

Creating and Capturing Total Value of Agricultural Resources for Biofuels and Biobased Products

Biorefining research at WRRC features an “out of the box” view of the biorefinery that reaches beyond the refinery walls to include the farm and forest. Hence our research (a) identifies and creates or induces the plant chemical and physical architecture that is the foundation of the biorefining enterprise and (b) defines the ways that alter the crop architecture to capture its inherent value.

In our non-traditional view the farm becomes one step in the chain of events leading to biofuels and biobased product. To the “new biorefiner”, the farm takes on the role of a large flat plate reactor with a crop of corn or wheat as the culture, just as the fermenter is a microbiological crop in a bottle. To the “new biologist, the biorefining system becomes a complex network of biochemical and chemical pathways. Farm-based and city-based components of biorefining are intimately linked through foundations in genetics and molecular biology. These determine the chemical and physical architecture of the crop resource and the kinetics of the chemical and biological separations and conversions.

Biorefining, in the crop plant and in the field or the refinery, is crucial to the full development of biofuels and biobased industrial products. The successful implementations of biorefining enterprises reduce imports, stabilize economies, create markets for surplus agricultural production, improve the balance of payments, create rural employment, and reduce the impact of unstable petroleum supplies.

WRRC uses genomics, separation science, process integration, novel solvent properties, and polymer formulation in its research. There is a partial focus on nationally important small grains such as wheat and rice, and on physical and genetic methodologies with generic utility to all grains and grain straws. The research reflects the efforts of 15 full time PhD scientists and approximately 40 technical staff.



Inductive Biorefining... Finding and Inducing New Chemical and Physical Architectures:

Changing the Genetic Resource for Biofuels. WRRRC scientists seek to develop the knowledge and genetic technologies to improve the use of renewable plants as a resource for chemical feedstocks and fuels. The focus is on the cereal crops (especially wheat and rice) and other high yielding grasses with potential to be biofuel resources such as switch grass (shown). Plant molecular biology, genomics, bioinformatics, and plant transformation are being marshalled to alter these crops. The research is defining (1) factors for expression of polysaccharides and proteins in plant leaf and stem tissue, (2) the potential for new plant architecture and cell wall structure for more efficient light gathering, storage capacity, and post-harvest refining; and (3) the potential for polysaccharide production to enhance ethanol production as well as existing and novel polymer structures. Drs. Christian Tobias, John Vogel, Olin Anderson.



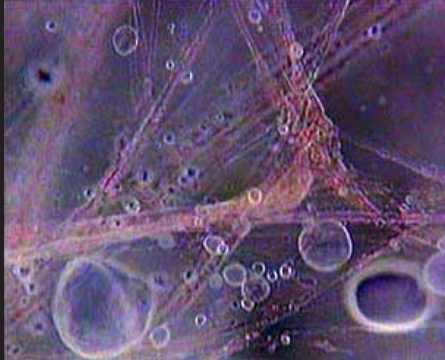
Toxin-Free Castor as a New Oilseed Resource:

WRRRC research is leading to a new domestic crop for castor oil, a key industrial oil that is now imported because the traditional castor plant is hazardous to grow. The castor seed produces a unique oil with up to 90% ricinoleate (12-hydroxy-oleate). The oil is used to produce lubricants, coatings and plastics. Albany researchers have invented the means to transform the castor plant, *Ricinus communis*, the source of castor oil, to eliminate the toxin ricin and allergens from castor. This research could lead to the re-introduction of castor in a non hazardous form as an energy and chemical crop for bio-based products. Dr. Thomas McKeon.

Domestic *Guayule* as a New Source of Industrial and Hypoallergenic Rubber: Natural rubber is vital to United States commerce, defense, transportation and medicine. The entire domestic supply is imported and is subject to shortages and high cost due to declining production, increasing global demand, international politics, and crop disease. Life-threatening "latex allergy" to *H. brasiliensis* rubber products makes development of an alternative, safe source imperative. WRRRC is developing alternative rubber-producing domestic crops. The research has led to an exclusive patent license for commercial production of the perennial *guayule*, as a source of high-value, hypoallergenic latex. Expanded research will lead to higher-yielding *guayule* lines as well as to production of non-*guayule* annual rubber-producing crops. Dr. Colleen McMahan.

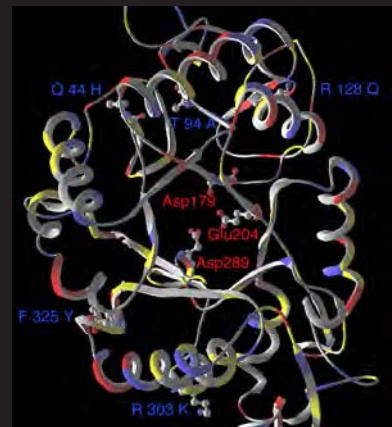


Reductive Biorefining...Capturing Native Values



Wheat Grain Separation by Cold ethanol displacement to starch and protein platforms. The separation of wheat precedes all refining options for wheat grain. WRRRC has developed the cold-ethanol separation method that improves on both the separation and the subsequent drying technology (shown at left are starch grains being displaced from developed gluten fibrils). The cold ethanol method is capable of producing a gluten that meets or exceeds the properties of freeze-dried gluten, a conventional standard of gluten vitality, by enabling low temperature drying. This lab-scale technology is now being adapted for scaleup. New efforts include evaluation of gluten subfractionation to new protein platforms. Dr. George Robertson.

Reductive Biorefining: Separation / Disassembly: Starch-to-sugar platform by enzyme evolution/ cold hydrolysis: Processes for the conversion of crops or crop components into fuel or chemical products are inefficient and have high energy use. WRRRC is utilizing molecular evolution to create enzymes to help overcome these limitations. In one example a process called cold hydrolysis could reduce energy and capital needs for grain-to-ethanol conversions. Enzymes with enhanced properties for this purpose have already been produced at WRRRC and plans are underway to begin evaluation at larger scale. Shown in the color example is one highly-active mutant amylase enzyme created in the lab. Drs. Dominic Wong, George Robertson, Charles Lee.



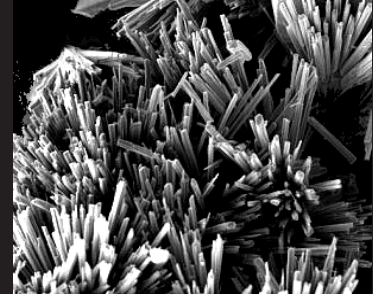
Reductive Biorefining: Separation / Disassembly: Hemicellulose-to-C5 and cellulose platform by enzyme evolution: The conversion to fermentable substrates of hemicellulose-rich crop components such as grain fiber, mill feeds, straws, etc. depends on chemically and energy-intensive acid hydrolysis to produce cellulase susceptible substrate. WRRRC research is defining new enzymatic resources that can enzymatically disassemble these substrates at temperatures that reduce energy usage. Key to this effort is the identification of new parent enzymes and the optimization of these by molecular evolution. Drs. Charles Lee, Dominic Wong, Kurt Wagschal, George Robertson.

New Separation Initiatives.....



Separation and Recovery of Nano Fibers:

Reductive refining to fractionate and modify fibers from agricultural straws, and determine their physical, chemical, and mechanical properties will lead to novel composites and nanocomposites, plastic materials reinforced with ag-derived nanoparticles, silicates, clays, or cellulose whiskers (rice straw and silicate whiskers shown). Fiber refining methods such as organo-solv fractionation, hot water/mechanical pulping and micro-fibrillation will be used to isolate straws components. These high-value added materials will help to alleviate straw disposal problems in the western states. Drs. Gregory Glenn, Maria Inglesby, William Orts,

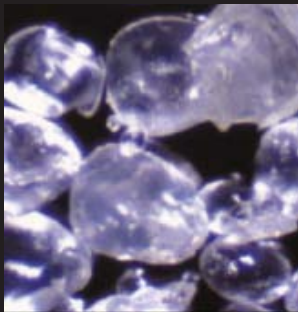


Separation and Recovery of Ethanol: Reductive refining is also applied at a molecular level using barrier or membrane-based methods and novel barrier compositions for ethanol from water separation. This is important to the recovery of ethanol from fermentation broths to increase fermentation efficiency but also reduces the energy used in the separation; thereby reducing the need for inefficient and energy-demanding distillation. Drs. Richard Offeman, George Robertson, William Orts.

Inductive Biorefining at the Refinery... New Post-Harvest Values

Conversion from Starch Platform: Bio-Based Disposable Polymers: Various enzymatic and chemical methods are being applied to modify properties of starches and isolated fibers to improve their functionality, processability and compatibility within films, composites and nanocomposites. These characterized starches, fibers and other additives are being processed into packaging and building materials using extruders, baking molds, expanded bead cup molders, compression molders and injection molders. Performance and biodegradability of specific biomaterials derived within the project will be examined, focusing on their biodegradability.

One example is the clamshell fast food container using potato starch, cellulose fiber, and limestone that is currently being test-marketed at large fast food restaurant chains. Research using wheat starch to reduce brittleness, processing losses, and cost is underway. Wheat starch containers are opaque, low-density foams with excellent thermal insulating properties, high tensile strength and improved flexibility.



Another example, lightweight concrete, is made by first swelling a starch aggregate in water. Starch-based lightweight concrete is then made by mixing the starch gel with cement. The mixture is poured and allowed to harden. During the curing and subsequent drying process, water migrates out of the gels causing them to shrink and form voids within the concrete product. These void spaces make the concrete lightweight. Lightweight concrete has many uses in floors, walls, roofing tile and other products that need to be durable, lightweight and provide sound and thermal insulation. Dr. Gregory Glenn, William Orts, Syed Imam, Bor-sen Chou.

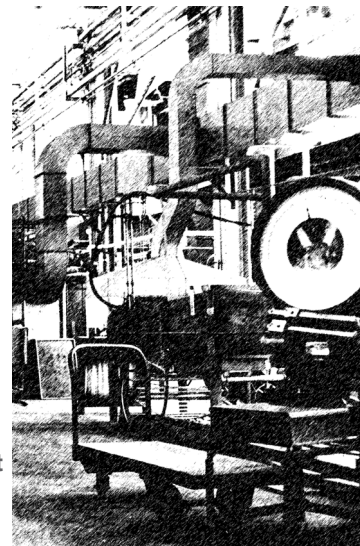


Selected Publications

1999 to 2003 (July)

Scientists
in current
biofuels, biobased products,
and biorefining projects

For on-line updates,
see
the WRRC Scientists Publication Database at
<http://www.pw.usda.gov/publications>



Research units represented:

Bioproduct Chemistry and Engineering, Crop Improvement Utilization Genomics and Gene Discovery

Inductive Biorefining: Creating new genetic resources

Anderson O.D., Blechl AE. Transgenic Wheat - Challenges and Opportunities. In : O'Brien L, Henry RJ editors. Transgenic Cereals. St. Paul, MN.: American Association of Cereal Chemists, 2000: 1-27.

Anderson OD, Hsia CC, Torres V. The wheat gamma-gliadin genes: Characterization of ten new sequences and further understanding of gamma-gliadin gene family structure. Theoretical and Applied Genetics 2000; 103(2/3):323-30.

Weeks JT, Koshiyama KY, Maier-Greiner U, Schaeffner T, Anderson OD. Wheat transformation using cyanamide as a new selective agent. Crop Science 2000; 40:1749-54.

Anderson OD. Wheat biotechnology. 9th edition. New York: McGraw-Hill, 2001.

Anderson OD, Hsia CC, Adalsteins AE, Lew EJL, Kasarda DD. Identification of several new classes of low-molecular-weight wheat gliadin-related proteins and genes. Theoretical and Applied Genetics 2001; 103(2/3):307-15.

Hsia CC, Anderson OD. Isolation and characterization of wheat w-gliadin genes. Theoretical and Applied Genetics 2001; 103(1):37-44.

Laudencia-Chingcuanco D, Lazo GR, Miller R, Carollo V, Chao S, Anderson OD. Endosperm ESTs from *Triticum aestivum* Cv. Cheyenne. Plant & Animal Genome IX. 2001: 68.

Lazo GR, Tong J, Miller R *et al.* Software Scripts for Quality Checking of High Throughput Nucleic Acid Sequencers. Biotechniques 2001; 30(6):1300-5.

Anderson, OD, Carollo, V, Lazo, GR, Matthews, DM. GrainGenes web pages and databases. REV 2002. [Web Page]. 2002; Available at <http://wheat.pw.usda.gov>.

Anderson OD, Larka L, Christoffers MJ, McCue KF, Gustafson JP. Comparison of orthologous and paralogous DNA flanking the wheat high molecular weight glutenin genes: sequence conservation and divergence, transposon distribution, and matrix-attachment regions. Genome 2002; 45:367-80.

Inductive Biorefining: New Crop Resources: Rubber

Cornish K. Biochemistry of natural rubber, a vital raw material, emphasizing biosynthetic rate, molecular weight and compartmentalization, in evolutionarily divergent plant species. Nat Prod Rep 2000; 1:1-8.

Cornish K, Castillón J, Scott DJ. Substrate-dependent rubber molecular weight regulation, in vitro, in plant species that produce high and low molecular weights in vivo. Biomacromolecules 2000; 632-41.

Cornish K, Chapman MH, Nakayama FS, Vinyard SH, Whitehand LC. Latex quantification in guayule shrub and homogenate. Industrial Crops and Products 2000; 10:121-36.

Cornish K. Similarities and differences in rubber biochemistry among plant species. Phytochemistry 2001; 5:1123-34.

Cornish K, Brichta JL, Yu P, Wood DF, McGlothlin MW, Martin J.A. Guayule latex provides a solution for the critical demands of the non-allergenic medical products market. Agro-Food-Industry Hi-Tech. 2001; 12:27-31.

Krishnakumar R, Cornish K, Jacob J. Rubber biosynthesis in tapping panel dryness affected Hevea trees. Journal of Rubber Research 2001; 131-9.

Cornish K, Brichta JL. Purification of hypoallergenic latex from guayule shrub. Janick J, editor. Trends in New Crops and New Uses. Alexandria, VA.: ASHS Press, 2002: 214-21.

Cornish K, Brichta JL. Rheological properties of latex from *Parthenium argentatum* Gray compared with latex from other Rubber-Producing Species. *Journal of Polymers and the Environment*. 2002; 10:13-8.

Cornish K., Brichta JL, Chapman MH *et al.* Biological and physical characteristics of *Parthenium argentatum* (guayule) latex in comparison with latex from *Hevea brasiliensis* and *Ficus elastica*. *Proceedings of the 5th International Latex Conference*. 2002: 1-15.

Inductive Biorefining: New Crop Resources: Castor

Lin JT, Lew KM, Chen JM, Iwasaki Y, McKeon TA. Metabolism of 1-acyl-2-oleoyl-sn-glycero-3-phosphoethanolamine in castor oil biosynthesis. *Lipids* 2000; 35:481-6.

Lin JT, Lew KM, Chen JM, McKeon TA. Separation of the molecular species of intact phosphatidylethanolamines and their N-monomethyl and N-dimethyls by high-performance liquid chromatography on a C8 column. *Journal of Chromatogr. A* 2000; 891:349-53.

Lin JT, McKeon TA. Biosynthesis of castor oil studied by high-performance liquid chromatography. In : Pandalai SG editor. *Recent Research Developments in Lipids Research*. Vol. 4. 2000: 361-8.

Lin JT, McKeon TA. Separation of intact phosphatidylcholine molecular species by high-performance liquid chromatography. *Journal of Liquid Chromatogr. and Relat. Technol.* 2000; 23:813-29.

McKeon TA, Chen GQ, Lin JT. Biochemical aspects of castor oil biosynthesis. *Biochem Soc Trans* 2000; 28(8):972-4.

McKeon TA, Chen QG, Lew KM, Stafford AE, Lin JT. Domestic production of castor oil. *Proceedings of the 49th Oilseed Conference*. 2000: M1-M4.

McKeon TA, Chen G, He X, Lin JT. Two acyltransferases that make triacylglycerol in castor bean. *Proceedings of 30th Annual Meeting of the United States Japan Natural Resources Cooperative Program*. 2001: 149-53.

Lin JT, Chen JM, Chen P, Liao LP, McKeon TA. Molecular species of PC and PE incorporated from free fatty acids in castor oil biosynthesis. *Lipids* 2002; 37:991-5.

Lin JT, Chen JM, Liao LP, McKeon TA. Molecular species of acylglycerols incorporating radiolabeled fatty acids from castor (*Ricinus communis* L.) microsomal incubations. *J Agric Food Chem* 2002; 50:5077-81.

McKeon TA, Lin JT. Biosynthesis of ricinoleic acid for castor oil production. in: Kuo TM, Gardner HW editors. *Lipid Biotechnology*. New York: Marcel Dekker, Inc, 2002: 129-39.

McKeon TA, Lin JT, Chen G. Developing a safe source of castor oil. *Inform* 2002; 13:381-5.

Turner C, McKeon TA. The use of immobilized *Candida antarctica* lipase for simultaneous supercritical fluid extraction and in-situ methanolysis of cis vaccenic acid in milkweed seeds. *Japan American Oil Chemists Society* 2002; 79:473-8.

Lin JT, Turner C, Liao LP, McKeon TA. Identification and quantification of the molecular species of acylglycerols in castor oil by HPLC using ELSD. *Journal of Liquid Chromatography & Related Technologies* 2003; 26:759-66.

Reductive Biorefining: Biofuels enzymes for biopolymer disassembly

Lee CC, Wong DWS, Robertson GH. Cloning and characterization of two cellulase genes from *Lentinula edodes*. *FEMS Microbiol Lett* 2001; 205:355-60.

Lee CC, Wong DWS, Robertson GH. An *E. coli* expression system for the extracellular secretion of barley alpha-amylase. *J Protein Chem* 2001; 20(3):233-7.

Tibbot BK, Wong DWS, Robertson GH. A Functional raw starch-binding domain of barley alpha-amylase expressed in *Escherichia coli*. *J Protein Chem* 2000; 19(8):663-9.

Tibbot BK, Wong DWS, Robertson GH. Studies on the C-terminal region of barley alpha-amylase 1 with emphasis on raw starch-binding. *Biologia, Bratislava* 2002; 57(Suppl. 11):229-38.

Whitaker J.R., Voragan AGJ, Wong DWS, editors. *Handbook of Food Enzymology*. New York, NY: Marcel Dekker, Inc., 2002.

Whitaker JR, Wong DWS. Catalase. Whitaker J.R., Voragan AGJ, Wong DWS, editors. *Handbook of Food Enzymology*. New York, NY: Marcel Dekker, Inc., 2002: 389-402.

Wong DWS. Lipase. Whitaker J.R., Voragan AGJ, Wong DWS, editors. *Handbook of Food Enzymology*. New York, NY: Marcel Dekker, Inc., 2002: 667-80.

Wong DWS. Nucleic acid biosynthesis. Whitaker J.R., Voragan AGJ, Wong DWS, editors. *Handbook of Food Enzymology*. New York, NY: Marcel Dekker, Inc., 2002: 211-20.

Wong DWS. Recent Advances in Enzyme Development. Whitaker J.R., Voragan AGJ, Wong DWS, editors. *Handbook of Food Enzymology*. New York, NY: Marcel Dekker, Inc., 2002: 379-88.

Wong DWS, Batt SB, Lee CC, Robertson GH. Direct screening of libraries of yeast clones for alpha-amylase activity on raw starch hydrolysis. *Protein and Peptide Letters* 2003; 10(5):459-68.

Wong DWS, Batt SB, Lee CC, Robertson GH. Increased expression and secretion of recombinant alpha-amylase in *Saccharomyces cerevisiae* by using glycerol as the carbon source. *J Protein Chem* 2002; 31:419-25.

Wong DWS, Batt SB, Robertson GH. Characterization of active barley a-amylase 1 expressed and secreted by *Saccharomyces cerevisiae*. *J Protein Chem* 2001; 20(8):619-23.

Wong DWS, Batt SB, Robertson GH. Microassay for rapid screening of alpha-amylase activity. *J Agric Food Chem* 2000; 48:4540-3.

Wong DWS, Batt SB, Tibbot BK, Robertson GH. Isolation of a raw starch-binding fragment from barley alpha-amylase. *J Protein Chem* 2000; 19(5):373-7.

Wong DWS, Robertson GH. Alpha-amylases. 56. in: Whitaker JR, Voragen AGJ, Wong DWS. Handbook of Food Enzymology. New York: Marcel Dekker, 2002: 707-18.

Wong DWS, Robertson GH. Beta-amylases. 57. in: Whitaker JR, Voragen AGJ, Wong DWS. Handbook of Food Enzymology. New York: Marcel Dekker, 2002: 719-26.

Wong DWS, Whitaker JR. Catalase. In: Whitaker et al. editors. Handbook of Food Enzymology. New York: Marcel Dekker, Inc., 2002: 389-402.

Reductive Biorefining: Biorefining: Separation to starch and gluten

Robertson GH, Cao TK, Irving D. Effect of morphology of mechanically developed wheat flour and water on starch from gluten separation using cold ethanol displacement. *Cereal Chemistry* 2000; 77(4):439-44.

Robertson GH, Cao TK. Farinograph responses for wheat flour dough fortified with wheat gluten produced by cold-ethanol or water displacement of starch. *Cereal Chemistry* 2001; 78:538-42.

Robertson GH, Cao TK. Mixograph responses of gluten and gluten-fortified flour for gluten produced by cold-ethanol or water displacement of starch from wheat flour. *Cereal Chemistry* 2002; 79(5):737-40.

Robertson GH, Cao TK. Effect of processing on functional properties of wheat gluten prepared by cold-ethanol displacement of starch. *Cereal Chemistry* 2003; 80(2):212-7.

Inductive Biorefining: Biorefining: New Values

Nobes GAR, Orts WJ, Glenn GM. Synthesis and properties of water-resistant poly(glucaramides). *Ind. Crops & Products*. 2000; 12:125-35.

Orts WJ, Sojka RE, Glenn GM. Biopolymer additives to reduce erosion-induced soil losses during irrigation. *Ind. Crops & Products*. 2000; 11:19-29.

Glenn GM, Orts WJ. Mechanical and physical properties of starch-based foams. Gross RA, Leatham GF editors. *Polymers from Renewable Resources: Carbohydrates and Agroproteins*. Washington, DC: American Chemical Society, 2001: 42-60. (ACS Symp. Series; 786).

Glenn GM, Orts WJ. Properties of starch-based foam formed by compression/explosion processing. *Industrial Crops and Products* 2001; 1:135-43.

Glenn GM, Orts WJ, Buttery RG, Stern DJ. Mechanical and physical properties of starch-based foams formed from gels. Chapter 2. In : Gross RA, Leatham GF editors. *Biopolymers from Polysaccharides and Agroproteins*. Washington, DC: American Chemical Society, 2001: 42-60. (ACS Symposium Series; 786).

Glenn GM, Orts WJ, Nobes GAR. Effect of Starch, Fiber and CaCO₃ on the Properties of Foams Made by a Baking Process. *Ind. Crops & Products*. 2001; 14:201-12.

Glenn GM, Orts WJ, Nobes GAR, Gray GM. In Situ Laminating Process for Baked Starch-Based Foams. *Industrial Crops and Products* 2001; 1:125-134.

Nobes GAR, Orts WJ, Glenn GM, Gray GM, Harper MV. Blends of starch with poly(vinyl alcohol)/ethylene copolymers for use in foam containers. ANTEC 2001 Proc. Soc. Plastics Engineers. Society of Plastics Engineers, 2001.

Orts WJ, Sojka RE, Glenn GM, Gross RA. Biodegradable soil additives for the reduction of erosion losses during irrigation. In : Gross RA, Leatham GF editors. *Biopolymers from Polysaccharides and Agroproteins*. Washington, DC: American Chemical Society, 2001: 102-16. (ACS Symposium Series; 786).

Wood DF, Edwards RH, Berrios J *et al.* The effects of moisture and amylose content on the microstructure of starch gels. UJNR Symposium Proceedings. 2001: M1-M8.

Glenn GM, Klamczynski AP, Takeoka GR, Orts WJ, Wood DF, Widmaier R. Sorption and vapor transmission properties of uncompressed and compressed microcellular starch foam. *J Agric Food Chem* 2002; 50:7100-4.

Klamczynski A, Glenn GM, Orts WJ. Flavor retention and physical properties of rice cakes prepared from coated grain. *Cereal Chemistry* 2002; 79:387-91.

