

Separating Protein from Switchgrass During Biomass Refining

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Overview

- Introduction
 - Switchgrass Protein
 - Integration into Biorefinery
 - Methods
- Results
 - Optimization
 - Integration
- Conclusions and Further Study

Switchgrass

- Model herbaceous bioenergy crop
 - Dense crop
 - Grown anywhere east of Rocky Mountains
- Potential for proteins?
 - 10-15% protein if harvested in spring
 - High cellulose yield



Importance of Protein

- Protein is highly valued as an animal feed
- Present in abundant amounts
- Two advantages
 - Utility – interested in using as much of the plant as possible
 - Economics – significantly reduce wholesale ethanol selling price
- Alkali or Alkali/alcohol extraction

Amino Acid Profile

	SG	Soy*	Corn*
Arg	2.1	7.5	2.9
His	1.8	2.6	1.6
Ile	3.7	4.9	4.3
Leu	5.6	7.7	16.2
Lys	7.4	6.1	1.6
Met	0.6	1.6	2.3
Phe	9.1	5.1	5.9
Thr	4.9	4.3	3.1
Val	6.1	5.1	4.4

Essential Amino Acid
Ratio = 0.43

Allan, G. et al. "Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*" **186** *Aquaculture*, 293-310 (2000).

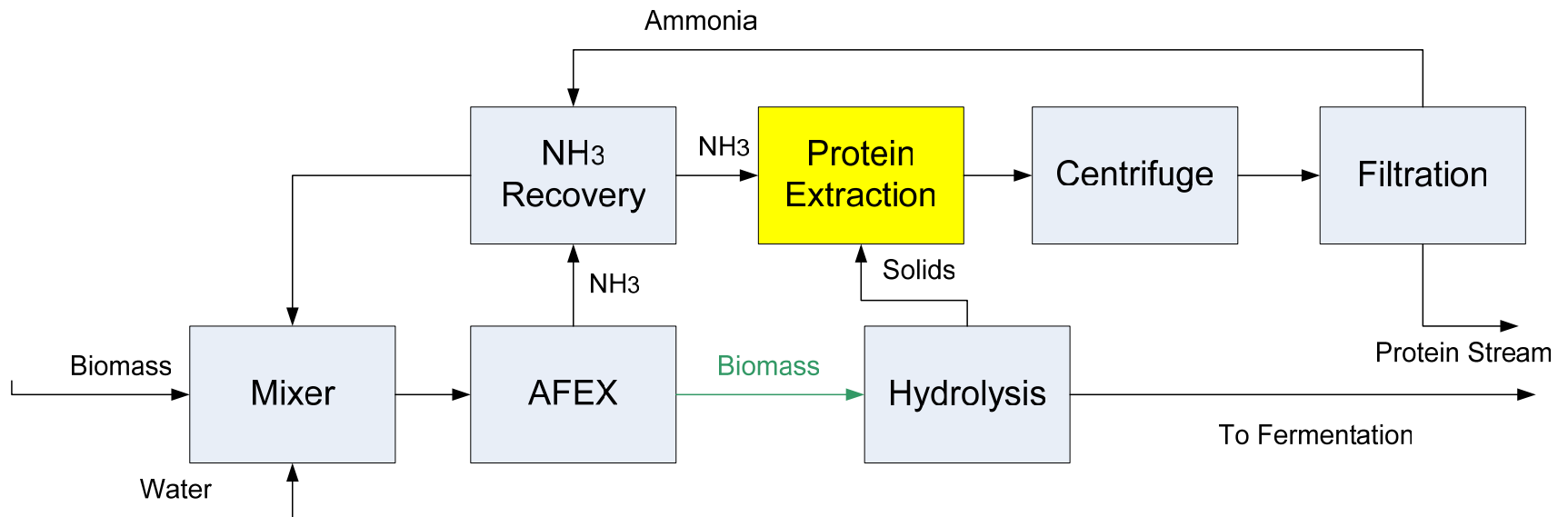
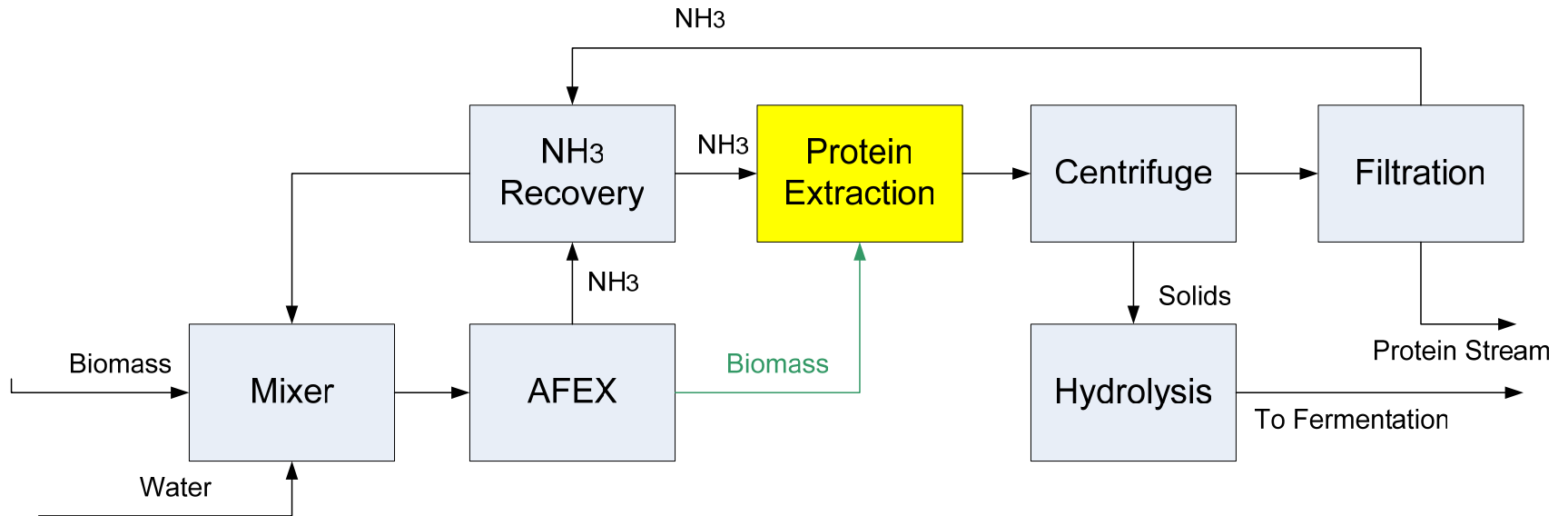
Integration

- Protein extraction is only one step!
 - Effect on ethanol yields?
 - What else is present in the streams?
- *Integrating a protein extraction step within a biorefinery to optimize profits or utilization is more important than simply looking at the protein stream alone*
- Combining processes may be advantageous

AFEX Integration

- Ammonia Fiber Explosion (AFEX) – pretreatment process for enzymatic hydrolysis of cellulose involving saturating the biomass with liquid anhydrous ammonia and explosively releasing the pressure
 - Decrystallizes cellulose, depolymerizes hemicellulose, removes lignin
 - Ammonia is recoverable
- Possible advantages for protein extraction?
 - Disrupts cell walls, facilitating protein diffusion
 - Ammonia may be used as the alkali in extraction

Potential Schemes



Previous Work

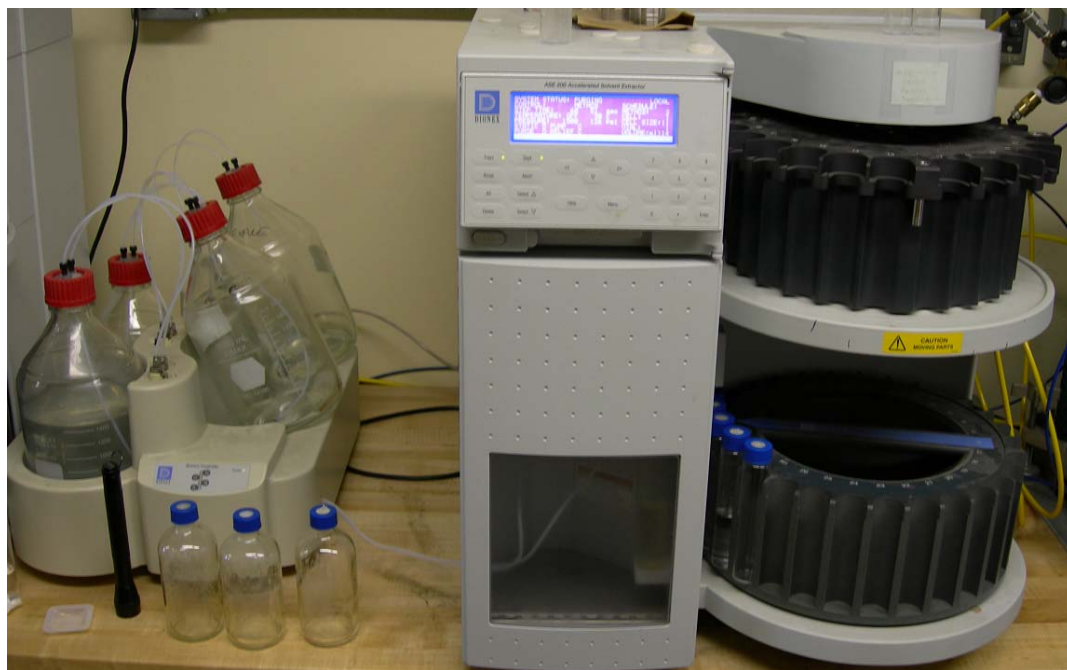
- Coastal Bermudagrass (10% CP)
 - Calcium Hydroxide Extraction
 - With and without AFEX
 - Hydrolysis of samples afterwards

	AFEX	Untreated
Protein	80 mg/g	34 mg/g
Sugar	714 mg/g (equiv.)	155 mg/g (equiv.)

De la Rosa, L. et al. "Integrated Production of Ethanol Fuel and Protein from Coastal Bermudagrass." **45-46** *Applied Biochemistry and Biotechnology*, 483-497 (1994).

Protein Extraction

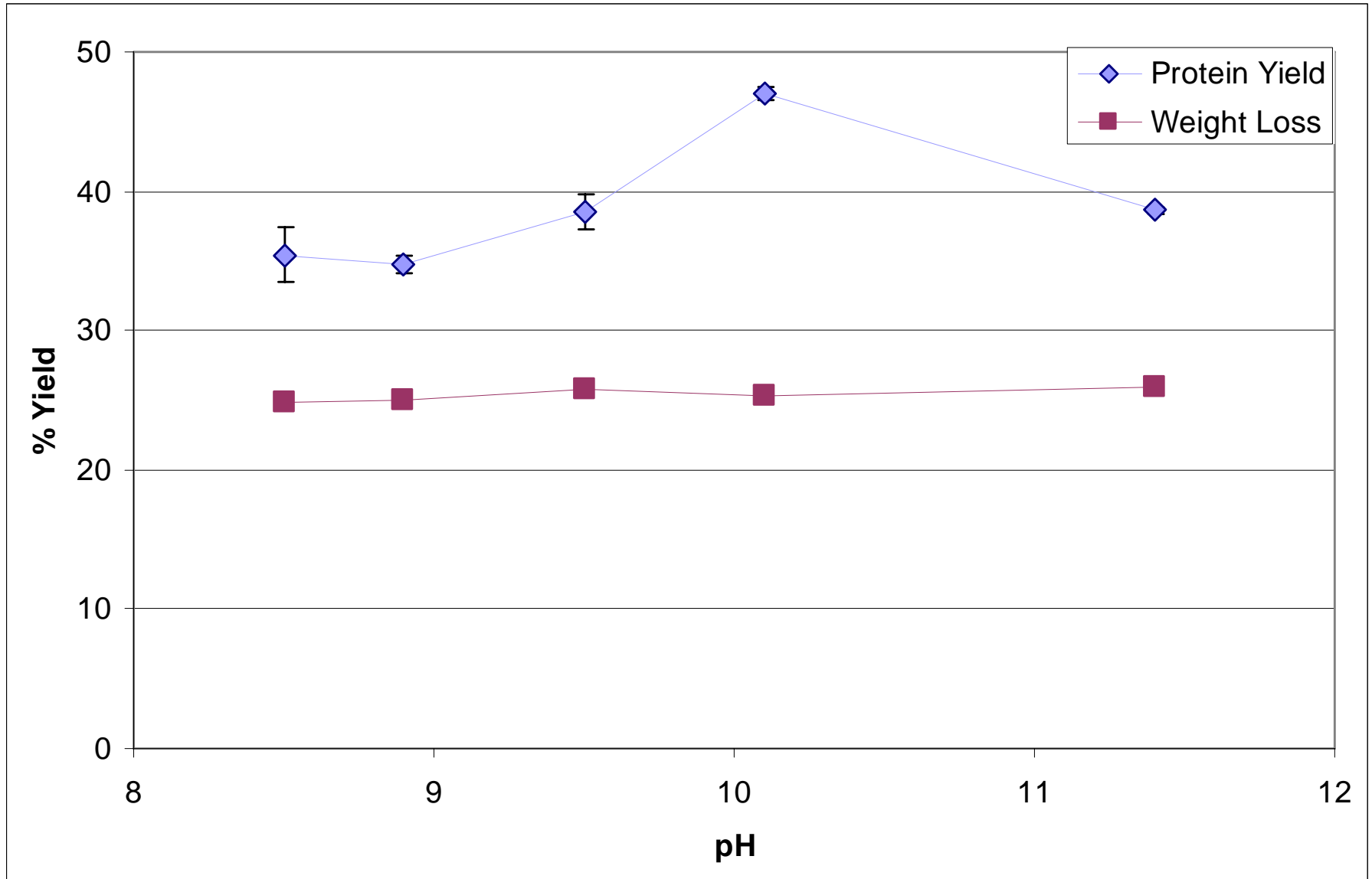
- Dionex Accelerated Solvent Extractor
 - High pressure – more efficient extraction
 - Low residence time
- 10:1 liquid/solid ratio



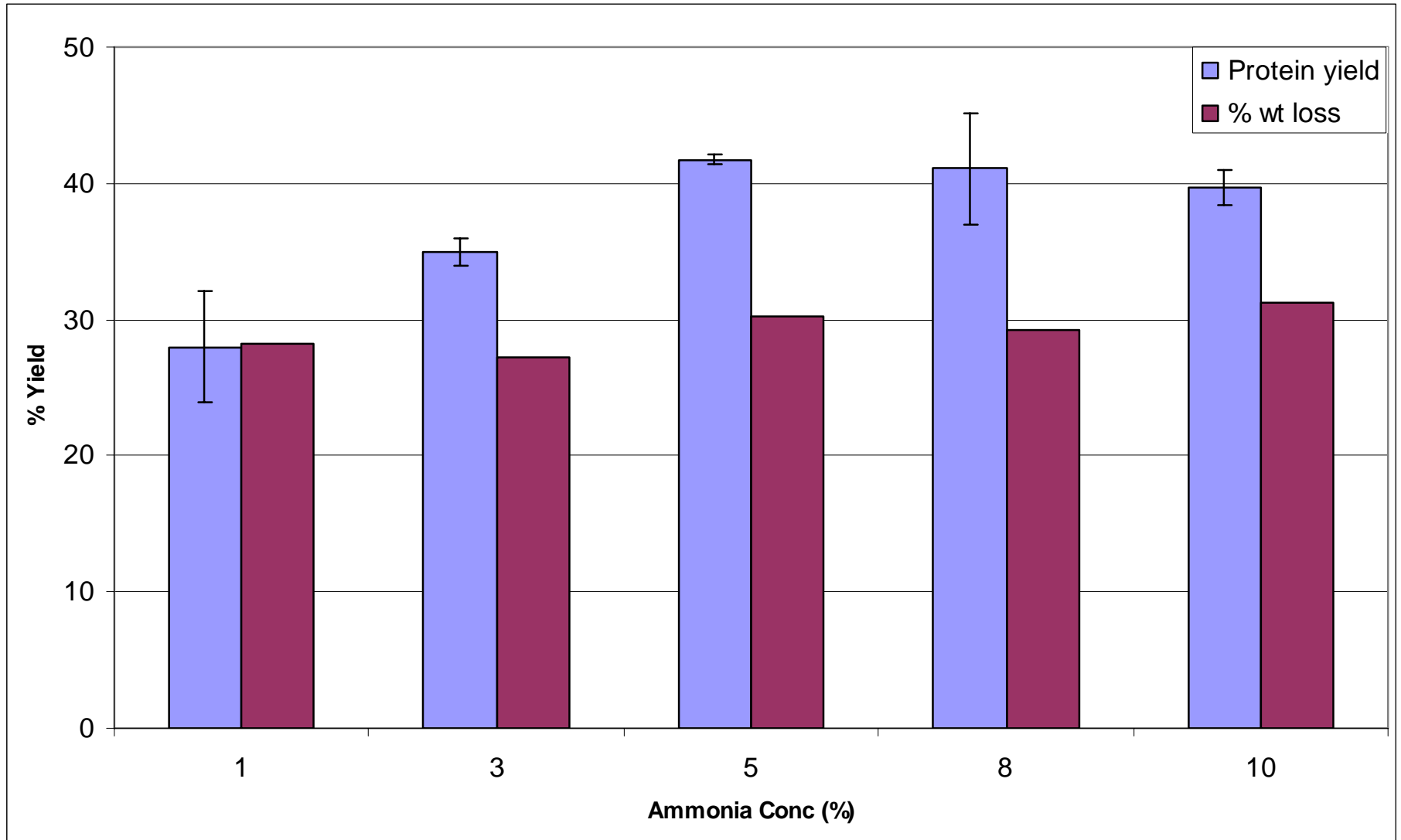
What are we looking for?

- Yield
- Concentration
- Purity
 - Presence of toxic materials?
 - Presence of beneficial components?
- Sugar yields
- Protein degradation and quality

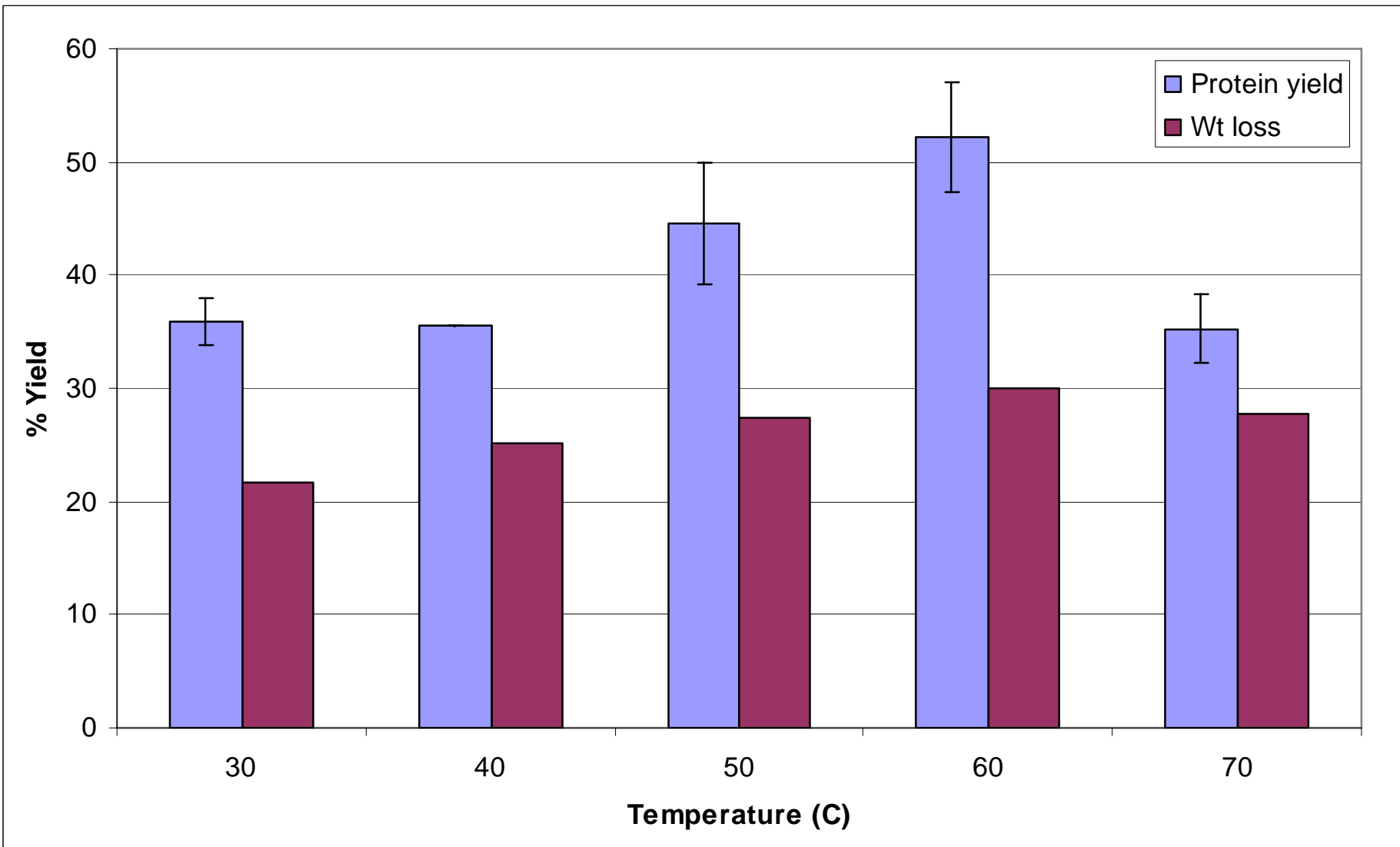
pH Optimization



Concentration Effect



Temperature Effect



Integration

- AFEX(A), Grind(G), Hydrolysis(H), Extraction(E)

	Extract		Hydrolysate		Total	
	Gluc. g/kg	Protein g/kg	Gluc. g/kg	Protein g/kg	Gluc. g/kg	Protein g/kg
AGHE	n.d.	10.6	309	23.9	309	34.5
GAHE	n.d.	3.5	293	34.6	293	38.1
AGEH	n.d.	33.2	326	7.1	326	40.3
GAEH	n.d.	28.5	302	11.5	302	40.0

Protein extraction before hydrolysis gives optimal results for both glucose and protein yields

Conclusions

- Switchgrass has potential as source for protein and ethanol
- Yields in excess of 50% can be obtained using ammonia extraction
- AFEX process improves protein extraction
- Highest sugar and protein yields achieved if extraction before hydrolysis

Further Study

- Fully optimize extraction
 - Reducing agents
 - Surfactants
 - Multiple solvents
- Concentration and purification
 - Ultrafiltration
 - Other compounds present
- Protein Quality
- Cost analysis
 - Solid/Liquid ratio vs residence time

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Protein Assays

- Total Nitrogen Content
 - + Reasonably accurate
 - + Can test both solid and liquid samples
 - Cannot work with ammonia
 - Non protein nitrogen
 - Amino Acid Analysis
 - + Most accurate
 - Time consuming, expensive
 - **BCA Colorimetric Assay**
 - + Quick and easy
 - Masked by other chemicals present
 - Low precision and accuracy
- Acetone Precipitation