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Development of a high-throughput fermentation assay using colorimetric measurement of gas production.

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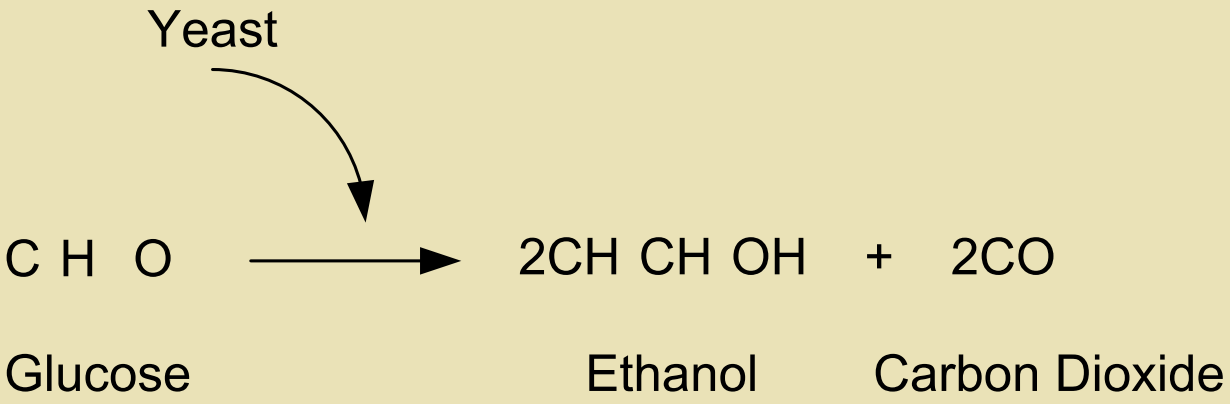
Current Ethanol Measurements

- Fermentation +
  - High Performance Liquid Chromatography (HPLC)
  - Gas Chromatography (GC)
- Drawbacks
  - Expensive equipment and with high operating costs
  - Time consuming (HPLC - 30 min/sample)

CO_2 Generation from ethanol Production



- CO_2 production is stoichiometrically related to amount of ethanol produced

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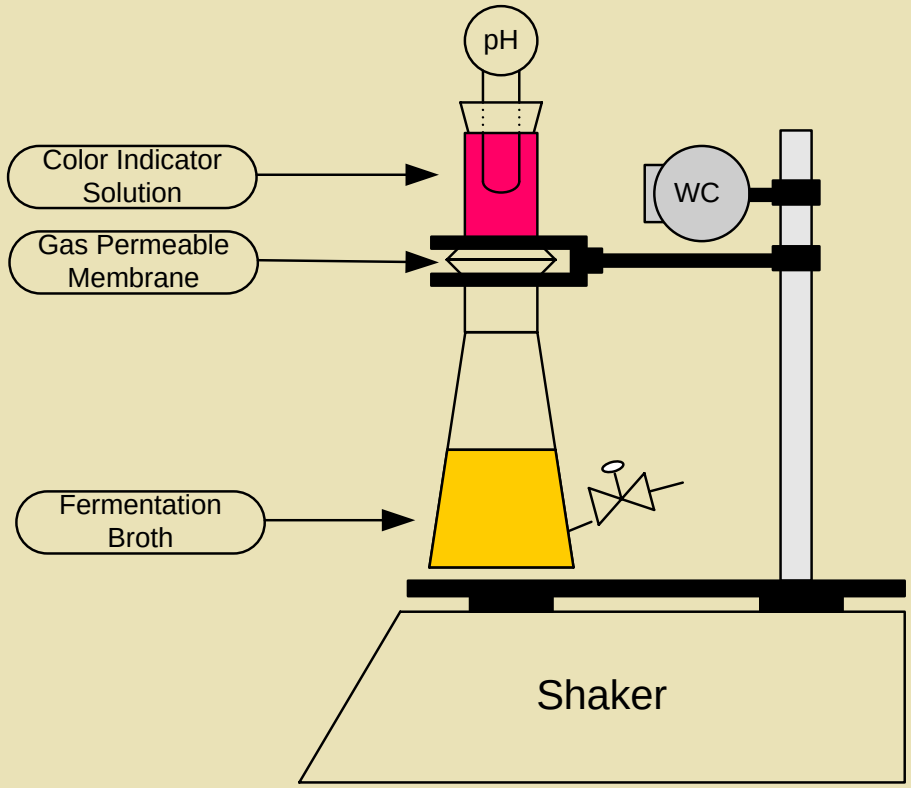
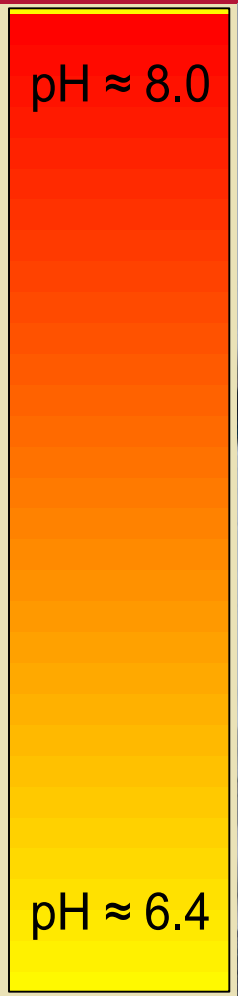
Chemi-visual Sensor

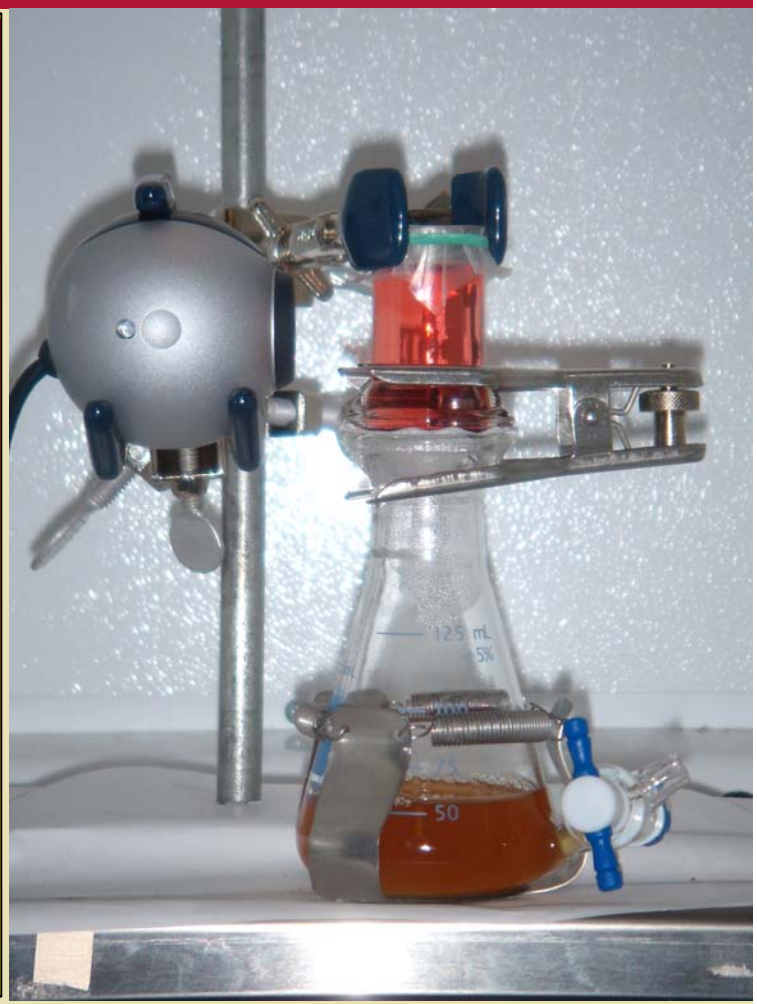


- Color indicator solution containing a buffer, D.I. H_2O, and phenol red indicator solution
- Membrane supports the solution while allowing gas transfer
- Color signal captured by CCD camera and processed in software

Chemi-visual Sensor (cont.)

- Indicator changes from red to yellow with decrease in pH
- CCD camera detects value of individual color signals (R,G,B)
- Green signal has largest response to color change

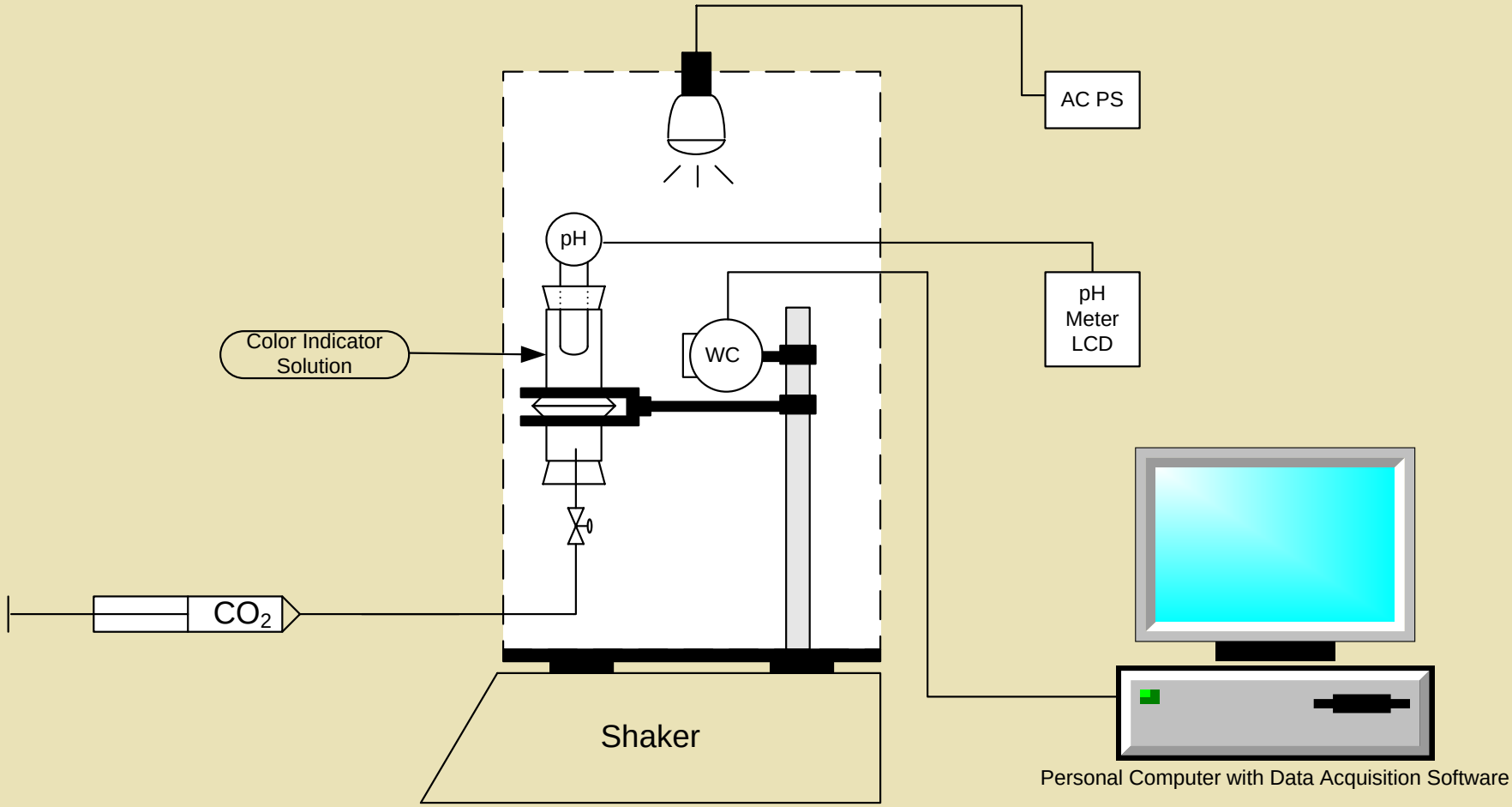




Sensor Development

- Generate correlations between:
  - pH vs. CO_2
  - Green Signal (RGB value) vs. pH
  - Green Signal (RGB value) vs. CO_2

Sensor Development (cont.)




GLASS
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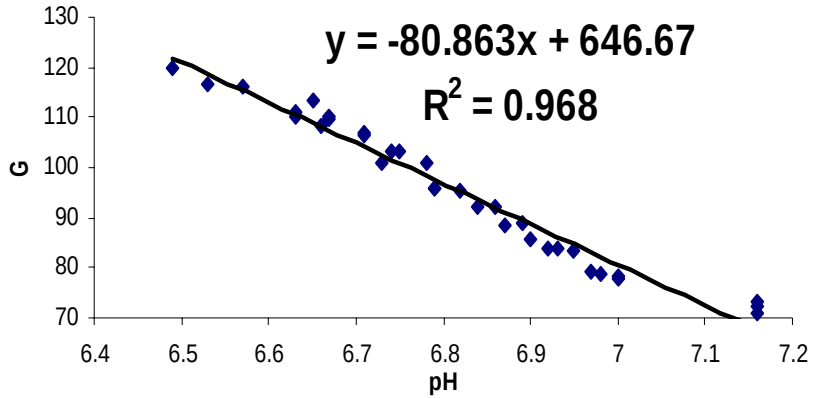
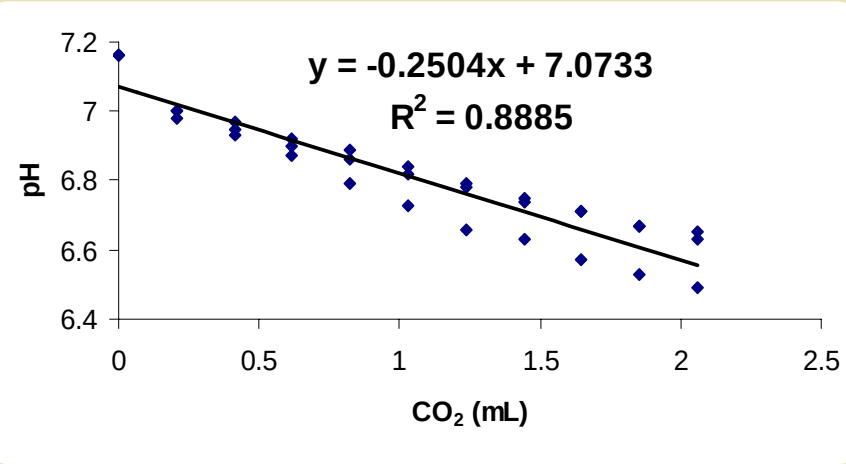
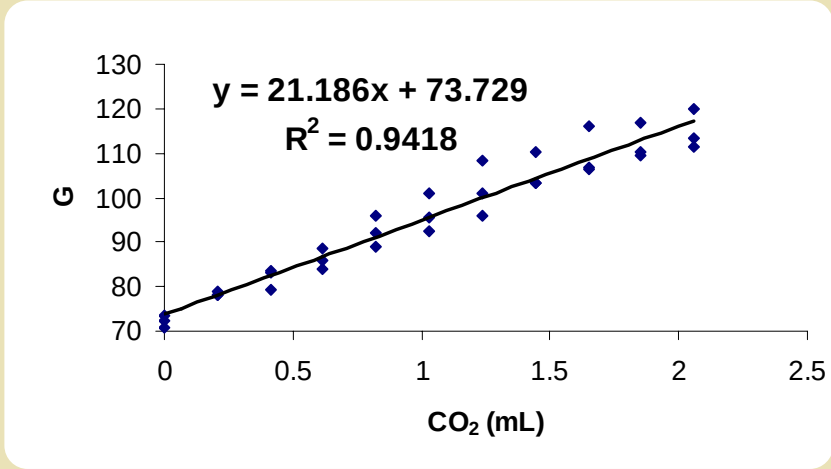
Correlations







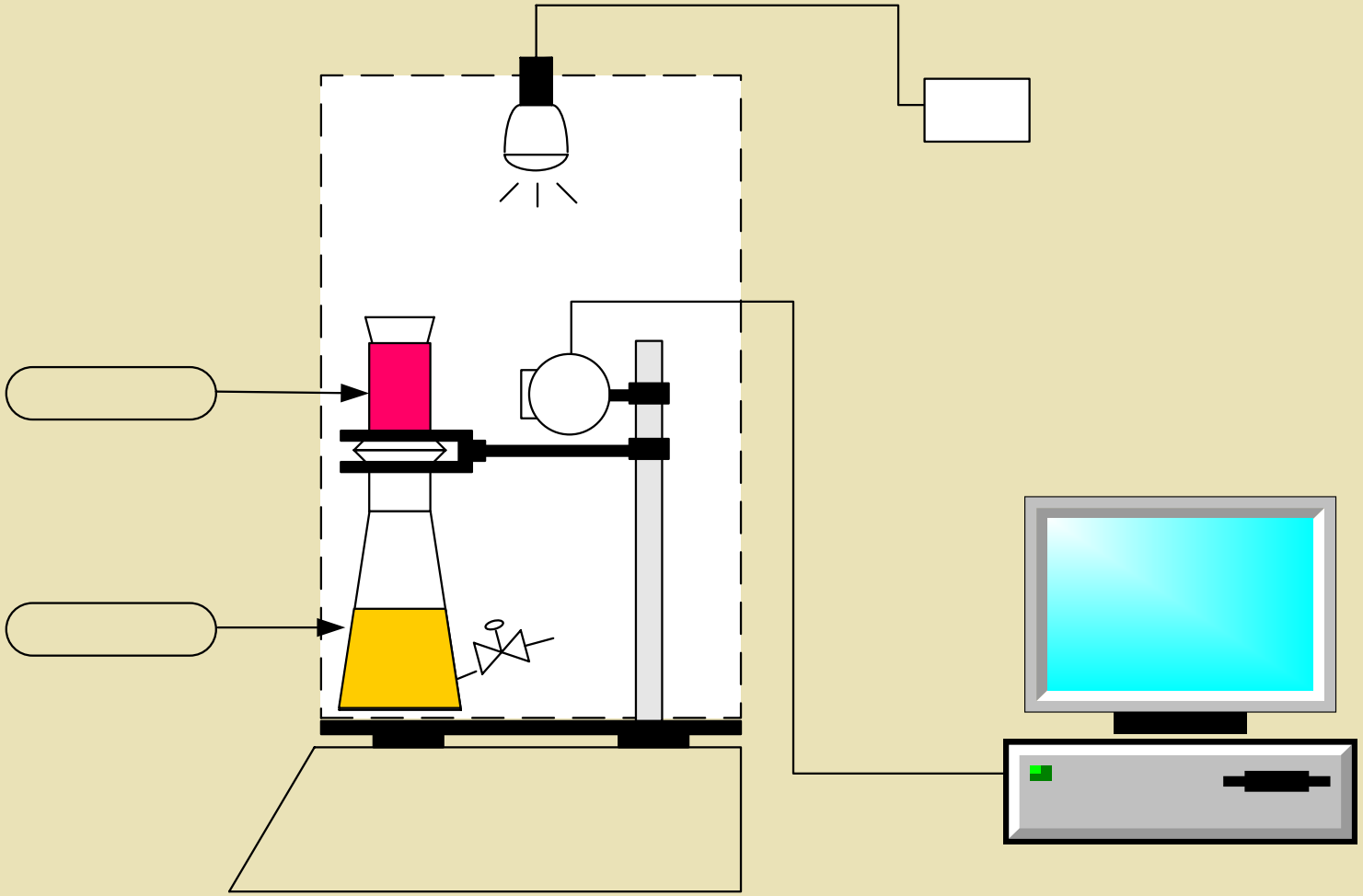
Glucose Fermentation

- Ethanol concentration determined by sampling at defined time intervals and HPLC analysis
- Green signal recorded and matched with corresponding ethanol concentration.
- Replicated 3 times

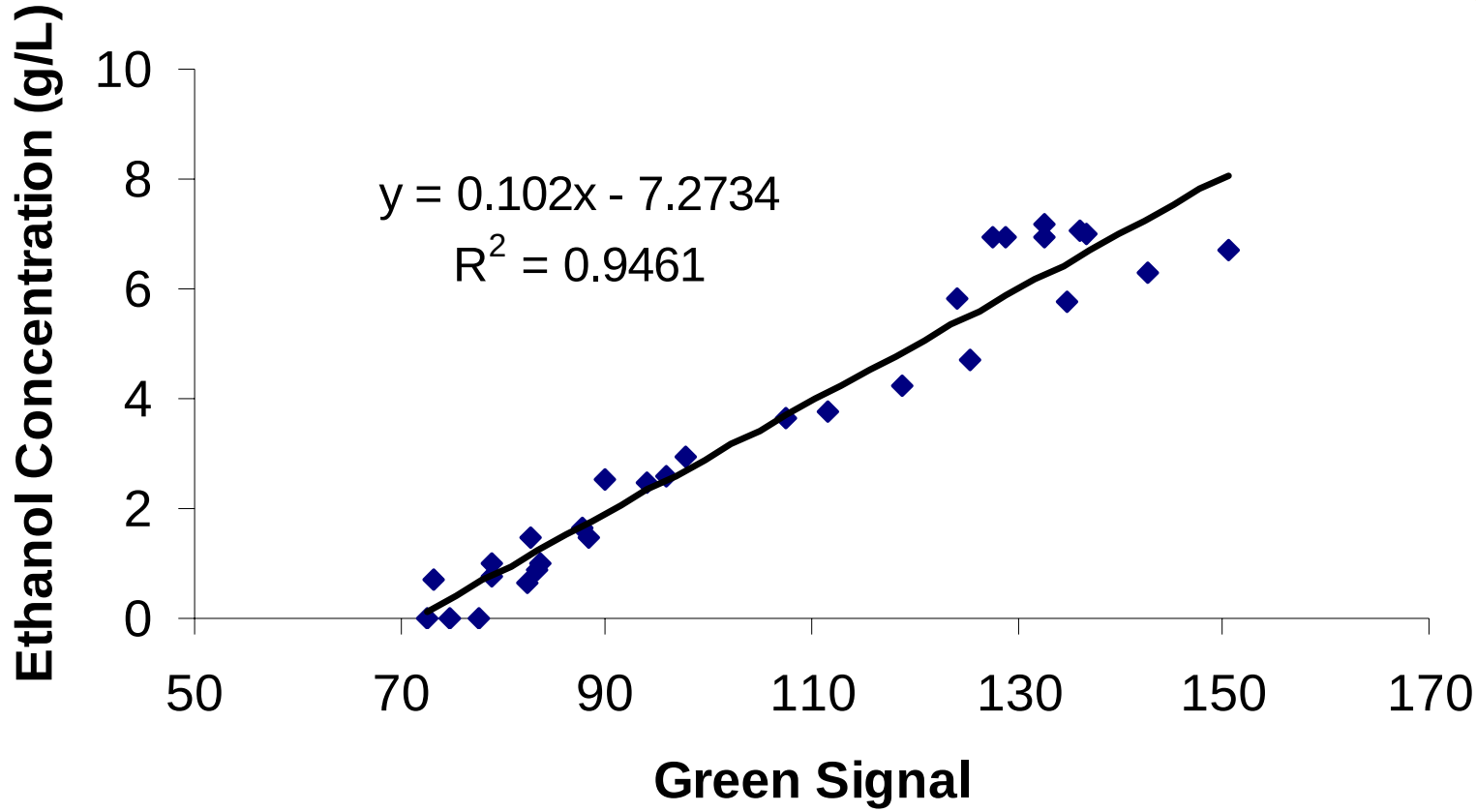
Fermentation Broth Recipe

Glucose	15.4 g/L
CO_2 Production (@ 90% theoretical conversion)	272 mL
Citrate Buffer (1 M)	50 mM
Peptone	20 g/L
Yeast Extract	10 g/L
Red Star Yeast	1.5 g/L

Glucose Fermentation



Glucose Fermentation - Results



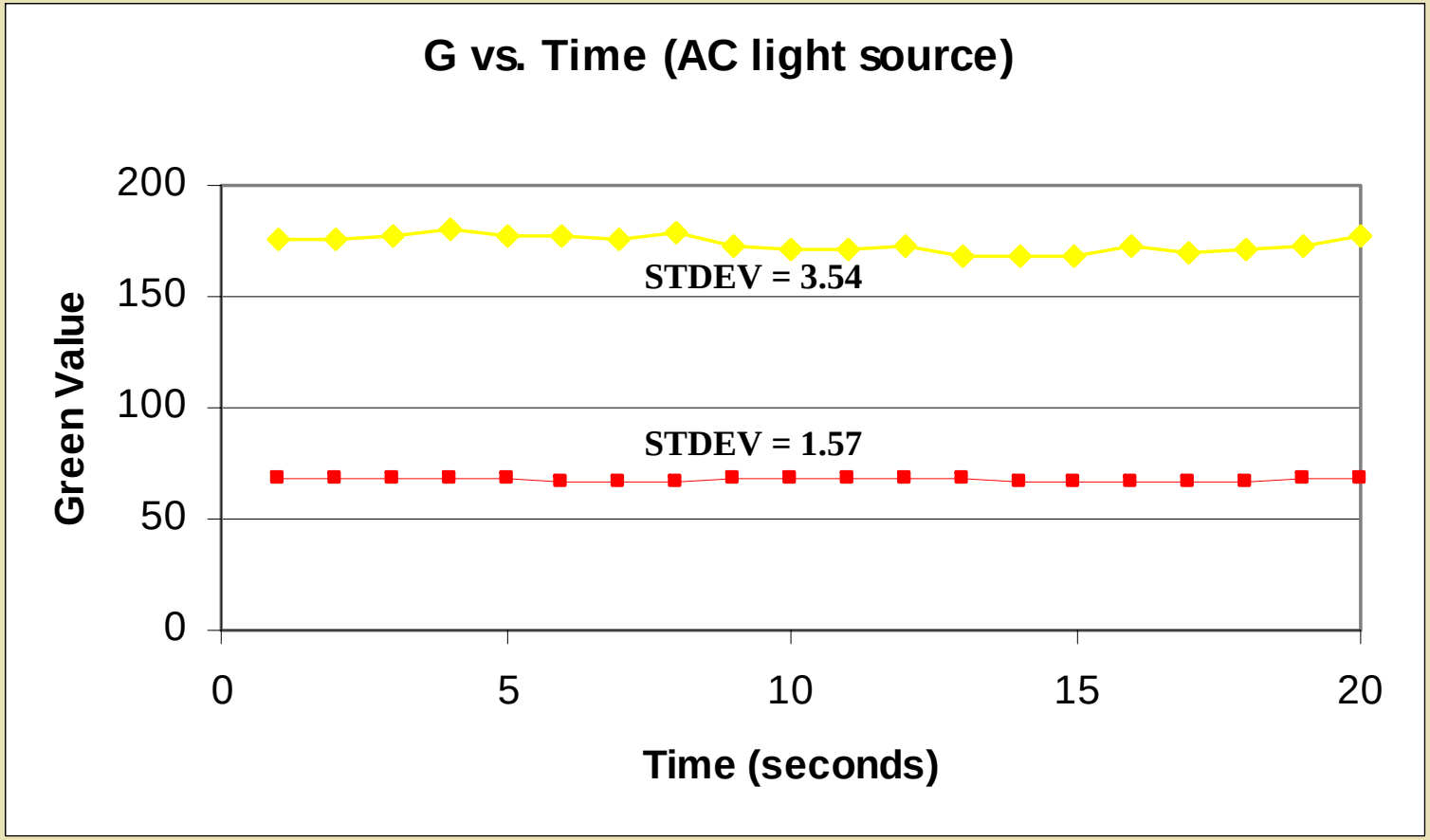
Glucose Fermentation – Results (cont.)

- Fermentations achieved 90.0%, 90.3%, and 87.4% of theoretical ethanol yield
- The use of the green signal appears to be a good predictor of ethanol production

Potential Sources of Variability

- Uncertainty in HPLC measurement of ethanol concentration
- "Noise" in the green signal
  - Sensitive to lighting, reflection, …
  - Sinusoidal signal due to AC light source

Light Source



Future Work

- Model the interactions between the following variables on system response:
  - Indicator solution volume
  - Indicator solution buffering capacity
  - Initial pH of indicator solution
  - Substrate concentration
  - Yeast inoculation level
  - Fermentation headspace volume
- Miniaturize to enable monitoring of 24 – 96 fermentations simultaneously

Intended Applications

- Evaluate enzyme combinations
- Evaluate effectiveness of pre-treatment methods
- Determine fermentability of biomass feedstocks

Acknowledgements

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- Special thanks to Dr. Robert Anex, and Dr. Raj Raman for their guidance with this project

Thank you for your time

Questions???