

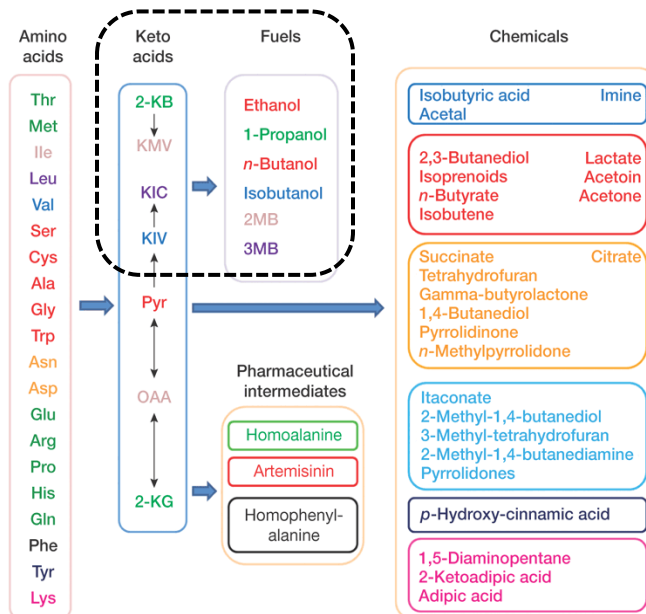
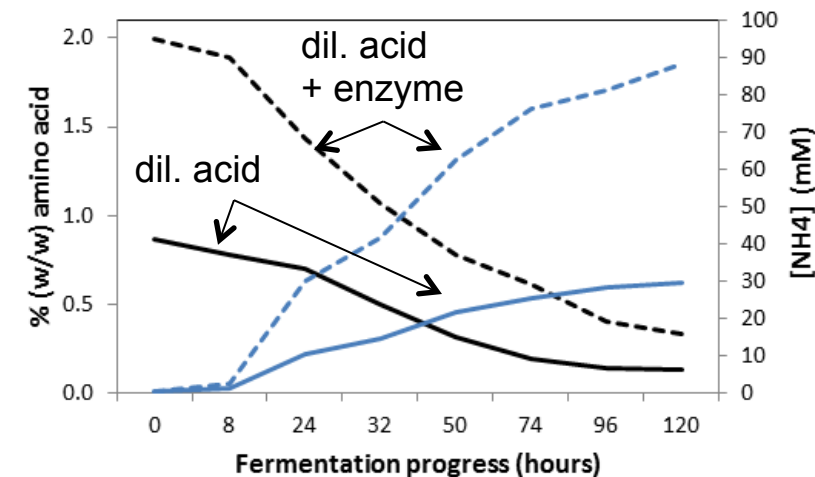
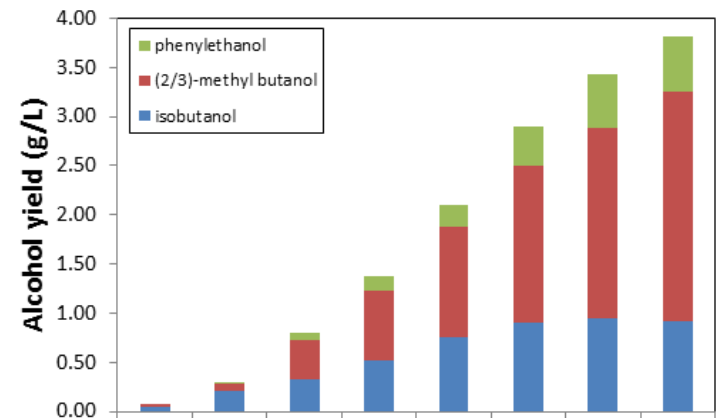
Biochemical Upgrading of Dried Distillers Grains

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Biochemical conversion: integrated sugar & protein fermentation

- Dilute acid (2-10% H_2SO_4 , 90-145°C, 0.5-6 hr) & enzymatic (0.5-2 g/L, 37-55°C, 12-48 hr) pretreatments being evaluated
- Sugar fermentation strain: *E. coli* KO11 for conversion of sugars to EtOH
- Protein fermentation strain: *E. coli* YH83 for conversion of amino acids to >C2 alcohols + NH_4

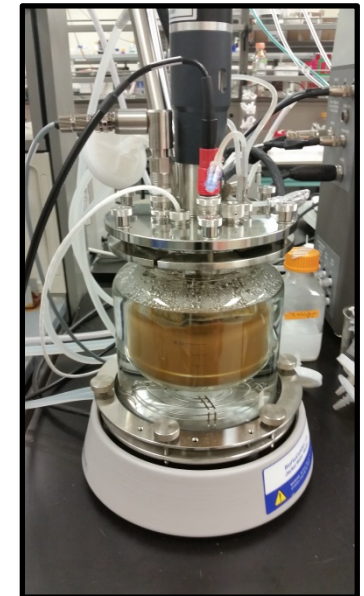
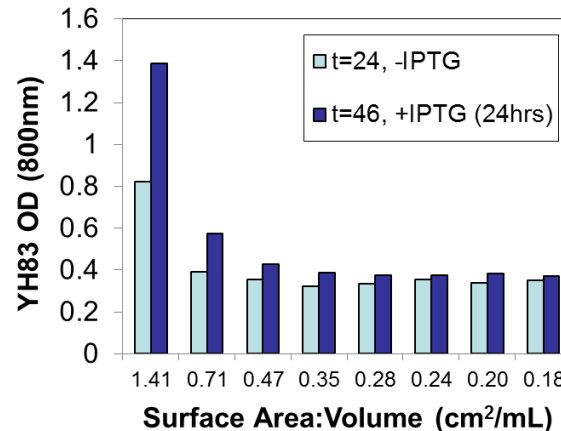
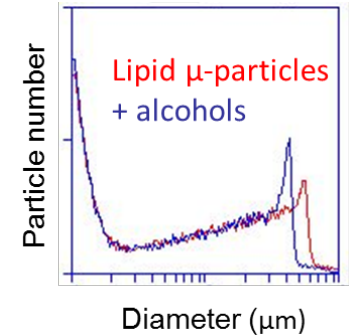
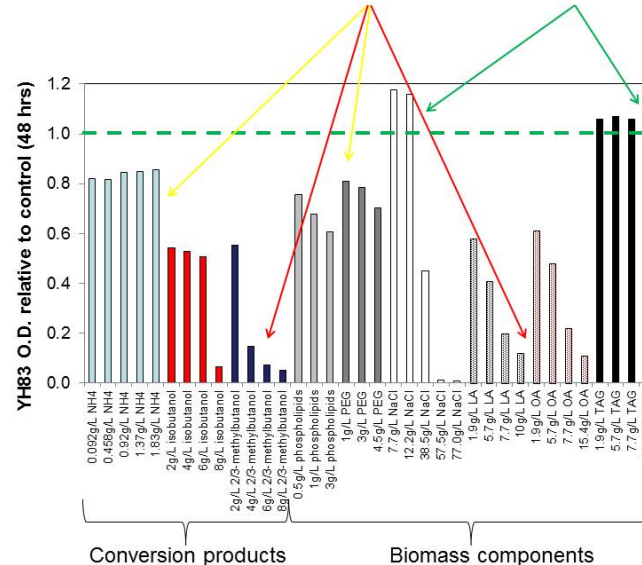


(Huo *Nat. Biotech* 2011)

Optimizing yield of biochemical conversion processes

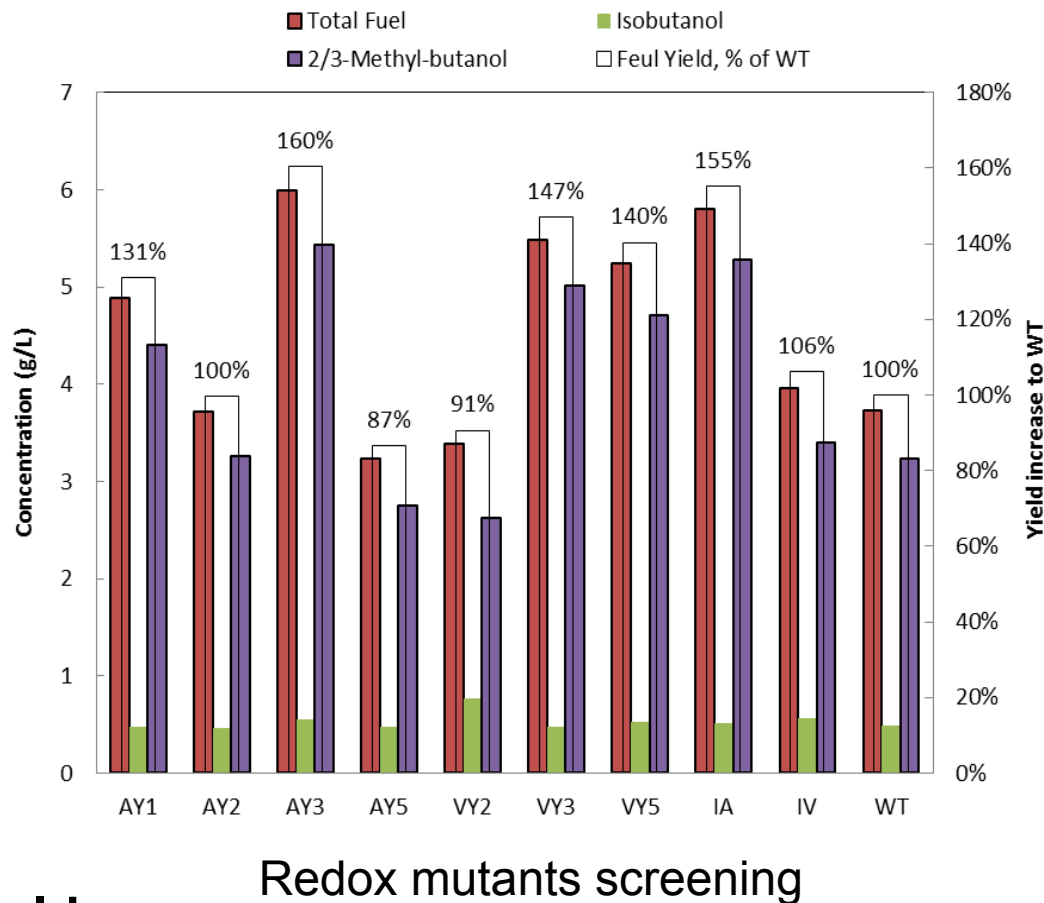
- Optimization performed at bench scale (1L) prior to scale-up
- No enhancement observed for addition of M9 salts or vitamins
- Product inhibition minimized by retaining lipids
- Microaerobic conditions, kinetic enhancement from air bubbling at inoculation
- Co-extraction of alcohol & lipid biofuel intermediates following fermentation

Fermentation inhibitors & enhancers



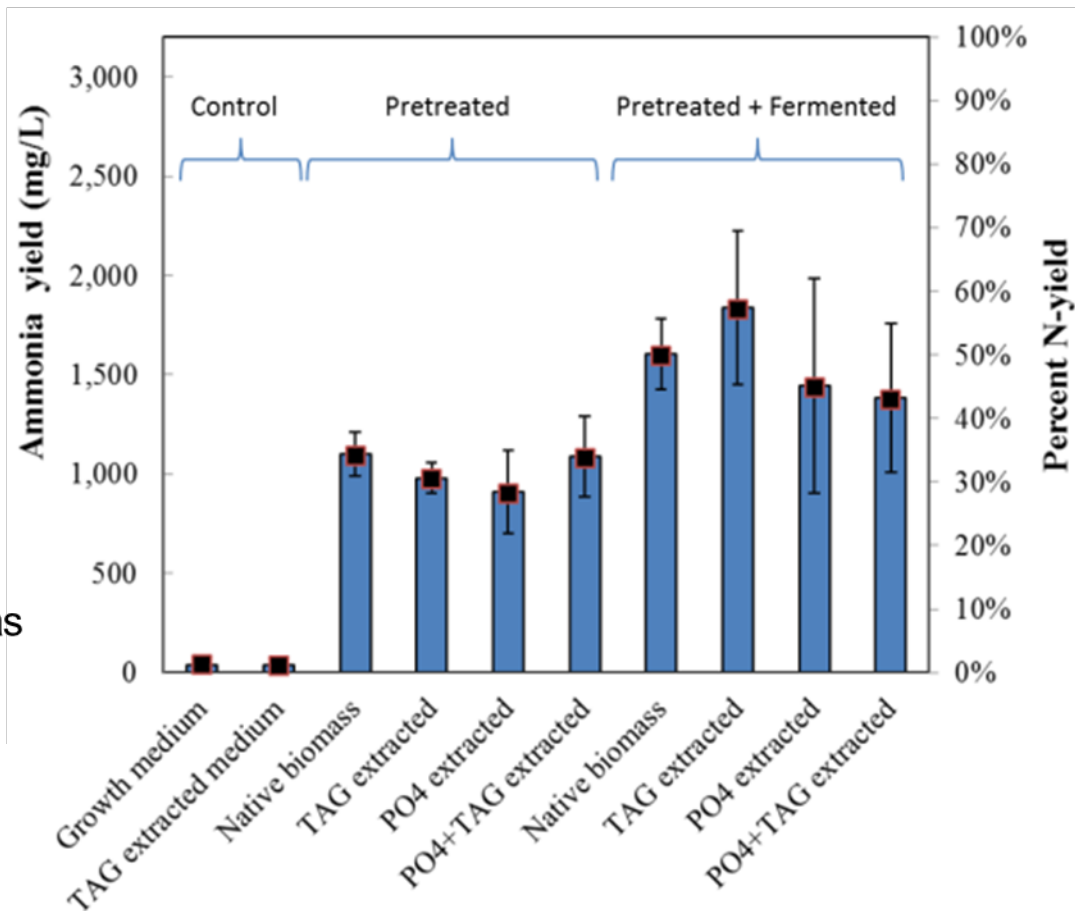
Redox cofactor engineering provides 60% yield improvement

- NADPH depletion observed during time course studies
- IlvC and ADH keto-acid pathway enzymes modified for NADH specificity
- 5 new *E. coli* strains showed increased conversion yield relative to YH83
- **Key to achieving FY15 milestone of 80% of net theoretical conversion yield**



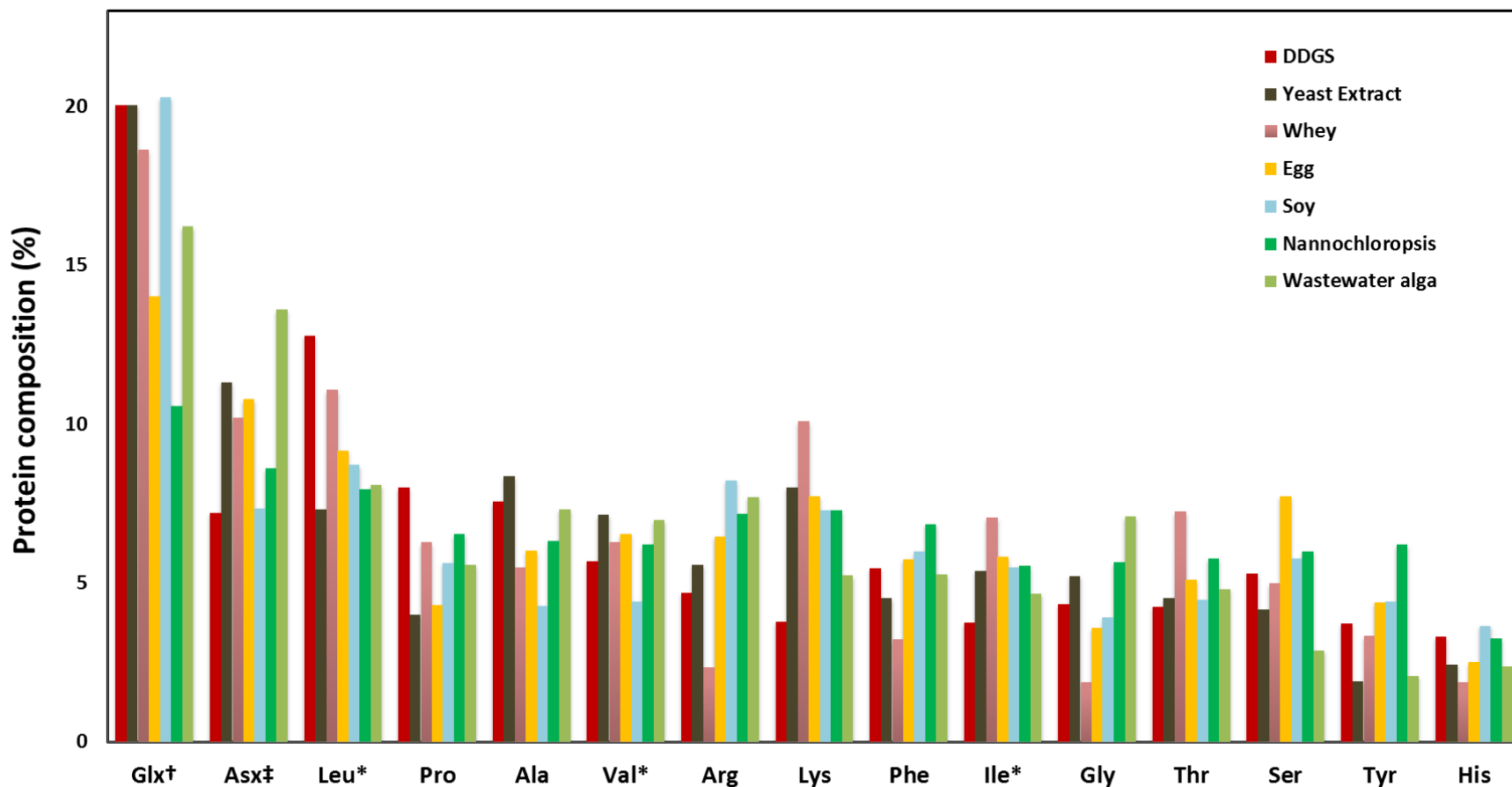
Fuels Production + Nutrient Recycling*

- Dilute acid + enzymatic pre-treatment converts ~35% of the total N to ammonia
- Protein fermentation converts ~27% of total N to ammonia
- Extraction of TAG or PO₄ did not significantly alter the ability to remineralize biological N to ammonia
- The N-remineralization yield was 57% ($\pm 14\%$) of theoretical



**Experimental data based on microalgae feedstocks*

Comparative Protein Composition



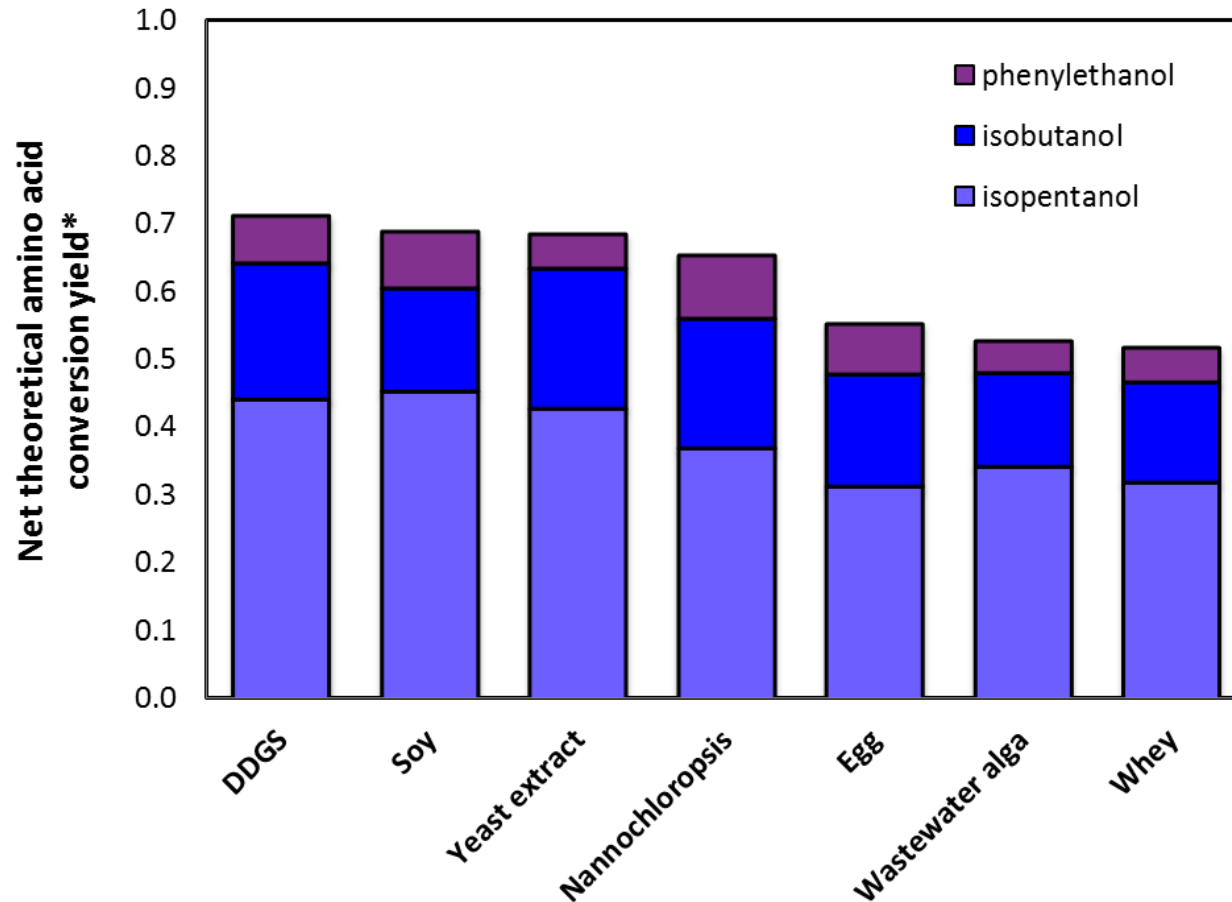
DDGS spec's: 44% solids (as recieved), 29-32% proetin, 32-37% NDF, 8-12% lipids, 4-5% ash

† Glu + Gln

‡ Asp + Asn

* Brached chain amino acids

Comparative Technoeconomic Potential of High Protein Feedstocks



* Remineralized ammonia (as NH_4MgPO_4) is included in the mass balance

Process Flow and Yield Potential

