sample were 2.2%, 0.8%, and 0.6% for starch, protein, and oil/lipids (data not sown). Therefore, NIR analysis appears to be accurate for compositional assessment of corn kernels infected with ear rot.

**Ethanol Fermentation Results**

To prepare for ethanol fermentation, the corn was ground using a hammer mill. For the conventional dry grind process, the milled corn was prepared as a 25% (w/w)
slurry, liquefied at 85°C for 90 min in the presence of alpha-amylase while being stirred at 60 rpm; a rotary IR oven was used for temperature control. Liquefied mash was transferred to a fermentation bottle and mixed with urea and glucoamylase and inoculated with dry active *S. cerevisiae*. Fermentation was allowed to proceed for 72 hr at 30°C and was mixed at 100 rpm. Gas production was monitored continuously using a wireless gas...
production module attached to the top of each fermentation bottle. Fermentations were sampled for final ethanol and residual glucose concentrations, which were measured by an HPLC equipped with a refractive index detector and an organic acid column. Spent grains were processed to produce DDGS by heating them at 90°C for 3 hr followed by 60°C until dry. Corn was processed using the granular starch hydrolyzing amylase with the following modifications: corn was mixed with water at 30% w/w solids, there was no liquefaction step, granular amylase was substituted for glucoamylase, and spent grains were not processed into DDGS.

The final ethanol concentrations and yields were similar for all six samples processed in the conventional manner (Figure 3). Ethanol yields were calculated in gallons per normalized bushel; the bushel weight was normalized to 55 lb at 15% (w/w) moisture. The final yield was 2.77 ± 0.07 gal/bu, which is very close to the expected industrial yield of 2.8 gal/bu. Likewise the starch conversion efficiencies (e.g., g ethanol/g starch content) did not vary across samples and was 95.6 ± 0.2% of theoretical. This is probably close to the upper limit for what can be achieved with S. cerevisiae when also considering carbon from starch used to generate yeast biomass. Ethanol concentrations and yields observed using the newer granular starch amylase generally followed the same trends observed for the conventional process, though the most infected sample had a 7% lower yield than the control. Overall final ethanol concentrations were higher than that observed for the conventional process because of the higher solids (30 vs. 25%) used for the latter. The mean ethanol yield (2.72 ± 0.11 gal/bu) and efficiency (95.3 ± 1.5% of theoretical) were similar to the results observed for the conventional process. (The means were calculated excluding the results for the most infected samples.) These results all lead to the same conclusion: the fungal infected corn does not interfere with processing and fermentation of the corn starch. The fermentation rates for all these samples were continuously monitored by measuring gas (e.g., CO₂) production. All the fermentation rates were similar (data not shown). This result confirms that the fungus is not producing chemicals that affect yeast metabolism, otherwise the growth and fermentation rates, which are intertwined, would have been slowed for the infected corn fermentations.

The spent grains produced from the conventional process were processed into DDGS. DDGS yield did not vary for the control versus infected samples and had a mean value of 353 ± 8 kg/ton corn (db), which is similar to the industry average of 17 lb/bu. DDGS was analyzed for crude protein, fat, fiber and ash (Table 2). Protein was slightly elevated for the fungal infected corn and changes in fiber and ash were inconclusive. However, crude fat was dramatically reduced, from 10.9% in the control to as low as 3.83% in the most infected sample. As discussed, crude fat is an important energy
component of DDGS, especially for dairy animals. Therefore, DDGS marketers would be sensitive to changes in fat content. This is not to suggest that corn processors will accept truckloads of noticeably infected corn but even the partial inclusion of infected corn would result in a change in the DDGS fat content.

Table 2. DDGS compositions (% w/w, db).

<table>
<thead>
<tr>
<th>Corn Class</th>
<th>DDGS Yield, kg/t</th>
<th>Protein Crude %</th>
<th>Fat Crude %</th>
<th>Fiber Crude %</th>
<th>Ash %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>342.8±14.1</td>
<td>32.3±0.9</td>
<td>10.9±1.6</td>
<td>7.7±0.4</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>2</td>
<td>352.7±9.3</td>
<td>35.0±0.2</td>
<td>6.6±0.9</td>
<td>7.5±0.3</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>3</td>
<td>349.4±9.9</td>
<td>35.1±1.2</td>
<td>6.1±0.9</td>
<td>8.6±0.2</td>
<td>3.9±1.3</td>
</tr>
<tr>
<td>4</td>
<td>348.6±4.5</td>
<td>36.0±0.5</td>
<td>5.4±1.1</td>
<td>8.9±0.5</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>5</td>
<td>366.6±3.3</td>
<td>36.2±0.9</td>
<td>5.5±0.3</td>
<td>8.5±0.9</td>
<td>3.9±0.5</td>
</tr>
<tr>
<td>6</td>
<td>355.6±6.9</td>
<td>34.5±1.1</td>
<td>3.8±0.4</td>
<td>7.6±0.2</td>
<td>4.2±0.3</td>
</tr>
</tbody>
</table>

Summary

*Stenocarella* ear rot is becoming more widespread in the U.S. Corn Belt and it is of interest to determine its effect on production of corn ethanol. *Stenocarella* ear rot dramatically reduced test weights and kernel sizes compared to the uninfected control corn. While relative starch content remained unchanged, oil content declined progressively with increased severity of infection. Corn was processed to ethanol using conventional and newer granular amylase processes. Results demonstrated the same trends; infection did not affect either the rate or yield of ethanol production. Nor did it influence the DDGS mass yield. However, the infected corn reduced the quality of the DDGS by significantly lowering its fat content.

Acknowledgements

Patricia O’Bryan, Jacob Brown, and Michael Powell for excellent technical help. Bulk and compositional analysis were performed by Illinois Crop Improvement Association (Champaign, IL) and Midwest Laboratories (Omaha, NE).
BEYOND HPLC: ADDITIONAL TECHNIQUES FOR THE ANALYSIS OF STARCH DERIVED PRODUCTS AND TRANSFORMATION PROCESSES

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HPLC currently is the most used chromatographic method for the analysis of starch components and to monitor their evolution during starch transformation processes such as saccharification and fermentation. HPLC is capable of rapid measurements without the need for the sample to be derivatized. Depending on the detection method (i.e., Refractive Index), HPLC can be limited in sensitivity, but still can provide specific and precise information on components of complex heteropolymers such as starch. Moreover, by the use of good reference compounds and appropriate calibration curves, quantification of different components is amenable with good accuracy.

A more in depth characterization of the transformation and fate of different components in starch requires the use of other techniques. Analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy (Kiemle et al 2004, Gidley 1985), gas chromatography coupled to mass spectroscopy (GC-MS) (Carpita and Shea 1988), matrix assisted laser desorption ionization mass spectroscopy (MALDI-MS) (Harvey 2003), fluorescent assisted capillary electrophoresis (FACE) (O’Shea et al 1998), HPLC anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) (Hanashiro et al 1996) or evaporative light scattering detection (HPLC-ELSD) (Dreux and Lafosse 1995), size exclusion chromatography with multiangle light scattering (SEC-MALS) (Fishman et al 1996), near infrared spectroscopy (NIR) (Chung and Arnold 2000), high resolution ultrasonic spectroscopy (HR-US) (Lehmann et al 2004), high performance thin layer chromatography (Lovsin-Kukman et al 1998), quartz crystal microbalance (QCM) (Nishino et al 2004) and other commonly used simpler methods (calorimetry, viscosity, iodine number, brix) have been employed with this purpose and their applications documented in the scientific literature.

We will illustrate the application and utility of some of these techniques to increase understanding of the factors involved in starch processing. Particular attention will be given to 1H NMR spectroscopy, MALDI-MS and FACE.
1H NMR spectroscopy has been used for many years for the characterization of carbohydrates (Duus et al 2000); its applications extend from detailed analysis of the equilibrium and kinetics of individual saccharides to studies of starch retrogradation or gelatinization kinetics using pulse sequence or solid state NMR (Angyal 1984, Kiemle et al 2004, Kulik and Haverkamp 1997). In a standard, aqueous phase, it is possible to identify changes in the anomeric region of the NMR spectra despite the overall spectra complexity. This allows evaluation of the changes on the reducing and nonreducing C-H bonds in the anomeric center. Moreover, it is possible to differentiate the anomeric, nonreducing signals corresponding to H-1,4 linkage from those that form the H-1,6 linkages (Figure 1). This allows the evaluation of branching degree and its change during enzymatic modification of starch substrate.

![Figure 1. NMR spectra depicting the anomeric region and differentiation of reducing and nonreducing sugars.](image)

Our interest in using 1H NMR was to monitor changes on the oligosaccharides present in the extractable soluble part of liquefact upon the addition of different enzymes. For instance, in the case of saccharification with a glucoamylase, it was possible to get an in situ view of the transformation by programming an acquisition time point sequence which upon analysis can be translated to the kinetics of saccharification with a particular enzyme (Figure 2).

An additonal application of 1H NMR is the identification of components in heterogeneous mixtures. 1H NMR was used to identify some of the components in the end of fermentation liquor produced during simultaneos saccharification and
fermentation of corn liquefact (Figure 3). A single anomeric signal was observed and characterized by 1H NMR as the nonreducing disaccharide trehalose. The absence of nonreducing anomeric signals can be correlated to the depletion of fermentable sugars.

Figure 2. Acquisition time point sequence during saccharification with glucoamylase.

Figure 3. Use of 1H NMR to identify components in fermentation liquor during simultaneous saccharification and fermentation of corn liquefact.
Another technique that allows a better resolution of the components of a heterogeneous polysaccharide mixture is capillary electrophoresis (CE). In general, the analysis of carbohydrates is hampered by the lack of chromophoric or fluorophoric functional groups and low extinction coefficients for both UV and fluorescent detection (Figure 4). These facts restrict detection by methods, such as refractive index, with relative insensitive mass responses. As a consequence, without derivatization of these analytes, high resolution separation techniques such as electrophoresis and reverse phase HPLC are not feasible. The use of CE requires the components be separated to bear an electrical charge. To that end, proper derivatization, such as a reductive amination with a charged moiety, allows for separation by electrophoresis. The charged molecule can be chosen to have fluorescent properties which facilitate a much higher sensitivity in the detection. The technique known as fluorescence assisted capillary electrophoresis (FACE) has been applied to the analysis of complex heterogeneous mixtures of carbohydrates (ie, starch) as well as the accurate determination of individual saccharides in mixtures (O’Shea et al 1998). We present the use of FACE in the analysis of high dextrose syrups and its correlation to 1H NMR results.

Finally, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was developed originally for measuring the mass of large molecules such as proteins (Figure 5). MALDI-MS also has been applied to carbohydrates since 1991 (Mock et al 1991). The technique is relatively simple, requires minimum amount of sample and has been used as an alternative to HPAE-PAD for chain length distribution analysis of amlopectin or starch (Broberg et al 2000).

We employed MALDI-TOF-MS as a tool to follow change in chain length distribution of liquefact during enzymatic saccharification (Figure 6). This technique allowed us to evaluate quickly the effect of different enzymes in changing the polydispersity profile of liquefact.
Figure 4. Capillary electrophoresis with laser induced fluorescence.
Figure 5. Matrix assisted laser desorption/ionization mass spectrometry for measuring chain length distribution of carbohydrates.

Figure 6. Monitoring change in chain length distributions using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS).
LITERATURE CITED


RESIDUAL STARCH MEASUREMENT IN DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS)

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INTRODUCTION

Ethanol production in the US was greater than 12 billion gal during the first 11 months of 2010 (EIA 2011). Carbon in ethanol is derived from starch in the cereal grain via a biochemical transformation involving fermentation with *Saccharomyces cerevisiae*. The yeast, *S. cerevisiae*, requires the cereal starch to be hydrolyzed into glucose prior to fermentation (Figure 1). A byproduct of ethanol production is known as distillers dried grains with solubles (DDGS) and contains residual starch that failed to transform into ethanol. As such, accurate measurement techniques are needed to allow proper evaluation and optimization of ethanol production from cereal grain (Figure 1).

There are no reported accurate quantitative methods available to measure starch directly. Starch must first be hydrolyzed into glucose, the glucose can be measured quantitatively, and then the glucose measurement is used to calculate the starch value. Indirect measurements such as those used in starch analysis are problematic. Various groups reporting on the correlation of starch content of feedstock and the ethanol yield all had conflicting results (Kindreda et al 2008, Lacerenza et al 2008, and Swanston et al 2007). As others have concluded, this is due to inaccurate starch analysis (Kindreda et al 2008 and Zhao et al 2009). Another consideration needs to be made for other non-starch polysaccharide (NSP) polymers in cereal grains that are made of glucose; namely cellulose and β-glucan.

Enzymatic approaches have targeted specifically the α-bonds in starch molecules, thereby eliminating the cleavage of β-bonds present in cellulose and β-glucan. While this is a definite positive to the enzymatic approach, DDGS starch already has escaped enzymatic attack during the saccharification stage of fermentation. The official AOAC method for measuring total starch (AOAC 996.11 – Megazyme T-TSTA kit) consists of a thermostable α-amylase (from *Bacillus licheniformis*) and an amyloglucosidase (from
Aspergillus niger). These are the same enzyme classes utilized in the grain ethanol industry across North America. For this reason, the AOAC total starch analysis used for measurement of residual starch in DDGS potentially is underestimating starch values due to the presence of resistant starch in the DDGS.

![Diagram](image)

**Figure 1.** Simplified process diagram for ethanol production and DDGS production. Includes a closed loop diagram showing how analysis supports process evaluation and optimization.

Heating and cooling of starch can lead to enzyme resistant starch formation. Above the starch gelatinization point of starch, the granule swells allowing amylose to leak out of the granule. Upon cooling the amylose becomes insoluble and precipitates out of solution, as a result it becomes resistant starch (type 3). Other researchers (Xu et al 2006, Zhao 2009) have reported high amylose content to be correlated negatively with ethanol yield. We exposed DDGS to heating and cooling at two different steps, jet cooking and oven drying.

Chemical approaches are designed to protonate the glycosidic bond making the bond unstable and susceptible to hydrolysis. Dilute mineral acids, such as HCl, commonly are used. The drawback is there is less specificity as to what type of bond is
cleaved. That leaves the potential for an overestimation of starch due to any cleavage of NSP glucose polymers like cellulose and β-glucan. However, this method is worth pursuing because it may be able to access enzyme resistant starch for hydrolysis into glucose.

The objective of our research was to identify an accurate, reliable, timely and cost effective residual starch analysis method for DDGS. We attempted to find novel approaches to solubilize DDGS resistant starch prior to enzymatic hydrolysis. Also, we studied the impact of chemical hydrolysis on other nonstarch polysaccharides (NSP) in cereal grains.

MATERIALS AND METHODS

DDGS Samples

Wheat, corn, triticale and barley DDGS samples tested were from previous fermentations completed in our laboratory. High temperature (jet cooking) and low temperature (raw starch hydrolyzing enzyme) hydrolysis techniques were used prior to fermentation. During jetcooking, mash was heated to 110 to 120°C by direct injection of high pressure (50 lb/in²) clean steam at an approximate rate of 150 lb/hr. Stargen 001 (Genencor) was used for the low temperature hydrolysis. More detailed methodology can be found in by Gibreel et al (2009).

DDGS was freeze dried following fermentation; however, in an attempt to mimic industrial drying practices, a portion of this DDGS was rehydrated to 30% solids and dried in a convection oven at 105°C for 24 hr.

Enzymatic Analysis Methods

Total starch was determined using a Megazyme International Total Starch Kit - AOAC standard method no. 996.11 (AOAC 2003). Thus far, two assay variants have been tested, including standard method B and standard method D. Method B used thermostable α-amylase (from Bacillus licheniformis) and amyloglucosidase (from Aspergillus niger). Method D used the same enzymes, but dimethylsulfoxide (DMSO) was used to solubilize resistant starch prior to enzyme exposure. Aliquots of these digested DDGS samples were reacted with glucose oxidase peroxidise reagent (GOPOD); absorbance was read at 510 nm against a known glucose standard. Method B and D were each completed using triplicate analysis.
Chemical Analysis Methods

Total starch was determined using an altered acid hydrolysis method (Kim et al 1988). DDGS samples were boiled in 2N HCl for 35 min; upon completion an equivalent amount of 2N NaOH was added to stop the reaction and neutralize the sample. Aliquots of hydrolyzed DDGS samples were reacted with GOPOD; absorbance was read at 510 nm against a known glucose standard. Acid hydrolysis of DDGS was completed in triplicate.

RESULTS AND DISCUSSION

Drying Effects on Analysis Values

Oven dried DDGS had a lower total starch value than freeze dried DDGS for nearly every grain type and hydrolysis method (Table 1). Megazyme analysis measured oven dried DDGS samples total starch values at 40 to 66% of their freeze dried counterparts, the only exception being Stargen 001 treated corn. High temperature, high moisture drying conditions created DDGS residual starches resistant to enzymatic attack. It was unclear if resistant starch formation or Maillard reaction products were the cause of this trend.

DMSO is recognized as a standard method addition for the Megazyme K-TSTA total starch kit when resistant starch is present (AOAC method no. 996.11). Megazyme analysis without DMSO can measure only soluble starch, so using this method had low total starch values for the oven dried DDGS, probably due to more resistant starch as compared to freeze dried DDGS. This trend was supported by the larger increase in total starch for oven dried samples when DMSO was used prior to enzyme addition (Figure 2, Table 1). DMSO is thought to solubilize resistant starch and make it available for enzymatic cleavage.

An even greater total starch value was determined for acid hydrolyzed samples (Figure 2, Table 1). The level of improvement depended on grain type of the DDGS. Acid hydrolysis increased the measured starch value for every DDGS sample; however, the extent of increase varied. For example, in Stargen 001 corn samples, acid hydrolysis increased measured starch by 35%, but in a jet cooked barley sample the increase was 32x higher (Table 1). This was unexpected given previous studies reporting the inability of dilute acid to hydrolyze crystalline regions of starch, the same regions expected to escape enzymatic analysis. Acid hydrolysis is not specific to the α-glucose bonding in starch and could be hydrolyzing β-glucose bonds in cellulose and β-glucan.
Effects of High vs Low Temperature Hydrolysis

High temperature cooking prior to hydrolysis, as is used in jet cooking, is expected to create resistant starch and/or Maillard reaction products similar to high temperature drying. However, in this case cereal grain was heated and more starch was present than for high temperature oven drying of DDGS. Jet cooking uses high temperature and sheer, along with the addition of thermostable α-amylase, to liquefy the starch potentially preventing the formation of resistant starch. We confirmed the production of resistant starch during jet cooking, based on lower values for every DDGS type using the Megazyme method (Table 1). More importantly, the analysis methods capable of hydrolyzing resistant starch raise jet cooking total starch values above those of Stargen 001 DDGS in all cases.
Table 1. Total starch % (db) of various laboratory generated DDGS samples using enzymatic and chemical analysis methods.

<table>
<thead>
<tr>
<th>Hydrolysis Method</th>
<th>Drying</th>
<th>Analysis Method</th>
<th>Wheat DDGS</th>
<th>Corn DDGS</th>
<th>Triticale DDGS</th>
<th>Barley DDGS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Starch % (dwb) ±</td>
<td>Starch % (dwb) ±</td>
<td>Starch % (dwb) ±</td>
<td>Starch % (dwb) ±</td>
<td></td>
</tr>
<tr>
<td>Jet Cooking</td>
<td>Freeze</td>
<td>Megazyme</td>
<td>1.07 0.00</td>
<td>1.63 0.01</td>
<td>1.02 0.02</td>
<td>0.76 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSO</td>
<td>2.49 0.04</td>
<td>1.82 0.05</td>
<td>1.57 0.02</td>
<td>1.60 0.01</td>
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<tr>
<td></td>
<td></td>
<td>Acid</td>
<td>6.77 0.19</td>
<td>8.39 0.45</td>
<td>5.15 0.07</td>
<td>11.14 0.34</td>
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<tr>
<td></td>
<td>Oven</td>
<td>Megazyme</td>
<td>0.62 0.01</td>
<td>1.02 0.03</td>
<td>0.66 0.04</td>
<td>0.31 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSO</td>
<td>1.72 0.03</td>
<td>1.70 0.01</td>
<td>1.30 0.04</td>
<td>1.16 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acid</td>
<td>5.10 0.16</td>
<td>4.34 0.16</td>
<td>4.03 0.07</td>
<td>10.02 0.32</td>
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<tr>
<td>Stargen 001</td>
<td>Freeze</td>
<td>Megazyme</td>
<td>1.60 0.04</td>
<td>11.05 0.32</td>
<td>1.80 0.06</td>
<td>0.82 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSO</td>
<td>1.75 0.06</td>
<td>14.04 0.27</td>
<td>2.00 0.02</td>
<td>0.89 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acid</td>
<td>4.91 0.04</td>
<td>19.94 0.79</td>
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<td>11.95 0.60</td>
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<tr>
<td></td>
<td>Oven</td>
<td>Megazyme</td>
<td>0.90 0.04</td>
<td>14.41 0.45</td>
<td>0.88 0.00</td>
<td>0.40 0.02</td>
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<tr>
<td></td>
<td></td>
<td>DMSO</td>
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<td>14.85 0.19</td>
<td>1.22 0.03</td>
<td>0.55 0.03</td>
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<tr>
<td></td>
<td></td>
<td>Acid</td>
<td>4.14 0.06</td>
<td>19.45 0.18</td>
<td>3.40 0.11</td>
<td>8.07 0.24</td>
</tr>
</tbody>
</table>

CONCLUSIONS

To date, the methods tested had differences in the reported starch values. Acid hydrolysis reported high starch levels and Megazyme reported low levels (Figure 2). It is yet to be determined why this was the case. DMSO did increase the amount of starch measured by the Megazyme kit, but it was not a multiple fold increase as seen for the acid hydrolysis method. This same trend was seen across all DDGS samples, independent of both hydrolysis and drying type.

To advocate acid hydrolysis as the best methodology, it is important to determine if any other nonstarch polysaccharides are contributing to the high reporting values. Pure microcrystalline cellulose was analyzed with acid hydrolysis and minimal cleavage was seen; equal to 1 to 2% starch for 100 mg of cellulose (results not shown). Cellulose content in corn DDGS samples ranges from 7 to 16% (Loy and Wright 2003, Xu et al 2009), this would correlate to <0.3% starch if all of the cellulose was hydrolyzed into glucose. The other cereal grains in this study had higher cellulose contents than corn, but it still did not explain the increase in total starch when acid hydrolysis was used. β-glucan has not been tested, nor have any other NSP’s. Barley grain has the highest
content of β-glucan and also has a greater total starch increase when measured by acid hydrolysis. Going forward it will be important to find out what other carbohydrates are hydrolyzed during acid hydrolysis.

LITERATURE CITED


BIOPOLYMERS, COMPOSITES AND NANOCOMPOSITES
DEVELOPED AS PART OF BIOREFINERY STRATEGIES

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SUMMARY

The Albany, CA, USDA research team is developing biorefinery strategies relevant to the Western US to go beyond corn ethanol, including 1) new biomass feedstocks for bioenergy production, 2) development of novel enzymes and microbes for cellulose to ethanol conversion from feedstocks prevalent in the West, 3) novel separation engineering for ethanol and bioproduct isolation and 4) production of complementary products. We will focus on production of complementary products, including biodegradable polymers, sustainable monomers and novel nanocomposites. We will highlight specific examples, including new starch based foams for single use items, composites and nanocomposites, specifically cellulose based nanocomposite biomaterials with novel properties.

INTRODUCTION

The US corn ethanol industry has been under pressure with criticisms that utilizing corn as a feedstock raises food prices, ethanol contains relatively less energy per liter than gasoline and the industry does not integrate well into the existing petroleum infrastructure. The contrasting argument is this so called first generation fuel introduces important new changes into the existing infrastructure and the knowledge base paves the way toward markets for cellulosic ethanol and “third generation” advanced fuels using nonfood feedstocks.

One strategy is to provide multiple value added products from an existing biorefinery. Starch is an abundant, biodegradable, renewable low cost commodity that has been used as a biofuel feedstock but also can be used, for example, as a thermoplastic...
replacement for petroleum based polymers. By itself, starch is a poor replacement for plastics because of its moisture sensitivity and brittle properties. By introducing different processing technologies and adding fibers or nanoparticles to create composite materials, promising new materials emerge. For example, starch based foam composite materials with unique and useful properties are being made using extrusion, puffing and solvent extraction processing techniques. Composite materials, including pulped cellulose fibers and nanoparticles, help to improve the properties of starch bioproducts.

HEAT EXPANSION: PUFFING, EXTRUSION AND NOVEL PROCESSING

Starch has been processed into single use foamed products that appear similar to polystyrene foam but are fully compostable and sustainable. Using starch as a substitute for packaging foams is challenging because starch is hydrophilic and requires a water barrier coating (Glenn et al 2001a, Glenn et al 2007, Glenn et al 2008, Shey et al 2006, Shogren et al 1993, Swanson et al 1993, Tiefenbacher 1993). By combining starch with other biodegradable components, starch based composites with excellent functional properties have been developed (Anderson and Hodson 1996, Anderson and Hodson 2000). We will describe technologies for making starch based foam and composite materials as well as details on some of their promising applications.

One issue in replacing polystyrene based food containers with starch materials is processing cost. Expanded bead polystyrene (EPS) items can be heat expanded or extruded in a matter of seconds, while starch requires at least 3 times the processing time. Processing costs for starch can be minimized by using a “drop in” replacement that can be processed with existing equipment. Starch softens and expands into a foam product similar to EPS using water/steam as a blowing agent. Typically, this is accomplished by heating the starch in a pressurized vessel to soften the starch matrix and superheating steam until the pressure is released. Puffing or foaming occurs when the pressure is released explosively; moisture within the starch matrix instantaneously expands into steam and creates a foam product. However, starch can be converted to a foam product at atmospheric pressures if heated rapidly (Anderson and Hodson 1996, Anderson and Hodson 1997a, Anderson and Hodson 1997b, Anderson and Hodson 2000, Glenn and Orts 2001).

Extrusion is another processing technology used for making starch into single use foamed containers. Extrusion is an energy efficient cooking system capable of breaking down starch granule structure through a combination of high shear, temperature and pressure. High amylose starches extruded with 13 to 19% moisture and at temperatures
of 160 to 210°C form a closed cell foam similar to that of polystyrene foam (Anderson and Hodson 1996, Anderson and Hodson 1997a, Anderson and Hodson 1997b, Glenn et al 2001b, Glenn et al 2001c). Chemically modifying starch with propylene oxide to produce starch ethers improves expansion and resiliency of the extruded product. This technology has been used to produce commercially starch based loose fill packing material as a replacement for polystyrene loose fill. One way to increase strength and resiliency of extruded starch based foam products without chemically modifying the starch is by incorporating pulped cellulose fiber as a reinforcing agent (Nobes et al 2001, Orts and Glenn 2002). Starch based foam/fiber composites were made by dispersing uniformly fiber in a viscous, gelatinous starch melt before mixing in other additives. Clay nanoparticles were added to starch to improve toughness and reduce water uptake. Such materials, with moisture levels as low as 2%, can be microwaved without damage thus opening the possibility of using these items as hot fill cups and microwaveable cups and bowls (eg, packaging for instant noodles).

Industrially, molds for mass producing single use items are similar to those used to make waffles; they have been adapted to making starch based food containers (Anderson and Hodson 1996, Anderson and Hodson 1997a, Anderson and Hodson 1997b, Anderson and Hodson 2000). The process involves baking an aqueous starch slurry in a compression mold. Steam vents are machined along the seam where upper and lower halves of the mold join together. The cycle starts by depositing a measured amount of a starch slurry in the mold which is closed. The slurry rapidly heats and becomes a viscous, molten liquid foam that expands as steam forms. A skin forms against the inner mold surfaces as the molten starch completely fills the mold cavity. Steam formed within the molten core forges its way through the molten mass enroute to the vents thereby creating an irregular foam core structure consisting of channels and voids. The starch foam is baked until the skin and porous core structure completely solidify (ca 5% moisture). The finished, baked product is removed from the mold but remains brittle, moisture sensitive and poorly suited for its intended use as food containers.

Bowls and plates laminated with a biodegradable polyester film function well as single use food service containers and currently are being marketed in the US. These products appear similar to polystyrene foam containers but degrade in about 35 days in a composting facility. Further improvements in baking technology and reduction in product costs will help baked starch based foam products gain greater market share. Production costs could be reduced by developing starch slurries with less expensive material costs (ie, cheaper starches) or by lowering moisture content. Reducing slurry moisture content would reduce energy costs associated with the baking process and would allow higher production rates by decreasing cycle times.
One of our intriguing uses of these starch nanofoams is for controlling parasitic mites in honeybee colonies by the controlled release of natural plant oils as an agent. Starch foam particles ranging in diameter from 2 to 10 μm were prepared by atomizing a gelatinized starch melt and using an air classification method to select for particles in the size range of pollen grains. Particles were collected in ethanol, dried into a porous foam and loaded with up to 25% (w/w) of the natural oils. The starch particles were small enough to be ingested by the honeybee or to attach to the hairs on the bee body where they could provide protection against parasitic mites. Field tests have proven they reduce mite populations in hives >95%.

STARCH NANOCOMPOSITES

Early efforts to incorporate starch into plastics included adding native starch granules as a filler in a plastic matrix (Chiou et al 2005, Chiou et al 2007, Orts et al 1998, Wood et al 2008). Although adding starch as a filler increased the renewable content, mechanical properties of these plastic composites were inferior. In contrast to many traditional fillers, nanoparticle fillers are known to improve mechanical properties of plastics and enhance physical properties such as gas or solvent permeability. The effectiveness of nanoparticle fillers has been attributed, in part, to their high surface area compared to typical fillers. Starch nanocrystals have been made by acid hydrolysis using HCl or H2SO4. Starch nanocrystals are about 45% crystalline depending on the botanical source. Starch nanocrystals typically increase tensile strength and elastic modulus of nanocomposite materials. Some investigators indicate that water vapor permeability is decreased by incorporating starch nanocrystals in composite materials. The porous starch particles or xerogels described earlier could be effective fillers for plastic composite materials because of their high surface area due to the distribution of nanosized pores and interconnecting matrix materials within the particles.

Other starch nanocomposites have been made using starch as the matrix material and incorporating nanoparticles from other sources. Starch composites made with only 5% montmorillonite clays have higher dynamic elastic moduli and tensile strength. The water vapor transmission rate was lowered by nearly one half. The low water vapor transmission rate was thought to be due to the tortuous diffusion pathway formed as the stacked platelets that form each clay particle become intercalated and dispersed in the matrix material. Starch nanocomposites also have been made using cellulose microfibrils. Cellulose microfibrils are long crystalline particles with diameters from 10 to 20 nm and aspect ratios from 20 to 100. Starch plastic composites reinforced with 10%
cellulose microfibrils derived from cotton had a 5 fold increase in young’s modulus compared to control samples without microfibrils (Glenn et al 2010). Potato starch plastics reinforced with microfibrils had improved thermomechanical properties and lower water sensitivity.

CONCLUSIONS

The use of starch in industrial products will continue to grow as long as it continues to be an abundant, inexpensive and renewable feedstock and to add value as part of biorefinery strategies. New bioproducts from starch based foam and foam composite materials, including nanocomposites, provide viable alternatives for some petroleum based products such as single use foam food containers. Further development of mechanical and chemical processes and composite materials will continue to improve the performance of starch based bioproducts and make them more effective replacements for petroleum based products.

LITERATURE CITED


INTELLIGENT ENZYME DESIGN BASED ON STARCH ANALYTICS DURING THE DRY GRIND ETHANOL PROCESS

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ABSTRACT

Three industrial paradigms were questioned and tested in the laboratory. Dry grind whole corn fermentations were not complete at drop and produced more ethanol and glucose over an extended fermentation time. Also, while HPLC is a valuable instrument for monitoring different aspects of the fermentation, it does not predict accurately the end of fermentations. This is due mostly to the insoluble phase providing additional substrate for the production of more glucose and ethanol. Liquefaction was not 100% effective at solubilizing all starch present in corn. Using this information, better enzyme blends were created for liquefaction and fermentation parts of the industrial process. These enzyme blends were tested for robustness across different ethanol plants at both laboratory and industrial scales. This ensured the optimal blends identified would perform well in customer plants.

Three main paradigms exist within the dry grind ethanol process: 1) fermentations are complete at drop, 2) HPLC determines the fermentation end point and 3) liquefaction is efficient at solubilizing starch present in corn. However, are these paradigms valid? Are there data to support these paradigms or do they need to be challenged? How can data which support or refute these paradigms influence new enzyme design to better address the needs in liquefaction and fermentation?

The first paradigm was fermentations are complete at drop. Some parameters measured to determine if fermentation was complete were glucose and residual sugars concentrations, ethanol concentration, DP4+ concentration and sometimes Brix. All of these have upper limits below which fermentations are considered finished. However, what happens to these parameters during an extended fermentation time, either in the
laboratory or during a temporary plant shutdown? Are there any further glucose or ethanol increases?

To better understand this, a laboratory fermentation experiment was conducted. Two different industrially liquefied mashes were obtained. Urea and antibiotic were added to 1000 and 2 ppm, respectively. To start fermentations, glucoamylase and rehydrated yeast were dosed on g dry solids basis. Samples were fermented for the standard 54 hr and for 112 hr (Figure 1, top). Although fermentation kinetics were slower after 54 hr, ethanol was produced for the extended fermentation times (4.5 and 6.2%, Figure 1, top.). Not all substrate was utilized during the standard fermentation time.

Verifying the laboratory data, two plants that had shut down temporarily with full fermentors continued collecting ethanol and glucose data. There were increases (9.3 and 6.0%) in ethanol yields for both plants (Figure 1, bottom). Glucose concentrations also increased during this time. The yeast health was declining over time leading to increased glucose concentrations. Not all of the starch substrate was utilized during the standard fermentation time. There was continued enzymatic dextrin breakdown and further ethanol production.

The second paradigm was that HPLC can be used to predict the fermentation end point. There were higher ethanol and total residual sugar concentrations at longer fermentation times (Figure 2). Substrate was hydrolyzed enzymatically and moved from the insoluble to the soluble phase. Although HPLC can be a useful tool for monitoring DP4+ conversion, yeast metabolism, potential microbial contamination and ethanol production; there is a major limitation for predicting fermentation endpoints. HPLC can measure only material in the soluble phase, thus missing any potential starch substrate which is still in the insoluble phase. This insoluble material, if not converted enzymatically during the standard fermentation time, passed into the DDGS and represented lost yield. Therefore, HPLC may not be the ideal indicator of starch conversion efficiency.
Figure 1. Results of the laboratory extended fermentation experiment (darker bars, top) and increases in ethanol produced compared to the standard 54 hr fermentation time (lighter bars). Results from two plants for extended fermentations are depicted in plot at bottom. Lighter bars represent ethanol concentrations and darker bars are glucose concentrations.
The third paradigm was the liquefaction process is efficient at solubilizing all starch present in corn. Liquefaction is the process that involves starch hydrolysis which is solubilized during gelatinization by an alpha-amylase. It produces a dextrin profile necessary for glucoamylase activity. During liquefaction, starch is brought into the soluble phase; enzymes break it down into smaller dextrins (average length of 6 to 12) and reduce the viscosity to improve heat transfer and handling. Liquefaction parameters vary from plant to plant. Enzyme dosage, particle size distribution, backset utilization and composition, jet use and hold time all affect liquefaction efficiency and starch solubilization.

Looking further into plant to plant variation, a series of liquefied mashes collected from 40 industrial plants were centrifuged and separated into 3 phases. There was a liquid phase, containing the soluble materials, a gel phase containing suspended solids and a solids phase with fiber, proteins and insoluble starch residuals. Solid phases were washed to remove trapped soluble material and hydrolyzed enzymatically to quantify the amount of starch and dextrins present in each mash. There were great variations in liquefaction efficiency (Figure 3).
We used scanning electron microscopy to examine starch after the liquefaction process; it was not in a granular form. DDGS residual starch was measured using an enzymatic assay. It varied in a similar fashion to that depicted in Figure 3. When the amount of residual carbohydrate was measured using an HCl assay (Ebell 1969) even more glucose was measured in DDGS. Liquefaction was not 100% effective in solubilizing starch and it varied amongst ethanol plants.

Intelligent enzyme design can leverage these paradigm shifting experimental results to generate the best enzyme solutions for the ethanol industry. To this end, individual Novozymes enzymes and enzyme mixtures were screened for their abilities to solubilize and convert the maximal amount of starch to glucose within an industry relevant fermentation time (Figure 4 left plot). Evaluation of different enzyme blends on a panel of industrial mashes is depicted in Figure 4 right panel.

To be beneficial to the industry as a whole, selected blends need to perform well across a series of different mashes. A similar enzyme design strategy was applied to the
evaluation of enzymes and blends in liquefaction. Enzymes and blends were tested against one set of industrially ground corn and backset. Top performing enzymes or blends were tested against a series of different corn and backsets to ensure robustness (results not shown).

For ease of screening, enzyme development was done initially at the laboratory scale. Once promising blends had been identified, proof of concept work was carried out at different customer plants or at pilot scale. Personnel at selected plants were approached about testing a new enzyme. Baseline information and samples were gathered prior to the trial. The enzyme trial was conducted; samples and data were gathered. Upon completion of the trial, results were analyzed and provided to the customer. This scale of testing provided relevant data for both the customer and Novozymes.

Three industrial paradigms were questioned and tested in the laboratory. Fermentations produced ethanol and glucose when allowed to continue past the standard industry time. HPLC, while a valuable instrument for monitoring different aspects of fermentation, did not predict accurately the end of fermentations. This was due to the insoluble phase, not quantifiable using HPLC, which provided additional substrate to make more glucose and ethanol. Liquefaction was not 100% effective at solubilizing all of the corn starch. Using this information, better enzyme blends can be created for the liquefaction and fermentation portions of the industrial process.

LITERATURE CITED

Figure 4. Effects of different enzyme blends on producing ethanol (top panel). Effects of different blends on a panel of industrial mashes to check for robustness (bottom panel). For each Plant, each group of bars are for Control and Blends J through N, respectively, left to right.
USE OF REVERSE OSMOSIS CONCENTRATE IN ETHANOL FERMENTATION

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ABSTRACT

Reducing water consumption in bioethanol production conserves an increasingly scarce natural resource, lowers production costs and minimizes effluent management issues. The suitability of reverse osmosis (RO) reject water for reuse in fermentation was investigated as a means to lower water consumption. Extensive chemical characterization of the reject water revealed low concentrations of toxic elements and total dissolved solids. Fermentation carried out with RO reject water resulted in similar levels of ethanol, most sugars, organic acids and glycerol as a control study using deionized water. The only exception was the slightly elevated levels of DP4+ in the runs using RO reject. Reasons for this elevation are unclear at present but may be due to limitations of analytical methodology. Under appropriate conditions, reuse of RO reject water in fermentation is feasible.

INTRODUCTION

The conversion of biomass to energy, under the current state of the art processes, requires the use of copious amounts of water. Recently constructed dry grind ethanol plants utilize 3 to 4 m³ H₂O/m³ EtOH produced and lignocellulosic plants are projected to use between 6 to 10 m³ H₂O/m³ EtOH (Wu et al 2009). This level of water abstraction for bioethanol production can place an undue burden on local water supplies. In some instances, the competition for water among various end uses, including biofuel production, is sparking debate on the merits of large scale bioethanol production (Meersman 2008, Clayworth 2007). This has generated intense interest in identifying opportunities to reduce water consumption in bioethanol production.
Dry grind plants use water for many purposes. These include the use of water to slurry corn, propagate yeast, generate steam, cool fermentation broth and condense process vapor. Dry grind plants utilize a variety of strategies to reduce water consumption. Among these are the use of treated municipal waste water as a substitute for ground or surface water (Manning and Craddock 2008) and extensive use of water recycling within the process (Ingledew 2003). Process streams commonly recycled include thin stillage, syrup evaporator condensate, boiler blow down and equipment wash water. Extensive use of heat exchange within the plant also is carried out to decrease the amount of process heat dissipated to the environment (Pfeffer et al 2007). Heat integration reduces cooling tower load resulting in water savings. Recently designed plants also use highly treated water for use in cooling towers in conjunction with advanced cooling tower monitoring techniques to reduce water consumption (Stanich 2007, Stanich and Van’t Hul 2009). In a recent study, Rajagopalan et al (2010) reported that under certain circumstances, the use of cooling tower blow down in fermentation may be feasible. We examined the feasibility of using RO reject water in fermentation.

METHODS

A dry grind ethanol plant in Illinois, USA, using RO for pretreatment was chosen for this study. City water was used as input to the RO. The water was pretreated with acid, scale inhibitor (Nalco PC191T) and a chlorine scavenger prior to RO. RO reject was concentrated from 2.85 to 4 fold with reference to city water, indicating recoveries of 65 to 75%. RO reject was collected in prerinsed plastic buckets and transported to the Illinois Sustainable Technology Center, Champaign, IL, within 3 hr. It was stored at 4°C prior to analysis.

Aliquots of RO reject were filtered through a 0.22 µm membrane to exclude suspended solids prior to analysis. B, Mg, Al, Si, P, Cr, Mn, Ni, Cu, Zn, As, Sr, Cd, Tl, Ba and Pb were analyzed via an inductively coupled plasma mass spectrometer (PQ Excell, Thermo Scientific, Waltham, MA) using USEPA method 6020A (USEPA 1998). Na, K, Ca and Fe were analyzed using USEPA method 7000B (USEPA 1998).

EPA Method 300.1 Revision 1.0 (USEPA 2000) was used for anion analysis. Total organic carbon (TOC) analysis was carried out as per EPA Method 415.3 (Potter and Wimsatt 2005). Total suspended solids and total dissolved solids (TDS) were determined in accordance with Standard Methods 2540 D and C (Clesceri et al 1998).

Yellow dent corn (34M78, Pioneer Hi-Bred International, Johnston, IA) grown in 2007 at the Agricultural and Biological Engineering Research Farm, University of Illinois
at Urbana-Champaign, was used. Samples were sieved using a 4.8 mm round hole screen to remove broken corn and foreign material. Cleaned corn samples were ground in a 165 mm diameter hammer mill (model MHM4, Glen Mills, Clifton, NJ) at 500 rpm using a 0.5 mm round hole screen. The ground corn moisture content was measured by oven method (AACC International 2000).

One hundred g milled corn (12.95% moisture) were slurried in water to obtain a solids content of 30% and adjusted to pH 4 with 10\(N\) \(\text{H}_2\text{SO}_4\). Water composition was the main experimental variable. Water composition was varied by mixing DI water and RO reject water in various proportions as shown in Table 1. Each experiment was carried out in triplicate.

<table>
<thead>
<tr>
<th>Experiment Label</th>
<th>DI Water mL (%)</th>
<th>RO reject water mL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>190 (100)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>RO 100</td>
<td>0 (0%)</td>
<td>190 (100%)</td>
</tr>
<tr>
<td>RO 50</td>
<td>95 (50%)</td>
<td>95 (50%)</td>
</tr>
<tr>
<td>RO 20</td>
<td>152 (80%)</td>
<td>38 (20%)</td>
</tr>
</tbody>
</table>

Granular starch hydrolyzing enzymes (GSHE) (0.4 mL), Stargen™ 001 (Genencor International, Palo Alto, CA), was added to the mix along with 0.04 mL GC 212, a commercial acid fungal protease (Genencor International, Palo Alto, CA). The media was supplemented with 0.4 mL urea. Yeast inoculum (2 mL), prepared by dispersing 1 g activated dry yeast (Ethanol Red, Lesaffre Yeast Corp., Milwaukee, WI) in 5 mL water and incubating at 30°C for 20 min, was added to conduct simultaneous saccharification and fermentation (SSF). SSF was carried out at a temperature of 32°C in a 120 rpm shaking water bath (Model SHKA 7000, Barnstead/Lab-line, Melrose Park, IL).

Glucose, maltose, fructose, glycerol, succinic acid, lactic acid and DP4 were monitored during fermentation by taking 2 mL samples at 2, 4, 6, 24, 48 and 72 hr. From each 2 mL sample, clear supernatant liquid was obtained by centrifuging the sample at 13,000 rpm (Model 5415 D, Brinkmann-Eppendorf, Hamburg, Germany). Supernatant was passed through a 0.2 \(\mu\)m syringe filter into 150 \(\mu\)L vials. Filtered liquid was injected into an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) maintained at 50°C. Analytes were eluted from the column with HPLC grade water containing 5 mM sulfuric acid. The elution rate was 0.6 mL/min. Separated components were detected
with a refractive index detector (model 2414, Waters Corporation, Milford, MA). Data were processed using HPLC software (Waters Corporation, Milford, MA).

RESULTS AND DISCUSSION

The composition of RO reject is presented in Table 2. TDS of this water was 1100 mg/L. Ca and Mg constituted the major cations while bicarbonate was the primary anion. RO reject water was moderately high in silica. In addition to these metals, RO reject water contained a slew of trace elements and the scale inhibitor. Heavy metals or elements toxic to yeast were detected but at levels that were not of concern.

Table 2. Composition of RO reject.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Value (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>36</td>
</tr>
<tr>
<td>Potassium</td>
<td>8.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>220</td>
</tr>
<tr>
<td>Magnesium</td>
<td>120</td>
</tr>
<tr>
<td>Strontium</td>
<td>1.27</td>
</tr>
<tr>
<td>Barium</td>
<td>0.91</td>
</tr>
<tr>
<td>Chloride</td>
<td>10</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>1,214</td>
</tr>
<tr>
<td>Sulfate</td>
<td>14</td>
</tr>
<tr>
<td>Nitrate</td>
<td>2</td>
</tr>
<tr>
<td>Fluoride</td>
<td>1.5</td>
</tr>
<tr>
<td>Silica</td>
<td>44</td>
</tr>
</tbody>
</table>

Results of fermentation experiments carried out with 100% RO reject water are presented in Figure 1. Glucose concentration increased, in all cases, during the initial phases of SSF and subsequently decreased rapidly. In all instances, glucose concentration was negligible after 24 hr, indicating complete fermentation. Profiles of the sugars, glucose, maltose and fructose, during fermentation with RO reject were similar to the control. The only profile that did not follow this trend was that of DP4+. Compared to the control, slightly elevated concentrations of DP4+ were noted in the fermentation with RO reject.
Ethanol concentration (Figure 2) increased slowly initially, then rapidly and finally at a decreasing rate after 24 hr. These follow the typical fermentation patterns associated with yeast. Final ethanol concentrations were the same for both control and runs using 100% RO reject water.

Glycerol production (Figure 2) is an indicator of yeast stress. A small amount of glycerol (0.8 to 1.5%) is produced in dry grind ethanol fermentations (Ingledew 2003). Final concentrations of glycerol were observed to be low (< 1.00 % (w/v)) and comparable among all treatments. Therefore, yeast was not under stress due to the use of RO reject water. The scale inhibitor specific to this study at levels normally encountered in RO reject water had no adverse effect on ethanol production. Production of lactic and succinic acids also were not different between controls and runs with 100% RO reject water.

The primary difference between runs using 100% RO reject and the control run is noticed only in DP4+ analysis. DP4+ nominally refers to residual sugars. An increase in DP4+ can, therefore, be interpreted as incomplete sugar hydrolysis. However, it should be noted, the HPLC method of analysis for DP4+ measures a mixture of all soluble components from corn, yeast and enzymes left over from fermentation. While dextrins may be a fraction of this mixture, other components such as proteins, lipids and salts may be present in the DP4+ peak. Using ion chromatography, it has been shown that less than 10% of the DP4+ peak is attributable to dextrins. Given this, it appears more prudent to use ethanol yield and residual glucose as indicators of enzyme and fermentation performance. If such a perspective is adopted, the use of RO reject appears to have little effect on ethanol fermentation.

CONCLUSIONS

A water source with elevated TDS, Ca and Mg content, such as that generated during RO water treatment, can be used for ethanol fermentation. In this particular study, TDS levels were 1100 mg/L; Ca and Mg were present at 220 and 120 mg/L respectively. The general applicability of the use of RO reject in ethanol fermentation will, however, be dictated by source water quality and degree of recovery, both of which are site specific. Some uncertainty remains with respect to the effect of RO reject on sugar hydrolysis that can be resolved through adopting a different analytical technique for measuring DP4+. If these results can be replicated in plant trials, it would open up another pathway to reduce water consumption. The costs of implementing this option would be less than using cooling tower blow down as RO reject does not have to be cooled.
Figure 1. Concentrations of sugars during ethanol fermentation with RO reject.
Figure 2. Concentration profiles of ethanol, organic acids and glycerol during ethanol fermentation with RO reject.

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LITERATURE CITED


LOGISTICAL AND PROCESSING CONSIDERATIONS RELATED TO CORN STOVER AS A FLEXIBLE FEEDSTOCK FOR INDUSTRIAL AND BIOCONVERSION PROCESSES

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ABSTRACT

We will describe handling and processing of corn stover bales collected in a mid scale harvest project. A total of 6,096 bales were collected in eastern Iowa in 2009; 606 net wrapped bales (round and square), averaging 21.6 ± 7.9% moisture, were selected for storage and processing. Bales were stored under roof (barn), stacked and tarped, plastic wrapped (tube or sleeve) or left exposed outdoors. Bales entered storage in November, 2009, and subsets were processed in February, April or July, 2010. Square and round bales had similar average moisture content and throughput (kg/min) when ground by a commercial grinder. Bales stored in barns or under tarp had stable moisture content; whereas, exposed net wrapped bales and tube wrapped squares bales became wet and difficult to process. Exposed round bales averaged 35% moisture; whereas, round bales stored under roof or under tarp had 17% moisture. Protecting bales resulted in numerically better grinding capacity and larger mean particle size compared with minimal protection. Bale moisture reduced geometric mean particle size of ground material, especially when bales were >30% moisture. Fines in ground material increased, possibly because of partial screen plugging and extended grind action. Storage method affected bale moisture content which in turn affected physical processing.

INTRODUCTION

Size reduction of corn stover is critical for downstream conversion processes. Grinding increases surface area for thermal or chemical reactions and enables densification processes. Size reduction characteristics of biomass feedstocks have been studied using laboratory scale (Igathinathane et al 2008, Mani et al 2004) and commercial
scale (Hoque et al 2007) equipment. Moisture affects shearing characteristics of stover and other biomass and the productivity and performance of size reduction equipment (Womac et al 2005). Therefore, one of the objectives of the collaborative ADM-Monsanto joint stover project in 2009-2010 was to evaluate the effects of storage on moisture content and processing characteristics of corn stover collected in a large scale harvest in eastern Iowa.

MATERIALS AND METHODS

Corn Stover Collection and Storage

Corn stover was baled by a custom harvester near Cedar Rapids, IA, during the 2009 harvest season. Round bales (3 net wraps and 5 net wraps) were made using John Deere balers and Vermeer’s 605 Super M Cornstalk Special; square bales were made using Case IH’s LB433 Rotor Cutter large square baler. Stover was raked at an oblique angle to the corn rows without mowing or shredding. A total of 6,084 bales were made; 606 round and square bales collected from one field were selected for the storage and processing trial. Bales were core sampled before entering the storage trial; core moisture content averaged 21.6 ± 7.9%.

Bales entered storage in November, 2009. Storage methods for round and square bales were:

1) stacked in barn
2) stacked outside but tarped (with “protective” outer bales)
3) individually wrapped with black plastic sleaves
4) wrapped as a continuous plastic tube or
5) outdoors with no cover in rows (round bales).

Tarped and barn stored bales were placed at a common depot west of Cedar Rapids; whereas, exposed round bales were stored at three different locations. Bales stored outdoors were placed on prepared surfaces intended to minimize moisture infiltration from surface contact.
Bale Grinding and Sampling

Bales were removed from storage and ground in February, April and July, 2010. A total of 296 round and square stover bales were transported from storage and ground by commercial grinders at a common grinding site. Bales were weighed and fed individually into grinders; grab samples were collected and composited from the discharge stream. Grinding time was recorded. Samples were assayed for moisture by drying at 105°C. Additional samples of ground stover were collected from 113 bales and geometric mean particle size (MPS) and percent mass distribution was calculated according to ANSI/ASAE S424.1 Standard.

RESULTS AND DISCUSSION

Unprotected net wrapped bales increased in moisture during storage (mean moisture of 32.6%) (Table 1) compared with initial mean moisture of 21.6% at harvest. However, bales stored in a barn environment exhibited a slight decrease in moisture content compared with initial moisture. Tarping provided good protection but plastic tube wrapping was less effective because tears in the material compromised the integrity of the wrap thus permitting moisture accumulation (mean moisture of 35.3%).

Table 1. Effect of storage method on moisture content of bales and mean particle size (MPS) of bales ground using commercial grinders.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Bale type</th>
<th>% moisture</th>
<th>MPS, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barn</td>
<td>Round</td>
<td>15.9 a</td>
<td>14.2 cd</td>
</tr>
<tr>
<td>Tarp</td>
<td>Round</td>
<td>18.1 ab</td>
<td>15.8 d</td>
</tr>
<tr>
<td>Sleeve</td>
<td>Round</td>
<td>29.4 d</td>
<td>13.2 bc</td>
</tr>
<tr>
<td>Tube wrapped</td>
<td>Round</td>
<td>26.7 cd</td>
<td>13.0 abc</td>
</tr>
<tr>
<td>Net wrapped</td>
<td>Round</td>
<td>32.6 de</td>
<td>10.6 a</td>
</tr>
<tr>
<td>Barn</td>
<td>Square</td>
<td>17.0 ab</td>
<td>13.2 bc</td>
</tr>
<tr>
<td>Tarp</td>
<td>Square</td>
<td>22.2 bc</td>
<td>13.6 cd</td>
</tr>
<tr>
<td>Tube</td>
<td>Square</td>
<td>35.3 e</td>
<td>11.1 ab</td>
</tr>
<tr>
<td>Standard error of mean</td>
<td></td>
<td>2.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a, b, c, d, e = means having uncommon letters differ (P < .05)
Storage method affected MPS of ground stover with an indication that protecting bales resulted in greater MPS. For example, MPS for round bales stored under tarp was 49% greater compared with exposed round bales (15.8 vs 10.6 mm). Likewise, square bales in barns or under tarps had lower moisture content and greater MPS compared with tube wrapped square bales.

MPS of ground stover linearly decreased with increased bale moisture (Table 2). When bales were stratified into classes ranging from dry (ie, <15% moisture) to wet (>35% moisture), MPS decreased, particularly when bales had >30% moisture. Decreased particle size may have been caused by extended grind times and reduced throughput for wetter bales. Grinding higher moisture bales resulted in partial plugging of screens and extended grinding action required to discharge particles from the grinder, which likely contributed to a greater percentage of fines (particles <1 mm).

Table 2. Effect of bale moisture on mean particle size (MPS) of ground stover.

<table>
<thead>
<tr>
<th>Bale Moisture</th>
<th>MPS, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15%</td>
<td>15.4a</td>
</tr>
<tr>
<td>15 – 20%</td>
<td>14.0a</td>
</tr>
<tr>
<td>20-25%</td>
<td>13.9a</td>
</tr>
<tr>
<td>25-30%</td>
<td>13.2a</td>
</tr>
<tr>
<td>30 - 35%</td>
<td>10.7b</td>
</tr>
<tr>
<td>&gt;35%</td>
<td>10.9b</td>
</tr>
</tbody>
</table>

Bale moisture affected percentages of coarse (>19 mm) and fine (<1 mm) fractions, which subsequently affected MPS and mass distribution of ground material. It was difficult to pinpoint the particular moisture content at which these phenomena occurred because we measured average bale moisture content but very wet bottom or outside ring material (eg, >50% moisture) likely existed in “wet” bales.

Downstream applications for ground stover ultimately will dictate optimal particle size and mass distribution. For densification, a mixture of course and fine particles may be desirable for agglomeration and briquetting, with fine particles filling the void space surrounding coarse particles. For biochemical conversions, a uniform small particle may
be desirable for maximizing surface exposure for enzymatic hydrolysis. Coarse particles may be desirable in certain reactions for which physical properties of the resulting residue may affect further processing (e.g., paper pulping or cattle food). Size reduction is energy intensive and accounts for a large proportion of feedstock cost; improving efficiency of this step will depend on matching equipment and conditions to the specific feedstock application.

The 2009-2010 season was exceptionally wet. Bales stored outdoors gained considerable moisture; these high moisture bales created problems for the grinders and also for particle size distribution analysis. Grinder screens occasionally partially plugged or fouled, reducing open area. The reduced open area of the screens increased grinding times and altered particle size distribution. Also, while conducting the stover particle size distribution analysis in the laboratory, clumping of damp stover particles made sieving a challenge.

As grinding date proceeded, moisture contents were not different across processing dates but a numeric increase was observed in moisture contents for bales ground in April and July compared to February (Table 3). Mean particle size also was not affected by processing date; nor was throughput.

Table 3. Effects of grinding date on characteristics of ground stover using a Vermeer tub grinder.

<table>
<thead>
<tr>
<th>Grind Date</th>
<th>Bale Moisture, %</th>
<th>MPS*, mm</th>
<th>Throughput, dry kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Bales</td>
<td>Vermeer</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>23.0</td>
<td>23.7</td>
<td>14.4</td>
</tr>
<tr>
<td>April</td>
<td>24.9</td>
<td>27.0</td>
<td>14.9</td>
</tr>
<tr>
<td>July</td>
<td>26.0</td>
<td>27.8</td>
<td>13.0</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.3</td>
<td>2.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*MPS: mean particle size

Barn stored or tarped bales had stable moisture contents; whereas, exposed, net wrapped bales and tube wrapped square bales became wet and more difficult to grind (Table 4). Exposed round bales averaged 35% moisture with throughput of 197 dry kg/min; whereas, round bales stored in barns or under tarp had 17% moisture and throughput of 274 or 295 dry kg/min. Protecting bales by storing under roof or tarp generally resulted in numerically better grinding capacity and larger MPS compared to bales stored outside with no protection.
Table 4. Grinding characteristics of stored bales processed by a Vermeer tub grinder.

<table>
<thead>
<tr>
<th>Storage type</th>
<th>Bale type</th>
<th>Moisture, %</th>
<th>SEM*</th>
<th>MPS*, mm</th>
<th>Throughput, dry kg/min</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barn</td>
<td>Round</td>
<td>16.9 ab</td>
<td>4.0</td>
<td>15.1</td>
<td>274</td>
<td>40</td>
</tr>
<tr>
<td>Tarp</td>
<td>Round</td>
<td>17.2 a</td>
<td>3.0</td>
<td>16.0</td>
<td>295</td>
<td>26</td>
</tr>
<tr>
<td>Sleeve</td>
<td>Round</td>
<td>31.7 cd</td>
<td>2.8</td>
<td>14.6</td>
<td>255</td>
<td>26</td>
</tr>
<tr>
<td>Tube wrapped</td>
<td>Round</td>
<td>25.8 bc</td>
<td>2.8</td>
<td>14.5</td>
<td>247</td>
<td>26</td>
</tr>
<tr>
<td>Net wrapped</td>
<td>Round</td>
<td>35.0 d</td>
<td>2.6</td>
<td>11.9</td>
<td>197</td>
<td>26</td>
</tr>
<tr>
<td>Barn</td>
<td>Square</td>
<td>16.8 a</td>
<td>3.0</td>
<td>13.2</td>
<td>236</td>
<td>26</td>
</tr>
<tr>
<td>Tarp</td>
<td>Square</td>
<td>25.1 abc</td>
<td>3.0</td>
<td>14.0</td>
<td>286</td>
<td>26</td>
</tr>
<tr>
<td>Tube</td>
<td>Square</td>
<td>37.1 d</td>
<td>3.6</td>
<td>14.3</td>
<td>223</td>
<td>31</td>
</tr>
</tbody>
</table>

*SEM = standard error of mean; MPS = mean particle size
a,b,c,d = means having uncommon letters differ (P < .05)

Storage affected moisture content and processing characteristics of baled stover. Protecting bales from moisture accumulation can improve grinder capacity and uniformity of ground material.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Koopman Hay and Forage, Peterson Biomass and Amana Farms for assistance in collection, storage and processing of bales. Special thanks are extended to Kevin Shinners for allowing the use of laboratory equipment for particle size analysis and to Heather Dan, Purdue University, for assistance in statistical analysis.

LITERATURE CITED


INTRODUCTION

To account for uncertainties in process conditions, physical properties, heat transfer and pressure drop correlations, fouling, as well as the risk associated with the heat exchanger not meeting the process requirements, a margin commonly is used when designing heat exchangers. Among these parameters, fouling generally has been associated with a design margin due to its financial impact in terms of energy losses.

Better heat exchanger designs with more appropriate margins can help reduce energy losses due to fouling. This applies across process industries, including refining and petrochemical, as well as the bioprocess industries. As reported by Rausch et al (2010), more than 200 US fuel ethanol plants use evaporators that are affected negatively by heat transfer fouling. By improving the heat exchanger designs as well as its associated maintenance, the environmental footprint and costs of biofuel production can be reduced.

TRADITIONAL APPROACH

There are several methods that can be used to apply a margin due to fouling or other factors. The method most commonly used for shell and tube heat exchangers is to apply the Tubular Exchanger Manufacturers Association (TEMA) fouling resistances in the form of a constant that is added to the overall thermal resistance. Although TEMA (2007) states there are different approaches to provide for fouling allowance in designing shell and tube heat exchangers and provide added heat transfer surface area, fouling resistances to be specified should be those that reflect the value at the time just before the exchanger is expected to be cleaned. It goes on to state the purchaser of a heat exchanger “should attempt to select an optimal fouling resistance that will result in a minimum sum
of fixed, shutdown and cleaning costs” and provides a list of typical fouling resistances for industrial fluids, chemical processing streams and oil refinery streams.

Since the majority of heat exchanger designers today have limited experience and are no longer specialists in the field, there is an increased tendency to rely on TEMA fouling resistances. While TEMA’s original intentions were good, providing fouling resistance values for typical streams were only to be used when the designer did not have better information based on past experience with a similar service. Unfortunately, it is rare that designers have better information, unless they are part of a major organization that has the capability and enforces the use of good design guidelines. If we review fouling resistances published by TEMA since its first publication in 1941, except for a few minor adjustments in the 1980s and replacing the word “factor” with the word “resistance”, values have remained almost unchanged.

There are two problems with this traditional approach: the values themselves and treating them as an additive resistance. The values themselves are a problem because at the time such resistances initially were published, there was much more uncertainty in the prediction of heat transfer coefficients, so the published values not only considered fouling but also took into account other uncertainties that may no longer be relevant with more advanced fouling reduction design methods. The problem with using a constant additive resistance is that it penalizes a good exchanger design (one with lower fouling tendencies) more heavily than a bad design (one with higher fouling tendencies). Furthermore, such approach discourages the use of more rigorous prediction methods as well as adequate fouling mitigation strategies since the additive fouling resistance cancels out any improvements obtained from good design or fouling mitigation.

IMPROVED METHODOLOGY

In addition to the TEMA fouling resistance approach, other methods may include the specification of an experience based design margin combined with good design practices or the use of risk based design margins requiring knowledge of plant operation to assess appropriate risk and associated values (Shilling 2011). The latter two approaches require specific experience with the service and thus are more applicable to plant engineers who may have access to such information. Since plant engineers are less likely to be involved in design of heat exchangers, such approaches are not as practical. Therefore, we propose the use of a fouling “resistance factor” which multiplies the thermal resistance instead of adding to it. The use of a “resistance factor” approach
encourages good heat exchanger design and the use of proper fouling mitigation strategies.

The resistance factor approach would use the following expression to calculate the overall thermal resistance in a heat exchanger.

\[
\frac{1}{U} = \frac{r_o}{h_o} + \frac{A_o x_w}{A_m k_m} + \frac{A_o r_i}{A_l h_i}
\]

For proposed new methodology to be effective, it is preferable to replace information currently published in TEMA (2007). As a starting point, we can convert TEMA additive fouling resistances to an equivalent resistance factor by determining the heat transfer coefficient at the time original fouling resistances were established. This work is underway within the activities of HTRI’s Exchanger Design Margins Task Force, which is comprised of a group of individuals who brings together industrial participants with extensive experience in process heat transfer and heat exchanger design. The published list of resistance factors will be evaluated further using experience within the HTRI task force and expanded and updated as new information and experience becomes available. Adoption of the new methodology will encourage better heat exchanger designs and the use of adequate fouling mitigation methods.

SUMMARY

An improved methodology is proposed for specifying margins when designing heat exchangers. The new approach is based on the use of resistance factors instead of traditional TEMA fouling resistances, which encourages the use of more rigorous and accurate heat exchanger calculation methods, good design practices and application of appropriate fouling mitigation techniques. The proposed methodology results in the design of heat exchangers with less excess surface area as well as preventing the promotion of fouling due to poor designs.
Nomenclature

\[ A_i \quad \text{Inside surface area, m}^2 \]
\[ A_m \quad \text{Mean tube wall area, m}^2 \]
\[ A_o \quad \text{Outside heat transfer area, m}^2 \]
\[ h_i \quad \text{Inside heat transfer coefficient, W/m}^2 \text{K} \]
\[ h_o \quad \text{Outside heat transfer coefficient, W/m}^2 \text{K} \]
\[ k_m \quad \text{Metal thermal conductivity, W/m K} \]
\[ r_i \quad \text{Resistance factor based on the inside fluid} \]
\[ r_o \quad \text{Resistance factor based on the outside fluid} \]
\[ U \quad \text{Overall heat transfer coefficient, W/m}^2 \text{K} \]
\[ x_w \quad \text{Wall thickness, m} \]

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Poster Presentations
(alphabetically listed by lead author)
EFFECT OF AMMONIUM HYDROXIDE TREATMENT ON DEGRADABILITY OF CORN BRAN

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Removal of fiber from corn during dry grinding can improve fermentation efficiency but results in another coproduct stream (corn bran) to be marketed. Corn bran is largely cell wall material, contains a high proportion of pentosans and is quite refractory (Belyea et al 2009). Ammonia treatment can increase the degradability of fibrous materials. The objective was to determine the effect ammonium hydroxide treatment on corn bran degradability.

Corn bran was exposed to different concentrations (15, 30 or 45%) ammonium hydroxide (Sendich et al 2008) and/or heat; samples of treated material were degraded with two commercial cellulases for different periods of time at 50°C and pH 5.0. Samples also were degraded by ruminal bacteria using an in situ technique. Purified wood cellulose was included as a reference material. Degradability was estimated from weight loss following exposure to enzymatic activity.

Native (untreated) corn bran was 20 to 25% degraded by cellulases, similar to earlier work (Belyea et al 2009). Treatment with ammonium hydroxide increased degradability 60 to 80%, while heating had a small effect. Treatment of corn bran with 30% ammonium hydroxide (Sendich et al 2008) resulted in the greatest increase in degradability. Most of the degradation of corn bran occurred within 24 hr; there was little additional degradation at 48 and 72 hr. By comparison, wood fiber was degraded more gradually over 72 hr. Ruminal bacteria were able to degrade both untreated (65 to 70%) and treated (80 to 90%) corn bran extensively.

Ammonium hydroxide could be used to increase the availability of fiber in corn bran for utilization in subsequent processes or applications. The method is simple and requires no special equipment.

LITERATURE CITED
INFLUENCE OF STENOCARPELLA MAYDIS INFECTED CORN ON THE DRY GRIND ETHANOL PROCESS

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Widespread epidemics of Stenocarpella ear rot (formerly Diplodia ear rot) have occurred throughout the central US corn belt in recent years with reports of some fields containing more than 50% mummified ears. Ears infected within 2 wk of silking may be mummified completely with white to grayish brown mycelium covering light weight, shriveled and lusterless light brown kernels. The severity of ear rotting decreases sharply with later infection dates during kernel development. Ears infected later in the growing season may appear normal with disease symptoms seen as discolored kernel embryos after the ear is broken in the middle. There have been no reports of S. maydis toxicity to livestock in North America. The influence of S. maydis infected grain on corn ethanol production is unknown.

Approximately 200 S. maydis infected ears of variety ‘Heritage 4646’ were hand harvested in 2010 from a production field in central Illinois and segregated into 1 of 5 levels of ear rot severity based upon visual symptoms. The concentration of ergosterol, a sterol produced by fungi but not plants, increased with the severity of ear rot (127 to 306 µg/g) and none was detected in control corn. These corn samples were characterized initially for bulk properties and chemical composition. Corn test weights declined with progression of the disease and were 42.6% lower for the most severely rotted grain sample. Accompanying changes in composition also were apparent. Oil content decreased (4.7 to 1.5%) and fiber increased (6.6 to 9.6%) but starch content remained largely invariant. Oil composition varied among infected samples.

Corn samples were converted to ethanol using a laboratory scale process that mimics the typical corn dry grind ethanol process using simultaneous saccharification and fermentation (SSF). Ethanol yields were similar on an equivalent weight basis (2.77 to 2.85 gal/bu) and starch to ethanol conversion efficiencies were 91.5 to 98.3%. However, DDGS composition was modified and reduced in oil content (10.9 to 3.8%). Based upon these results, we concluded that Stenocarpella ear rot will affect DDGS composition but not ethanol yield on an equivalent weight basis.
CELLULOSIC ENZYMES AS PROCESSING AID FOR BIOFUELS PRODUCTION

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The importance of energy efficiency for biofuels production has become increasingly scrutinized as governments set higher and higher standards for greenhouse gas emissions. Water consumption also is being criticized as supply and availability is reduced in many locations. During the last two decades, energy use for fuel ethanol production has decreased through improved heat integration, high gravity fermentations and increased sale of wet distillers grains.

Enzymes also have played a role in the improvements by helping to increase ethanol yields. During the last 4 yr, we have been developing the use of cellulosic enzyme preparations to aid in downstream coproduct separations, with the ultimate goal of improving energy efficiency and decreasing water consumption. Laboratory data will be presented to demonstrate that cellulosic enzyme preparations improve solid liquid separation of whole stillage.

Enzyme treatment increases the water (stillage) going to the more energy efficient evaporator, thereby decreasing the amount going to the dryer. Additionally, more water is recycled through the evaporator, decreasing the total clean water requirements of the process. Using data gathered during full scale plant trials, process and cost models were developed to compare the base case conventional process with the enzymatic dewatering process. There was a decrease in utility consumption for the enzymatic treatment compared to the conventional model. Using sensitivity analysis, we showed energy, water and economic benefits for a wide range of enzyme and natural gas costs.
TROUBLESHOOTING FERMENTATION IN CORN WET MILLING ETHANOL PRODUCTION

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To convert starch to ethanol, continuous fermentation processes are employed by corn wet milling plants all over the world. Contaminations by bacterial microorganisms like \textit{Lactobacillus} and wild yeasts like \textit{Brettanomyces} are common and result in lower ethanol yields (Abbott and Ingledew 2005, Skinner and Leathers 2004). Contaminants compete with inoculated yeast for nutrients and produce inhibitory end products, leading to stuck fermentations and expensive down times required for cleaning unit operations (Skinner and Leathers 2004).

Low ethanol yields and poor yeast viabilities in continuous fermentations for ethanol production were investigated. For hydrolyzate preparation, starch liquefaction and saccharification steps at a commercial ethanol facility were reproduced in the laboratory. Fermentations with hydrolyzates prepared in the laboratory were compared with plant hydrolyzate for final ethanol concentrations and total yeast counts. Fermentation controls were prepared using hydrolyzates (plant and laboratory) but were not inoculated with yeast. Hydrolyzates prepared in the laboratory resulted in higher final ethanol concentrations (15.8\% v/v) than plant hydrolyzates (13.4\% v/v). Controls resulted in ethanol production from both laboratory (12.2\% v/v) and plant hydrolyzates (13.7\% v/v), indicating the presence of a contaminating microorganism. Upon further experimentation, involving yeast colony counts on cycloheximide and virginiamycin plates, we confirmed the presence of a contaminant. DNA sequencing and fingerprinting studies conducted also were indicative of a number of dissimilar communities in samples obtained from fermenters, coolers, saccharification tanks and thin stillage.

LITERATURE CITED


PROCESS MODELING OF THE QUICK GERM/QUICK FIBER PROCESS: ENERGY, WATER AND ECONOMICS

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Long term sustainability of ethanol production from corn has been debated widely. Environmentally, the major concern is that producing ethanol from corn demands intensive water and energy consumption. Economically, the ethanol industry is vulnerable to periods of economic weakness because its product value varies with oil prices, while its feedstock corn cost varies with food prices (Rodriguez et al 2010). In response this challenge, the quick germ/quick fiber (QQ) process was developed to improve the profitability of ethanol production. By recovering nonfermentable components (germ and fiber) using a wet fractionation process before fermentation, improvements have been observed on processing efficiency and nutritional characteristics of coproducts at the lab scale (Singh et al 2005); however, analysis of energy and water consumption, coupled with economic performance at the commercial scale is lacking.

A process simulation model was developed on the SuperPro Designer® platform for a modified dry grind ethanol facility using the QQ process. The facility was designed for a capacity of 50 million gallons of annual production. Germ and fiber recovery at the front end improved ethanol production capacity of a conventional dry grind ethanol facility by 24%. The QQ process reduced energy demand by 32% (31,342 vs 42,081 BTU/gal). Major energy savings sources were reduced steam demand in distillation and liquefaction steps. The QQ process reduced water demand by 17.9% (3.49 vs 4.25 gal/gal), because of savings of cooling water resulting from higher ethanol concentration after fermentation. The QQ process had lower ethanol yield (2.72 vs 2.78 gal/bu) because of starch loss during the front end separation. Based on market prices observed in April 2009, the QQ process had a lower payback period (6.5 yr) than the conventional dry grind process (9.2 yr). Increased ethanol production, more value added coproducts, as well as reduced utility costs were contributions improving economic performance of the QQ process (Lin et al 2011).
LITERATURE CITED


INDUSTRIAL EVALUATION OF A DYNAMIC CONTROLLER FOR A SIMULTANEOUS SACCHARIFICATION AND FERMENTATION PROCESS

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The corn dry grind industry is the largest ethanol producer in the US (RFA 2011). The simultaneous saccharification and fermentation (SSF) process is one of the critical process steps that determines ethanol yields and conversion efficiencies of the entire process. Due to its complexity, the SSF process is not controlled completely in corn dry grind processing plants. To control the fermentation process, a dynamic optimal controller (DC) was developed and demonstrated on a 15 L laboratory scale system (Murthy et al 2011). The DC was developed by formulating the SSF process as a Bolza problem and using gradient descent methods (Murthy and Singh 2011). The DC was used to determine fermenter temperature, pH and amount of glucoamylase to achieve optimum performance.

Use of DC during the SSF process resulted in lower peak glucose concentrations, similar ethanol yields (13.38±0.36 and 13.50±0.15% v/v for SSF process with DC and without DC, respectively; Figure 1). Experiments were conducted to simulate temperature and pH disturbances that are likely to occur in practice (Murthy 2006). The optimal controller improved final ethanol concentrations as compared to process without optimal controller under conditions of temperature (13.35±1.28 and 12.52±1.19% v/v for SSF process with DC and without DC, respectively) and pH disturbances (12.65±0.74 and 11.86±0.49% v/v for SSF process with DC and without DC, respectively).

Validation of this optimal controller was conducted in a commercial corn dry grind plant. During commercial trials, DC maintained an average peak glucose concentration of 3.15±0.58% (w/v) for the SSF process compared to 7.9±0.36% (w/v) for conventional SSF process with no DC. There were no differences in final ethanol concentrations with or without the use of DC. Live and budding yeast cell numbers were higher in fermenters with DC. Based on 39 runs over a 3 mo period (Figure 2), use of DC resulted in a 25% reduction in glucoamylase usage.
Installation costs for the DC were estimated to be $10,000 to 15,000 for a 40 million gal/yr ethanol plant. The estimated savings in enzyme costs (based on assumed glucoamylase cost of $0.006/L ($0.024/gal) of ethanol) for a 151 million L/year (40 million gal/yr) ethanol plant were $240,000 (Figure 3). Based on results in industrial scale fermenters, DC use could reduce operating costs and improve fermentation efficiencies by maintaining low glucose concentrations during SSF.

![Graph showing concentration profiles](image_url)

Figure 1. Glucose and ethanol concentration profiles for dynamic controller (DC) in a 15L fermenter.
Figure 2. Final ethanol concentrations in long term industrial evaluation trials.

Figure 3. Estimated cost savings for different ethanol plant sizes.
LITERATURE CITED

Murthy, G.S. 2006. Development of a controller for fermentation in the dry grind corn process. Dissertation. Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, Urbana, IL.


Mention of brand or firm names does not constitute an endorsement by University of Illinois, Oregon State University, USDA or Golden Triangle Energy above others of similar nature not mentioned.
INTEGRATION OF LIQUID FUELS AND VALUE ADDED CHEMICALS PRODUCTION IN BIOREFINERIES

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During the last several years, many attempts have been made to develop technology for production of ethanol as a liquid fuel from lignocellulosic biomass feedstocks. Microbial strains have been isolated or developed for production of ethanol from both biomass derived C5 and C6 sugars. Microbial conversion of C5 sugars to ethanol normally has low efficiencies. In addition, production of ethanol, which is a commodity chemical with small profit margin, as the only main product in a commercial plant with high capital costs results in highly unfavorable overall process economics.

Thus, we proposed a different strategy where only glucose is used for ethanol production and the C5 sugars are used for production of higher value added coproducts. In the first step, biomass is pretreated by soaking in aqueous ammonia. The pretreated biomass is hydrolyzed first with commercial xylanases to generate xylose rich streams, which are used for coproduct production. Residual solids are hydrolyzed with commercial cellulases to generate glucose rich streams, which are used for ethanol production. Fermentation of glucose is performed with the yeast *Saccharomyces cerevisiae*, which is the most efficient ethanol producing organism widely used in the fuel ethanol industry. Results of biomass pretreatment, fractionation and fermentation will be presented.
COMPARISON OF
RESIDUAL STARCH DETERMINATION METHODS
IN DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS)

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Cereal grains across the US and Canada are being utilized to make ethanol. Carbon in ethanol is derived from starch in cereal grain via enzymatic cleavage into sugars followed by biochemical transformation involving fermentation with \textit{Saccharomyces cerevisiae}. A coproduct of ethanol production, known as dried distillers dried grains with solubles (DDGS), contains residual starch that failed to transform into ethanol. Accurate measurement of residual starch is necessary to ensure complete starch hydrolysis in bioethanol plants utilizing cereal grains as a feedstock.

Starch measurement methods are enzymatic or chemical in nature and are designed to cleave starch into glucose. Glucose is measured quantitatively and back calculated to determine starch content. Complications arise when using these methods for DDGS residual starch measurement due to both starch structure (resistant starch) and presence of interfering compounds, such as nonstarch polysaccharides. It is imperative the proposed methodology be able to cleave resistant starch without creating favorable conditions for the hydrolysis of glucose based nonstarch polysaccharides, which could lead to an overestimation of residual starch.

We attempted to optimize and compare directly various starch detection methods for DDGS. Established methods fail to determine quantitatively accurate starch content. Methods reporting low residual starch values are equated with high starch hydrolysis efficiency; however, industry recognizes a gap between theoretical and actual ethanol yields. Correct measurement of residual starch is expected to narrow this gap, thereby allowing industry to improve their process based on the metric of starch hydrolysis efficiency.
ECONOMICS OF PROCESSING
DISTILLERS DRIED GRAINS WITH SOLUBLES
TO HIGH PROTEIN FOOD, BIOFUELS AND ZEIN

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Distillers dried grains with solubles (DDGS) has become an accepted and desirable food for numerous livestock producers; however, DDGS often sells for just 91% the price of corn. However, laboratory and pilot scale testing has been done to demonstrate that DDGS can be processed using ethanol as a solvent to extract water, antibiotic residues, zein, corn oil and glycerin, resulting in high protein distillers grains (HPDG) approaching 40% crude protein. This improvement from 28% crude protein results after corn oil, solubles and water has been extracted from conventional DDGS. An integrated process converts glycerin from the soluble fraction to additional ethanol by bacterial fermentation. Another integrated process converts extracted corn oil to biodiesel or jet fuel. Zein also can be collected from DDGS for use as a food preservative. As little as 1% of the ethanol used in extraction is lost in the extraction process, residing mostly in the high protein foodstuff.

In additional to the extra fuel and higher protein food, there may be qualitative advantages resulting from these integrated processes. The high protein grain product (HPDG) should be free of any residual traces of antibiotic, which may be important to some customers. In addition, residual traces of ethanol in the food serve to reduce biological degradation of the food while in storage. Because the protein found in the conventional DDGS has been concentrated in HPGD, there is 30% less mass to be shipped for the same amount of protein, an advantage when serving distant, international customers.

Economic analysis of this integrated biorefinery utilizes pilot scale estimates of capital costs for such a biorefinery, which could be colocated at an established ethanol plant to share facilities, scales, offices and staff. In addition to being a source of solvent ethanol, steam from an existing ethanol plant can provide thermal loads to heat ethanol used for extraction. Distillation capacity at an existing plant can be used to remove water from ethanol used for extraction, so solvent ethanol can be reused in another round of
extraction or sold as fuel. In terms of products to be sold, an additional 10% ethanol and 10% biodiesel or jet fuel beyond the amount of ethanol already recovered from starch fermentation can be sold from every bushel of corn processed.

Sensitivity analysis was performed using our economic model in an effort to understand the economic viability of this method of capturing more valuable coproducts from every bushel ground at a dry grind plant. Because the additional ethanol produced arises from glycerin, not starch, this fuel should be considered an advanced biofuel and should receive incentives in terms of payments and/or RINS certificates. This process may be important in capturing more value from each bushel ground by ethanol plants, resulting in gains for growers and processors of corn, while improving the life cycle performance of ethanol processing.
ECONOMIC EVALUATION OF KOREAN HIGH OIL CORN:
GENOMIC RESEARCH, DEVELOPMENT OF LOCAL HYBRIDS,
CONSIDERATION OF MILLING AND EXTRACTION METHODS

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Access to several ears of very high oil corn from North Korean permitted
University of Minnesota corn breeders the opportunity to study Korean High Oil (KHO)
corn, which was found to have oil levels approaching 20 vs 3.5% found in many US corn
hybrids. Research team members recognized this unusual line had kernels with twice the
percentage of oil in the germ and germ three times as big as those found in many varieties
of corn. Detailed genomic analysis was performed to determine the genes responsible for
production of higher amounts of oil. Insight also was gained with respect to genes
responsible for establishing the balance between oleic and linoleic acids, reflecting
quality attributes for different uses. Crosses with locally adapted lines of corn were made
to determine opportunities to increase the oil content of corn grown in the US Corn Belt,
resulting in crosses having 11 to 12% oil.

For hybrids carrying the KHO genes, milling characteristics have been evaluated;
the cost and effectiveness of various oil extraction technologies continue to be
considered. Oil extraction techniques considered include wet mill technology, quick
germ/quick fiber and back end extraction using solvents and centrifuge techniques
applied to distillers dried grains with solubles (DDGS) following dry grind processing.

To determine probable extraction amounts and quality of oil by a variety of
techniques, economic models have been utilized and continue to evolve. The value of
starch and bran constituents also are important when considering costs of applying
milling and extraction to locally adapted hybrids that may be released containing the
KHO genes. Another part of the economic research will require field scale production of
KHO hybrids to determine yields of these hybrids and the level of yield drag that may be
permissible for farmers with interest in growing high oil corn hybrids. Large scale
utilization of KHO technology resulting in 12% oil corn would be transformative in the
US Corn Belt and around the world because corn fields could produce over 1000 pounds
of oil per acre or 70% more than soybeans.
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