Phibro ETHANOL™ | EXPERIENCE PERFORMANCE

The critical role of pKa and pH in bacterial contamination and yeast inhibition

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Outline

pH/pKa basics

pH direct effects on yeast

Types of pKa chemicals found in a fuel ethanol plant

Using pKa to lower inhibition on yeast

How to raise pH at the plant

Case studies on raising pH

Phibro Animal Health Corporation

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Division of Phibro Animal Health Corporation (PAHC)

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PAHC has over 1700 products presentations and 1720 employees world-wide

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PhibroMat™

Cleaning

PhibroClean™
PhibroAC™
PhibroSI™
PhibroDC™

Yeast

PhibroADY™
FortiPhi™
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Corn Oil Recovery

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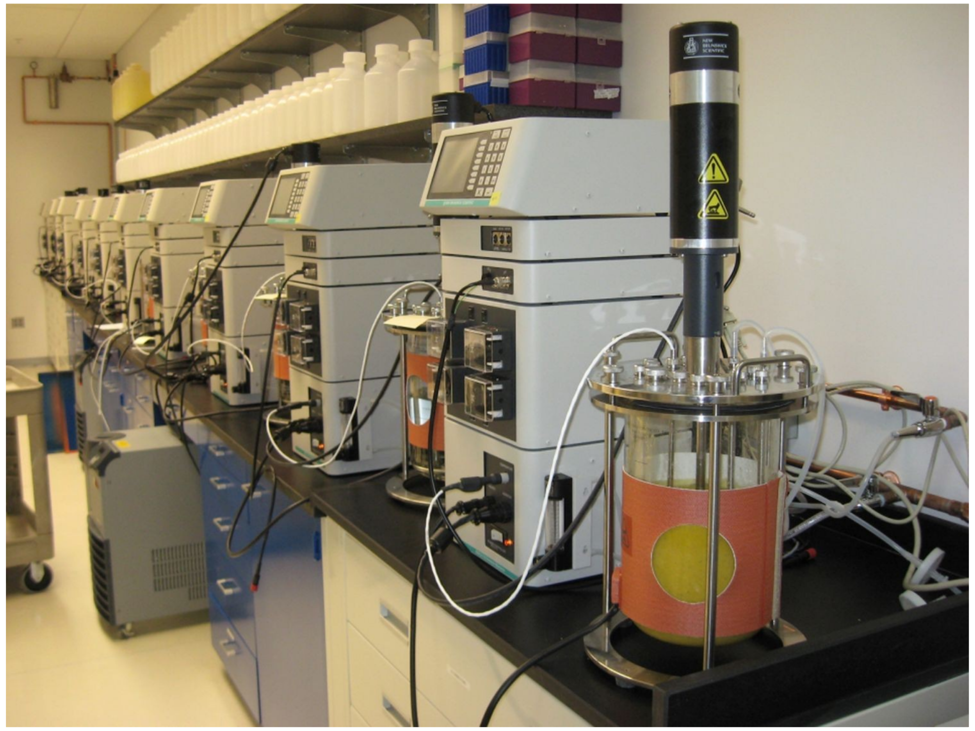

Technical Services

- Phibro's Universal Enterprise Labs (UEL) St. Paul, MN
  - Microbiological Testing & Evaluation (Diagnostic Kits & Omnilog/MIC)
  - Chemical Analysis
  - Fermentation Experimentation
  - New Product Development
  - Analysis of Antimicrobial Systems
- Phibro Animal Health Regulatory Services Teaneck, NJ
- Field Technical Service
  - Root cause and change analysis utilizing JMP statistical analysis software
  - Plant audits
  - Fermentation reviews
  - New product trial support
  - FAN calculator
  - On-site and off-site training

A contaminated Fuel ethanol plant

Traditionally in the fuel ethanol industry, when a plant is determined to be contaminated with bacteria, the plant usually adds antibiotics and lowers the pH in the mash.

The thinking is that the antibiotics together with pH will inhibit bacterial growth (which is true)

But is lowering the pH when contaminated always the best course of action?

pH and pKa Basics

pH and pKa Basics

Fully dissociated acids/bases

These chemicals fully dissolve in water and fully dissociate into their ionic components (i.e. “one-way” reaction). The pH of the resulting solution is directly proportional (logarithmically) to the concentration of the hydronium ions released from the dissociated chemical.

e.g. Hydrochloric acid

$$HCl \rightarrow H^{+} + Cl^{-}$$

$$pH = -\log\left[H^{+}\right]$$

Undissociated Dissociated

pH and pKa Basics

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$$HCl \rightarrow H^{+} + Cl^{-}$$

$$pH = -\log[H^{+}]$$

Undissociated   Dissociated

Sample calculation

Mol weight hydrochloric acid	36.46 g/mol
Test solution	0.5 % (w/v) 0.5 g/100ml 5 g/L
Concentration of HCL	0.14 mol/L
Concentration of H+	0.14 mol/L
pH	**0.86**

Wt% HCl	pH
3.647	0.00
2.500	0.16
2.000	0.26
1.500	0.38
1.000	0.56
0.500	0.86
0.3647	1.00
0.2500	1.16
0.1150	1.50
0.03647	2.00
0.01150	2.50
0.003647	3.00
0.001150	3.50

pH and pKa Basics

Partially dissociated acids/bases

These are chemicals that fully dissolve in water but only partially dissociate into their ionic components (i.e “bidirectional” reaction). The pH of the resulting solution is dependent on the pKa of the chemical and the logarithmic concentration ratio of the dissociated and undissociated species.

pKa is simply defined as the pH where 50% of the total chemical is undissociated and 50% is dissociated. pka values are specific to a particular chemical

e.g. acetic acid

$$CH_3COOH \Leftrightarrow CH_3COO^- + H^+ \quad pKa = 4.76$$

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

Undissociated Dissociated

pH and pKa Basics

If you create a 0.5% w/v acetic acid solution, it will not fully dissociate but will equilibrate between its undissociated and dissociated forms based on pH and it’s pKa.

$$CH_3COOH \Leftrightarrow CH_3COO^- + H^+ \quad pKa = 4.76$$

Undissociated Dissociated

pH and pKa Basics

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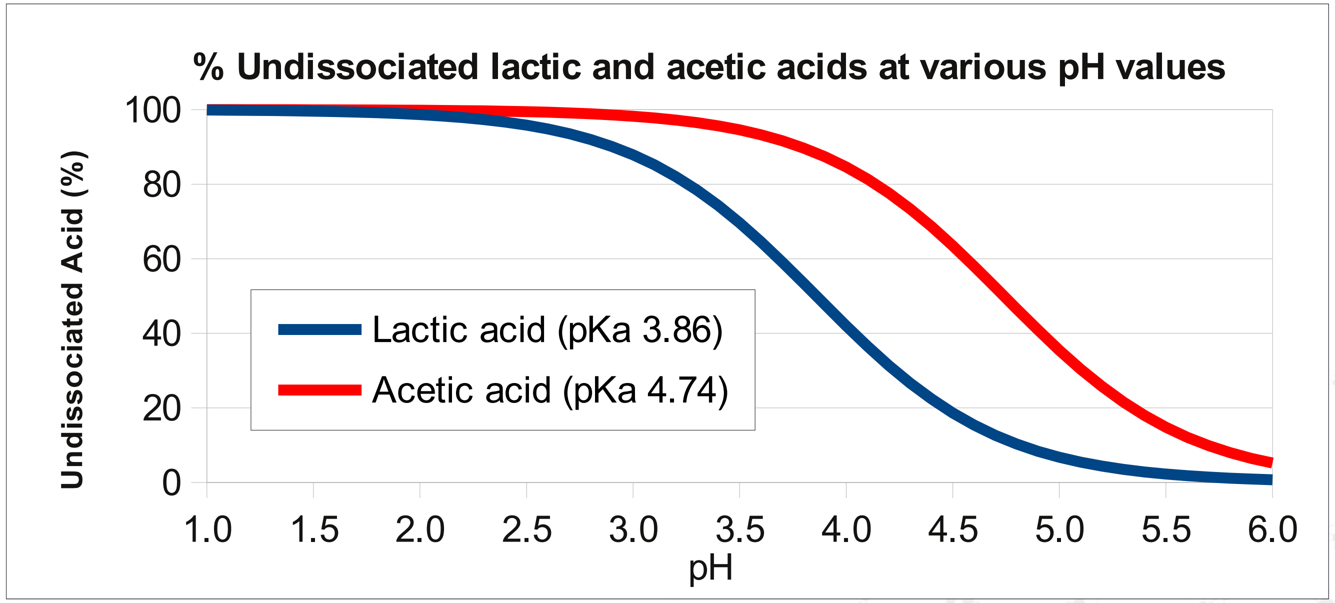
Undissociated | Dissociated



e.g. After equilibration, you chemically detect the undissociated form at 0.0272 w/v (dissociated form must be 0.5-0.0272 = 0.4727 %w/v)

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

$$pH = 4.76 + \log\left[\frac{0.4727}{0.0272}\right]$$

$$pH = 2.5$$

pH and pKa Basics

If you create a 0.5% w/v acetic acid solution, it will not fully dissociate but will equilibrate between its undissociated and dissociated forms based on pH and it's pKa.

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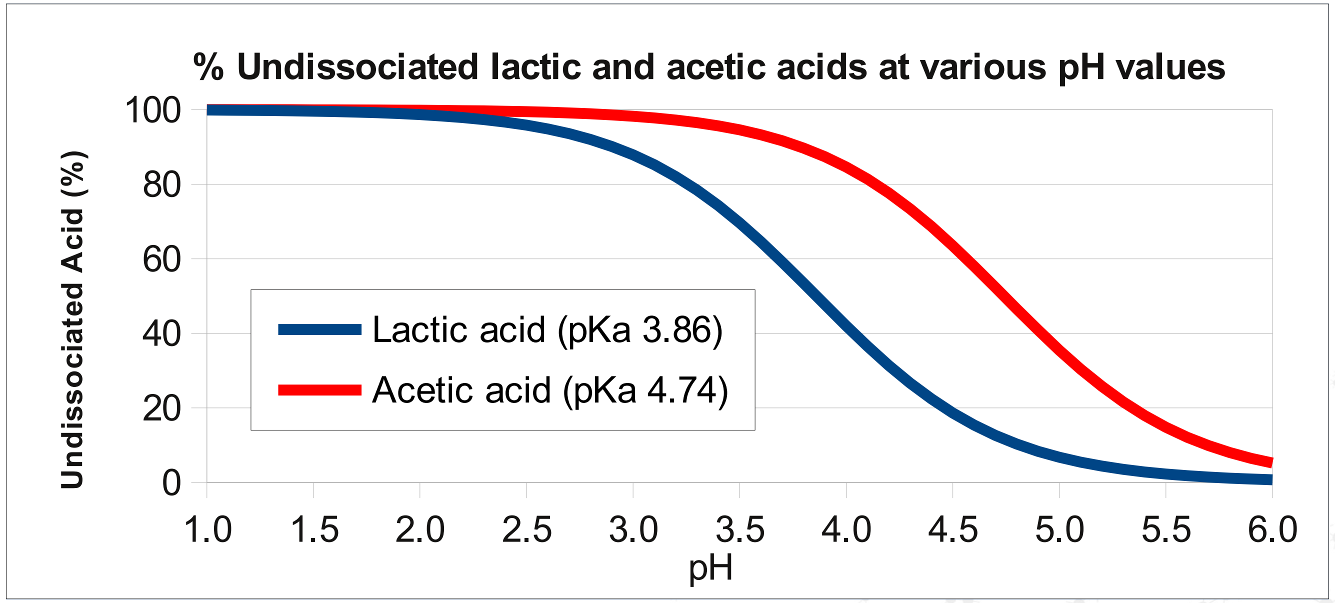


Can calculate in "reverse". If you know the:

pKa (for chemical)
pH (pH probe)
Total amount of acetic acid (by HPLC)

You can determine the amount of undissociated and dissociated forms of any pKa chemical.

Yeast Stress Factors

pH effect on yeast inhibition

Operational factors

Glucose content
(10% w/v max)

Sulfite
(100 ppm)

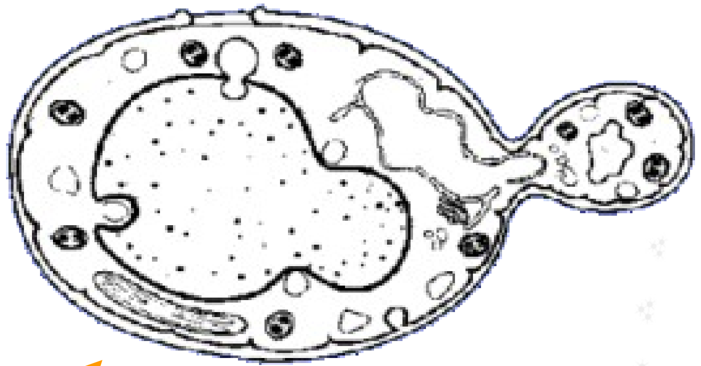
Temperature
(35ºC = 95ºF)

Sodium
(500 ppm)

CIP
Chemicals

Nutritional factors
(media formula)

Lack of: Sterols, Nitrogen,
Oxygen, UFA, Minerals/vitamins



Microbial factors

Chemical

Acetic acid
(0.05% w/v)

Ethanol
(23% v/v max)

Lactic acid
(0.8% w/v max)

Mycotoxins
(10-100 ppm)

pH
(<4.0 or >6.0)

Fusel volatiles
(0.1–1 % w/v)

Competition

Bacterial nutrient depletion
(trace nutrients)

Yeast Stress Factors

pH direct effect on yeast metabolism

Direct consequences on yeast growth/fermentation

pH is a signal for yeast in itself . In general, efficient yeast multiplication occurs at pH >5.0 while efficient fermentation is achieved at pH <5.0.

A pH of 2.8 is the lowest absolute pH discovered for yeast growth yet yeast metabolic activity still continues (at a l lower rate than normal).

Yeast multiplication rate increases (non-linearly) as the pH is increased to an optimal pH of 5.5-6.0 for most yeasts.

Yeasts multiply and ferment optimally when there is the ability for the yeast to produce a pH change of 1-2 pH units from the start to the end of propagation/fermentation. Cut short this difference either by starting at a lower absolute pH, or preventing the pH from reaching this difference and yeast multiplication is significantly curtailed.

Yeast transport of nutrients are dependent on pH. Many of the yeast cell transporters that transport materials (e.g. carbohydrates, ions, amino acids) not only requires that a pH gradient be maintained across the membrane (pHout < pHin) but each transporter have optimal pH's. Moving the pH away from these optimal transport values decreases uptake of nutrients.

Increased genetic mutation frequency has been observed in yeasts cultured at very low pH (<3.5)

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at a fuel ethanol plant

Fatty acids	
	pKa
Acetic acid	4.74
Butyric acid	4.82
Capric acid	4.92
Caproic acid	4.88
Caprylic acid	4.89
Formic acid	3.77
Isobutyric acid	4.86
Isovaleric acid	4.98
Lauric acid	5.30
Proprionic acid	4.87
Valeric acid	4.82

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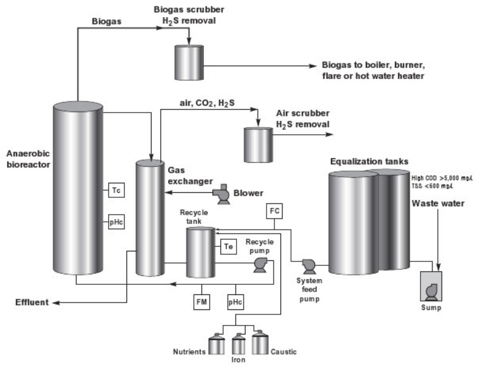
Part of normal yeast and bacterial physiology. Many non-LAB bacteria such as soil bacteria, *Bacillus* sp, *Clostridia* sp. Methanogenic bacteria regularly make fatty acids

Overproduction of fatty acids by stressed bacteria (prolonged shutdown of a fuel ethanol plant)

Microbial breakdown of corn oil by bacteria (prolonged shutdown of a fuel ethanol plant).

Chemical breakdown of oil (e.g. saponification of mash by caustic)

Unbalanced Anaerobic Digestor. Consortium of bacteria "doing the work" are many:



Enterobacteriasiae (30 species)

Clostridium aceticum, *Clostridium termoautotrophicum, Clostridium thermoaceticum,Clostridium formiaceticum*

Methanobacterium bryantii, Methanobacterium formicum, Methanobrevibacter arboriphilicus, Methanobrevibacter gottschalkii, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanocalculus chunghsingensis, Methanococcoides burtonii, Methanococcus aeolicus, Methanococcus deltae, Methanococcus jannaschii, Methanococcus maripaludis, Methanococcus vannielii,Methanocorpusculum labreanum, Methanoculleus bourgensis, Methanoculleus marisnigri, Methanofollis liminatans, Methanogenium cariaci, Methanogenium frigidum, Methanogenium organophilum, Methanogenium wolfei, Methanomicrobium mobile, Methanopyrus kandleri, Methanoregula boonei, Methanosaeta concilii, Methanosaeta thermophila, Methanosarcina acetivorans, Methanosarcina barkeri, Methanosarcina mazei, Methanosphaera stadtmanae, Methanospirillium hungatei, Methanothermobacter defluvii, Methanothermobacter thermautotrophicus, Methanothermobacter thermoflexus, Methanothermobacter wolfei, Methanothrix sochngenii

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

	pKa
Acetic acid	4.74
Lactic acid	3.86

pKa chemicals within a fuel ethanol plant

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Fatty acids

Bacterial contamination

	pKa
Acetic acid	4.74
Lactic acid	3.86

Lactic Acid Bacteria (LAB) family of bacteria regularly infect fuel ethanol plants. Primary metabolic products are acetic and lactic acid but can produce hundreds of chemicals in minor amounts.

Lactobacillus sp

paracasei, plantarum, casei, brevis, fermentum, rhamnosus, delbrueckii, buchneri, pentosus, acidophilus, gasseri, jenserii, amylovorus, reuteri, cornyeformis, divergens, carnis, piscicola, sake, sharpeae, bavaricus, curvatus, hamsteri, amylophilus, agilis, homohiochii

Production yeasts can make acetic acid when stressed (heat, FAN depletion, osmotic, salt)

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

Yeast production

		pKa
Acetic acid		4.74
Succinic acid	pKa1	4.20
	pKa2	5.60

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

Yeast production

		pKa
Acetic acid		4.74
Succinic acid	pKa1	4.20
	pKa2	5.60

Yeasts can produce many organic acids including acetic acid, succinic acid, oxaloacetate, citric acid, and others.

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

Yeast production

Amino acids

		pKa			pKa			pKa
Alanine	pKa1	2.34	Glutamine	pKa1	2.17	Methionine	pKa1	2.28
	pKa2	9.69		pKa2	9.13		pKa2	9.21
Arginine	pKa1	2.17	Glycine	pKa1	2.34	Phenylalanine	pKa1	1.83
	pKa2	9.04		pKa2	9.60		pKa2	9.13
	pKa3	12.48	Histidine	pKa1	1.82	Proline	pKa1	1.99
Asparagine	pKa1	2.02		pKa2	9.16		pKa2	10.60
	pKa2	9.10		pKa3	6.00	Serine	pKa1	2.21
Aspartic acid	pKa1	1.88	Isoleucine	pKa1	2.36		pKa2	9.15
	pKa2	3.65		pKa2	9.60	Threonine	pKa1	2.09
	pKa3	9.60	Leucine	pKa1	2.36		pKa2	9.10
Cysteine	pKa1	1.96		pKa2	9.60	Tryptophan	pKa1	2.83
	pKa2	8.18	Lysine	pKa1	2.18		pKa2	9.39
Glutaminc acid	pKa1	4.25		pKa2	8.95	Valine	pKa1	2.32
	pKa2	9.67		pKa3	10.53		pKa2	9.62

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Fatty acids

Bacterial contamination

Yeast production

Amino acids

		pKa			pKa			pKa
Alanine	pKa1	2.34	Glutamine	pKa1	2.17	Methionine	pKa1	2.28
	pKa2	9.69		pKa2	9.13		pKa2	9.21
Arginine	pKa1	2.17	Glycine	pKa1	2.34	Phenylalanine	pKa1	1.83
	pKa2	9.04		pKa2	9.60		pKa2	9.13
	pKa3	12.48	Histidine	pKa1	1.82	Proline	pKa1	1.99
Asparagine	pKa1	2.02		pKa2	9.16		pKa2	10.60
	pKa2	9.10		pKa3	6.00	Serine	pKa1	2.21
Aspartic acid	pKa1	1.88	Isoleucine	pKa1	2.36		pKa2	9.15
	pKa2	3.65		pKa2	9.60	Threonine	pKa1	2.09
	pKa3	9.60	Leucine	pKa1	2.36		pKa2	9.10
Cysteine	pKa1	1.96		pKa2	9.60	Tryptophan	pKa1	2.83
	pKa2	8.18	Lysine	pKa1	2.18		pKa2	9.39
Glutaminc acid	pKa1	4.25		pKa2	8.95	Valine	pKa1	2.32
	pKa2	9.67		pKa3	10.53		pKa2	9.62

Hydrolysis of proteins by proteases

Lysis of yeast and bacteria

Yeast foods

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

Yeast production

Amino acids

Process chemicals

	pKa
Ammonia	10.50
Sulfuric acid	1.99
Sulfamic acid	1.0
	pKb
Urea	13.9

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

Yeast production

Amino acids

Process chemicals

	pKa
Ammonia	10.50
Sulfuric acid	1.99
Sulfamic acid	1.0
	pKb
Urea	13.9

Chemicals that are regularly added to the fuel ethanol process to change pH, provide additional FAN for yeast nutrition, and cleaning.

pKa chemicals within a fuel ethanol plant

Multiple types of pKa chemicals can exist at the plant

Fatty acids

Bacterial contamination

Yeast production

Amino acids

Process chemicals

Fusels

	pKa
Major components	
Isoamyl alcohol	n/a
2-methyl-1-butanol	n/a
isobutyl alcohol	n/a
1-propanol	n/a
Minor components	
Ketones	Yes
Esters	Yes
FA	Yes
Aldehydes	Yes
Organic acids	Yes

pKa chemicals within a fuel ethanol plant

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Fatty acids

Bacterial contamination

Yeast production

Amino acids

Process chemicals

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Minor components	
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Esters	Yes
FA	Yes
Aldehydes	Yes
Organic acids	Yes

Fusels are a mixture of higher alcohols, aldehydes, ketones, esters, FA, and organic acids.

Although the higher alcohols do not have a pKa, the other components present in fusel oils do.

Primarily produced by yeast during fermentation.

Bacterial production of fusels documented

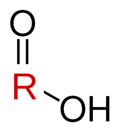
pKa chemicals within a fuel ethanol plant

- Fatty acids
- Bacterial contamination
- Yeast production
- Amino acids
- Process chemicals
- Fusels

pKa chemical presence is ubiquitous at all fuel ethanol plants

Fatty Acid Inhibition on Yeast

Fatty Acid Inhibition on Yeast



SCFA (Short Chain Fatty Acids)

R	Chemical	pKa
C1:0	formic acid	3.77
C2:0	acetic acid	4.76
C3:0	propionic acid	4.87
C4:0	butyric acid	4.82
	isobutyric acid	4.86
C5:0	valeric acid	4.82
	isovaleric acid	4.98

C1-C5 SCFA do not readily pass thru the yeast cell membrane in dissociated form

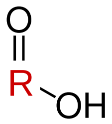
Inhibition on yeast pH dependent

Methanator bacteria can produce butyric, valeric, and isovaleric fatty acids especially if excess acetic acid is detected leaving the methanator

Mechanism of inhibition on yeast:

Disruption of pH gradient across yeast cell membrane

Fatty Acid Inhibition on Yeast



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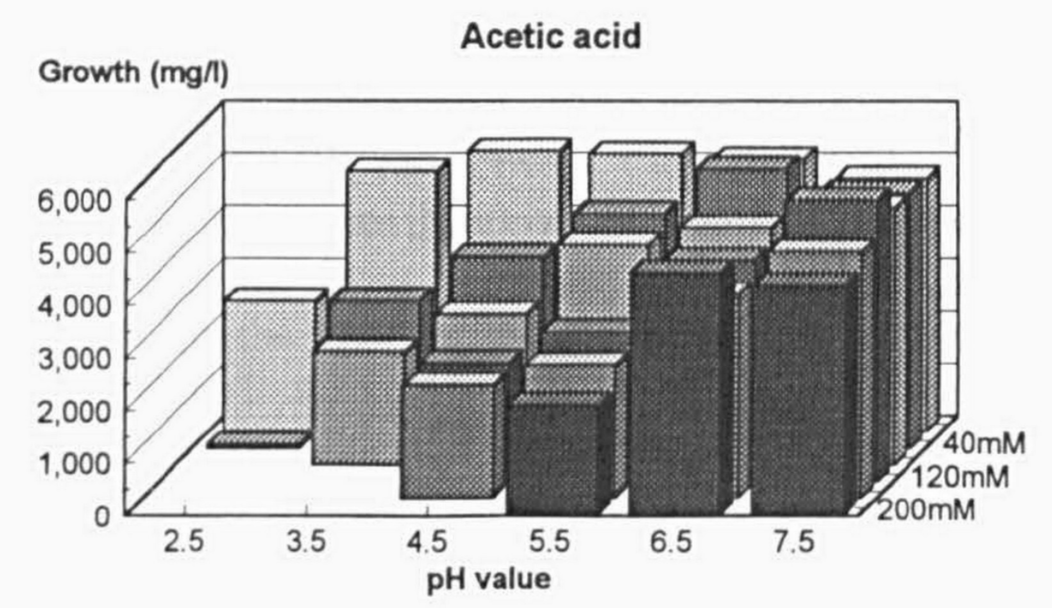
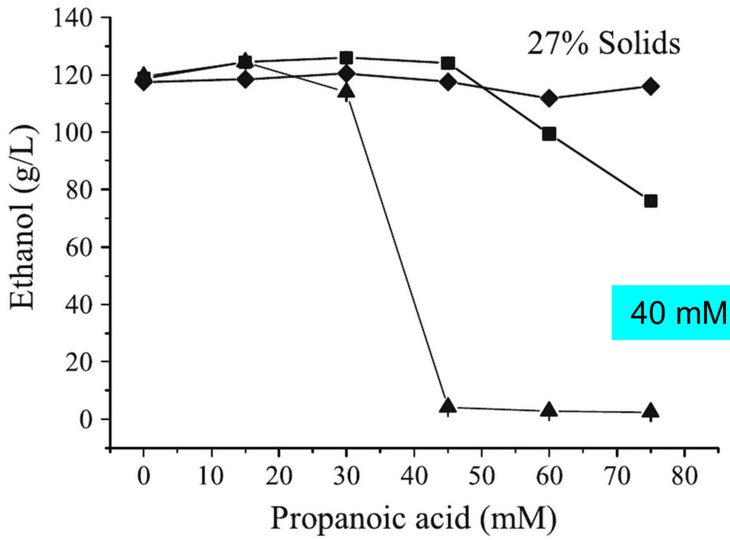


40 mM ~ 0.24% w/v

(Stratford and Anslow, 1996)



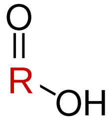
40 mM ~ 0.29 % w/v

(Zhang *et al*, 2011)

Fig. 2 Final ethanol concentrations produced by S. cerevisiae from cassava mash fermentation at 30 °C with various solids contents and propanoic acid concentrations and adjusted to different pH values. Symbols: triangle pH 4.0, square 5.0, and diamond 6.0. Error bars, too small to show; standard deviation, <2.5%

Inhibition of yeast dependent on pH and concentration of fatty acid

Fatty Acid Inhibition on Yeast



MCFA (Medium Chain Fatty Acids)

R	Chemical	pKa
C6:0	caproic acid	4.88
C8:0	caprylic acid	4.89
C10:0	capric acid	4.92
C12:0	lauric acid	5.30

C6-C12 MCFA generally insoluble in water, soluble in fats/oils.

Inhibition on yeast not as dependent on pH

Manufactured by yeast/bacteria as part of their membrane lipids.

Hydrolysis of corn oil

Mechanism of inhibition on yeast:

- Disruption of the yeast cell membrane via insertion into membrane
- Disruption of pH gradient across yeast cell membrane

Fatty Acid Inhibition on Yeast

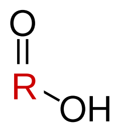


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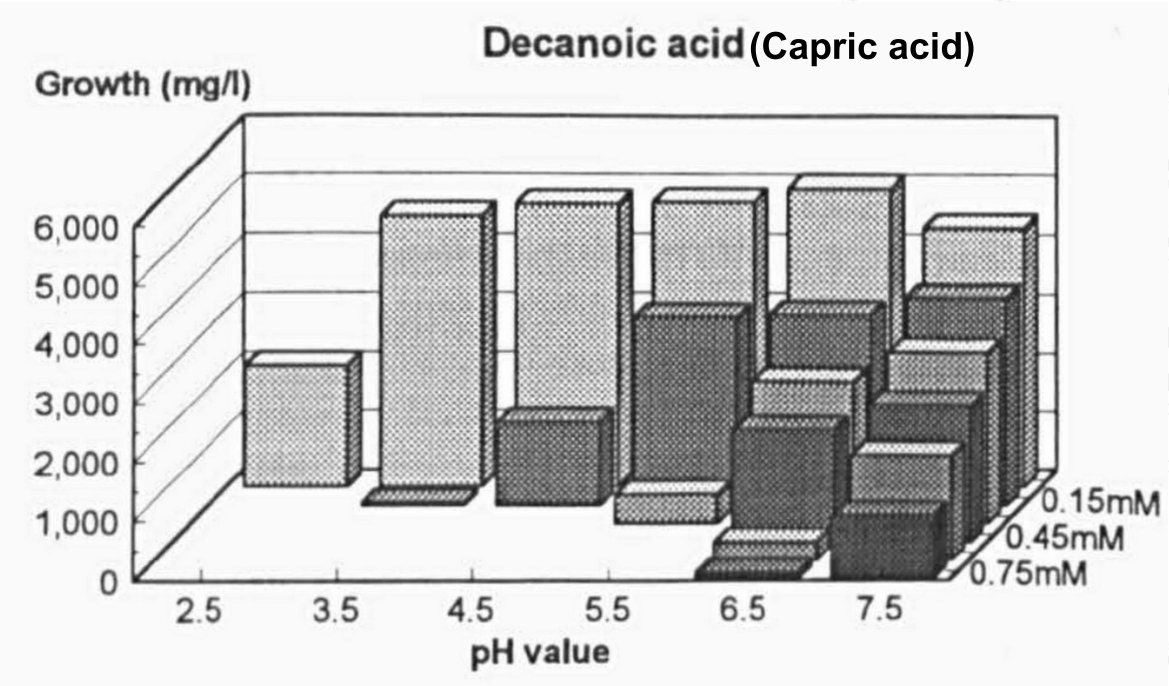
Table 2. Inhibition constants, *k*, of fermentation rate for fatty acids and their ethyl esters at pH 3.8 and 6.4

Compound	[Ethanol] (%, v/v)	pH	*k* (l/mg)	*k* (mM)
Octanoic acid	4	3.8	0.00822	1.19
Ethyl octanoate	4	3.8	0.00000	0.00
Decanoic acid	4	3.8	0.02090[a]	3.62[a]
			0.40300[b]	69.6[b]
Ethyl decanoate	4	3.8	0.00721	1.44
Decanoic acid	4	6.4	0.00997[c]	1.72[c]
Decanoic acid	12	6.4	0.00921[c]	1.59[c]
Ethyl decanoate	12	6.4	0.00355[c]	0.709[c]

[a] Measured in the concentration range 0–24 mg/l
[b] Measured in the concentration range 24–32 mg/l
[c] Measured in the concentration range 0–100 mg/l

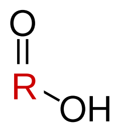
(Stevens and Hofmeyr, 1993)



(Stratford and Anslow, 1996)

0.75 mM ~ 0.013% w/v

Fatty Acid Inhibition on Yeast



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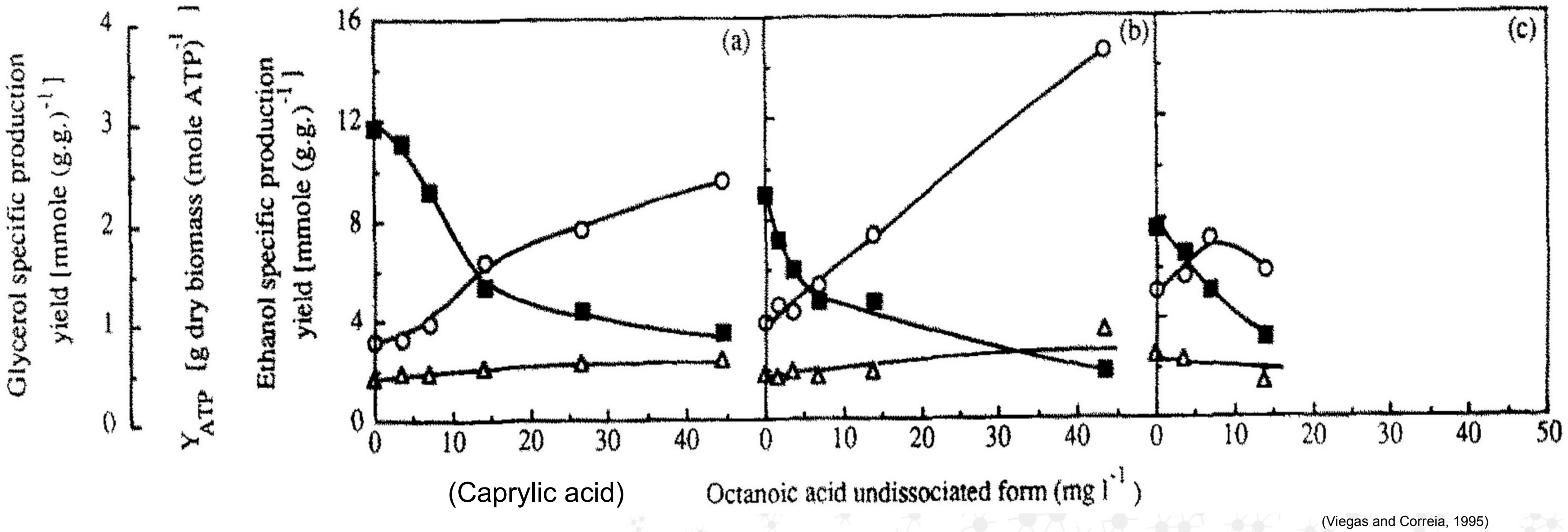


(Viegas and Correia, 1995)

Figure 3: Effects of increasing concentration of the undissociated form of octanoic acid on the Y_{ATP} (■) and on the specific production yields of ethanol (○) and glycerol (◊) by *Saccharomyces cerevisiae* IGC 3507111 grown at 30°C (a), 15.5°C (b), and 8.5°C (c)

40 mg/L ~ 0.004 %w/v

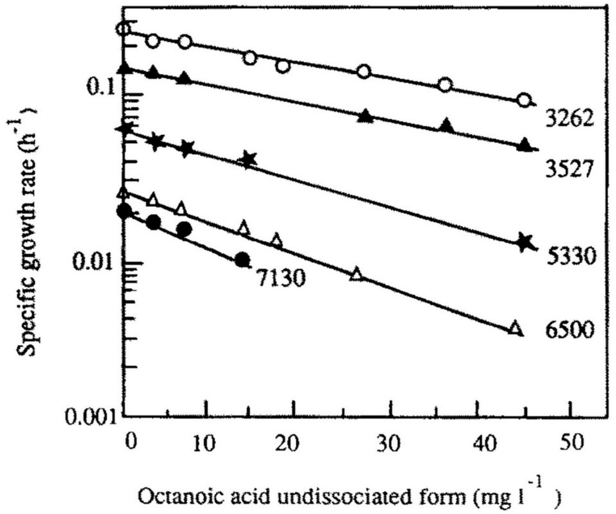
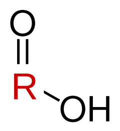


Figure 1: Effects of increasing concentrations of octanoic acid (undissociated form) on the maximal specific growth rate of *Saccharomyces cerevisiae* IGC 3507111 at 30°C (A). 25°C (B), 15.5°C (C), 10°C (D), and 8.5°C(E)

(Viegas and Correia, 1995)

Fatty Acid Inhibition on Yeast



LCFA (Long Chain Fatty Acids)

R	Chemical	pKa
C16:0	palmitic acid	n/a
C16:1	palmitoleic acid	n/a
C18:1	oleic acid	n/a
C18:2	linoleic acid	n/a
C20:4	arachidonic acid	n/a

C13-C21 LCFA insoluble in water, soluble in fats/oils.

Inhibition on yeast not as dependent on pH

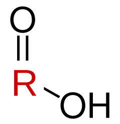
Many LCFA make up the yeast cell membrane, hydrolysis of corn oil

Manufactured by yeast/bacteria as part of their membrane lipids.

Mechanism of inhibition on yeast:

- Disruption of the yeast cell membrane via insertion
- Disruption of pH gradient generally does not occur.

Fatty Acid Inhibition on Yeast



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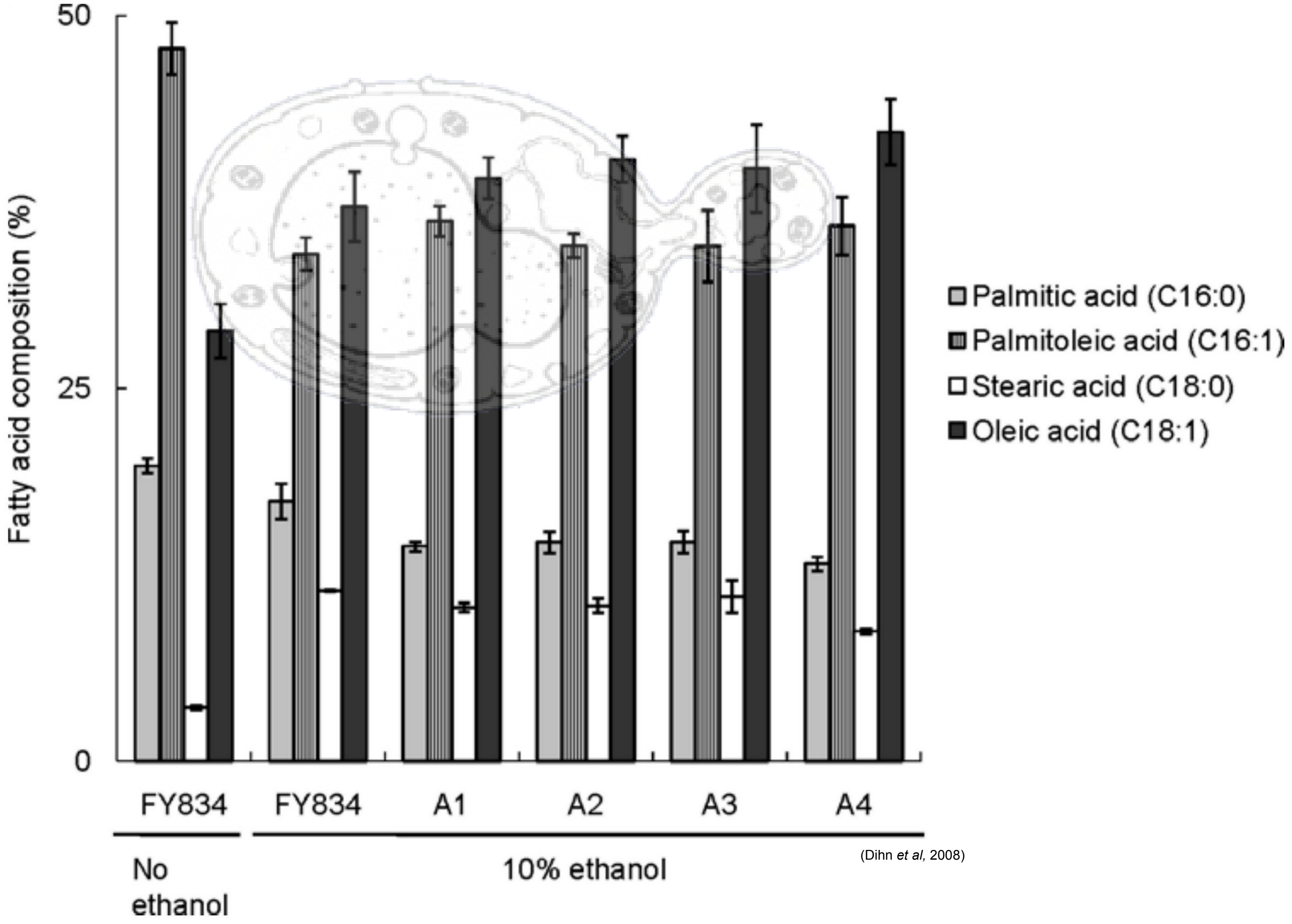

Fatty Acid Inhibition on Yeast

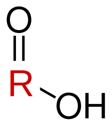

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Table II. Effect of palmitic acid (PA) and Tween 20 on the growth of *S. cerevisiae* on YP medium[a]

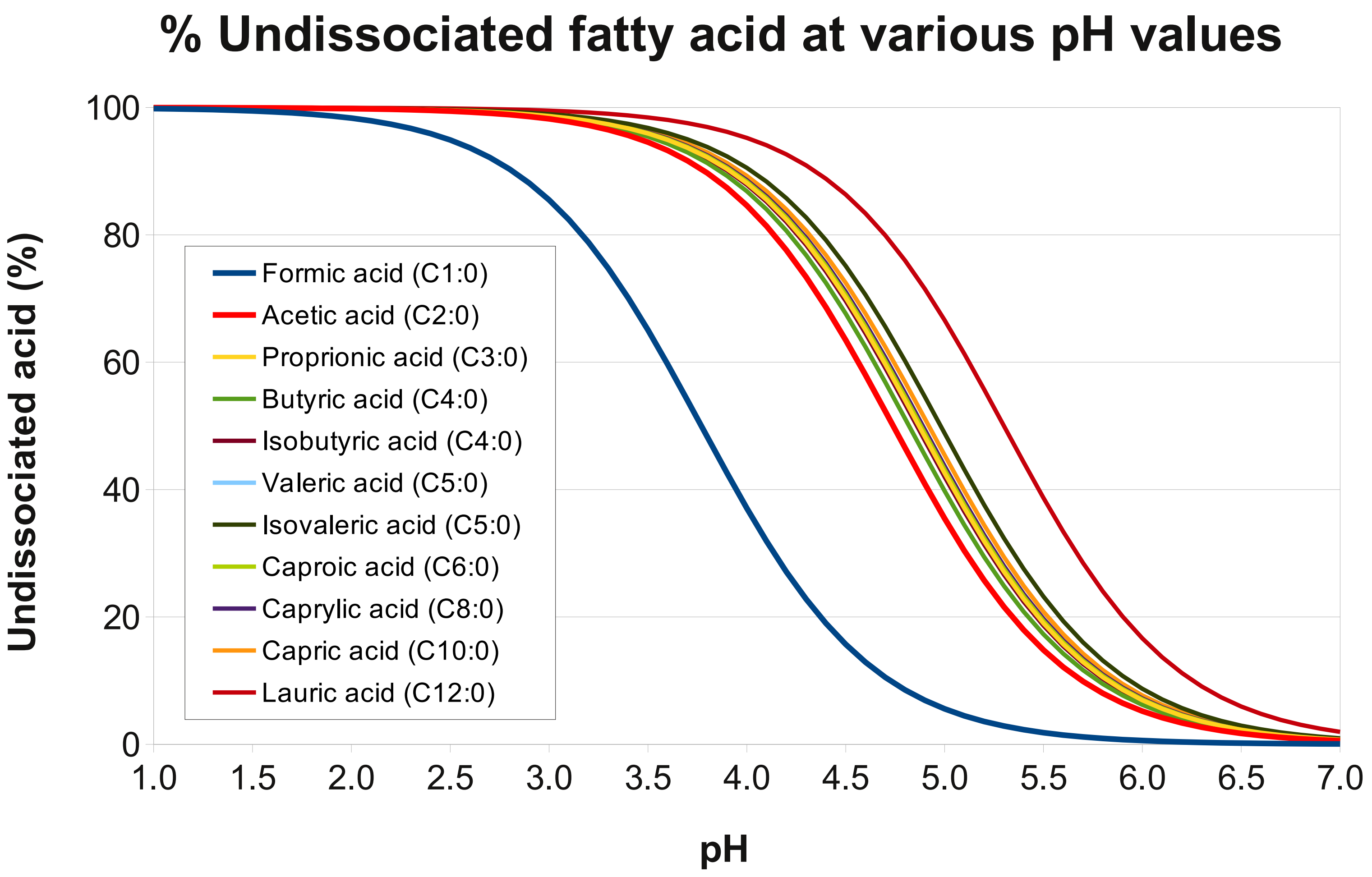
Addition	*n*	Cell dry mass mg/L medium
None	10	410 ± 50
0.1 mmol/L PA	3	180 ± 25*
0.3 mmol/L PA	3	151 ± 23*
3 mmol/L PA	11	189 ± 48*
3 mmol/L PA + 0.5 % Tween 20	4	263 ± 35* **

(Dell'Angelica *et al*, 1993)

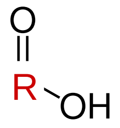
0.1 mM ~ 0.0026% w/v

[a]Cells were grown for 24 h. Each value represents mean -/+ SD; n = number of experiments. Student's t-test analysis: *p < 0.0001 vs. YP alone, **p < 0.01 vs. YP + 3 mmol/L PA

Fatty Acid Inhibition on Yeast



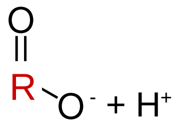
Fatty Acid Inhibition on Yeast



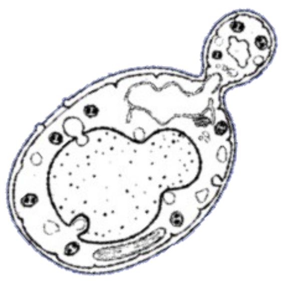
Undissociated fatty acid
(uncharged)

pH decrease

pH increase

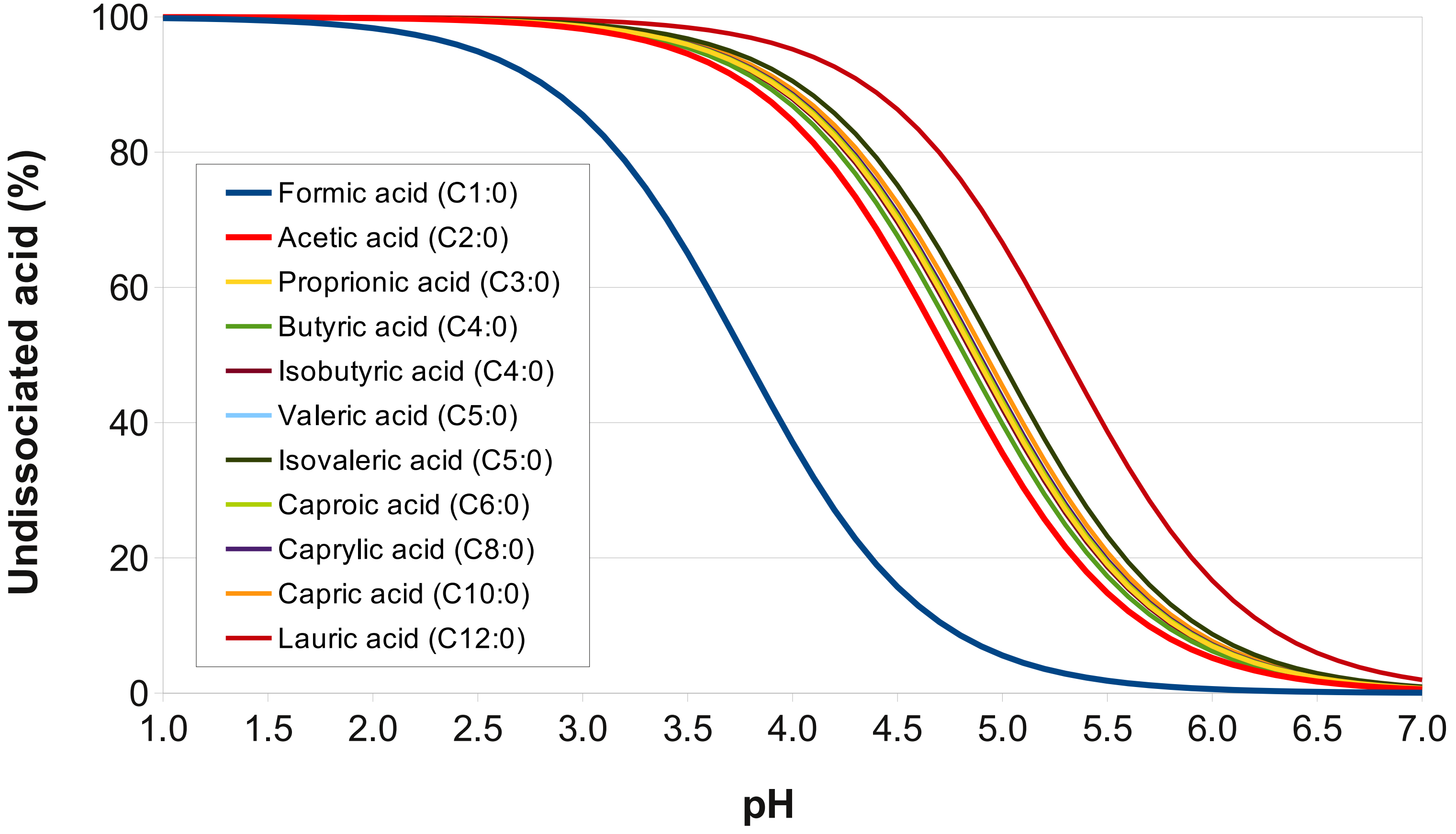


Dissociated fatty acid
(charged)

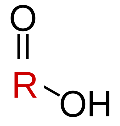


Inhibition on yeast

% Undissociated fatty acid at various pH values



Fatty Acid Inhibition on Yeast



Undissociated fatty acid
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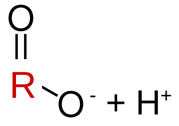
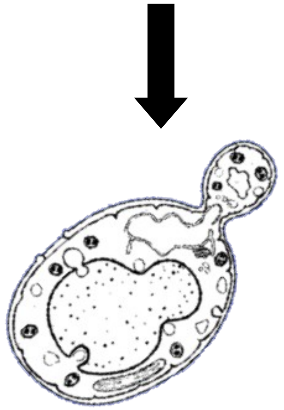
pH decrease

pH increase

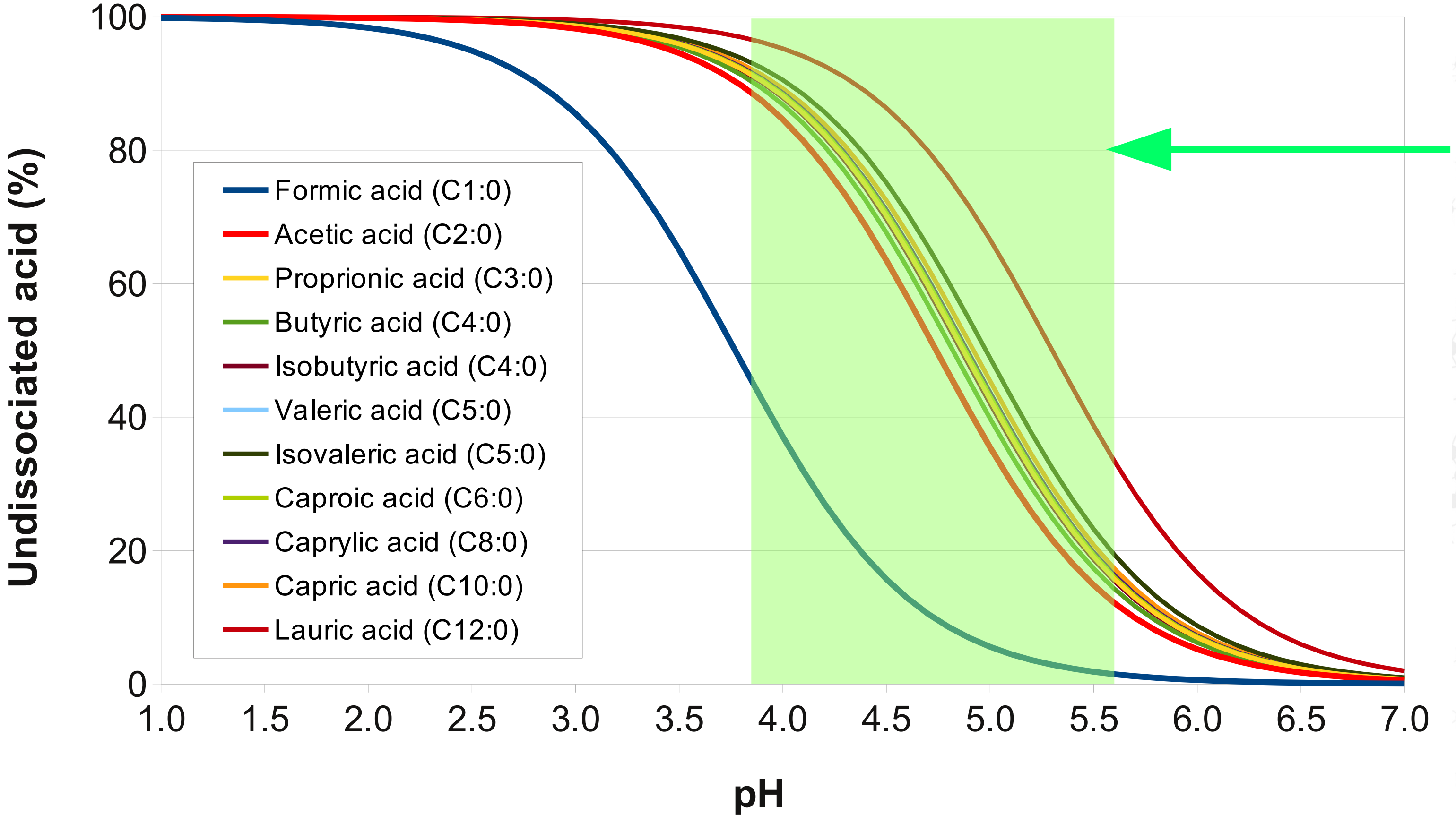


Dissociated fatty acid
(charged)



Inhibition on yeast

% Undissociated fatty acid at various pH values



Maximum delta in undissociated fatty acids occurs over fermentation/propagation pH range

Smallest change in pH in this range drastically changes ratio of undissociated and dissociated fatty acids

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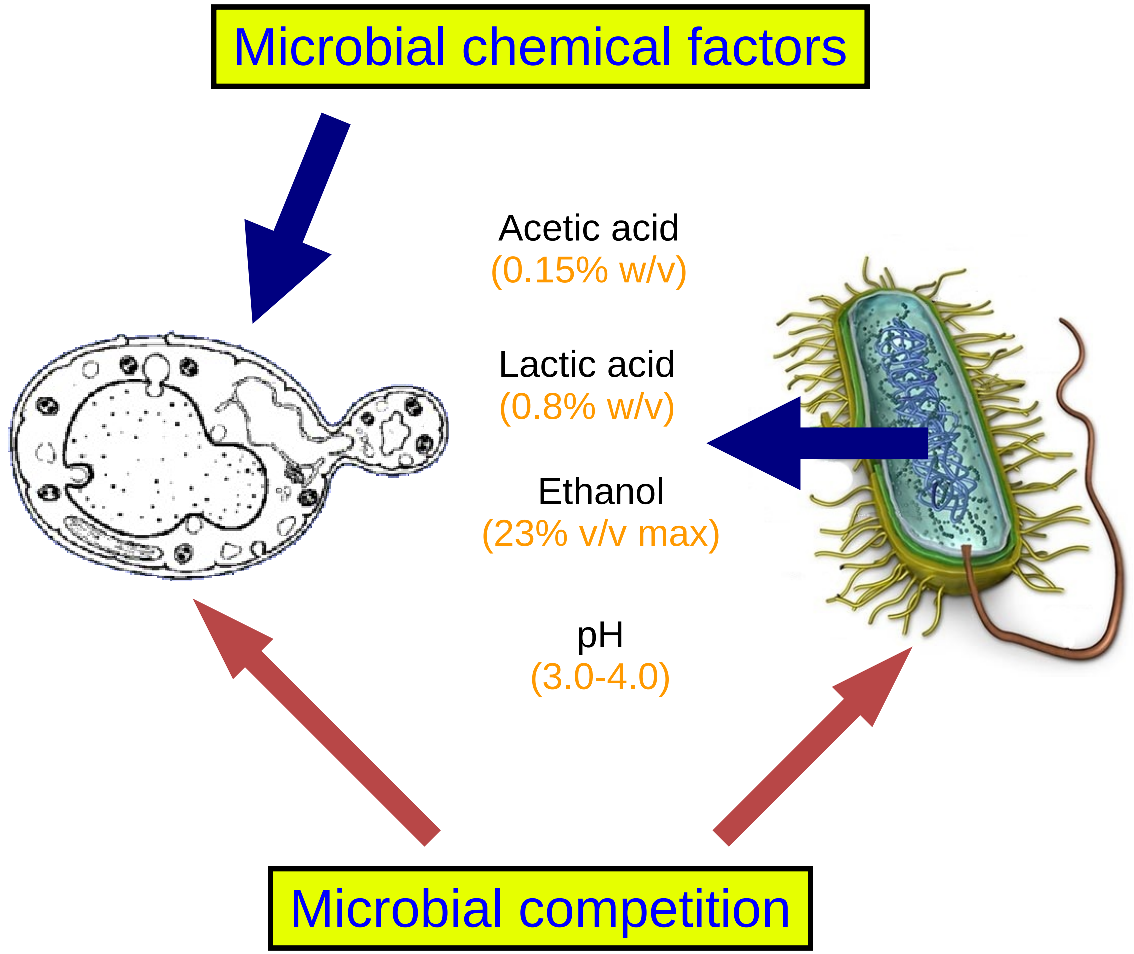
Combating contamination

Combating contamination



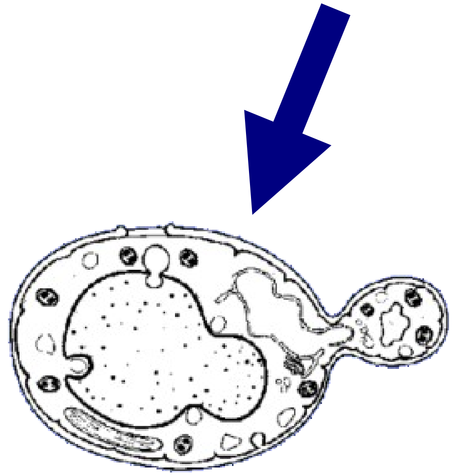
In severe contaminated plants, you have **both** bacterial production of acids and bacterial competition for nutrients as major issues that affect yeast.

At some plants, recycle of organic acids are alone responsible for yeast inhibition.

Can we somehow reduce the effect of one or both of these of these to provide the yeast some “breathing room”?

Combating contamination

Microbial chemical factors



Acetic acid
(0.15% w/v)

Lactic acid
(0.8% w/v)

Ethanol
(23% v/v max)

pH
(3.0-4.0)

Microbial competition

In severe contaminated plants, you have **<u>both</u>** bacterial production of acids and bacterial competition for nutrients as major issues that affect yeast.

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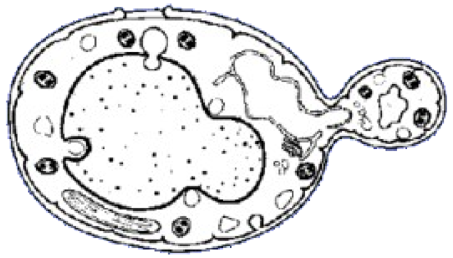
Combating contamination

Microbial chemical factors



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At some plants, recycle of organic acids are alone responsible for yeast inhibition.

Can we somehow reduce the effect of one or both of these of these to provide the yeast some "breathing room"?

1. Add antibiotics to limit growth of bacterial population and microbial competition

2. Raise pH to reduce inhibition of pKa chemicals

Permeability of yeast membrane

Acetic acid (dissociated state)

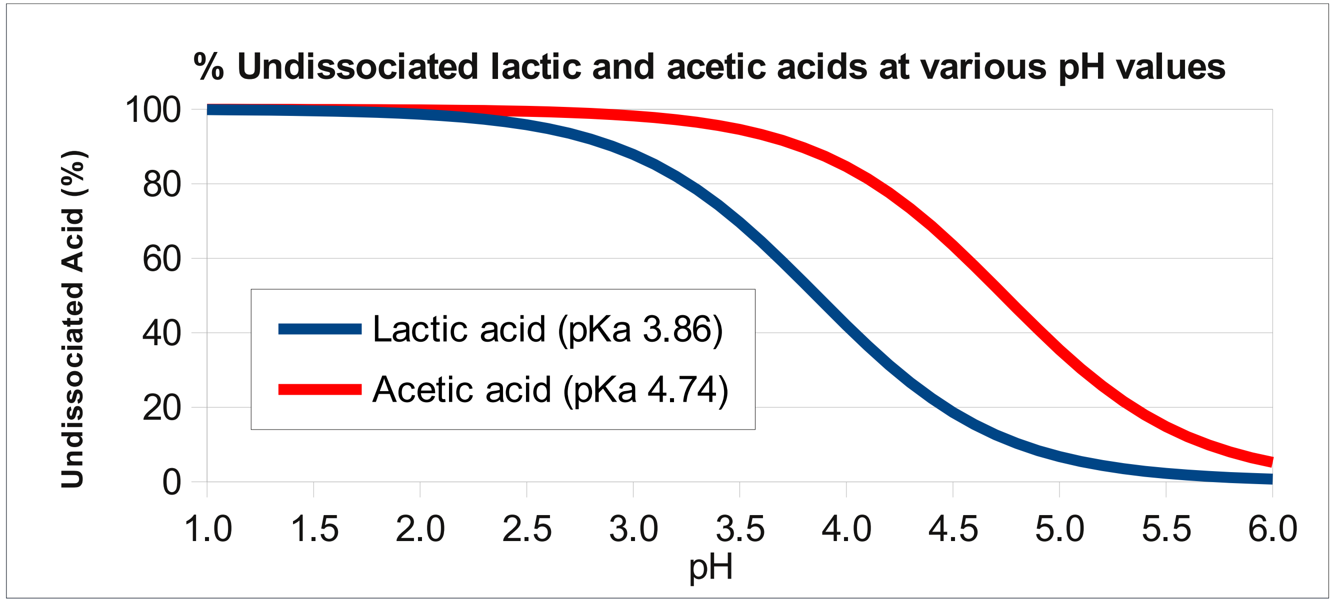
pH down ↓ ↑ pH up

Acetic acid (undissociated state)

Lactic acid (dissociated state)

pH down ↓ ↑ pH up

Lactic acid (undissociated state)



Lactic and acetic acid states dependent on pH and pka

HPLC/GC determines total amounts of lactic/acetic acids

Permeability of yeast membrane

Acetic acid (dissociated state)

pH down ↓ pH up ↑

Acetic acid (undissociated state)

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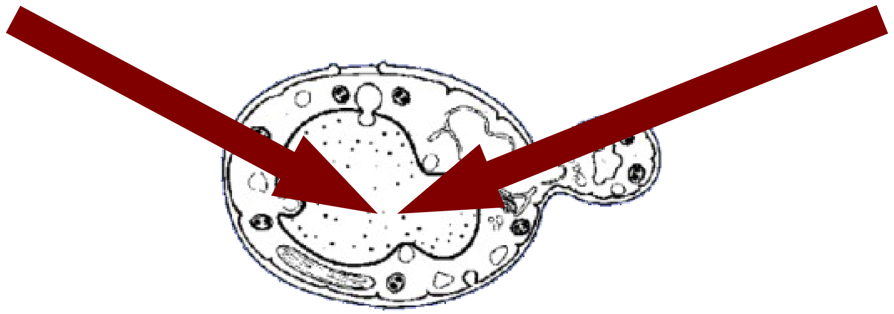
Lactic acid (undissociated state)



Lactic and acetic acid states dependent on pH and pka

HPLC/GC determines total amounts of lactic/acetic acids

Only the undissociated form of pKa chemicals can enter into the yeast cell

Permeability of yeast membrane

Acetic acid (dissociated state)

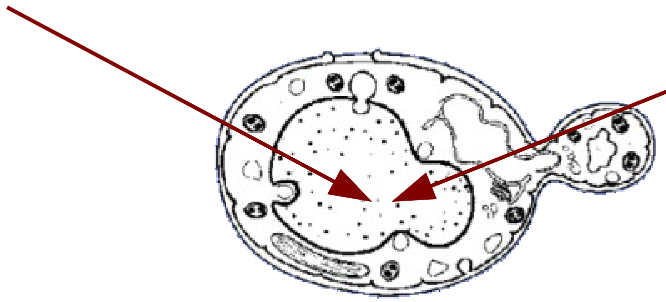
pH down ↓ ↑ pH up

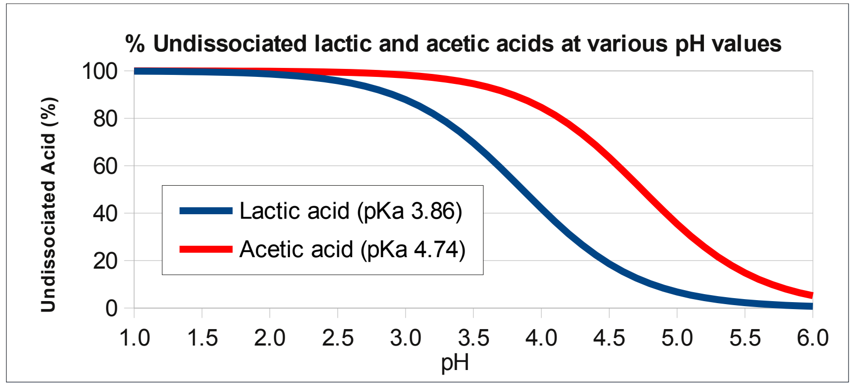
Acetic acid (undissociated state)

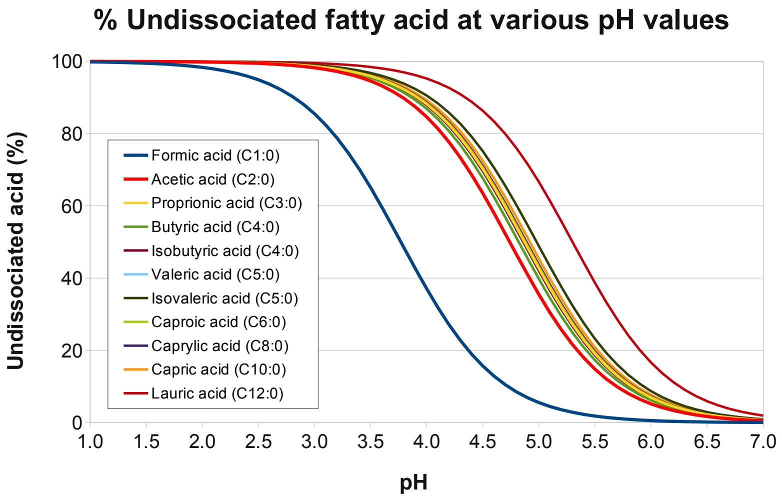
Lactic acid (dissociated state)

pH down ↓ ↑ pH up

Lactic acid (undissociated state)







Lactic and acetic acid states dependent on pH and pka

HPLC/GC determines total amounts of lactic/acetic acids

Only the undissociated form of pKa chemicals can enter into the yeast cell

Raising the pH reduces the concentration of the undissociated form of pKa chemicals which consequently lowers the inhibitory effect of the pKa chemical

Total amount of chemical remains the same (HPLC)

Raising the pH with contamination

What's the catch?

Raising the pH with contamination

What's the catch?

Higher pH	Least inhibitory to yeast and bacteria growth (bacteria have advantage of growth rate) pka chemicals least inhibitory
0.2 pH Absolute Increase	Less inhibitory to yeast and bacteria growth rate pka chemicals less inhibitory
Lower pH	Most inhibitory to yeast and bacteria growth rate pka chemicals most inhibitory

Raising the pH with contamination

What’s the catch?

Higher pH

Least inhibitory to yeast and bacteria growth (bacteria have advantage of growth rate)

pka chemicals least inhibitory

0.2 pH Absolute Increase

Less inhibitory to yeast and bacteria growth rate

pka chemicals less inhibitory

Lower pH

Most inhibitory to yeast and bacteria growth rate

pka chemicals most inhibitory

“Balancing act” between reducing organic acid stress on yeast and bacterial growth.

“A little is good, more is better” does not apply here. If pH is overshot, remaining viable bacterial contamination will have competitive advantage over yeast (game over).

Must monitor pH change after additions to ensure pH is not overshot.

Must add antibiotics to counter any increase in bacterial growth due to increase in pH

Potential foaming issues (i.e. mash reacting with caustic)

Challenges on what to add to raise pH and how

Does not always work. Cannot shift all pKa chemical to the dissociated form. Remaining undissociated form may be at inhibitory levels for the yeast.

Raising the pH with contamination

What to raise it with?

Raising the pH with contamination

What to raise it with?

1. Chemicals

Caustic	Strongest base
Urea (pKb 13.9, pKa 0.1)	↓
Ammonia (aq) (pKa 10.5)	Weakest base

Raising the pH with contamination

What to raise it with?

1. Chemicals

Caustic	Strongest base
Urea (pKb 13.9, pKa 0.1)	↓
Ammonia (aq) (pKa 10.5)	Weakest base

Since Ammonia and Urea have pKa/b, they are classified as weak bases. As such not all of each chemical is present in full dissociated form. Consequently, more of each is needed than caustic (which fully dissociates) to do the same job.

Estimates place the amount of ammonia at 5-8 fold and urea at 3-5 fold to do the same job as caustic at 1 fold.

May not have enough room in vessels to add increased amounts of urea/ammonia

Caustic fully dissociates which means that all of the chemical influences the pH and thus the least volume of a pKa chemical is needed.

Raising the pH with contamination

What to raise it with?

1. Chemicals

Caustic	Strongest base
Urea (pKb 13.9, pKa 0.1)	↓
Ammonia (aq) (pKa 10.5)	Weakest base

2. Process changes

Reduce backset addition to front end

Reduce sulfuric acid use at front end

Yeast Stress Factors

Inhibition

Operational factors

Glucose content
(10% w/v max)

Sulfite
(100 ppm)

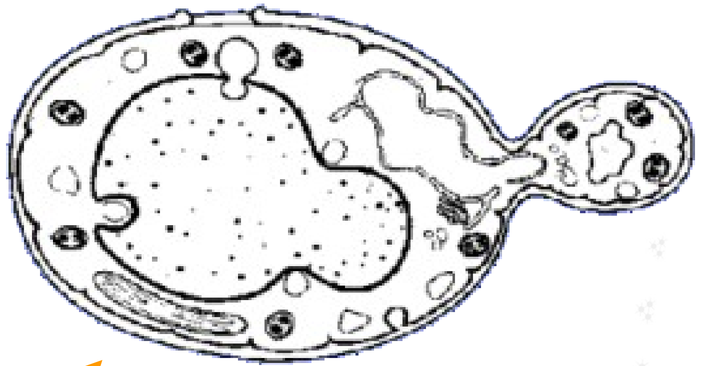
Temperature
(35ºC = 95ºF)

Sodium
(500 ppm)

CIP
Chemicals

Nutritional factors
(media formula)

Lack of: Sterols, Nitrogen,
Oxygen, UFA, Minerals/vitamins



Microbial factors

Chemical

Acetic acid
(0.05% w/v)

Ethanol
(23% v/v max)

Lactic acid
(0.8% w/v max)

Mycotoxins
(10-100 ppm)

pH
(<4.0 or >6.0)

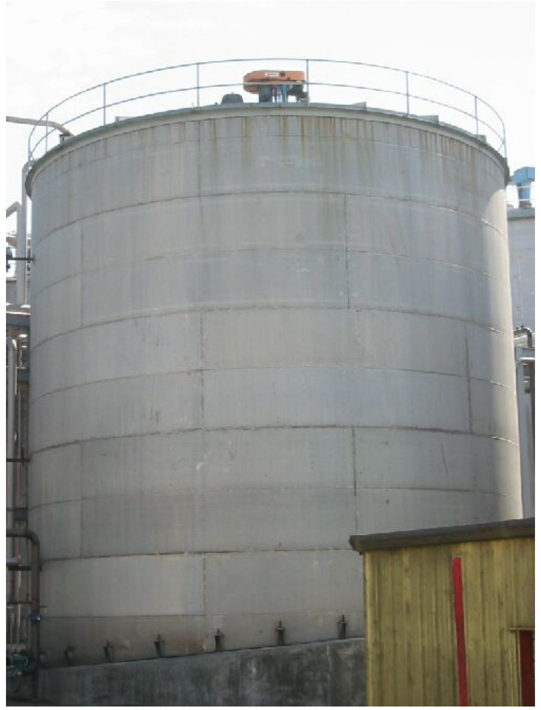
Fusel volatiles
(0.1–1 % w/v)

Competition

Bacterial nutrient depletion
(trace nutrients)

Raising the pH with Caustic

Adding sodium to the fermentor



Fermentation

To raise the pH by 0.2 absolute units in a fermentor requires ~3000 gal 5% caustic

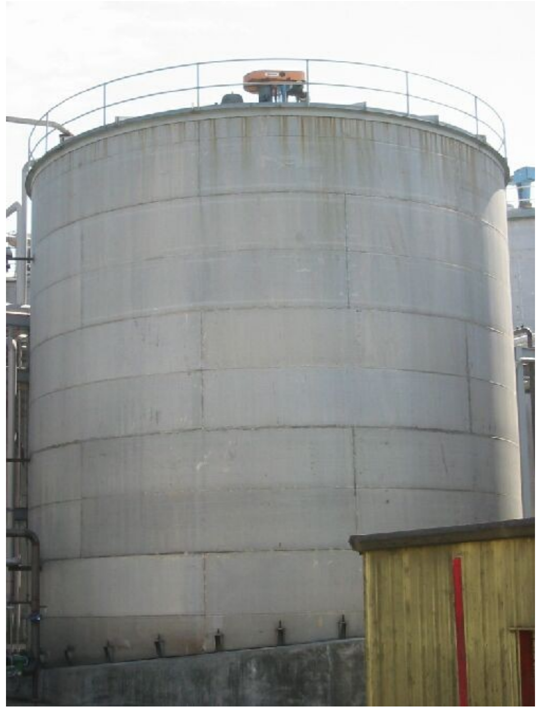
pH verified by monitoring sampling port on fermentor recycle every 15 minutes for 2 hours

Calculations (plant example)

Size of Fermentor	750,000 gal
	2,839,050 L
% Fill in Fermentor	95 %
Working volume in Fermentor	2,697,098 L
Strength of Caustic	5 % (w/v)
	5 g/100ml
	50 g/L
Volume of Caustic to add	3,000 gal
	11,356 L
Amount of Caustic added to Fermentor	567,810 g
	567,810,000 mg
Molecular weight of NaOH	39.9971 g/mol
Percent sodium in NaOH	57.48 %
Amount of Sodium added	**121 ppm**

Raising the pH with Caustic

Adding sodium to the fermentor



Fermentation

To raise the pH by 0.2 absolute units in a fermentor requires ~3000 gal 5% caustic

pH verified by monitoring sampling port on fermentor recycle every 15 minutes for 2 hours

Calculations (plant example)

Current cost of caustic	\$560.00 per ton \$0.28 per pound \$0.13 per kg
Amount of Caustic added to Fermentor	567,810 g 567.81 kg
Cost of 3000 gal 5% (w/v) caustic	**\$72.12**

When to raise pH in contaminated plant

What situations at a plant have been tested?

When to raise pH in contaminated plant

What situations at a plant have been tested?

1. Temporarily in blend tank once contamination discovered in plant (Emergency situation).
2. Temporarily after contamination successfully controlled with antibiotics (Post-infection*).

* Has not yet been tested at a plant but symptoms similar to 1 in recycle of pKa chemicals

When to raise pH in contaminated plant

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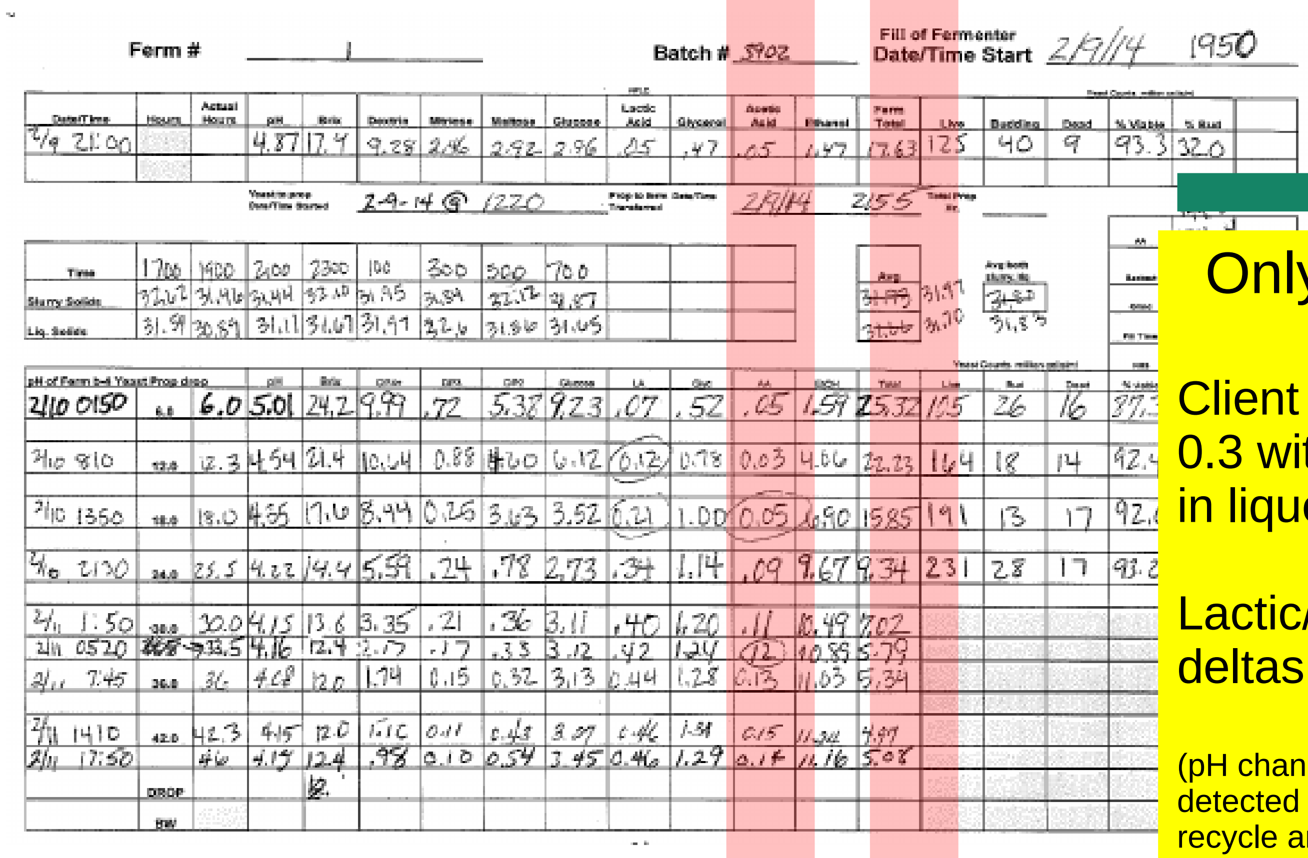
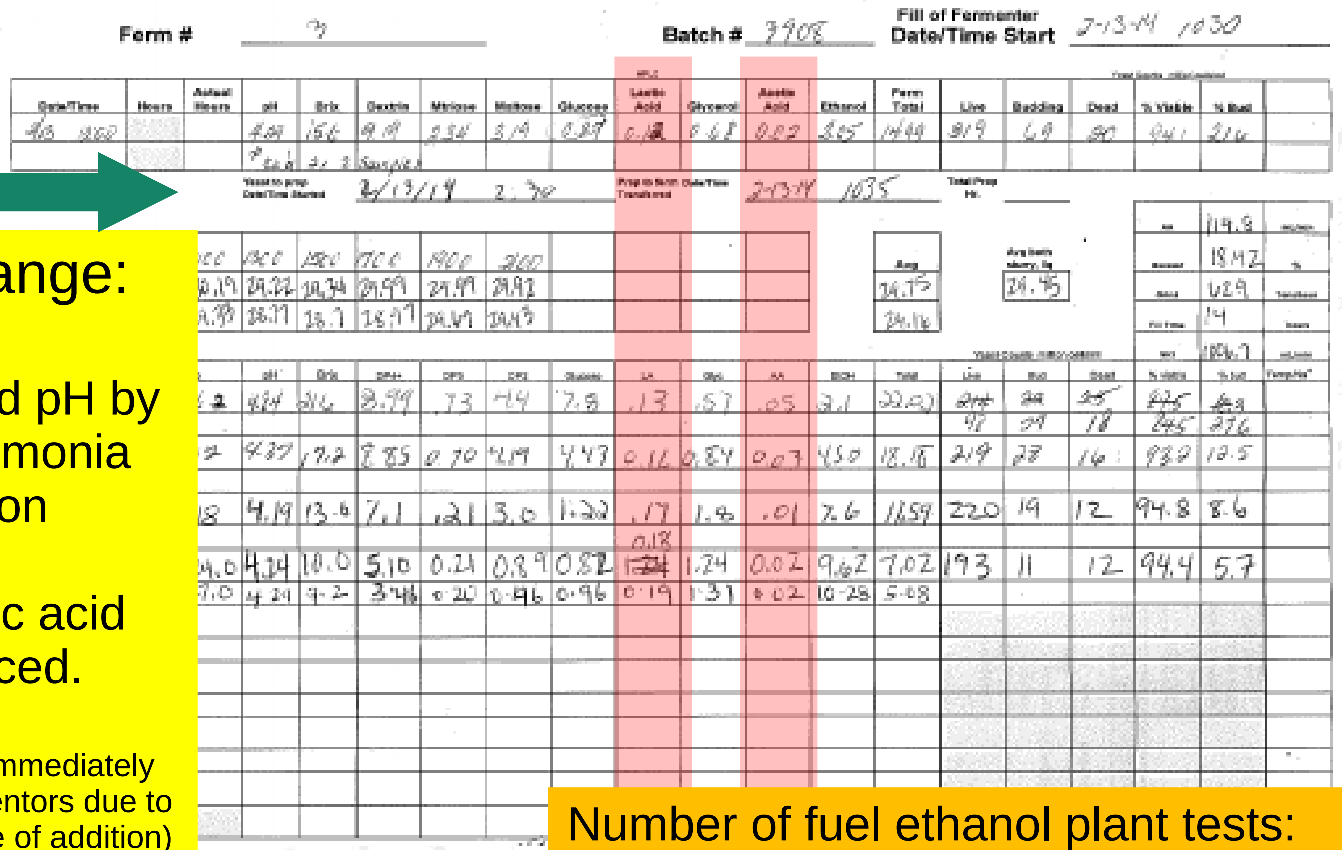




Only change:

Client raised pH by 0.3 with ammonia in liquefaction

Lactic/Acetic acid deltas reduced.

(pH change not immediately detected in fermentors due to recycle and place of addition)

Number of fuel ethanol plant tests:	6
Number of successes:	2

* Has not yet been tested at a plant but symptoms similar to 1 in recycle of pKa chemicals

When to raise pH in contaminated plant

What situations at a plant have been tested?

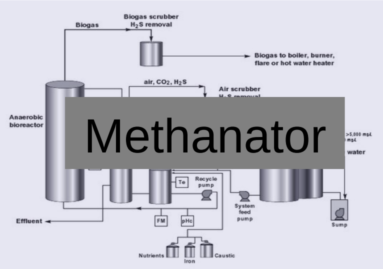
3. Temporarily in blend tank or fermentor with Methanator process upsets (Emergency situation).

When to raise pH in contaminated plant

What situations at a plant have been tested?

3. Temporarily in blend tank or fermentor with Methanator process upsets (Emergency situation).

Recall that Methanator process upsets can spew out a variety of chemicals



What chemicals that may be present in methanator effluent that may be inhibiting yeast growth/metabolism?

What levels of methanator effluent needed for inhibition?

Fatty acids (mix)	Inhibition on yeast well studied in literature but not at ethanol plants
Simple sugars (mix)	Sugars from starch carbohydrate hydrolysis not inhibitory to yeast
Amino acids (mix)	Not inhibitory to yeast as can be used as source of FAN nutrition
Hydrogen	Volatile, not inhibitory to yeast
Acetic acid	Inhibitory to yeast starting at 0.05% w/v
Volatile fatty acids (VFA)	Inhibition on yeast well studied in literature but not at ethanol plants
Alcohols (mix)	Fusels compounds inhibitory to yeast starting at 0.04% w/v
Ammonia	Not inhibitory to yeast as can be used as source of FAN nutrition
Carbon dioxide	Not inhibitory to yeast at operating conditions in plant
Hydrogen sulfide	Volatile, inhibitory to yeast

Number of fuel ethanol plant tests:	12
Number of successes:	8

When to raise pH in contaminated plant

What situations at a plant have been tested?

4. Temporarily in stalled fermentors that show no yeast activity that have high organic acids (>0.5% w/v acids), >30hr fermentation time, 6-10% total sugars remaining) (Emergency situation).

When to raise pH in contaminated plant

What situations at a plant have been tested?

4. Temporarily in stalled fermentors that show no yeast activity that have high organic acids (>0.5% w/v acids), >30hr fermentation time, 6-10% total sugars remaining) (Emergency situation).

Most times, plant attempts to add more yeast and urea to the fermentor as quick first steps to try to polish off fermentors with marginal effect.

Additional yeast added to same environment as current inhibited yeast – no benefit to the yeast! Must change the environment for the yeast to make a difference.

At plants where yeast activity was resumed with an increase in pH, Ethanol yield and total sugars did not reach their respective (drop) levels as per "Good" fermentors but reached approximately 80-90% of their respective drop (Fully stalled fermentors with no intervention remained at 80%)

Number of fuel ethanol plant tests:	20
Number of successes:	14

When to raise pH in contaminated plant

What situations at a plant have been tested?

5. Temporarily in vessels when starting up a plant after a prolonged shutdown (Planned startup).

When to raise pH in contaminated plant

What situations at a plant have been tested?

5. Temporarily in vessels when starting up a plant after a prolonged shutdown (planned startup).

Antibiotics and antimicrobials have a difficult time keeping bacteria at bay for extended periods of time (weeks). Most antimicrobials and antibiotics do not have chemical ½ life spanning weeks.

Many non-LAB bacteria such as *Bacillus sp/Clostridia sp* (soil spore-forming bacteria) have an opportunity to grow (slowly) and produce many chemicals similar to what is seen with Methanator effluents that have process upsets.

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Ammonia	Not inhibitory to yeast as can be used as source of FAN nutrition
Carbon dioxide	Not inhibitory to yeast at operating conditions in plant
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Fermentors essentially become slow anaerobic digestors

Number of fuel ethanol plant tests:	3
Number of successes:	2

When to raise pH in contaminated plant

What situations at a plant have been tested?

6. Temporarily in fermentors with confirmed stalling due to fusel inhibition

When to raise pH in contaminated plant

What situations at a plant have been tested?

6. Temporarily in fermentors with confirmed stalling due to fusel inhibition

Table 1 Kinetic constants for the inhibition of growth of *Saccharomyces cerevisiae* by alcohols

Alcohol	α	P_m (g/l)	I_{20} (g/l)	I_{50} (g/l)
Ethanol	1.11	103.5	24.29	53.4
n-Propanol	0.64	14.5	1.21	4.9
n-Butanol	0.69	18.1	1.76	6.6
Isoamyl alcohol	0.93	8.1	1.44	3.8
2-phenylalcohol	1.96	3.1	1.37	2.1

Yeast Growth

Ethanol concentration

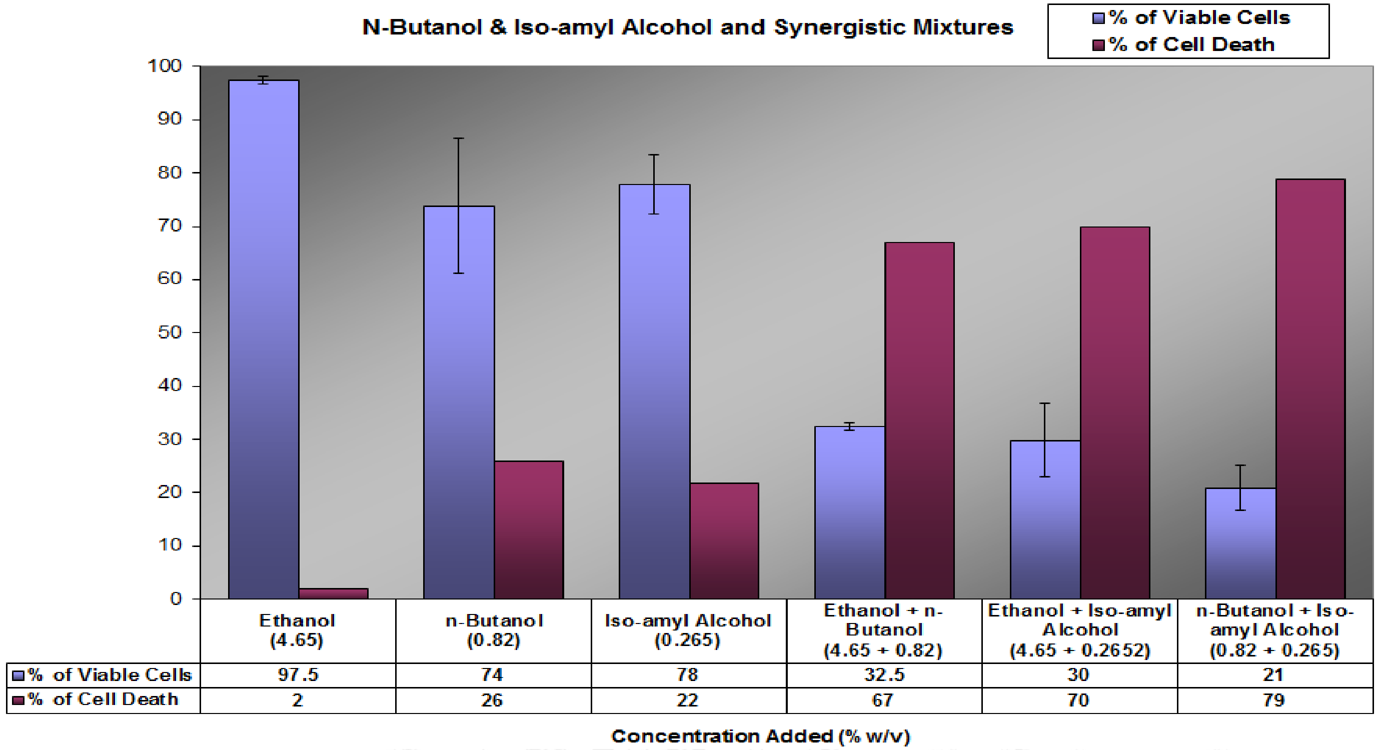
Conversions
2.1g/l
0.21 g/100ml
0.21% w/v

Table 2 Kinetic constants for cell death of *Saccharomyces cerevisiae* in suspensions containing various alcohols

Alcohol	β	P'_m (g/l)	LD_{20} (g/l)	LD_{50} (g/l)
Ethanol	3.05	79.6	49.96	63.42
n-Butanol	1.50	18.79	6.42	11.83
Isoamyl alcohol	1.09	8.17	1.87	4.32

Yeast Death

(Okolo et al, 1990)



Number of fuel ethanol plant tests: 4
Number of successes: 2

Isoamyl alcohol more toxic to yeast than ethanol by ~15 fold

Summary

pH and pKa both play a significant role in determining overall yeast inhibition by chemicals present at a fuel ethanol plant

pKa chemicals are ubiquitous at all fuel ethanol plants

Both the pH and the concentration of an inhibitory pKa chemical must be known in order to determine the concentration of the undissociated form of the pKa chemical which is the primary form that can enter the yeast cell to cause inhibition.

Lowering the pH at a contaminated plant has direct consequences on yeast health and will make existing pKa chemicals more inhibitory to the yeast. Raising the pH under certain circumstances will reduce pKa chemical inhibition on the yeast while proper antibiotics will reduce the inhibition of viable bacteria on the yeast.

Raising the pH at a contaminated plant is a viable option to counter the effects of contamination but must be implemented on a case by case basis at contaminated plants.

QUESTIONS?

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