Anaerobic Fermentation to Produce Carboxylic Acids and Inactivate *Ascaris* Eggs

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Our Goal:

Inactivate pathogens in fecal solids through *in-situ* production of carboxylic acids

Outline

Introduction

- What are carboxylic acids?
- Carboxylic acids and pathogens
- Production of carboxylic acids

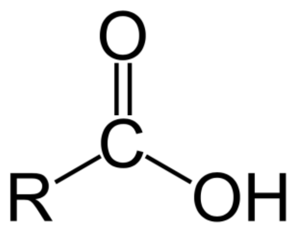
Methods and Results

- Batch fermentation
- Inactivation of *Ascaris* eggs

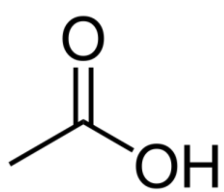
Summary and Future Work

What is a carboxylic acid?

Carboxylic acid: a weak organic acid containing a carboxyl group

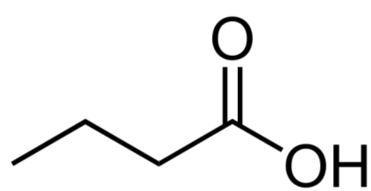


We most often think of ***fatty acids*** (hydrocarbon chains followed by a carboxyl group).



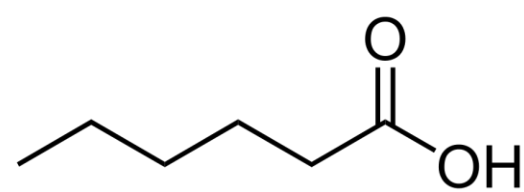
Acetic acid

C2



n-Butyric acid

C4

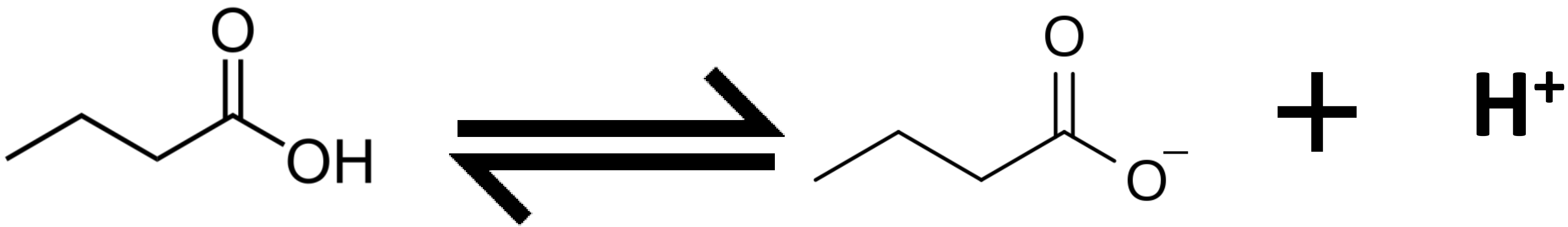


n-Caproic acid

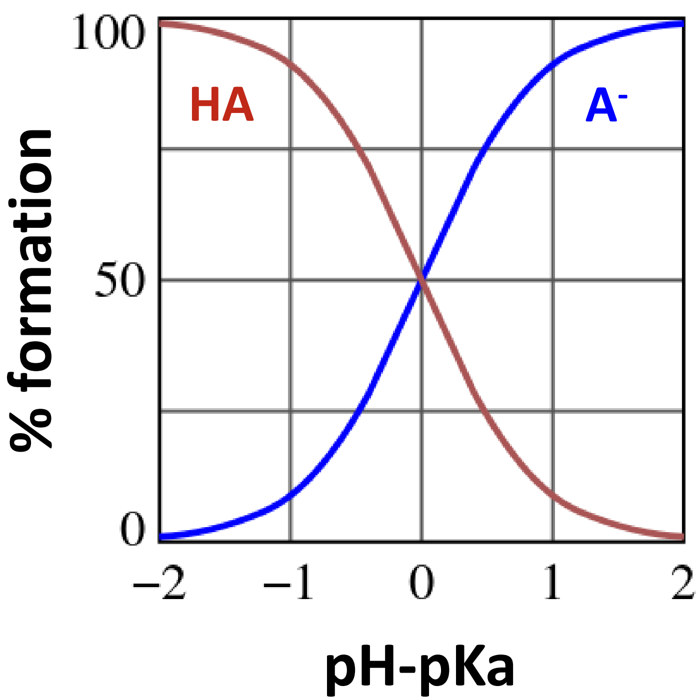
(Hexanoic Acid)

C6

A note about weak acids



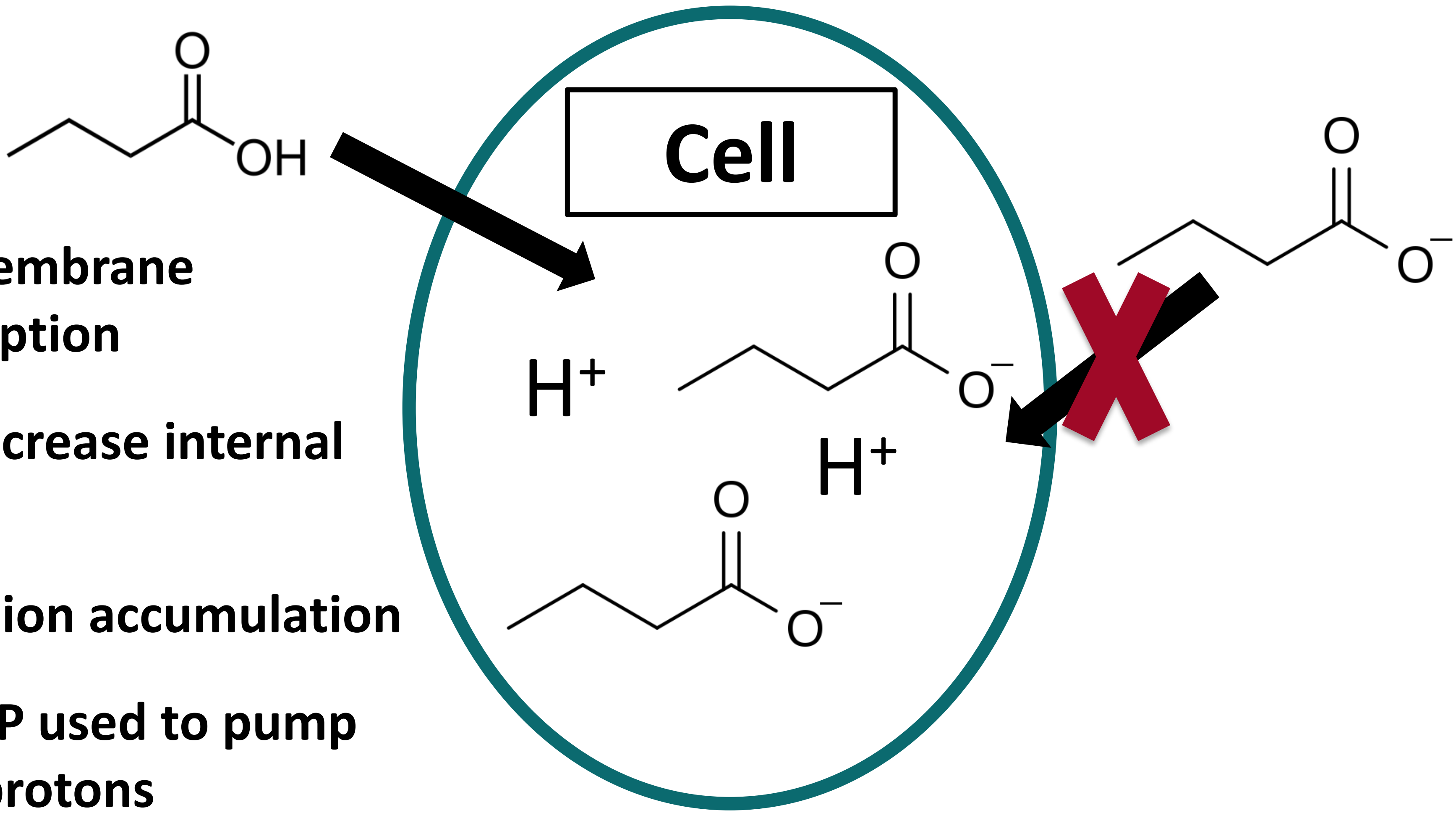
n-Butyric Acid $\underset{K_a}{\rightleftharpoons}$ *n*-Butyrate



$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

pK_a=4.8

Carboxylic acids and pathogens



1. Membrane disruption

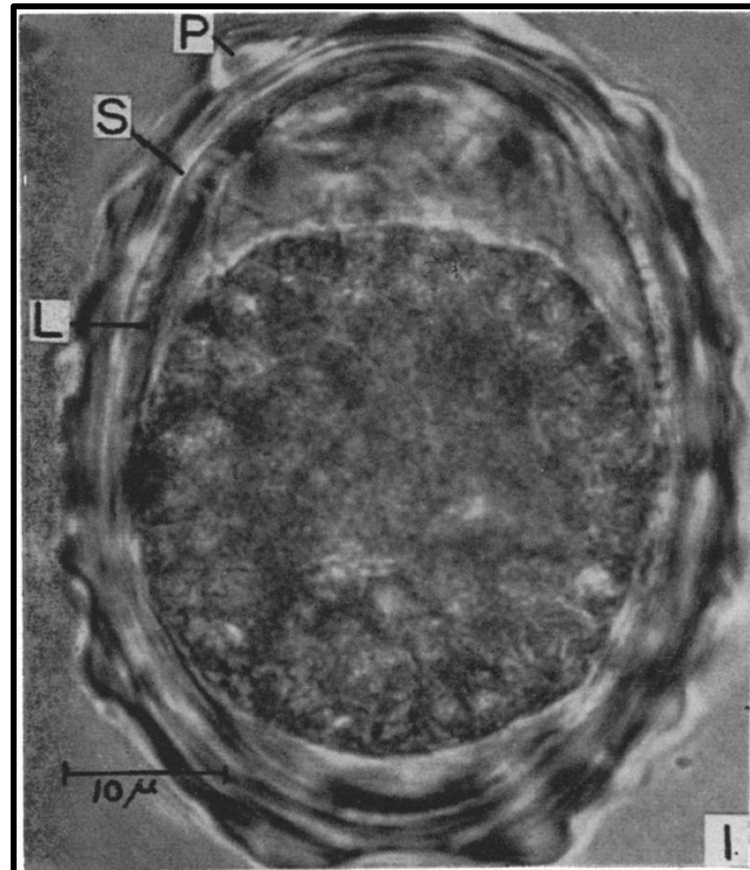
2. Decrease internal pH

3. Anion accumulation

4. ATP used to pump out protons

Ascaris as a model pathogen

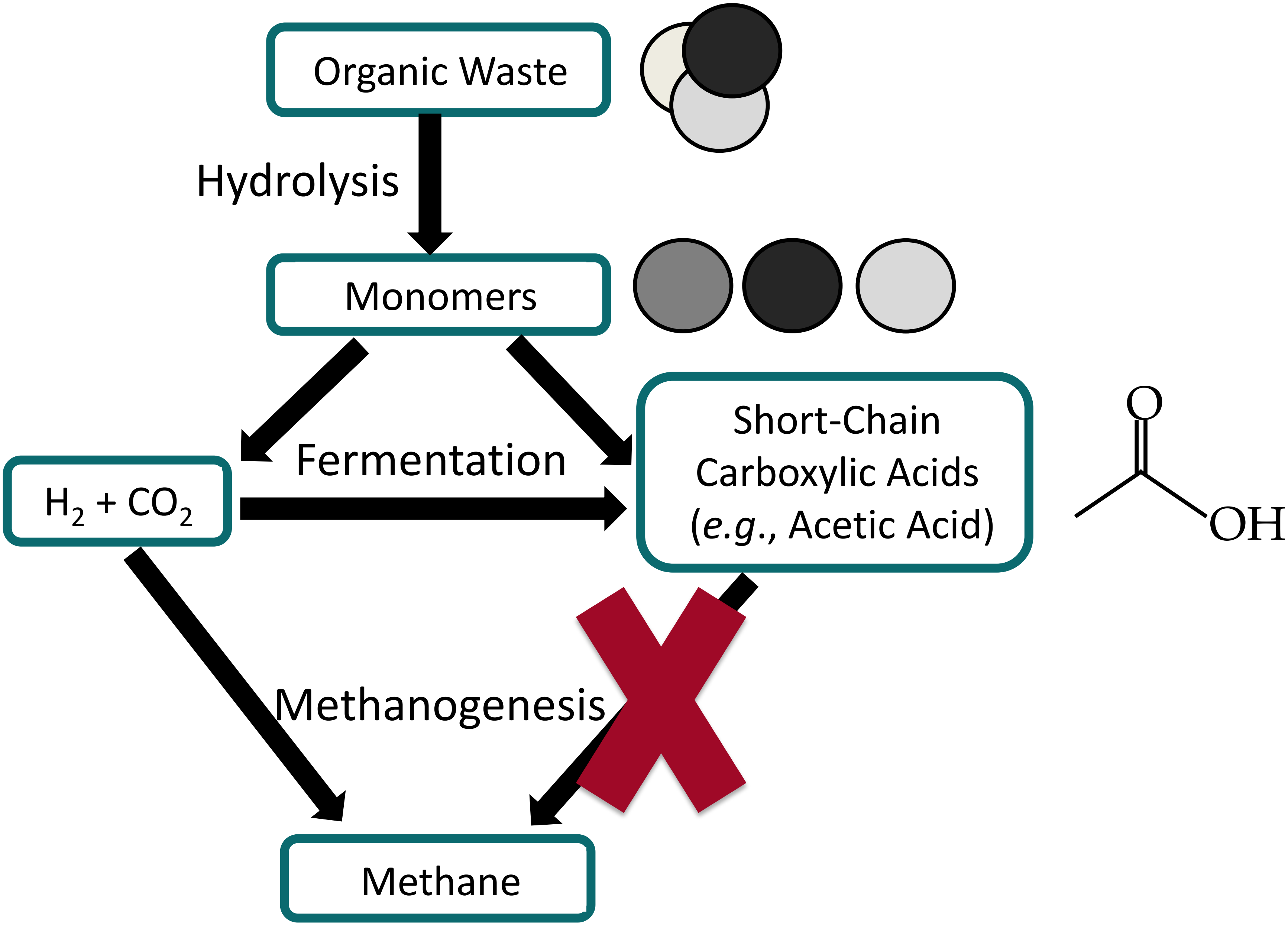
medicalstate.tumblr.com



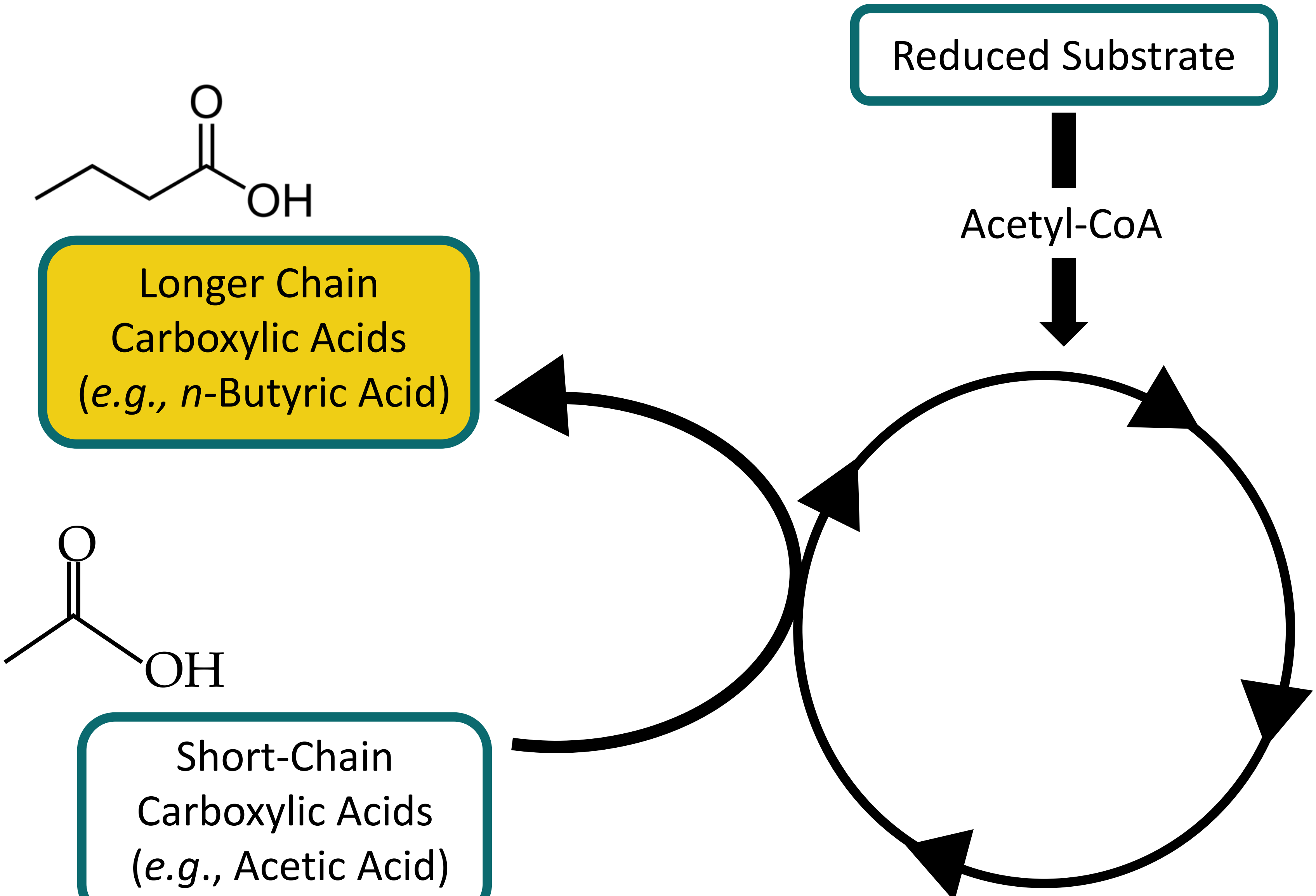
Rogers, 1956

Butkus, M. A., *et al*. 2011. Inactivation of *Ascaris suum* by short-chain fatty acids. *Applied and environmental microbiology, 77*(1), 363-366.

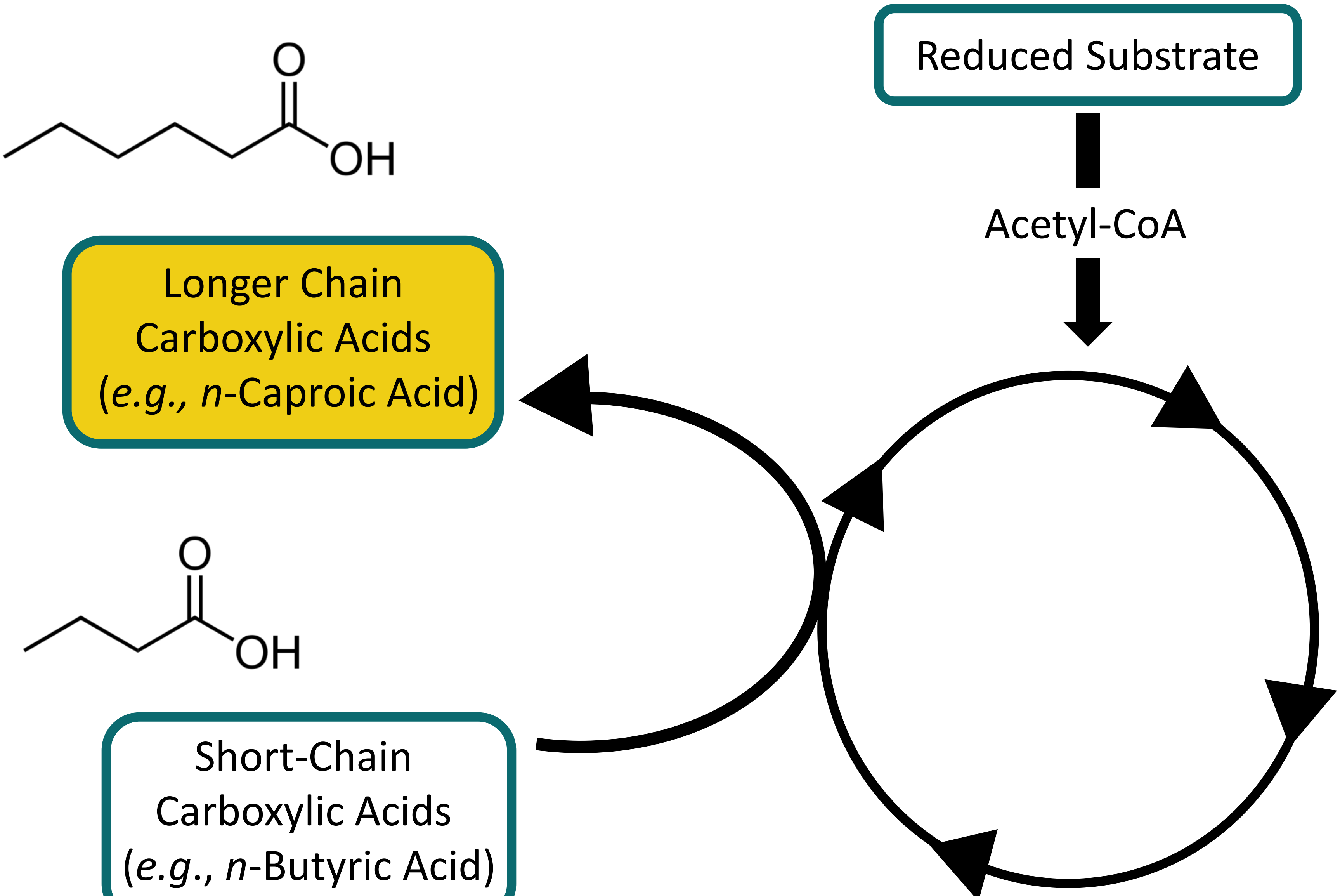
Carboxylic acid production and chain elongation



Chain Elongation



Chain Elongation



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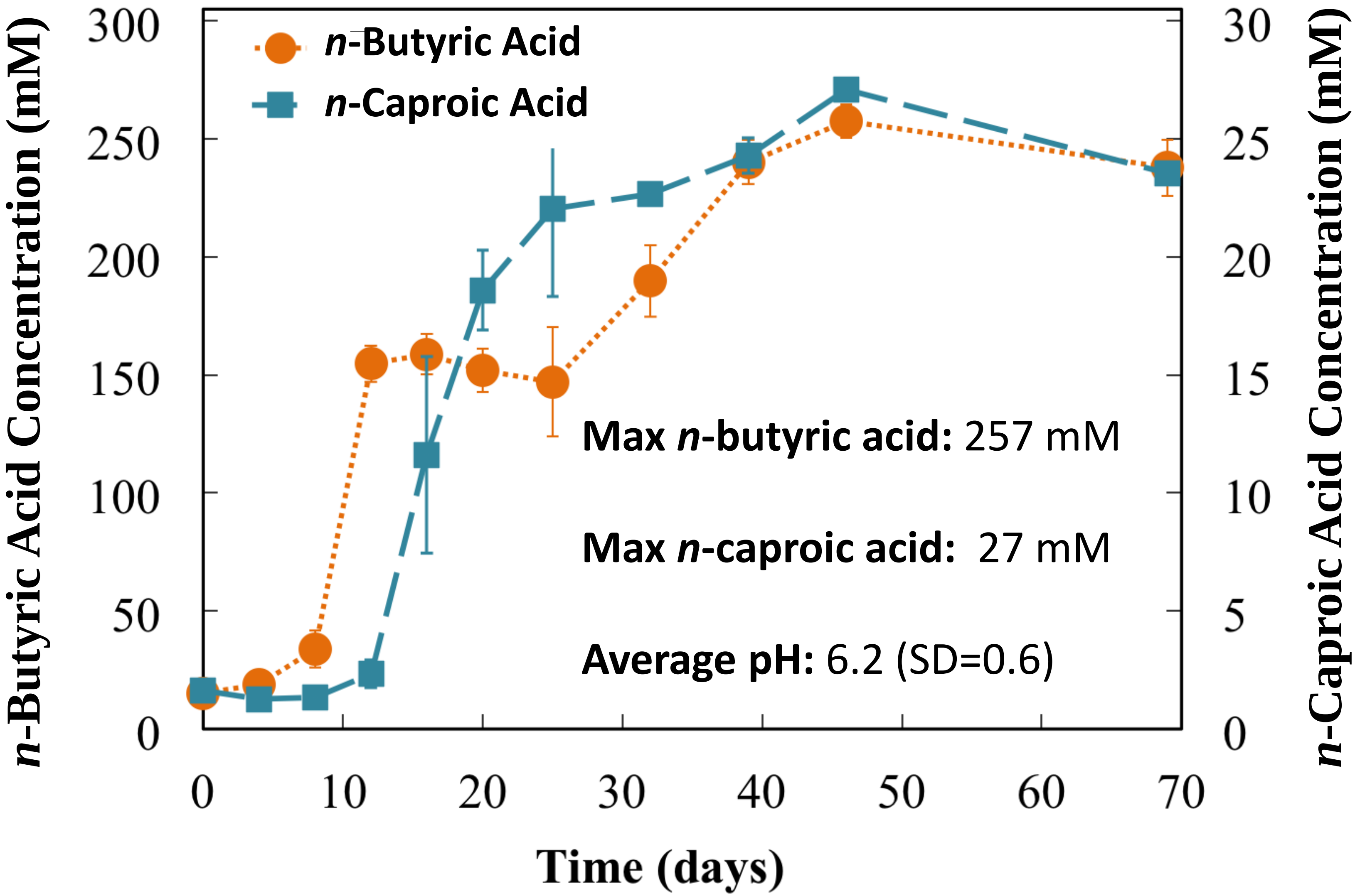
Batch fermentation to produce carboxylic acids

- Fecal solids and inoculum from a carboxylate-producing reactor
- Incubated anaerobically at 30°C
- Sampled every 4-7 days for 69 days

Batch fermentation to produce carboxylic acids

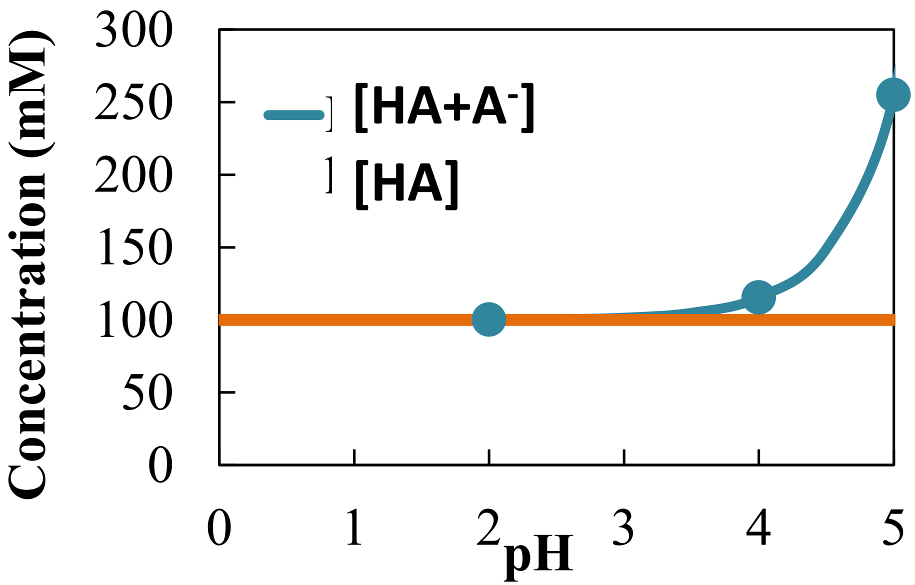


Parameters controlling *Ascaris* viability

- Concentration
- pH
- Carbon chain length
- Exposure time
- Temperature

Ascaris viability: Carboxylic acid concentration and pH

- Three pH levels tested (pH= 2,4,5)
- Same concentrations of uncharged acids (HA) tested at each pH



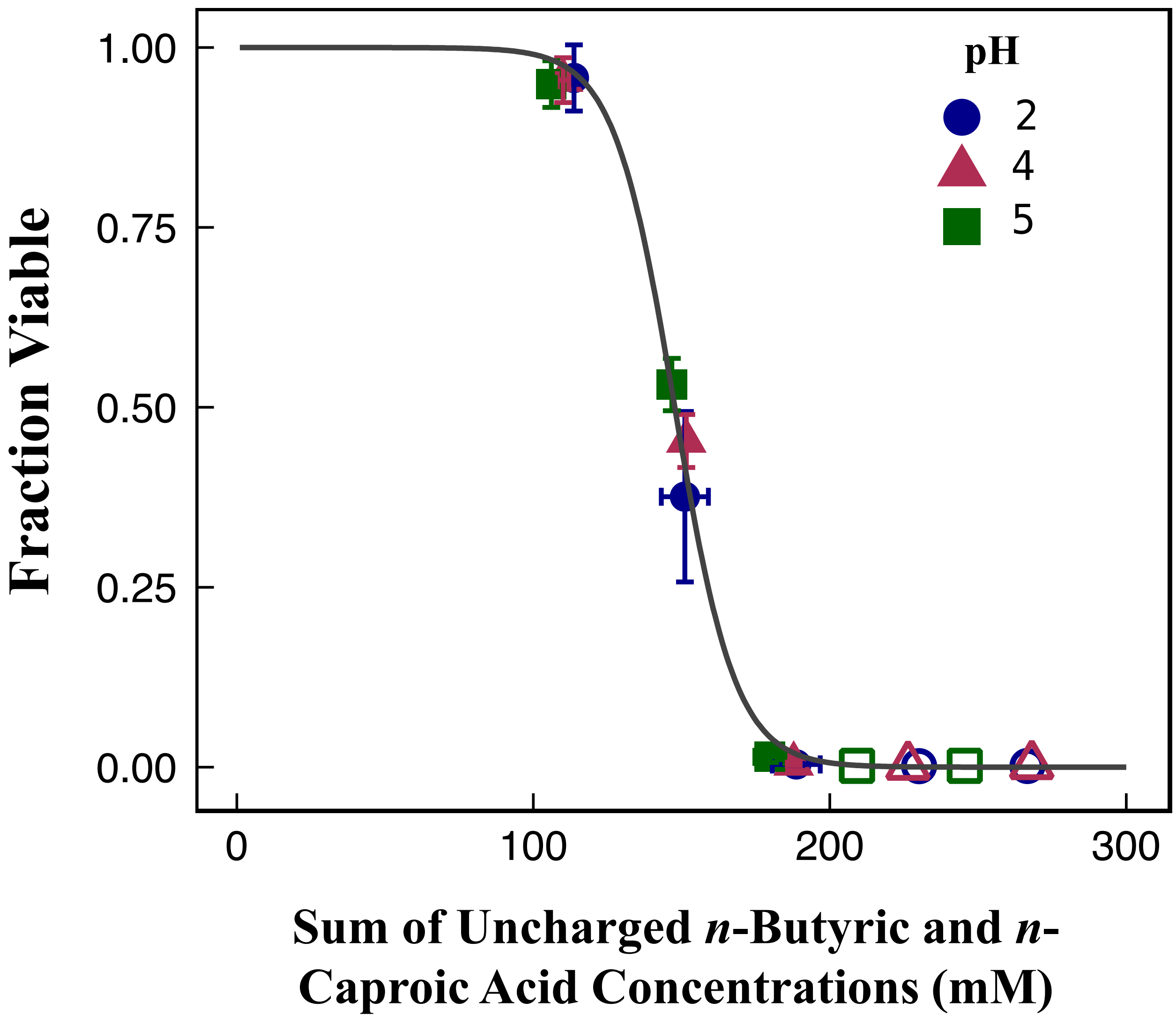
$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

$$[HA + A^-] = [HA]\,(10^{(pH-pKa)} + 1)$$

Ascaris viability: Carboxylic acid concentration and pH

- Exposed for 3 days at 37°C
- Washed and incubated for 21 days at 30°C
- 500 eggs examined for viability

Ascaris viability not dependent on anion concentration or pH



$$y = \frac{1}{1 + \exp(-a(x - b))}$$

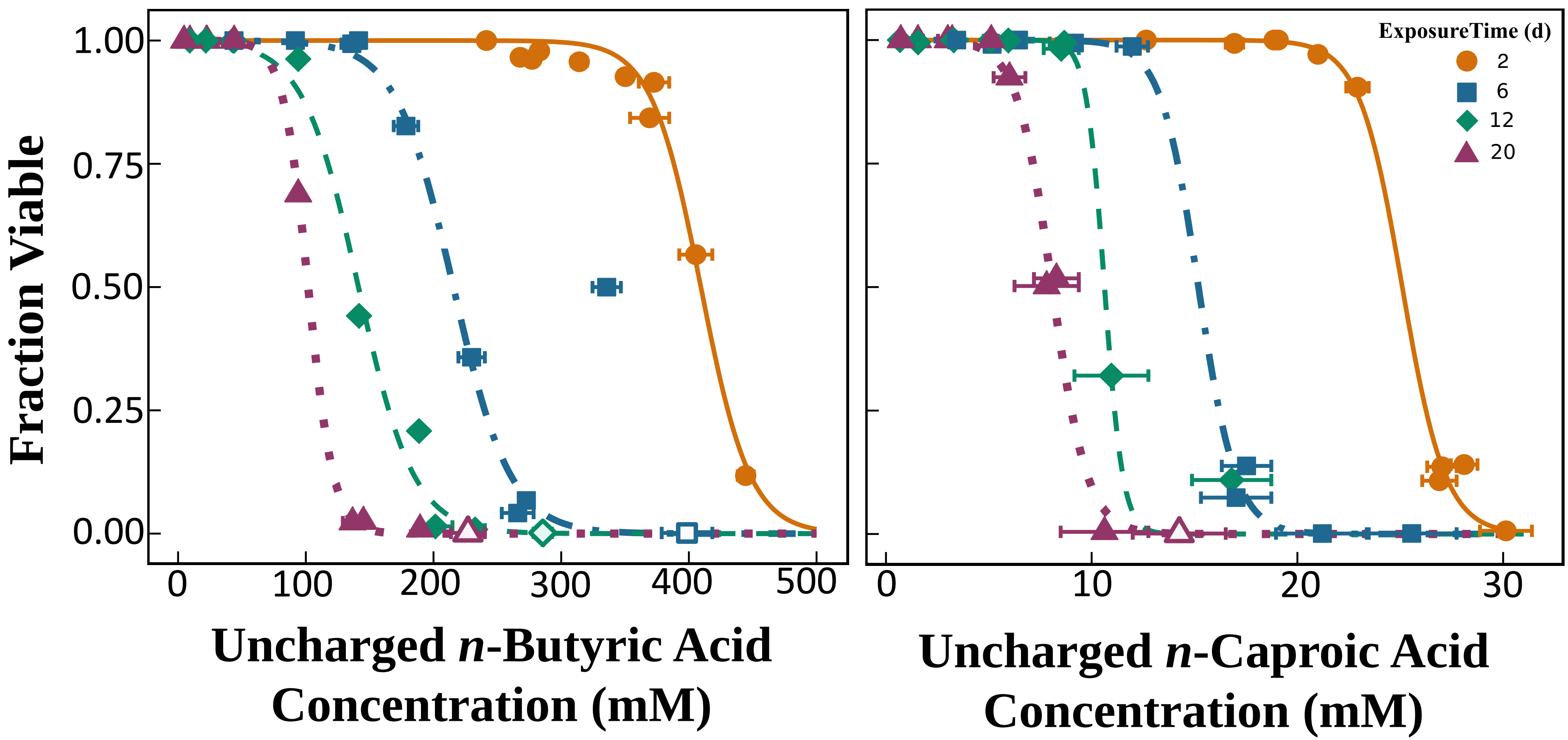
Parameters controlling *Ascaris* viability

- Concentration
- pH
- Carbon chain length
- Exposure time
- Temperature

Ascaris viability: Concentration, chain length, and exposure time

- *A. suum* eggs exposed to *n*-butyric acid and *n*-caproic acid individually
- pH 2
- Concentration and exposure time varied
- T=30°C
- Anaerobic

Ascaris viability dependent on concentration, chain length, and exposure time



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Conclusions

- **We can produce carboxylic acids from fecal solids.**
- **The concentration of uncharged acid is important.**
- **For individual acids, we can predict *Ascaris* inactivation based on exposure time and concentration.**

Future Work

- **Decrease pH through fermentation**

- **Understand different parameters relating to inactivation:**
    - **Temperature**
    - **Mixtures of acids**
    - **Matrix**

- **Scale up**

Acknowledgements

Angenent and Bowman Labs

Ascaris inactivation

- Dr. Michael P. Labare, PhD
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- Zhu Dan, MEng

Statistics

- Dr. Lynn Johnson, PhD

  Cornell Statistical Consulting Unit

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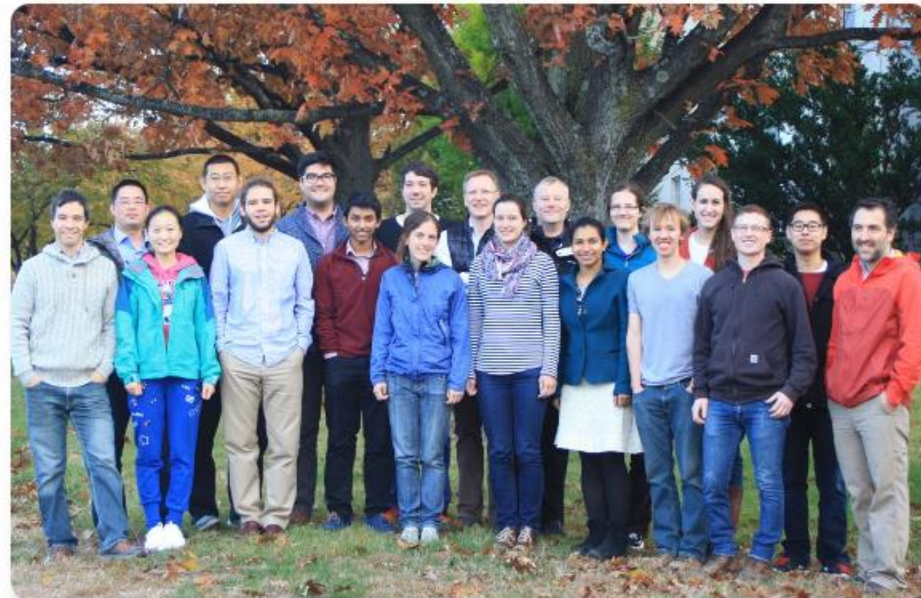

Atkinson Center for a Sustainable Future

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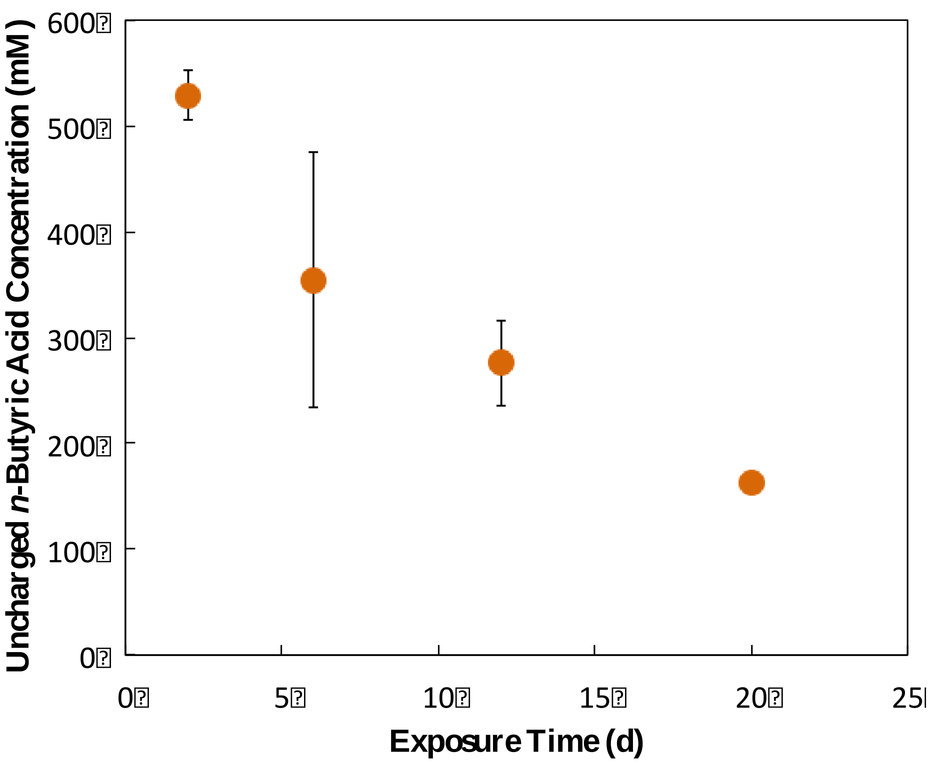
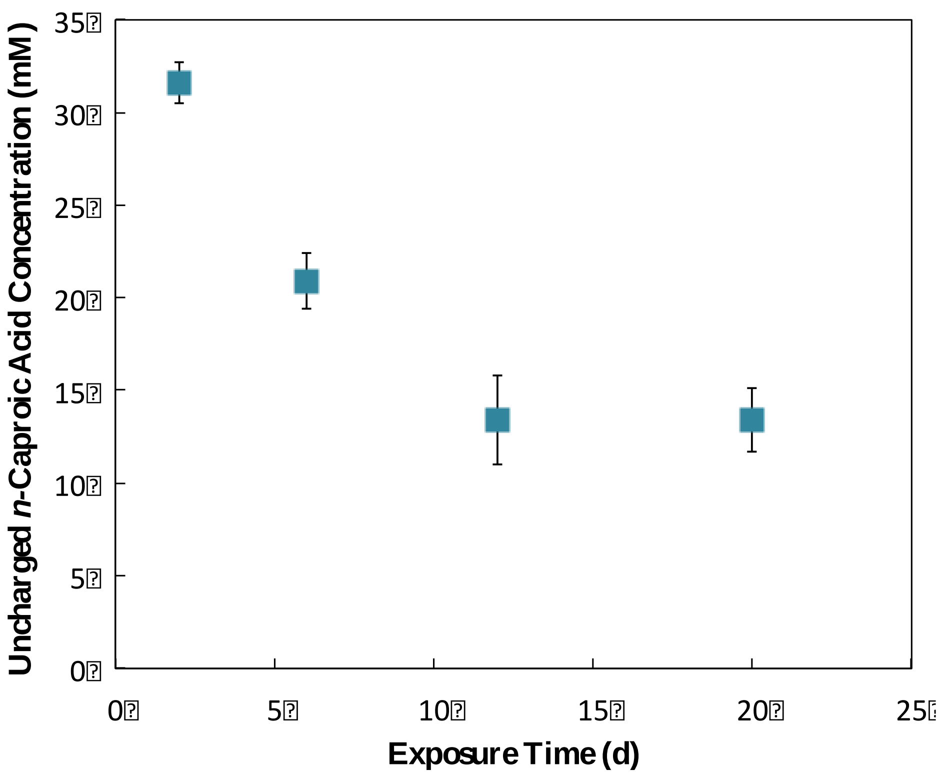
Conclusions

- We can produce carboxylic acids from fecal solids.
- The concentration of uncharged acid is important.
- For individual acids, we can predict *Ascaris* inactivation based on exposure time and concentration.

Predict time and concentration requirements for complete inactivation





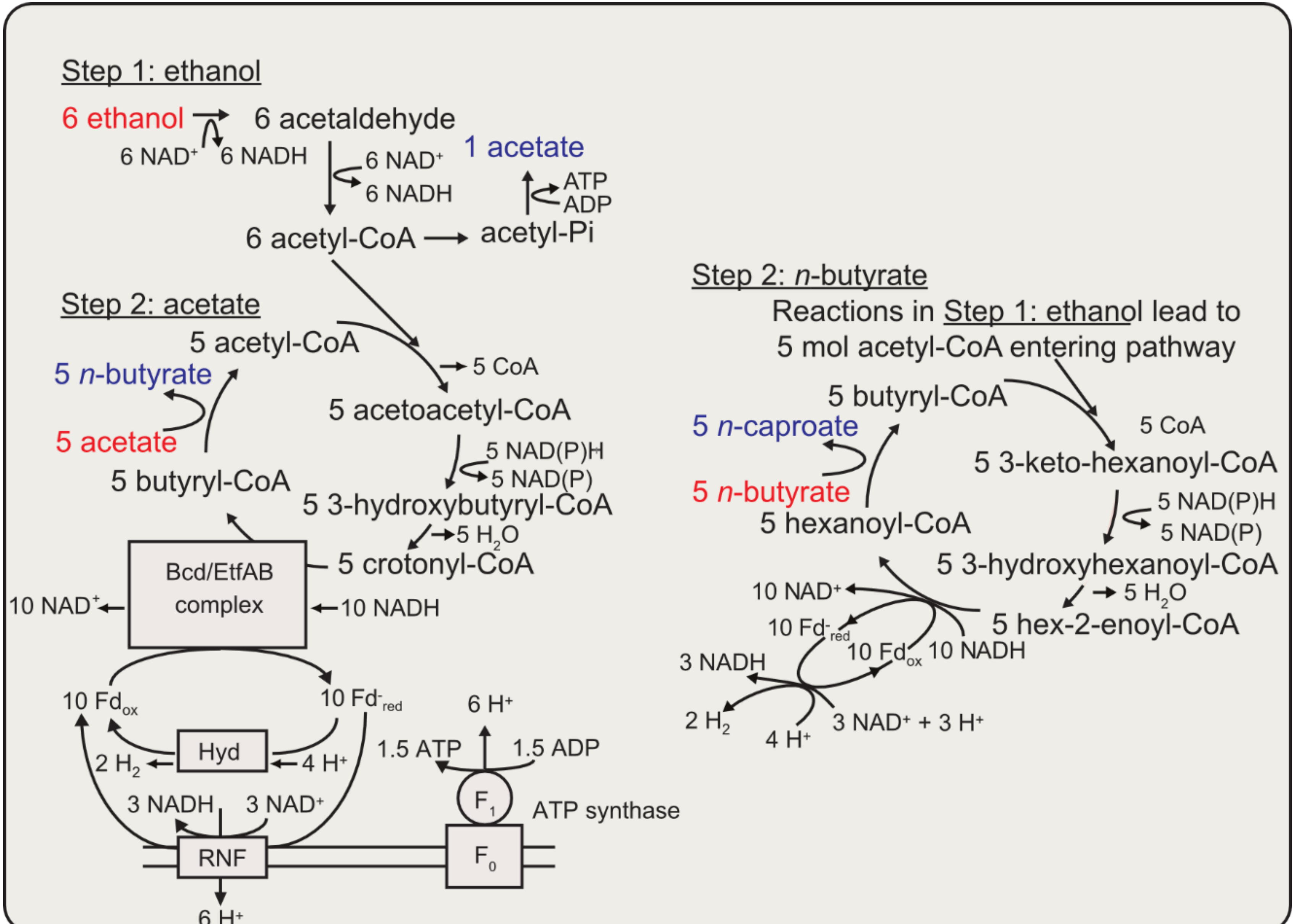
Predict time and concentration requirements for complete inactivation

Acid	Exposure Time (d)	Predicted Uncharged Concentration Required to Reduce Viability Below Detection (0.2%) (mM)	95% Confidence Interval (mM)
n-Butyric	2	529	506-553
n-Butyric	6	354	234-475
n-Butyric	12	276	236-316
n-Butyric	20	163	158-169
n-Caproic	2	32	31-33
n-Caproic	6	21	19-22
n-Caproic	12	13	11-16
n-Caproic	20	13	12-15

Model parameters

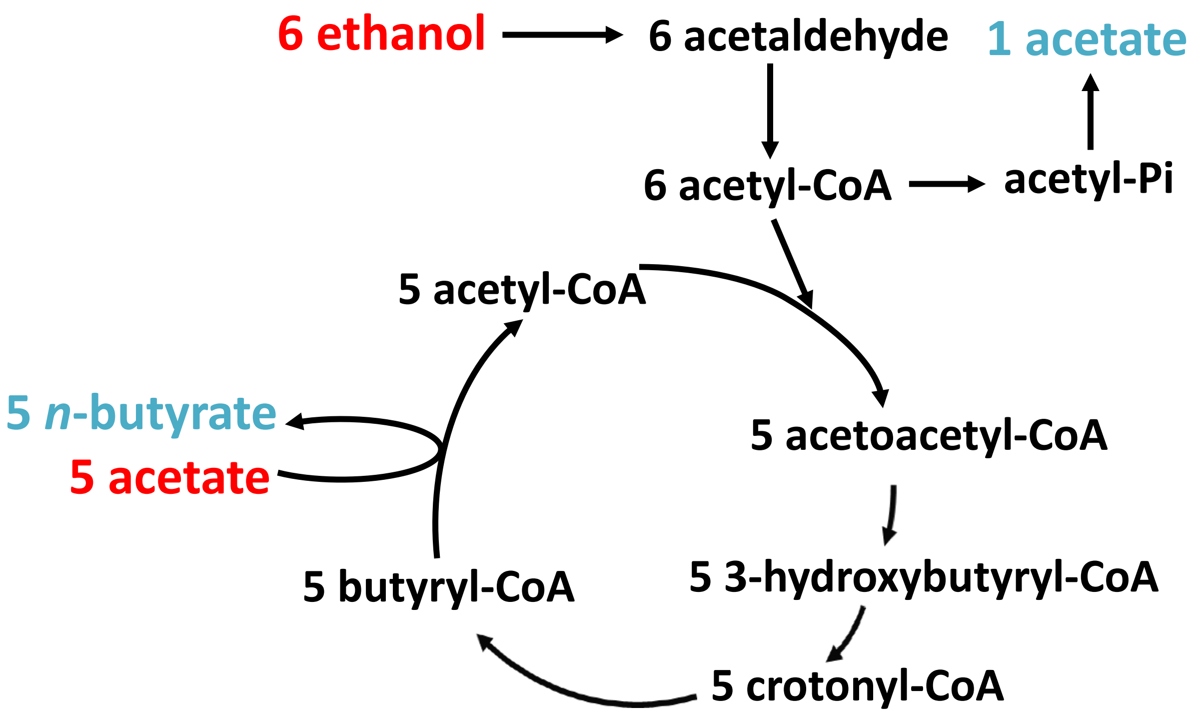
Acid	Exposure Time (d)	a (std error)	p-value a	b (std error)	p-value b	Mean Square Error	Mean Absolute Error
Butyric	2	-0.0519 (0.00480)	4.71*10^(-6)	409 (2.23)	8.75*10^(-16)	0.000981	0.0282
Butyric	6	-0.0448 (0.0203)	0.0582	216 (14.1)	3.23*10^(-7)	0.025	0.0641
Butyric	12	-0.0462 (0.00707)	0.000182	141 (3.85)	3.36*10^(-10)	0.00207	0.0303
Butyric	20	-0.0101 (0.00418)	9.26*10^(-9)	102 (0.388)	2*10^(-16)	2.58* 10^(-5)	0.00315
Caproic	2	-0.954 (0.0927)	6.87*10^(-6)	25.1 (0.200)	1.83*10^-14)	0.000987	0.0171
Caproic	6	-1.09 (0.199)	0.000585	15.2 (0.377)	1.58*10^(-10)	0.000676	0.0142
Caproic	12	-2.24 (1.11)	0.0784	10.6 (0.179)	7.22*10^(-12)	0.00121	0.013
Caproic	20	-1.17 (0.188)	0.000254	8.08 (0.103)	7.61*10^(-13)	0.00146	0.024

Reverse Beta-Oxidation



Slide by: C. Spirito

Reverse Beta-Oxidation: C2 to C4

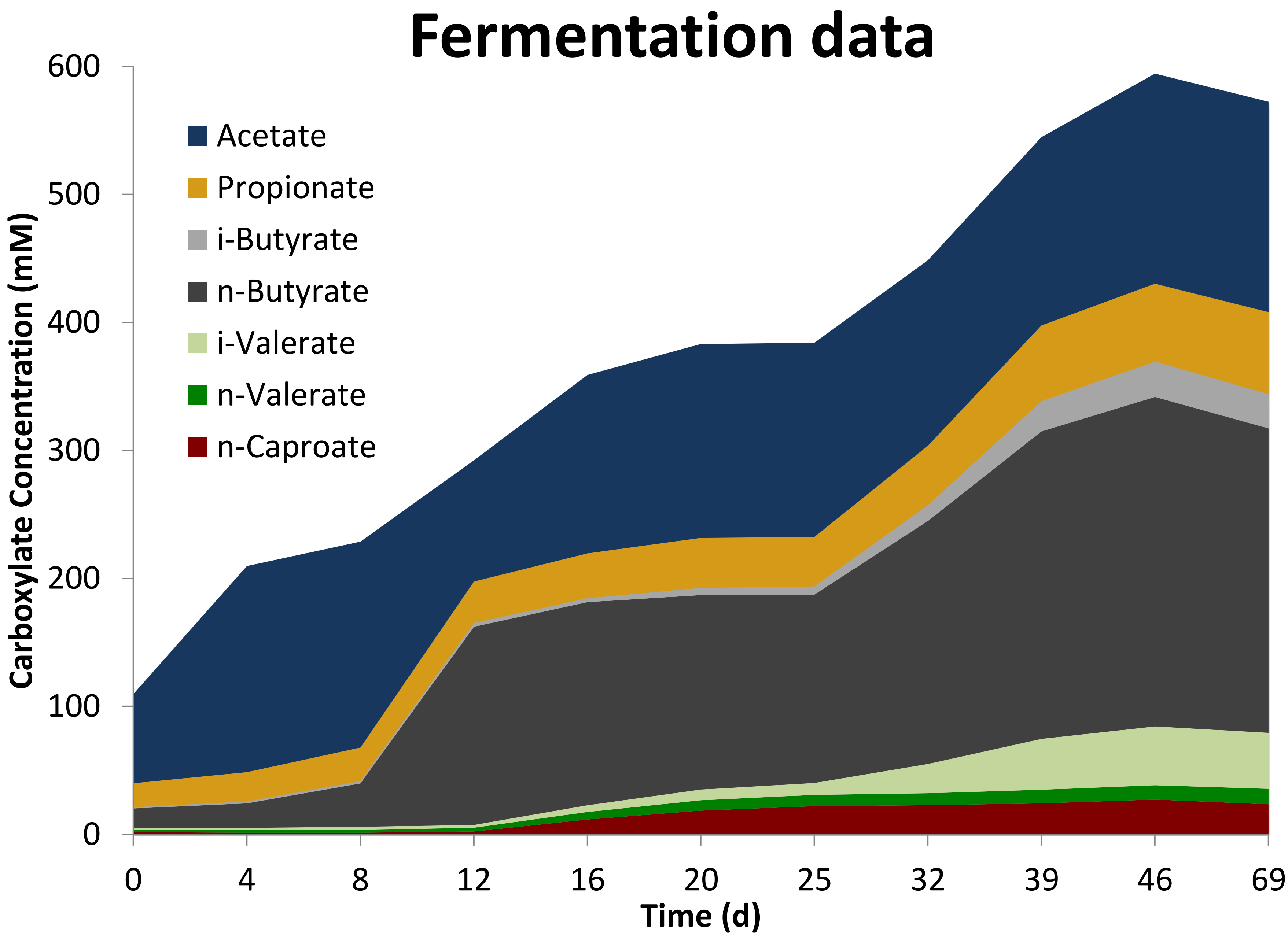


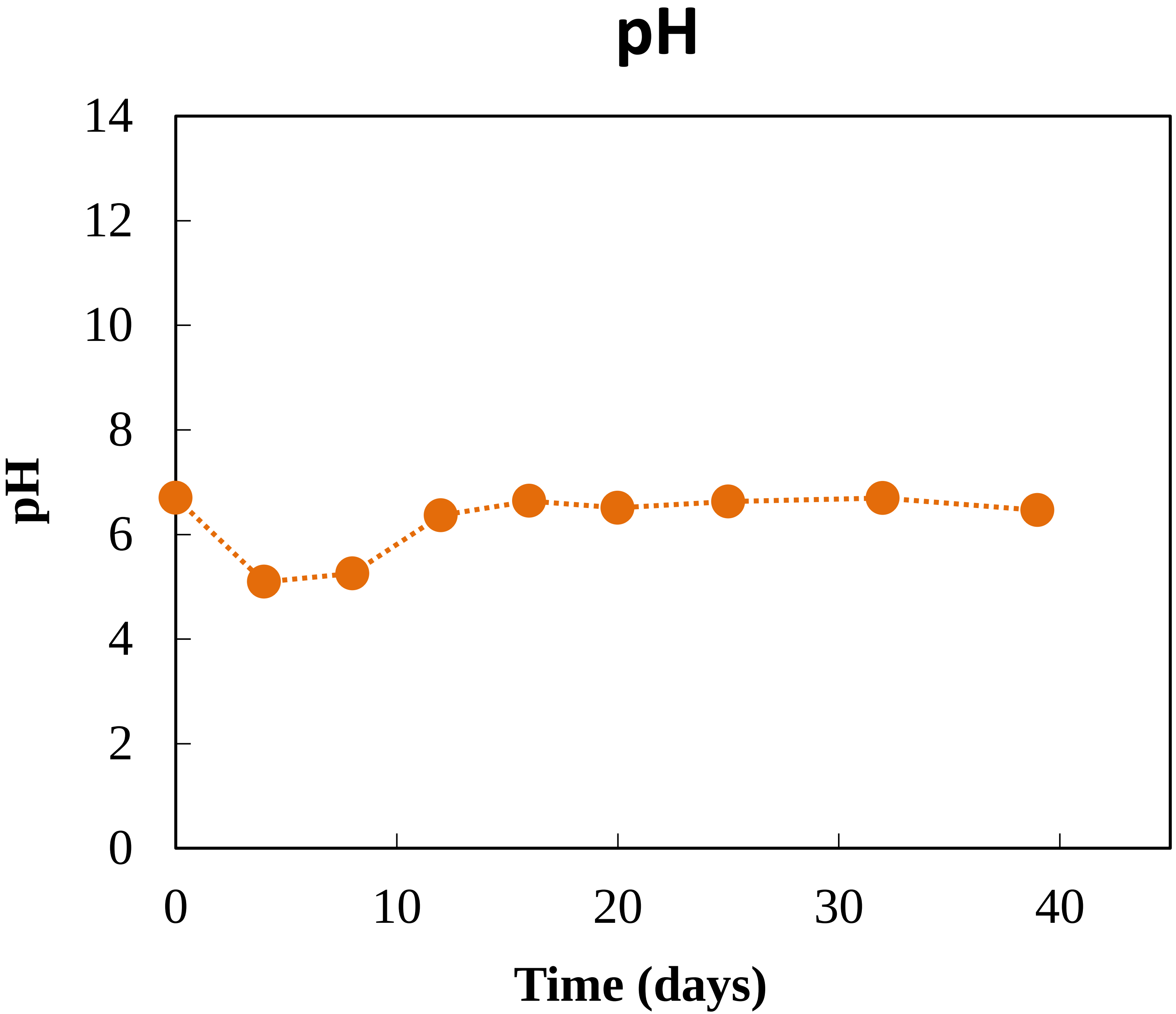
$$6 \text{ ethanol} + 4 \text{ acetate}^{-} \rightarrow 5\ n\text{-butyrate}^{-} + H^{+} + 2H_2 + 4H_2O$$

Slide by: C. Spirito

Concentrations for uncharged acid data

Treatment #	pH	Uncharged Butyric Acid (mM)	Uncharged Caproic Acid (mM)	Total Butyrate (mM)	Total Caproate (mM)
1	2	100	10	100	10.0
2	2	135	13.5	135	13.5
3	2	170	17	170	17.0
4	2	205	20.5	205	20.5
5	2	240	24	240	24.0
6	4	100	10	115	11.3
7	4	135	13.5	155	15.3
8	4	170	17	196	19.2
9	4	205	20.5	236	23.2
10	4	240	24	276	27.2
11	5	100	10	251	23.2
12	5	135	13.5	339	31.3
13	5	170	17	427	39.4
14	5	205	20.5	515	47.5
15	5	240	24	603	55.6


Fermentation data
Acetate
Propionate
i-Butyrate
n-Butyrate
i-Valerate
n-Valerate
n-Caproate
Carboxylate Concentration (mM)
0
100
200
300
400
500
600
Time (d)
0
4
8
12
16
20
25
32
39
46
69


pH
pH
0
2
4
6
8
10
12
14
0
10
20
30
40
Time (days)

Ascaris eggs exposed to *n*-butyric acid and *n*-caproic acid in fecal sludge