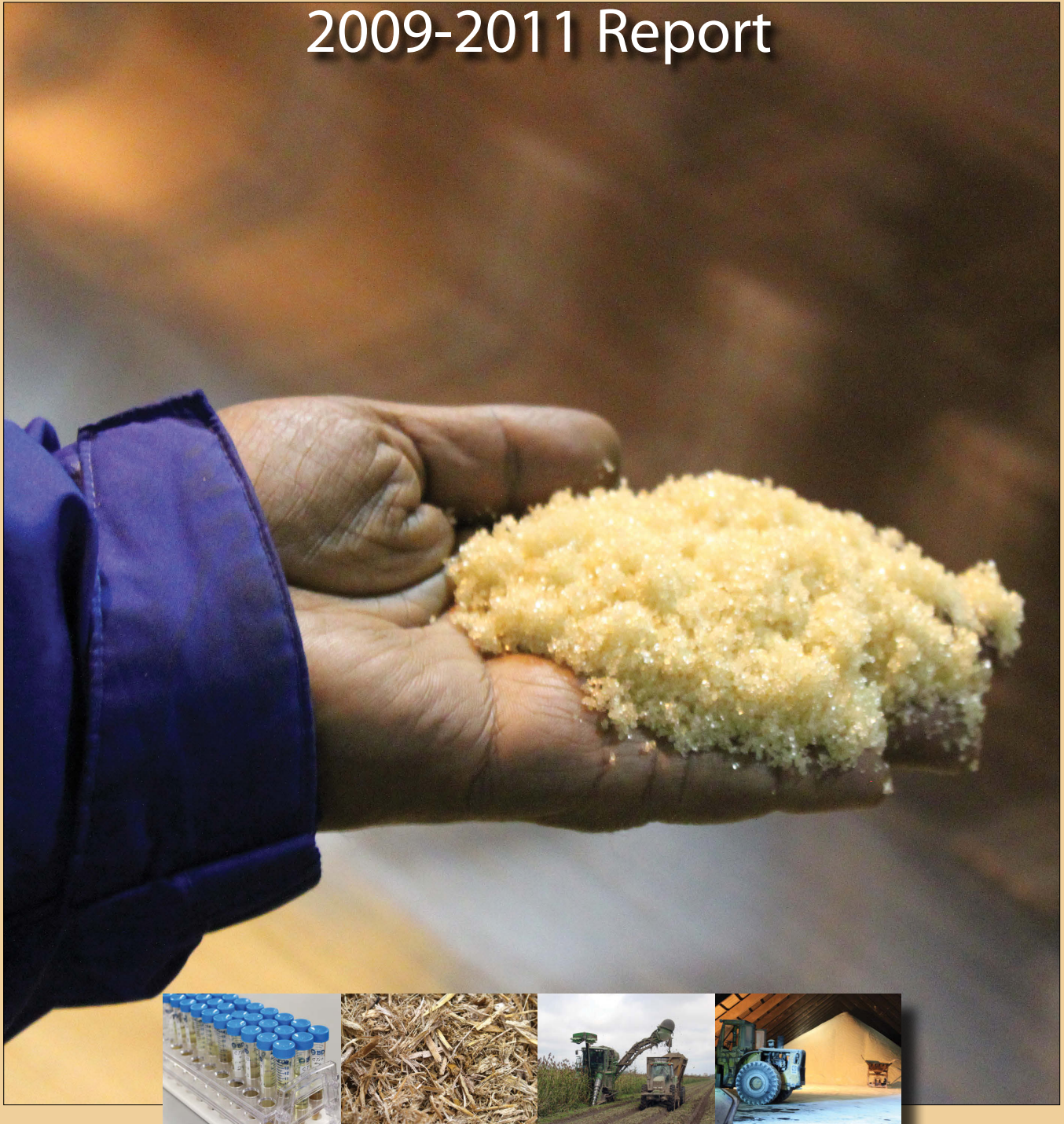




# Audubon Sugar Institute 2009-2011 Report



Sugar and Biofuel Research for Louisiana





**Audubon Sugar Institute**  
Louisiana State University Agricultural Center  
3845 Highway 75, Saint Gabriel, LA 70776, USA

Tel 225-642-0135 Fax 225-642-8790  
[www.LSUAgCenter.com/audubon](http://www.LSUAgCenter.com/audubon)

## Table of Contents

Audubon Sugar Institute . . . . .	2
Vice Chancellor's Foreword . . . . .	3
Head of Institute's Report . . . . .	4
Research Reports . . . . .	5
Extension Work . . . . .	20
Analytical Capabilities . . . . .	22
Education . . . . .	23
Publications . . . . .	24
Faculty and Staff . . . . .	27

### Audubon Sugar Institute Advisory Board

Dr. John Russin – LSU AgCenter  
Dr. Kenneth Gravois – LSU AgCenter  
Windell Jackson – American Sugar Cane League  
Lonnie Champagne – Louisiana Sugar Cane Products, Inc.  
David Thibodeaux – St. Mary Sugar Cooperative, Inc.  
Will Legendre – M.A. Patout & Sons Ltd.  
Mike Combs – Louisiana Sugar Cane Cooperative  
Lance Weber – Cajun Sugar Cooperative  
David Stewart – Alma Plantation Ltd.  
Charlie Schudmak – Cora Texas Manufacturing Co.  
Neville Dolan – Raceland Raw Sugar  
Duane Legendre – Lafourche Sugars LLC  
Stephen Bergeron – Lula/Westfield LLC  
Dr. Benjamin Legendre – LSU AgCenter

### Goals of the Audubon Sugar Institute

- To enhance the productivity and profitability of the Louisiana sugar industry and other sugar-processing-related industries.
- To improve the practice of sugar manufacturing through education and technology transfer.
- To conduct research toward a diversified sugar-processing industry.
- To attract, retain and develop a world-class staff to serve our stakeholders.
- To encourage use of low-environmental-impact technologies in sugar processing.



[www.LSUAgCenter.com/audubon](http://www.LSUAgCenter.com/audubon)

**Louisiana State University Agricultural Center**  
William B. Richardson, Chancellor  
**Louisiana Agricultural Experiment Station**  
John S. Russin, Vice Chancellor and Director  
**Louisiana Cooperative Extension Service**  
Paul D. Coreil, Vice Chancellor and Director

The LSU AgCenter is a statewide campus of the LSU System and provides equal opportunities in programs and employment.





## Vice Chancellor's Foreward

*As the new vice chancellor for research in the LSU AgCenter and director of the Louisiana Agricultural Experiment Station, I am honored to provide comments for this issue of the Audubon Sugar Institute Report.*

*Second only to forestry in overall value, sugar is the leading row crop in the state and contributed in excess of \$1.081 billion to Louisiana's agricultural economy in 2011. The LSU AgCenter is proud of its role in helping to maintain the profitability of this industry. In strategic partnership with the American Sugar Cane League, which provided much of the support for the projects overviewed in this report, we plan to provide effective research and outreach for years to come.*

*Scientists from the Audubon Sugar Institute maintain a strong presence at Louisiana's sugar factories. Led by Drs. Harold Birkett and Vadim Kochergin, these teams provide valued assistance in all phases of factory activities beginning with mill-yard storage and culminating with quality of the finished product. New clarification technologies developed at the Audubon Sugar Institute show great promise, while ongoing work to reduce entrainment sugar loss and enhance boiler efficiency are just part of the LSU AgCenter's efforts to improve the industry's bottom line.*

*Adding new value to the existing sugar industry, indeed all agricultural industries in Louisiana, remains a strong focus of the LSU AgCenter. Through some major awards from U.S. Department of Agriculture and the U.S. Department of Energy, we are expanding our biofuels research program to develop biomass-based fuels and specialty chemicals from bagasse and sweet sorghum. If successful, our efforts will provide a sound, science-based foundation for developing these new industries in Louisiana. The newly established Louisiana Institute for Biofuels and Bioprocessing, whose activities include some of the programs at the Audubon Sugar Institute, will play a leading role in these initiatives.*

*The LSU AgCenter, through its various sugar research programs, is committed to a strong, effective footprint in Louisiana's sugar industry. Allied closely with the American Sugar Cane League and the USDA-ARS Sugarcane Research Unit in Houma, we are committed to the long-term stability and prosperity of Louisiana's sugar producers and processors.*



Dr. John R. Russin

**Louisiana is the oldest and most historic of the U.S. sugarcane producing areas. Sugarcane arrived in Louisiana with the Jesuit priests in 1751, and the first sugarcane was planted within the city limits of New Orleans near where their church now stands on Baronne Street. Then, in 1795, Etienne DeBoré first granulated sugar on a commercial scale at Audubon Park. Except for disastrous production years during the Civil War, the disease epidemics of the 1920s and after a severe freeze of 10 degrees Fahrenheit during December 1989 that affected the 1990 crop, the Louisiana sugarcane industry has continued to prosper, mainly due to improved varieties, cultural practices, pest control and sugar processing innovations.**



Dr. Benjamin L. Legendre

The Louisiana sugarcane industry is in its third century of uninterrupted sugar production. During the period covered by this report the total value of the sugarcane crop to Louisiana producers, processors and landlords at the first processing level has increased to \$1.081 billion; sugarcane is ranked first as the leading agricultural row crop produced in Louisiana.

Although seven factories have closed in the past nine years, the remaining 11 factories have increased their capacity to absorb the cane supply that was crushed at the closed factories. At the same time, the remaining factories have become more efficient due to the dedicated research activities of the faculty of the LSU AgCenter and the Audubon Sugar Institute (ASI). ASI has taken the lead in assisting factories in improving pol extraction (pol percentage in cane), reducing pol losses in bagasse and in filter cake, reducing molasses purities and increasing total sugar recovered as a percent of the total sugar in cane.

As demanded by our stakeholders, the faculty of ASI has made a concerted effort to visit each of the factories throughout the year to assist in design and installation of new equipment and to provide consultation in mill yard storage, cane preparation, milling, boiler operations, steam balance, clarification, crystallization, exhaustion of final molasses and sugar storage and quality. These visits are well received and slow-but-steady improvements in processing

efficiency are evident. Further, many research projects conceived by ASI are funded by the industry through recurring appropriations, the dedicated research funding of the American Sugar Cane League and onsite research conducted at and funded by the 11 factories.

A significant factor in increased efficiency has been the consolidation of the factories, which meant the remaining factories have an ample cane supply to make the necessary improvements to bolster their efficiency. Our faculty has been proactive in diagnosing possible problems in processing that might otherwise go unnoticed. For instance, the results from a preliminary study in 2009 to investigate high levels of entrainment were enough to warrant continued investigations in 2010. It was found that sugar losses in condensates generally are insignificant (average 0.06 pounds per ton of cane). However, losses in condenser water are significant, averaging 1.0-1.4 pounds per ton of cane, but can be as high as 8.6 pounds per ton. The entrainment at each factory was measured, and recommendations were made to reduce or eliminate sugar losses due to entrainment.

In another instance, there was a significant increase in the starch content of the juice and consequently the sugar during the 2010 crop. This necessitated many visits by our faculty to diagnose the cause of the problem and to make recommendations to correct it. With the assistance of ASI faculty, factories also have made great strides in improving boiler efficiency and steam utilization thereby minimizing natural gas consumption and greatly lowering operating costs.

Since 2001, the ASI's analytical lab has analyzed the final molasses provided weekly by the Louisiana sugar factories to determine the efficiency at which each factory is recovering sugar from their massecuites. ASI faculty members have led the way in efforts to produce VHP and VLC sugar and to understand the changes to raw sugar in storage, which is of critical importance for the future competitiveness of the Louisiana sugar industry. An innovative way to ventilate the sugar pile in storage has been evaluated by ASI faculty for the past two years and is showing some promise in

helping to hold or maintain the color of the sugar placed in storage.

Research also was conducted in cooperation with the USDA-ARS, Sugarcane Research Unit at Houma to assess stalk cold tolerance of commercial and candidate varieties. This information has led to better harvest management of the different varieties following a killing freeze. ASI faculty is also involved in studying the efficacy of glyphosate as a chemical ripener. It has been found that glyphosate can increase the yield of recoverable sugar per ton by 5-30 percent, especially early in the harvest season.

Research is under way at ASI and the factories to improve use of biocides, particularly amylase, for control of starch after the unprecedented levels reached during the 2010 crop. Other research conducted the past two years involved the control of dextran in process streams, plan and testing of new, less expensive clarifier designs, proper seeding of pans, crystallization, centrifugation and exhaustion of molasses. The outcome of these studies should result in further improvements in efficiency as well as lower costs of production such that the Louisiana sugar industry cane remain competitive in a global economy.

A two-year grant was awarded to the ASI by USDA in 2009 to generate nontoxic, biodegradable polyesters from sugarcane, in particular from molasses and bagasse. Further, ASI faculty received funding from the U.S. Department of Energy to develop technology for a bagasse-based biorefinery that will give sufficient economic advantages when integrated with a sugarcane factory to support low-cost ethanol and other biofuels production. Research conducted in 2010 and 2011 was to lay the foundation for use of new grassy crops such as sweet sorghum and possibly Miscanthus that can be used in a sugarcane factory-based biorefinery. Although significant progress has been made in determining the feasibility of using the sugarcane factory as a biorefinery, much remains to be done.

Finally, ASI faculty and staff would like to express appreciation to the American Sugar Cane League and the Louisiana sugar industry for their continued financial support.





## Giovanna M. Aita

Dr. Giovanna M. Aita is an assistant professor at the Audubon Sugar Institute with adjunct appointments in the Department of Food Science and the Department of Biological and Agricultural Engineering in the LSU AgCenter.

She heads the renewable fuels and byproducts research laboratory at the Audubon Sugar Institute. Her research work focuses on handling and storage of lignocellulosic biomass (e.g. sugarcane, energy cane, sorghum), development of processing technologies for the breakdown of lignocellulosic biomass into its corresponding monomers (e.g. sugars, phenolic compounds), conversion of monomeric compounds into bio-based fuels and byproducts and fuel production from algae.

## Pretreatment of Sugarcane, Sorghum and Energy Cane Bagasse by a Dilute Ammonia Process

Pretreatment of lignocellulosic biomass is required to make cellulose accessible to enzymes for conversion into fermentable sugars and further bioconversion into fuels and chemicals. The purpose of a pretreatment is to reduce cellulose crystallinity, remove lignin and/or hemicellulose and increase biomass porosity.

Bagasse (sugarcane, sorghum or energy cane) was pretreated with water and ammonium hydroxide (28 percent v/v) in mass proportions of 1:8:0.5 at 160 degrees C for 60 minutes (PCT/US2009/033173). After pretreatment, a 37 percent, 44 percent and 55 percent delignification (g/100g dry biomass) was observed in dilute ammonia treated bagasse, respectively (Table 1).

Alkali pretreatments (sodium, calcium, potassium and ammonium hydroxide) are very effective in lignin solubilization, exhibiting a lesser effect on cellulose and hemicellulose as compared to acid pretreatment. But complete biomass delignification is difficult because of the location of lignin within the lignin-carbohydrate complex, strong poly-ring bonds of C-O-C, C-C, and hydrophobicity.

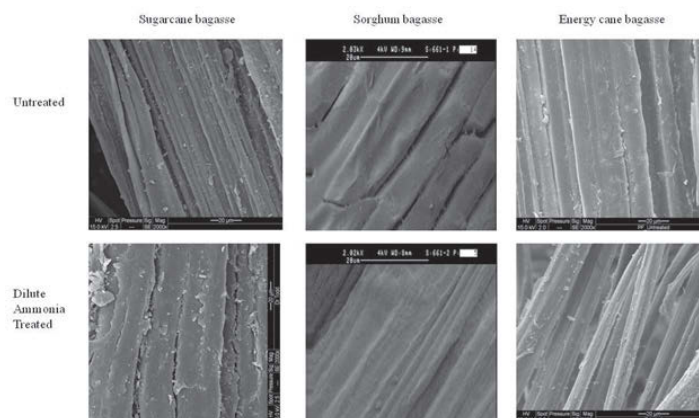
Most of the cellulose (more than 90 percent) was retained in the biomass regardless of the type of crop. However, a higher loss of hemicellulose (22 percent, 35 percent and 30 percent) was detected in sugarcane, sorghum or energy cane, respectively.

Significant morphological changes were observed in this study as shown in Figure 1. Bagasse changed from compact and rigid to loose and swollen after treatment with dilute ammonia. Monitored enzymatic hydrolysis yields (cellulose only) were 89 percent (sugarcane), 84 percent (sorghum) and 75 percent (energy cane).

It is speculated that the improvements in enzyme hydrolysis in the dilute ammonia treated samples were due to lignin removal, increased surface area and the presence of pores that can be

accessed by enzymes. Theoretical ethanol yields (cellulose only) averaged 24 grams ethanol per 100 grams dry biomass. (G. Aita, M. Walker, D. Salvi, T. Ramachandran, D. Robert) ■

Figure 1. Scanning electron microscopy images of untreated and dilute ammonia treated sugarcane, sorghum and energy cane bagasse.



## Research Studies at the Renewable Fuels and Byproducts Laboratory

Biorefineries are processing facilities that use renewable plant materials (i.e., carbohydrate, protein, oil and lignin) as biomass. These plant materials are converted in the biorefinery into higher-value products.

This opportunity can become available in Louisiana if biomass feedstocks are grown on fallow or marginal land. The recent USDA Roadmap on Biofuels calls for the production of 13.4 billion gallons of advanced biofuels from grassy crops with the southern region expected to supply 50 percent of these crops.

Energy cane and sweet sorghum have similar gross structure to sugarcane and are considered the most appropriate crops for this area. In a modified sugar mill operation, they could be handled by traditional sugarcane harvest and delivery systems and processed into green fuels and chemicals without interfering with the sugarcane season. Water supplies, carbon dioxide and land are unexploited assets at sugarcane mills and could potentially be used for the growth and processing of algae as an additional biofuel feedstock.

Progress has been made in overcoming some of the technical barriers for the conversion of biomass into higher-value products, but much still remains to be done. Some of the barriers that must be overcome on the technical side include biomass handling, storage and distribution; biomass recalcitrance and pretreatment; cellulosic enzymes and microbial development; separation technologies; and process integration. (G. Aita) ■

Table 1. Biomass composition before and after pretreatment with dilute ammonia.

Biomass Components *	Sugarcane Bagasse		Sorghum Bagasse		Energy Cane Bagasse	
	Untreated	Dilute Ammonia	Untreated	Dilute Ammonia	Untreated	Dilute Ammonia
Ash	3.71	2.14	0.31	0.38	0.69	0.75
Glucan/Cellulose	43.19	40.96	44.52	40.18	45.53	41.63
Xylan/Hemicellulose	23.41	18.3	27.72	18.01	24.67	17.24
Arabinan	1.49	0.83	2.79	1.79	2.73	2.61
Mannan	ND	ND	ND	ND	ND	ND
Lignin	25.33	16.09	22.04	12.36	24.95	11.27
Total	97.13	78.32	97.38	72.72	98.57	73.5

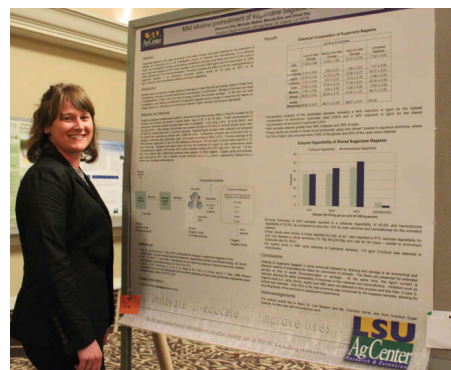
\*= g/100 g dry biomass; ND: none detected.



## Soaking of Sugarcane Bagasse in Dilute Ammonia during Storage

Biofuels require processing of large volumes of biomass to match the fuel and energy output of fossil fuels. Consequently, biomass typically is stored prior to processing in a biorefinery.

Storage of biomass can result in biodegradation of fibers, which can cause loss of energy content and possible spoilage. A mild ammonia-soaking or preconditioning of sugarcane bagasse during storage (one month at 30 degrees C) was investigated to determine the effects on energy content (i.e., cellulose and hemicellulose retention) and lignin removal.



Dr. Michelle Walker with her poster entitled, "Mild alkaline pretreatment of sugarcane bagasse during storage" at the 33rd Symposium on Biotechnology for Fuels and Chemicals, Seattle, Wash., May 2, 2011.

The fibers resisted spoilage over the 30-day period, retained most of the energy content (100 percent cellulose, 70 percent hemicellulose) and lost 44 percent of lignin. The loss of lignin actually is beneficial in the conversion of biomass to biofuels because that allows better access for enzymes to hydrolyze sugar polymers to monomers for eventual fermentation to fuels and value-added products. In addition, only 30 percent of the free ammonia was consumed by the bagasse samples, allowing for recycling of the ammonia in subsequent studies. (G. Aita, M. Walker) ■

## Microwave-Assisted Dilute Ammonia Pretreatment of Sorghum Bagasse

Microwaving is an alternative heating method to conventional conduction and convection heating. While convection and conduction are based on superficial heat transfer; microwaving uses the ability of an applied electromagnetic field to directly interact with the molecular structure of the heated object.

When microwaves are used to treat lignocellulosic biomass, they selectively heat the more polar parts and creates a "hot spot" within the material. It is hypothesized that this unique heating feature results in an "explosion" effect among the particles and improves the disruption of the recalcitrant structures of lignocellulose.

Sorghum bagasse with particle sizes ranging from 9.5 to 18 millimeters, 4 to 6 millimeters and 1 to 2 millimeters were pretreated with 28 percent (v/v) ammonium hydroxide and water at a ratio of 1:0.5:8 at five temperatures (100 degrees C, 115 C, 130 C, 145 C and 160 C) for one hour by microwave heating. The best ethanol yield among all pretreatment conditions was  $22 \pm 1.1$  grams per 100 grams dry biomass for 1-2 millimeter bagasse pretreated at 130 C. (G. Aita, D. Boldor, C. Chen) ■

## Effect of Surfactants on the Enzymatic Hydrolysis of Dilute Ammonia Pretreated Sugarcane Bagasse

The polymers cellulose, hemicellulose and lignin are not readily available for conversion into fuels and chemicals in their native form.

It is widely accepted that lignin acts as the "glue" that binds cellulose and hemicellulose, giving both rigidity and resistance to the lignocellulosic structure. The use of nonionic surfactants during pretreatment can help alter the structure of lignocellulosic biomass to improve cellulose digestibility and ethanol yields.

Tween 80 has been demonstrated to improve lignin solubility and lignin removal during the pretreatment stage. Tween 80, Tween 20, PEG 4000 or PEG 6000 were used in combination with ammonium hydroxide for the pretreatment of sugarcane bagasse. The pretreatment was carried out by mixing sugarcane bagasse, ammonium hydroxide, water and 3 percent (w/w) surfactant based on the weight of dry biomass and heating the mixture to 160 degrees C for 1 hour.

PEG 4000 and Tween 80 were the most effective surfactants, increasing cellulose digestibility and ethanol yield by 78 percent and 73 percent, respectively, over dilute ammonia pretreatment without the addition of surfactants. None of the surfactants caused additional loss of cellulose, but in some cases, an increase in lignin removal was observed. Tween 80 has been chosen for future studies because of its lower production cost compared to PEG 4000. (G. Aita, S. Cao) ■

## Bioplastics from Sugarcane

This study aims at generating nontoxic, biodegradable polymers from sugarcane by making use of molasses, bagasse, glycerol and lignin-containing waste streams generated during the conversion of sugarcane bagasse into ethanol.

Polyesters will be made from aconitic acid (to be isolated from molasses), glycerol and cinnamic acid (to be isolated from pretreatment wastes associated with the production of ethanol

Figure 2. Polyester prepared from commercially available glycerol, citric and cinnamic acids.







using dilute ammonia pretreatment technology). Polyester matrices have been prepared from commercially available glycerol, citric and cinnamic acid (Figure 2). An economical approach for the isolation and/or purification of glycerol and precursors (aconitic acid and cinnamic acid) via ion-exchange, addition of salts or continuous liquid-liquid extraction is under evaluation.

In addition, it is expected that in-vivo the gelled polyester matrices will support cellular growth. This will result in a matrix impregnated by oriented cells that can serve to speed healing of severe wounds while degrading into nontoxic compounds easily absorbed and excreted by the human body. (G. Aita, L. Madsen, A. Kanitkar) ■

## Isolation, Identification and Lipid Characterization of Algal Strains for Biofuel Production

Microalgae are photosynthetic organisms that produce and store lipids. They also fix carbon dioxide, which makes them attractive candidates for the production of fuels in the area of sugarcane mills because they could benefit from the supply of carbon dioxide and energy generated at these sites.

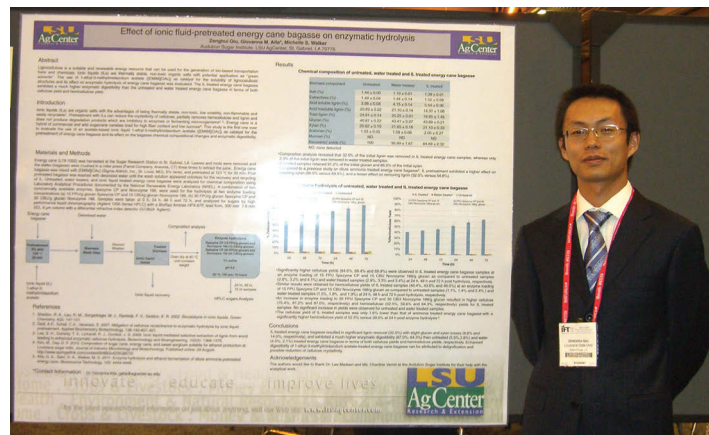
Seventeen isolates were obtained from both saltwater and freshwater sources within the state of Louisiana's sugar belt. Through morphological differences and microscopic observations, four microorganisms were chosen and identified as *Synechococcus sp.*, *Sellophora pupula*, *Chlorella sorokiniana* and *Scenedesmus abundans* by PCR amplification of 16S rRNA or 18S rRNA genes (Figure 3).

The predominant fatty acids were palmitic, myristic, oleic, linoleic and linolenic acids as confirmed by GC-FID lipid analysis. Palmitic, stearic, oleic and linoleic acids are recognized as the most common fatty acids contained in biodiesel. The fatty acid profiles of the identified and analyzed microorganisms make them potential candidates for biodiesel production. The predominant fatty acids were palmitic, myristic, oleic, linoleic and linolenic acids as confirmed by GC-FID lipid analysis. Palmitic, stearic, oleic and linoleic acids are recognized as the most common fatty acids contained in biodiesel. The fatty acid profiles of the identified and analyzed microorganisms make them potential candidates for biodiesel production. (G. Aita, R. Moreno) ■

## Ionic Liquid as Catalyst during Pretreatment of Energy Cane Bagasse

Ionic liquids are thermally stable, nontoxic organic salts with potential applications as "green solvents."

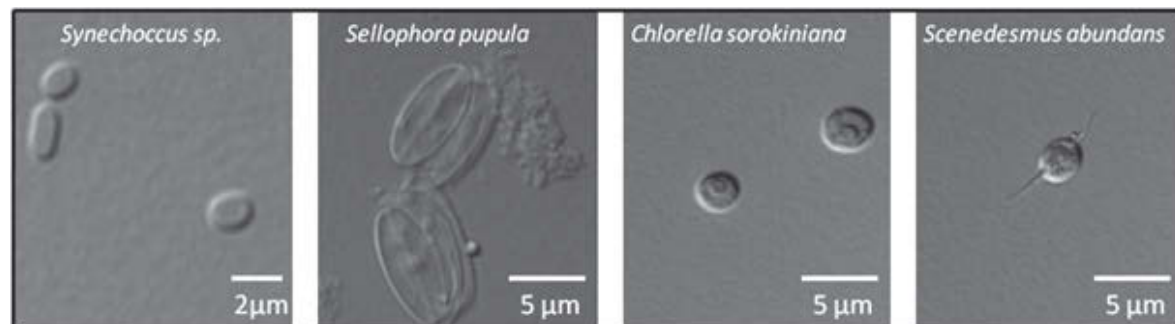
In this research, the use of 1-ethyl-3-methylimidazolium acetate as a catalyst for the solubility of lignocellulosic structures and its effect on biomass composition and enzymatic hydrolysis of energy cane bagasse was evaluated.



Zenghui Qiu was a finalist for the Biotechnology Division Poster Competition at the 2011 Institute of Food Technologists' Annual Meeting & Food Expo, held at New Orleans, La., June 11-14, 2011. His poster was entitled, "Effect of ionic fluid-pretreated energy cane bagasse on enzymatic hydrolysis."

Ionic liquid treated energy cane bagasse resulted in significant lignin removal (32 percent) with slight glucan and xylan losses (9 percent and 14 percent) respectively and exhibited a much higher cellulose and hemicellulose digestibility (87 percent, 64 percent) than untreated (6 percent, 3 percent) and water (4 percent, 2 percent) treated energy cane bagasse, respectively. Enhanced digestibility of 1-ethyl-3-methylimidazolium acetate treated energy cane bagasse can be attributed to delignification and possible reduction of cellulose crystallinity. (G. Aita, Z. Qui) ■

Figure 3. Spectral confocal microscopy images of microorganisms identified for potential use in biodiesel production.





## Harold Birkett

*Dr. Harold Birkett has more than 40 years sugar, ethanol and sugar byproducts experience.*

*He has worked 10 crops in sugar factories both in Louisiana and overseas as chief chemist, fabrication superintendent and rum distillery manager. He holds a B.S., M.S. and Ph.D. in chemical engineering and is a registered professional engineer. He developed computerized material and energy programs for the sugar and process industries that facilitate the design of plants and the analysis of factory operation and the checking and analysis of laboratory reports.*

*Birkett has served as the chief process design engineer and process specialist for Schaffer and Associates for the past 33 years. He also is an LSU AgCenter associate professor with the Audubon Sugar Institute where his main area of interest is improving the efficiency of the Louisiana sugar industry through teaching, extension and research.*

*His interests include the testing of commercial factory equipment, final molasses exhaustion, milling efficiency and boiler efficiency improvements. He specializes in core lab (cane quality determinations) operations, overall sugar processing and energy efficiency including cogeneration of electricity.*

## Brief Summary of Project to Determine Evaporator Heat Transfer Coefficients

The capacity and configuration of the evaporator station are key considerations in sugar factory design. The capacity is affected by vapor bleeds, level of imbibition, syrup Brix, steam pressure and frequency of cleaning. The configuration of the evaporator station (number of effects, number of effects bled and quantity of vapor bled) controls the exhaust steam requirements of the factory.

The objective of this study, originally conducted in the late 1990s and early 2000s, was to gather data from actual factory operations and use the data to calculate heat transfer coefficients, evaporation rates and effects of scaling on the heat transfer coefficients. During processing of the past season's crop, data were collected again in conjunction with a project investigating heat transfer coefficients of plate heaters. The data required included duty that the evaporator performs, operating conditions of the evaporator and evaporator equipment installed. Actual data for the grinding rate and boiling house operations were used to calculate the steam requirement for the heaters and pans operating on vapors. A rigorous heat balance calculation for the evaporator station was performed to calculate the evaporation rates and heat transfer coefficients for the individual evaporator effects.

Results show the high heat transfer coefficients measured for the first, second and third (unless the last) effects indicates increased vapor bleeding from these bodies is practical. The high heat transfer coefficients measured for all evaporator effects (350-550 Btu/hr/sq. ft./°F), with the exception of the last effect (120-200 Btu/hr/sq.ft./°F), indicate increasing the number of effects to achieve greater steam economy will require less additional equipment than generally believed. The improved heat transfer coefficient of the first evaporator effect when a clarified juice heater is installed indicates that the benefits of clarified juice heating may be greater (6 percent) than generally assumed.

We would like to express appreciation to the American Sugar Cane League and all Louisiana sugar factories for their support of this project. (H. Birkett, J. Stein) ■

## Entrainment Losses at Louisiana Sugar Factories

Several Louisiana factories experience high levels of entrainment, which results in the loss of recoverable sugar. Results from a preliminary study in 2009 to investigate these losses were enough to warrant continued investigation in 2010.

Condensate and/or condenser water samples were collected at every Louisiana factory and analyzed for trace sugar using the phenol-sulfuric acid method (Dubois et al 1956; SASTA, 1985).

Table 2 shows the average, high and low sugar concentrations in the condensate leaving the individual evaporator bodies.

Table 2. Sugar in evaporator body condensates.

	Evaporator Body Condensates, ppm sugar				
Factory	First	Second	Third	Fourth	Average
Average	6.74	20.62	39.90	108.77	48.04
High	29.83	139.75	558.73	1653.79	641.43
Low	0.21	1.76	0.92	2.12	2.53

For evaporators with no leaking tubes, the first effect condensate should be sugar free since this is condensed exhaust. The condensates from the subsequent bodies generally contain sugar resulting from entrainment.

The second, third and fourth body condensates contained an average of 21, 40 and 109 parts per million of sugar, respectively. The increasing level of entrainment down the evaporator set is expected as the vapor velocity increases at the progressively lower pressures in the effects while the sugar concentration of the syrup also is increasing.

The quantity of evaporator condensate produced typically is in the range of 1,300 to 1,700 pounds per ton of cane. The average sugar concentration in the evaporator condensates of 48 ppm represents a loss of about 0.06 pounds of sugar per ton of cane.

Table 3 shows the average, high and low sugar losses in once-through, closed (with intermittent blowdown) and recirculation systems with continuous blowdown.

Table 3. Sugar losses in condenser water (pounds of sugar per ton of cane).

	Once-through Systems	Closed (Assuming 2 Percent Blowdown)	Recirculating With Continuous Blowdown
Average	1.14	0.94	1.11
High	8.57	2.42	3.29
Low	0.01	0.01	0.01

For the once-through systems, the sugar concentration in the condenser water varied from 1 to 857 ppm. Assuming 10,000 pounds of water per ton of cane for the once-through injection water system, the sugar loss varied from 0.01 to 8.57 pounds of sugar per ton of cane and averaged 1.14 pounds of sugar per ton of cane. This indicates there is considerable scope for reducing sugar losses in the condenser water.





For the closed systems (cooling tower or spray pond) or recirculating systems with continuous blowdown, it is not possible to calculate the sugar losses without knowing the quantity of blowdown. The sugar concentration in the water in the cooling tower and spray pond systems varied from 26 to 7,121 ppm and averaged 2,764 ppm. Assuming the blowdown from the closed systems is about 2 percent, the average sugar losses at these factories would be comparable to the 1.14 pounds per ton of cane losses estimated for the once-through systems.

For recirculation systems with continuous blowdown, the sugar losses varied from 3 to 1,533 ppm and averaged 1.11 pounds of sugar per ton of cane.

A routine, and preferably continuous, monitoring system for measuring entrainment losses is highly recommended. Entrainment losses from evaporator, vacuum pan and filter condensers all are subject to periodic high levels of entrainment. High levels of sugar in the condenser water at several factories indicate improved entrainment separators would be highly cost-effective.

Sugar losses in condensates generally are insignificant (average 0.06 pounds per ton of cane). Sugar losses in condenser water are significant, averaging 1 to 1.4 pounds per ton of cane but can be as high as 8.6 pounds per ton of cane. (H. Birkett, J. Stein) ■

## ACKNOWLEDGMENTS

We would like to thank all of the factories and personnel who participated in the study – particularly for so kindly assisting us with sample collection.

## REFERENCES

- Dubois, Michel, K.A. Gilles, J.D. Hamilton, P.A. Rebers and Fred Smith, 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* 28:350-356.  
South African Sugar Technologists' Association. 1985. *Laboratory Manual for South African Sugar Factories*, pp. 366-367.

**Efforts to minimize U.S. dependence on foreign oil are pushing the country toward the development and use of biologically derived fuels and feedstock chemicals.**

## Mill Performance Summary

The LSU AgCenter's Audubon Sugar Institute has tested the performance of milling tandems in Louisiana for many years. Mill performance tests are an independent evaluation of how well individual mills and the tandem are extracting pol (sucrose) from the sugarcane being milled.

Seven mill extraction tests (five tandems) were conducted during the 2010 crop. Prepared cane and bagasse samples leaving each mill in the tandem were collected over a 30-60 minute period and analyzed for pol, moisture, fiber and ash content using standard methods. The data were used to calculate preparation index (pol percentage open cells) and pol extraction.

A brief summary of milling data for the past crop is shown in Table 4. Of note for the 2010 crop is the high average fiber percentage in cane of 14.38. Also of interest are the highest individual tandem and reduced extractions (96.70 percent and 96.95 percent, respectively) ever measured by Audubon Sugar Institute.

Summary data from 2005-08 is compared to that of 2009 and 2010 in Table 5 (page 10). Again, of note, is the higher fiber percentage of cane of 14.38 for 2010 versus 12.27 percent for 2005-08. Pol percentage of cane of 12.88 this past crop was higher than 12.02 percent during 2005-08. Moisture percentage of bagasse averaged 50.60 in 2010 compared with the previous 2005-08 average of 52.92. Average tandem pol extraction this past crop was 93.53 percent, which was 0.75 points higher than the 2005-08 average of 92.78. The ash percentage of cane in 2010 of 1.96 was lower than for the 2005-08 period (2.83 percent) and for 2009 (2.76 percent).

While mill performance has improved over the years, there is still work to be done to improve pol extraction and reducing bagasse pol losses (average 14.92 pounds 96 sugar/ton cane or \$4.5 million per factory in 2010). Running a weekly moisture tests can indicate which mill or mills in the tandem need adjustment and is an easy method to help improve extraction.

Table 4. Summary of mill performance data for 2010.

Run Number:	1	2	3	4	5	6	7	Average
Preparation index	80.21	74.94	82.94	81.05	76.67	77.22	78.49	78.79
Fiber percentage of cane	14.55	13.11	14.55	14.72	13.40	16.04	14.29	14.38
Pol percentage of cane	12.33	12.24	14.39	14.29	12.71	12.06	12.14	12.88
Imbibition percentage of cane	31.55	25.93	20.60	21.31	28.57	25.48	26.25	25.67
Moisture percentage of bagasse	51.91	54.37	45.96	46.09	48.41	54.83	52.65	50.60
Mill 1 extraction, percentage	59.47	58.33	69.97	67.79	74.51	27.11	46.55	57.68
Tandem extraction, percentage	90.82	91.21	95.00	94.62	96.70	92.41	93.97	93.53
Reduced extraction, percentage	92.30	91.68	95.80	95.55	96.95	94.33	94.84	94.49



Table 5. Summary data from 2005-08 and 2009 versus 2010.

	2005-08	2009	2010
Prepared cane			
Preparation index	79.73	80.74	78.79
Pol percentage	12.02	12.01	12.88
Fiber percentage	12.27	12.26	14.38
Ash percentage	2.83	2.76	1.96
Mill 1			
Moisture percentage	60.82	58.53	59.34
Pol extraction	60.83	65.30	57.68
Last Mill			
Moisture percentage	52.92	51.89	50.60
Pol percentage	2.68	2.60	2.36
Ash percentage	4.88	5.70	3.70
Tandem			
Pol extraction	92.78	92.77	93.53
Reduced extraction	92.59	92.60	94.49
Number of tests	53	8	7

(H. Birkett, J. Stein) ■

## ACKNOWLEDGMENTS

We would like to express appreciation to the American Sugar Cane League and all participating Louisiana sugar factories for their support of this project.

## Bagasse Boiler Performance Summary

An efficiently run boiler station is essential to the operation of a well-run raw sugar mill. During processing of the 2010 crop, 41 boiler tests were conducted at seven factories on 24 different boilers. The objective was to investigate boiler operations.

Operating conditions such as steam pressure, steam flow and boiler feed-water temperature were noted. Flue gases were analyzed for temperature and oxygen concentration using a Testo 300 XL flue gas analyzer. Preheated air temperature was determined using an Omega handheld digital thermometer and thermocouple probe. Bagasse samples were collected during each test and were analyzed for moisture and ash content. Excess air, boiler efficiency and pounds steam produced per pound of bagasse burned were calculated using a program written for a similar study conducted during the early 1990s.

A brief summary of 2010 operating data and results are presented in Table 6 along with boiler operating data from 2005-08 (287 tests) and the early 1990s (320 tests). Preheated air temperature increased slightly in 2010 from that of the previous years. Flue gas temperature decreased slightly from that of 2005-08 but was higher than in 1990-92. Moisture percentage of bagasse showed a steady decrease over time while efficiency increased along with pounds steam produced per pound bagasse burned.

Table 6. Comparison of boiler performance tests in 2010 with those in 2005-08 and 1990-92.

	1990-92	2005-08	2010
Number of runs	320	287	41
Steam, pounds per hour	69,318	107,920	101,799
Preheated air temp, Fahrenheit	433	445	453
Flue gas temp, Fahrenheit	418	467	430
Oxygen, percentage	9.8	8.2	8.9
Bagasse moisture, percentage	54.48	53.41	51.23
Bagasse ash, percentage	2.91	4.55	3.53
Excess air, percentage	107.67	75.04	77.74
Efficiency, percentage	55.14	56.33	59.93
Pounds of steam per pound of bagasse	1.98	1.96	2.28
Bagasse percentage of cane	36.83	31.81	34.80

Boiler efficiencies have been improving over time and currently average about 60 percent with an average flue gas temperature of 430 degrees Fahrenheit. A large increase in boiler efficiency can be achieved by lowering the flue gas temperature to 300 F. The only practical way to achieve this is with an economizer (boiler feed-water heater using flue gas). This would increase boiler efficiency to about 65.4 percent and produce 9 percent more steam from the same quantity of bagasse. (H. Birkett, J. Stein) ■

## ACKNOWLEDGMENTS

Appreciation is extended to the American Sugar Cane League and all Louisiana sugar factories for their support of this project.



*The Louisiana Institute for Biofuels and Bioprocessing (LIBBi) is a research, education and outreach initiative within the LSU AgCenter. The proposal to form LIBBi was approved by the Louisiana Board of Regents in December 2009 to serve as a source for reliable, science-based information to support planning and decision making by emerging biofuels and bioprocessing industries within the state. The institute links the Louisiana agricultural base with emerging bioenergy initiatives, thereby expanding and strengthening local, state and regional economies. For details on LIBBi's objectives and the collaborating researchers, please visit [www.LSUAgCenter.com/libbi](http://www.LSUAgCenter.com/libbi).*

*Established in tandem with LIBBi, is Sustainable Bioproducts Initiative (Subi), a regional program developed to reinvigorate the Southeastern United States sugar and chemical industries. This project is funded by a five-year grant from the United States Department of Agriculture for the study of producing biomass, such as energy cane and sweet sorghum, and an economically viable conversion to biofuels and bioenergy using existing infrastructure. The program involves a team of university and industry partners led by the LSU AgCenter. Visit [www.LSUAgCenter.com/subi](http://www.LSUAgCenter.com/subi) for information.*





**As part of our extension educational efforts, the Audubon Sugar Institute's faculty members conducted a seminar for approximately 60 sugarcane producers in 2010 to explain the influence of cane and juice quality on sugarcane factory and refinery operations. Audubon Sugar Institute also continues to conduct its annual Factory Operations Seminar that is attended by representatives of all 11 Louisiana factories and focuses on our faculty members reporting directly to the factories the results of research projects conducted during the previous crop year.**

## Donal F. Day

*Dr. Donal Day is the A. Wilbert and Sons Endowed Professor for Bio-fuels. He has spent 38 years in process research, 32 of them at the Audubon Sugar Institute, specializing in applying microbial physiology and industrial microbiology to research and development (laboratory, bench and pilot scale) for sugar production, diagnostic and pharmaceutical industries. Day has published more than 200 technical publications and he holds 18 patents. He has expertise in the areas of biofuels, fermentation, new product development and industrial application, carbohydrates, polysaccharides, enzymes and cell culture. Recently he has been heading an LSU AgCenter and Audubon Sugar Institute program on conversion of sweet sorghum and energy cane to ethanol and butanol – with the goal of expanding opportunities for the sugar processors of Louisiana.*

## Department of Energy Funded Biofuels Program

There is opportunity to produce biofuels in Louisiana if biomass feedstocks can be produced on fallow or marginal land and these feedstocks can be harvested and delivered in a manner that can be processed in a modified sugar mill operation.

Two such feedstocks are a variety of sugarcane (energy cane), which produces higher biomass and lower sugar than commercial varieties, and sweet sorghum, which was investigated for sugar production in this area in 1985.

Both of these crops can be harvested after or before commercial sugarcane. Energy cane will withstand the freezing weather in south Louisiana and should be available from January to March. Sweet sorghum normally would be harvested between August and September. The sugarcane harvest is October through December. One experimental crop, Miscanthus, also shows promise as a biomass crop and it normally would be harvested in April to June.

All of these crops are similar in gross structure and could be handled by the traditional sugarcane harvest and delivery system.

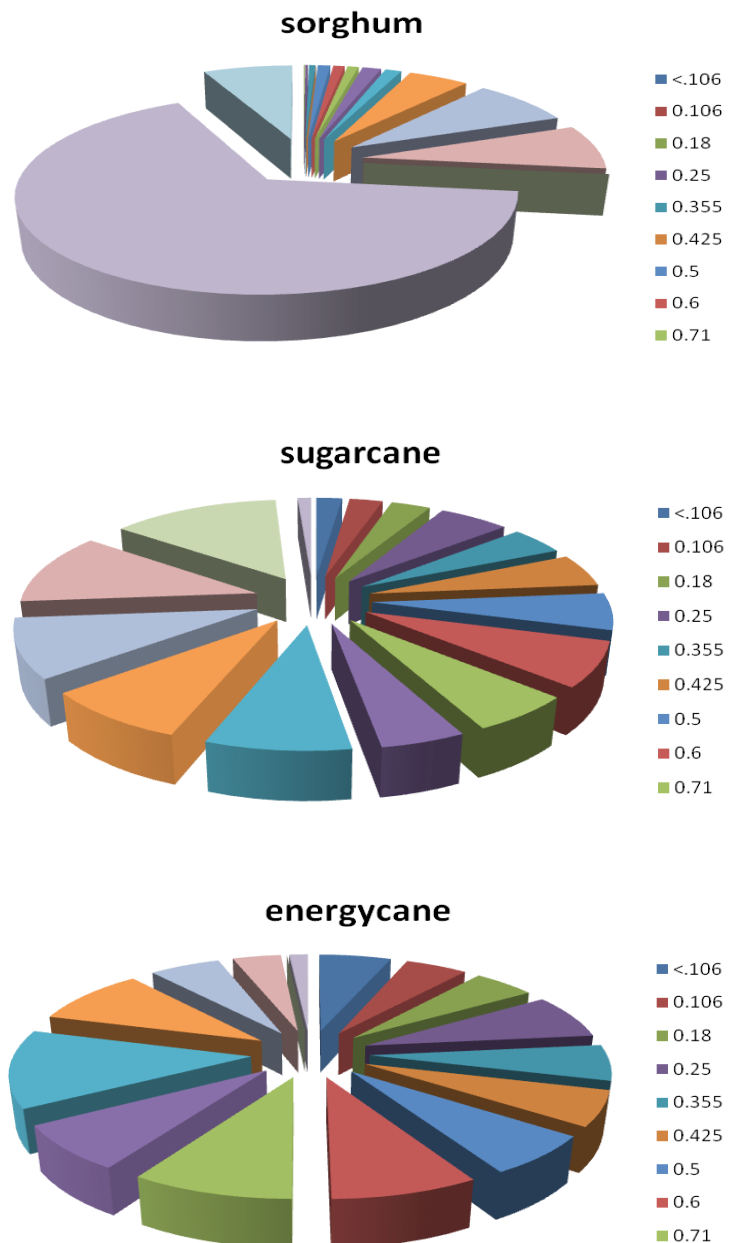
The following technical barriers to implementation for the chosen crops were addressed in the 2009-2010 period. The possibility of fuel production from algae also is being investigated.

## Biomass Physical State Alteration (i.e., grinding, densification and blending)

The initial sizing and grinding of biomass affects efficiencies and quality of all the downstream operations.

As the proposed process is mated with a sugar mill, the effect of preprocess milling on the size of the “bagasse” from the various feedstocks was studied (Figure 4). Particle size analysis indicates some changes in mill settings may be required depending on the crop processed.

Figure 4. Fragmentation patterns by crop, after milling, showing relative percentage of each particle size.



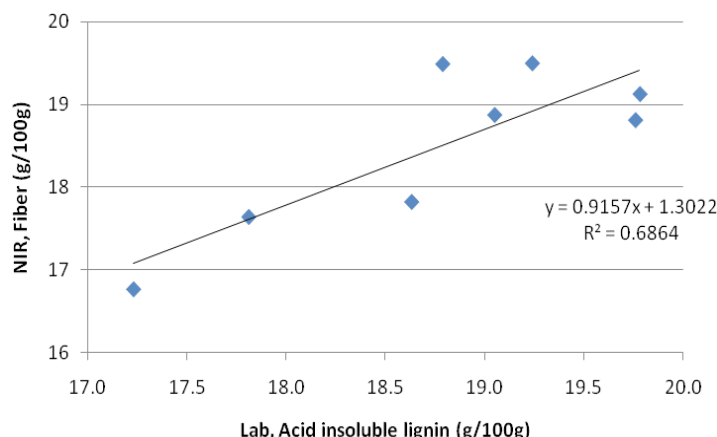


## Feedstock Quality and Monitoring

Near infrared spectroscopy is a passive, noninvasive analytical tool being used for rapid and/or real-time compositional analysis in the sugarcane breeding program at the LSU AgCenter.

We used this equipment for our chosen biomass crops and determined that cellulose composition for each of the biomass crops can be determined along with simple sugar contents (Figure 5). This is the start for developing processor standards and specifications for these feedstocks.

Figure 5. Fibers predicted for energy cane using the sugarcane MPLS for NIR versus acid insoluble lignin determined by wet chemical analyses.



## Pretreatment (prehydrolysis)

Biomass normally undergoes a thermochemical process to prepare it for hydrolysis. Three alkaline pretreatment processes were tested for their ability to convert the test biomass to a readily enzyme hydrolysable form. The pretreatment processes were dilute ammonia, lime and AFEX (Table 7).

Table 7. Efficacy of pretreatment method of crop bagasse (percentage of cellulose hydrolyzed by enzymes after pretreatment).

Pretreatment	Sugarcane	Sweet sorghum	Energy cane	Miscanthus
AFEX*	68	79	57	43
Dil. ammonia (160°C)	90	89	70	ND
Dil. ammonia (121°C)	49	66	39	ND
Lime (121°C)	90	90	67	ND

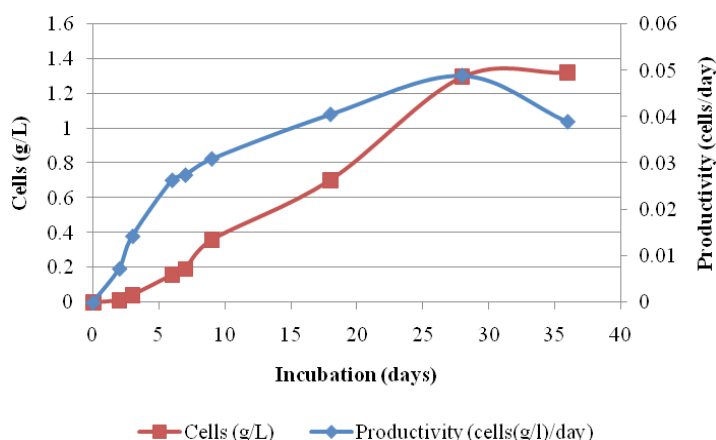
\*Changes in conditions improved AFEX results.

## Algae

There is some potential in growing algae near sugar mills to produce lipids for biofuels. It would allow use of available energy from the mills for algal processing.

Figure 6 below illustrated the productivity of a common alga (*Chlorella vulgaris*) in response to light. The project is designed to determine the feasibility of algae as a supplemental biorefinery product.

Figure 6. Cell mass and productivity of *C. vulgaris* during incubation with a fluorescent light.



## Butanol Production

The energy difference between a gallon of gasoline and a gallon of ethanol make the production of an alternative to ethanol an attractive possibility.

With an industrial partner, we have been investigating the production of butanol, rather than ethanol, by fermentation. A continuous butanol production system has been in operation in the laboratory for eight months. Work is focused on scaling this system up, in conjunction with an efficient solvent separation system. (D. Day, D. Kim, G. Aita, V. Kochergin, L. Madsen) ■



Bench scale butanol production system





## Vadim Kochergin

Dr. Vadim Kochergin is a professor at Audubon Sugar Institute with over 25 years of extensive experience in research and development from conception to commercialization in the sugar, biorefining and pharmaceutical industries. His background is in chemical engineering with an area of expertise in unit operations involving all aspects of new process development and implementation: membrane filtration, industrial chromatography, solvent extraction, ion exchange, distillation, filtration, crystallization and bulk solids handling. He is a member of the International Society of Sugar Cane Technologists, American Society of Sugar Beet Technologists, American Society of Sugar Cane Technologists and National Society of Professional Engineers. He is a referee of the International Sugar Journal and has served as chairman of the research advisory committee to Sugar Processing Research Institute, an organization sponsored by over 20 international companies.

He is currently an adjunct professor at the LSU Chemical Engineering and the Biological and Agricultural Engineering departments and also the director in process technology of the newly formed Louisiana Institute of Biofuels and Bioprocessing (LIBBi). He is an active participant of the Agricultural Leadership program at LSU AgCenter and is a registered professional engineer in the state of Idaho.

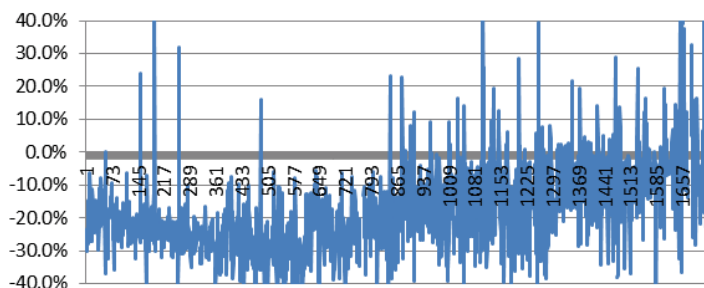
## Design and Operation of Low Turbulence Clarifier

A pilot rig has been constructed to address potential air bubble entrainment in the clarifier pre-distribution system. Water tests during summer 2010 demonstrated that air entrainment could occur in case of free waterfall in the pre-distribution lines. With the proper pre-distribution, existing design of turbulence reduction devices did not show any problems with flows up to 140 gpm per device (design flow is 100 gpm).

A trough pre-distribution system was designed together with the specialists from Sterling mill. The newly designed 20-foot clarifier with nine turbulence reductions devices has been constructed and water tested during the summer.

The new clarifier has been operated reliably at flow rate of 750 gpm with a very short 35-minute residence time. Performance of the new clarifier exceeded an SRI clarifier's performance with 45-minute residence time by an average 20 percent. The results are shown in Figure 7 below.

Figure 7. CJ Turbidity, Percent Difference (from SRI).



Toward the end of season, the difference in turbidity tended to decrease as the absolute values approached 30-40 NTU. Two special tests have been run, where the clarifier throughput was increased to 1,000-1,100 gpm without the loss of efficiency. The capital cost of new design was estimated at 30-40 percent of a conventional design. (V. Kochergin, C. Gaudet) ■

## Belt Mud Filter Performance at Raceland

Performance of a belt-type Technopulp mud filter was evaluated for mud de-sweetening, water use, etc. Analytical information and mass balance calculations and comparisons with conventional rotary drum filters are being summarized. Representatives of the Raceland sugar mill will present the results at the ASSCT meeting. conventional design. (V. Kochergin, J. King, S. Goudeau) ■

## Sugars™ modeling

A training session has been carried out for Audubon Sugar and Louisiana mill specialists regarding the Sugars™ modeling software. It has been used by specialists from LASUCA to evaluate various options for raw color improvements. Work will continue during the next crop. (V. Kochergin, M. Suhr) ■

## Production and Storage Aspects of VHP (very high pol) and VLC (very low color) Sugar

Monitoring of quality of VHP and VLC sugar produced during 2008 grinding season at St. Mary was accomplished with a final report on conclusions distributed to all Louisiana the mills. Results were also reported in the sugar quality meeting in September 2009.

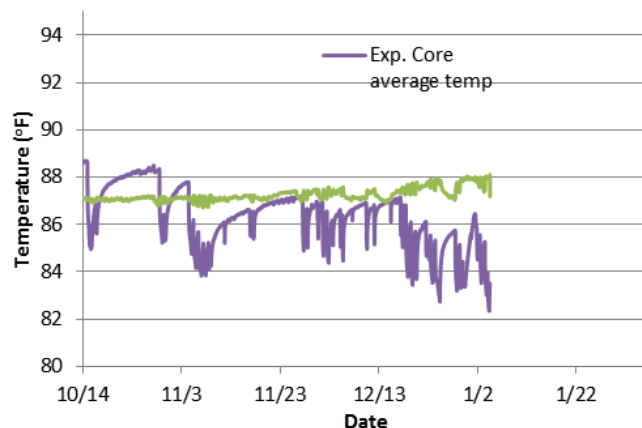
It was concluded that sugar temperature was the main factor affecting the storage. Surface layer of the sugar pile (as much as 10 feet deep) follows the ambient temperature trend and the sugar quality does not change significantly. Sugar in the core of the pile lacks the ability to release the heat resulting in additional color formation. Further ideas have to be explored in cooling sugar to below 90 degrees F prior to storage and releasing heat during storage if needed.

A new set of temperature-humidity sensors have been installed at St. Mary mill to accumulate additional data on changes in sugar quality during storage. Sampling procedure has been modified to obtain sugar from the middle of the pile. Monitoring will continue throughout the 2010 storage season.

## Heat release from sugar pile at Cora-Texas

It has been proposed to use ambient air when the conditions are right to release heat from the core of a sugar pile. A system was designed and installed at a Cora-Texas warehouse. It included a Roots blower and a control system that activates the blower at certain air parameters.

Figure 8. Comparison of temperature trends in sugar pile at Cora Texas





Air temperature and relative humidity were continuously measured in seven different points across the pile. The conditions were compared with the unventilated portion of the pile in the same warehouse.

Figure 8 (page 13) indicates that some cooling takes place in the experimental section while there is a slight upward trend in the non-ventilated portion of the pile. The results will be reported to stakeholders at the ASSCT meeting and after the completion of the study. (V. Kochergin, C. Lohrey) ■

### Reduction of Sugar Losses in Boiling House Operation

Boiling studies have been performed in July-August 2009 using the ASI pilot vacuum pan. The effect of seed quantity and uniformity on the grain crystal properties was investigated under controlled boiling conditions to minimize process variations. The pilot tests have been attended by specialists from five sugar mills. Twelve drums of Alma syrup (86.6 purity) collected at the end of December 2008 and stored in the freezer room were used as the feed material. Seeding was performed with slurries currently used in the mill operations - powdered sugar and ball-milled sugar. Pan vacuum and steam pressure were tightly controlled. A standard boiling operation was simulated to the equivalence of the addition of 4 pounds of sugar per 1600 cubic feet graining pan. Doubling the amount of slurry resulted in slightly smaller crystal size (about 10 micron less) and a little less uniformity. Less seed addition (2-4 times less than the standard operation) resulted in formation of second grain with high level of non-uniformity. In the mill operations, we routinely add more slurry than required from theoretical crystal growth calculations. It was observed that crystal size grew linearly with time during boiling, but the non-uniformity was increasing in the beginning of the boiling cycle. This raises the question if full pan seeding actually occurs or it is accompanied by partial “shock seeding” that provides additional surface area for crystal growth when insufficient amount of crystals are added.

Based on the studies, it is recommended to calculate the amount of sugar in the seed slurry not less than 1 pound of sugar per 1000 cubic feet of final C- massecuite. Using ball-milled slurry is safer in terms of preventing the second grain formation, since seed slurry contains more crystals. Using excessive quantities of seed slurry result in smaller grain with higher degree of non-uniformity.

### Crystal Size Analysis

Technical assistance to the mills in analyzing crystal size and uniformity in massecuites and product sugar has been provided throughout the season. A total of 240 crystal size measurements have been performed during the 2009 grinding season and 315 more samples in 2010.

Tests results allowed mills to evaluate the target crystal size in boiling house operations and make appropriate adjustments to crystallization procedures. Several special projects have been supported, where the “snapshot” of boiling house operations was taken with crystal size measurements for C-massecuites, crystallizers, reheaters and C-sugar accompanied by Nutsch purities. Crystal size analysis has proven to be a useful troubleshooting tool for the mills in the recent seasons, and it is

planned to provide it on the routine basis. Final molasses purities appeared to lowest in the mills when the C-massecuite size was in the range of 280-300 micron with the coefficient of variation (uniformity) below 0.48.

Multiple measurements of crystal developments in the continuous vacuum pans were made. The importance of growing seed magma for feeding the pans with the sufficient uniformity was emphasized.

Analysis of target purity difference curves during the grinding season showed that it takes mills some time to reach the desired results in the boiling house optimization. Reducing this length of “transitional” period to the optimized boiling house operation will result in significant savings for the industry. Providing fast feedback on the crystal size of C-massecuites will decrease the “tuning” time for boiling house. Several special projects requiring crystal size measurements were carried out at managers’ requests at the Lula, Alma, Westfield and Lafourche mills.

### Crystallizer Performance Study

A pulse tracer test was carried out at Alma mill in 2009 to evaluate the performance of the vertical and Blanchard crystallizers. A small quantity of zinc sulphate was injected into C-massecuites before the massecuiteflow was split between the vertical and Blanchard crystallizers. Concentration of zinc was then measured in the hourly samples that were taken after the crystallizers. Crystal size measurements and Nutsch purities were recorded for corresponding samples. Residence time was evaluated based on the measured response curves and compared with the times estimated from the massecuite flowrates and tank volumes. Presence of the peaks earlier than the expected residence time indicated the shortcutting through the crystallizer. Long “tail” in the curves are explained by the presence of dead zones.

Analysis of the test results indicated that the massecuite has made a shortcut through the vertical crystallizer, thus effectively reducing its efficiency and residence time. Blanchard crystallizer demonstrated better performance, which was corroborated by analysis of crystal size and Nutsch purities. Crystals grew by about 20 microns during more than 30 hours. Coefficient of variation (CV) indicating the crystal uniformity was observed to decrease as crystals grew. Most likely it is related to the way there are calculated. Larger CVs are an indicator of second grain formation. It was concluded that no second grain was being formed during cooling crystallization. Cooling curves followed approximately linear fashion. It may be beneficial to explore the cooling curves where massecuite is held at a constant temperature for the last few hours before reheating. This will allow mass transfer to take place and further exhaust molasses. The efficiency of the mixer arrangement in the vertical crystallizer should be completed. The following parameters should be evaluated for vertical crystallizer operation- it may be the case that the mixers are not adequately stirring the massecuite next to the tower walls. In such case, the ambient temperature outside the towers may be cooling the massecuite near the perimeter of the towers, creating an area of cool, highly viscous massecuite which is not flowing through the towers efficiently. (V. Kochergin, I. Tishechkina) ■





## Audubon Sugar Institute Team Wins LSU AgCenter's Tipton Team Research Award

The sugar processing and biofuels team at the Audubon Sugar Institute was the 2010 winner of the LSU AgCenter's Tipton Team Research Award. The award is supported through an endowment honoring LSU AgCenter Vice Chancellor and Director Emeritus Kenneth W. Tipton.

The Tipton Team Research Award was established to recognize significant contributions to Louisiana agriculture by a team of at least three Louisiana Agricultural Experiment Station scientists who have participated in exceptional collaborative research efforts. The selection is based on contributions as a team during the most recent five years with collaborative efforts that include intra- and inter-unit research activities along with joint efforts with other universities, government agencies and the private sector.

The Audubon Sugar Institute group received this award as an acknowledgment of its many efforts and accomplishments in helping Louisiana's sugar industry remain profitable through factory improvements and technological innovations. It also recognized the development of the concept of a sugarcane biorefinery using cane and byproducts of the sugar processing as an economically viable approach for the industry to diversify and advance.

This research group has been involved in bringing new technologies that can be integrated at the sugar mills using the fibrous residue of sugarcane and other similar grassy feedstocks to produce ethanol, butanol and other special chemicals.

The nomination of the Audubon Sugar Institute team was seconded by the American Sugar Cane League, Lula-Westfield LLC and Louisiana Sugar Growers and Refiners Inc. ■

## Benjamin Legendre

*Dr. Ben Legendre is the Denver T. Loupe/ASSCT Sugar Heritage Professor and has 42 years of research and extension experience in state and federal organizations in the genetic improvement of sugarcane, selection of improved sugarcane cultivars, cane and juice quality and is an international authority on sugarcane breeding and sugarcane quality. He is recognized for his work on plant growth regulators as chemical ripeners.*

*He served as research leader for the USDA-ARS, Sugarcane Research Unit, Southern Regional Research Center in Houma, La. for 11 years prior to accepting a position as the sugarcane specialist/professor with the LSU AgCenter. He was appointed department head of Audubon Sugar Institute in 2008.*

*Legendre has authored or co-authored 221 publications and made over 200 presentations before professional societies. He is a member of honorary societies such as Alpha Zeta, Gamma Sigma Delta and Phi Kappa Phi and an honorary life member of both the American and the International Society of Sugar Cane Technologists. He also received numerous awards in recognition of his invaluable contributions to the sugar industry.*

## A Look at Alternative Chemistry to Glyphosate for Use as a Sugarcane Ripener during 2009 Harvest Season

Researchers have been on a quest to find an alternative ripener to glyphosate that displays potential to increase the yield of theoretical recoverable sugar per ton of cane (TRS/TC) and overall sugar yield per acre as well as glyphosate with little or no effects on the subsequent stubble crops' ratooning ability. One such product, Palisade (trinexapac-ethyl), manufactured by Syngenta Crop Protection, is used in Brazil and other Latin American countries as a sugarcane ripener. Palisade differs from glyphosate in that it is classified as a plant growth regulator, unlike the herbicide glyphosate. This report deals with the results of experiments in which trinexapac-ethyl was tested as a sugarcane ripener in Louisiana in comparison to glyphosate and control plots where no ripeners were used.

### Efficacy of Palisade as a Chemical Ripener

In 2008, the commercial sugarcane varieties, HoCP 96-540, L 99-226, L 99-233, HoCP 00-950, and L 01-283, were planted on Aug. 26, 2008, at St. Gabriel, La., to evaluate their response to the sugarcane ripeners Touchdown Total (glyphosate) and Palisade EC (trinexapac-ethyl). The objectives of this study were: 1) to compare the effectiveness of the two ripeners, glyphosate and trinexapac-ethyl, on the five varieties listed above; and 2) to evaluate sucrose storage within the top, middle and bottom section of the stalk following treatment with glyphosate and trinexapac-ethyl.

It appears that glyphosate is a more effective ripener in increasing the yield of theoretical recoverable sugar per ton of cane (TRS/TC) for all varieties than trinexapac-ethyl at the two rates of trinexapac-ethyl tested at both 28 and 56 days after treatment (data not shown). It also appears that because of a trend in the reduction in mean stalk weight that there is little or no increase in the yield of theoretical recoverable sugar per acre (TRS/A) for both glyphosate and trinexapac-ethyl.

The top, middle and bottom third of the stalks treated with glyphosate, as an average of all varieties, yielded more



LSU AgCenter's sugarcane processing and biofuels research team received the Tipton Team Research Award at a ceremony held at Lod Cook Alumni Center on Dec 3, 2010.

L-R: Dr. Don Day, Dr. Kenneth Tipton, Dr. Vadim Kochergin, Mrs. Kenneth Tipton, Dr. Lee Madsen, Dr. Kun-Jun Han, Melati Tessier, Dr. Harold Birkett, Dr. Misook Kim, Dr. Ben Legendre, Dr. Giovanna Aita, Dr. David Boethel.



TRS/TC than respective controls (Figure 9). Higher TRS/TC in the top segment was only achieved for trinexapac-ethyl at 0.276 pounds per acre and middle segment at 0.312 pounds per acre.

## 2010 Spring Shoot Counts

Beginning on March 14, 2010, shoot emergence data were collected weekly on the 2009 ripener-by-variety experiment conducted at the LSU AgCenter's Sugar Research Station. These first-stubble plots were evaluated to assess possible residual effects of glyphosate or trinexapac-ethyl on shoot re-emergence following fall treatment in 2009 with either glyphosate or trinexapac-ethyl on five varieties – HoCP 95-540, L 99-226, L 99-233, HoCP 00-950 and L 01-283. The data show that both glyphosate- and trinexapac-ethyl-treated plots had higher shoot populations when compared to nontreated plots (Figure 10). Some of the shoots from the plots treated with glyphosate were white and stunted, but the trinexapac-ethyl-treated plots showed no whitening or growth reduction.

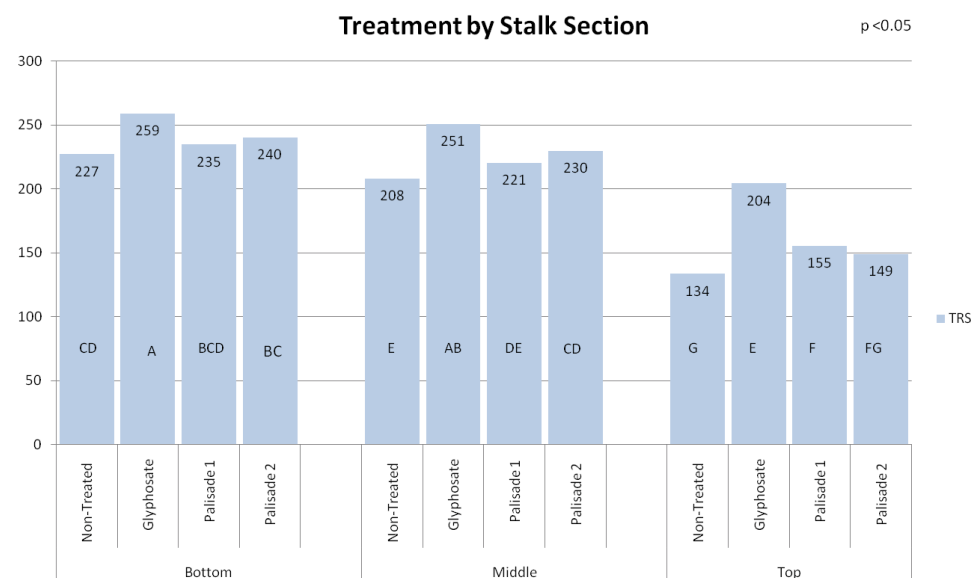
## Short-term Residual Effect of Ripeners

For the 2009 ripener-by-variety experiment, plots were scheduled to receive the same ripener treatments as the previous year in late August 2010. This was to determine the residual effect of continuous ripener treatment from the plant-cane crop through the crop cycle for the five varieties listed in the previous section. Severe lodging occurred, and treatments were not applied, however. But the plots were harvested in 2010 to evaluate residual effects of the ripener treatments in the first-stubble crop. No significant interactions were found, suggesting that there were no negative residual effects for any of the varieties due to the ripener treatment in the first-stubble crop (data not shown).

## Effect of Nitrogen Rate on Ripeners

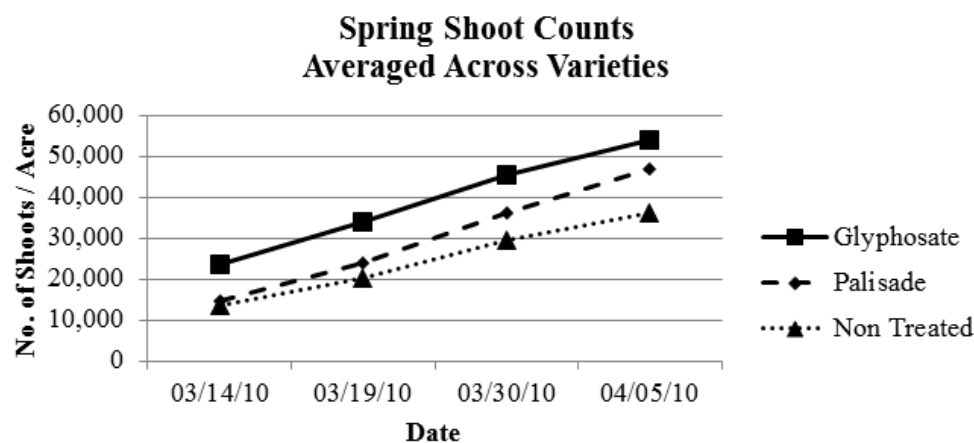
In late April 2010, a plant-cane crop of HoCP 96-540 was fertilized with UAN at rates of 60, 100 and 140 units of nitrogen per acre. Glyphosate or trinexapac-ethyl was applied to plots on Aug. 20, 2010, to investigate the response of sugarcane to ripeners with differing nitrogen levels. The experiment was arranged as a split block with three replications. Plots were hand-sampled and processed. No significant differences were observed for the ripener-by-nitrogen rate interaction as well as for nitrogen rate plots. Averaged across nitrogen rates, both glyphosate- and trinexapac-ethyl-treated plots yielded statistically more TRS/TC of cane than the nontreated plots. The TRS/TC for glyphosate, trinexapac-ethyl and the nontreated plots were 228, 221 and 207 lb, respectively (data not shown).

Figure 9. Effect of chemical ripener treatments on yield of theoretical recoverable sugar per ton of cane (TRS/TC) for the bottom third, middle third and top third of the stalk<sup>1</sup>.



<sup>1</sup> Touchdown Total applied at 5.7 ounces per acre; Palisade 1 applied at 0.276 pounds per acre; Palisade 2 applied at 0.312 pounds per acre; Applied, August 28, 2009.

Figure 10. Shoot counts in the spring of 2010 as an average of five varieties following ripener treatment in the fall of 2009.



## Harvest Interval

Many of the previous techniques used by researchers to evaluate potential ripeners were based on results observed for glyphosate. Trinexapac-ethyl, unlike glyphosate, is not a herbicide; therefore, protocols previously used may have been biased in favor of glyphosate. Both glyphosate and trinexapac-ethyl were applied to HoCP 96-540 plots on Aug. 9, 2010. The experiment was arranged as a randomized complete block with four replications. Sugarcane was hand-harvested at six, eight, 10 and 12 weeks post-treatment. TRS/TC was significantly greater for glyphosate over the control for all harvest sample intervals and significantly greater for trinexapac-ethyl over the nontreated at eight, 10 and 12 weeks following treatment (data not shown). The response in TRS/TC for trinexapac-ethyl-





treated cane doubled at 10 weeks after treatment compared to the six- and eight-week sampling dates. This suggests that a longer treatment interval may be beneficial to improving effectiveness of trinexapac-ethyl as a ripener.

## Surfactants and Spray Volumes

During September 2010, a field of third-stubble L 99-226 was used to evaluate the value of additional surfactant with loaded glyphosate products as well as application spray volumes. Plots were arranged in a randomized complete block design with four replications. Sugarcane ripeners included glyphosate applied at 5.3 ounces per acre and trinexapac-ethyl at 19 ounces per acre. Spray volumes included 8 and 16 gallons per acre. Addition of the nonionic surfactant Induce® to the spray mixture at rates of 0 and 0.25 percent volume/volume also was evaluated. A nontreated control was included for comparison. For each of the glyphosate and trinexapac-ethyl treatments, tonnage and sugar yield were not different from the nontreated control (data not shown). TRS/TC was greater than the nontreated control plots when glyphosate was applied at 8 or 16 gallons per acre spray volume whether or not surfactant was added. For trinexapac-ethyl, TRS/TC was increased compared with the nontreated control when applied at 16 gallons per acre spray volume with or without surfactant addition, but not when applied in 8 gallons per acre. Application of trinexapac-ethyl at the higher spray volume would not be practical when considering aerial application. This data indicates, however, that the efficacy of trinexapac-ethyl is enhanced by the higher spray volume. (A. Orgeron, B. Legendre, J. Griffin, K. Gravois, M. Pontif) ■

## ACKNOWLEDGMENTS

We would like to express appreciation to the American Sugar Cane League and Syngenta Crop Protection for their support of this research project.

**Louisiana has produced more than 1 million tons of sugar per year since 1994. The greatest production was in 1999 when 1.698 million tons of sugar were produced. For the years covered by this report, annual production of sugar in Louisiana has remained essentially the same, approximately 1.4 million tons. Cane yield per harvested acre for the 2010 crop (31.1 tons) was lower than for the 2009 crop (35.8 tons), but the yield of recoverable sugar per ton of cane was considerably higher in 2010 (226 pounds) when compared to the 2009 crop (204 pounds), offsetting the lower cane yields. The cane yield per harvested acre did not change significantly for the 2011 crop (31.3 tons), however, the recoverable sugar per ton of cane in 2011 increased to 231 tons.**

**Dry weather in 2010 from September through much of the harvest made for excellent harvesting conditions, generally resulting in lower trash in harvested cane, which contributed to the excellent factory sugar yields. This was just the opposite of the 2009 crop – when rainfall was excessive and field conditions horrible during the harvest, contributing to the lower factory sugar yields. The lower tonnage in 2010 was likely the result of damage from the wet harvest of 2009 followed by a cold winter and spring. The yield of recoverable sugar per acre for this reporting period ranked the production in the top five in the history of the Louisiana industry.**



Energy cane, left, and sweet sorghum, right, are two Louisiana crops being investigated by the research team at Audubon Sugar Institute in the development of an economically viable production of green fuels.





## Lee R. Madsen II

*Having held every sort of position ranging from laboratory glass-washer to laboratory director, Dr. Lee R. Madsen II has worked as a chemist for 16 years and has spent the past decade authoring grants, performing research and managing the analytical laboratory at the LSU AgCenter's Audubon Sugar Institute in St. Gabriel, La. There, he has served to guide and execute a wide range of multidisciplinary research including chemistry, biochemistry and engineering.*

*He currently manages personnel, instrumentation, method development, training and QA/QC protocol for the analytical laboratory. During his time at the Audubon Sugar Institute, he has authored grants leading to the award of more than \$1 million in funding for research and instrumentation. He is involved with research in the following areas:*

*Development and testing of methods and/or products that can lead toward the direct production of white sugar in Louisiana.*

*Adding value to the cane-sugar industry (and/or integrated biorefinery) via derivation of useful chemicals and precursors from biobased feedstock and waste from processing of such material.*

*Conversion of biofuel processing waste/byproducts (e.g., glycerol) into polymeric materials with specially engineered electronic and mechanical properties.*

*Calibrating a near infrared spectrometer to provide real-time compositional analysis of biomass feedstock, including sweet sorghum, sugarcane and energy cane.*

## The 2010 Molasses Survey and the Effect of Starch on Target Purity Difference

Since 2001, the LSU AgCenter's Audubon Sugar Institute has analyzed the molasses provided weekly by each of the Louisiana raw sugar factories. The results of our analyses are used to calculate a target purity and a true purity for the molasses.

The target purity is the theoretical concentration of sucrose (sugar) where, regardless of effort, no further sugar can be crystallized. The model used to calculate the target purity originates from South Africa (Rein, 2007) and has been confirmed as representative of the Louisiana industry (Saska et al., 2010).

The true purity is determined by HPLC and is free of the interferences that can offset polarimetric determinations. The formula for target purity is given below, where RS is the total reducing sugar (glucose + fructose) via HPLC (ICUMSA, 2002) and Ash is the approximate sulfated ash via conductivity (Saska et al., 1999).

$$TP = 33.9 - 13.4 \cdot \log_{10} \frac{RS}{Ash}$$

The target purity is subtracted from the true purity to give a target purity difference. The target purity difference is used to determine how efficiently sugar is removed from massecuite. "True purity" is the sum of the noncrystallizable sugar and that which was crystallized but was lost across the centrifugals. For this reason, the nutsch purity should be assayed to determine how much sugar is lost across the centrifugals.

Evaluation of sample replicates and weekly quality control demonstrated an overall precision for true-purity measurements of  $\pm 0.19$  percent. The overall error, propagated across all tests needed to calculate the target purity difference, was  $\pm 0.32$  percent.

Although 2009 demonstrated marked improvement in target purity difference, 2010 was marked by a significant and industrywide increase in target purity difference. In all but one case, factory target purity difference increased by, on average, +1.7 points (the greatest increase was +3.3) to give an industrywide yearly average target purity difference of 9.3. It is interesting to note, however, that both the order and spread between individual factories remained more-or-less consistent.

The differences were attributed to a wide range of factors that included favorable weather and harvest conditions, the introduction of a new cane variety, cane maturity and increased awareness at the cane delivery/mill level. The quantity of reducing sugar observed between 2007 and 2009 was examined to better understand what happened with the target purity differences.

In general, there has been a significant downward trend, relative to time, in the amount of reducing sugar in final molasses. This was attributed to the observation that the mills were grinding more cane than ever and had installed evaporation capacity sufficient to minimize the sucrose inversion that would take place. This is evidenced by the decrease in reducing sugar over time. Although the total reducing sugar was greater during the 2010 season, it was very close to the average values established between 2007 and 2009. This was attributed to evaporators running hotter, which led to a greater extent of sucrose inversion.

While ash remained statistically consistent from 2007 through 2009 and was not a significant contributing factor to differential target purity difference (unlike reducing sugar in 2009), it was significantly lower ( $-2.5 \pm 0.7$  percent) in 2010. Lower ash leads to lower target purities, which, when all else is equal, will lead to higher target purity differences.

Calculated values, relative to the established average, account for approximately 40 percent of the observed increase in target purity difference. On average, 0.68 points of target purity difference can be accounted for as decreased ash content. The lower ash was attributed to the especially dry season, which resulted in less field soil entering the factories. This means an increase of at least one point of the target purity difference must be attributed to some other industrywide perturbation (cannot be explained using the target purity formula), possibly to purging difficulties related to the high levels of starch that were observed in 2010. (L. Madsen, C. Verret) ■

## REFERENCES

- Baddley Chemical. (2001). Octapol and Octapol Plus. <http://www.baddley.com/octapol.htm>
- ICUMSA. (1994). Method GS4-13: The determination of refractometric dry substance (RDS %) of molasses – Accepted. ICUMSA Methods Book. Verlag Dr. Albert Bartens KG-Berlin. Supplement 2003. ISBN 3-87040-550-0.
- ICUMSA. (2002). Method GS7/4/8-23: The determination of sucrose, glucose and fructose by HPLC in cane molasses and sucrose in beet molasses – Official. ICUMSA Methods Book. Verlag Dr. Albert Bartens KG-Berlin. Supplement 2003. ISBN 3-87040-550-0.
- ICUMSA. (1994). Method GS1/3/4/7/8-13: The determination of conductivity of ash in raw sugar, brown sugar, juice, syrup and molasses – Official. ICUMSA Methods Book. Verlag Dr. Albert Bartens KG-Berlin. Supplement 2003. ISBN 3-87040-550-0.
- Rein, P. and Smith, I. (1981). Molasses exhaustibility studies based on sugar analysis by gas-liquid chromatography. Proc. S. Afr. Sug. Technol. Ass., 55, pp. 85-91.





- Rein, P. (2007). Cane sugar engineering. Verlag Dr. Albert Bartens KG-Berlin. ISBN 978-3-87040-110-8. pp. 459.
- Saska, M., Goudeau, S. and Andrews, L. (1999). Molasses exhaustion and target purity formulas. Sug. J. 62, pp. 7, 20-24.
- Saska, M., Goudeau, S. and Beyene, F. (2010). Exhaustibility of Louisiana final molasses and the target purity formula: The 2009-2010 season results. Annual Meeting of the American Society of Sugarcane Technologists, Louisiana Division. Lafayette, Feb. 1-3.

## High Levels of Starch in the Sugar Factory

Although the use of intermediate-temperature amylase is widespread in the Louisiana raw sugar industry (Madsen and Day, 2002; Eggleston and Montes, 2009), but unlike dextranase (Morel du Boil and Wienese, 2002), there is very little widespread knowledge of how enzymes are saving money (Cuddihy and Day, 1999; Day, 1994).

In many cases, changing conditions (temperature or pH) or lack of attention to detail can lead to incorrect application and or inefficacy of the enzyme. Both situations can occur simultaneously and result in higher levels of starch in final product (Saska, 2002). Once mitigated, the continued application of excessive amounts of enzyme can lead to residual activity in sugar and molasses.

Because penalties are not currently levied against starch in raw sugar or molasses (and highly viscous molasses frequently contains more sugar), quantifiable evidence of related losses were not evident. In 2011, however, the levels increased dramatically (to greater than 3,000 mg/g DS in clarified juice), which, when coupled with unforeseen difficulties, led to large amounts of starch propagating through multiple-boiling to final molasses. That final molasses may have contained starch in excess of 2 percent w/w on dry solids. At such a concentration, the gel-point (perhaps greater than 1 g/100g at amylose DP >110, Morris 1990) for many plant starches has been exceeded, and massecuite consistency will behave accordingly. Gelation of molasses leads to difficulties in pan boiling (hard to boil massecuites) and purging, both of which can lead to unacceptable losses of sugar.

The evaporators were found to be running hotter than usual. Because of this, the injection point now exceeded the temperature range for the enzyme. Running the evaporators cooler solved the problem, but residual enzyme activity was noted in the sugar.

The source of the starch was not determined. Fortunately, there are not, at present, penalties for starch in sugar, so the financial consequences will be limited to sugar lost to molasses as a consequence of increased massecuite consistency.

It was noted by Kaur, et al. 2002, that the consistency index,  $\mu$ , increased with addition of starch and that the shear viscosity obeyed the power law. It was also noted that while molasses containing less starch than that required for gelation, e.g., 0.6 percent, demonstrated no net change in viscosity. Samples containing 1.3 percent and 1.9 percent obeyed a power law (demonstrating a 60 percent increase in viscosity between 0.6 percent and 1.9 percent starch at 50 degrees C). This was in keeping with the observation by Morris (1990) that the gel point for starch is frequently near 1 percent and that this value holds

true in molasses, at least for the CDH-grade water-soluble starch that Kaur used in the reported experiments.

This was, to our knowledge, the first time in a decade when starch levels in final molasses had reached 1.5-2.0 percent. At these levels, we expect that the molasses becomes increasingly recalcitrant as gelation proceeds through retrogradation of the starch. While this is happening, particularly with materials with very low water activity such as massecuite, free water is bound into the gel structures.

The bound water cannot be easily removed by boiling and may result in hard-to-boil massecuite. Extended heating can result in the formation of color bodies (melanoidins) at the expense of reducing sugar and contributes to melassigenesis. In molasses, flow characteristics will be affected, making the material difficult to wash from the crystals during centrifugation. We suppose this would elevate TPD as excessive amounts of sugar are washed into the molasses. When starch is allowed to accumulate in molasses to a point where its concentration exceeds the gel point, excess molasses will be formed and it will contain much more sugar than it should. (L. Madsen, D. Day) ■

## REFERENCES

- Madsen, L.R. and Day, D.F. (2003). Enzyme use in Louisiana sugar mills. Audubon Sugar Institute, LSU AgCenter Factory Operations Seminar Research Presentations – 2002, pp. 82-83.
- Eggleston, G. and Montes, B. (2009). Optimization of amylase applications in raw sugar manufacture that directly concern refiners. Sug. Inst. Technol. #973, pp. 243-252.
- Morel du Boil, P.G. and Wienese, S. (2002). Enzymic reduction of dextran in process-laboratory evaluation of dextranases. S. Afric. Sug. Technol. Ass. 76, pp. 435-443.
- Cuddihy, J.A. and Day, D.F. (1999). The process and financial impact of dextran on a sugar factory. Sug. J. March, pp. 27-30.
- Day, D.F. (1994). Dextran induced sugar loss to molasses (The Louisiana experience). J. Am. Soc. Sug. Cane. Technol. 14, pp. 53-57.
- Saska, M. (2003). High temperature amylase. Audubon Sugar Institute, LSU AgCenter Factory Operations Seminar Research Presentations – 2002, pp. 14.
- Morris, V.J. (1990). Starch gelation and retrogradation. Trends Food Sci. Technol. July, pp. 2-6.
- ICUMSA. (2007). Method GS1-17: The determination starch in raw sugar - tentative. ICUMSA Methods Book. Verlag Dr. Albert Bartens KG-Berlin. Supplement 2005. ISBN 978-3-87040-562-5.
- Kaur, S., Kaler, R.S.S. and Aamarपाली. (2002). Effect of starch on the rheology of molasses. J. Food Eng. 55, pp. 319-322.

**Since 2001, the Audubon Sugar Institute has analyzed the final molasses provided weekly by Louisiana sugar factories. The results are used to calculate "target purity" and a "true purity." The target purity is the theoretical concentration of sucrose where, regardless of effort, no further sugar can be crystallized. The true purity is the sum of the noncrystallizable sugar and that which was crystallized but was lost across the centrifugals. This information is used by the factories to determine the efficiency at which they are recovering sugar from their massecuite.**



Taking information gained from research to those who need it is one of the functions of the LSU AgCenter's Audubon Sugar Institute and its partnership with the AgCenter's Louisiana Cooperative Extension Service.

Extension functions performed by the Audubon Sugar Institute's faculty are listed below.

### Boilers:

Monitored several boiler compliance tests during the 2009 and 2010 crops. Assisted sugar factories in recording test data and ensuring calculations and final reports by independent test companies were reasonable and accurate.

Provided an emissions testing protocol to the Louisiana Department of Environmental Quality to compare portable combustion analyzers used at the fan with that of traditional equipment used on the stack.

Spent a significant amount of time assisting the American Sugar Cane League and their attorneys in responding to newly proposed EPA boiler rules. Some success was achieved when the final rule was published this past March and no limits were set for carbon monoxide emissions on biomass boilers. Other concerns remain, and work is continuing to request modifications or exemptions to one or more of the rules issued.

### Oil Well Blowout:

Participated in discussions regarding an oil well blowout and its effects on nearby sugarcane crops – in conjunction with the LSU AgCenter's Dr. Kenneth Gravois, Westfield Sugar Factory personnel and growers, Louisiana Department of Ag and Forestry personnel, Louisiana Department of Environmental Quality personnel, farm consultants and other AgCenter Sugar Station personnel.

Was responsible for running hand-cut cane samples for routine core lab analyses at the LSU AgCenter's Sugar Station. Arranged for sample analyses of potassium and heavy metals to be run in the Agricultural Chemistry lab. In addition to results of these analyses, provided published information on cane and molasses constituents in an effort to provide FDA officials with enough information to determine if and when the damaged cane could be processed.

### Miscellaneous:

Provided information and/or training on a number of issues. Training was conducted for three chief chemists. Factories requested help with various methods of analyses, including dextran, pol, moisture, sugar color, phosphate, starch, reducing sugars and trace sugars in water. Information was provided on core lab operations, mill testing, microscope cameras, evaporator cleaning and entrainment, inversion and molasses foaming.

Assisted several factories applying for the Biomass Crop Assistance Program.

Collaborated with U.S. Department of Agriculture personnel conducting cold-tolerance testing of various cane varieties following a potentially killing freeze in 2010. The article follows. ■

## Stalk Cold Tolerance of Commercial and Candidate Varieties during the 2009-2010 and 2010-2011 Harvest Seasons

The exposure of sugarcane to damaging frosts occurs in more than 20 of the 79 sugarcane-producing countries but is most frequent on the mainland of the United States. The frequent winter freezes in the sugarcane-producing area of Louisiana forced the industry to adapt to a short growing season (seven to nine months) and a short milling season (about three months).

To measure the post-freeze deterioration of stalks of commercial and candidate varieties, a collaborative study was conducted by the LSU AgCenter's Audubon Sugar Institute in St. Gabriel, La., and the USDA-ARS Sugarcane Research Unit at Houma and New Orleans, La., at the USDA-ARS Ardoyne Farm on Bull Run Road at Chacahoula, La.

Variety trials for estimating stalk cold tolerance by measuring post-freeze deterioration of stalks of commercial and candidate varieties in the field are routinely planted at the Ardoyne Farm near Houma, La. Commercial varieties of known cold tolerance are grown as controls. Those include, but are not limited to, these varieties: LCP 85-384 for "good" stalk cold tolerance and TucCP 77-42 for "poor" cold tolerance.

The cane is allowed to remain in the field until the first severe freeze (generally  $<27^{\circ}\text{F}$ ) of the harvest season of the year following planting (plant-cane crop). Just prior to or immediately following a freeze, samples are removed serially along the center row of each plot. Normally, from one to five post-freeze samples are taken, depending on the severity of the freeze and post-freeze weather conditions. Each sample consists of 10 stalks cut at the ground by hand but not stripped or topped. All samples are weighed and passed once through a pre-breaker and a subsample of 2.2 lb of the prepared cane is pressed through a hydraulic press. The juice is analyzed for Brix and pol and the residue for moisture (by drying). The Brix, sucrose, purity and fiber content of the cane are then calculated from these analyses.



*Prepared post-freeze cane is pressed through a hydraulic press to separate the juice for analyses.*





From the data, the estimated yield of theoretical recoverable sugar per ton of cane (TRS/TC) is calculated. Mean stalk weight is calculated by dividing the sample weight by the number of stalks per sample. Juice samples also are analyzed for pH, titratable acidity, mannitol and dextran by the ASI II Method and total soluble polysaccharide. When possible, visual ratings are made for both leaf and stalk cold tolerance in the field.

In these experiments, 9 and 10 commercial and 2 and 1 candidate varieties were planted at the Ardoyne Farm during the late summer of 2008 and 2009, respectively. The commercial varieties included in both studies were LCP 85-384, HoCP 96-540, L 97-128, L 99-226, L 99-233, HoCP 00-950, L 01-283 and TucCP 77-42 (Argentina). Candidate varieties included in the study were L 03-371 in 2008 and HoCP 04-838 in 2008 and 2009. L 03-371 was planted in 2009 as a commercial variety. The commercial variety Ho 95-988 was planted in 2008 only while L 01-299 was planted for the first time in 2009.

For the 2009-2010 harvest season, freezing temperatures that affected the Louisiana sugar industry occurred for 11 consecutive nights from Jan. 2 through Jan. 12, 2010. A low of 18°F was reported at Ryan Airport at Baton Rouge on Jan. 11 and that followed two nights when the temperatures dipped to 19°F. Freezing conditions prevailed for more than 12 hours of each of these three nights. An inspection of the stalks on the morning of Jan. 9 revealed that all internal stalk tissue had been damaged by the previous night's freeze. It was noted that longitudinal cracks appeared on all stalks for each of these three nights when, in fact, all stalk tissue was frozen solid. After thawing, all internal tissue appeared ruptured with a brownish and watery appearance.

For the 2010-2011 harvest season, freezing temperatures that affected the Louisiana sugar industry occurred Dec. 13 and Dec. 26, 2010, when the temperatures at the field site at the Ardoyne Farm dipped to 23.7°F and 22.9°F, respectively. Subfreezing temperatures occurred for 11.5 hours on Dec. 13 and 13.5 hours on Dec. 26. Subfreezing conditions also actually occurred on the nights of Dec. 6, Dec. 8 and Dec. 9, leading up to the damaging freeze the night of Dec. 13, but field inspections revealed the earlier freezes only affected the growing point (terminal bud) of the stalks so sampling was not conducted. An inspection of the stalks on the morning of Dec. 14 revealed that all internal stalk tissue had been damaged by the previous night's freeze. The subfreezing conditions of Dec. 26 exacerbated the damage seen following the initial freeze event of Dec. 13, and it was noted that some stalks had evidence of longitudinal (freeze) cracks appearing on the stalks. Average temperatures for December and January were below normal, undoubtedly, helping to reduce the rate of deterioration following the freeze events.

Samples were taken the mornings of Jan. 8, 15 and 23, 2010 and Dec. 14, 20 and 28, 2010 and Jan. 4 and 11, 2011 for the 2009-2010 and 2010-2011 study, respectively. Data were obtained for mean stalk weight, brix percentage of cane, sucrose percentage of cane, purity percentage of cane, fiber percentage of cane, yield of theoretical recoverable sugar per ton cane (TRS), pH, titratable acidity (milliliter 0.1 N NaOH) and total polysaccharides (parts per million/brix). When considering all the data collected and reported thus far, the reaction (rating) for the commercial and candidate are found in Table 8.

From the data, it appears HoCP 04-838 has significantly better stalk cold tolerance than any of the other varieties in the two



*The post-freeze stalks are prepared for juice extraction.*

Table 8. Reaction of commercial and candidate sugarcane varieties to subfreezing temperatures based on current tests (based primarily on percentage reduction in TRS and percentage increase in total soluble polysaccharide).

Resistant	Intermediate	Susceptible
HoCP 00-950	LCP 85-384†	L 99-226
	Ho 95-988	L 99-233
	HoCP 96-540†	TucCP 77-42**
	L 97-128	
	L 01-283	
	L 03-371*†	
	HoCP 04-838*†	

\*Candidate varieties

\*\* Argentine commercial variety resistance

† Subcategory of Intermediate to Susceptible

tests and was rated as "Resistant or Very Good." LCP 85-384, Ho 95-988, HoCP 96-540, L 97-128, HoCP 00-950, L 01-283 and L 01-299 were rated as "Intermediate or Good," while L 99-226, L 99-233, L 03-371 and TucCP 77-42 were rated as "Susceptible or Poor" (Table 8). These ratings are similar for all varieties for the two tests; however, the rating of one variety, HoCP 00-950, was switched from resistant to intermediate with additional data obtained following the freeze events of Dec., 2010. The ratings as seen in Table 1 are still subject to change with additional data. Other changes included L 03-371 being switched from Intermediate to Susceptible following the freeze events of 2010-2011; all other varieties remained with the same classification. (B. Legendre, T. Tew, A. Hale, H. Birkett, G. Eggleston, M. Duet, J. Stein) ■

## ACKNOWLEDGMENTS

We would like to express appreciation to Dr. Edward Richard Jr., research leader, and the laboratory and field staff at the USDA-ARS Sugarcane Research Unit at Houma, La., for making this collaborative project possible.

# Analytical Capabilities



The LSU AgCenter Audubon Sugar Institute's analytical staff currently is comprised of two associate chemists and a postdoctoral researcher.

The Audubon Sugar Institute has six operational HPLC systems. The Louisiana Board of Regents and the American Sugar Cane League provided the funding for two Agilent 1200 HPLCs. Configured with differential refractive index detectors, these isocratic instruments are used for the routine quantification of the carbohydrates commonly found in either molasses or lignocellulosic hydrolysates. These instruments are operated using BioRad Aminex mixed-mode columns (HPX-87) in either potassium or lead-form. Both of these systems are automated using Agilent LC-Chemstation software and CAN interfaces via TCP.

Another Board of Regents enhancement grant was awarded to purchase an Agilent 1100 HPLC with quaternary gradient pump and a diode array/evaporative light scattering tandem. This instrument can be operated in either reversed or normal phase. We have a wide range of columns available (NH<sub>2</sub>, C18, phenyl, cyanopropyl, etc.). This system is equipped with an Agilent 1200 automated fraction collector that is software controlled (LC-3D Chemstation). It also is equipped with an autosampler capable of handling 100 samples.

An Agilent 6890 gas chromatograph also has been purchased and installed. The 6890 gas chromatograph has been used mainly in analyses of ethanol and fuels. In addition, Lyondell donated three Agilent 5890 gas chromatographs. Two of the donated gas chromatographs have been installed, and a license for the Agilent software has been purchased to control them. The third is equipped with an automated gas sampling loop and tandem thermal conductivity and helium ionization detectors.

The most recent acquisitions include an Agilent 7890 gas chromatograph equipped with a 5975C inert mass-selective detector. Funded by the Louisiana Board of Regents, this instrument is equipped with a turbomolecular pump for minimal downtime and an autosampling robot capable of handling 100 samples. The instrument is controlled via Agilent MS-Chemstation software and is updated to include both the NIST and Wiley spectral libraries.

Spectroscopy-related instruments include a variety of UV-Vis instruments including Spectronic Genesys models 2 and 5 and Beckman-Coulter models DU 730 and 800. A SpectraCane NIR integrated with a Dedini shredder is available for use with whole sugarcane, sorghum and energy cane.

A Shimadzu AA-6650 graphite furnace atomic absorption spectrophotometer is available for use. Lamps are acquired on an element-as-needed basis.

In addition, we have recently acquired both a Varian Cary Eclipse spectrofluorometer (thanks to the Board of Regents) and a Thermo-Nicolet 6700 fourier transform infrared spectrophotometer (through LSU AgCenter support funds). The Eclipse can handle up to four cells for sequential (kinetic) experiments with programmable temperature and in-cell stirring. The instrument is capable of performing experiments involving chemiluminescence, photobleaching and photon-counting. The fourier transform infrared spectrophotometer is equipped with a single-bounce diamond-anvil ATR, KBr beam splitter (350 cm<sup>-1</sup> or 28571 nm) and a gas flow cell with heated lines suitable for interfacing with a thermogravimetric analyzer.

A Foss Soxtec 2050 automated soxhlet extractor is available for use. It can extract up to six samples simultaneously and is equipped for automated solvent addition and recovery. This

apparatus is suitable for general use, including analytical protocols for the compositional analysis of biomass.

A Parr 6200LE semi-automated isoperibol oxygen bomb calorimeter with two 1008 bombs, pellet press and loading station is available for the determination of high heating values (gross calorimetry) and S,N content in both solid and liquid samples.

We have a Mettler Toledo TGA/SDTA851 TGA equipped with an autosampler capable of handling 34 samples.

Other instrumentation includes temperature compensated critical angle refractometers (Bellingham and Stanley) with 0.01 g/100g (brix) resolution, a Rudolph Autopol 880 polarimeter with 0.01°Z resolution (589 or 880 nm), and a conductivity meter with temperature compensation. ■



Old and new gas chromatographs used in analyses of various applications.





## Short Courses

Audubon Sugar Institute continues to offer short courses designed to provide training and professional development for the sugar and sugar-related industry personnel. A few training courses such as Bench Chemist, Chief Chemist and Introduction to Sugar Boiling are conducted around late August to early September to prepare new sugar factory personnel with theoretical knowledge and practical experience prior to the grinding season.

The bench chemist course provides the sugar mill lab trainee with an overview of the raw sugar manufacturing process and hands-on quantitative analyses on bagasse, juice, syrup, molasses, massecuite and product sugar. The chief chemist course on the other hand is designed for a mill chemist, process engineer or manager to become familiar with daily, weekly and crop manufacturing reports. They also learn how to check the data against theoretical considerations. The sugar boiling class trains students with little or no experience in sugar boiling to gain an understanding of the principles involved in sugar crystallization and the effects of steam, vacuum and purity on sugar boiling. Students learn how to boil sugar themselves in a 15 gallon vacuum pan at ASI's pilot facility.

On July 20, 2010, a seminar covering an overview of sugar processing was conducted at ASI for the sugar cane producers. It was well-attended with over 60 individuals representing Louisiana sugar cane growers.

Introduced in 2007 is a short course focusing on separation technologies. It was offered in May of 2011 in Montreal, Canada to target attendees at the Sugar Industry Technologists' annual meeting held there. The course provides basic information required for understanding, operation and process design related to affinity-based separation technologies utilizing fine-granulated media such as ion exchange resins. Case studies discuss a broad range of applications in sugar, sweetener, renewable energy, food, chemical, pharmaceutical and water treatment industries. Emphasis is made on the challenges posed by the emerging industry of biomass conversion to fuels and chemicals and the practical aspects of affinity-based technologies required for successful integration into industrial processes. ■

## Degree Course for Sugar Engineers at LSU

The educational program of the Audubon Sugar Institute is offered at both graduate and undergraduate levels in the form of sugar engineering as an area of specialization or a minor in conjunction with other major areas for engineering degrees.

Audubon Sugar provides part-time work in the analytical lab and factory for undergraduate students minoring in sugar engineering and graduate assistantships to LSU students pursuing their master's or doctoral degrees in chemical engineering, biological engineering, chemistry, mechanical engineering and food science. With the increased number of research projects approved for funding and added space at the new facility, Audubon has enhanced its capability for more graduate students to attain expertise in various aspects of sugar processing.

## Sugar Engineering Courses

Two sugar engineering courses introduced in 2002 by Audubon Sugar have continued to be offered by the LSU College of Engineering. They are a course in sugar process engineering,

which teaches all the background to sugar processing (BE 4342), and sugar factory design (BE 4347), which introduces the students to the details of equipment design in a factory. Prerequisites to these courses are listed on our website at [www.lsuagcenter.com/audubon](http://www.lsuagcenter.com/audubon).

## Minor in Sugar Engineering

The objective of this program is to produce a graduate ideally suited to the operation and management of a sugar-producing facility. In all cases, the student is encouraged to work as a part-time student at the Audubon Sugar Institute, with maximum exposure to sugar processing at every opportunity.

A set of courses has been prescribed so students studying biological, chemical or mechanical engineering may earn a minor in sugar engineering by choosing the two sugar courses described above and their electives to meet the remaining requirements. If possible, the students should also choose his design project in a topic related to sugar engineering, which is overseen by the ASI staff. Sugar process engineering should preferably be taken during the junior year.

To earn the minor, students in the College of Engineering must complete 18 hours of required courses with a grade of C or better. Visit our website at [www.lsuagcenter.com/audubon](http://www.lsuagcenter.com/audubon) for the list of required courses.

Another option involves spending the fall semester at an operating sugar mill, using this period as an internship, which can yield three credit hours (allowed for in BE 3249). This could substitute for one of the required courses.

## Master's Degree in Chemical, Mechanical or Biological Engineering Specializing in Sugar Engineering

Students are required to take a master's degree in chemical, mechanical or biological engineering. This is targeted at people who have a first degree and wish to gain specific sugar-processing expertise through further study.

Following the thesis option, the master's academic requirements involve 24 credit hours in the nominally 21-month program. The course requirements include the sugar process engineering and sugar factory design courses listed above. Depending on the first degree of an individual, he or she would be encouraged to choose electives appropriate to supporting a strong expertise with sugar.

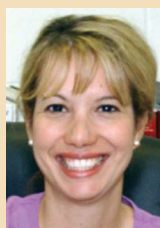
A thesis is required related to a relevant sugar-processing topic. In all cases, the student would be encouraged to take on a research assistantship in the Audubon Sugar Institute for the duration of the course. He or she would be given maximum exposure to sugar processing at every opportunity.

## Options for Presenting the Sugar Engineering Courses as Condensed, Intersession or Continuing Education Courses

In 2005, BE 4342 was offered as an intersession course for the first time as an alternative to the normal course arrangement. This intersession schedule involves an intensive 14-day period of lectures. This course also is open to others through a continuing education program. Audubon Sugar has successfully conducted this course for five years as a spring intersession course at the end of May. ■



## Giovanna M. Aita Publications



- Aita, G., Salvi, D. and Robert, D. (2009). Ethanol production from sorghum by a dilute ammonia pretreatment. *Industrial Microbiology and Biotechnology*. 37: 27-34.
- Aita, G. and Salvi, D. (2009). Lignocellulose as a source for fuels and chemicals. *Louisiana Agriculture*. 53, 4: 12-13.
- Aita, G. and Kim, M. (2010). Pretreatment Technologies for the Conversion of Lignocellulosic Materials to Bioethanol. In: *Sustainability of the Sugar and Sugar-Ethanol Industries*. ACS Press, Available on-line, Dec 10. ISBN13: 9780841225985; eISBN: 9780841225992; DOI: 10.1021/bk-2010-1058. Dec 14.
- Salvi, D., Aita, G., Robert, D. and Bazan, V. (2010). Dilute ammonia pretreatment of sorghum and its effectiveness on enzyme hydrolysis and ethanol fermentation. *Applied Biochemistry and Biotechnology*. 161: 67-7.
- Kim, M., Aita, G. and Day, D. (2010). Compositional changes in sugarcane bagasse on low temperature, long-term diluted ammonia treatment. *Applied Biochemistry and Biotechnology*. 161: 34-40.
- Salvi, D., Boldor, D., Ortego, J., Aita, G. and Sabliov, C. (2010). Numerical modeling of continuous flow microwave heating: a critical comparison of COMSOL and ANSYS. *Journal of Microwave Power and Electromagnetic Energy*. 44, 4: 1-11.
- Aita, G. (2010). Sucrose loss during milling and methods for prevention, *Annual ASI Factory Operations Seminar*. Apr 29.
- Aita, G., Salvi, D. and Walker, M. (2011). Enzyme hydrolysis and ethanol fermentation of dilute ammonia pretreated energy cane. *Bioresource Technology*. 102, 6: 4444-4448.
- Aita, G. and Madsen, L. (2011). Added value through bioplastics from sugarcane. *Annual ASI Factory Operations Seminar*. Apr 19.
- Presentations and Posters**
- Aita, G. (2009). Current use of lignocellulosics. Extension and Research Sugarcane Training. Sugar Research Station, LSU AgCenter, St. Gabriel, La. May 13.
- Aita, G. (2009). Overview on the current status of lignocellulosics. *Annual ASI Factory Operations Seminar*. Apr 28.
- DeQueiroz (Aita), G., Robert, D. and Salvi, D. (2009). Ethanol production from sweet sorghum by a dilute ammonia solution Symposium on Biotechnology for Fuels and Chemicals SIM. San Francisco, Calif. May 3-5.
- Kim, M., DeQueiroz (Aita), G. and Day, D. (2009). Compositional changes in sugarcane bagasse on low temperature, long-term ammonia treatment. Symposium on Biotechnology for Fuels and Chemicals SIM. San Francisco, Calif. May 3-5.
- DeQueiroz (Aita), G. (2009). Biomass, biofuels and the biorefinery. Louisiana 25 X 25 State Alliance. New Orleans, La. Jan 29.
- Chen, C., Boldor, D. and Aita, G. (2010). Enhancing enzymatic digestibility of sweet sorghum by microwave pretreatment. *Annual International Meeting ASAME*. Pittsburgh, Pa. Jun 20-23.

- Aita, G. (2010). Sucrose loss during milling and methods for prevention. *Annual ASI Factory Operations Seminar*. Apr 29.
- Aita, G. and Salvi, D. (2010). Enzyme hydrolysis and ethanol fermentation of ammonia treated energy cane." Symposium on Biotechnology for Fuels and Chemicals SIM. Clearwater Beach, Fla., Apr 19-22.
- Moreno, R., Aita, G., Gutierrez, D., Yao, S., Hurlburt, B. and Brashear, S. (2011). Identification of naturally isolated microalgae used for biofuel production by PCR amplification of 16S and 18S rRNA genes. *Annual Meeting. Society of Industrial Microbiologists*, New Orleans, La. Jun 24-28.
- Cao, S., Aita, G. and Walker, M. (2011). Impact of surfactants on dilute ammonia pretreatment of sugarcane bagasse. *Annual Meeting. Society of Industrial Microbiologists*, New Orleans, La. Jun 24-28.
- Qiu, Z., Aita, G. and Walker, M. (2011). Effect of ionic fluid-pretreated energy cane bagasse on enzyme hydrolysis. *Annual Meeting Institute of Food Technologists*. New Orleans, La. Jun 11-14.
- Aita, G., Kim, M., Walker, M. and Day, D. (2011) Mild alkaline pretreatment of sugarcane bagasse during storage. Symposium on Biotechnology for Fuels and Chemicals, SIM. Seattle, Wash. May 2-5.
- Aita, G. and Kanitkar, A. (2011). Added value through bioplastics from sugarcane. *Annual ASI Factory Operations Seminar*. Apr 19.
- Aita, G. (2010). Pretreatment technologies for the conversion of lignocellulosic biomass to fuels. *ACS 66th SWRM/62nd SERMACS*, New Orleans, La. Dec 1-4.

## D.F. Day Publications



- Lee, Y., Chung C. and Day, D. (2009). Sugarcane bagasse oxidation using a combination of hypochlorite and peroxide. *Bioresource Technology*. 100: 935-941. doi:10.1016/j.biotech.2008.06.043
- Madsen, L. and Day D. (2010). Iron mediated clarification and decolorization (FeMCaD) of sugarcane juice, *Proceedings of the XXVII Congress ISSCT*. 27: 1-13.
- Kim, M. and Day D. (2010). Butanol production from sugarcane juices, *Proceedings of the XXVII Congress ISSCT*. 27: 1-7.
- Kim, M. and Day, D. (2010). Compositional changes in sugarcane bagasse on low temperature, long-term diluted ammonia treatment. *Applied Biochemistry and Biotechnology*. 161: 34-40.
- Kim, M. and Day, D. (2010). Use of cellulase inhibitors to produce cellobiose. *Applied biochem and Biotechnol*. 162: 1379-1390, DOI:10.1007/s12010-010-8915-2.
- Kim, M., Han, K. and Day, D. (2010). Ethanol from sweet sorghum, a potential energy feedstock for bioethanol production. *J of Agricultural Food Chemistry*. submitted May 24.
- Kim, M. and Day, D. (2010). Composition of multiple feedstocks suitable for ethanol production at Louisiana sugar mills. *Journal of Industrial Microbiology and Biotechnology*. in press.
- Han, Pitman, W., Montgomery W., Harrell, D., Viator, H., Gravois, K., Kim, M. and Day, D. (2010). Agronomic considerations for sweet sorghum Biofuel production in South-Central USA. *Biomass and Bioenergy*. submitted Aug 18.
- Kim, M. and Day, D. (2010). Butanol production from sugarcane juices. *International Sugar J*. 112: 601-605.
- Day, D. (2009). Biorefinery development using multiple feedstocks. *Annual ASI Factory Operations Seminar*. Apr 28.
- Day, D. (2009). Ethanol and bio-oil from bagasse and other Louisiana natural resources. *Proceedings of the 2009 Louisiana Natural Resources Symposium. LSU AgCenter*, Baton Rouge, La. 72-83.
- Day, D. (2009). Producing biofuels, *Louisiana Agriculture*. 53, 4:14-15.
- Patents**
- Day, D. and Madsen, L. (2009). Power and Hydrogen Generation System. ser # 12/373,934
- Day, D. and Chung, C. (2009). Chemical oxidation for Cellulose Separation. US Patent 7,585,387. Sept 8.
- Day, D. and Kim, M. (2010). Antifungal and anti-cariogenic cellobio-oligosaccharides produced by dextranucrase, filed 12/988,343, Oct 18.
- Day, D. and Chung, C. (2010). Isomaltooligosaccharides from *Leuconostoc* as Nutraceuticals, Canadian Patent 2,467,695 Jan 6.
- Day, D. and DeQueiroz, G. (2010). Biocide Compositions and related methods. Australian Patent 2003270523, Feb 25.
- Day, D. and Chung, C. (2010). Isomaltooligosaccharides to inhibit avian pathogenic intestinal bacteria, US Patent 7,772,212 B2, Aug 10.
- Day, D., Hoogewind, A., Randhava, S., Oswald, J., Madsen, L. and Kim, M. (2010). Method for Producing Butanol and Isopropanol. File 61/380,747, Sep 8.
- Presentations and Posters**
- Day, D. (2009). Biorefinery development using multiple feedstocks. *Integrated Biorefineries Platform Peer Review*, Washington, D. C. Mar 19.
- Kim, M., Han, K. and Day, D. (2009). Ethanol from sweet sorghum, a potential energy feedstock for bioethanol production. *Annual World Congress of Industrial Biotechnology BIT*. Seoul, South Korea. Apr 5-7.
- Chung, C., Kim, M., Han, K. and Day, D. (2009). Evaluation of sweet sorghum for ethanol production. Symposium of Biotechnology for Fuels and Chemicals SIM. San Francisco, Cal. May 3-6.
- Cho, H., Kim, D., Kim, N., Kim, J., Kang, H., and Day, D. (2009). Rice straw oxidation using hypochlorite-hydrogen peroxide for Bioconversion to Ethanol. Symposium of Biotechnology for Fuels and Chemicals SIM. San Francisco, Cal. May 3-6.





Kim, M., DeQueiroz, G. and Day, D. (2009). Compositional changes in sugarcane bagasse on low temperature, long term ammonia treatment. Symposium of Biotechnology for Fuels and Chemicals SIM. San Francisco, Ca. May 3-6.

Day, D. (2009). Ethanol and bio-oil from gbagasse and other Louisiana natural resources. Louisiana Natural Resources Symposium. Baton Rouge, La., Jul 16-17.

Han, K., Kim M., Day, D., Alison, W., Pitman, W. and McCormick, M. (2009). Evaluation of sweet sorghum variety for biofuel feedstock and animal feed. ASA-CSSA-SSSA International Conference. Pittsburg, Pa. Nov 1-5.

Day, D. (2009). Ethanol, butanol and hydrogen, biofuels of the future. Annual Technical Review Meeting CPERC. Baton Rouge, La. Aug 28.

Hoogewind, A., Kim, M. and Day, D. (2009). Butanol production from sugarcane juices. Annual Technical Review Meeting CPERC. Baton Rouge, La. Aug 28.

Kim, M. and Day, D. (2009). Lime pretreatment of lignocellulosic biomass for bioethanol production. Annual Meeting South Central Branch ASM. Thibodeaux, La. Nov 6-7.

Day, D. (2009). Biomass fuels for Louisiana: Feedstocks and approaches. Annual Meeting South Central Branch ASM. Thibodeaux, La. Nov 6-7.

Day, D. (2010). Biofuels Program. CEPRC Meeting. New Orleans, La. Jan 29

Kim, M. and Day, D. (2010). Butanol from sugar juices. XXVII Congress ISSCT. Vera Cruz, Mexico. Mar 7-11.

Madsen L. and Day, D. (2010). Iron mediated clarification and decolorization of sugarcane juice. XXVII Congress ISSCT. Vera Cruz, Mexico. Mar 7-11.

Kochergin, V., Day, D., Suhr, M. (2010). Algal production and cane sugar mills: Is there a synergy? Symposium on Biotechnology for Fuels and Chemicals SIM. Clearwater Beach, Fla. Apr 19-22.

Kang, H., Kim, N., Kim, G., Kim, H., Day, D., Kim, J. and Kim, D. (2010). Enzymatic saccharification of rice straw pretreated using TiO<sub>2</sub>/UV for bioconversion to ethanol. Symposium on Biotechnology for Fuels and Chemicals SIM. Clearwater Beach, Fla. Apr 19-22.

Kim, M. and Day, D. (2010). Composition of multiple feedstocks suitable for extended ethanol production at Louisiana sugar mills. Symposium on Biotechnology for Fuels and Chemicals SIM. Clearwater Beach, Fla. Apr 19-22.

Madsen, L. and Day, D. (2010). Iron mediated clarification and decolorization of sugarcane juice. Annual ASI Factory Operations Seminar. Apr 29.

Kim, M. and Day, D. (2010). Butanol production from sugarcane juices. Annual ASI Factory Operations Seminar, Apr 29.

Madsen, L. and Day, D. (2010). Iron mediated clarification and decolorization of sugarcane juice: a pilot test. Joint Annual Meeting ASSCT. Panama City Beach, Fla. Jun 16-18.

Day, D. (2010). Why Ethanol? Butanol production and the raw mill. Joint Annual Meeting ASSCT. Panama City Beach, Fla. Jun 16-18.

Kochergin, V., Suhr, M. and Day, D. (2010). Biodiesel from algae as an alternative for sugar mill sustainability. Joint Annual Meeting ASSCT. Panama City Beach, Fla. Jun 16-18.

Kim, M. and Day, D. (2010). Comparative study of multiple energy crops for cellulosic ethanol production. Joint Annual Meeting ASSCT. Panama City Beach, Fla. Jun 16-18.

Han, K., Alison, W., Day, D. and Kim, M. (2010). Planting management impact on sweet sorghum as a biofuel crop. ASA-CSSA-SSSA International Annual Meeting. Long Beach, Calif. Nov. 2.

## Vadim Kochergin

### Publications

Kochergin, V. (2009). Affinity based separation technologies and their role in the current and future sugar industry. Proceedings of First Conference of European Society of Sugar Technologists, 168-175.

V.Kochergin, V. and Miller, K. (2010). Evaluation of target efficiencies for solid-liquid separation steps in biofuels production. Applied Biochemistry and Biotechnology, 163, 1: 90.

Kochergin, V. (2010). Studies of Long-Term Storage of High Quality Raw Sugar. Proceedings of the 26th Congress of the International Society of Sugar Cane Technologists, Veracruz, Mexico, March 7-11.

Kochergin, V. (2010). Reduction on Sugar losses in Boiling House Operations. Sugar Bulletin, 88, 7: 17-19.

Miller, K. and Kochergin, V. (2010). Dissolved Air Flotation (DAF) for recovery of suspended solids from pretreated bagasse streams. Applied Biochemistry and Biotechnology, submitted.

Kochergin, V. (2010). Studies of Long-Term Storage of High Quality Raw Sugar. Sugar Industry/Zuckerindustrie, 135, 7: 419-426.

Kochergin, V., Gaudet, C. and Robert, M. (2010). A Juice Clarifier with Turbulence Reduction Devices-Results of First Industrial Trials. Proceedings of the 83th Congress of SASTA, 315-325.

Kochergin, V. (2011). Summary report of funded proposals from the 2010 grinding season. , Sugar Bulletin, 89, 7: 11-12.

Kochergin, V. and Gaudet, C. (2011). A Juice Clarifier with Turbulence Reduction Devices-Results of First Industrial Trials. Int.Sugar J., 113, 1349: 348-355.

Lohrey, C. and Kochergin, V. (2012). Biodiesel Production from Microalgae: Co-location with Sugar mills. Bioresource Technology. 76-82 DOI: 10.1016/j.biotech.2011.12.035

**Presentations and Posters**

Kochergin, V., Gaudet, C., Robert, M. and Bergeron, S. (2010). Experience with new design of juice clarifier. Annual Meeting ASSCT, Louisiana Division. Lafayette, La.

Kochergin, V. and Tishechkina, I. (2010). Crystal size measurements - convenient tool for sugar mill tune-up. Annual Meeting ASSCT, Louisiana Division. Lafayette, La.

Kochergin, V. (2010). Studies of Long-Term Storage of High Quality Raw Sugar. Proceedings of the 26th Congress of the International Society of Sugar Cane Technologists. Vera Cruz, Mexico. March 8-11.



Miller, K. and Kochergin, V. (2010). Flow Characteristics of C-Masseccuite in Cooling Crystallizers. SPRI Conference. New Orleans, La. March 28-29.

Miller, K. and Kochergin, V. (2010). Dissolved Air Flotation (DAF) for recovery of suspended solids from pretreated bagasse streams. Symposium on Biotechnology for Fuels and Chemicals. Clearwater Beach, Fl. Apr 19-22.

Kochergin, V., Day, D. and Suhr, M. (2010). Algae production and sugar cane mills: is there a synergy? Symposium on Biotechnology for Fuels and Chemicals. Clearwater Beach, Fl. Apr 19-22

Kochergin, V. and Gaudet, C. (2010). New Approach to Fluid Distribution in the Industrial Clarifiers. Joint Annual Meeting ASSCT. Panama City Beach, Fl. Jun 16-18.

Kochergin, V. and Suhr, M. (2010). Biodiesel from Algae as an Alternative for Sugar Mill Sustainability. Joint Annual Meeting ASSCT. Panama City Beach, Fl. Jun 16-18.

Kochergin, V., Gaudet, C. and Robert, M. (2010). A Juice Clarifier with Turbulent Reduction Devices-Results of First Industrial Trials. Annual Congress SASTA. Durban, South Africa. Aug 25-27.

Kochergin, V. (2010). Sugar Industry: Pathways to non-ethanol fuels and carbohydrate based chemicals. Annual Congress SASTA. Durban, South Africa. Aug 25-27.

Lohrey, C. and Kochergin, V. (2011). Co-location of Algae Production Facilities and Sugarcane Mills for Commercial Biodiesel Production. National Algae Association Conference. Woodlands, Tx. Jan 13-14.

Kochergin, V., Gaudet, C., Acevedo, L., King, D., Willis, C. and Giraud, J. (2011). New LLT Clarifier: Lessons learned During Second Operational Season. Annual Meeting ASSCT, Louisiana Division. Lafayette, La. Feb 8-9.

Kochergin, V. and Lohrey, C. (2011). Heat Release from Raw Sugar Piles. Annual Meeting ASSCT, Louisiana Division. Lafayette, La, Feb 8-9.

Dolan, N., Goudeau, S. and Kochergin, V. (2011). Performance of a Technopulp Belt Filter at Raceland Sugar Mill. Annual Meeting ASSCT, Louisiana Division. Lafayette, La. Feb 8-9.

Kochergin, V., Suhr, M. and Lohrey, C. (2011). Utilization of beet sugar factory resources for production of Algal Biodiesel. Biennial Meeting ASSBT. Albuquerque, NM. Mar 2-6.

Kochergin, V. and Gaudet, C. (2011). Design of clarifiers with turbulence reduction devices. Biennial Meeting ASSBT. Albuquerque, NM. Mar 2-6.

Kochergin, V. (2011). Quality of Raw Sugar and Refining Possibilities in the Beet Sugar Factories. Annual Conference ESST. Bratislava, Slovakia. May 30.

Lohrey, C. and Kochergin, V. (2011). Heat Release from Raw Sugar Piles. Joint Annual Meeting ASSCT. New Orleans, La. Jun 8-9.

Gaudet, C. and Kochergin, V. (2011). LLT Clarifier Optimization and Performance. Joint Annual Meeting ASSCT. New Orleans, La. Jun 8-9.

**Patents**

Cross, W., Peterson, K., Kochergin, V. and Loescher, M. (2007). Fractal Distributor for Two-Phase Mixing. US Patent 029 7285 A1.

Kochergin, V. and Gaudet, C. (2010). Turbulence Reduction Device. US Patent filed on Sept 20



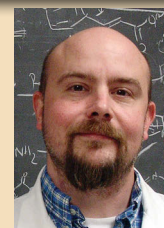
## Ben Legendre Publications



- Salassi, M. and Legendre, B. (2008). Sugarcane Outlook. In: 2008 Outlook For Louisiana's Agriculture. 20-22; Ed, Kurt Guidry, Louisiana State University AgCenter, Department of Agricultural Economics and Agribusiness Staff Paper SP 2008-03, March 2008, 55 pp.
- Reay-Jones, F., Wilson, L., Reagan, T., Legendre, B. and Way, M. (2008). Impact of an invasive stem borer. J. Econ. Entomol. 101, 2: 237-250.
- Johnson, R., Viator, H. and Legendre, B. (2008). Sugarcane fertilizer recommendations for the 2008 crop year. Sugar Bull. 86, 6:11-13.
- Legendre, B. and Gravois, B. (2008). The 2007 Louisiana sugarcane variety survey. Sugar Bull. 86, 8:18-22. Also, Sugarcane Research Progress Report, LSU Agricultural Center. 91-101.
- LaBorde, C., Robert, T., Legendre, B., Bischoff, K. and Gravois, K. (2008). Sugarcane variety identification guide - Louisiana 2008. Louisiana State University Agricultural Center. Pub. 3056. 24 pp.
- Eggleston, G., Karr, J., Parris, A. and Legendre, B. (2008). Viability of an enzymatic mannitol method to predict sugarcane deterioration at factories. Food Chemistry. 111: 476-482.
- Legendre, B. and Birkett, H. (2008). Sugar processing in Louisiana. Louisiana Agric. 51, 2: 8-9.
- Legendre, B. (2008). Audubon Sugar Institute: Keeping Louisiana sugar factories profitable. Louisiana Agric. 51, 2: 10-11.
- Day, D., DeQueiroz, G. and Legendre, B. (2008). Turning sugarcane cellulose into ethanol: Energy for the future? Louisiana Agric. 51, 2: 12-13.
- Viator, H., Bengston, R., Hall, S., Gaston, L., Selim, M., Wang, J., Legendre, B., Hymel, T., Flanagan, J., Hoy, J., Kennedy, C. and Prudente, J. (2008). Influence of sugarcane post-harvest residue management on yield, water quality. Louisiana Agric. 51, 2: 39-40.
- Legendre, B., Gravois, K., Bischoff, K. and Griffin, J. (2008). Use of glyphosate to enhance sugar production in Louisiana. Louisiana Agric. 51, 2: 42-43.
- Salassi, M. and Legendre, B. (2008). Economic importance of Louisiana sugarcane production: 2007 Agricultural Summary. Staff Report No. 2008-02, January 2008, Sugarcane Research Progress Report, LSU Agricultural Center. 1-5.
- Legendre, B. (2008). Sugarcane summary for crop year 2007. Sugarcane Research Progress Report, LSU Agricultural Center. 6-7.

- Tew, T., Dufrene, E., Garrison, D., White, W., Grisham, M., Pan, Y., Richard, E., Legendre, B. and Miller, J. (2009). Registration of 'HoCP 00-950' sugarcane. J. Plant Registration. 3, 1: 42-50.
- Salassi, M. and Legendre, B. (2009). Sugarcane Outlook. In: 2009 Outlook For Louisiana's Agriculture. 18-21; Ed, Kurt Guidry, Louisiana State University Agricultural Center, Publication No. 3048, April 2009.
- Salassi, M., Deliberto, M. and Legendre, B. (2009). Economic importance of Louisiana sugarcane production in 2008. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 1-3.
- Legendre, B. (2009). Sugarcane summary for crop year 2008. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 4-6.
- Legendre, B. and Gravois, K. (2009). The 2008 Louisiana sugarcane variety survey. Sugar Bull. 87, 10: 17-22.
- Legendre, B. (2009). The 2008 Louisiana sugarcane variety survey. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 91-104.
- Viator, H., Flanagan, J., Gaston, L., Hall, S., Hoy, J., Hymel, T., Kennedy, C., Legendre, B., Wang, J. and Zhou, M. (2009). The influence of post-harvest residue management on water quality and sugarcane yield in Louisiana. J. Amer. Soc. Sugarcane Technol. 28:76-85.
- Legendre, B. and Gravois, K. (2010). The 2009 Louisiana sugarcane variety survey. Sugar Bull. 88, 9: 23-28.
- Reagan, T., Wilson, B., Beuzelin, J., Gravois, K., Pollet, D. and Legendre, B. (2010). Developing best management practices for the Mexican rice borer. Sugar Bull. 88, 9: 29-31.
- Salassi, M., Deliberto, M., Westra, J. and Legendre, B. (2010). Economic importance of Louisiana sugarcane production in 2009. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 1-3.
- Legendre, B. (2010). Sugarcane crop summary for crop year 2009. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 4-6.
- Legendre, B. and Gravois, K. (2010). The 2009 Louisiana sugarcane variety survey. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 83-96.
- Legendre, B., Birkett, H., Stein, J., Duet, M., Tew, T. and Eggleston, G. (2010). Stalk cold tolerance of commercial and candidate varieties. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 146-149.
- Orgeron, A., Legendre, B., Pontif, M., Griffin, J. and Gravois, K. (2010). Ripener update. Sugarcane Research Annual Progress Report, LSU Agricultural Center. 152-158.
- Legendre, B. and Gravois, K. (2010). Variety trends and concern over the dependence of a single variety (Monoculture). Sugar Bull. 88, 10: 13-16.

## Lee Madsen Publications and Presentations



- Madsen, L., Polanco, S., Verret, C. and Dinu, I. (2010). A Survey of Louisiana Final Molasses Produced During the 2009-2010 Grinding Season. Annual Meeting ASSCT. Louisiana Division. Lafayette, La. Feb 3.
- Madsen II, L. and Day, D. (2010). Iron Mediated Clarification and Decolourisation of Sugarcane Juice. Proc. Int. Soc. Sugar Cane Technol. 27: 1-11; Abstract, p. 200.
- Madsen, L. and Day, D. (2010). Iron Mediated Clarification and Decolorization of Sugarcane Juice. Annual ASI Factory Operations Seminar. Apr 29.
- Madsen, L., Polanco, S., Verret, C. and Dinu, I. (2010). The Molasses and Juice Survey. Annual ASI Factory Operations Seminar. Apr 29.
- Madsen, L. and Day, D. (2010). Iron Mediated Clarification and Decolorization of Sugar Cane Juice: A Pilot Test. Joint Annual Meeting ASSCT. Panama City Beach, Fla. Jun 16-18.
- Madsen, L. and Day, D. (2011). Iron Mediated Clarification and Decolourisation of Sugarcane Juice. Int. Sug. J. 113, 1349: 335-342.
- Madsen, L., Day, D. and Verret, C. (2011). Amylase Efficiency in the Raw Sugar Factory: Discussed in the context of TPD. Annual Meeting ASSCT. Louisiana Division, Lafayette, La. Feb 8-9.
- Dorman, D. and Madsen, L. (2011). Illuminating Dye-Intercalated Biodegradable Polyester Made from Renewable Feed Stock. LSU-CAMD User Meeting, Baton Rouge, La. Apr 15.
- Madsen, L. and Verret, C. (2011). The 2010 Molasses Survey and the Effect of Starch on TPD. Annual ASI Factory Operations Seminar. Apr 19.
- Madsen, L. and Day, D. (2011). The Negative Impact Potential of Starch in the Sugar Factory: A Case Study. Annual ASI Factory Operations Seminar. Apr 19.
- Madsen, L. and Marceau-Day, M. (2011). Nanoparticle-Based Radiation Detectors and the Use of Radiation for Nanoparticle Detection. Annual Meeting Health Physics Society. West Palm, Fla. Jun 26-30.
- Marceau-Day, L., Hoover, M., Walker, S. and Madsen, L. (2011). Special Session: Emerging Opportunities for the Interaction(s) of Nanotechnology and Radiation Protection. Annual Meeting Health Physics Society. West Palm, Fla. Jun 26-30.
- Patents**
- Day, D., Hoogewind, A., Randhava, S., Oswald, J., Madsen, L. and Kim, M. (2010). Method for Producing Butanol and Isopropanol. US Patent filed.
- Madsen II, L.R. and Dorman, D.R. (2011). Biodegradable Photolithographic Matrices Made from by-products of the Production of Bio-based Diesel Range Organic Liquid Fuels. US Patent filed.

***Changes of sugar quality during the storage period may reduce the quality of raw sugar into a product that is less desirable to the refinery, resulting in additional processing costs and possible penalty in the price of the raw sugar paid to the producer of the raw sugar.***





## Faculty and Staff

### Administrative Staff

Dr. Ben Legendre. Professor and Head – B.S., M.S. and Ph.D., Agronomy (Louisiana State University)  
 Lanelle Mabile. Administrative Coordinator  
 Melati Tessier. Instructor – B.S., Chemical Engineering (Louisiana State University)  
 Jennifer Marceau. Assistant to Director of the Louisiana Institute for Biofuels & Bioprocessing (LIBBi) – B.A., Organizational Communications (University of Lafayette)

### Analytical Lab

Dr. Lee Madsen. Research Associate-Analytical Chemist – B.S. and Ph.D., Chemistry (Louisiana State University)  
 Dr. Derek Dorman. Post-Doctoral Researcher – B.S., Polymer Science (University of Southern Mississippi), Ph.D., Chemistry (Louisiana State University)  
 Chardcie Verret. Research Associate – B.S. Chemistry (Southern University)  
 Shyue Lu. Research Associate – B.S. Biology (Louisiana State University)

### Factory Staff

Julie King. Research Associate-Factory Manager – B.S. Mechanical Engineering (University of Arizona-Tucson)  
 Lamar Aillet. Maintenance Foreman

### Faculty and Staff

Dr. Giovanna Aita. Assistant Professor – B.S. and M.S., Food Science (Clemson University), Ph.D., Food Science (Louisiana State University)  
 Dr. Harold Birkett. Associate Professor – B.S., M.S. and Ph.D., Chemical Engineering (Louisiana State University)  
 Dr. Misook Kim. Assistant Professor – B.S. and M.S., Food Science and Nutrition (Dankook University, Korea), Ph.D., Food Science (Louisiana State University)

Dr. Young-Hwan Moon. Post-Doctoral Researcher – B.S., M.S. and Ph.D., Food Science (Chonnam National University, Korea)

Dr. Donal Day. Professor – B.S., Biochemistry (University of New Hampshire), Ph.D., Microbiology (McGill University, Canada)

Dr. Vadim Kochergin. Professor – M.S. and Ph.D., Chemical Engineering (Mendeleev Chemical Engineering University, Moscow, Russia)

Dr. Michail Granovskiy. Associate Professor – M.S., Chemical Engineering (Mendeleev University, Moscow, Russia), Ph.D., Chemical Engineering (Moscow State Lomonosov University, Moscow, Russia)

Dr. Michelle Walker. Research Associate – B.S. and Ph.D., Chemical Engineering (Louisiana State University)

Dr. Swetha Mahalaxmi. Research Associate – B.S., Chemical Engineering (Osmania University, Hyderabad, India), M.S. and Ph.D., Chemical Engineering (University of Mississippi)

Dr. Daira Aragon. Post-Doctoral Researcher – B.S., Chemical Engineering (Universidad de Antioquia, Medellin, Colombia), M.S., Environmental Engineering (Louisiana State University), Ph.D., Chemical Engineering (Louisiana State University)

Jeanie Stein. Research Associate – B.S., Plant Science (Nicholls State University)

Stuart 'Lenn' Goudeau. Research Associate – B.S. Industrial Technology (Louisiana State University)

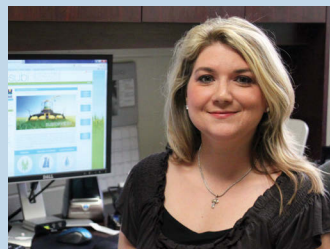
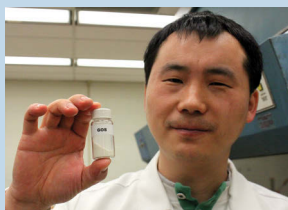
Iryna Tishechkina. Research Associate – M.S. Biology/Chemistry minor (Belarusian State University, Minsk, Belarus)

### Adjunct Faculty

Dr. Mike Inkson. SKIL – B.S., M.S. and Ph.D., Chemical Engineering (University of Manchester, United Kingdom)  
 Mary An Godshall. SPRI – B.S., Biological Science (Louisiana State University-New Orleans), M.S., Biochemistry (University of New Orleans)  
 Dr. Doman Kim. Chonnam National University – B.S. and M.S., Food Science (Seoul National University, Korea), Ph.D., Microbiology (Louisiana State University)

### Graduate Students

Stella Polanco. Graduate Student – B.S., Chemical Engineering (Universidad del Valle, Colombia), M.S., Chemical Engineering (Louisiana State University)  
 Akanksha Kanitkar. Graduate Student – B. Tech., Oils, Oleochemicals and Surfactants Technology (University Department of Chemical Technology, Mumbai, India), M.S. Biological and Agricultural Engineering (Louisiana State University)  
 Saeed Oladi. Graduate Student – B.S. Food Science (Tabriz University, Tabriz, Iran), M.S., Food Science (Isfahan University of Technology, Isfahan, Iran)  
 Keith Miller. Graduate Student – B.S., Biological Engineering (Louisiana State University), M.S., Biological and Agricultural Engineering (Louisiana State University)  
 Reynaldo Moreno. Graduate Student – B.S. Agriculture (Universidad de San Pedro Sula, Honduras), B.A., Music (Louisiana State University), M.S., Food Science (Louisiana State University)  
 Cong Chen. Graduate Student – B.S., Biological Engineering (Jiangnan University, China), M.S., Biological and Agricultural Engineering (Louisiana State University)  
 Christian Lohrey. Graduate Student – B.S., Chemical Engineering and Chemistry (University of Idaho), M.S., Biological and Agricultural Engineering (Louisiana State University)  
 Zenghui Qiu. Graduate Student – B.S., Food Science and Engineering (Nanchang University, Nanchang, China)  
 Adam Hoogewind. Graduate Student – B.S., Chemistry and Biology (Aquinas, Michigan)  
 Shuo Cao. Graduate Student – B.S., Bioengineering (Henan University, Kaifeng, China), M.S., Food Science (Louisiana State University)  
 Santiago Grimaldo. Graduate Student – B.S. Chemical Engineering (Universidad del Valle, Cali, Colombia)





**Audubon Sugar Institute**  
LSU AgCenter  
3845 Highway 75  
Saint Gabriel, LA 70776  
USA

### **Audubon Sugar Institute Mission Statement**

To foster a center of excellence for applied and original sugar research, which exceeds the expectations of our stakeholders in Louisiana and the international sugar industry, through innovative research, technology transfer and education.



*The gross farm value received by producers and landlords for the 2010 sugarcane crop for the sugar and molasses produced in Louisiana was \$503 million. Another \$343 million came from processing and marketing.*