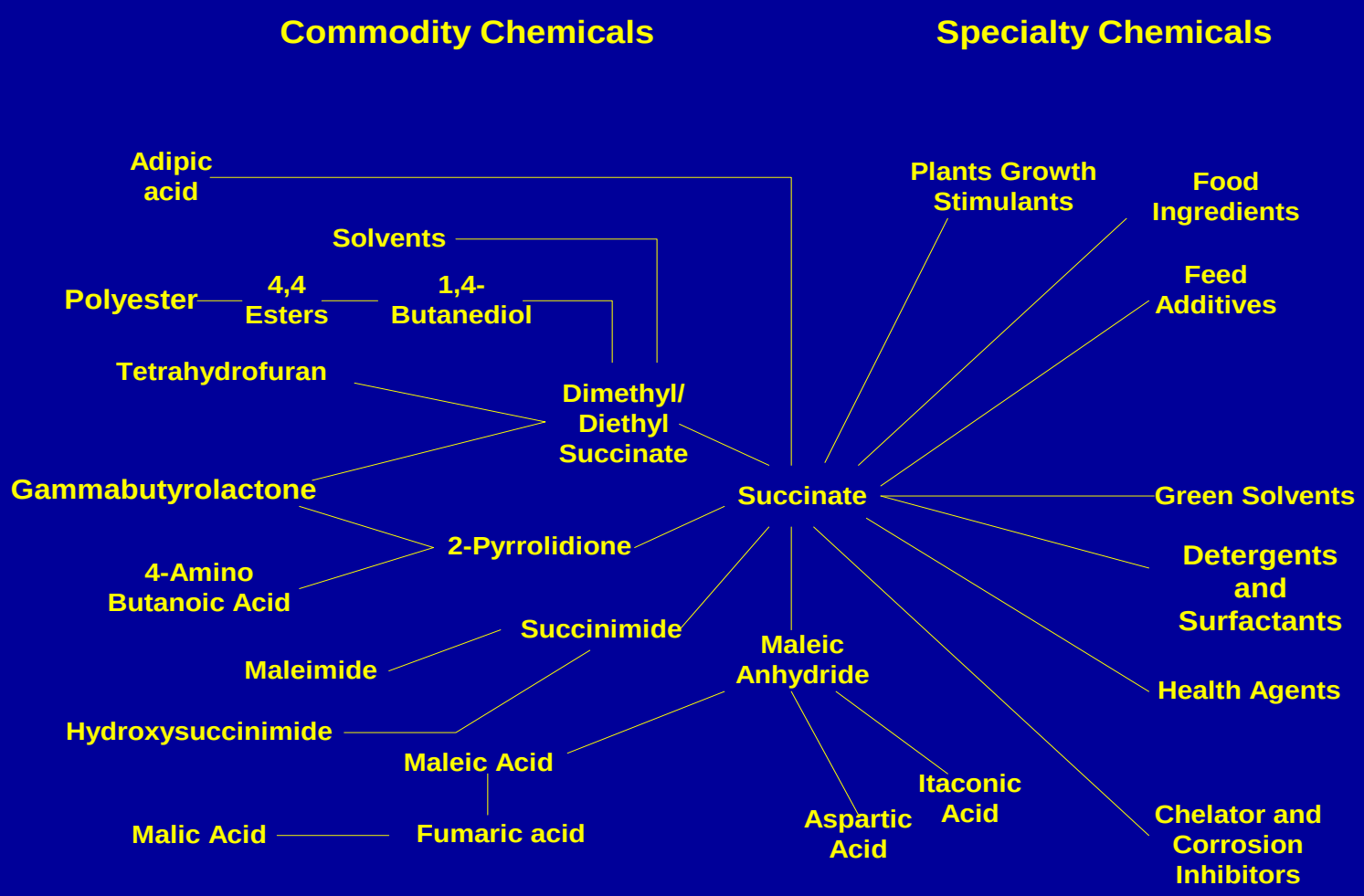
Effect of CO_2 on Succinate Production in *Escherichia coli* Fermentations

Shiying Lu, Mark A. Eiteman, Elliot Altman
Center for Molecular BioEngineering
University of Georgia

March 7, 2008

Why Succinate?



Appl Microbiol Biotechnol (1999) 51: 545-552

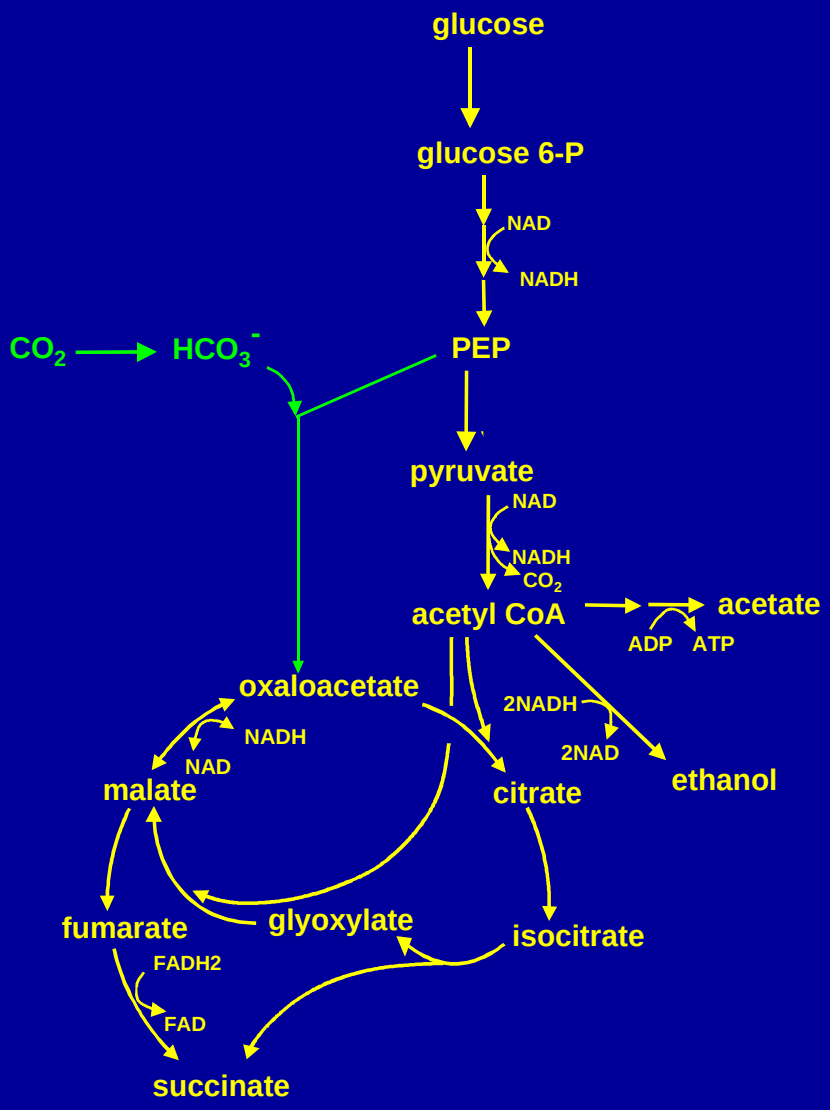
Succinate Biosynthesis

PEP Carboxylase (PPC)

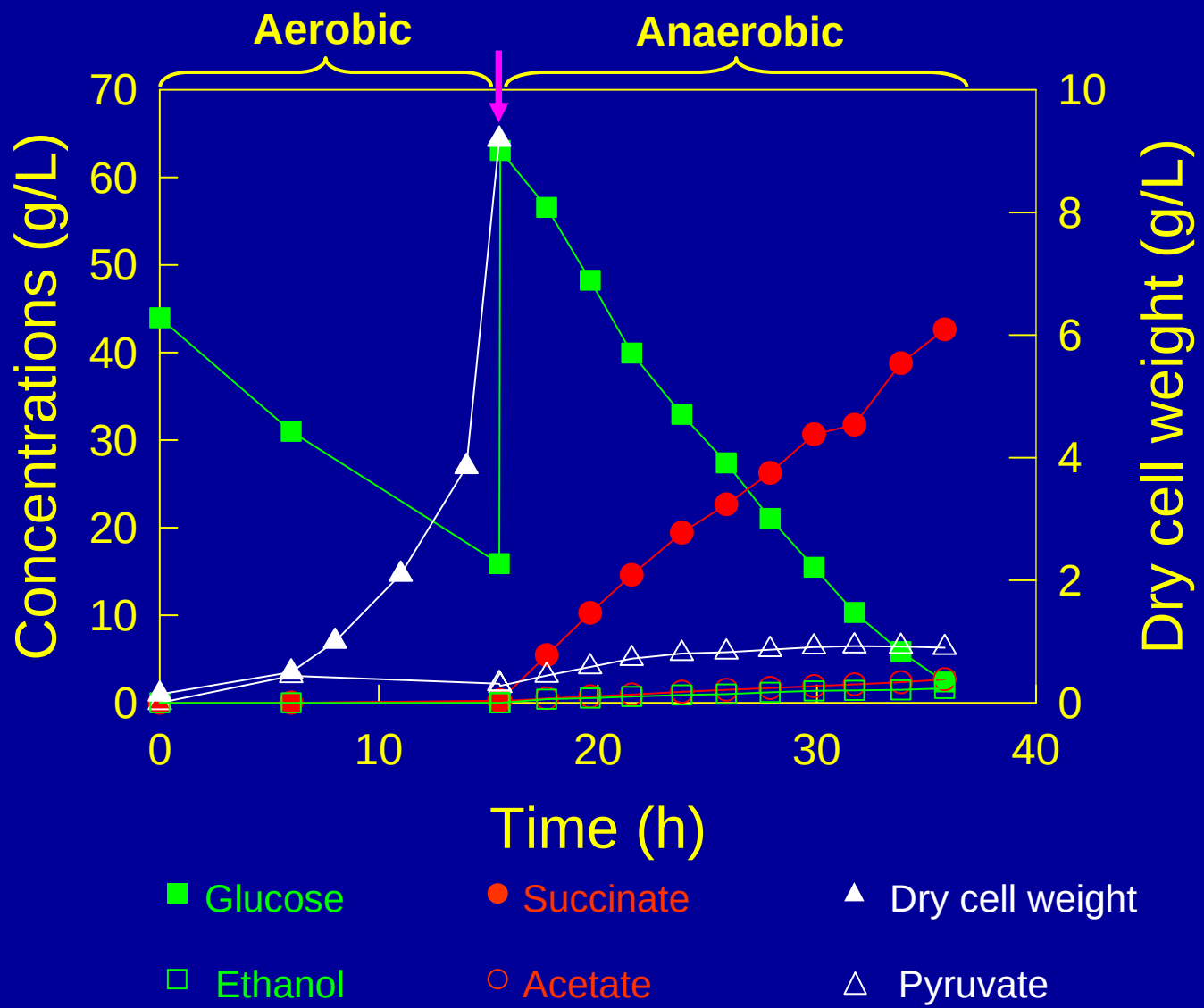
Mutations

1. lactate dehydrogenase (*ldhA*)
2. pyruvate formate lyase (*pfl*)
3. phosphotransferase system (*ptsG*)

AFP111 (*ΔpflAB ldhA ptsG*)



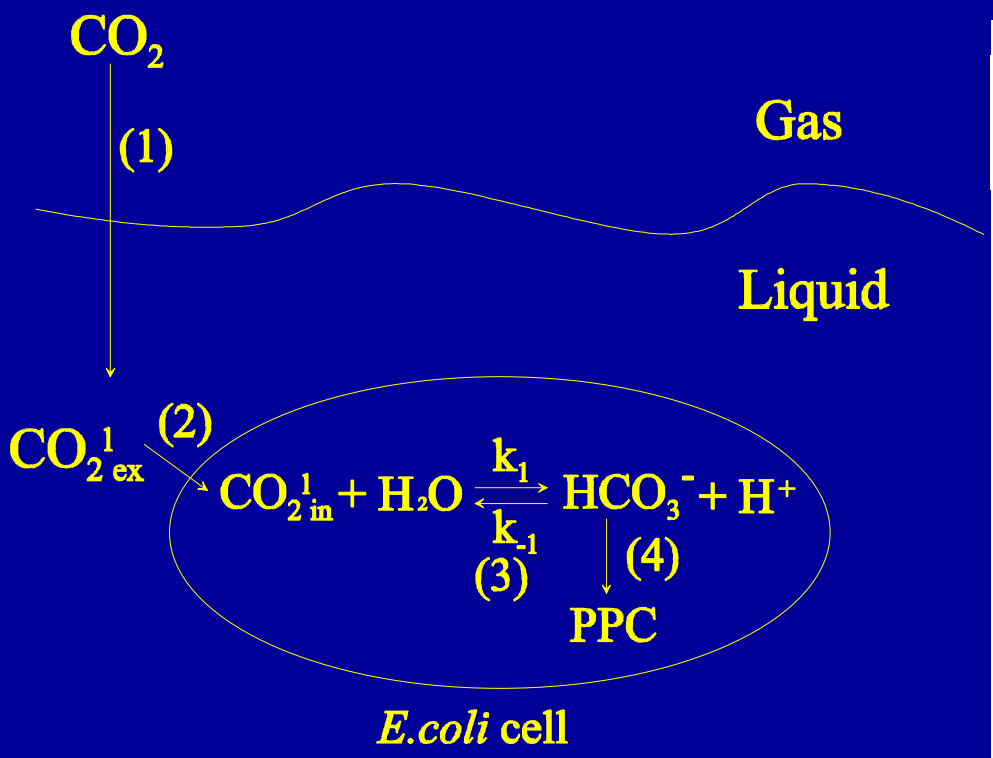
Dual-phase Fermentation



Effect of CO_2

Rationale

CO_2 gas may impact succinate production



$$J^{1}_{CO2} = \frac{d[CO_2]_{ex}}{(X/m_E)dt} = \frac{k_L a_{(CO2)}([CO^{*}_2] - [CO^{l}_2]_{ex})}{X/m_E}$$

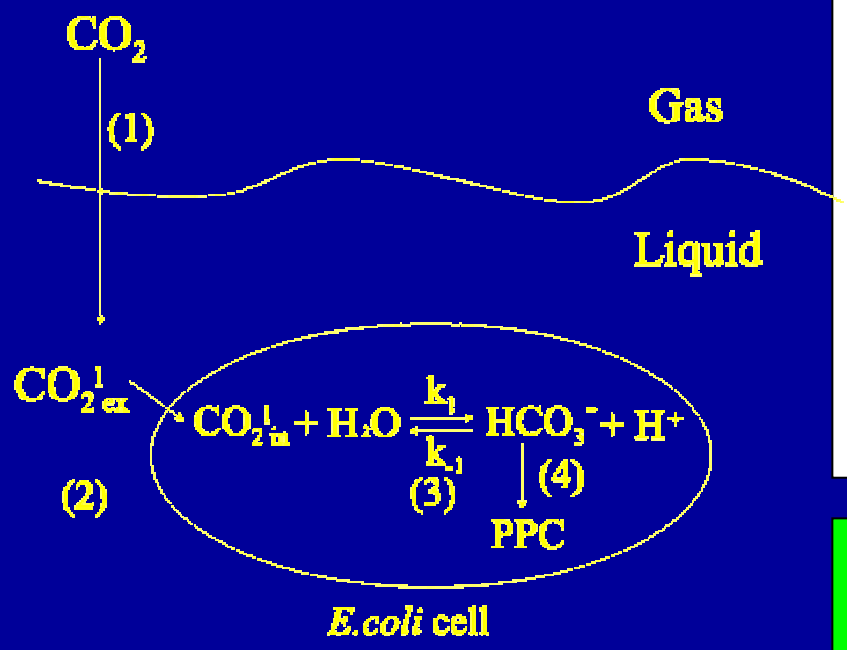
$$J^{2}_{CO2} = PA_E([CO^{l}_2]_{ex} - [CO^{l}_2]_{in})$$

$$J^{3}_{CO2} = (k_1[CO^{l}_2]_{in} - k_{-1}[HCO^{-}_3][H^{+}])V_E$$

$$J^{4}_{CO2} = \frac{V_{(PPC)}[HCO^{-}_3]}{(K_{m(PPC)} + [HCO^{-}_3])}$$

Effect of CO_2 (Cont.)

Rationale



$$J^{3}_{CO2} = (k_1[CO^{l}_{2}]_{in} - k_{-1}[HCO^{-}_{3}][H^{+}])V_E$$

$k_1 = 0.029\ s^{-1}$ (Pocker and Bjorkquist, 1977)

$k_{-1} = 2.0\times10^{4}\ L/mol\cdot s$

$V_E = 1.0\times10^{-15}\ L$ (Koppes et al., 1978)

$[H^{+}] = 10^{-7.5}\ mol/L$ (Olsen et al., 2002)

$J^{4}_{CO2(max)} = 1.29\times10^{-16}\ mmol/s$ (from experiments)

$$J^{3}_{CO2} = J^{4}_{CO2(max)} = 1.29\times10^{-16}\ mmol/s$$

$$[CO^{l}_{2}]_{in} = 4.5mmol/L + 0.02[HCO^{-}_{3}] > 4.5mmol/L$$

Effect of CO_2 (Cont.)

Summary of Model Results

With $k_L a_{(CO2)}$ of 29 h^{-1} and cell mass concentration of 8.4 g/L

- CO_2 gas is less than about 20%...
  system may become limited by CO_2 concentration.

- CO_2 gas is greater than about 20%...
  system may become limited by PEP carboxylase activity.

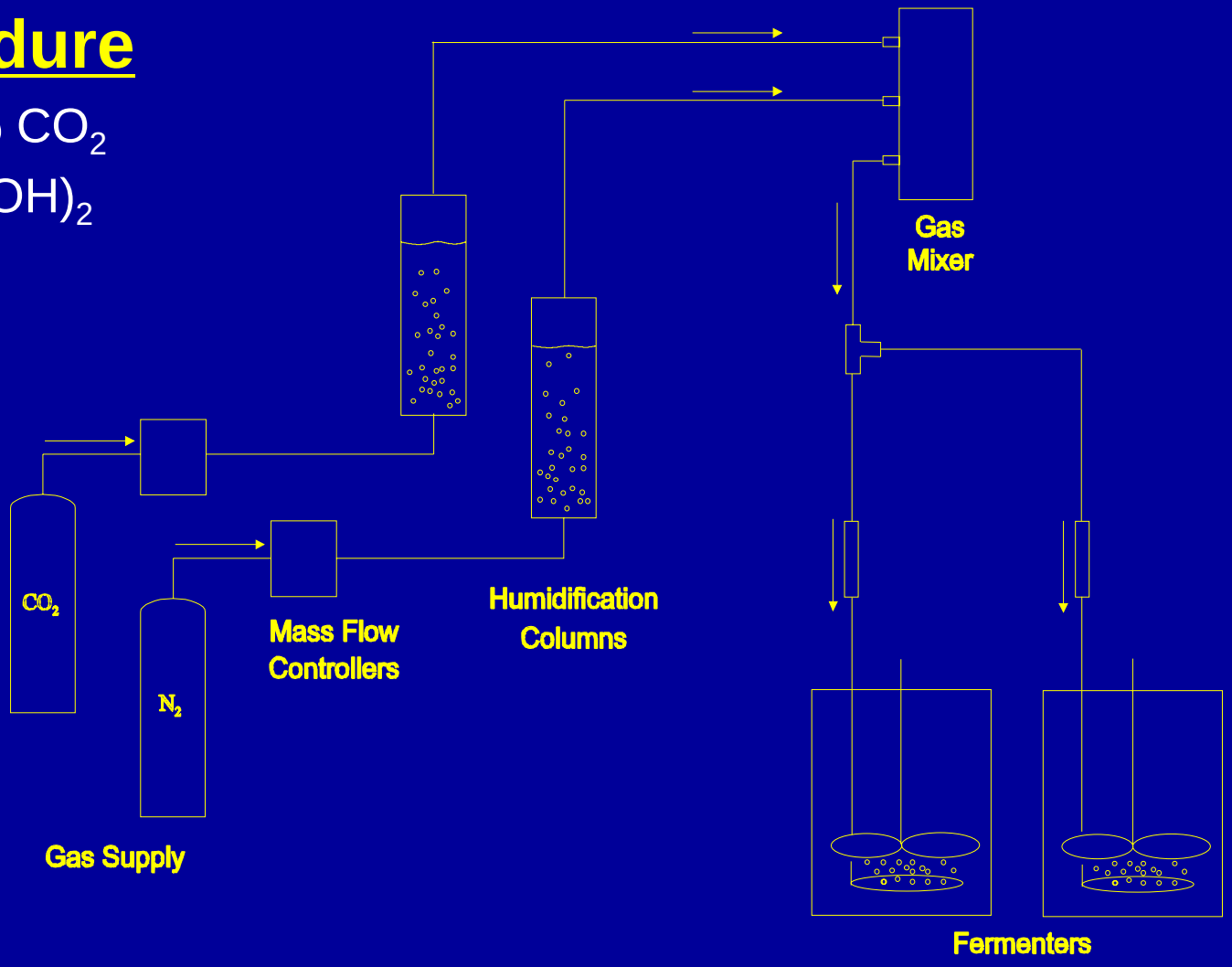
Effect of CO_2 (Cont.)

Procedure

0 – 100% CO_2

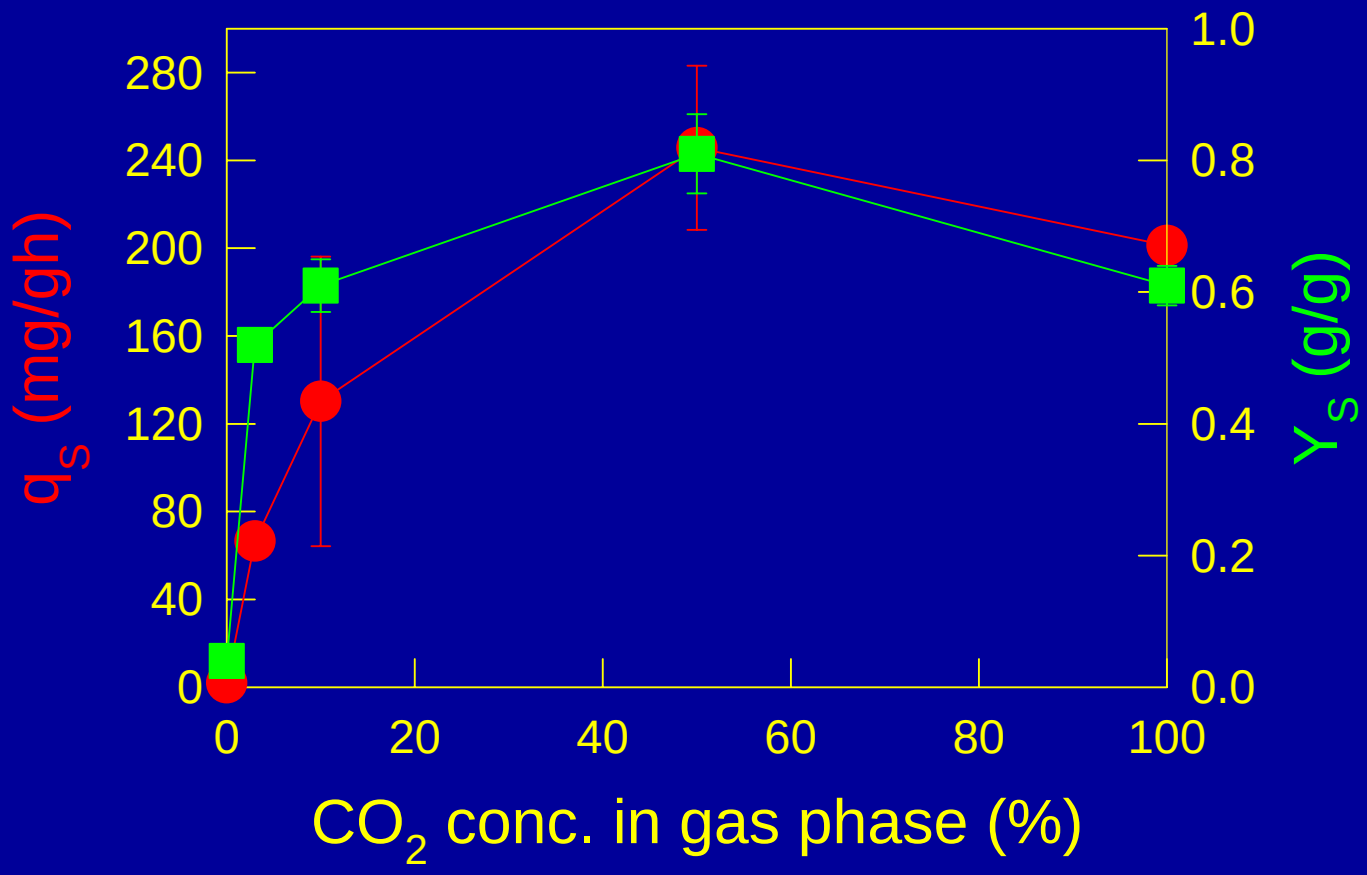
25% $Ca(OH)_2$

pH of 6.4



Effect of CO_2 (Cont.)

E. coli AFP111 (*ΔpflAB ldhA ptsG*)



Effect of Agitation

When CO_2 gas is less than about 20%, system may become limited by CO_2 concentration.

Increasing agitation rate may alleviate this limitation.

Procedure

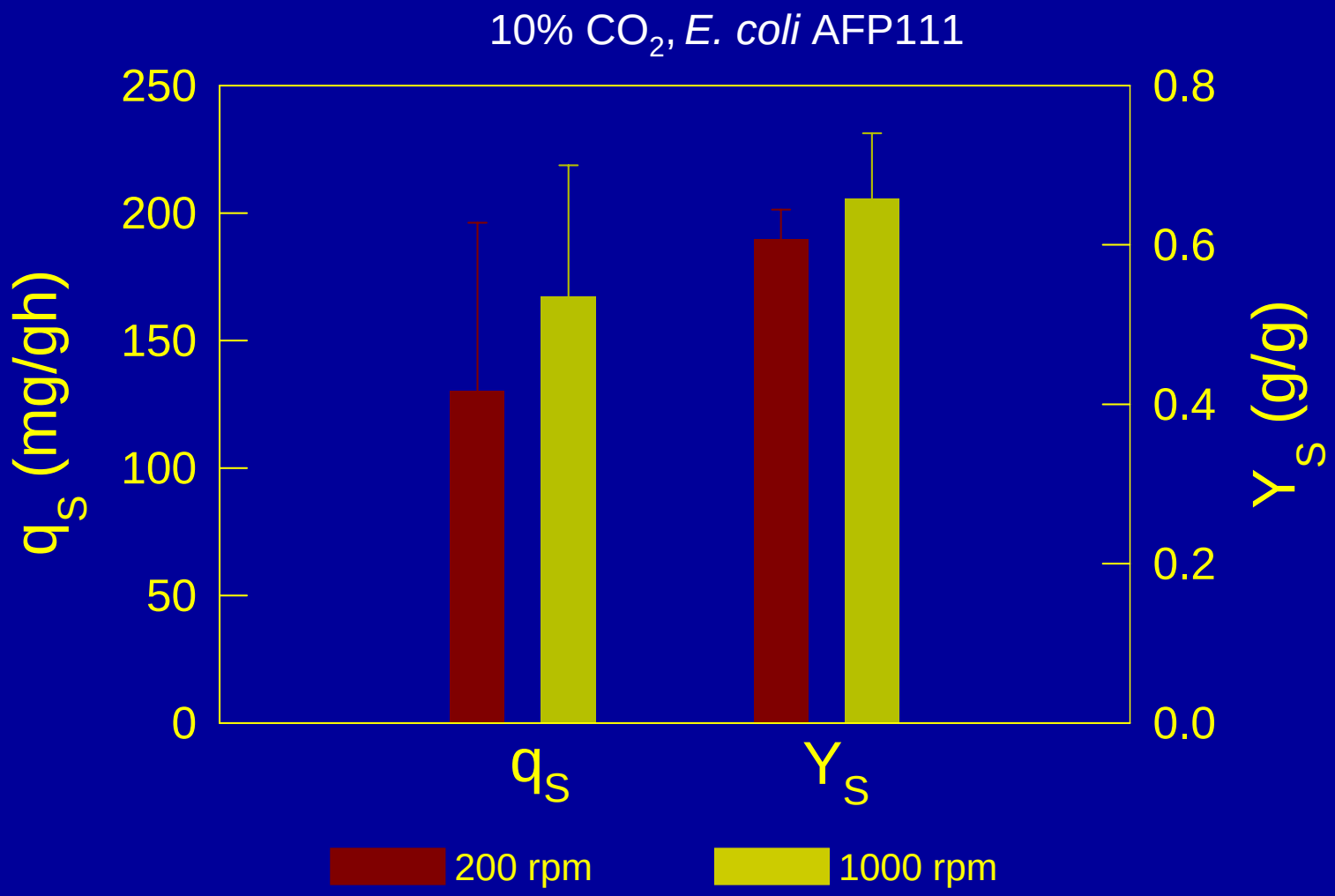
10% CO_2

200 rpm vs. 1000 rpm (k_La of 60 h^{-1})

pH of 6.4

25% $Ca(OH)_2$

Effect of Agitation (Cont.)



Effect of Pyruvate Carboxylase

When CO_2 gas is greater than about 20%, system may become limited by PEP carboxylase activity.

Overexpressing pyruvate carboxylase may increase succinate production.

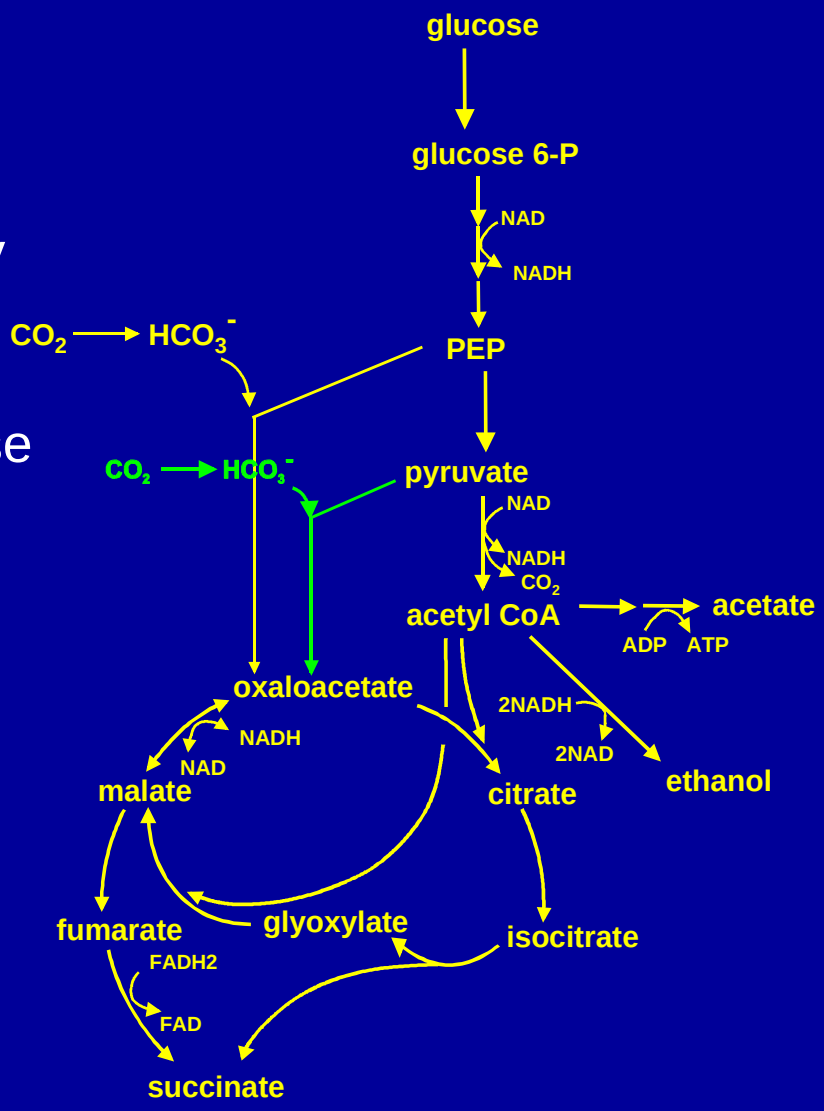
Procedure

50% CO_2

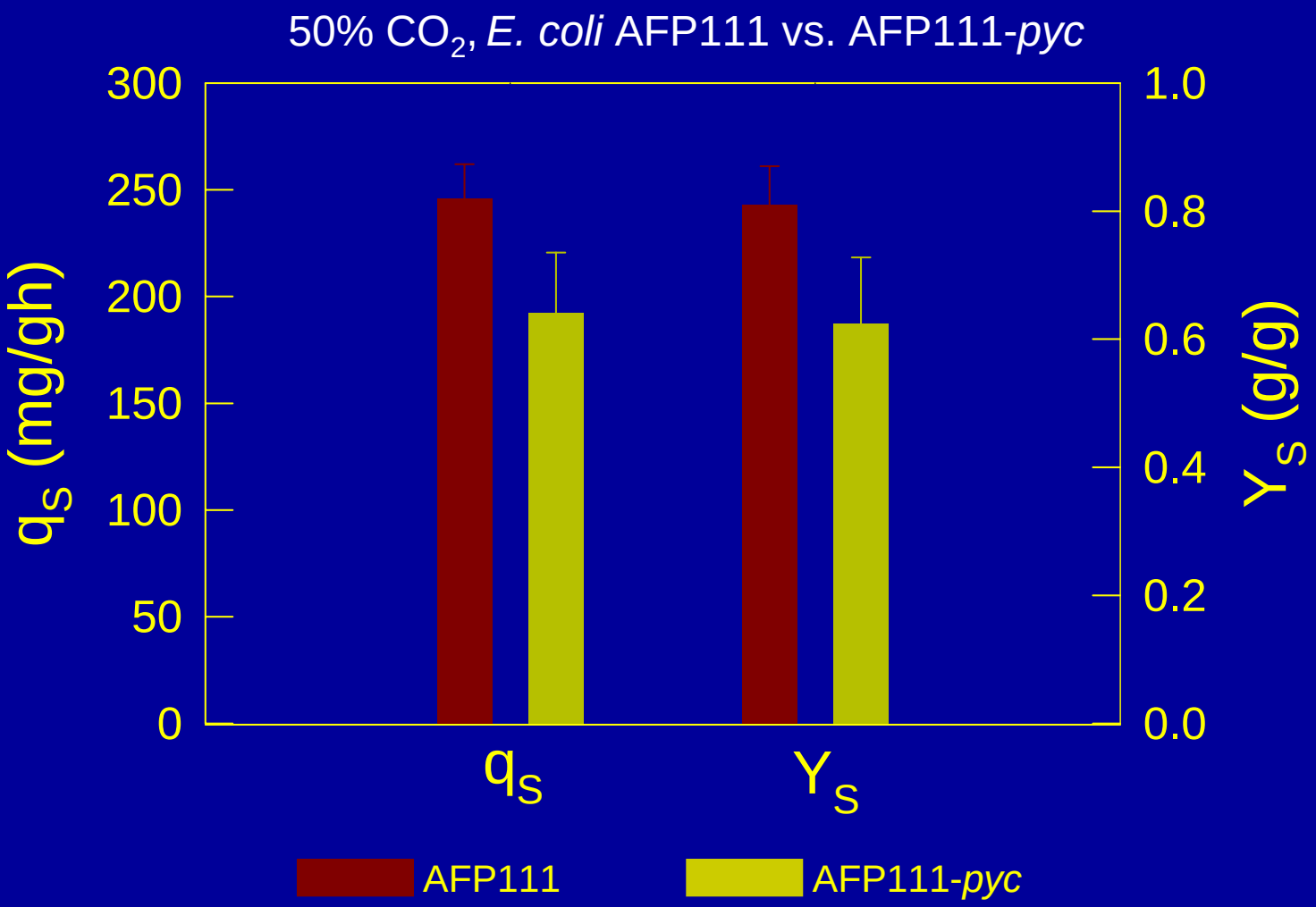
AFP111 vs. AFP111 pTrc99A-*pyc*

pH of 6.4

25% $Ca(OH)_2$



Effect of Pyruvate Carboxylase (Cont.)



Conclusions

- CO_2 was a rate-limiting factor at 10%; succinate formation did not increase with increasing CO_2 concentrations above 50%
- Higher agitation rate slightly increased succinate production at 10% CO_2
- With 50% CO_2, overexpressing pyruvate carboxylase did not improve succinate production

Acknowledgments

James Kastner
Sarah Lee
Greg Wylie
Yihui Zhu
Prabu Vijayan

Georgia Experiment Station