Thermal processing of acidified foods: pH 4.1 to 46 (and related data for acidified foods)

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Research priorities

- Prevent an outbreak of pathogenic bacteria in acid and acidified vegetable products…
  - What is the greatest threat?
  - What is the likelihood of occurrence?
- Science based regulation
  - Fill in knowledge gaps
    - Industry needs and regulatory questions
  - Novel ways of producing safe products?
- Fundamental knowledge about pathogens in acidified foods.
  - Acid resistance and survival of pathogens in acid and acidified foods

Current projects

- Applied research (*E. coli, Salmonella,* and *Listeria*)
  - Thermal processing at pH 4.6
  - Cold-Fill-Hold studies at pH 3.5
  - Alternative acids (citric, phosphoric, preservative acids)
  - Spore-forming bacilli, pH increase?
- Basic research (primarily *E. coli* O157:H7 and related serotypes)
  - Modeling internal pH, charge/ion balance
  - Internal cell metabolites
  - Acid resistance of alternate *E. coli* serotypes (O104:H4)
  - Modeling buffer capacity

Additional funding and support

- National Integrated Food Safety Initiative: *Bridging the Gap: Integrated Research and Extension in Support of Small Processors of Acidified Canned Foods*, 3Yr
  - Some funds for research to fill the knowledge gaps: cold fill hold, thermal processing, and bacillus spoilage (pH rise)
  - Project Investigators (PI's): Dr. Barbara H. Ingham (Lead) & Dr. Fletcher Arritt
- Collaborator with Dr. David Green: *Assisting the Integrated Food Safety System's National Food Training Program*, 3 Yr.
  - FDA Training (curriculum committee)
- Direct industry support

Three big questions

1. What conditions are needed for thermal processing acidified foods at 4.6?
   - Vegetative pathogen kill at pH 4.6
   - Thermal processing for spores with organic acids
2. Can bacillus spores germinate and raise pH under anaerobic conditions in a variety of acidified vegetables?
   - What is the mechanism of pH increase?
   - Role of oxygen
   - Buffering
3. Can ‘reasonable’ cold fill hold conditions at pH 3.5 and 10^{o}C (50^{o}F) be identified?
   - Different organic acids and concentrations

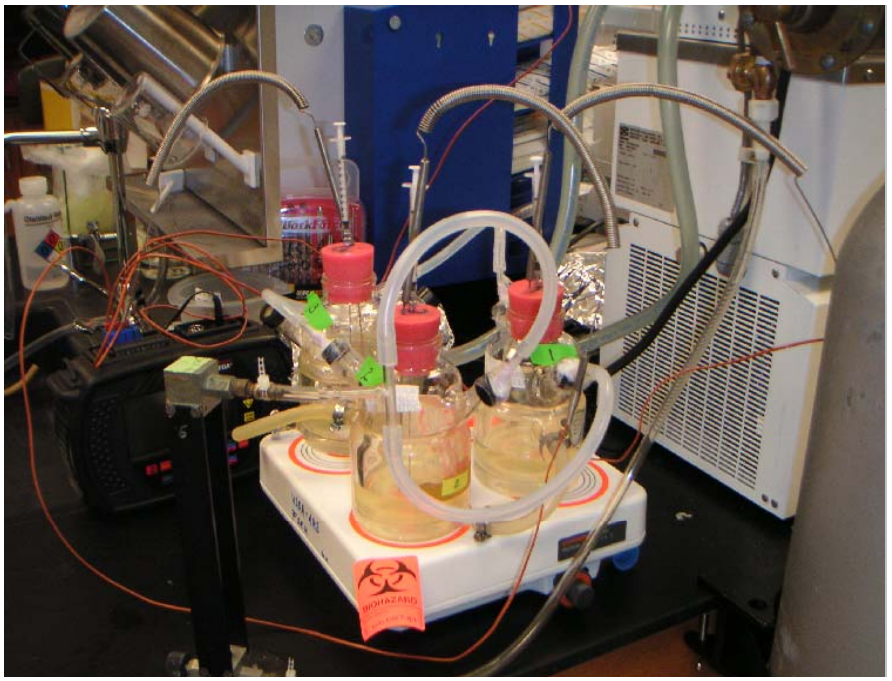
1. Thermal processing at pH 4.6

- Current published data is for pH 4.1
  - Acidified pickles
  - Cucumbers juice as a “generic” vegetable medium
- Vegetative cells *vs.* Spores (questions 1 & 2)
  - Tomato products, bad seals, and FDA concerns?
  - Industry knowledge? (Fred.Breidt@ars.usda.gov)
  - Effects of organic acids on 5D kill of vegetative pathogens
  - pH effect vs. organic acids
- Another important question: *Is a 5-D kill the right target to shoot for?*
  - Risk assessment approach?
  - Not just for thermal processing!

Thermal processing: microbiological methods

- Use a cocktail of acid resistant EHEC strains
  - Most heat/acid resistant in vegetable broth medium
- Induce acid resistance
  - Static growth at 37^{o}C
- Cucumber juice medium
  - Non-inhibitory
  - pH 4.6, 0.6% acetic acid
- Use non-selective media for plating cells
- Independent replication

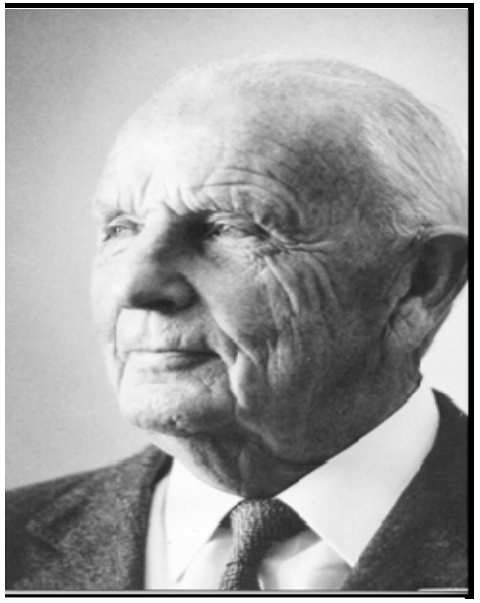
The *E. coli* inquisition…



Modeling approach

1. Generate Log CFU/ml *vs.* time data
2. Determine 5D reduction value and the standard error (SE) using a version of the Weibull model

   Note: Dr. Jason Osborne, NCSU statistics
3. Plot the $\log_{10}$ (5D + 5xSE) *vs.* Temperature to determine Z value
4. Determine the survival a reference temperature of 160^{o}F (F_{160})



Wallodi Weibull 1887-1979
Photo by Sam C. Saunders

pH 4.1 or lower:

TABLE 2. Z and F Values

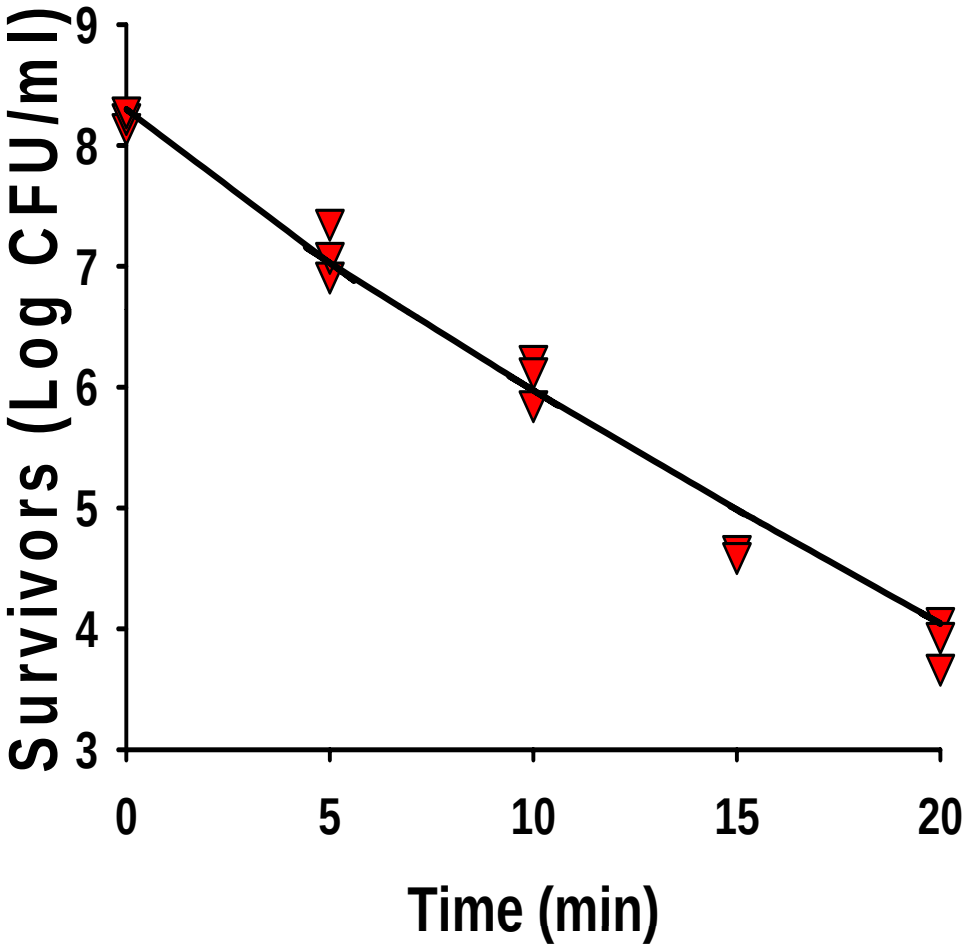
Model[a]	Z value (°F)	F_{160}[b]
Exp. Decay (5SE)	19.50	1.20
Exp. Decay	15.70	0.34
Five D Model	11.98	0.08
One D Model	11.98	0.02

[a]Models as described in the text: Exp. Decay (5SE), exponential decay model with five times the standard error added; Exp. Decay, exponential decay model; Five D Model, linear model based on a five log reduction; One D Model, linear model based on a one log reduction.

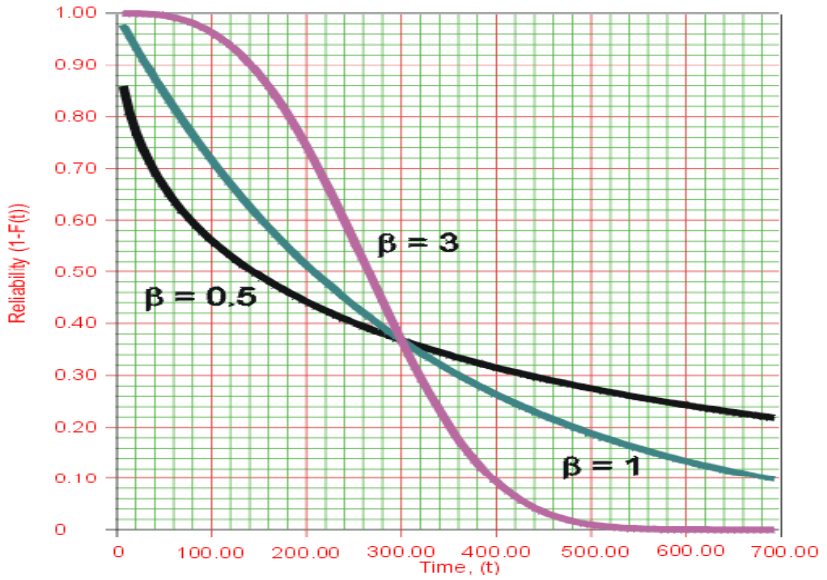
[b]F_{160}: Time in minutes (F value) needed to achieve the predicted reduction in cell numbers at a reference temperature of 160°F.

Breidt F, Sandeep KP, Arritt F. 2010. Use of Linear Models for Thermal Processing of Acidified Foods. *Food Prot Trends* 30(5):268-272.

TDT data 64ºC (147ºF)

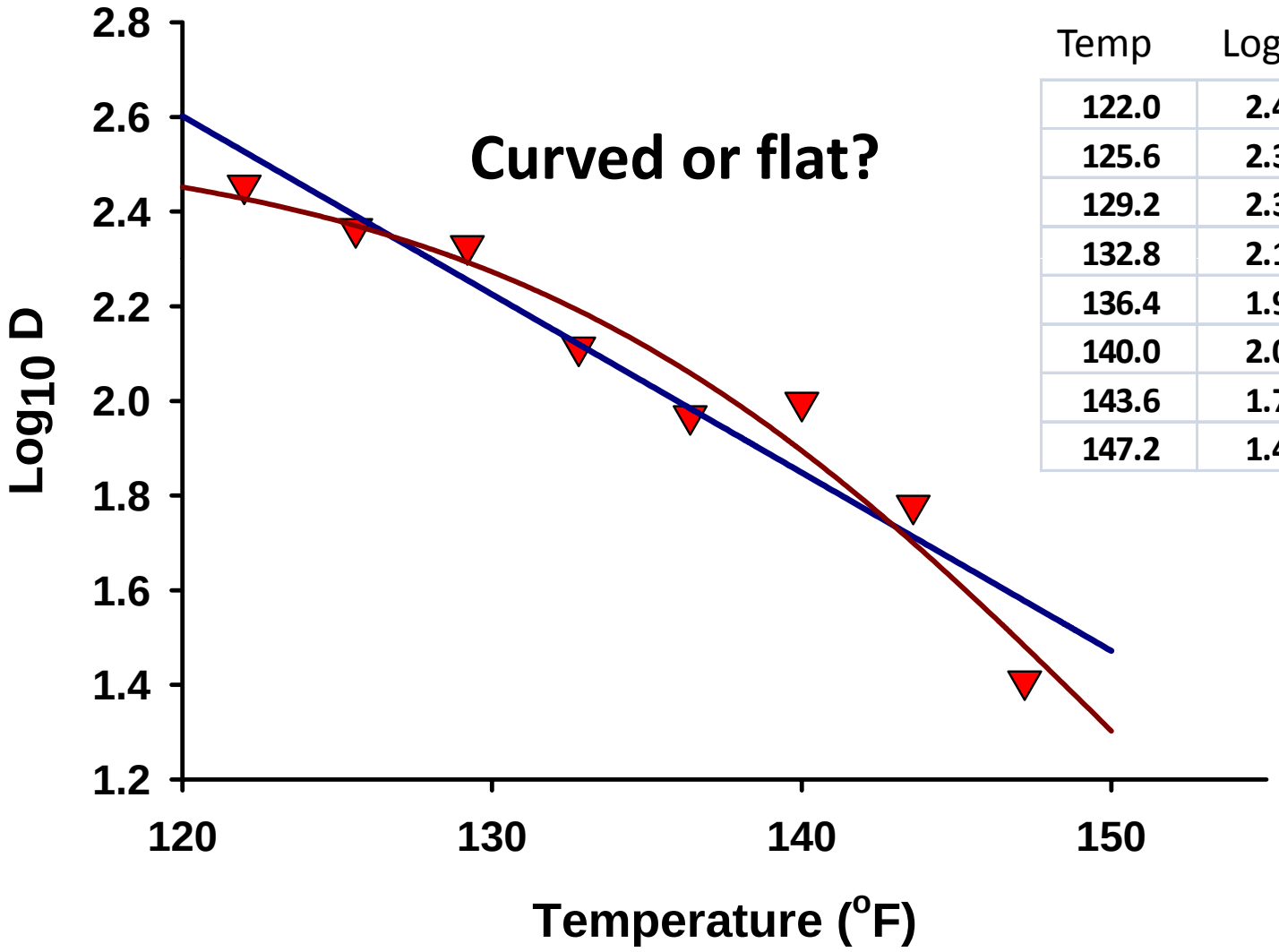


$$\log S = N_0 - 5(\tau/t^*)^{\beta}$$



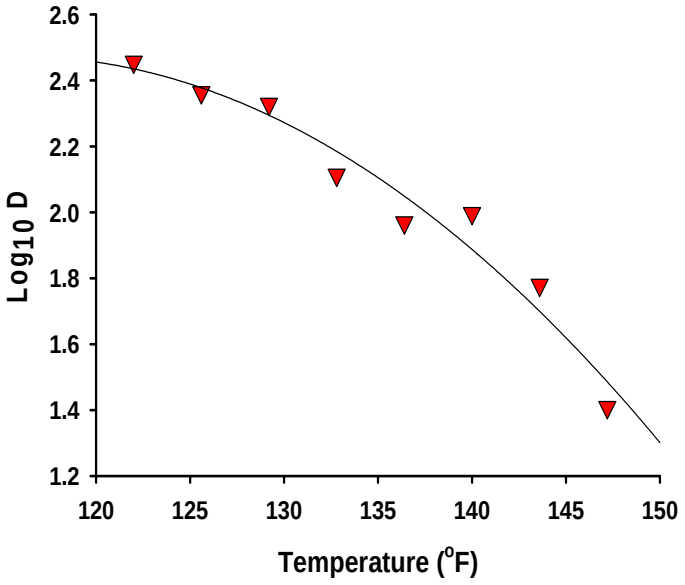
No	8.30	CFU/ml
B	0.872	
5D value (T*)	24.07	min
Log 5D	1.38	

pH 4.6 Data: Z value determination



Temp	Log(5D+5SE)
122.0	2.46
125.6	2.36
129.2	2.33
132.8	2.11
136.4	1.97
140.0	2.00
143.6	1.78
147.2	1.41

Linear Model, Non-Linear Data!



Temp F	pH 4.6	pH 4.1	pH 4.6 Poly
145	45.4	7.1	58.6
150	29.4	3.9	28.8
155	19.1	2.2	12.6
160	**12.4**	**1.2**	**4.9**
165	8.0	0.7	1.7
170	5.2	0.4	0.5
175	3.4	0.2	0.1
180	2.2	0.1	0.04

About 15 sec for cell survival at 176°F to get > 5-log reduction in water

Next steps…

- Times will be 5-10X greater than pH 4.1 data (F_{160} from 1.2 to 10 min)
- Significantly greater survival at lower temperatures (122^{o} F)
- TDT data: higher temperatures (150^{o} F or greater) needed for these experiments

Nice, but somewhat skewed

On the drawing board

- Alternative acids, pH 4.6
  - Gluconic acid: *acid independent* data
  - Citric, Phosphoric?
- *Listeria* and *Salmonella*
  - pH 4.1 data showed *Listeria* = EHEC and *Salmonella* was significantly more heat sensitive
- Spore cocktail
  - *Bacillus spp.* (*licheniformis, coagulans*)
  - *Alicyclobacillus*

2. *Bacillus* spores

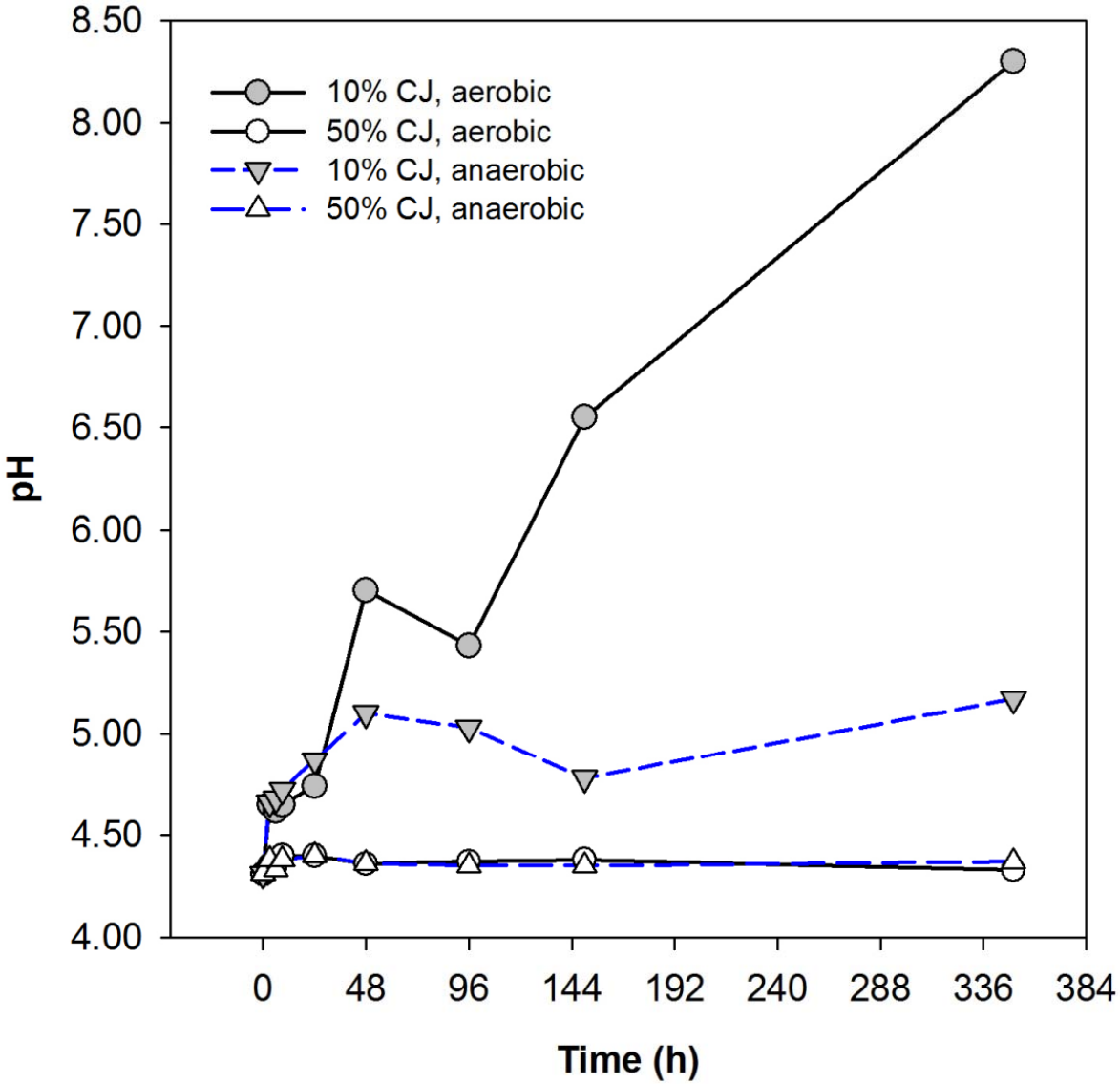
- Targeting acidified products with pH values between 4.1 and 4.6
- What are the limiting pH values for spore germination and growth?
- What is the mechanism of pH rise?
- How much buffer capacity is there to resist pH change in ‘typical’ product formulations?
- How much oxygen required for spore germination?
- TDT data for bacillus spores with conditions typical of acidified foods (not tomato products) at pH 4.6.

pH increase: microbiology and biochemistry

- Microorganisms of interest
  - *Alicyclobacillus* species
  - *Bacillus licheniformis*
  - *Bacillus coagulans*
- Non-inhibitory medium for studies
  - CJ broth
- Survey of pH elevation
  - *B. licheniformis*
- Mechanism: amino acid deamination?
  - HPLC, amino acid analyzer
- Titrations with standardized base to determine buffer capacity

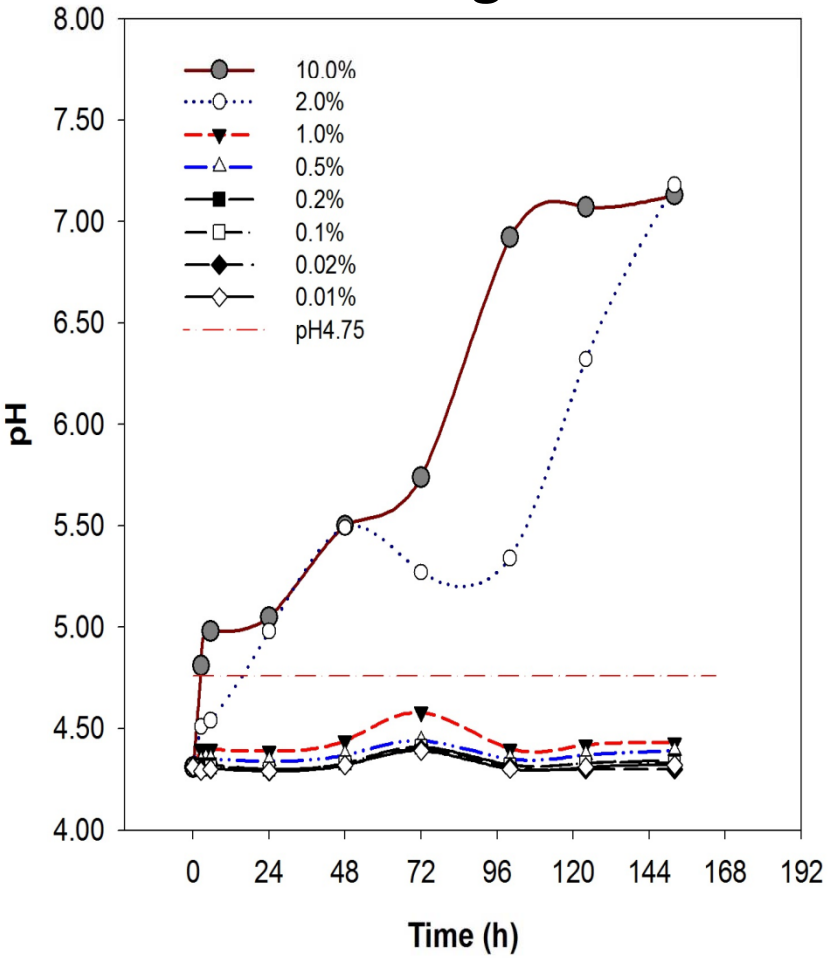
B. licheniformis: Sugar and pH elevation

- Fermentation *vs.* deamination
- CJ has 2% fermentable sugar
- Amino acids are present!

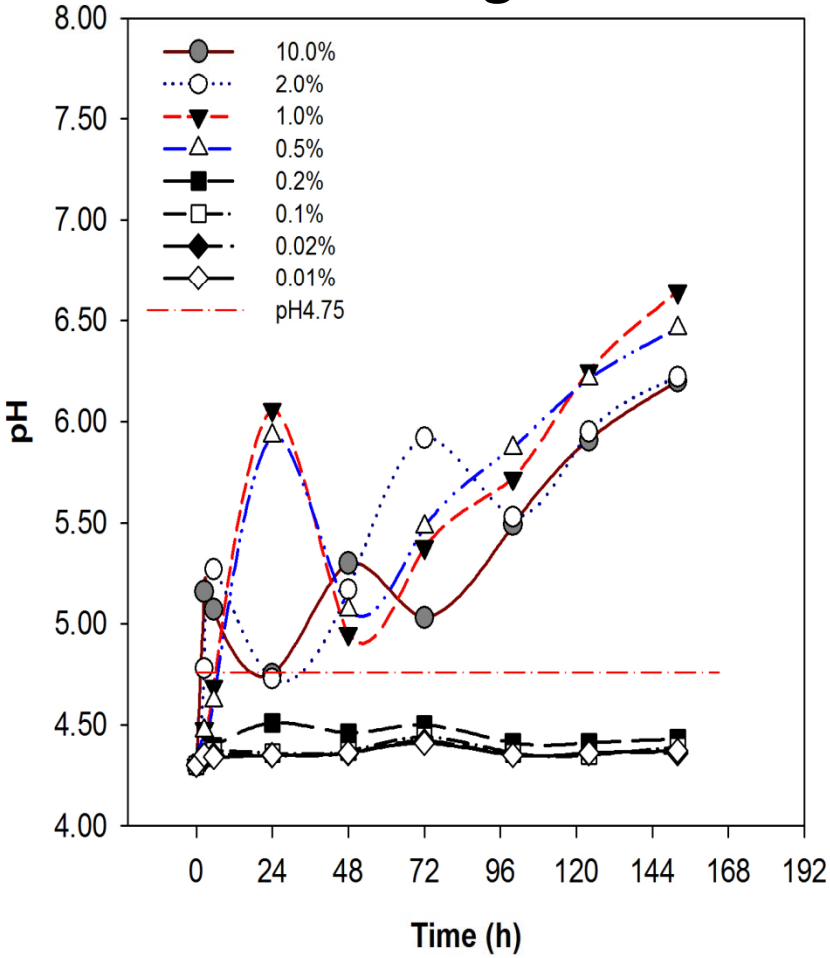


pH Elevation and Arginine

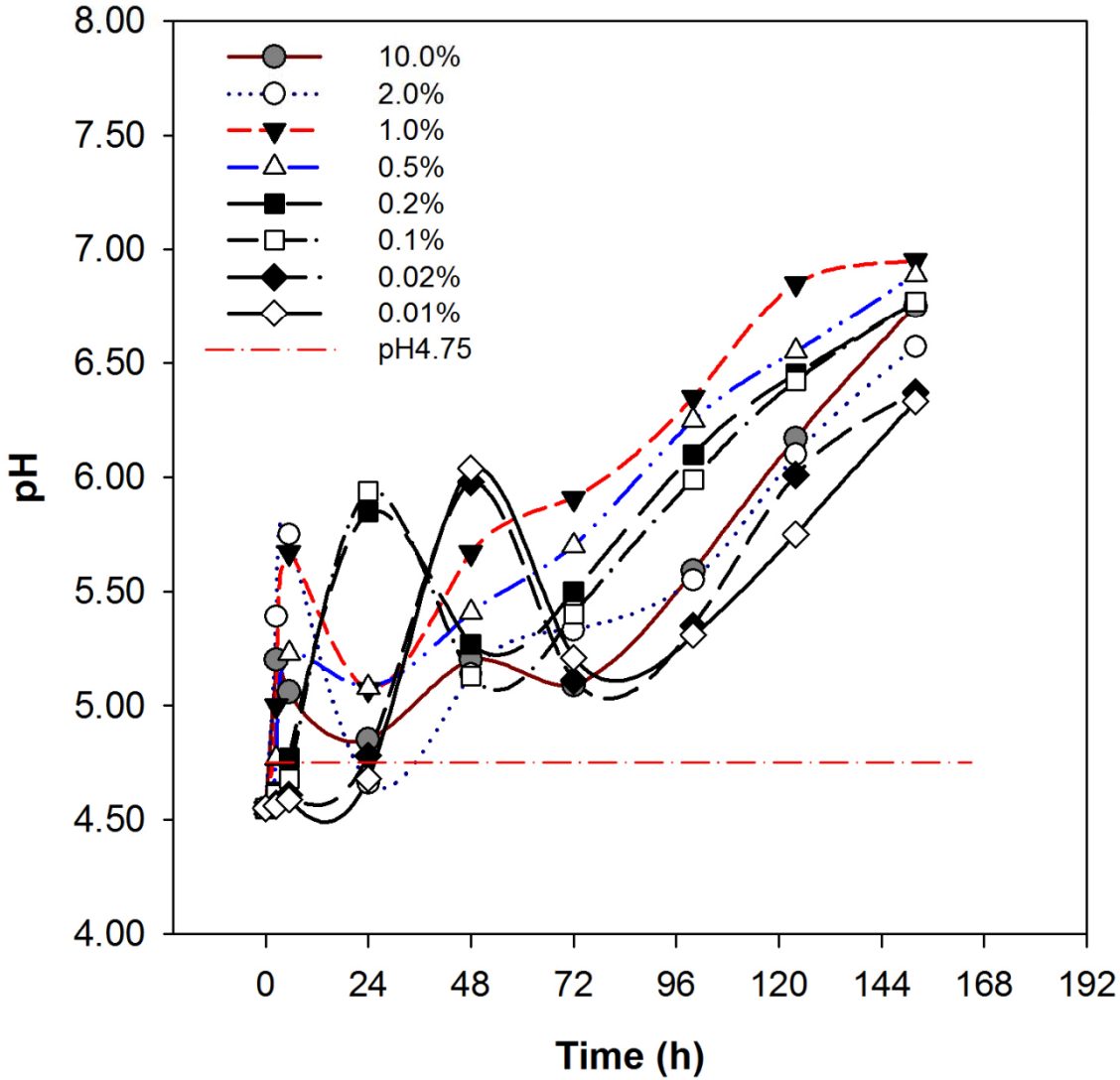
- added arginine



+ added arginine

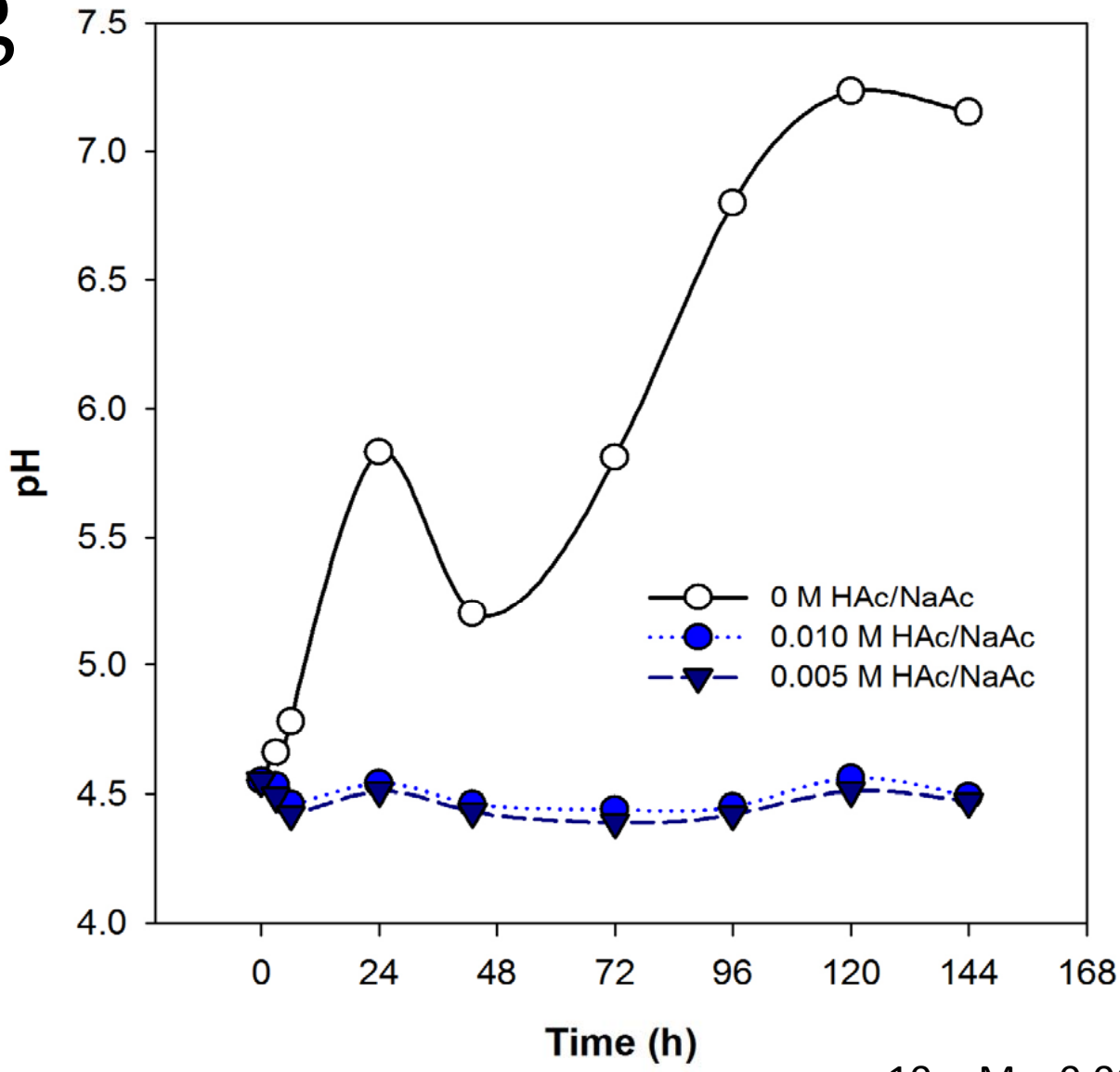


pH 5.5 with Arginine



Buffering

- 10% CJ
- pH 4.55



10 mM = 0.06%

Bacillus results

- Deamination of arginine can result in an initial pH rise
  - Aerobically AND anaerobically, but only if spores germinate and grow (we used vegetative cells)
- Arginine is not the predominant amino acid in CJ but sufficient amount is present
- Other amino acids can be deaminated as well!
- Buffering is important!

Buffer Capacity and pH

- Buffer capacity is the ability of a solution to resist a pH change.
- Cucumber juice has buffering due to acids, bases, and amphoteric compounds.
- Concentration and pKa values
  - Additive
  - Undefined
- Can be determined by titration?
  - Hypothesis: The complex buffering of CJ (and other vegetable based broths) can be modeled as a simple buffer with a single concentration and pK

Buffer Capacity

$$\beta = \frac{\partial C_b}{\partial \mathrm{pH}} = \frac{\partial C_b}{\partial [\mathrm{H}^+]} \frac{d[\mathrm{H}^+]}{d\mathrm{pH}} = -2.303[\mathrm{H}^+] \frac{dC_b}{d[\mathrm{H}^+]}$$

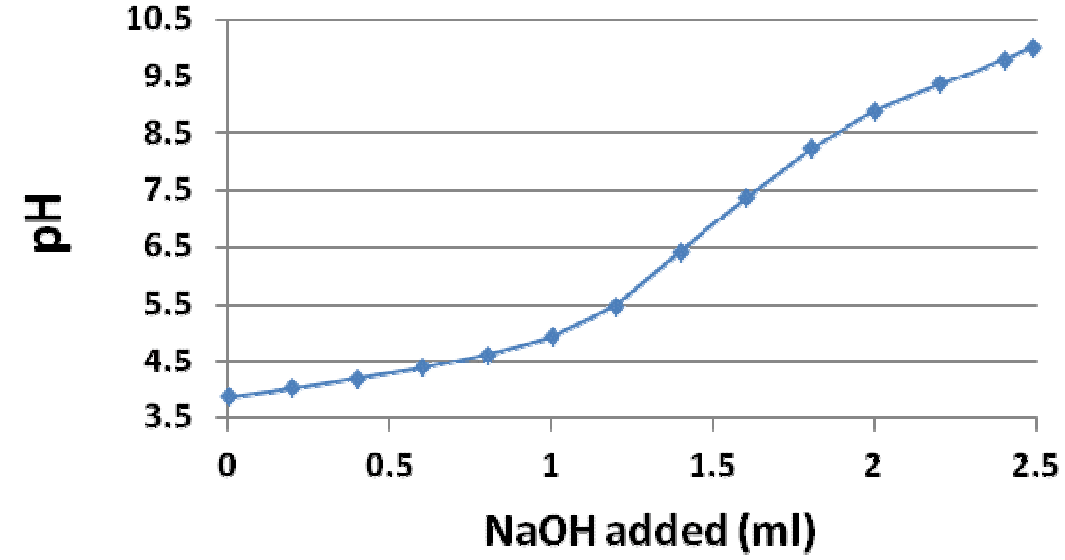
$$\beta = 2.303\left(\frac{CK_a[\mathrm{H}^+]}{([\mathrm{H}^+] + K_a)^2} + \frac{K_w}{[\mathrm{H}^+]} + [\mathrm{H}^+] \right)$$

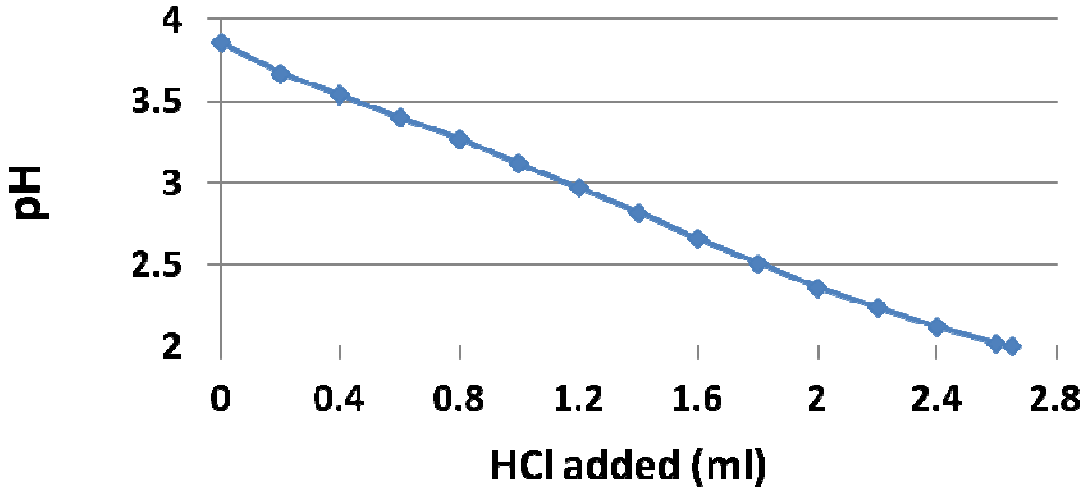
References

Dougherty DP, Ramos Da Conceicao Neta E, McFeeters RF, Lubkin SR, Breidt F Jr. 2006. Semi-mechanistic partial buffer approach to modeling pH, the buffer properties, and the distribution of ionic species in complex solutions. J Agric Food Chem 54:6021-6029.

Butler, J. N.; Cogley, D. R. Ionic Equilibrium: Solubility and pH Calculations; John Wiley and Sons: New York, 1998.

Titration of CJ with acetic acid





Acetic Acid

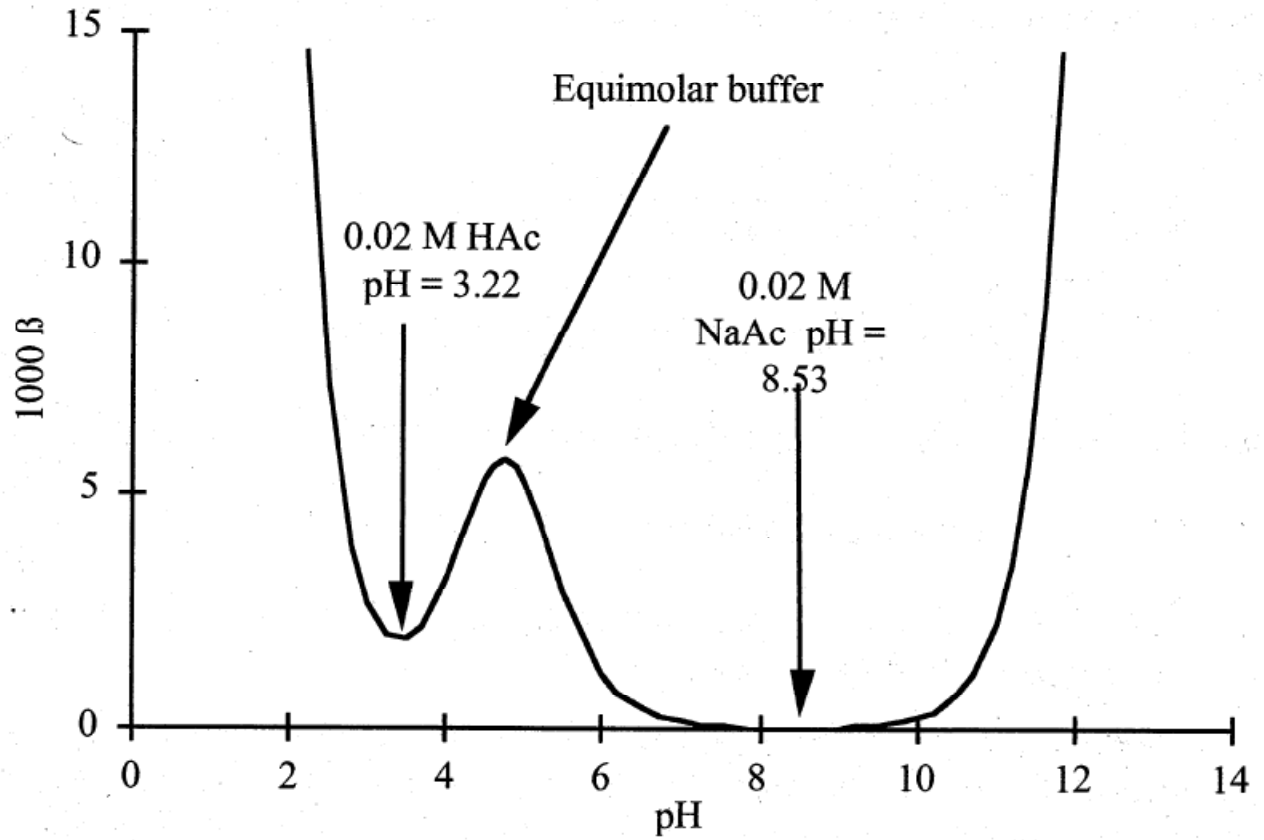
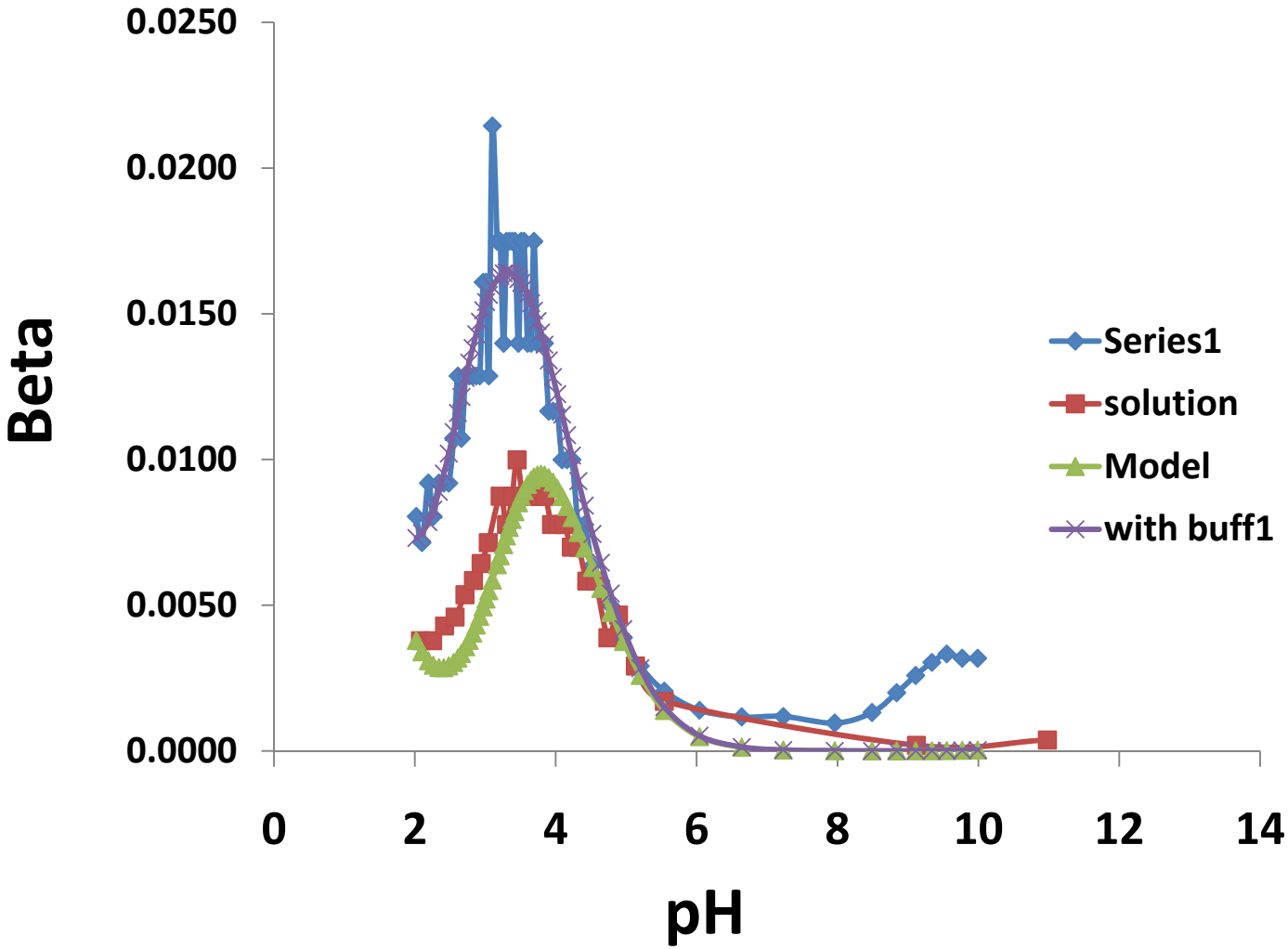


FIGURE 4.22. Buffer index of equimolar acetic acid–sodium acetate as a function of pH.

Butler, J. N.; Cogley, D. R. Ionic Equilibrium: Solubility and pH Calculations; John Wiley and Sons: New York, 1998. P. 135

Buffer capacity data and model



Buffer capacity with hypothetical buffer (pK apx. 3.0)

		M/L	mM	mM	
	pK	Conc	lactic	acetic	NaCl %
FF	2.997	0.125	103.3767	29.57	7.5
7day	2.909	0.066	55.72	27.69	5.2
New	2.843	0.036	11.53	0	6.88

Concentration is proportional to lactic acid concentration (Rsq = 0.98)

- Fermentation brines can be modeled using a buffer with a single pKa = 3.0
- NEXT: Allows predictions of pH change with bacillus growth?

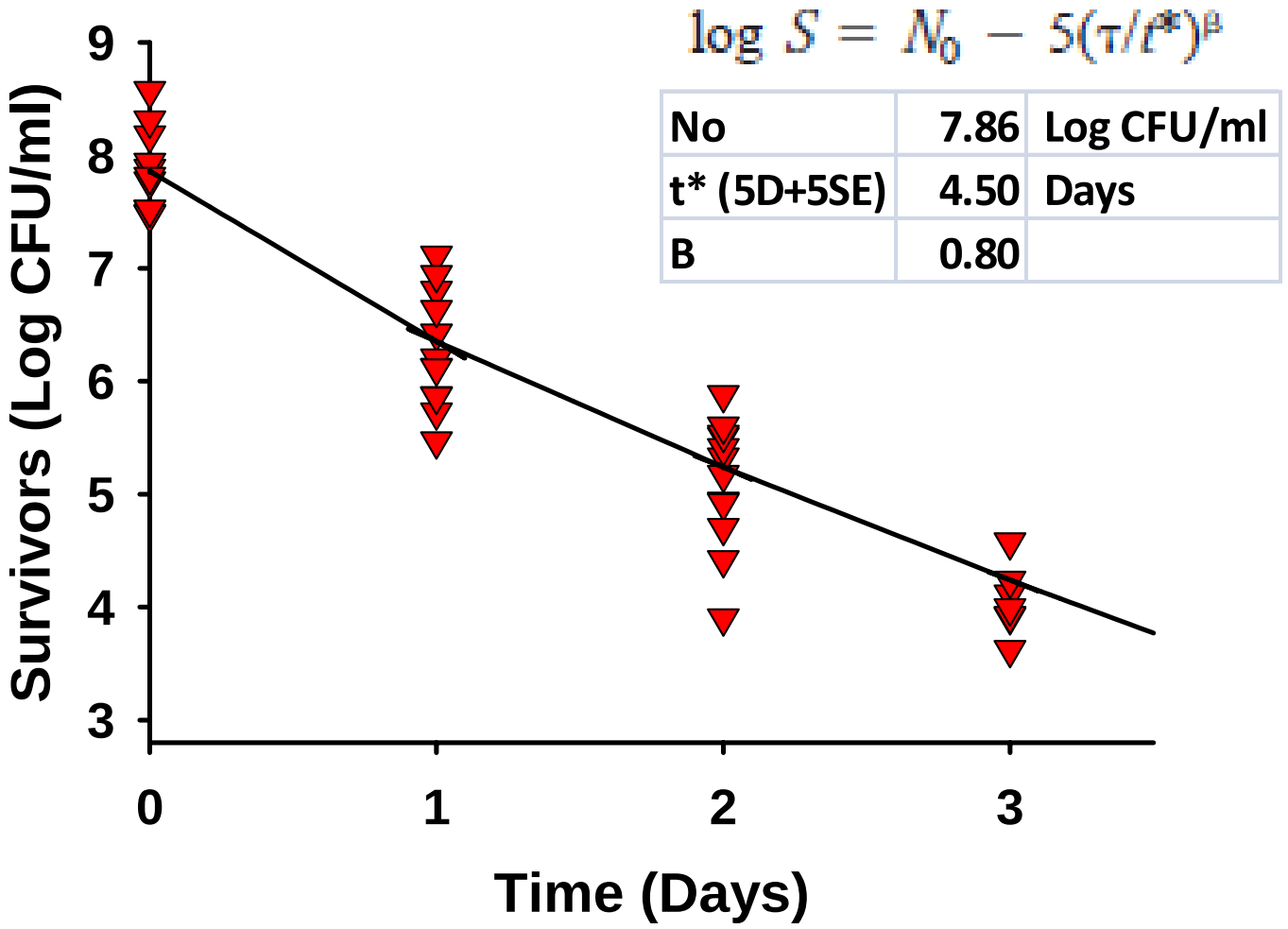
3. Cold fill hold at pH 3.5

- Current data shows requires pH 3.3 or below
  Breidt F, Hayes JS, McFeeters RF. 2007. J Food Prot 70(11):2638-2641
  - CJ as non-inhibitory medium
  - Acetic acid was used as the primary acidulent to get pH at or below pH 3.3
  - At 25^{o}C (77^{o}F): 48 hr.
  - At 10 C (50 F): 6 days
- Alternatives for acidified foods, pH 3.5
  - 2% and 2.5% acetic acid
  - Citric + acetic (1% each?)
  - Phosphoric
  - Preservative acids (benzoate, sorbate, etc.)
  - Fumaric acid

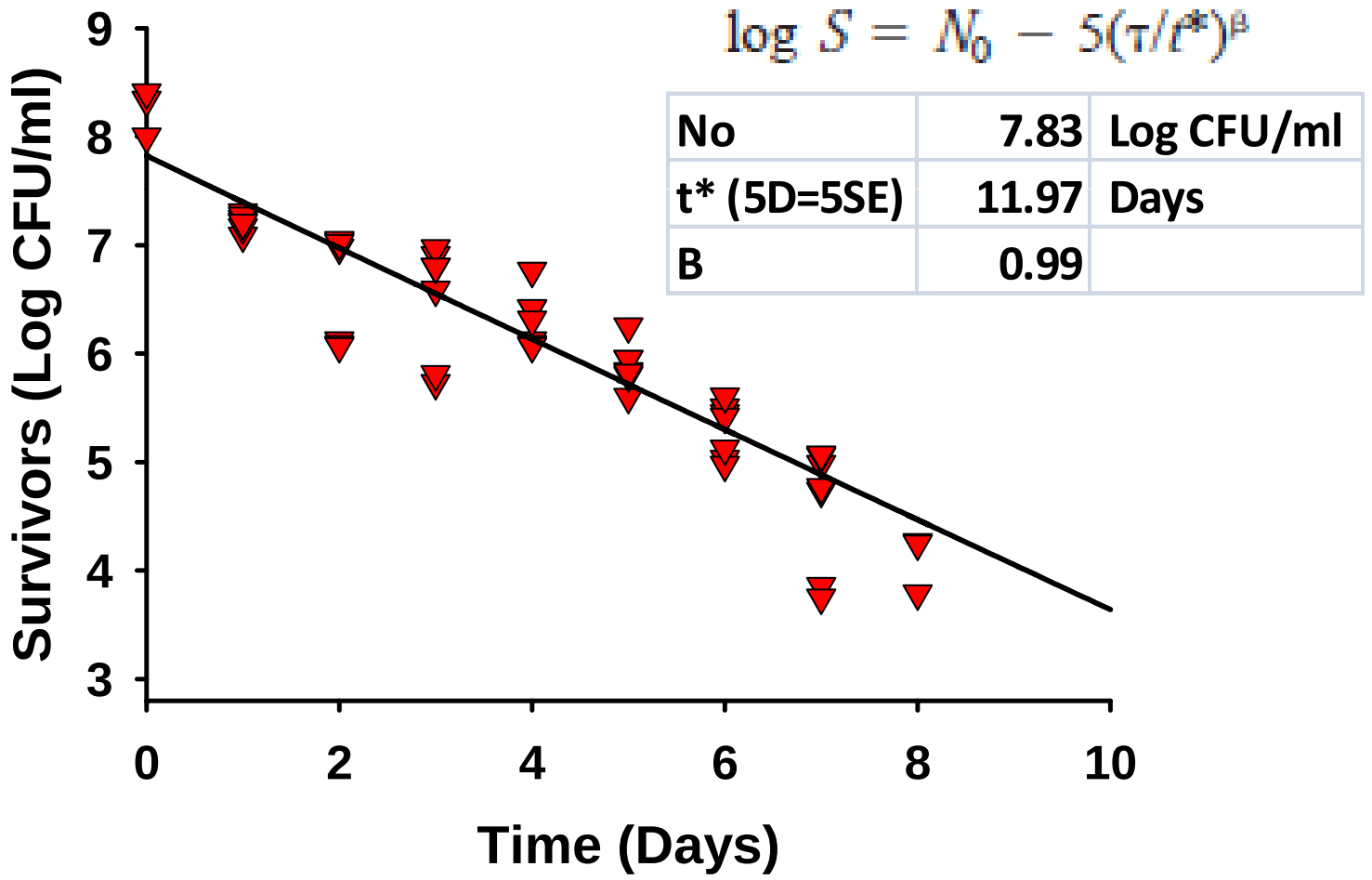
Microbiology

- Cocktail of 5 EHEC strains
  - Growth conditions to induce acid resistance
- Experiments done at 10^{o}C
  - Above refrigeration, low enough to prevent heating
- Inoculate brined cucumbers with indicated acid conditions
  - Sampling through septa using a syringe
  - Oxygen limited (essentially anaerobic)
  - Brined cucumbers are a non-inhibitory vegetable products that can be representative of a variety of products
- Plate on non-selective media
  - Recovery of injured cells
- Independent replication
- Weibull model for 5-log reduction and statistics

Cold-fill-hold pH 3.5, 2.5% Acetic



Cold-Fill-Hold pH 3.5, 2% Acetic



Conclusions

- Thermal processing at pH 4.6
  - TDT (120 – 150) for 100 mM acetic acid pH 4.6 Thermal processing data (equilibrated brine).
- Cold fill hold pH 3.5
  - Data for 2.5% acetic acid, apx. 5 days for holding!
  - 2%: apx. 12 days

  NOTE: pH 3.3 data, 6 days at 50^{o}F or 48 hr. at 77^{o}F
- Spore forming bacilli
  - IFT abstract: “pH elevation by *Bacillus licheniformis* in Acidified Vegetable Broth” by Meng et al.
  - pH 4.2 was lower limit for increase
  - Glucose represses pH rise, oxygen required!
  - Deamination of arginine responsible for early pH rise

More conclusions

- *Salmonella* and *Listeria* have previously been shown to be less acid resistant
  - Selected trials will be done to confirm this…
- pH 3.5 with 2.5% acetic acid
  - Holding 5 days at 10^{o}C or above
  - 2% acetic acid is probably not useful
- Additional acids and conditions will be done…
  - Suggestions? Fred.Breidt@ars.usda.gov
  - Objective is to meet a wide variety of products with least number of experiments

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