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MINI-REVIEW

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Biotechnological processes for conversion of corn into ethanol

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Abstract Ethanol has been utilized as a fuel source in the United States since the turn of the century. However, it has repeatedly faced significant commercial viability obstacles relative to petroleum. Renewed interest exists in ethanol as a fuel source today owing to its positive impact on rural America, the environment and United States energy security. Today, most fuel ethanol is produced by either the dry grind or the wet mill process. Current technologies allow for 2.5 gallons (wet mill process) to 2.8 gallons (dry grind process) of ethanol (1 gallon = 3.785 l) per bushel of corn. Valuable co-products, distillers dried grains with solubles (dry grind) and corn gluten meal and feed (wet mill), are also generated in the production of ethanol. While current supplies are generated from both processes, the majority of the growth in the industry is from dry grind plant construction in rural communities across the corn belt. While fuel ethanol production is an energy-efficient process today, additional research is occurring to improve its long-term economic viability. Three of the most significant areas of research are in the production of hybrids with a higher starch content or a higher extractable starch content, in the conversion of the corn kernel fiber fraction to ethanol, and in the identification and development of new and higher-value co-products.

Background

The production of ethanol from corn for use as a transportation fuel is a mature technology. It was first introduced in the United States in the early 1900s. The early Ford Model T had a carburetor adjustment that allowed the vehicle to run on either gasoline or ethanol produced by American farmers. Henry Ford's vision was to build a vehicle that was affordable to the working family and powered by a fuel that would boost the rural farm economy (Kovarik 1998).

Ethanol was used as a fuel source for cars well into the 1930s. Post World War II, however, little interest remained in using agricultural crops for liquid fuel production because of the abundant and cheap supply of fuel from petroleum and natural gas. Renewed interest in ethanol developed in the 1970s with oil supply disruptions from the Middle East and the phase-out of lead as an octane booster for gasoline (Hunt 1981). Additional Federal and State tax incentives helped fuel the revitalization of the ethanol industry from production volumes of 10×10^6 gallons in 1979 to 2.81×10^9 gallons in 2003 (1 gallon = 3.785 l; Anonymous 1999, 1981). Together, the passage of the Clean Air Act Amendments by Congress in 1990 (which mandated the use of oxygenated fuels), questions concerning the oxygen source methyl tert-butyl ether (MTBE), and the opportunity to spur rural development have further accelerated the growth of the domestic ethanol industry. With today's limited oil supplies and the ever-increasing United States' dependence on foreign oil (over 62% imported), the need for alternative energy sources is receiving a renewed focus. Fuel ethanol remains an attractive option. Ethanol has strategic value because it is a renewable energy source and reduces the United States' dependence on foreign oil imports. It benefits farmers by creating a substantial new market for corn supplies and by creating new jobs in economically depressed rural areas and small communities. As a fuel component, it burns cleanly and increases the octane level of gasoline. Because ethanol has a higher oxygen content than MTBE, only half the volume is required to produce the same oxygen level in gasoline; and it is biodegradable (DiPardo 2000).

Most of the current ethanol produced in the United States uses field corn as a feedstock. Corn is the most important and economical source of starch in the United States. Starch is the major carbohydrate storage product in corn kernels comprising 70–72% of the kernel weight on a

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dry weight basis. Starch is readily converted to glucose and fermented into ethanol. In 2003, conversion to ethanol accounted for nearly 10^9 bushels of corn, or 10% of the United States corn crop. Projections are for ethanol demand to reach 5×10^9 gallons by 2012. BBI International maintains records on current and proposed capacity. As of May 2004, they cite 76 currently running ethanol plants (with a capacity of over 3×10^9 gallons of ethanol) and 12 additional plants under construction, adding an incremental 0.5×10^9 gallons in commercial capacity. Tax incentives and commercial efficiencies are expected to keep pace with the expected future demand.

The United States Department of Agriculture and the Department of Energy at Argonne National Laboratories have conducted numerous studies on the energy balance of ethanol production. They both concluded that a gallon of ethanol produces more energy than the fossil inputs to produce it (Shapouri et al. 1996; Wang et al. 1997). The most recently published results by Shapouri indicate an average (across dry grind and wet mill processes) energy yield of 67% more than the fossil inputs required to produce it (Shapouri et al. 2004). This has been an area of significant improvement in ethanol production, with energy requirements at 50% less than what was required for ethanol production in the late 1970s. Alternatively, gasoline yields 20% less energy than the fossil inputs required to produce it (Shapouri et al. 1996; Wang et al. 1997).

Current ethanol production processes

Today, most fuel ethanol is produced from corn by either the dry grind (67%) or the wet mill (33%) process. The key distinction between wet mill and dry grind facilities is the focus of the resourcing. In the case of a dry grind plant, the focus is maximizing the capital return per gallon of ethanol. In the case of a wet mill plant, capital investments allow for the separation of other valuable components in the grain before fermentation to ethanol.

The wet milling process is more capital- and energy-intensive, as the grain must first be separated into its components, including starch, fiber, gluten, and germ. The germ is removed from the kernel and corn oil is extracted from the germ. The remaining germ meal is added to fiber and the hull to form corn gluten feed. Gluten is also separated to become corn gluten meal, a high-protein animal feed. In the wet milling process, a starch solution is separated from the solids and fermentable sugars are produced from the starch. These sugars are fermented to ethanol. Wet mill facilities are true “biorefineries”, producing a number of high-value products.

In the dry grind process, the clean corn is ground and mixed with water to form a mash. The mash is cooked and enzymes are added to convert starch to sugar. Then yeast is added to ferment the sugars, producing a mixture containing ethanol and solids. This mixture is then distilled and dehydrated to create fuel-grade ethanol. The solids remaining after distillation are dried to produce distillers’ dried grains with protein and are sold as an

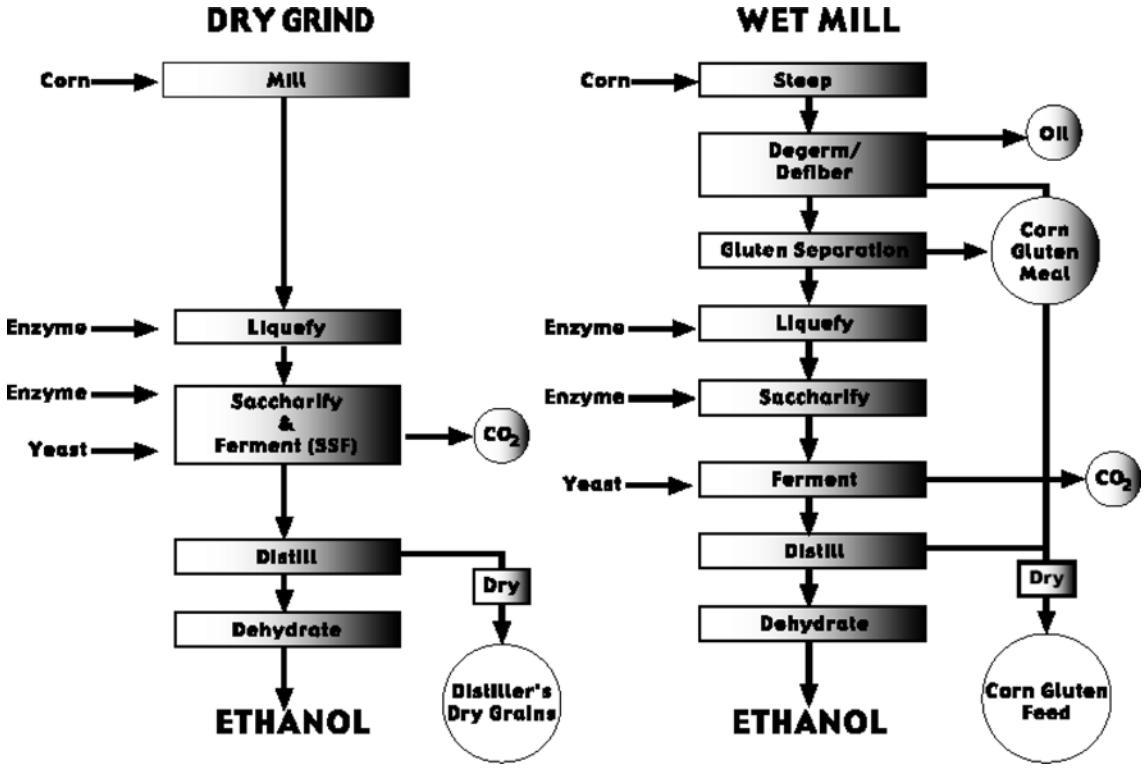

Fig. 1 Ethanol production processes

animal feed supplement. A schematic of both processes is illustrated in Fig. 1.

Current technologies allow for 2.5 gallons (wet mill) to 2.8 gallons (dry grind) of ethanol per bushel of corn. Recent growth in the industry has been predominantly with dry grind plants, because of lower (2× to 4×) capital costs per gallon and incentives for farmer-owned co-operatives (Shapouri et al. 1996; Wang et al. 1997). The wet mill industry has largely relied on expanding existing facilities rather than building new plants. Currently, 59 companies operate the 76 active ethanol plants in the United States today. The majority of these are farmer-owned cooperatives or limited liability corporation dry grind plants. The large-scale wet mill plants are concentrated among a few predominately publicly held companies with a long history of processing.

To be economically viable, both dry grind and wet mill plants must obtain value both from the ethanol resulting from the process and from the co-products generated. The most significant input cost is the cost of the starting corn. Commodity corn prices from the USDA database are U. S. $2.32 per bushel (10-year average). The coproducts of value are distillers dried grains with or without solubles (DDGS) from the dry grind process and corn oil, corn gluten meal, and corn gluten feed from the wet mill process. For every bushel of corn, 17 pounds of DDGS (1 pound = 0.4536 kg) are generated via the dry grind process and 1.6 pounds of corn oil, 2.6 pounds of gluten meal, and 13.5 pounds of gluten feed are generated via the wet mill process. The value of these coproducts fluctuates with available supply and the cost of competing commodity protein sources: primarily corn, soybeans, and soybean meal. According to the USDA, the 7-year average selling price (per ton) of DDGS, corn gluten meal, and corn gluten feed are U.S. $126, $357, and $88, respectively. The 10-year average price for corn oil (per pound) is U.S. $0.30. Carbon dioxide is generated via both processes. When it is captured, it is typically sold to the beverage industry for carbonation purposes.

This Mini-Review focuses on the dry grind fuel ethanol production process. Dry grind fuel ethanol production is where the majority of the fuel ethanol industry growth is today. Further, the majority of the efforts to improve ethanol production focus on taking the biorefinery concept of the wet mill and mimicking it in the dry grind process.

Dry grind ethanol production

The dry grind process is designed to ferment as much of the corn kernel as possible. There are five basic steps in the conventional dry grind ethanol process: grinding, cooking, liquefaction, saccharification, and fermentation. Little is wasted in the production of this fuel—in addition to ethanol, the manufacturing process also produces distillers grains, a high-quality livestock feed, and carbon dioxide, a food and industrial product. In the dry grind method of ethanol production, nothing is done to pre-separate the corn starch from the kernel. The entire corn kernel is ground into a coarse flour through a hammer mill, to pass through a 30 mesh screen, then slurried with water to form a “mash”. Each bushel of corn generates ~22 gallons of mash.

Starch conversion

Starch exists as insoluble, partially crystalline granules in the endosperm of the corn kernel. Corn starch is made up of individual units of glucose, linked together in chains by alpha 1–4 and occasional alpha 1–6 linkages. The 1–4 linkages produce linear chains that primarily comprise molecules called “amylose”, whereas the alpha 1–6 linkages serve as branching points to produce branched-chain molecules called amylopectin. Normal corn starch contains about 27% amylose, with the remainder being amylopectin. Starch cannot be metabolized directly by yeast, but must first be broken down into simple six carbon sugars prior to fermentation. To accomplish this conversion, the pH of the mash is adjusted to pH 6.0, followed by the addition of alpha-amylase. A thermostable alpha-amylase enzyme is added to begin breaking down the starch polymer to produce soluble dextrins by quickly and randomly hydrolyzing alpha 1–4 bonds. The mash is heated above 100°C using a jet cooker, which provides the high temperature and mechanical shear necessary to cleave and rupture starch molecules, especially those of a high molecular weight. The corn mash is kept at the elevated temperature for several minutes by pumping it through a holding tube equipped with a backpressure valve. The mash flows from the holding tube into a flash tank and the temperature is allowed to fall to 80–90°C. Additional alpha-amylase is added and the mash is liquefied for at least 30 min. Liquefaction greatly reduces the size of the starch polymer. The dextrinized mash is then cooled, adjusted to pH 4.5, and glucoamylase enzyme is added. Glucoamylase converts liquefied starch into glucose. Enough glucoamylase is added such that the saccharification of the starch to glucose, which occurs continually through the fermentation, does not limit the rate of ethanol production.

Fermentation

After cooking, the mash is cooled to 32°C and transferred to fermenters where yeast is added. Often, ammonium sulfate or urea is added as a nitrogen source for the growth of yeast. Recently, the ethanol dry grind mills have also begun to add proteases that break down the corn protein to free amino acids, which serve as an additional source of nitrogen for the yeast. The fermentation requires 48–72 h and has a final ethanol concentration of 10–12%. The pH of the beer declines during the fermentation to below pH 4, because of carbon dioxide formed during the ethanol fermentation. The decrease in pH is important for increasing the activity of glucoamylase and inhibiting the growth of contaminating bacteria. Dry grind plants can reduce the amount of glucoamylase added by saccharifying the liquefied starch at 65°C prior to fermentation. Many plants, however, have gone to simultaneous saccharification and fermentation (SSF) because it lowers the opportunity for microbial contamination, lowers the initial osmotic stress of yeast by avoiding a concentrated glucose solution, and is generally more energy-efficient. In addition, it can provide yields of up to 8% more ethanol per bushel of grain. Upon completion, the beer is distilled through the beer column.

Either batch or continuous fermentation systems may be used, although batch processing is more common. Some new fermentation systems are designed to minimize dilution water, which reduces the evaporation requirements and thus the energy required in the feed-processing stages after fermentation.

The carbon dioxide released during fermentation is often captured and sold, especially by larger dry grind facilities. The carbon dioxide is used in carbonating soft drinks and beverages, manufacturing dry ice, and in other industrial processes.

Distillation and dehydration

Distillation is the process of separating the ethanol from the solids and water in the mash. Alcohol vaporizes at 78°C and water at 100°C (at sea level). This difference allows water to be separated from ethanol by heating in a distillation column.

Conventional distillation/rectification methods can produce 95% pure (190 proof) ethanol. At this point, the alcohol and water form an azeotrope, which means further separation by heat cannot occur. In order to blend with gasoline, the remaining 5% water must be removed by other methods. Modern dry grind ethanol plants use a molecular sieve system to produce absolute (100%, or 200 proof) ethanol.

The anhydrous ethanol is then blended with approximately 5% denaturant (such as gasoline) to render it undrinkable and thus not subject to beverage alcohol tax. It is then ready for shipment to gasoline terminals or retailers.

Stillage processing

The solid and liquid fraction remaining after distillation is referred to as “whole stillage”. Whole stillage includes the fiber, oil, and protein components of the grain, as well as the non-fermented starch. This coproduct of ethanol manufacture is a valuable feed ingredient for livestock, poultry, and fish.

Although it is possible to feed whole stillage, it is usually processed further before being sold for feed. First, the “thin stillage” is separated from the insoluble solid fraction using centrifuges or presses/extruders. The stillage leaving the beer column is centrifuged with a decanter. Between 15% and 30% of the liquid fraction (thin stillage) is recycled as backset. The remainder is concentrated further by evaporation and mixed with the residual solids from the fermentation. After evaporation, the thick, viscous syrup is mixed back with the solids to create a feed product known as wet distillers grains with solubles (WDGS).

Feed products from stillage processing

WDGS, containing 65% moisture, can be used directly as a feed product. In fact, it is often favored by dairy and beef feeders because cattle seem to prefer the moist texture. However, WDGS has a shelf-life of only 1–2 weeks. Unless the feedlot is within about 50 miles (80 km) of the ethanol plant, handling and storage can be a challenge, especially in hot summer months when shelf-life is very limited.

To increase shelf-life and reduce transportation costs, WDGS is usually dried to 10–12% moisture, to produce DDGS. Drying WDGS is energy-intensive, consuming about one-third of the energy requirements of the entire dry grind plant. However, producing a uniform, stable, high-quality feed coproduct is essential to the profitability of the plant, resulting in most plants producing DDGS rather than WDGS.

Currently, dry grind ethanol plants produce over 3.8×10^6 tons of DDGS annually. It is projected that the volume of DDGS will increase to over 5.5×10^6 tons by the year 2005.

The dry grind ethanol production method is a rigorous biological process that requires stringent quality control. For example, bacterial contamination at mashing may result in the formation of acids that divert glucose from ethanol production and interfere with fermentation. Moldy grain, improper grain storage, faulty equipment, reintroduced stillage, and air are some of the major sources of contamination that can reduce ethanol yields or impact the value of the distillers grains.

Future directions

A number of surveys have been conducted by the Renewable Fuels Association, the Illinois Corn Growers Association, and the Iowa Corn Growers Association to determine the research priorities of the industry. The national priorities in ranked order are: new coproducts, plant emissions, fermentation, feedstocks, fiber recovery, DDGS, separation, pretreatment, saccharification, germ recovery, distillation, starch hydrolysis, and carbon dioxide. For the sake of this review, we highlightd three areas: high fermentable hybrids, conversion of fiber (biomass) component of the kernel to ethanol, and recovery of new and high-value ethanol coproducts with the best near-term opportunities to produce ethanol more cost-efficiently.

High fermentable hybrids

There is considerable interest today for seed companies to market specific hybrids bred for enhanced ethanol production. Corn hybrids are being developed either with higher extractable starch or with higher fermentable starch content, for wet mill or dry grind ethanol production, respectively. Early efforts are in selective breeding versus a transgenic approach. The two largest United States corn seed companies, Pioneer and Monsanto, both have ongoing research efforts to identify and develop new corn hybrids with these features and to understand the impact of agronomic practices and the environment on the hybrid characteristics. Additionally, both have commercial seed corn today specifically labeled for the ethanol industry. Not yet evaluated in either of these programs, but important research to be conducted, is the impact on the composition of coproducts from hybrids resulting in higher ethanol production.

Pioneer's research efforts include work to both generate hybrids more conducive to the ethanol production process and to understand how to optimize these characteristics under field conditions. Their efforts to generate hybrids for the dry grind industry have resulted in hybrids characterized by high total fermentables (HTF). Their research has shown that the HTF trait is a more accurate indicator of dry grind ethanol production than total starch or extractable starch. Pioneer has analyzed data from over 15,000 plot samples over 3 years to assign the HTF designation to specific brands. These hybrids, identified as Pioneer Industry Select, result in ethanol yields up to 4% greater than a bulk commodity. This could mean an increase of U.S. $\$1–2\times10^6$ in profitability for a 40×10^6 -gallon year^{-1} ethanol production facility. Additionally, Pioneer has developed a point-of-sale assay using whole-grain near-infrared (NIR) technology that allows ethanol plants to predict the value of corn for ethanol production by identifying HTF grain arriving at the plant. These instruments are provided to plants participating in the Pioneer program (Butzen et al. 2003; Bryan 2003a, b).

Pioneer's efforts to understand the impact of agronomic practices and environmental conditions in optimizing hybrid production for the ethanol industry are also progressing significantly. Their research to-date suggests an optimum exists for grain yield and extractable starch or total fermentables, based on plant population and applied nitrogen per acre. Pioneer recommends hybrid selection for yield and agronomics first, followed by the hybrid designation as either HTF or high extractable starch. Additionally, their research suggests that managing the field for optimum yield also maximizes extractable starch or total fermentables (O'Bryan 2004).

Monsanto's efforts focus on the dry grind industry. They have also developed a list of hybrids for ethanol production termed "Processor Preferred Fermentable Corn". For the 2004 crop year, these hybrids were offered in nearly 60 independent seed brands in addition to the Monsanto DEKALB and Asgrow brands. Additionally, Monsanto also provides NIR instrumentation to the 23 plants participating in their marketing program (Anderson 2003).

A third area of industrial interest is illustrated in the efforts of Syngenta Biotechnology to direct the accumulations of starch-hydrolyzing enzymes in the endosperm of transgenic corn kernels (Craig et al. 2004). Stable accumulations of enzymes, without detriment to grain viability and composition, allows "processing capability" to be built into the grain itself. Self-processing grains can be designed to meet specific and novel process constraints due to flexibilities in engineering enzymes with distinct biophysical properties and enzymatic specificities.

Longer-term efforts to create modified starches or other complex carbohydrates from genetically modified corn are intended to provide new functionalities that will make possible additional markets and products for corn. These hybrids may result in starch with improved gelling properties, viscosity, and temperature stability, improved flavor or flavor stability, improved adhesion or film formation, or properties that enhance the efficiency of processing.

Fiber conversion

Fermentation of the fiber fraction of the corn kernel can increase ethanol yield from a bushel of corn by 10% and subsequently yield a higher-value and higher-protein feed coproduct than is typically recovered in corn gluten feed and DDGS (Gulati et al. 1996). Expanding fuel ethanol production beyond 10% of our liquid transportation needs will require developing a lower cost feedstock and only feedstocks containing lignocellulosics are available in sufficient quantities to substitute for starch as an ethanol source.

Corn fiber is particularly attractive as a novel source of sugars for ethanol fermentation. Corn fiber has a high carbohydrate content that can be converted into fermentable sugars and is stockpiled at central locations—in many cases at existing fermentation facilities. Currently, most corn fiber is incorporated into low-value animal feeds which may face a limited market in the future as ethanol production continues to grow. In addition to using corn fiber as a feedstock for ethanol, it may serve well as a feedstock for such value-added fermentation products as lactic acid, xylitol, lycopene, etc. (Leathers 1998). In addition to the fibrous component of the kernel, the rest of the corn plant (e.g., corn stover) could also serve as a feedstock for ethanol (Wyman 2003). Corn stover contains 58% carbohydrates and 1.0–1.5 pounds of stover are produced per pound of harvested corn. Unlike DDGS and corn fiber, collecting and storing corn stover represents a formidable challenge. However, fermenting available corn stover could conceivably boost ethanol production 10-fold. To date, no commercial process is in operation for the conversion of corn fiber or corn stover into fuel ethanol.

Major technical constraints to commercialization exist in the conversion of lignocellulose to ethanol. These constraints are primarily in the areas of pretreatment of the substrate, enzymatic hydrolysis of the substrate to fermentable sugars and strain development for the fermentation of multiple sugars. Development of efficient substrate pretreatments that increase the susceptibility of crystalline cellulose and hemicellulose to enzymatic hydrolysis will reduce the cost of producing ethanol from lignocellulosic biomass. Pretreatment of lignocellulose, coupled to enzymatic hydrolysis, generates a stream of mixed sugars, including arabinose, glucose, and xylose (Grohmann and Bothast 1997). However, enzymes for the hydrolysis of biomass remain cost-prohibitive (~U. S. \$0.30 gallon^{-1}; Merino and Cherry 2004) and better enzymes are required. For example, commercial hemicellulase mixtures are ineffective for hydrolyzing corn fiber (Hespell et al. 1997). Improved microbial strains to ferment mixed sugars are also required (Bothast et al. 1999). Industrial yeast strains, used for fermenting corn starch, are unable to ferment arabinose and xylose; and the few naturally occurring strains that do ferment pentoses only grow slowly and produce low ethanol yields. Another challenge is to obtain strains that can tolerate the inhibitory compounds generated during pretreatment and hydrolysis. A fiber conversion process requires implementation of all unit operations used in biomass conversion, i.e. size reduction, pretreatment, enzymatic saccharification, fermentation, and product recovery. Last year, the United States Department of Energy and the United States Department of Agriculture announced several winners of their joint "Integrated Biomass Solicitation." Four of these multimillion dollar projects include conversion of the cellulosic and hemicellulosic components of corn fiber to ethanol. All of these projects require scale-up validation. It is this type of scale-up validation for which the National Corn-to-Ethanol Research Plant was constructed.

New and higher-value coproducts

As new technologies are implemented, adding value to coproducts is essential to the profitability of the fuel ethanol business (Anonymous 1999). This will require a more holistic approach to ethanol production in the dry grind plant. Optimization of coproducts and ethanol yield must be considered. A number of new processes have been developed in the laboratory. Examples include the quick germ (Singh and Eckhoff 1996), quick fiber (Singh et al. 1999), enzymatic milling (Johnston et al. 2003), and the COPE Process (Cheryan 2002). These process modifications may allow cost-effective removal of coproducts such as corn oil, zein, germ, pericap fiber, and endosperm fiber at the beginning of the process, prior to fermentation. Potential benefits of these processes are: (1) recovery of high quality corn germ oil and fiber for corn fiber oil, (2) an increase in protein quality of residual DDGS after fermentation, and (3) additional production of ethanol per batch. All await validation at the pilot scale. Within the concept of "biorefining", a cadre of products can be listed (Leathers 1998; Wyman 2003). Examples include: corn fiber oil, sweeteners, polysaccharides, pharmaceuticals, nutraceuticals, fibers, biodegradable films, organic acids, solvents, amino acids, pigments, enzymes, polyols, vitamins, etc. Also in the coproduct arena, today's DDGS feed customers are asking for more information than the traditional moisture, protein, fat, and fiber analyses. Animal nutritionists want complete nutrient profiles of the ingredients and they want to know the variability of these nutrients and to have the ability to select which nutrients they need. In Minnesota, a certification program for DDGS has been developed that is resulting in market premiums for certified DDGS (Bryan 2003a, b). Research projects are underway that could modify the amino acid composition, protein composition, or phosphorous content of DDGS. DDGS market expansion beyond cattle to swine, poultry, and aquaculture is dependent on improving the quality and consistency of the DDGS coproduct. In addition to feed uses, numerous other uses for DDGS are finding their way to the marketplace. Examples include: deicers, cat litter, "lick barrels", worm food, etc.

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