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)
CO₂ Generation from ethanol Production

Yeast

\[ \text{C H O} \rightarrow 2\text{CH CH OH} + 2\text{CO} \]

Glucose Ethanol Carbon Dioxide

- CO₂ production is stoichiometrically related to amount of ethanol produced
Chemi-visual Sensor

- Color indicator solution containing a buffer, D.I. H$_2$O, 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

pH ≈ 8.0

pH ≈ 6.4
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.)

- **CO₂**
- **pH**
- **WC**
- **Shaker**
- **Color Indicator Solution**
- **Personal Computer with Data Acquisition Software**

**Diagram Components:**
- **AC PS**
- **pH Meter LCD**

**Description:**
- CO₂ is connected to the Color Indicator Solution, which feeds into the pH meter.
- The pH meter is connected to the Shaker, which is also connected to the AC PS and the pH Meter LCD.
- The Personal Computer with Data Acquisition Software is connected to the system.
Correlations

\[ y = -80.863x + 646.67 \]
\[ R^2 = 0.968 \]

\[ y = 21.186x + 73.729 \]
\[ R^2 = 0.9418 \]

\[ y = -0.2504x + 7.0733 \]
\[ R^2 = 0.8885 \]

\[ y = 21.186x + 73.729 \]
\[ R^2 = 0.9418 \]
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

<table>
<thead>
<tr>
<th>Fermentation Broth Recipe</th>
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<tbody>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>CO$_2$ Production (@ 90% theoretical conversion)</td>
</tr>
<tr>
<td>Citrate Buffer (1 M)</td>
</tr>
<tr>
<td>Peptone</td>
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<tr>
<td>Yeast Extract</td>
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<tr>
<td>Red Star Yeast</td>
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</tbody>
</table>
Glucose Fermentation
Glucose Fermentation - Results

\[ y = 0.102x - 7.2734 \]

\[ R^2 = 0.9461 \]
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

G vs. Time (AC light source)

STDEV = 3.54

STDEV = 1.57
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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Thank you for your time

Questions???