New Technologies Fuel Ethanol

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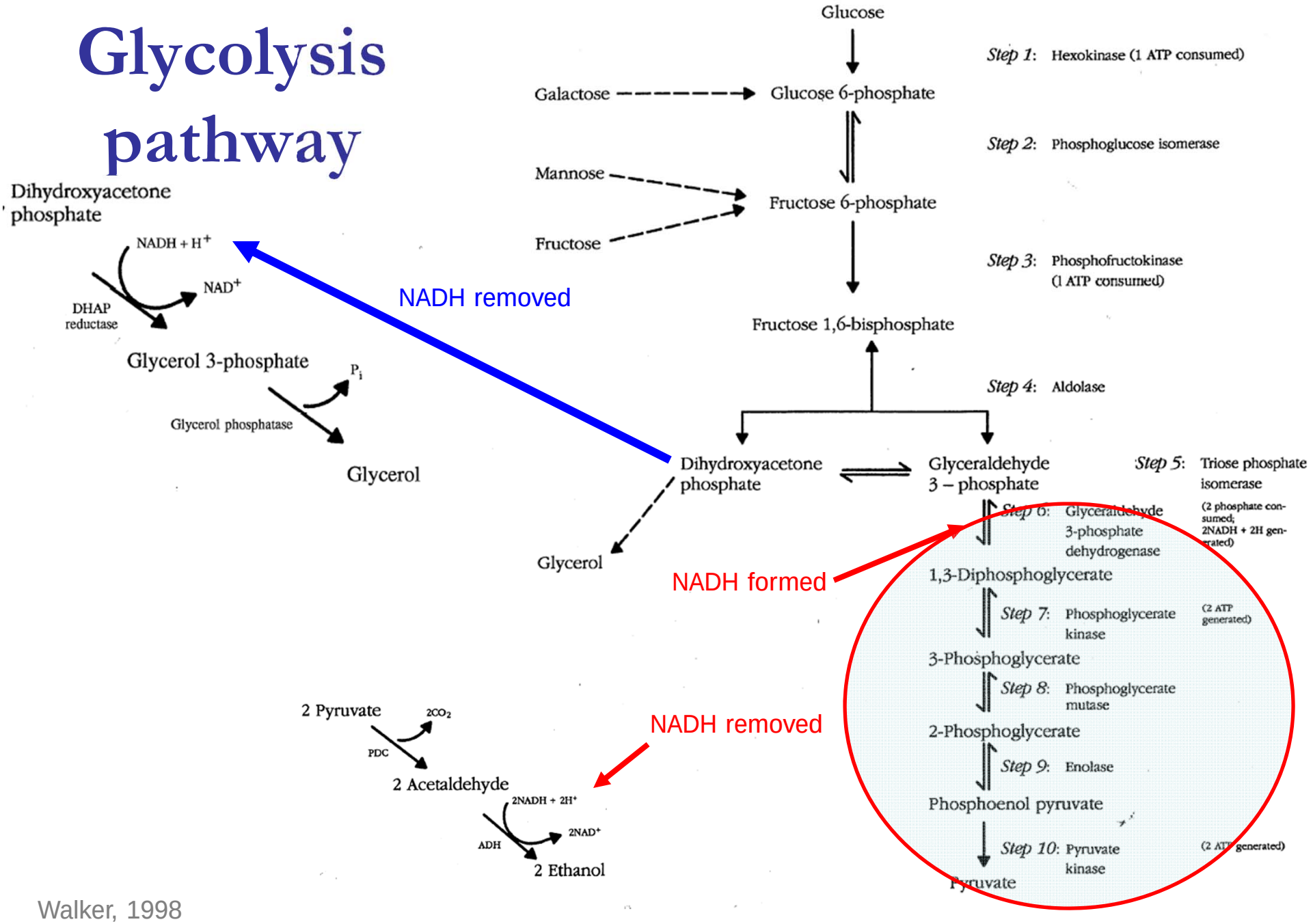
White Energy

Increasing Ethanol Yield by Lowering Glycerol Production

Agenda

- Reasons for glycerol production
  - Growth requires NAD/NADH rebalance
  - Compatible solute for osmotic stress
  - High pH of fermentation broth
- Reduce through nutrition
  - Amino acid
- Reduce through osmotic stress reduction
  - Novel yeast management strategy
- Reduce through new electron receptor
  - Genetic manipulation of pathway
- Industrial results

Glycolysis pathway



Walker, 1998

Compatible Solute

- Industrial fermentations are performed at high carbohydrate concentrations
  - Higher ethanol concentration at termination of fermentation process
  - Lower energy for distillation process
  - Higher hydraulic productivity (ethanol per unit flow)
  - Higher concentrations slow down bacterial competition
- SSF most common in batch fermentation
  - Dextrins from starch cook process pass to fermentation
  - Glucoamylase used to saccharify dextrins to glucose
- Low water activity
  - High solids decrease the effective water activity available to yeast
  - Osmostic stress pulls water out of the cell to balance osmolarity
  - Less than optimal water remains inside cell for cellular function
  - Compatible solute can "act like water" inside the cell
  - Compatible solute aids the internal cellular functions
- Glycerol
  - Preferred compatible solute for Saccharmyces cerevisiae
  - Over production of glycerol lowers ethanol yield

High pH Response

- High pH in fermentation medium
  - Aldehyde dehydrogenase enzyme activity increased at elevated pH
  - Increases production of acetic acid
  - Oxidation event generates molecule of NADH
  - Glycerol can be produced to rebalance NAD/NADH pathway
  - Ultimate effect is the same as the growth pathway mechanism
  - (ethanol per unit flow)
- An issue with early hours of fermentation in industry (pH 5.8 to 6.0 start)
  - Elevated pH early increases glycerol production
  - Lower pH alpha-amylases available
  - Many plants run lower pH in slurry and early fermentation today minimizing effect

Reduced Glycerol Can Increase Yield

- Glycerol used by yeast to reduce stress
- 2 wt% glycerol = 1 wt% ethanol
  - $C_3H_6O_3 + 2H \rightarrow \mathbf{C_3H_8O_3}$
  - $90_{(dihydroxyacetone)}$ **92 (glycerol)**
  - $C_3H_4O_3 \rightarrow CO_2 + \mathbf{CH_3CHO + 2H \rightarrow CH_3CH_2OH}$
  - $88_{(pyruvate)}$ 44 **46 (ethanol)**
- Example:
  - 12 wt/v% ethanol
  - Reducing wt/v% glycerol by 0.47% absolute will give 2% higher ethanol yield
    - 46/92 = 0.5
    - 0.5 * 0.47 = 0.235 wt% EtOH
    - (0.24%/12%) = 2% higher yield

Amino Acids

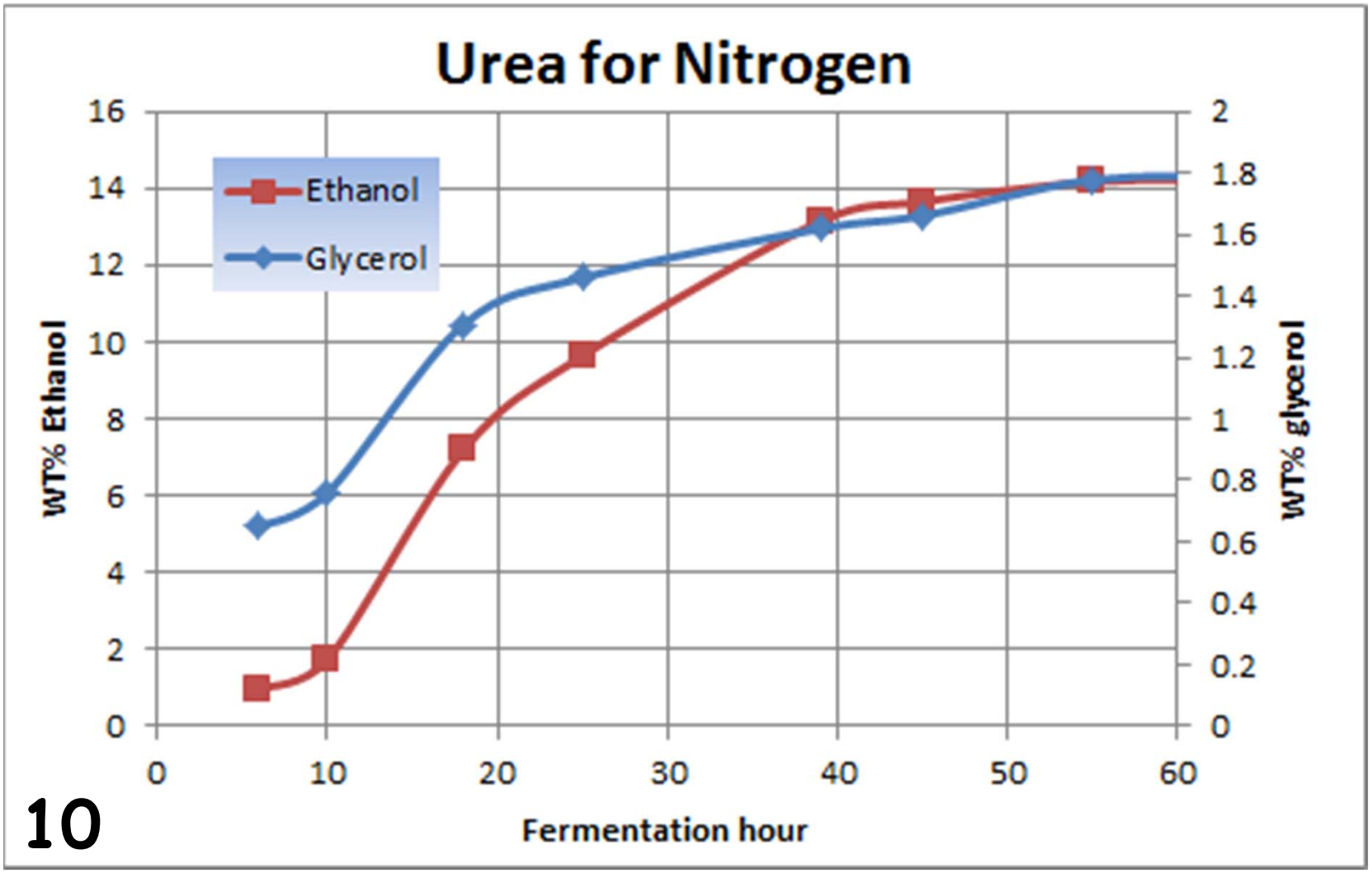
- Feed yeast with urea or ammonia
  - C, H, O required to complete amino acid synthesis
  - Glycerol production results from growth
- Feed yeast with amino acids
  - Amino acid will be assimilated into protein
  - No synthesis required
  - No redox imbalance so no glycerol production

GMO Modification

- Lower osmotic stress
  - Expression of glucoamylase has been added
  - Glucoamylase is expressed during fermentation
  - Slower release of glucoamylase lowers the peak glucose concentration
  - Less peak osmotic stress requiring less compatible solute
- New redox pathway
  - Two glycerol "pathways"
  - First pathway for osmotic stress
  - Second pathway for growth NAD/NADH balance
  - Second pathway has been altered
  - Formic acid now terminal electron acceptor rather than glycerol
  - Significant reduction in glycerol production

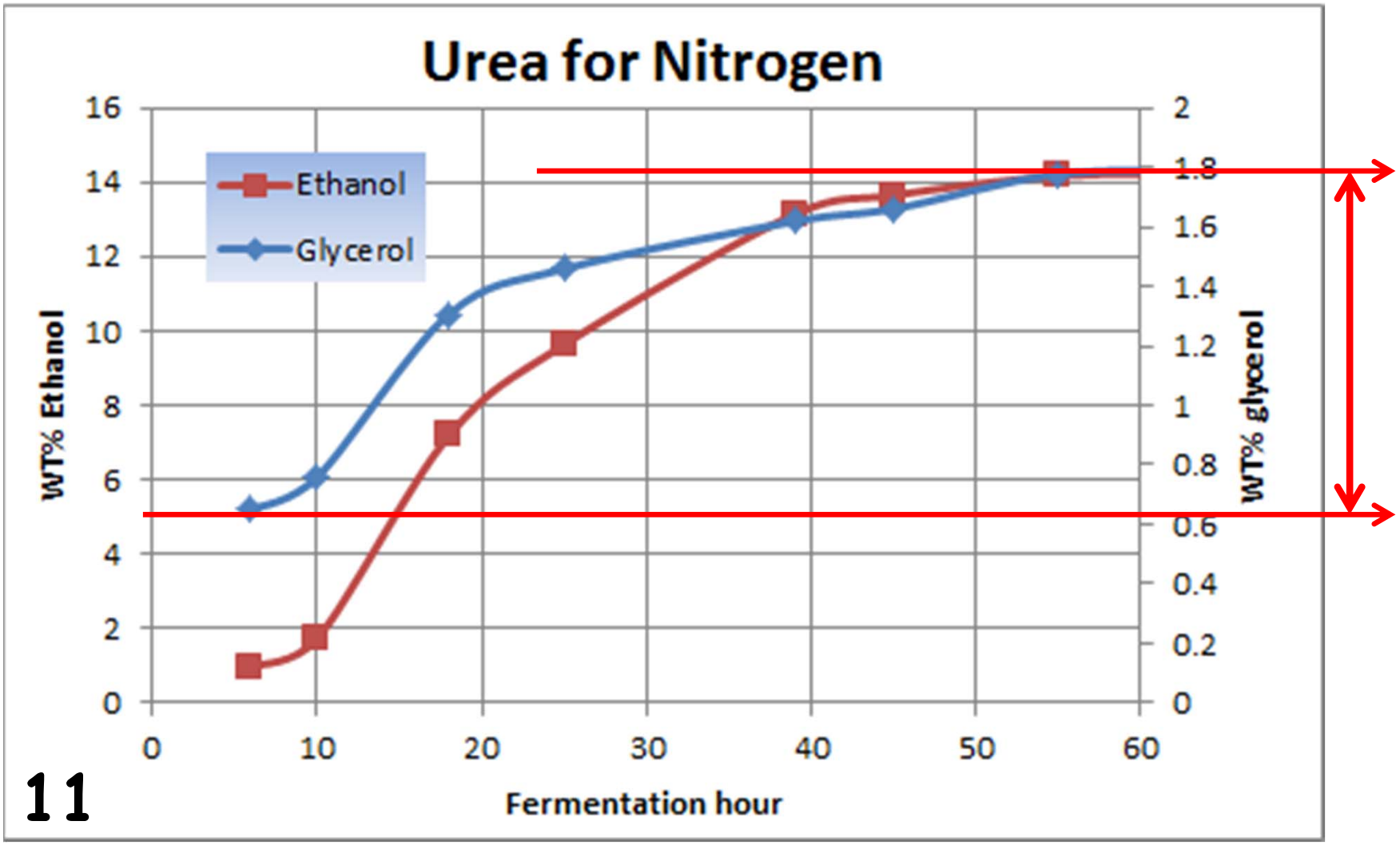
Industrial Results

“Standard” SSF

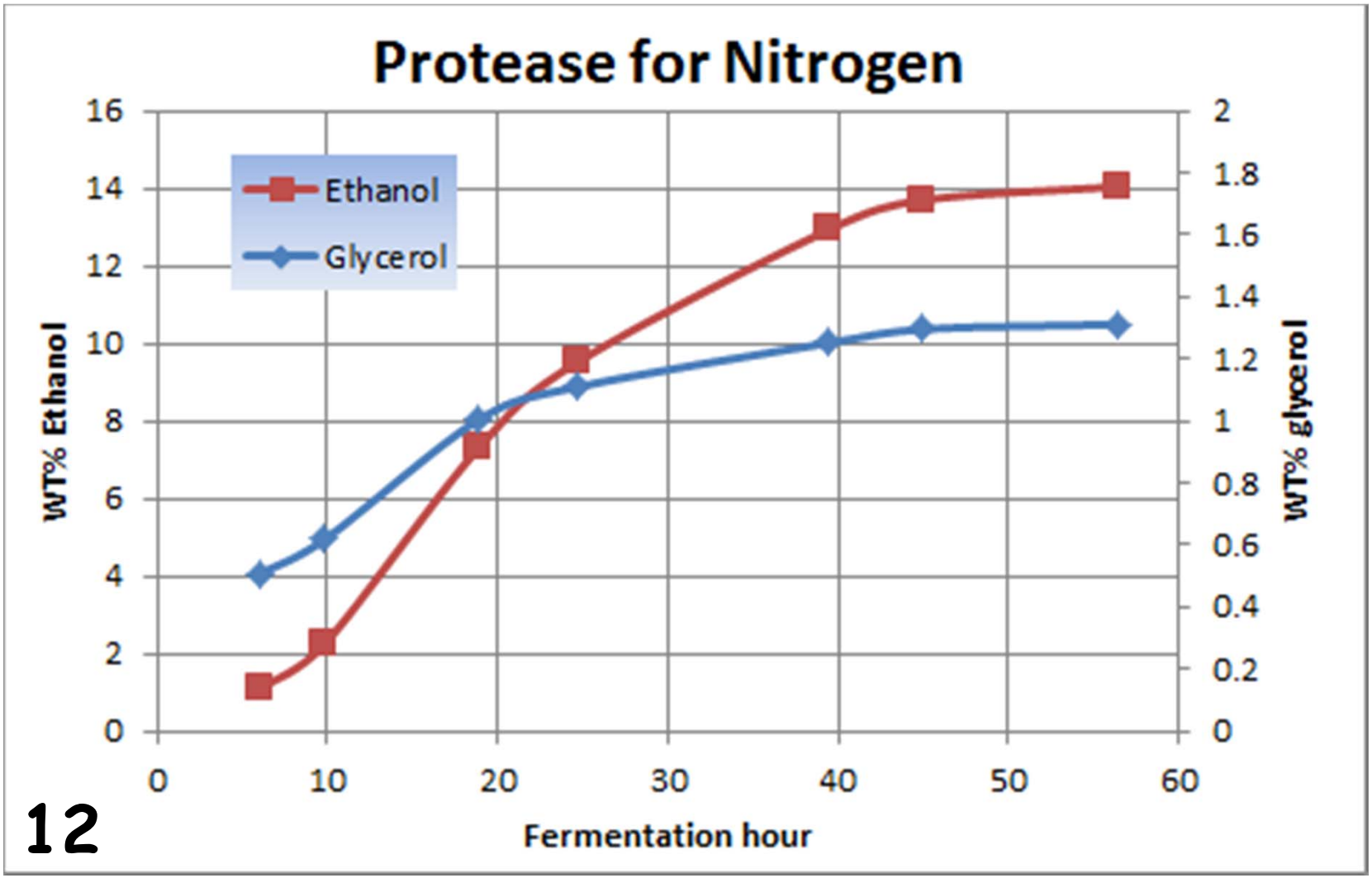


"Standard" SSF

d gly = 1.125%
d gly/d EtOH = 0.084

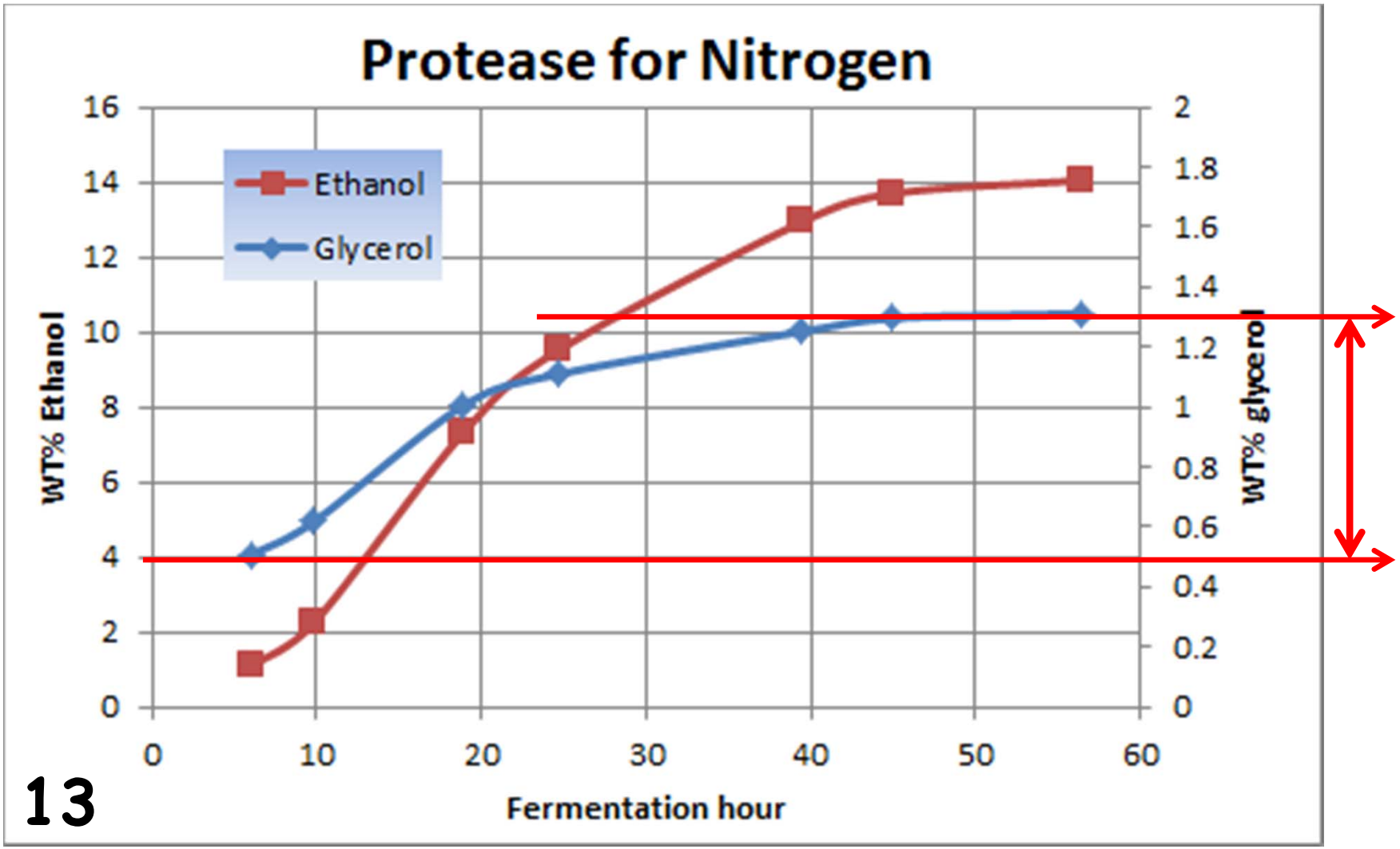


Protease for Nitrogen

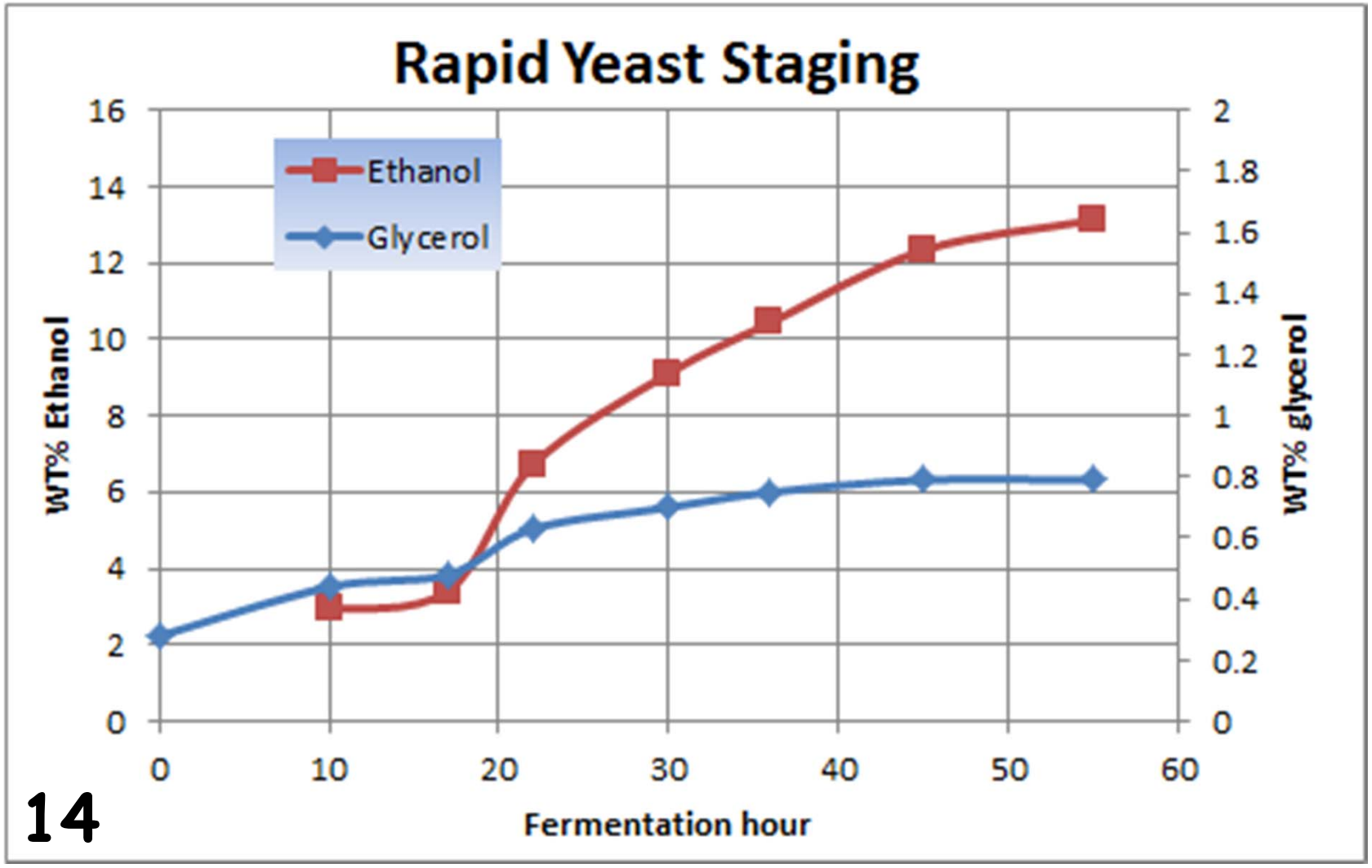


Protease for Nitrogen

d gly = 0.805%
d gly/d EtOH = 0.062

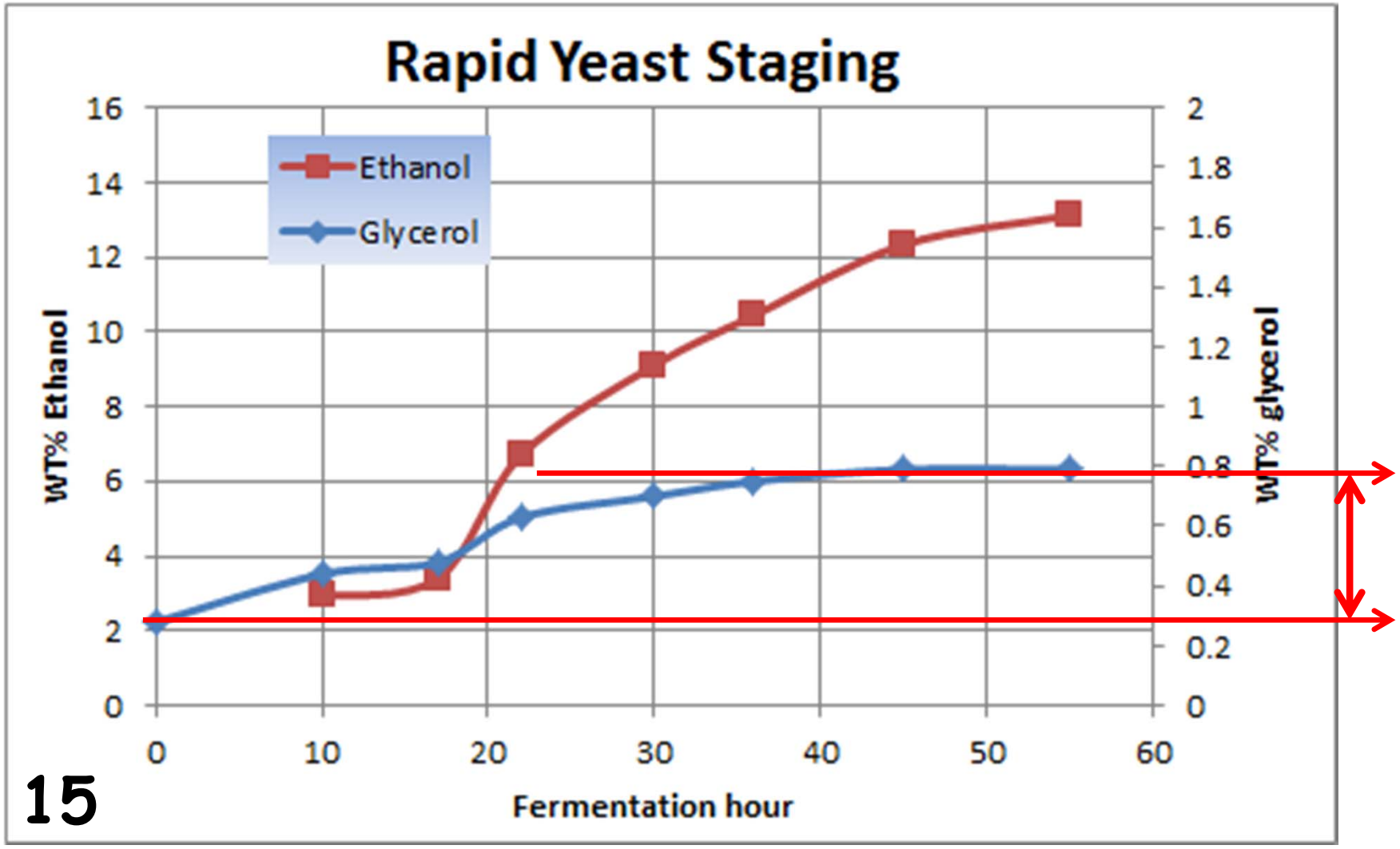


Rapid Yeast Staging

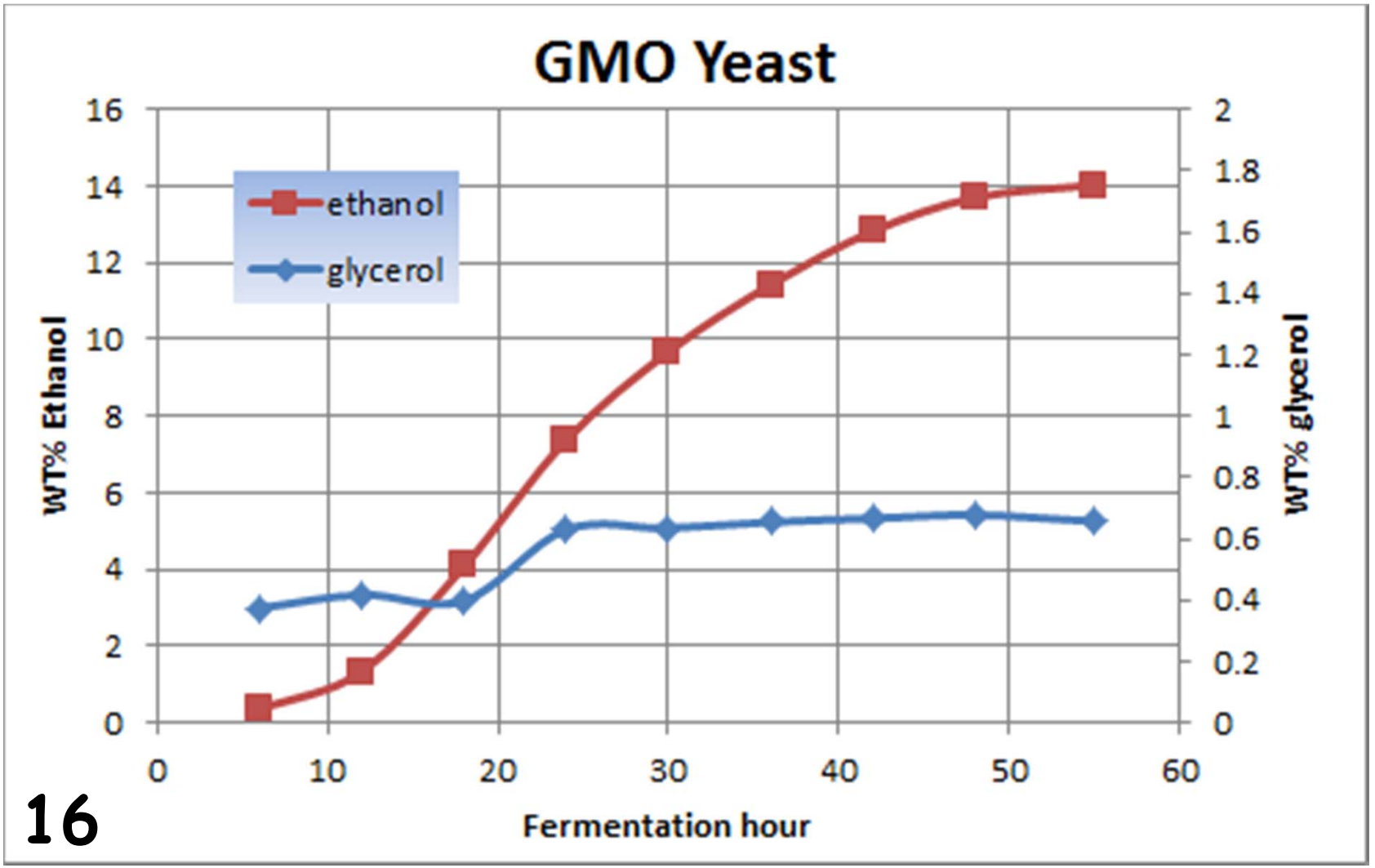


Rapid Yeast Staging

d gly = 0.51%
d gly/d EtOH = 0.039

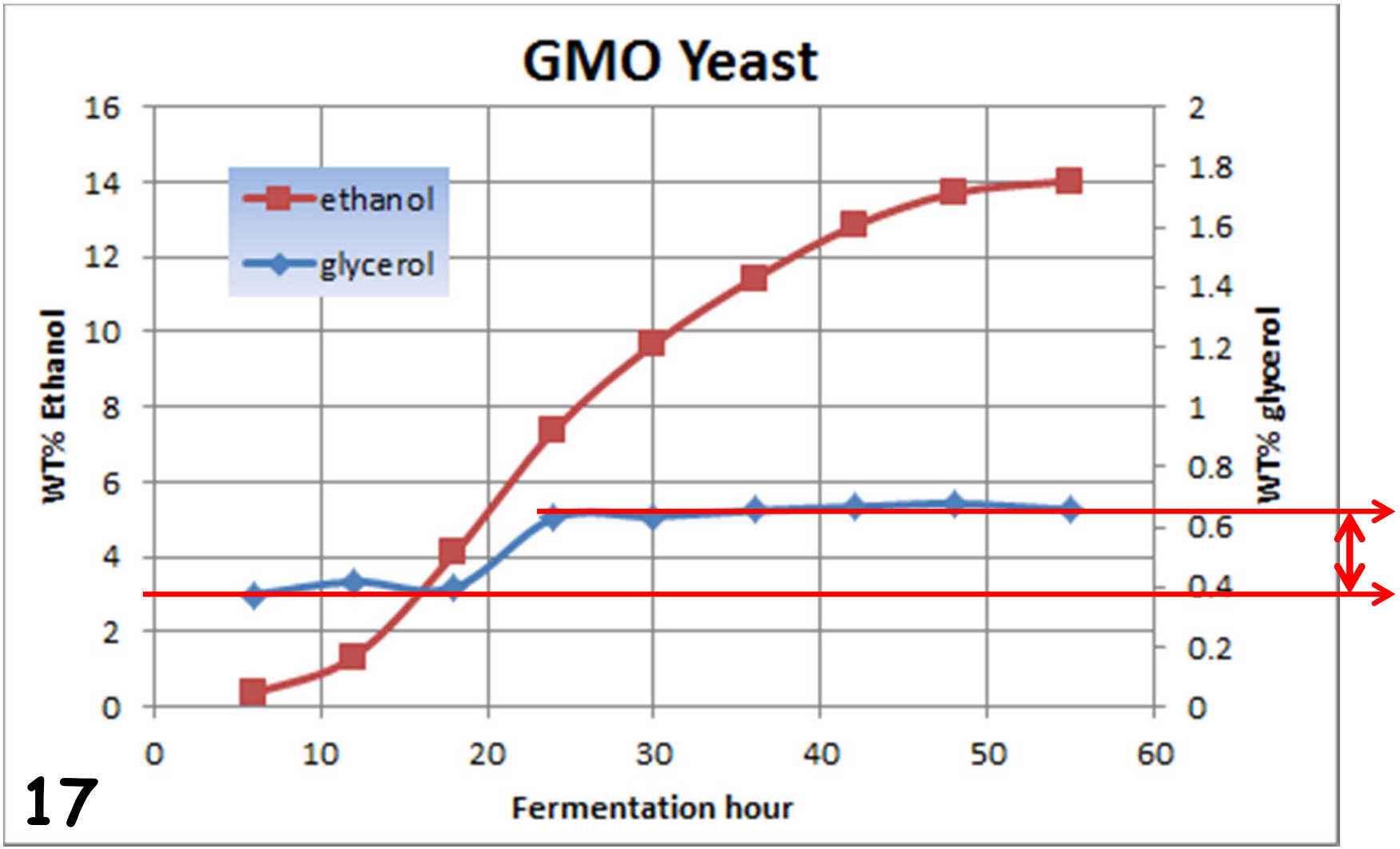


Formic Acid Pathway



Formic Acid Pathway

d gly = 0.303%
d gly/d EtOH = 0.022



Comparison

Method	d glycerol	d glycerol/d ethanol	% yield increase
Standard	1.125%	0.084	0%
Protease	0.805	0.062	1.1%
Rapid Staging	0.510	0.039	2.2%
Formic Acid	0.303	0.022	2.9%

Conclusions

- Glycerol is an undesired coproduct
  - Diverts sugar away from ethanol production
  - Makes drying more challenging as liquid at room temperature
- New methods are available for glycerol reduction
- "Best in class" applications of new techniques
  - Amino acid feed (protease)
    - Increases yield ~1%
  - Rapid yeast staging
    - Increase yield ~2%
  - Formic acid pathway + GA expression
    - Increase yield by ~3%

Questions

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