

The Effects of the Rate of Nitrogen Consumption on the Duration of Alcohol Fermentation Remain Unknown

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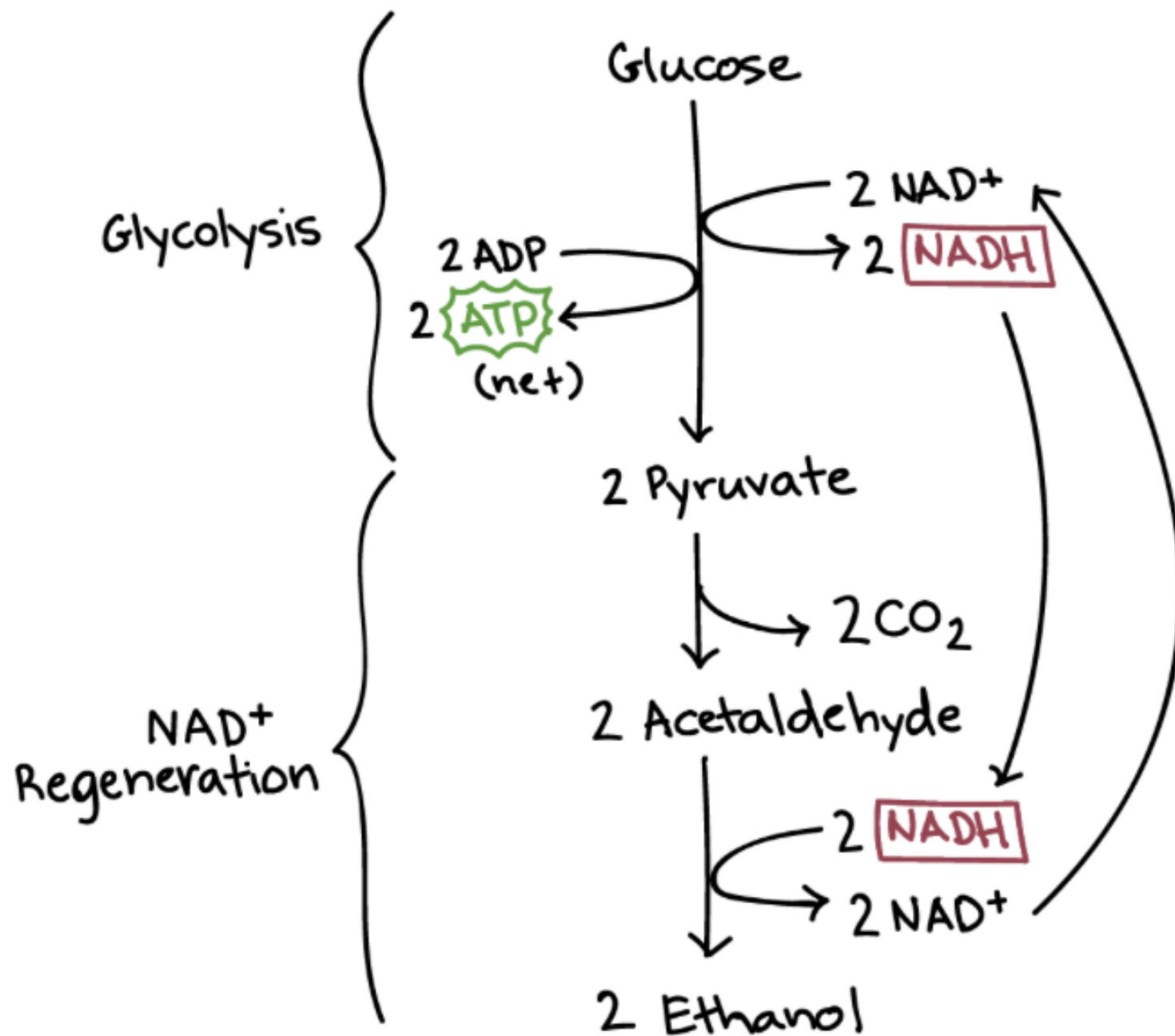
Outline

- **Background Info: Alcohol fermentation in *Saccharomyces cerevisiae***
- **Hypothesis: Rate of nitrogen consumption controls alcohol fermentation time**
- **Multiplicative Nutrient Model: Relating model to ter Schure (1995) and Albertin et al. (2011)**
- **Model fails to support results of ter Schure (1995) and Albertin et al. (2011)**
- **Further research into factors controlling rate and time of alcohol fermentation**

***Saccharomyces cerevisiae* play a vital role in ethanol production**

- **Used in food and biofuel industry to convert sugars to ethanol**
- **Done through the process of **alcohol fermentation (AF)****
 - **Main process yeast use to obtain energy** (Dombek & Ingram, 1987)
 - **Occurs in anaerobic conditions**
 - **Results from interaction of various environmental, genetic, and metabolic parameters** (Albertin et. al, 2011)

Alcohol fermentation begins with the breakdown of glucose



Glucose:

- Important macronutrient
- Used as source of sugar

Glycolysis:

- Produces ATP
- Uses NAD⁺ as electron acceptor

Fermentation:

- Results in ethanol & CO₂ production
- Regenerates NAD⁺

***S. cerevisiae* are facultative anaerobes**

- **Switch between anaerobic (fermentation) and aerobic (cellular respiration) pathways**
 - **Allows for maximization of ATP production**
- **When glucose scarce —> ethanol used as source of carbon, requiring the shift to cellular respiration**
 - **Converts sugar to ATP and CO₂** (Gasmi et. al, 2014)
- **Many experiments performed on how to increase the rate of fermentation in yeast**

Population size drives alcoholic fermentation in industrial *S. cerevisiae*

- **Albertin et. al (2011) evaluated relationship between fermentation kinetics and population dynamics**
 - **Used batch reactor model**

Key Results:

- **High fermentation speed (V_{max}) significantly correlated with increased population size (K)**
- **K and biomass positively correlated**
- **K and nitrogen consumption positively correlated**

Set of factors that regulate alcohol fermentation time remain unknown

Alcohol Fermentation (AF) time

- **beginning of CO₂ release to the point when CO₂ production rate drops below 0.5 g liter⁻¹ h⁻¹ (Albertin et. al, 2011)**

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The rate of nitrogen consumption governs alcohol fermentation time

Predictions:

Increase in nitrogen consumption will cause CO₂ production rate to reach V_{max} sooner then crash

Assumptions:

- As population size increases, nitrogen consumption increases**
- As population size increases, glucose decreases—> switch to cellular respiration**

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Nitrogen and glucose both required by yeast for alcohol fermentation

Two Nutrients:

1. Nitrogen:

- **Increase in ammonia concentration in feed from 29 to 61 mM —> increase in biomass, showing ammonia limitation**
- **Ammonium found to be limiting at NH_4^+ concentrations of 29 and 44 mM (ter Schure et. al, 1995)**

2. Glucose:

- **Source of carbon for alcohol fermentation (Albertin et. al, 2011)**

Multiplicative model acts as best representation

Nitrogen concentration (g/L)

$$\textcircled{1} \quad \frac{dc_1}{dt} = -y V_1 \frac{c_1}{K_1 + c_1} \cdot \frac{c_2}{K_2 + c_2}$$

Glucose concentration (g/L)

$$\textcircled{2} \quad \frac{dc_2}{dt} = -y V_2 \frac{c_1}{K_1 + c_1} \cdot \frac{c_2}{K_2 + c_2}$$

Concentration of yeast (g/L)

$$\textcircled{3} \quad \frac{dy}{dt} = y R \frac{c_1}{K_1 + c_1} \cdot \frac{c_2}{K_2 + c_2}$$

CO₂ production Rate (g/L.h)

$$\textcircled{4} \quad \frac{dV}{dt} = V_{\max} \left(1 - \frac{V}{y}\right) V$$

State Variables:

c₁= concentration of nitrogen

c₂= concentration of glucose

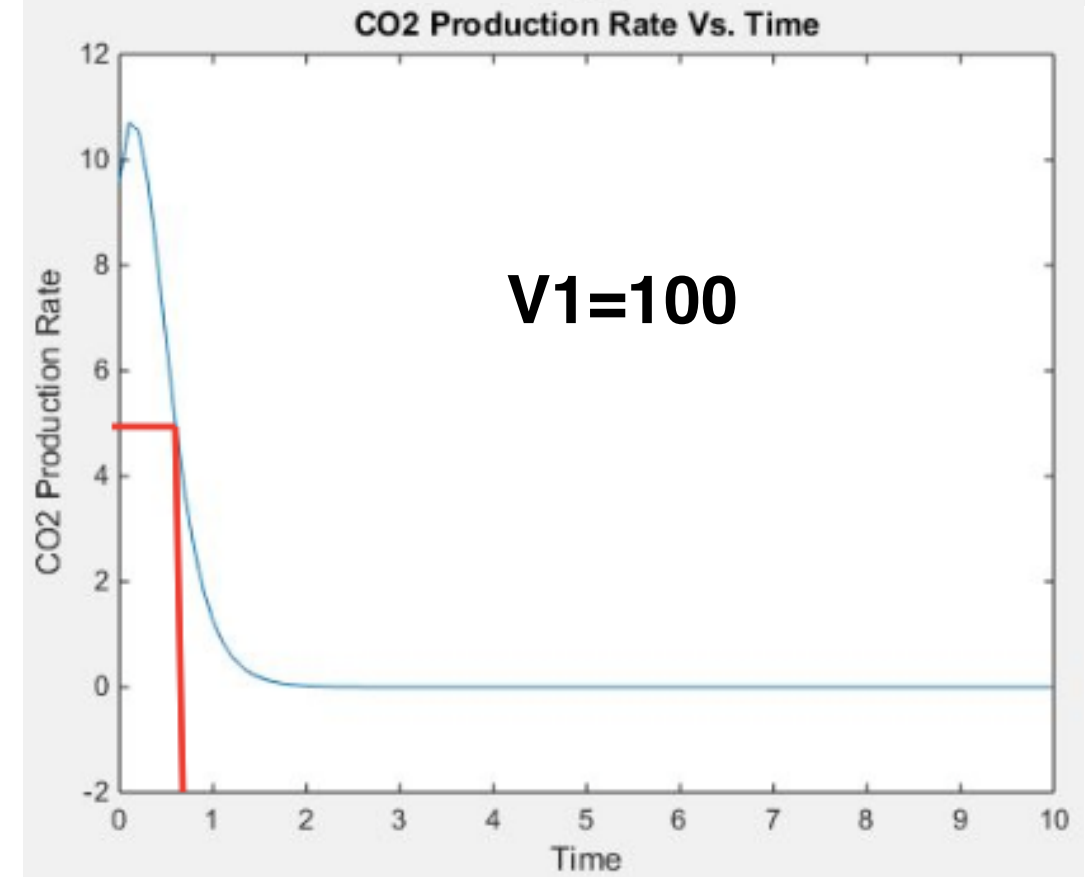
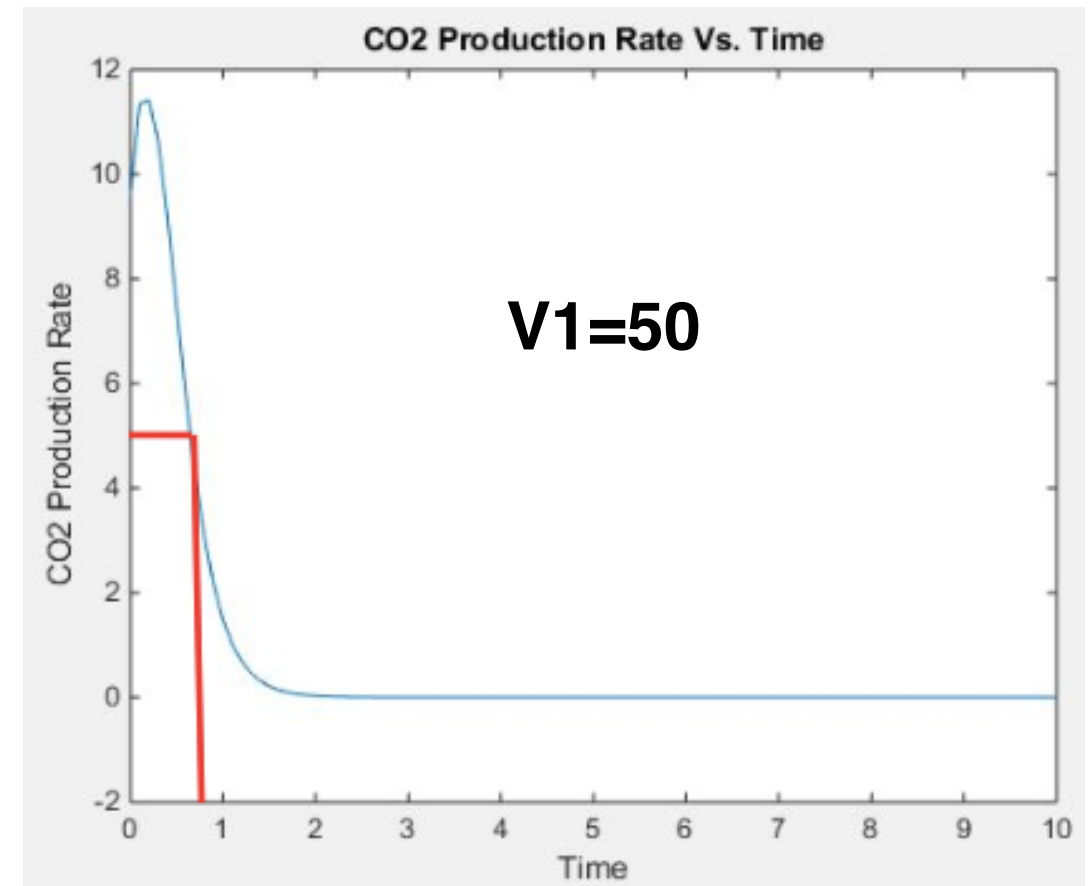
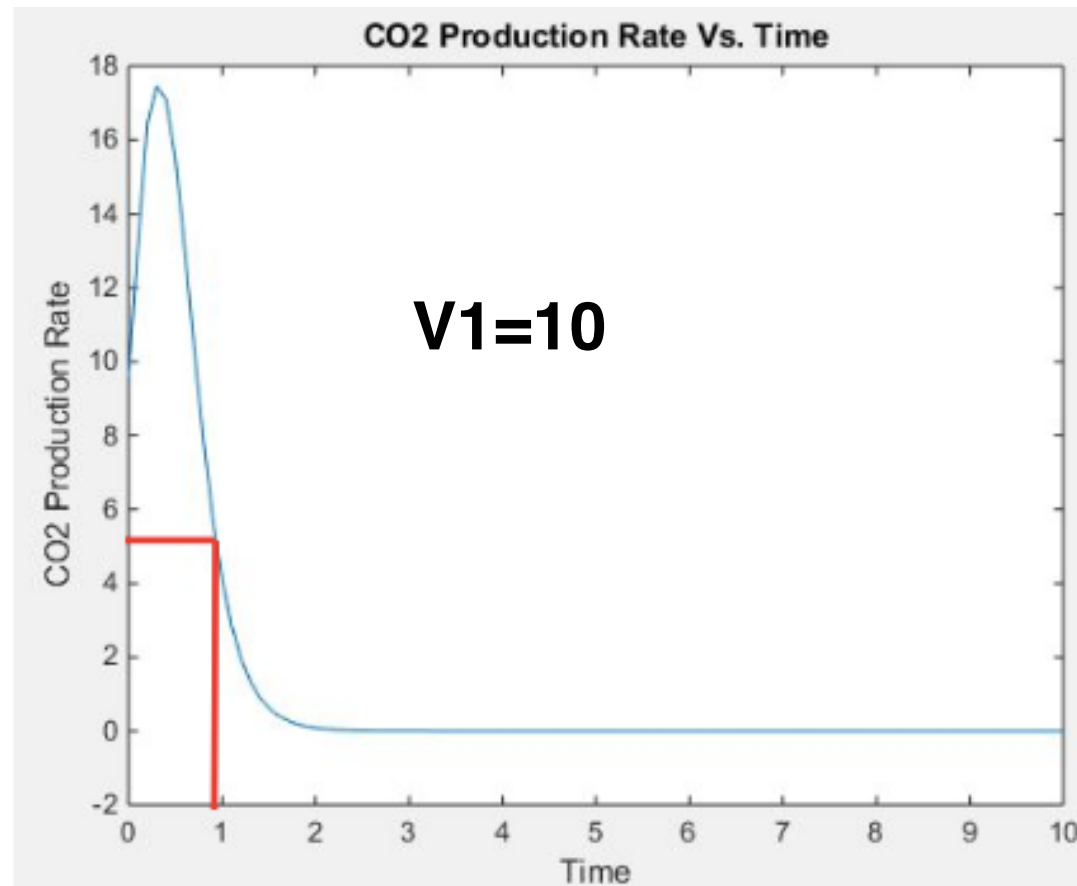
y= concentration of yeast

V= amount of CO₂ produced

Parameters:

- **V₁**= rate of nitrogen consumption
- **V₂**= rate of glucose consumption
- **K₁**= metabolic constant
(1/2 V_{max} of nitrogen)
- **K₂**= metabolic constant
(1/2 V_{max} of glucose)
- **R**= replaces relative efficiency
- **V_{max}**= max rate of CO₂ production

While CO₂ production rate seems to crash faster as V₁ increases, results remain inconclusive



All parameter values
(except V₁) remained constant at
V₂= 10, V₃= 4, K₁= 2, K₂= 2, R= 5

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Unlike model Albertin et. al (2011) and ter schure (1995) findings indicate that rate of nitrogen consumption plays a role in AF time

- **Nitrogen consumption and population size positively correlated**
- **Population size drives alcohol fermentation**

(Albertin et. al, 2011)

- **Increase in ammonia related to increase in biomass** (ter Schure et. al, 1995)
- **Biomass positively correlated to population size** (Albertin et. al, 2011)

Rate of nitrogen consumption is not the only factor that governs AF time

Nitrogen concentration (g/L)

$$\textcircled{1} \quad \frac{dc_1}{dt} = -y V_1 \frac{c_1}{K_1 + c_1} \cdot \frac{c_2}{K_2 + c_2}$$

Glucose concentration (g/L)

$$\textcircled{2} \quad \frac{dc_2}{dt} = -y V_2 \frac{c_1}{K_1 + c_1} \cdot \frac{c_2}{K_2 + c_2}$$

Concentration of yeast (g/L)

$$\textcircled{3} \quad \frac{dy}{dt} = y R \frac{c_1}{K_1 + c_1} \cdot \frac{c_2}{K_2 + c_2}$$

CO₂ production Rate (g/L·h)

$$\textcircled{4} \quad \frac{dV}{dt} = V_{\max} \left(1 - \frac{V}{y}\right) V$$

Changes in the rate of glucose consumption will have similar effects as changes in the rate of nitrogen consumption.

Summary

- **Identifying the factors that control the rate and duration of alcohol fermentation in *S. Cerevisiae* important issue**
 - **main source of energy production in yeast**
 - **allows for ethanol production for food and biofuel industry**
- **Set of factors that regulation the rate and time of alcohol fermentation remain unknown**
- **While model fails to support results of papers, possible rate of nitrogen consumption may still influence AF time**
- **Rate of glucose consumption may play a role as well**

Further study into the factors that affect AF needed

- **Results of model need verification**
 - **rate of nitrogen consumption may not be only factor affecting AF time —> verify by testing changes in rate of glucose consumption**
- **If rate of nitrogen consumption does influence AF time**
 - **further study into factors controlling rate of nitrogen consumption needed**
 - **since rate of nitrogen consumption can't be set by experimenter**

Acknowledgments

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References

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