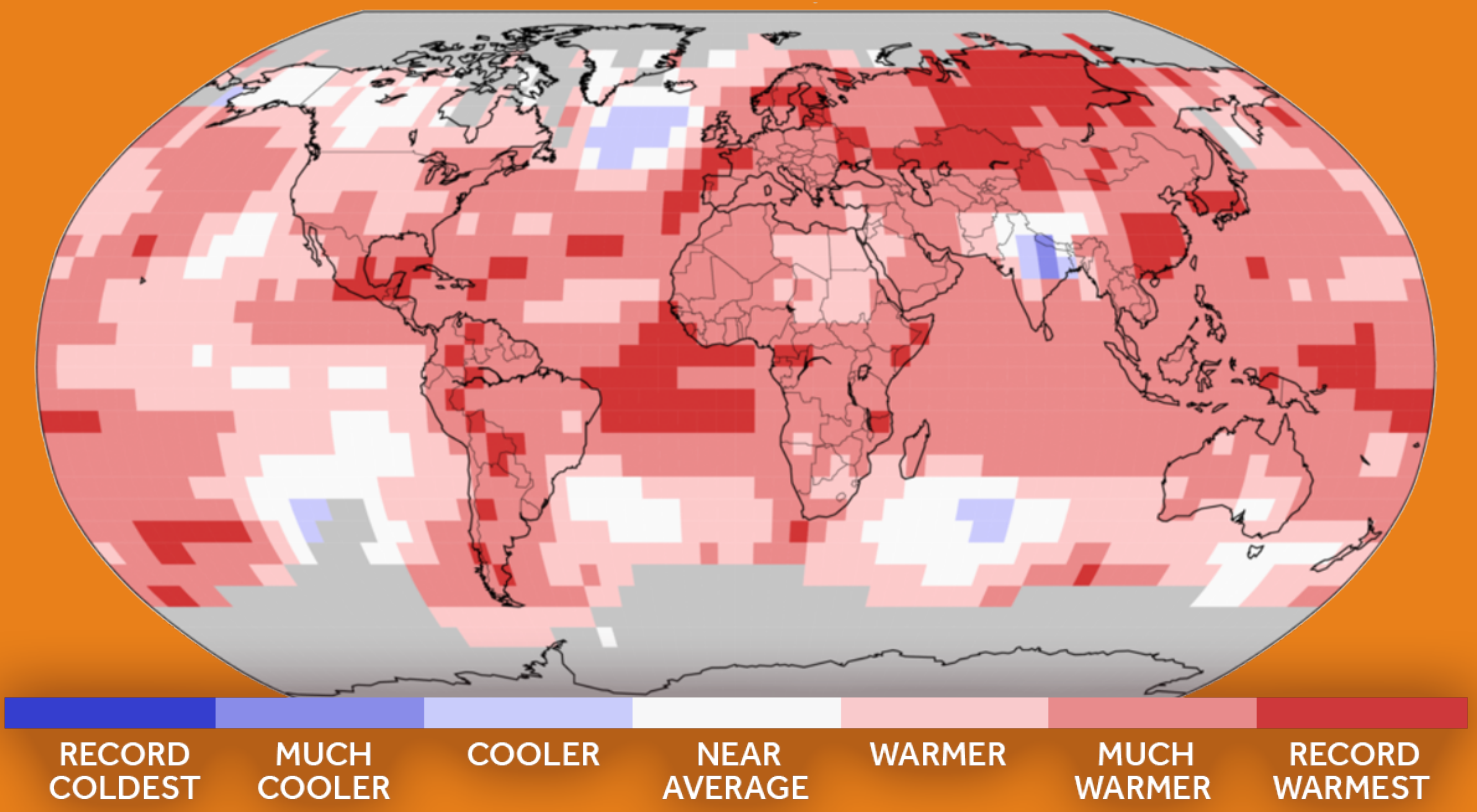
Engineering tolerance in yeast for cellulosic biofuels

Seminar Series for High School Teachers

Fall 2021

Felix Lam
Whitehead Institute
MIT Chemical Engineering

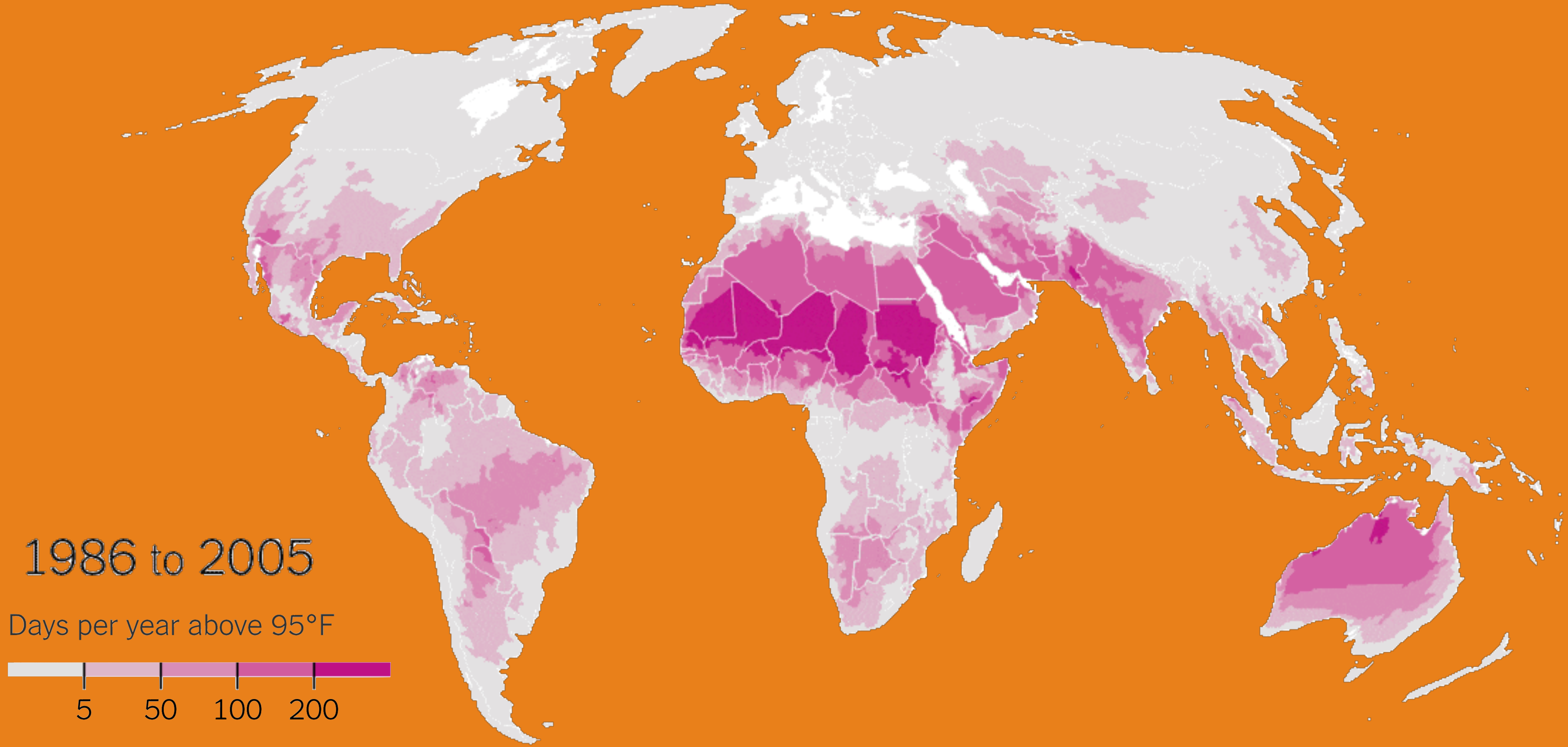
Global temperatures continue setting records



Jan – June 2020 Temperatures

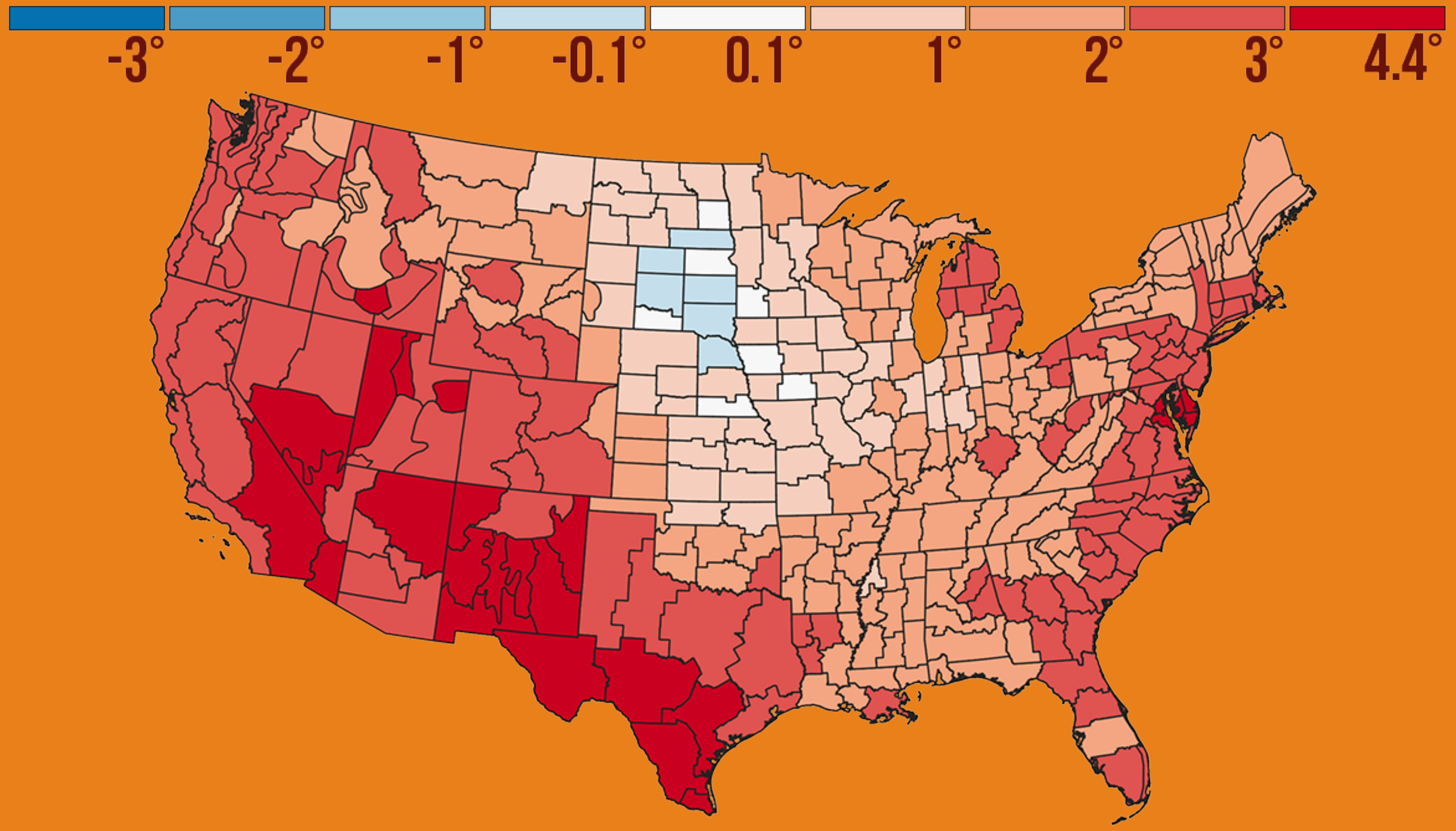
CLIMATE CENTRAL

Global temperatures continue setting records



Plumer *et al.*, *New York Times* (6/22/17)

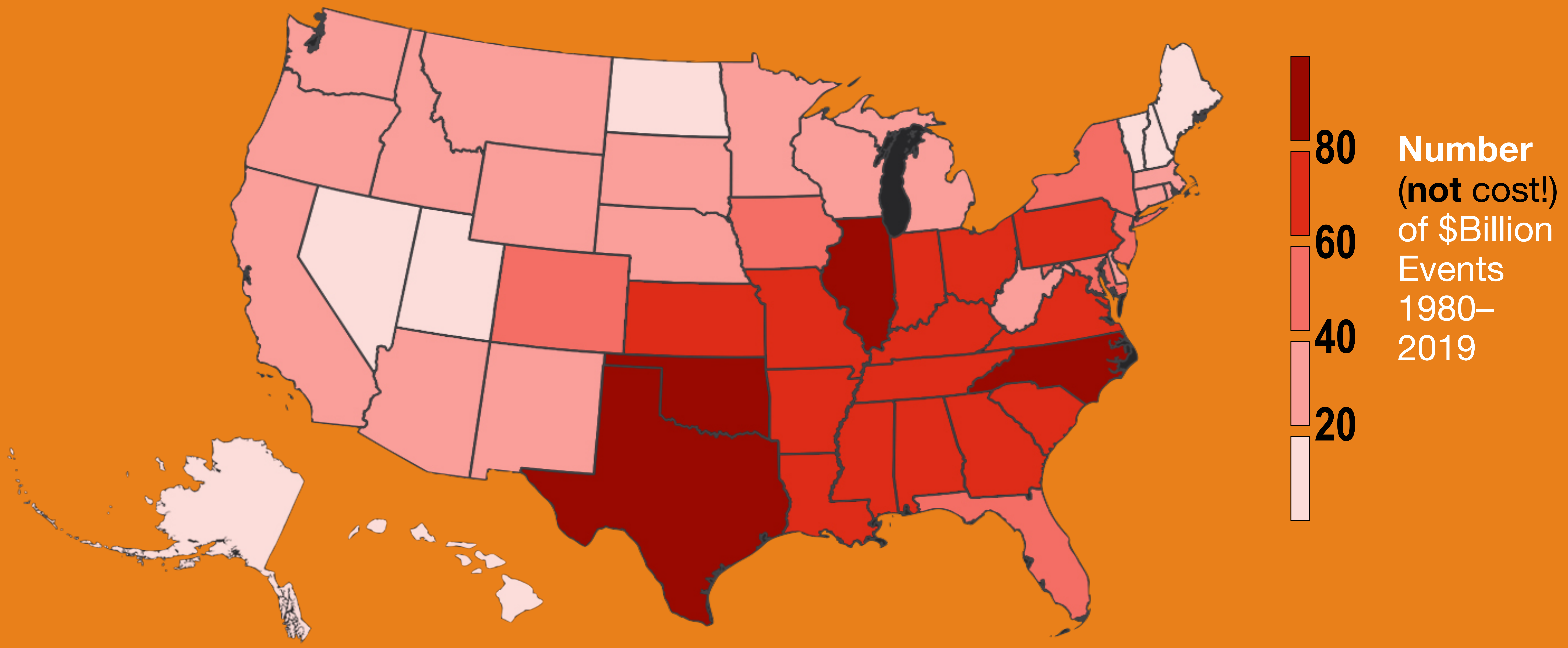
Global temperatures continue setting records



Summer Warmings Since 1970 (°F)

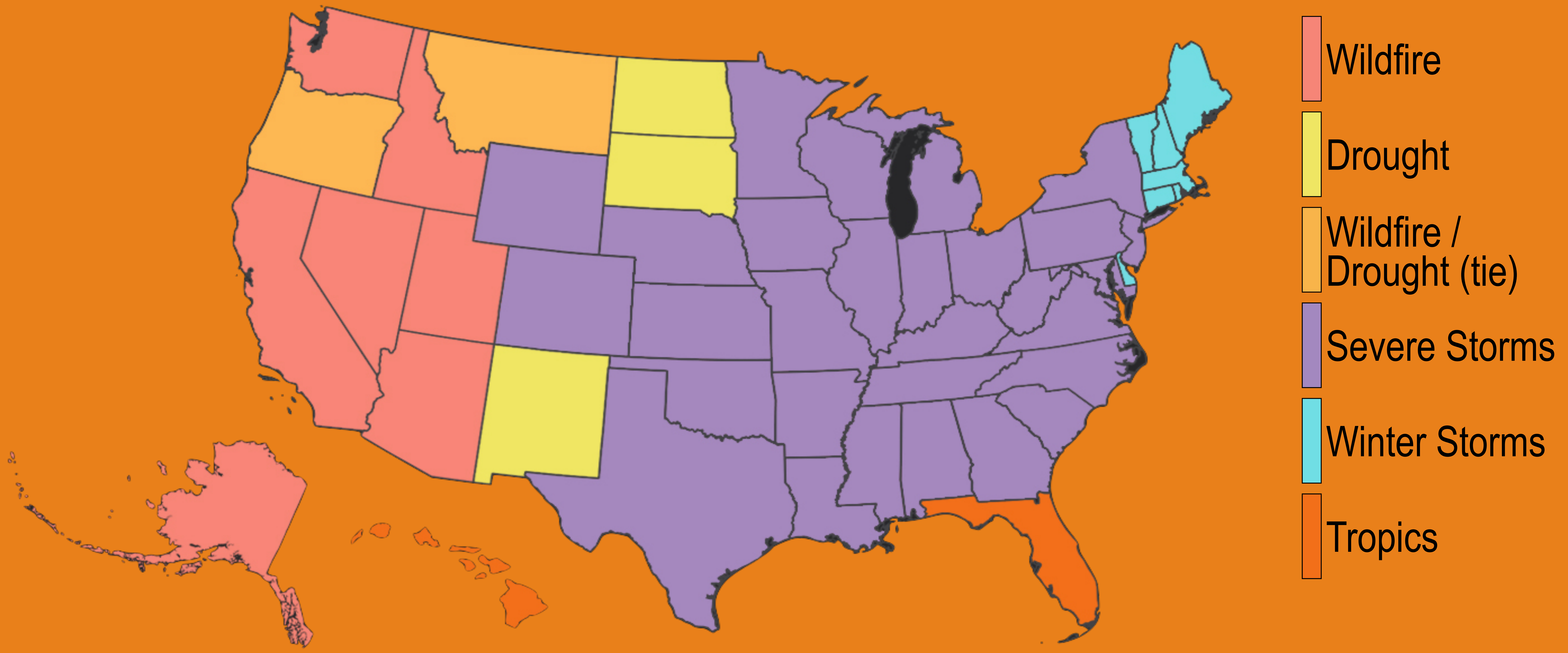
CLIMATE CENTRAL

Related $billion disasters continue setting records



CLIMATE CENTRAL

Related $billion disasters continue setting records



Most Frequent **Disaster Type** 1980–2019

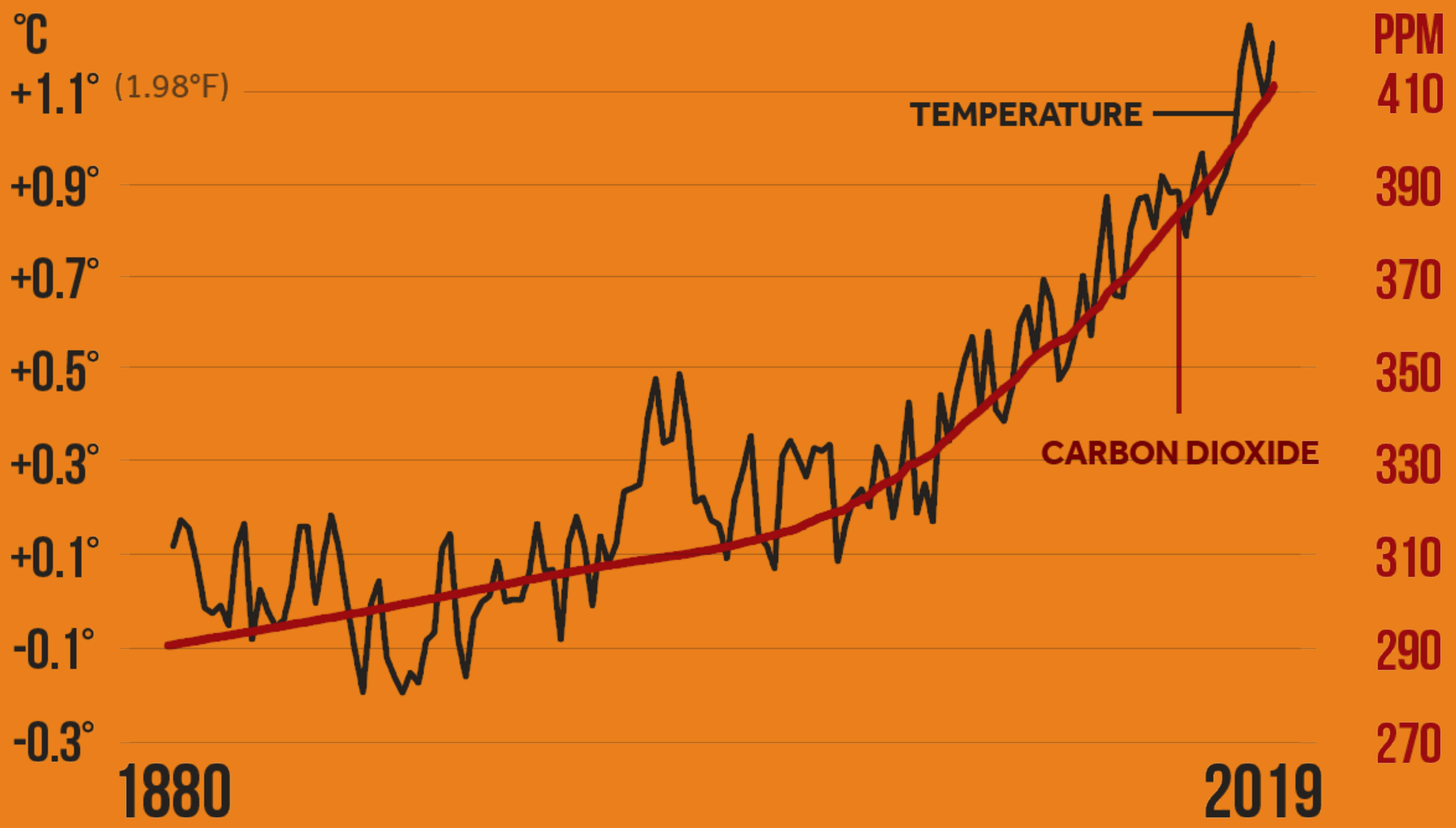
CLIMATE CENTRAL

Related $billion disasters continue setting records

Most Frequent **Disaster Type 1980–2019**

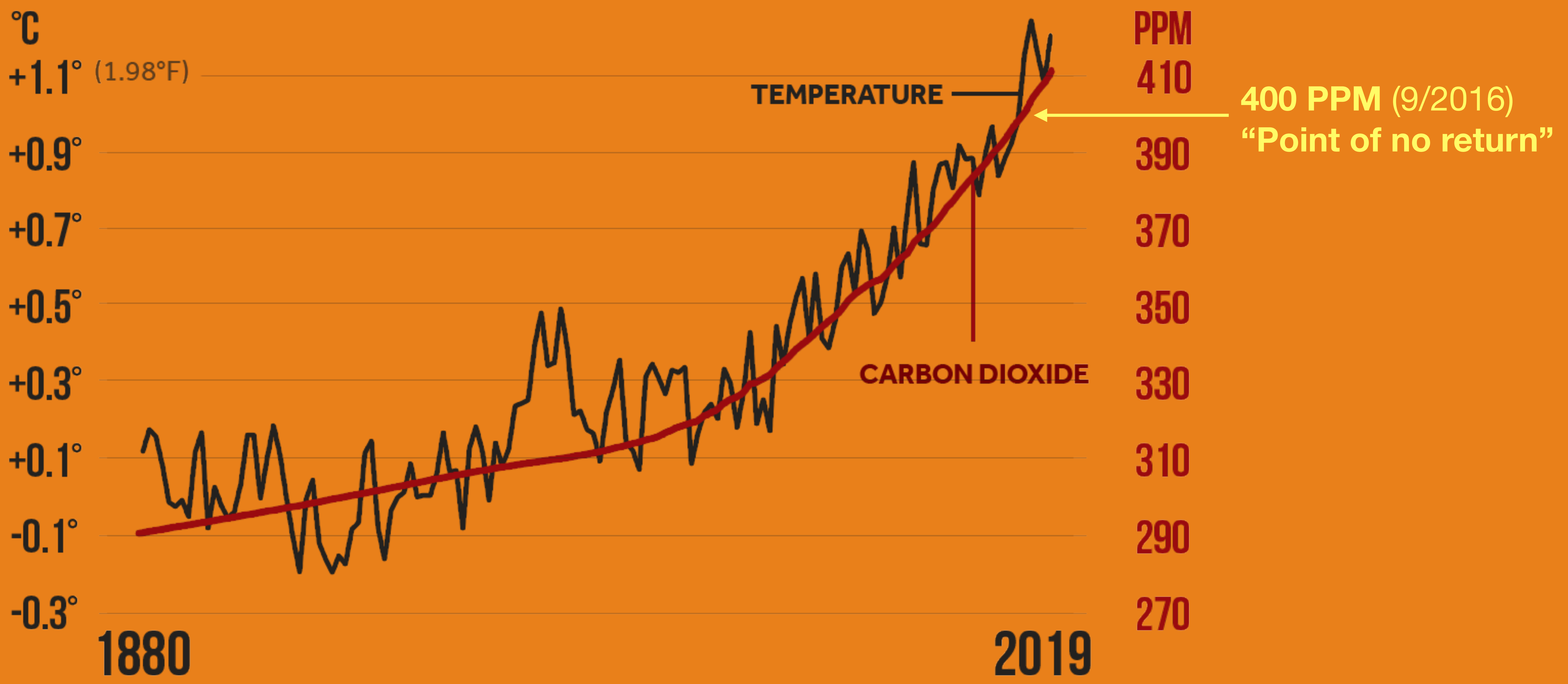
CLIMATE CENTRAL

Global temperatures directly linked to atmospheric CO_2



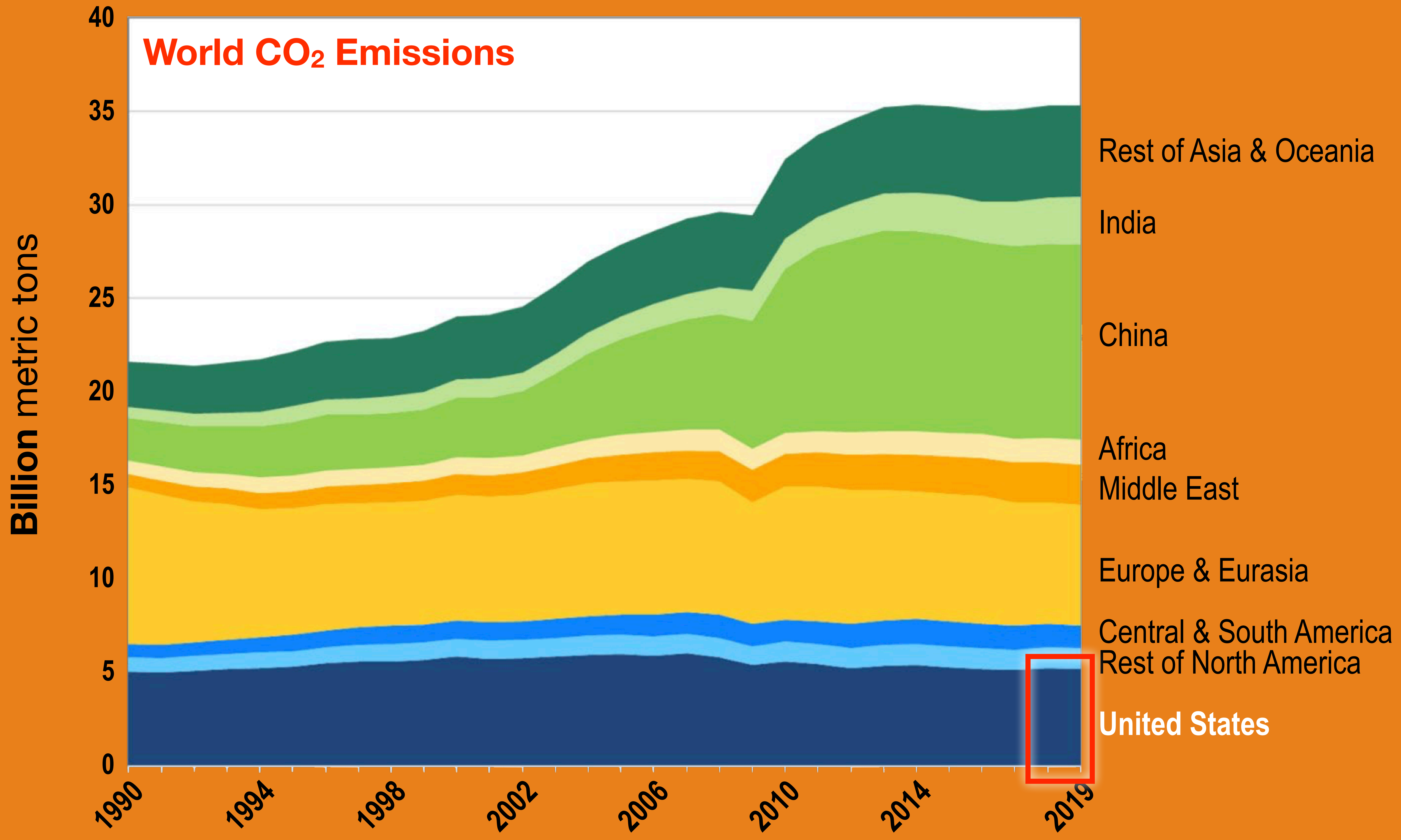
CLIMATE CENTRAL

Global temperatures directly linked to atmospheric CO_2



CLIMATE CENTRAL

Global temperatures directly linked to atmospheric CO_2



Transportation Energy Data Book, Ed. 39 (2021)

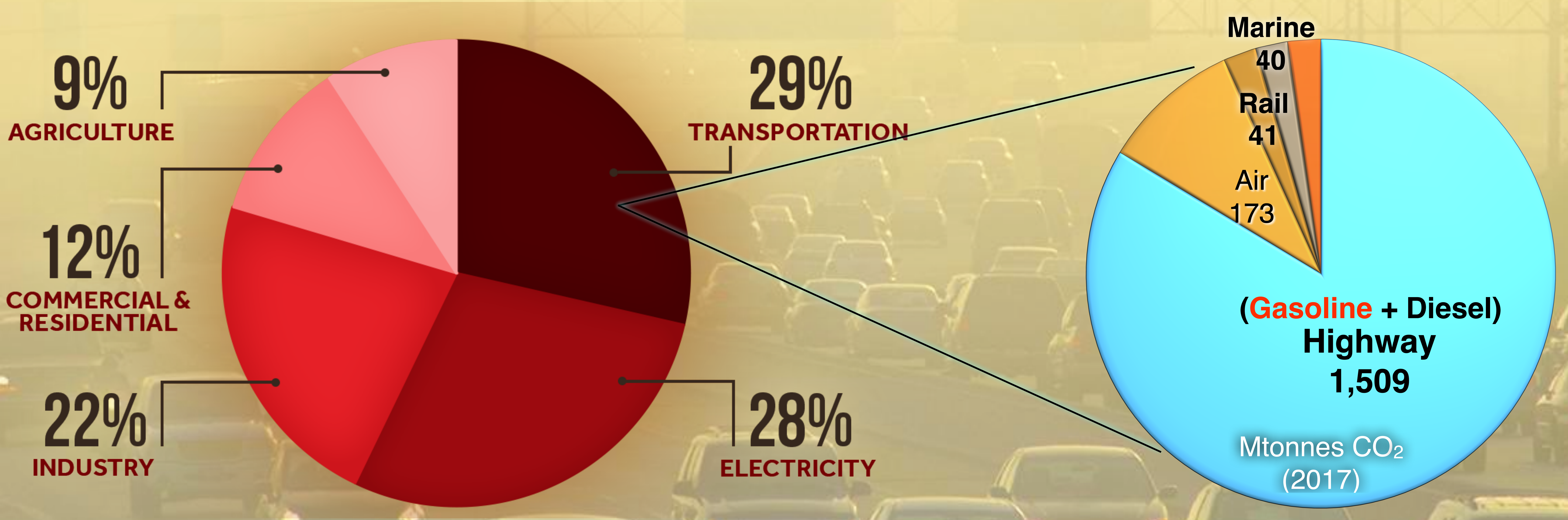
Highest US emissions from transportation

CLIMATE CENTRAL

Highest US emissions from transportation

CLIMATE CENTRAL

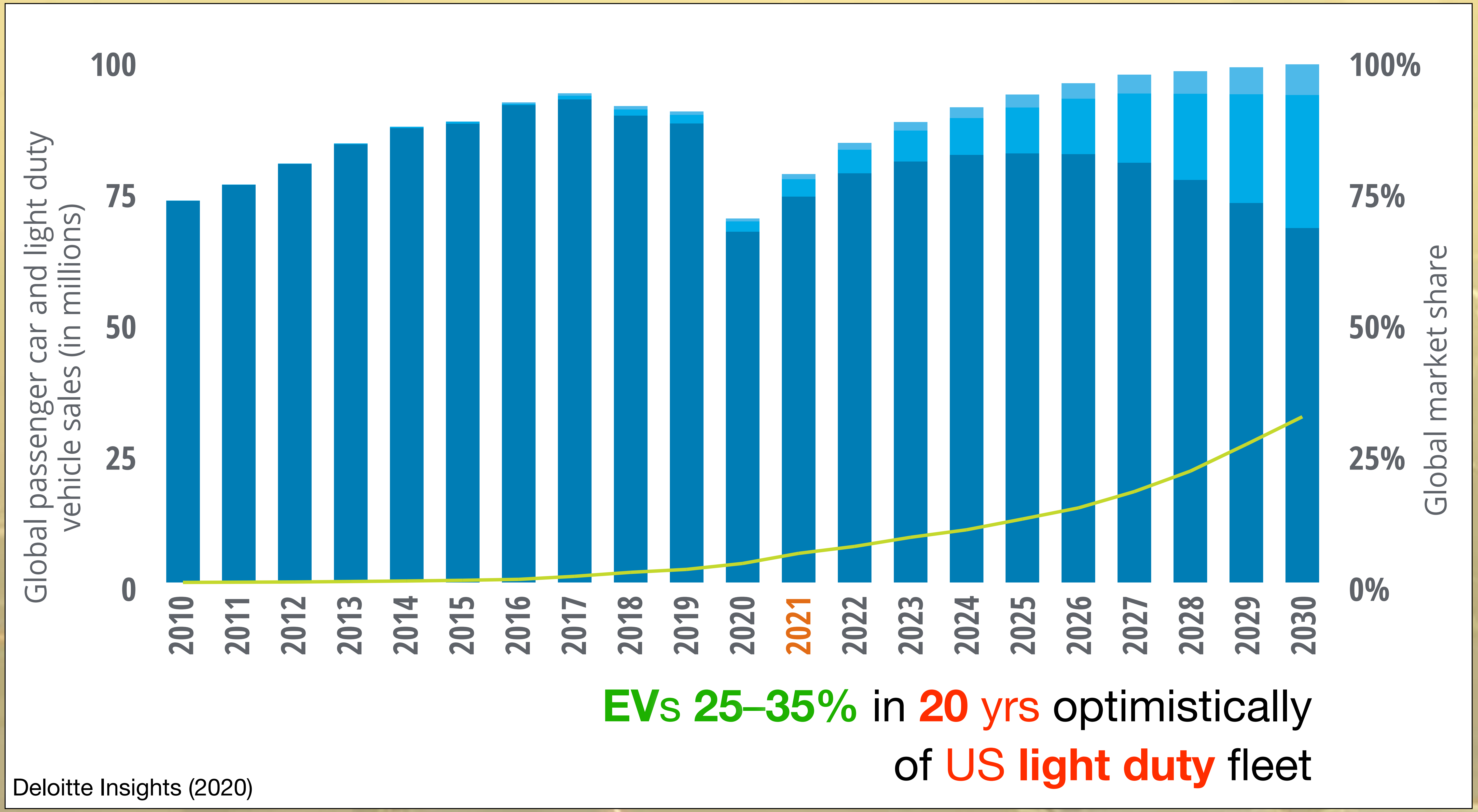
Highest US emissions from transportation

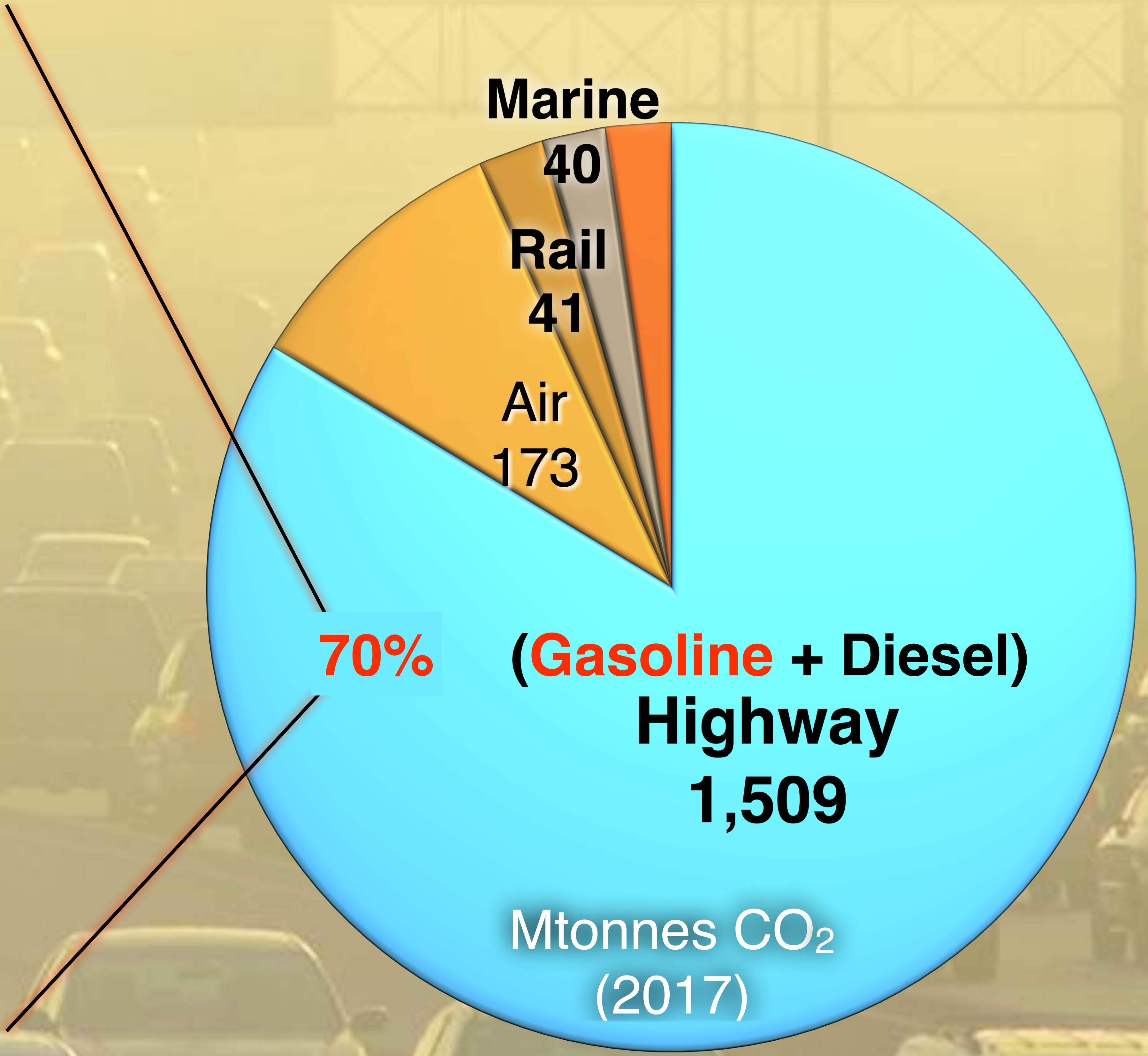


CLIMATE CENTRAL

Transportation Energy Data Book, Ed. 39 (2021)

Internal combustion fleet will predominate indefinitely





→ **Renewable liquid combustion biofuels**

critical to global CO_2 reduction

Energy: carbon oxidation cycle
CO_2
+4 (most oxidized)
O=C=O
C +4
oxidized
C 0
reduced
C −4
energy
ATP
(more) heat + work
sugar (0)
fats (-3 → -2)
CH_2OH
O
OH
OH
OH
OH
H_2C—O
HC—O
H_2C—O
O
O
O
heat + O_2
H
C
H
H
H
oil −4 (most reduced)

CO2
yeast
ethanol
X
oil (1B ton / yr)


EPA Renewable Fuel Standard (2007)
U.S. BIOFUELS PLAN
Biomass-based diesel
Non-cellulosic advanced
Cellulosic advanced
Conventional biofuels
Billions of gallons
40
35
30
25
20
15
10
5
0
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
corn (15B gal)
Growing gap. Energy legislation from 2007 mandates an increasing share of cellulosic ethanol (dark green). But the industry is already falling behind.
ALL TAXES INCLUDED
CONTAINS UP TO 10% ETHANOL
yeast
ethanol
10%
X
oil (1B ton / yr)


EPA Renewable Fuel Standard (2007)
U.S. BIOFUELS PLAN
Biomass-based diesel
Non-cellulosic advanced
Cellulosic advanced
Conventional biofuels
Billions of gallons
0
5
10
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30
35
40
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
cellulosic
corn (15B gal)
Growing gap. Energy legislation from 2007 mandates an increasing share of cellulosic ethanol (dark green). But the industry is already falling behind.
ligno-
cellulosic
biomass
yeast
ethanol
X
oil (1B ton / yr)


Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply
April 2005
U.S. Department of Energy
USDA
U.S. Department of Agriculture
EPA Renewable Fuel Standard (2007)
U.S. BIOFUELS PLAN
Billions of gallons
40
35
30
25
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15
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Biomass-based diesel
Non-cellulosic advanced
Cellulosic advanced
Conventional biofuels
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
cellulosic
corn (15B gal)
Growing gap. Energy legislation from 2007 mandates an increasing share of cellulosic ethanol (dark green). But the industry is already falling behind.
ligno-cellulosic biomass
(~1B ton / yr)
yeast
ethanol
X
oil (1B ton / yr)


EPA Renewable Fuel Standard (2007)
U.S. BIOFUELS PLAN
Billions of gallons
0 5 10 15 20 25 30 35 40
2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022
Biomass-based diesel
Non-cellulosic advanced
Cellulosic advanced
Conventional biofuels
cellulosic
corn (15B gal)
Growing gap. Energy legislation from 2007 mandates an increasing share of cellulosic ethanol (dark green). But the industry is already falling behind.
152 | NATURE | VOL 507 | 13 MARCH 2014
ENERGY
Cellulosic ethanol fights for life
Pioneering biofuel producers hope that US government largesse will ease their way into a tough market.
BY MARK PEPLOW
On the flat plains of Kansas, a stack of gleaming steel towers and pipes stretches 16 storeys into the sky. More than 1,000 construction workers toiled to complete the ethanol plant near the town of
included in the country's fuel supply. In its early years, the law emphasized the production of corn ethanol, considered ripe for early commercialization.
Yet corn ethanol comes with problems. It offers only modest savings in greenhouse-gas emissions compared to petrol (see Nature 499, 13–14; 2013). Production is vulnerable to poor harvests and can contribute to increased food prices because the maize must be grown on land that would otherwise be used for food. Tapping the storehouse of biomass left after the harvest is much less controversial. Ethanol made from corn stover produces at least 60% less greenhouse-gas emissions than petrol, and making it does not require any extra farmland.
MIT Technology Review
The Cellulosic Ethanol Industry Faces Big Challenges
The advanced-biofuels industry is in danger of withering away.
By Kevin Bullis on August 12, 2013
A series of cellulosic-biofuel plants are finally starting to come on line after years of delay. But the wave of plant openings, good news as it is for the emerging industry, also shows just how far it still has to go.
ligno-cellulosic biomass
(~1B ton / yr)
oil (1B ton / yr)

CO_2
ligno-
cellulosic
biomass
(~1B ton / yr)
yeast
ethanol
X
oil (1B ton / yr)


lignocellulose
yeast
ethanol


lignocellulose
yeast
ethanol

Origin of ethanol tolerance **unknown**

Yeast
Yeast 2006; **23**: 351–359.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/yea.1359

Research Article

Genome-wide identification of genes required for growth of *Saccharomyces cerevisiae* under ethanol stress

Frank van Voorst, Jens Houghton-Larsen, Lars Jønson#, Morten C. Kielland-Brandt and Anders Brandt*
Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark

DOI: 10.1534/genetics.110.121871

Exploiting Natural Variation in *Saccharomyces cerevisiae* to Identify Genes for Increased Ethanol Resistance

Jeffrey A. Lewis,[*,†] Isaac M. Elkon,[*,†] Mick A. McGee,[†,‡] Alan J. Higbee[†,‡] and Audrey P. Gasch[*,†,§,1]

[*]Laboratory of Genetics, [†]Great Lakes Bioenergy Research Center, [‡]Biotechnology Center, and [§]Genome Center of Wisconsin, University of Wisconsin, Madison, Wisconsin 53706

Manuscript received August 5, 2010

Applied and Environmental Microbiology, Mar. 2003, p. 1499–1503 Vol. 69, No. 3
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.3.1499–1503.2003

Ethanol Tolerance in the Yeast *Saccharomyces cerevisiae* Is Dependent on Cellular Oleic Acid Content

Kyung Man You,† Claire-Lise Rosenfield, and Douglas C. Knipple*

Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, New York 14456

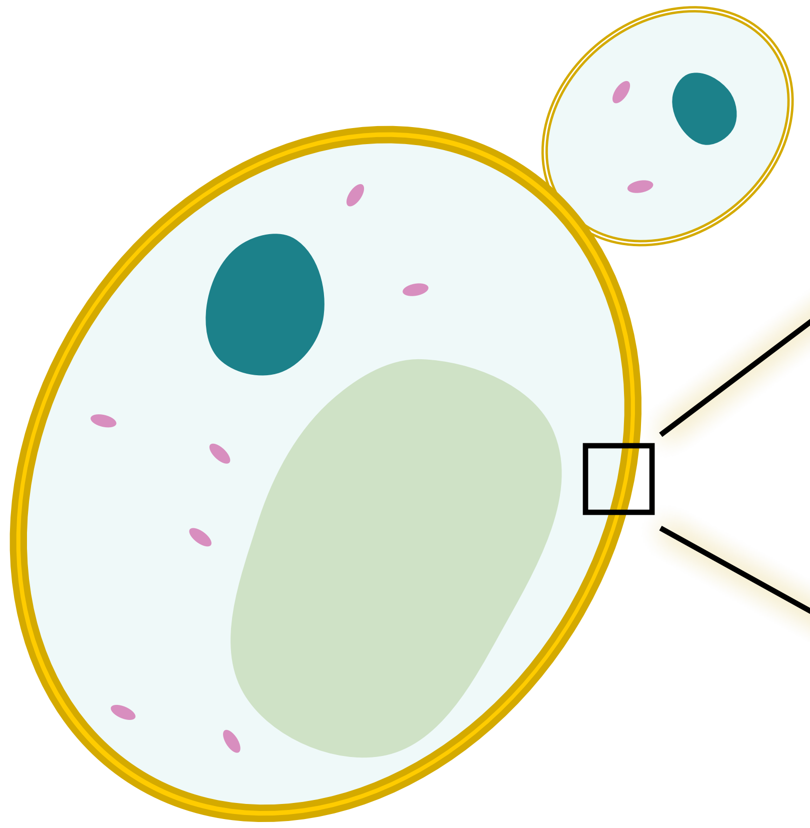
RESEARCH ARTICLE

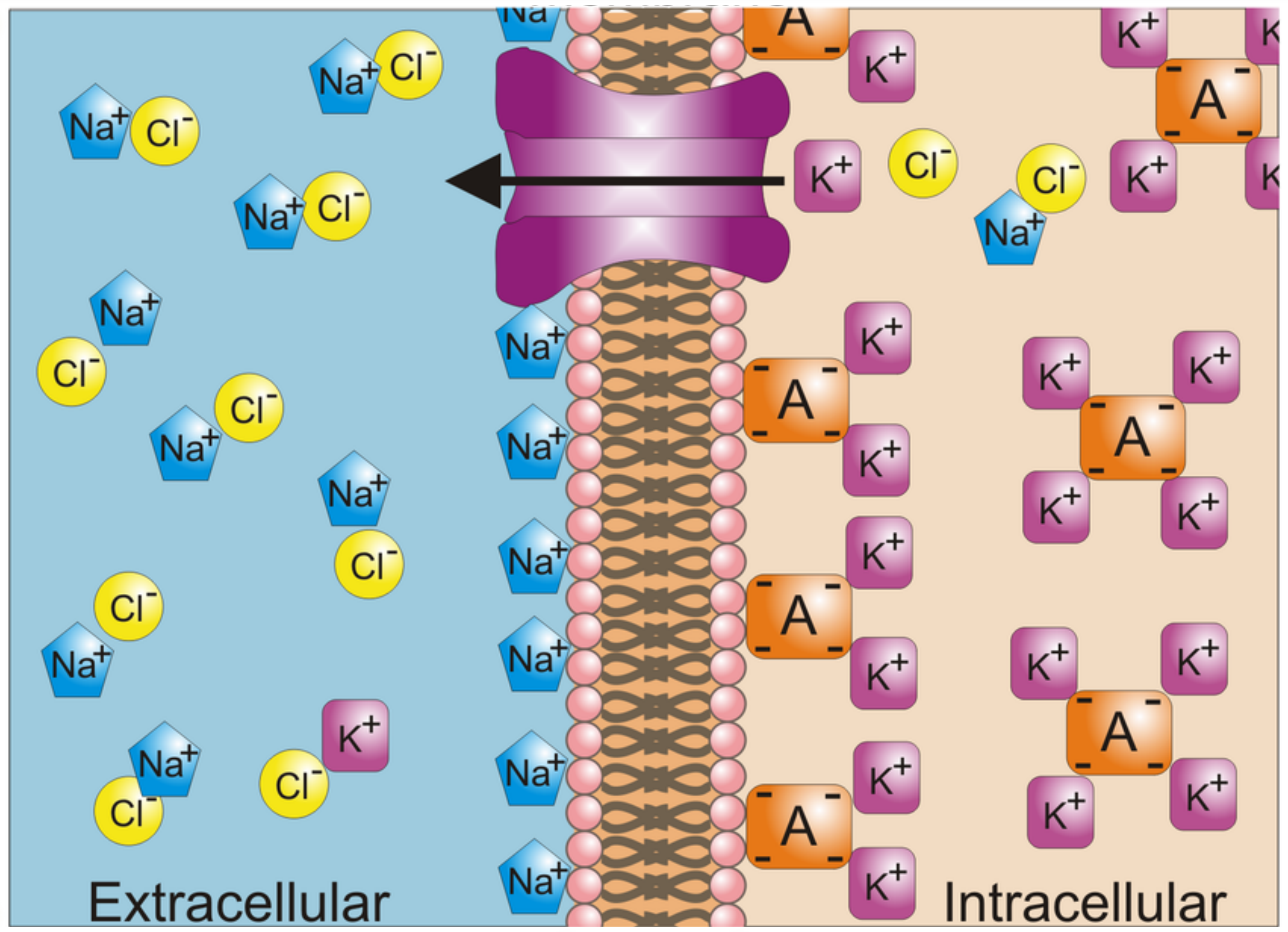
Trehalose promotes the survival of *Saccharomyces cerevisiae* during lethal ethanol stress, but does not influence growth under sublethal ethanol stress

Ajith Bandara[1], Sarah Fraser[1], Paul J. Chambers[2] & Grant A. Stanley[1]

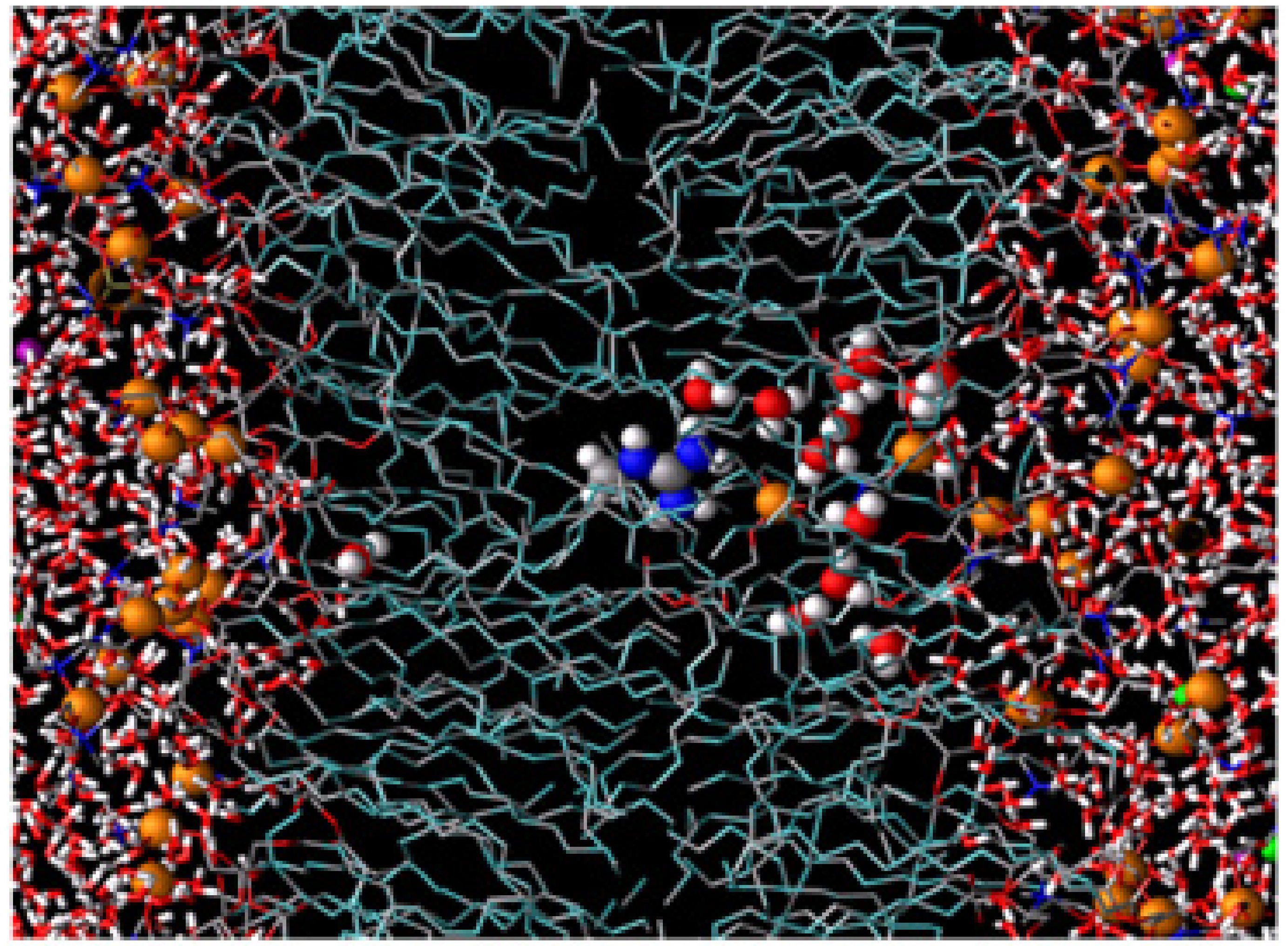
[1]School of Engineering and Science, Victoria University, Melbourne, Vic., Australia; and [2]The Australian Wine Research Institute, Glen Osmond, SA, Australia

Ethanol **disrupts membranes** → environmental **ions affect stability?**



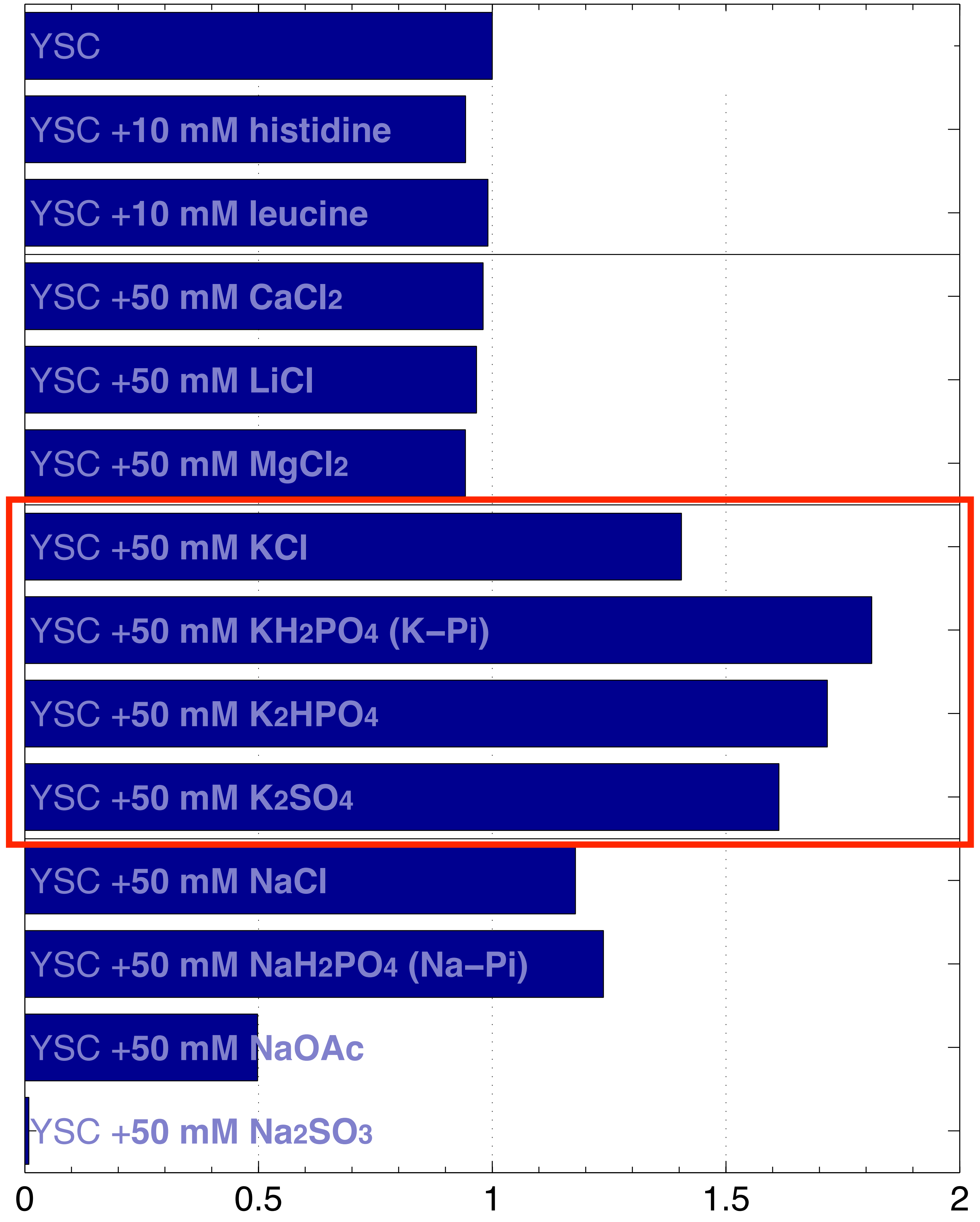


<u>Hypothesis</u>:
ethanol interacts /
disrupts lipids…

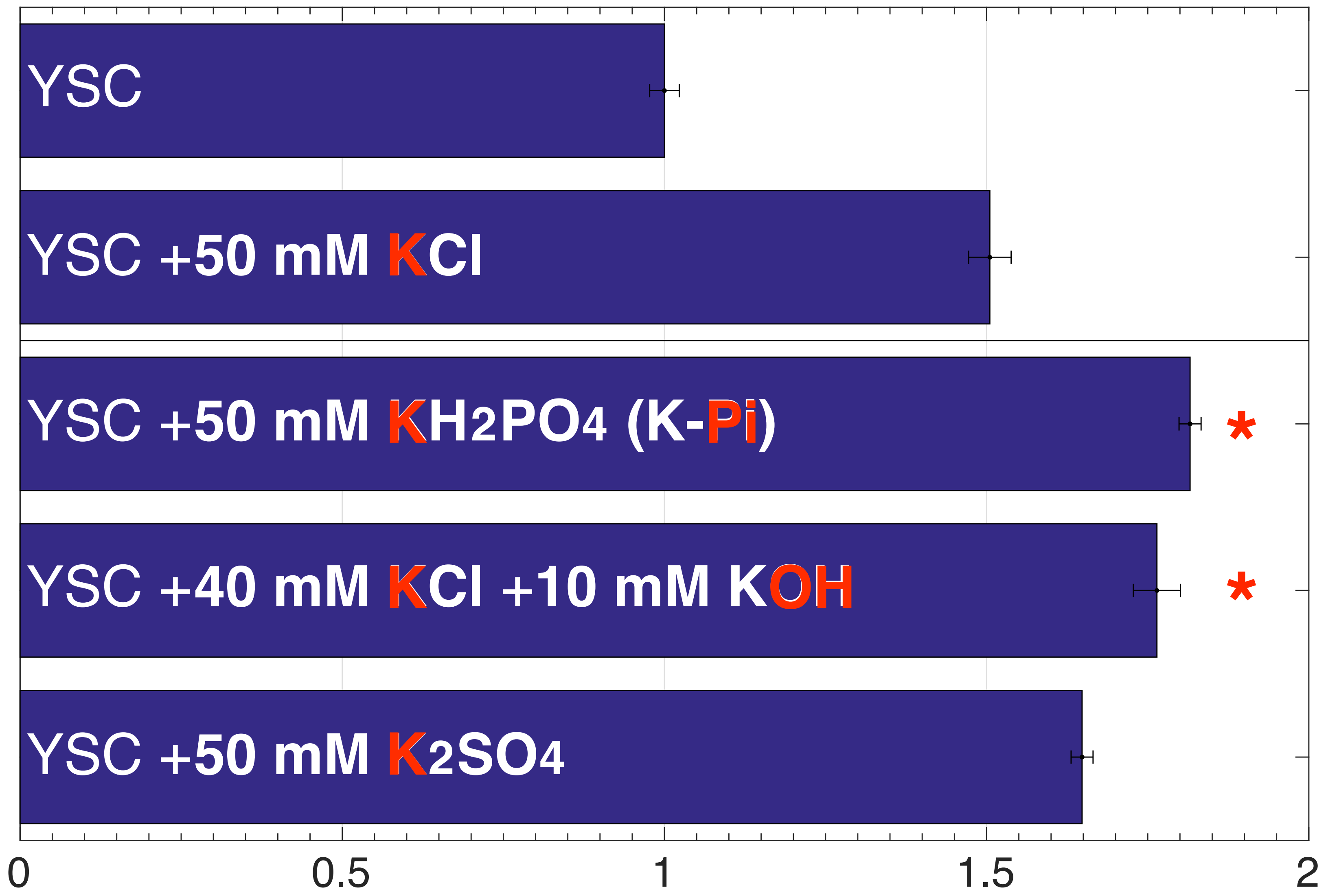


→ can
external ions
counteract?

Only external K^+ and **pH** counteract → **boost ethanol** output



YSC = yeast culturing medium

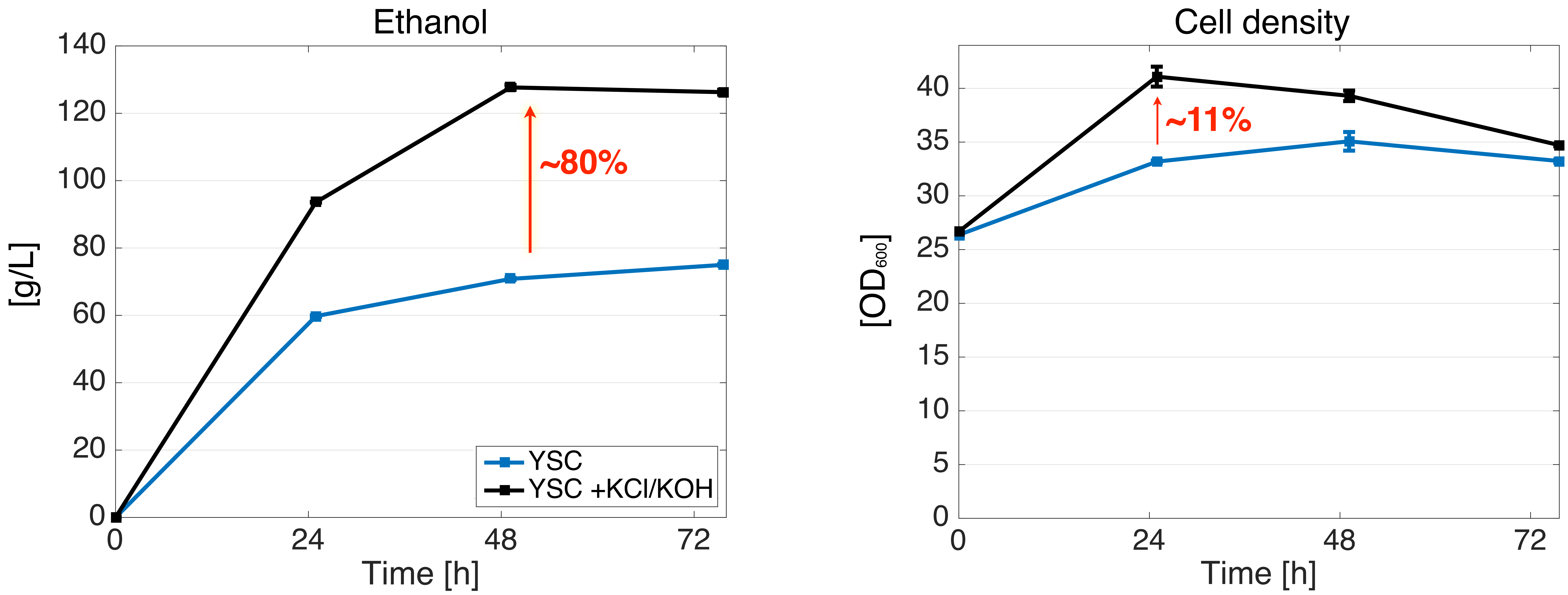


* statistically identical

…raising pH gives further boost

Largest increase from potassium (K^+) salts…

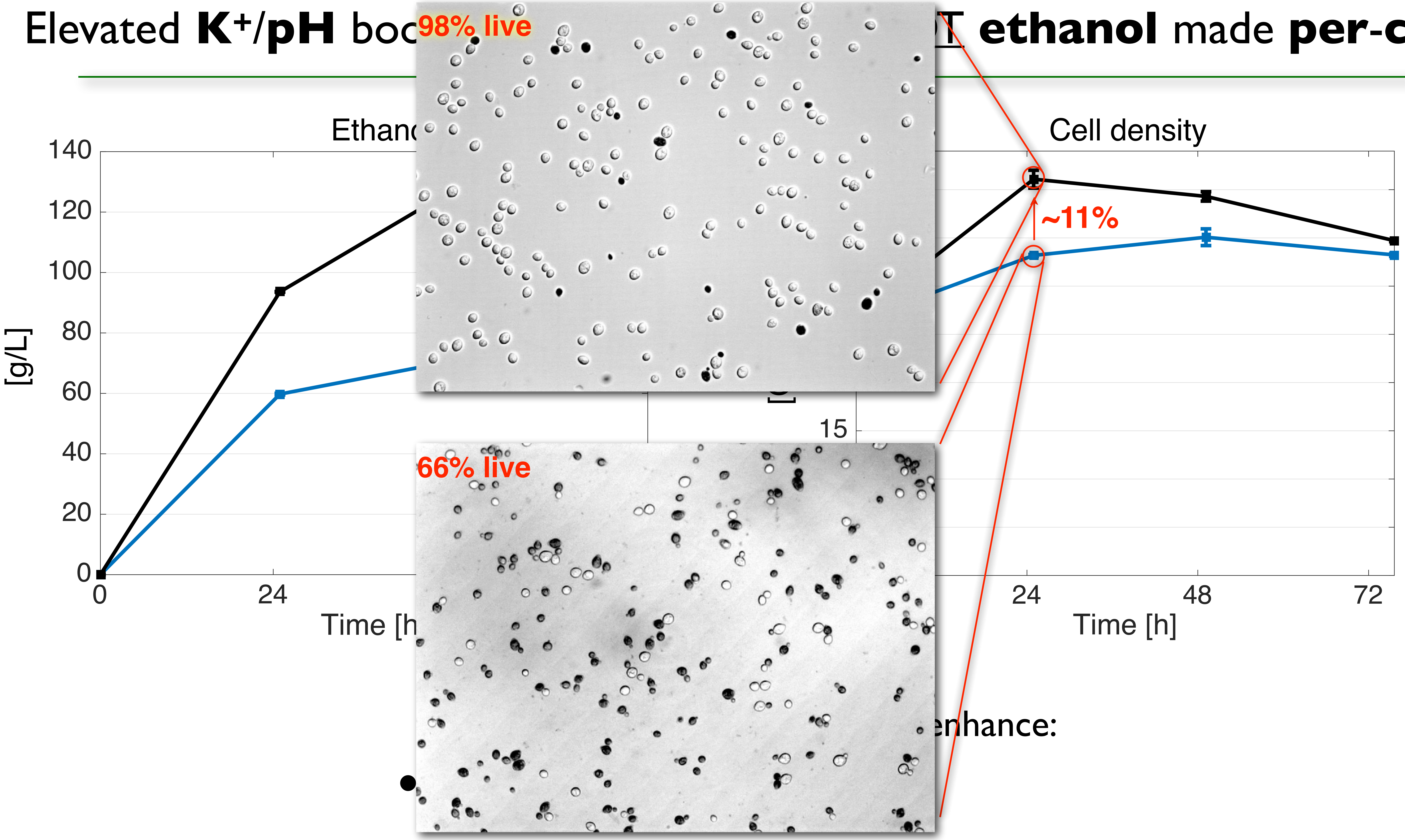
Elevated **K+/pH** boost **cell tolerance** — NOT **ethanol** made **per-cell**



Despite higher ethanol, **K+/pH** enhance:

- **Cell growth** (moderately)

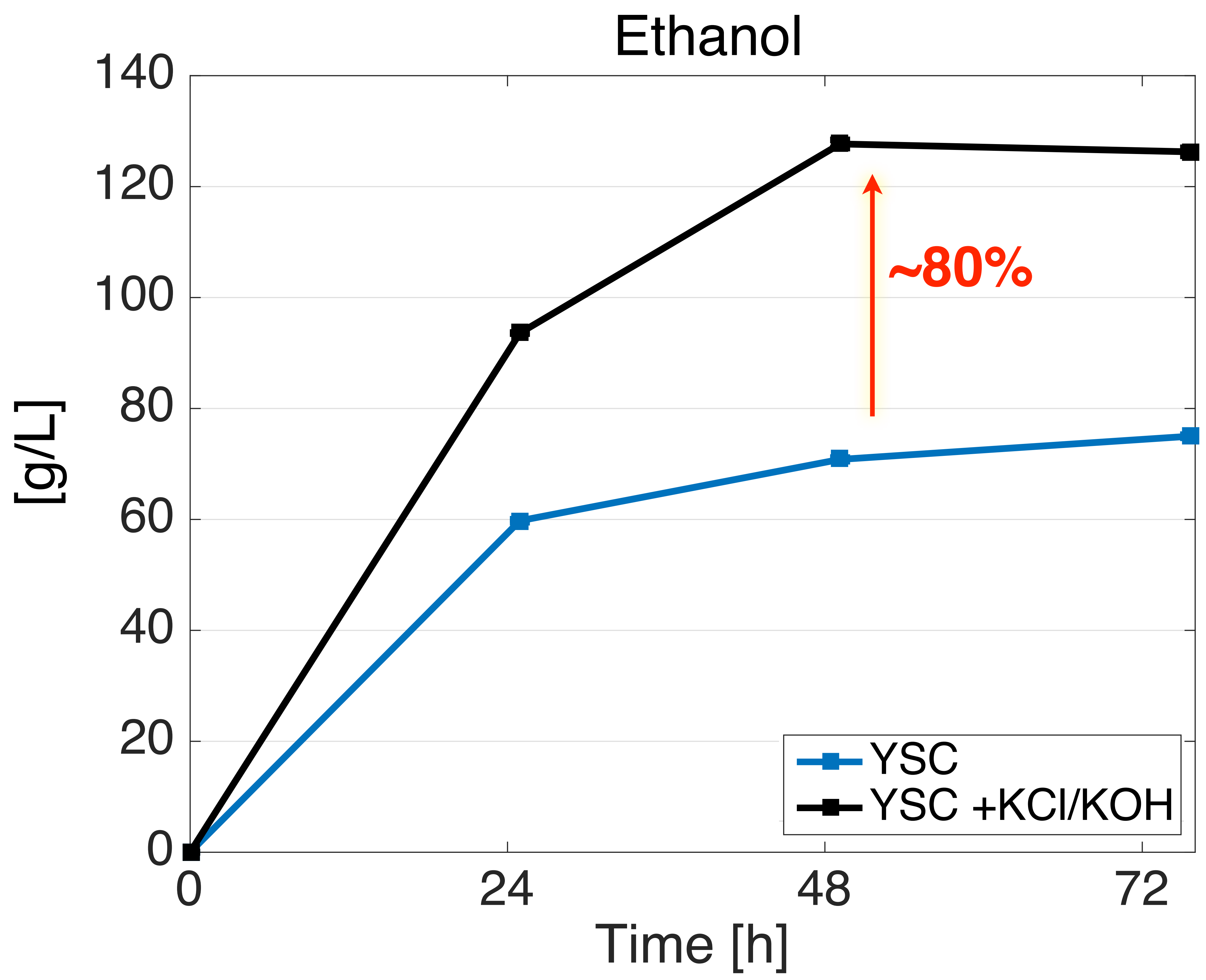
Elevated K^+/pH boo… …T **ethanol** made **per-cell**

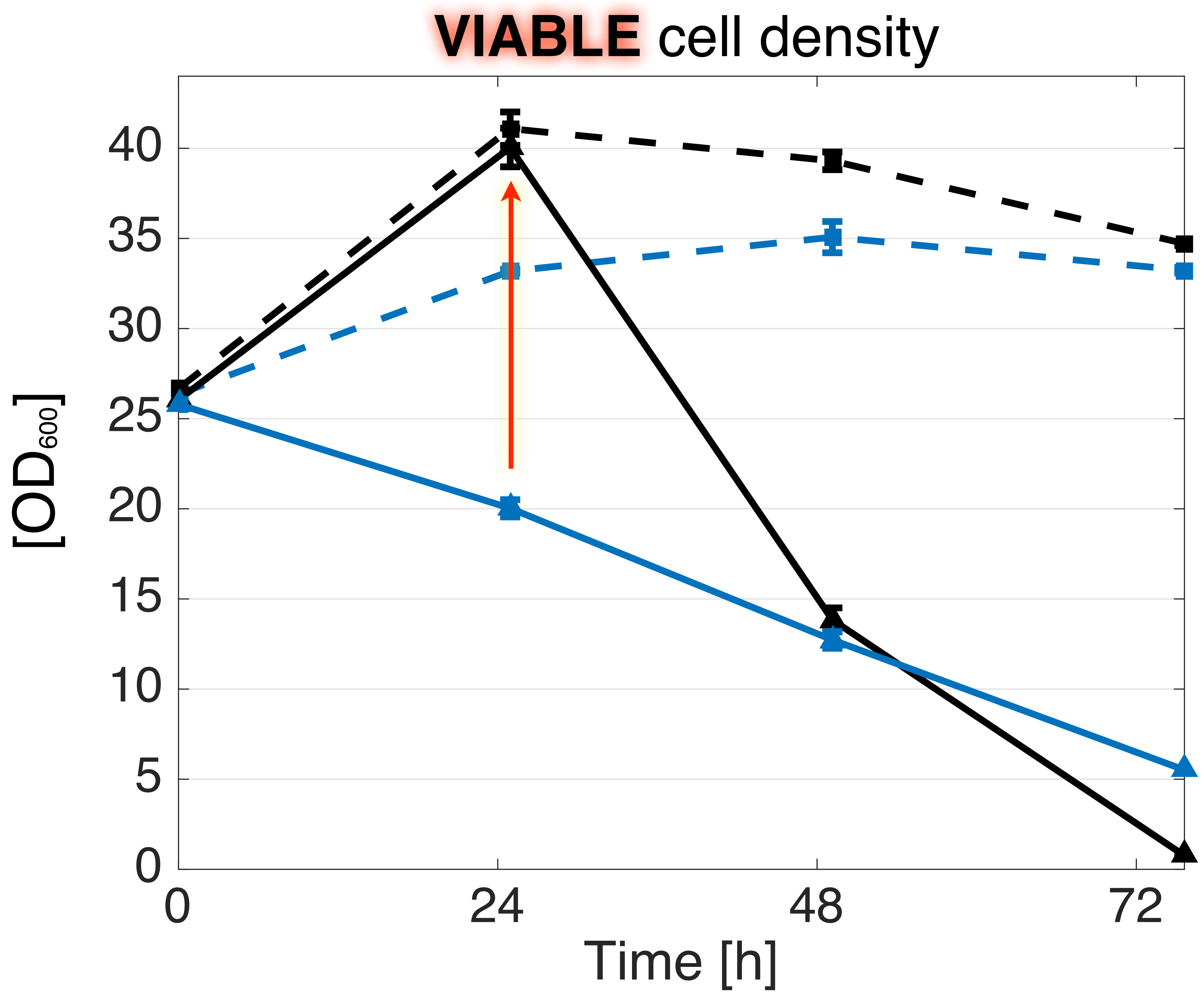


…enhance:

●

Elevated **K⁺/pH** boost **cell tolerance** — NOT **ethanol** made **per-cell**





Despite higher ethanol, **K⁺/pH** enhance:

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- **Tolerance**

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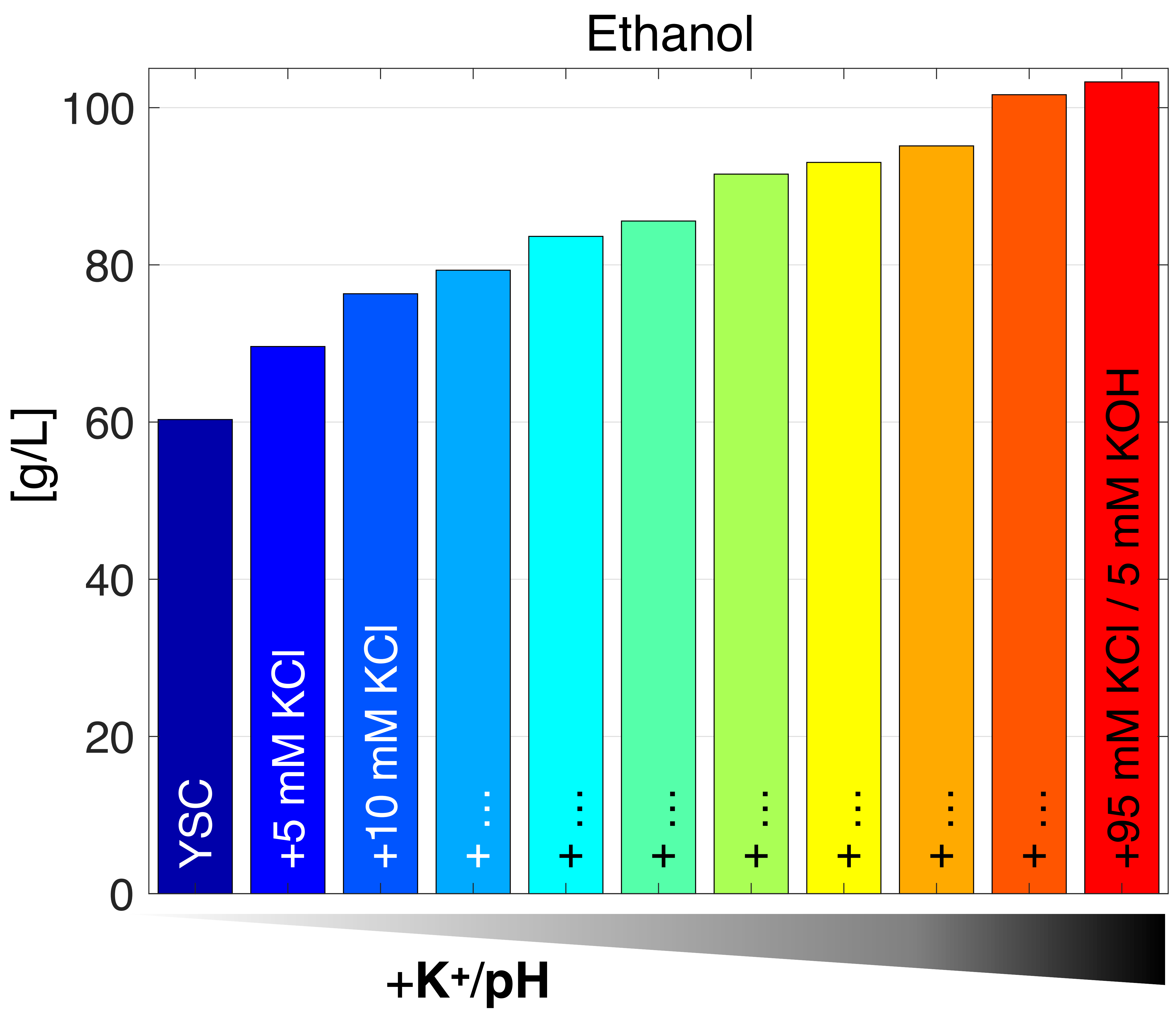

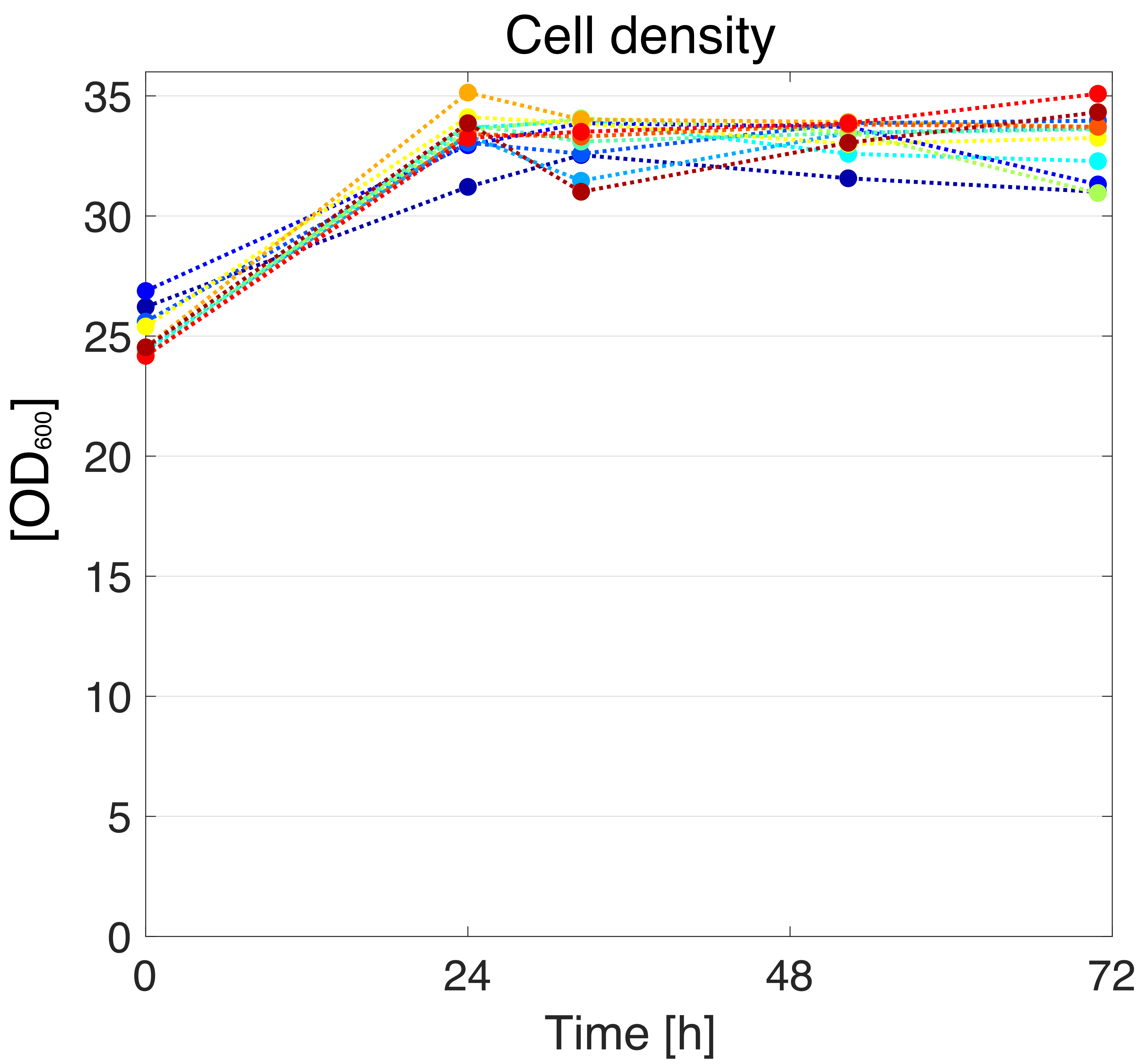

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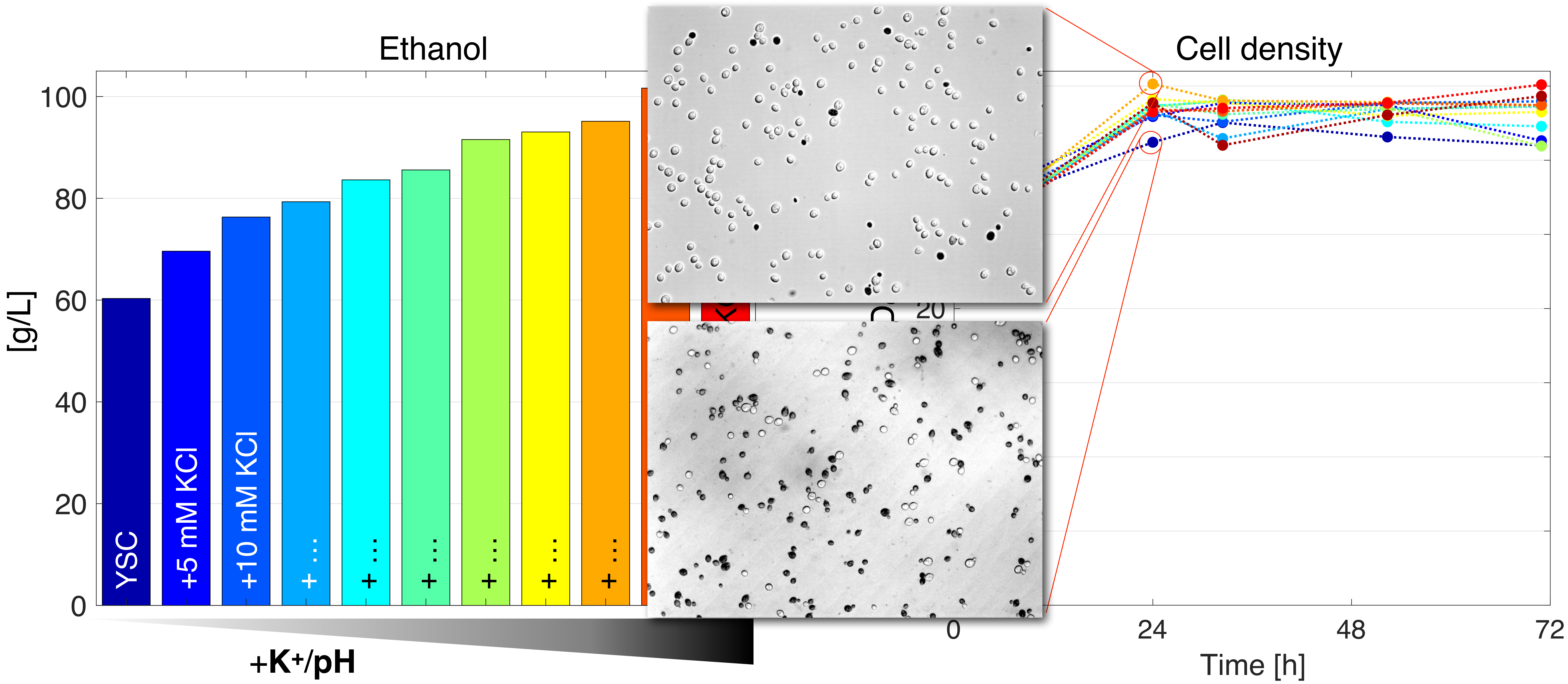

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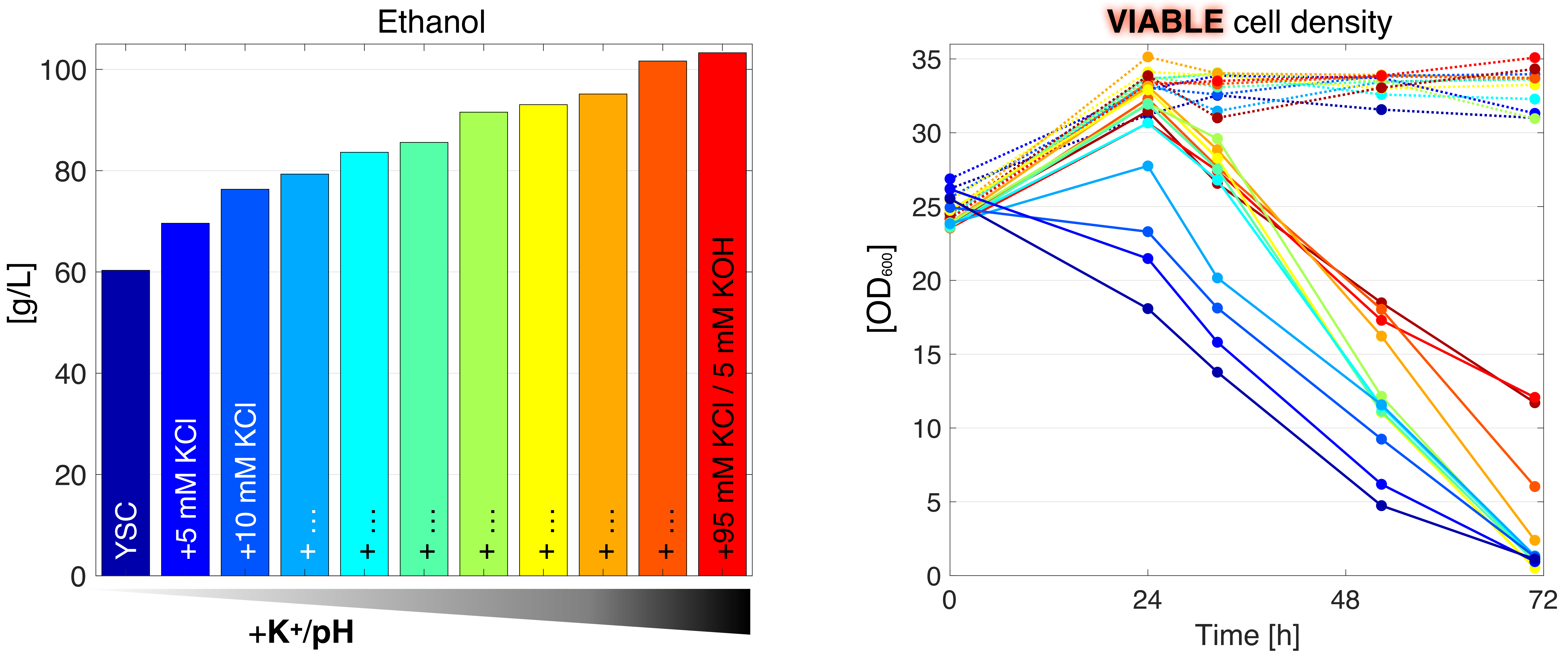

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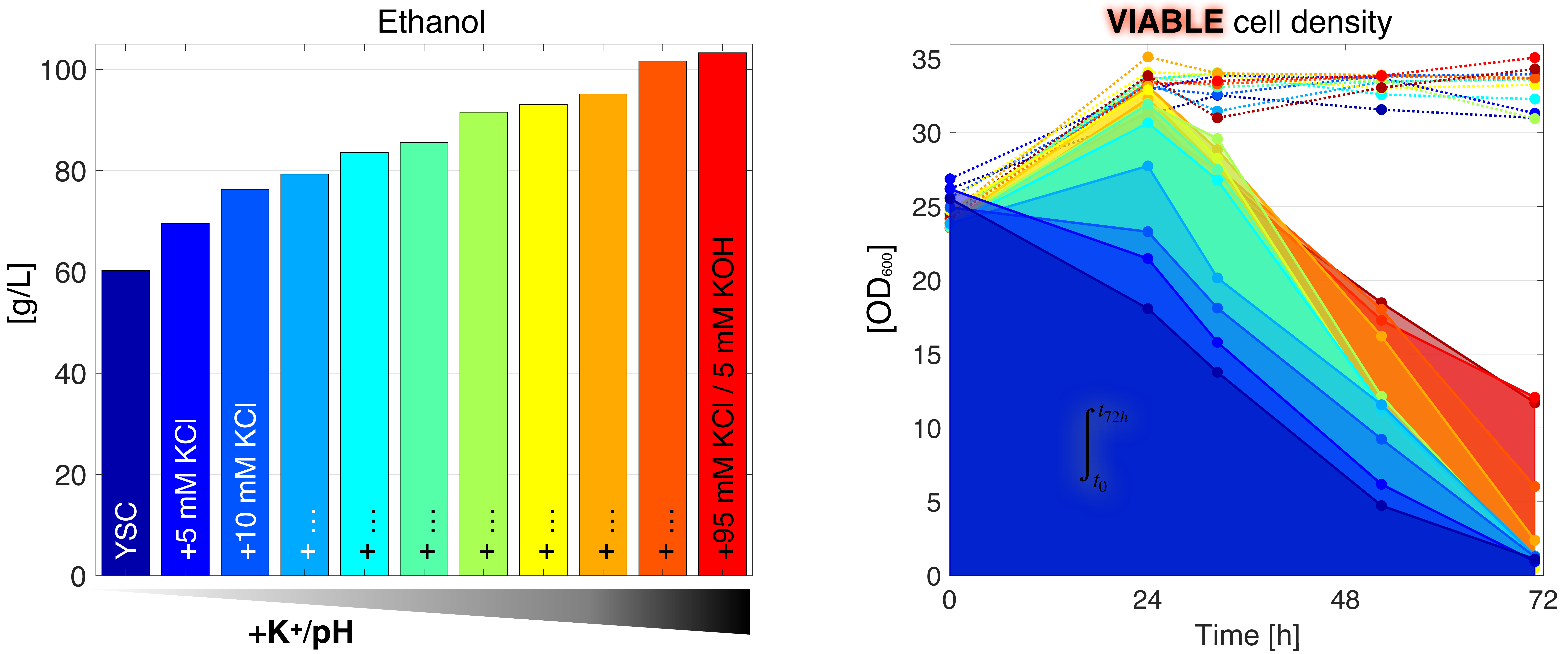
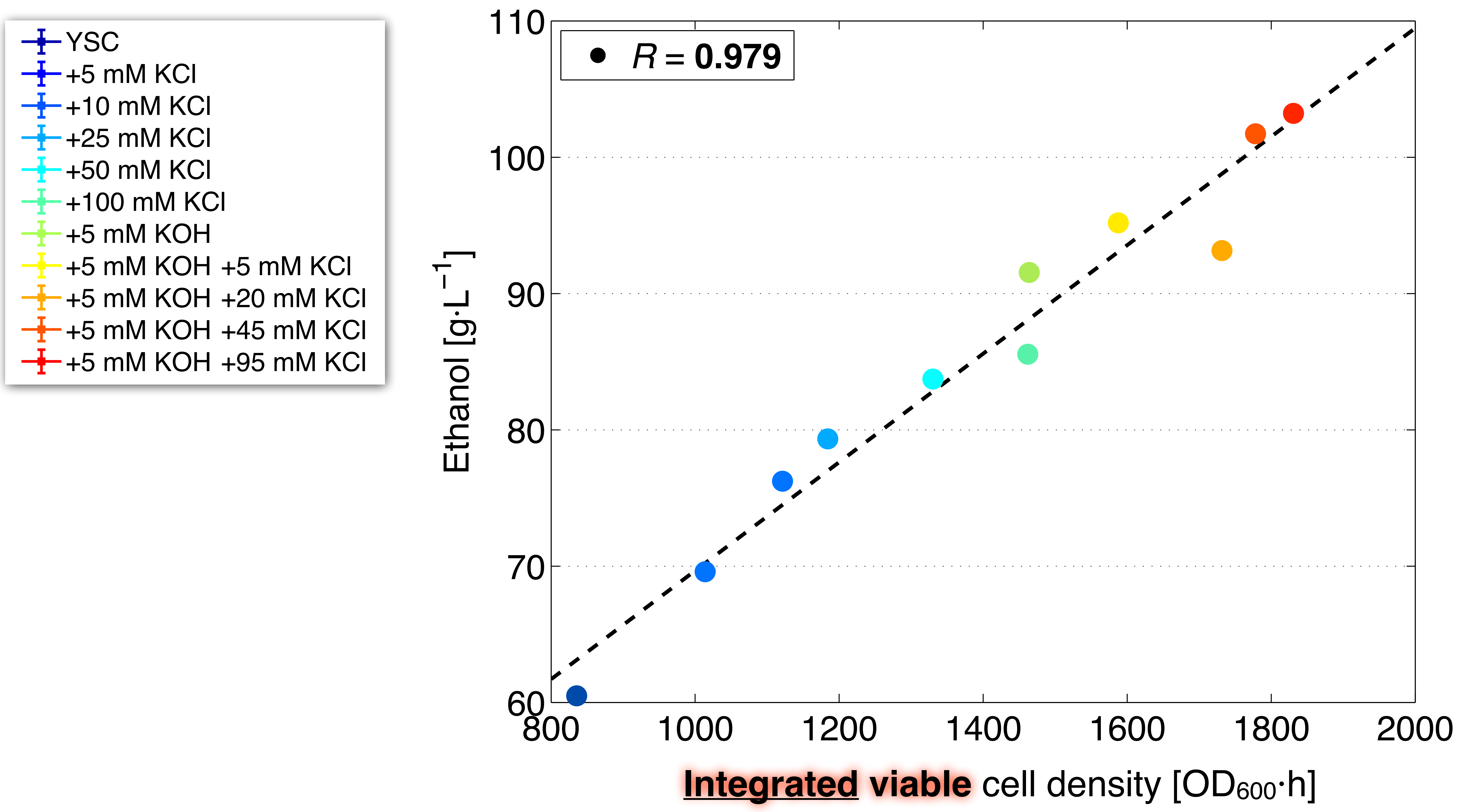


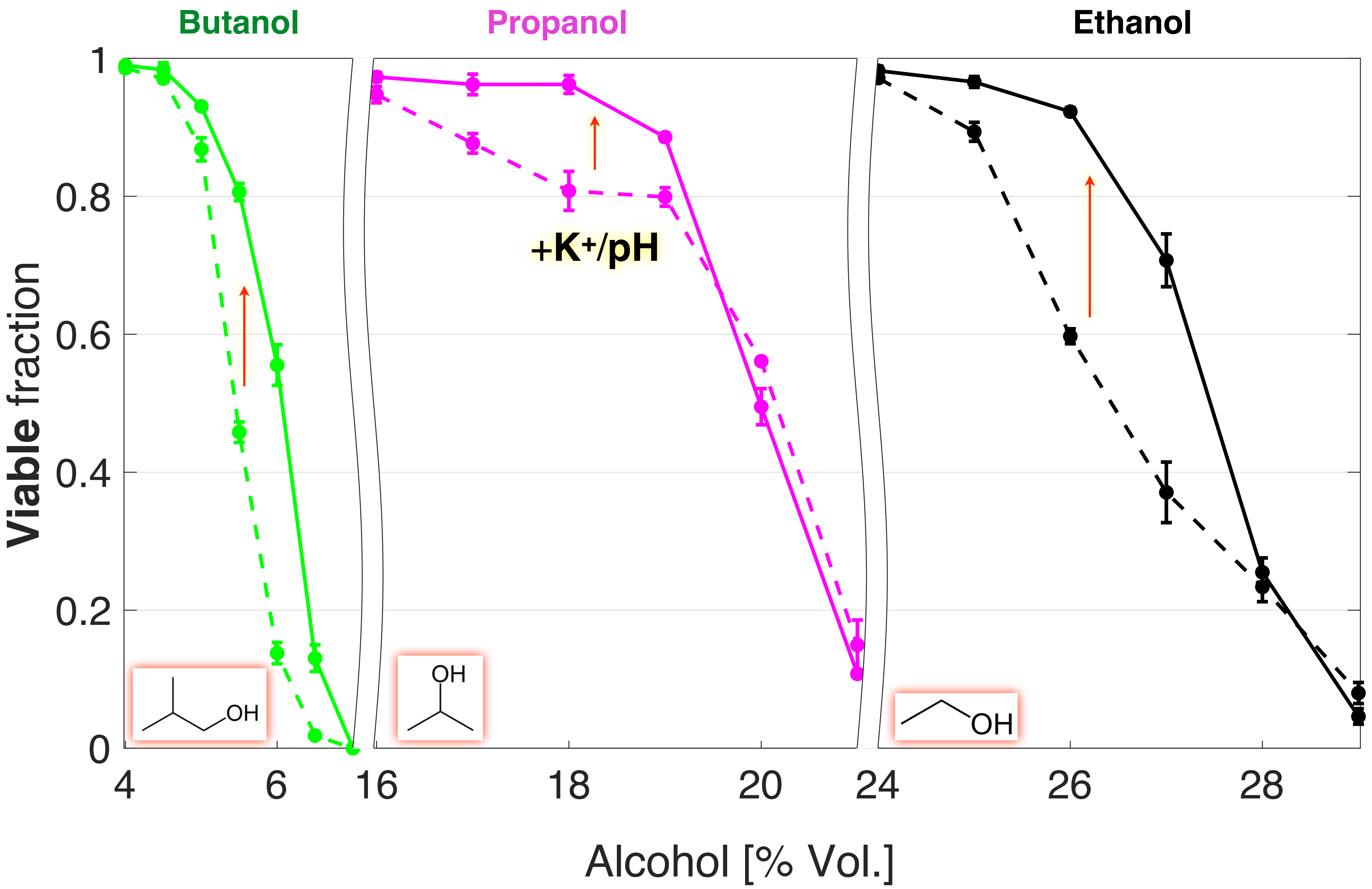
External K^+/pH directly control ethanol **tolerance** and **production**



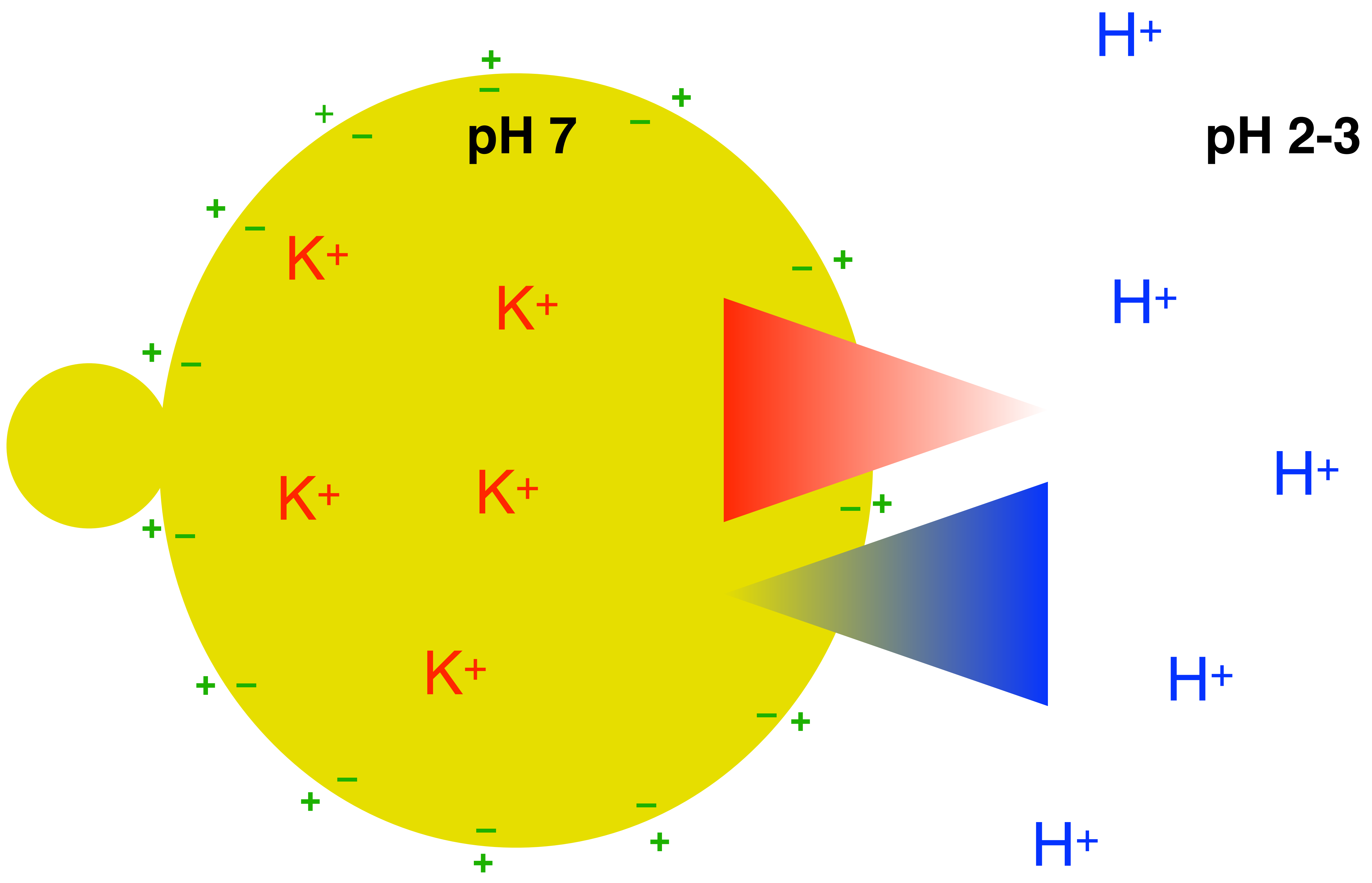
Straight line reveals:

- Ethanol **production per-cell** remains **same**
- **Only** population **tolerance** / endurance **is varied** → directly determines ethanol produced

K⁺/pH control yeast **tolerance** to ~~ethanol~~ **many alcohols**



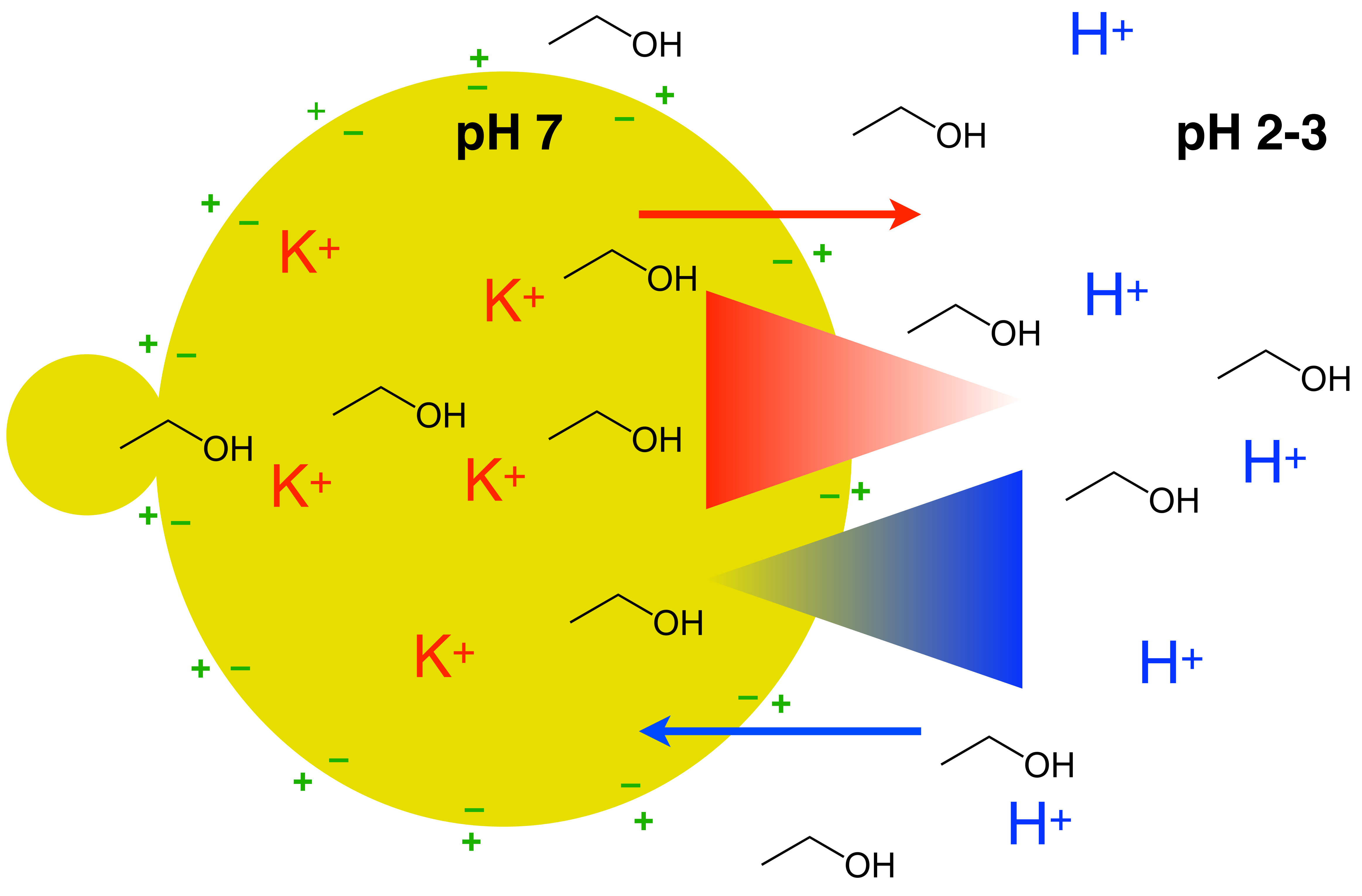
Why K^+/pH specifically? Form **gradients** of yeast **membrane potential**



Normally:

1. H^+ and K^+ membrane pumps maintain:
   Internal [pH 7 + high K^+]
   External [pH 3 + low K^+]
   → H^+ /K^+ gradients charge membrane

Why K^+/pH specifically? Form **gradients** of yeast **membrane potential**



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When ethanol accumulates:

2. Membrane becomes permeable to H^+ and K^+ → ions leak

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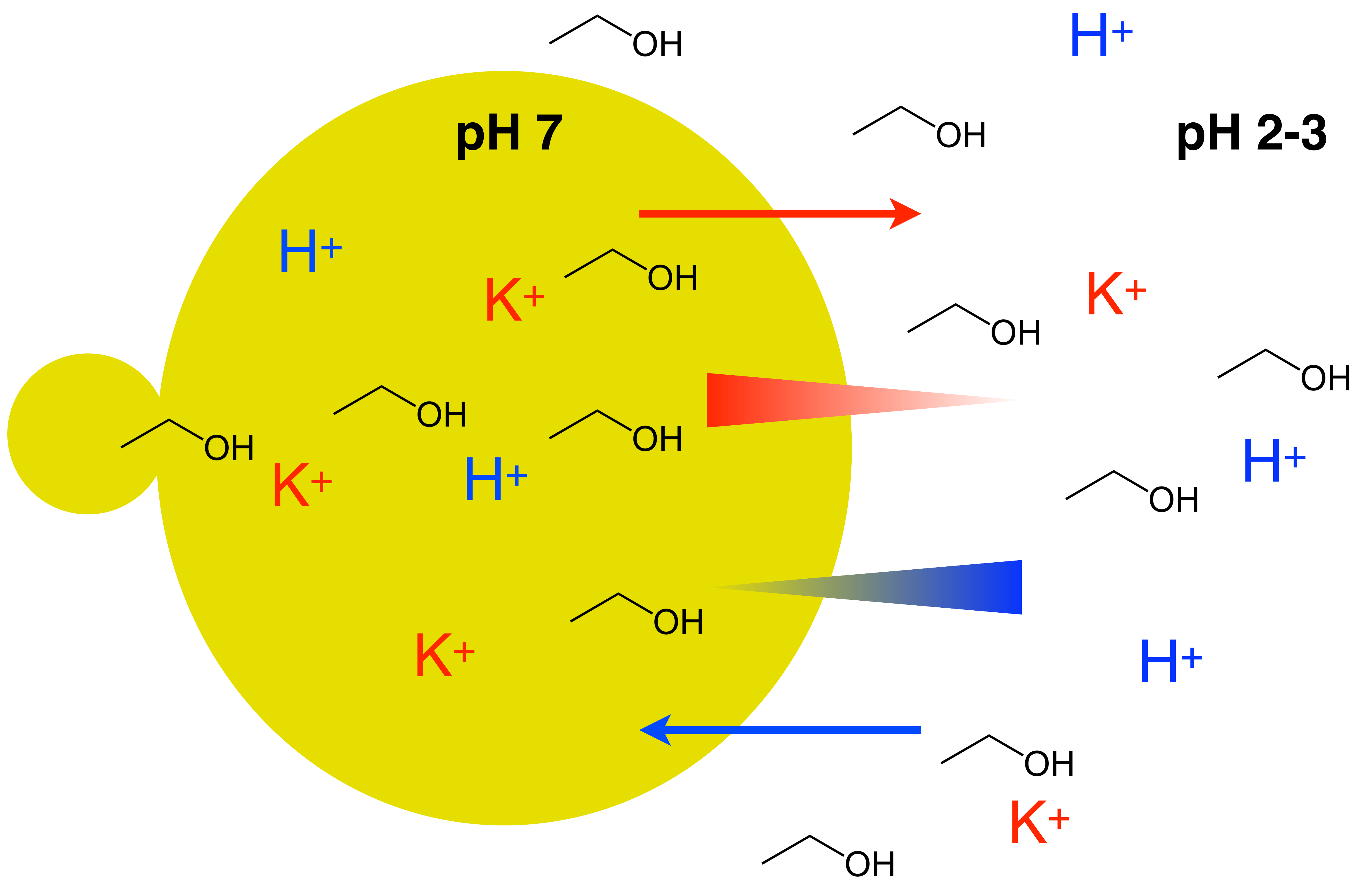

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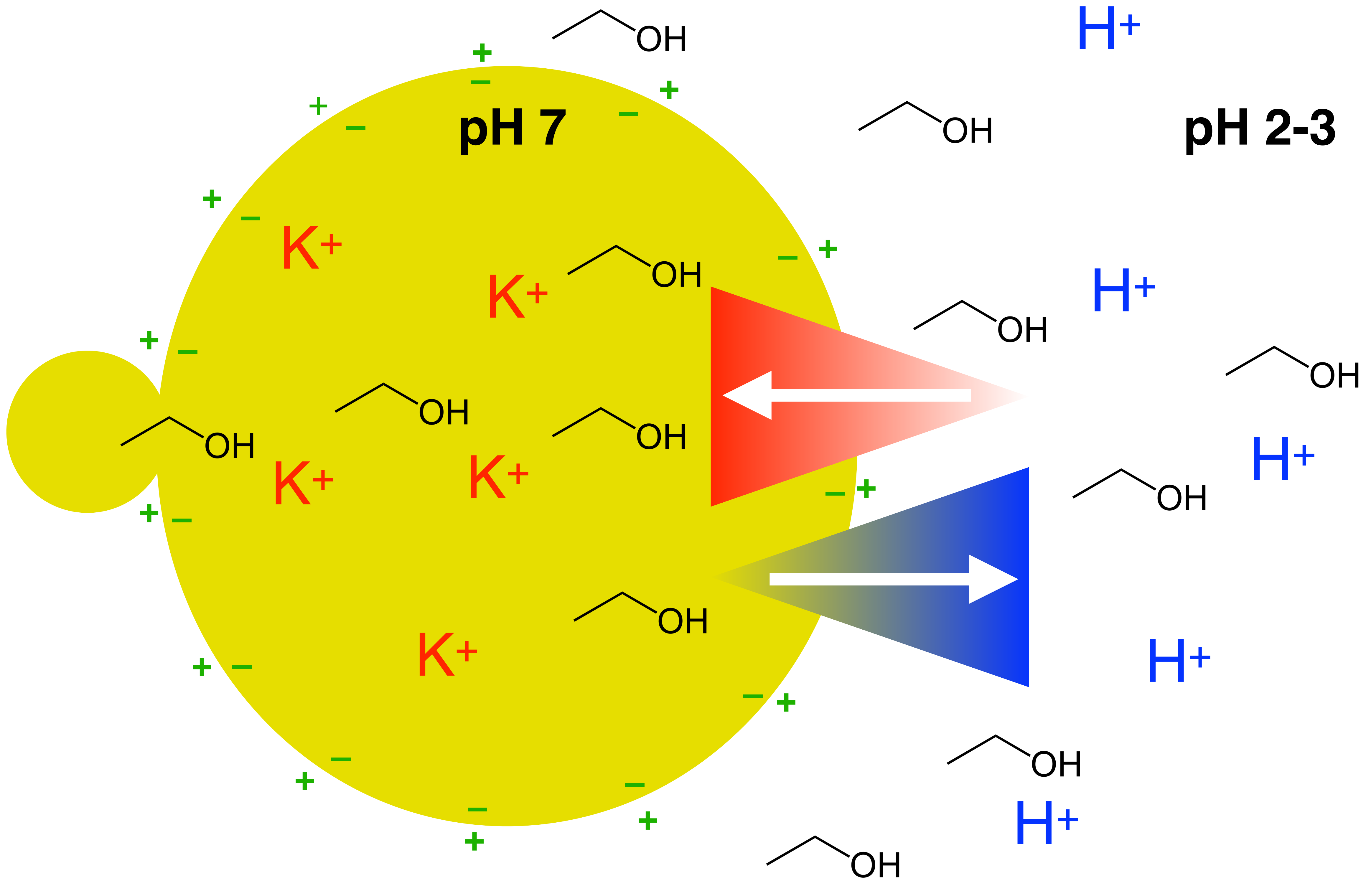

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When ethanol accumulates:

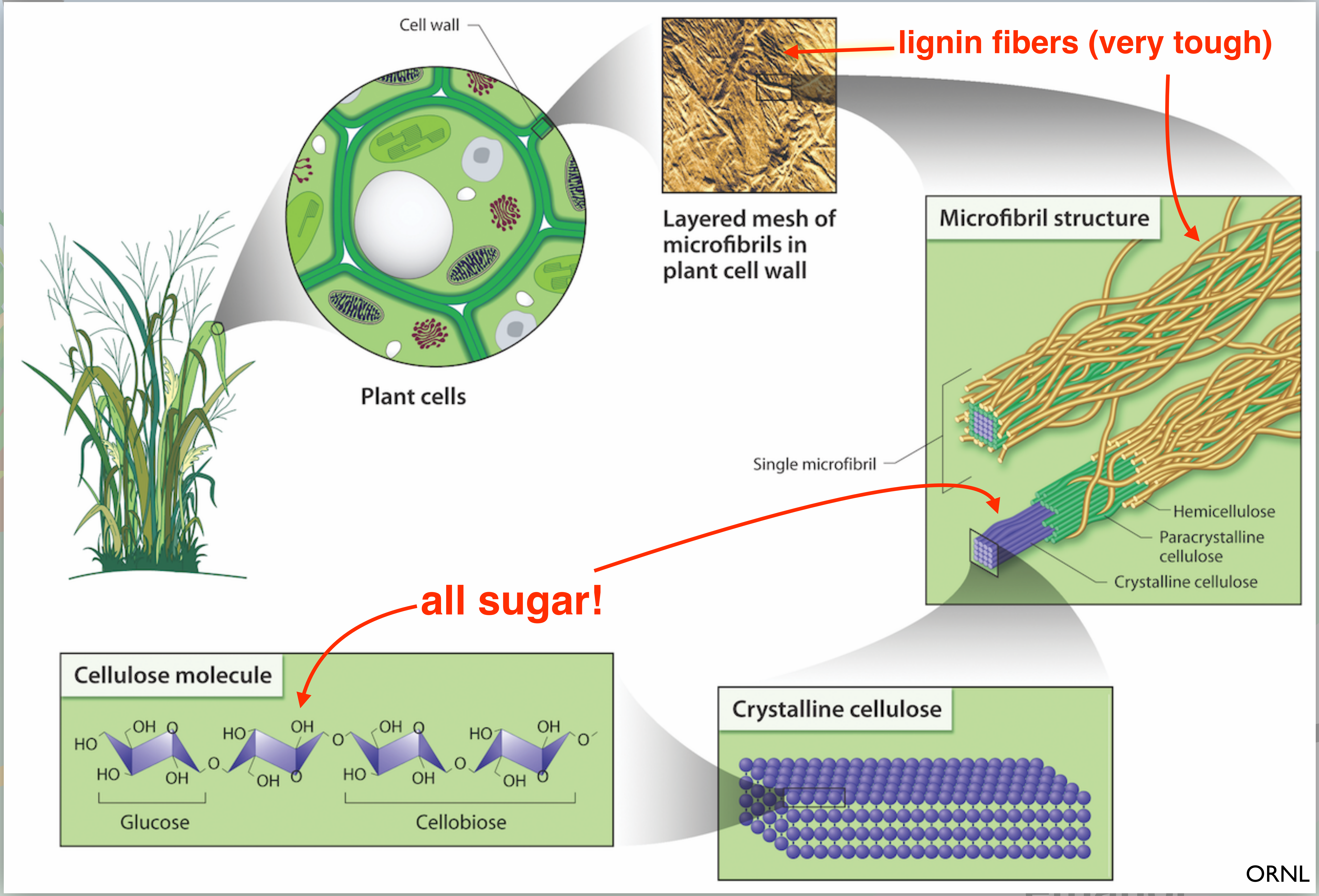
2. Membrane becomes permeable to H^+ and K^+ → ions leak → membrane gradients dissipate → **cell death**

Increasing external K^+/pH:

3. Assists membrane pumps with re-establishing gradients
4. Membrane charge is restored ✓


lignocellulose
yeast
ethanol

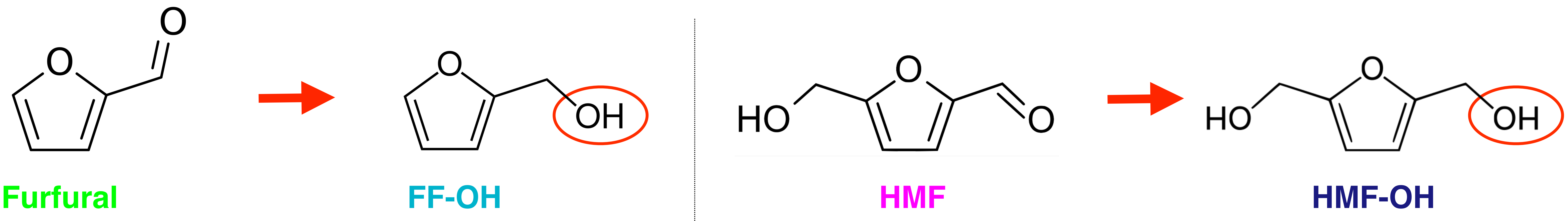

lignocellulose
yeast
ethanol



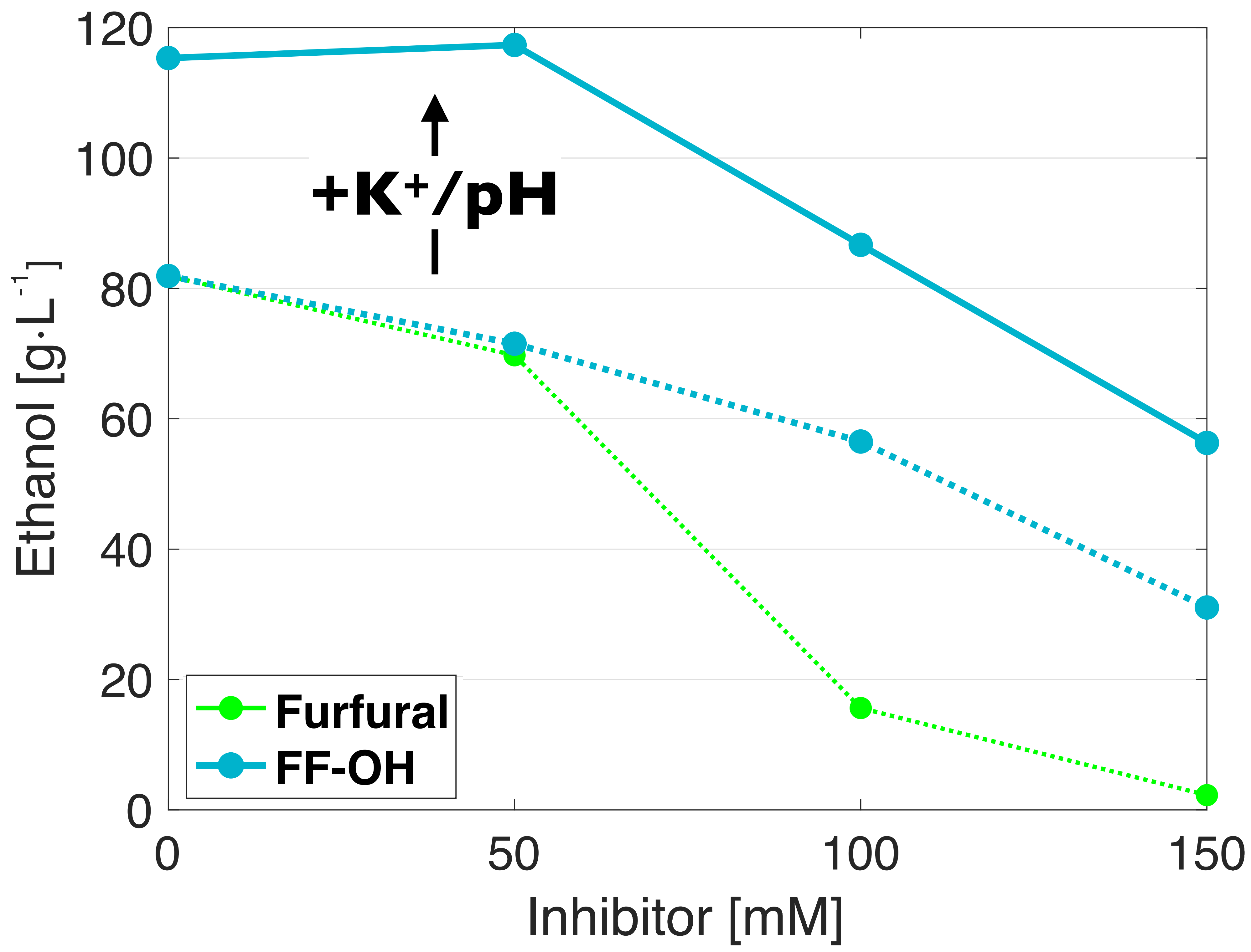
lignocellulose

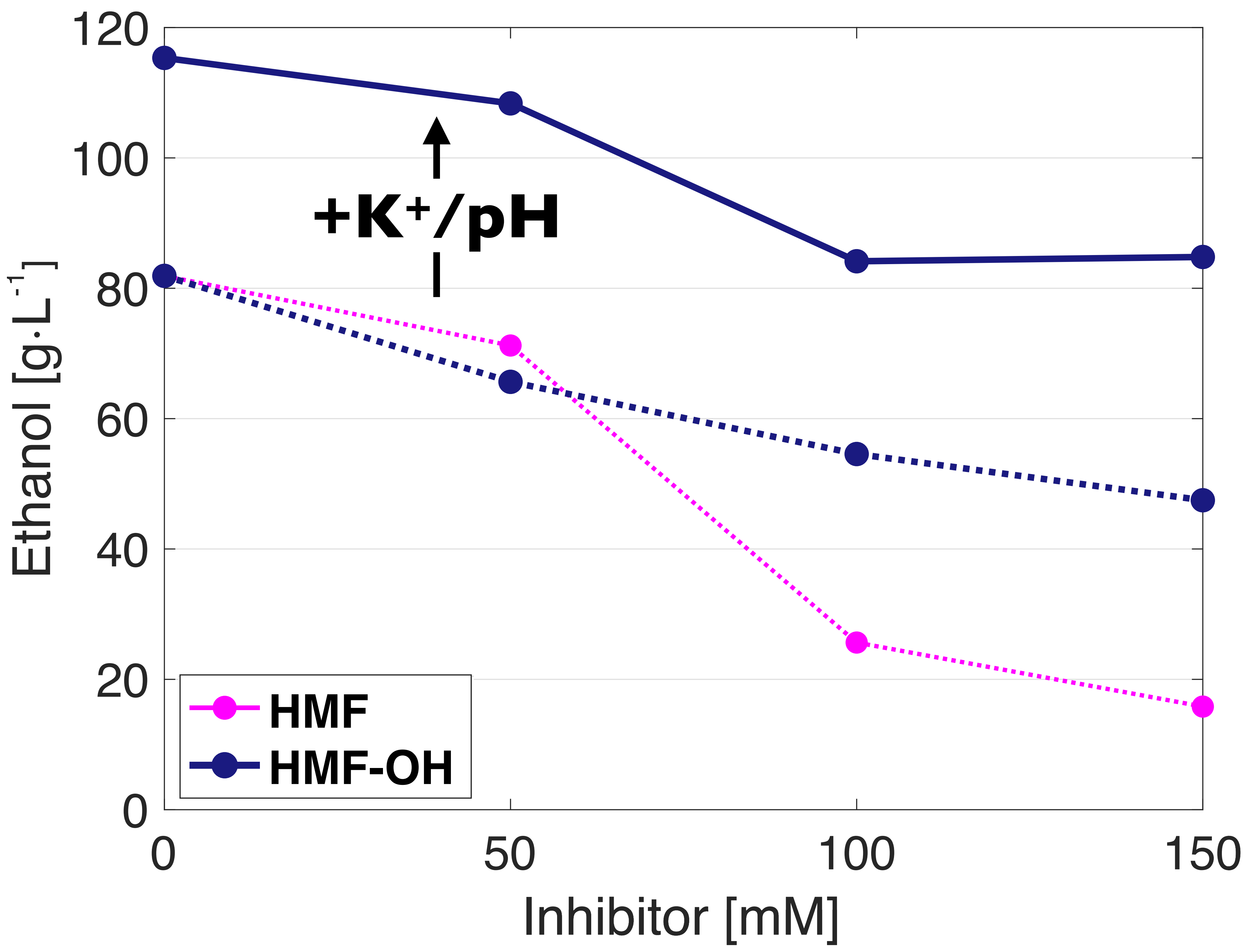

Inhibitors universal to all lignocellulosic sources
Cellulose
Hemicellulose
Lignins
Wood extractives
Glucose Mannose Galactose
Xylose Arabinose
HOH2C
O
CHO
Hydroxymethyl furfural (HMF)
Furfural
R
R'
OCH3
OH
Phenols
Levulinic acid
Formic acid
Acetic acid
lignin fibers (very tough)
Layered mesh of microfibrils in plant cell wall
Microfibril structure
Single microfibril
Hemicellulose
Paracrystalline cellulose
Crystalline cellulose
Cellulose molecule
Glucose
Cellobiose
Crystalline cellulose
ORNL
lignocellulose

Alcohol forms of furfural, HMF **less toxic** → **respond** to **K^+/pH**

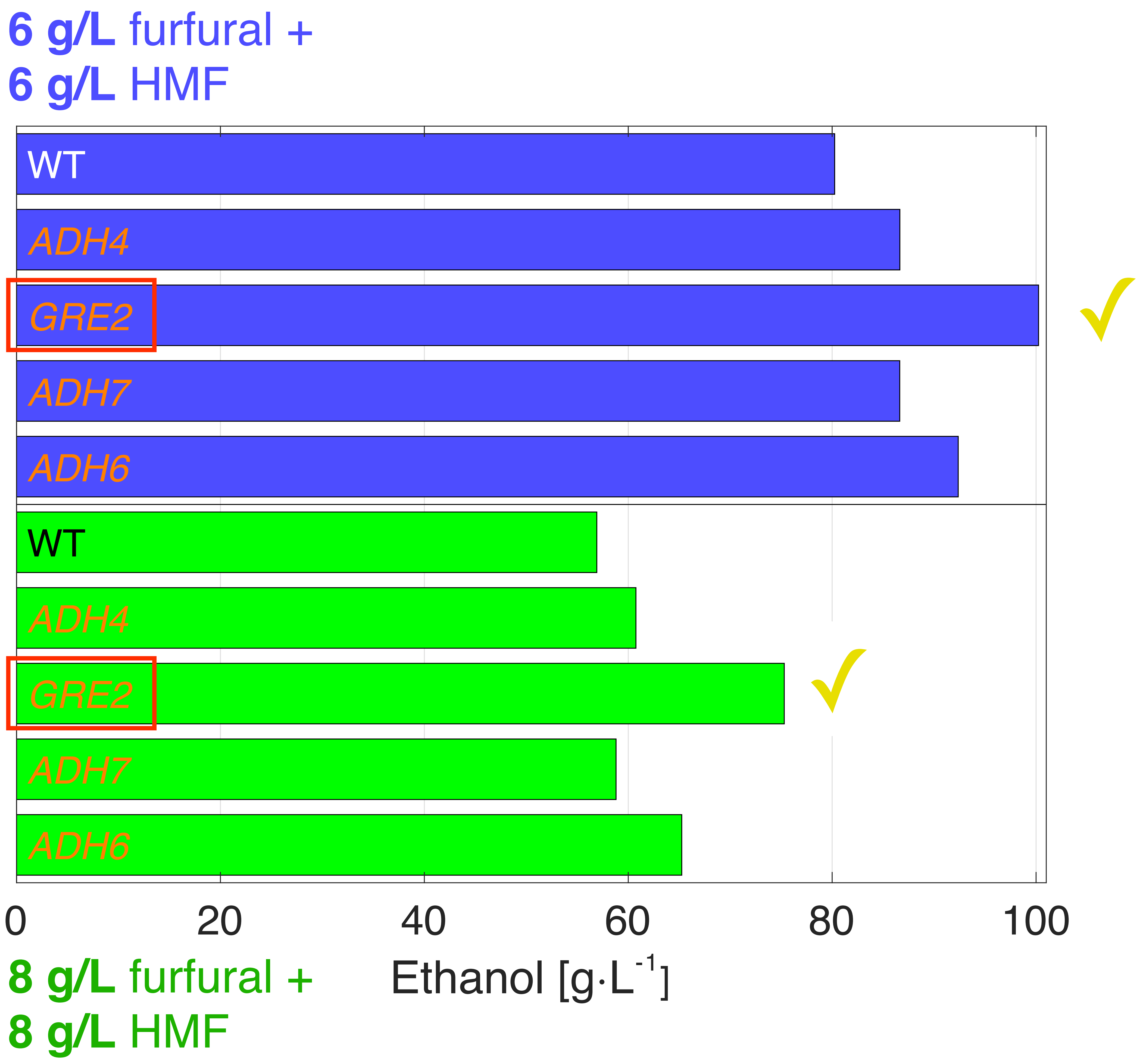


Can we **engineer** this **conversion within yeast?**



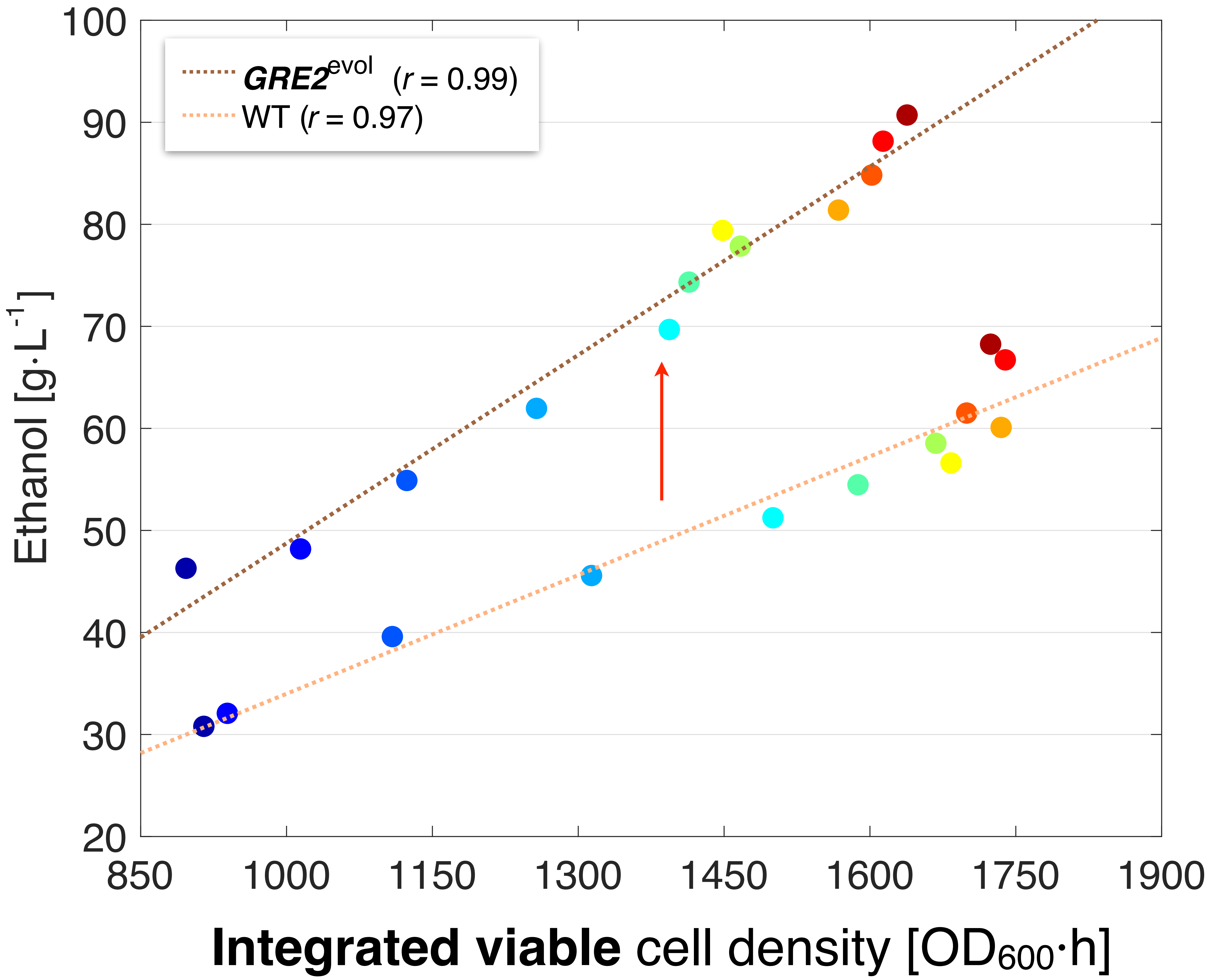


Reductase ***GRE2*** enhances **conversion** of **furfural**, **HMF**



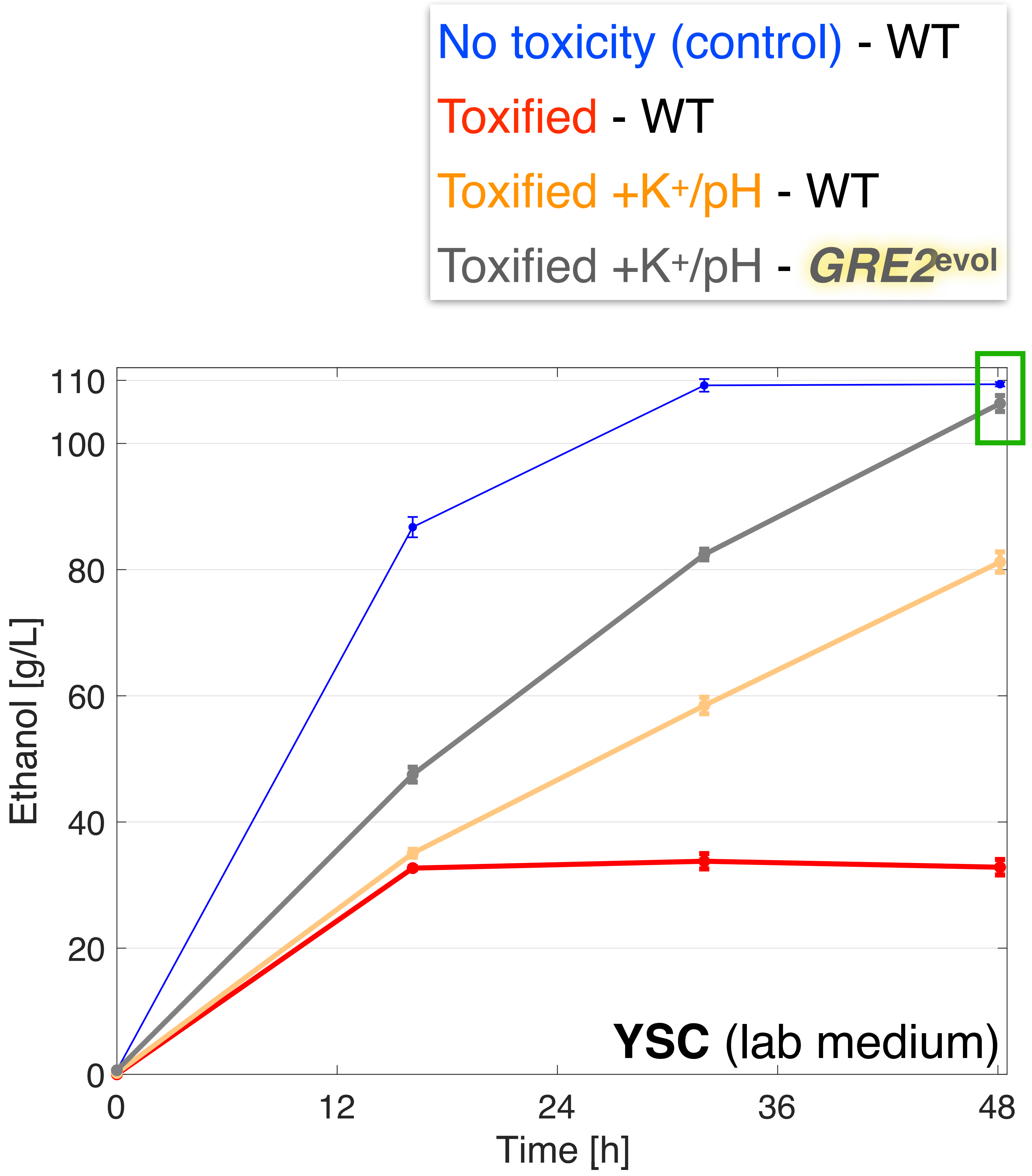
Evolved
GRE2

for even
higher
detoxification

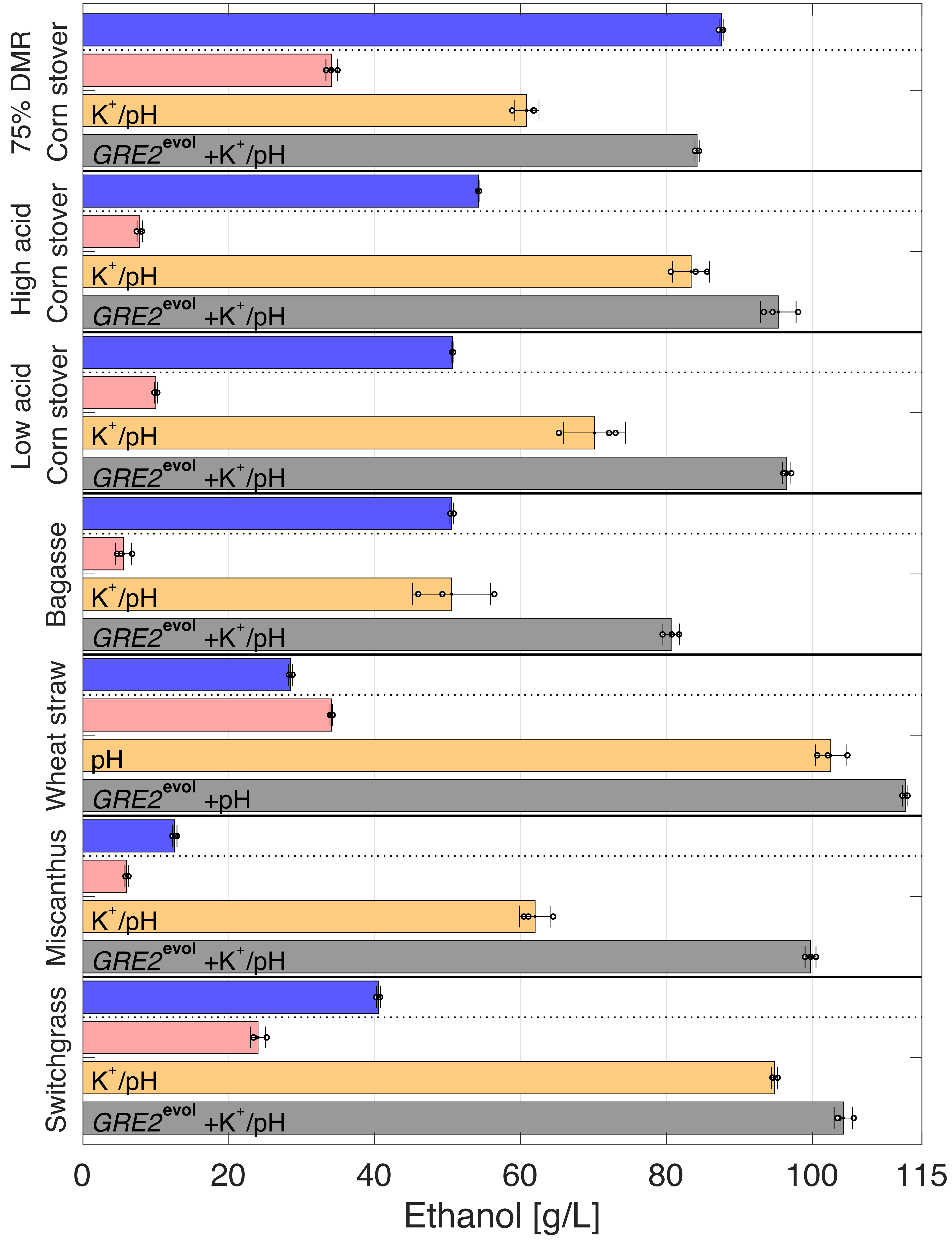


Increased **per-cell ethanol** production

GRE2 **evol** combined with **K+/pH** robustly ferments **genuine feedstocks***



→ **Matches performance in "clean sugar"**



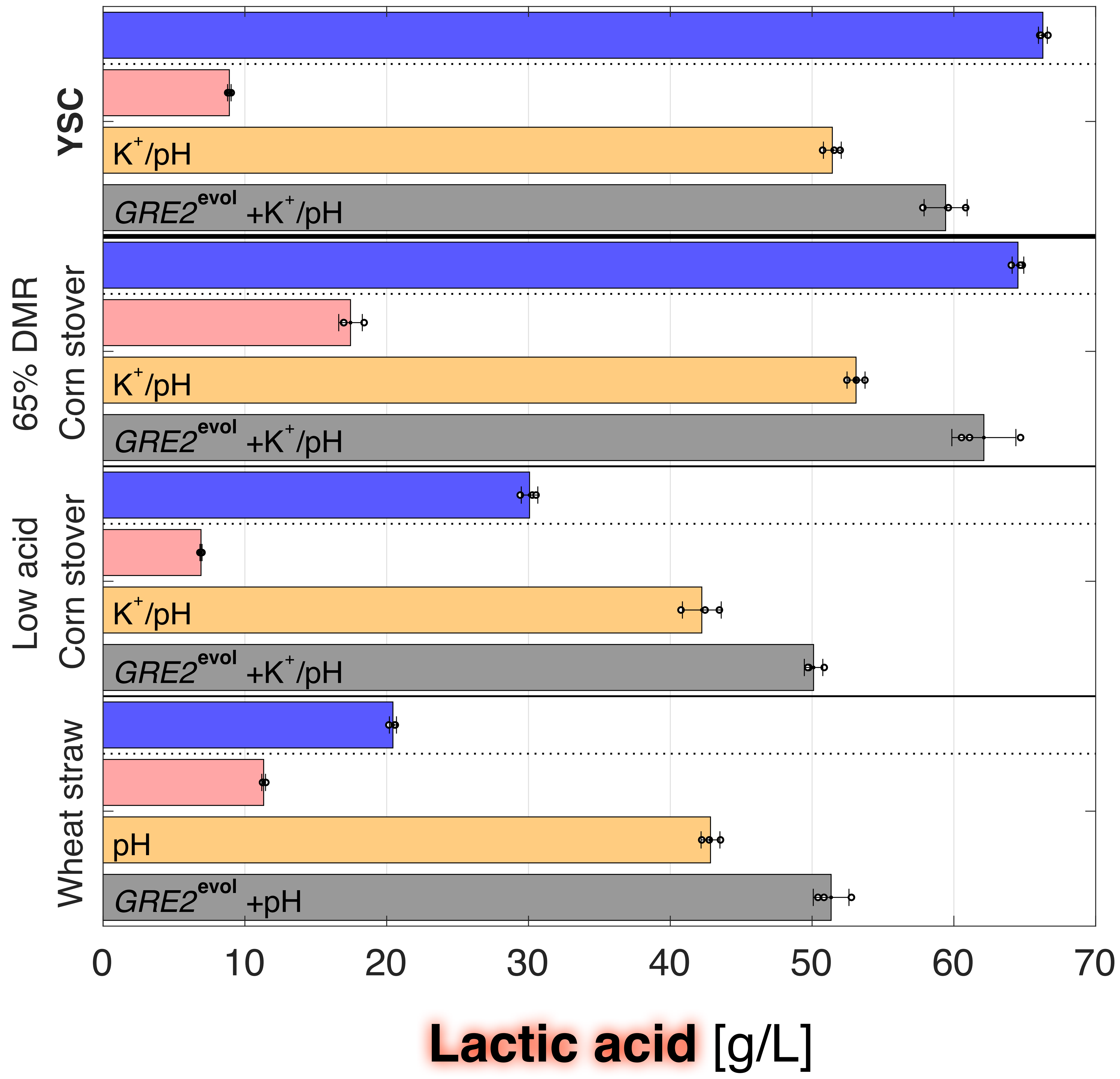
* All genuine feedstocks low in toxicity → toxification with furfural, HMF (and sugar) required

→ **Single strain handles diversity of cellulosic feedstocks**


lignocellulose
yeast
ethanol

***GRE2*evol** and **K+/pH** applied to production of **bio-plastic** precursor

PLA
polylactic
acid



→ Single strain

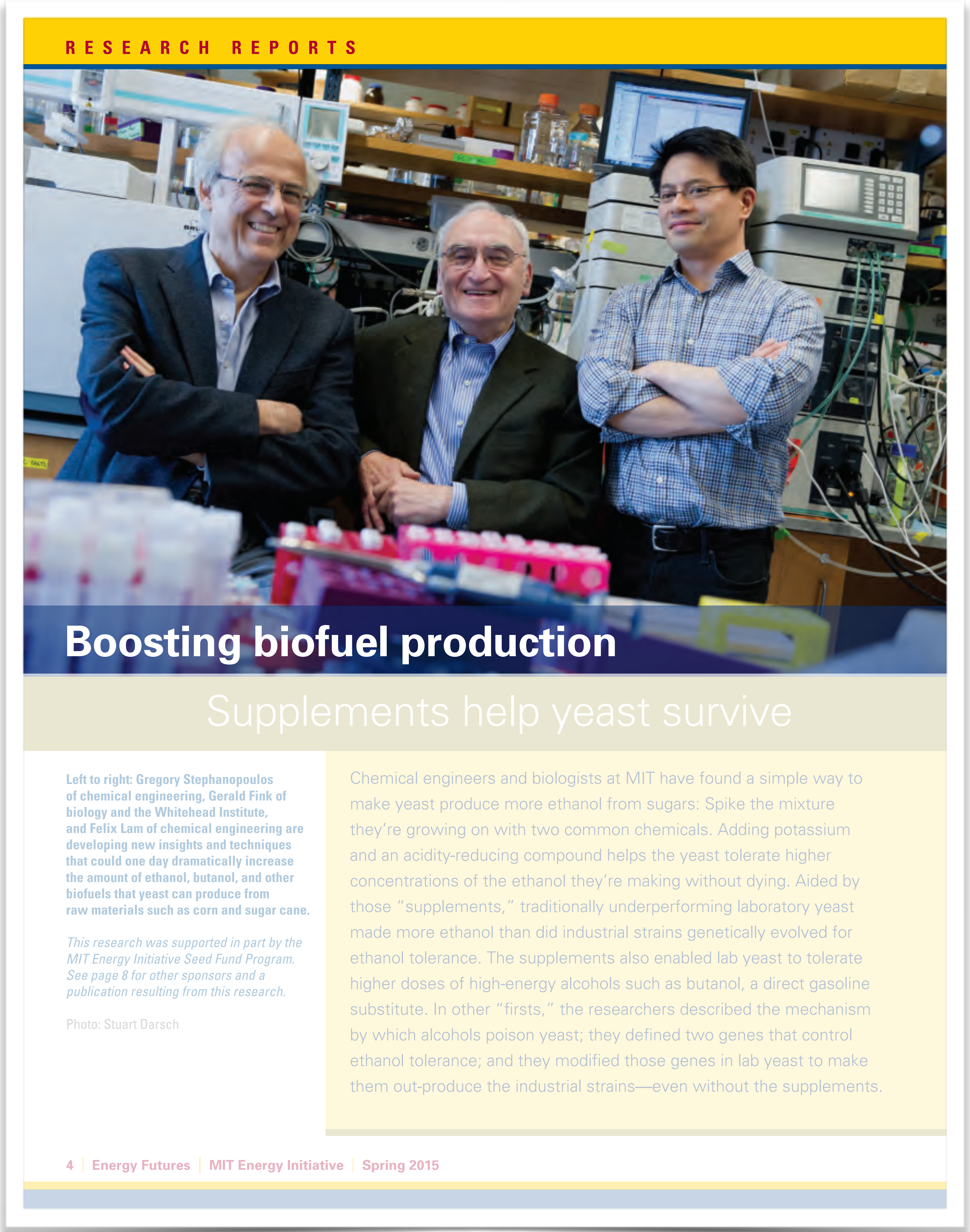
→ Diversity of cellulosic feedstocks

→ Matches "clean sugar" performance

CO_2
lignocellulose
(~1B ton / yr)
yeast
plastic
ethanol
X
oil (1B ton / yr)

CO_2
10 µm
oils
lignocellulose
(~1B ton / yr)
yeast
plastic
ethanol
biodiesel
oil (1B ton / yr)

Thanks!


RESEARCH REPORTS

Boosting biofuel production

Supplements help yeast survive

Left to right: Gregory Stephanopoulos of chemical engineering, Gerald Fink of biology and the Whitehead Institute, and Felix Lam of chemical engineering are developing new insights and techniques that could one day dramatically increase the amount of ethanol, butanol, and other biofuels that yeast can produce from raw materials such as corn and sugar cane.

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Photo: Stuart Darsch

Chemical engineers and biologists at MIT have found a simple way to make yeast produce more ethanol from sugars: Spike the mixture they're growing on with two common chemicals. Adding potassium and an acidity-reducing compound helps the yeast tolerate higher concentrations of the ethanol they're making without dying. Aided by those "supplements," traditionally underperforming laboratory yeast made more ethanol than did industrial strains genetically evolved for ethanol tolerance. The supplements also enabled lab yeast to tolerate higher doses of high-energy alcohols such as butanol, a direct gasoline substitute. In other "firsts," the researchers described the mechanism by which alcohols poison yeast; they defined two genes that control ethanol tolerance; and they modified those genes in lab yeast to make them out-produce the industrial strains—even without the supplements.

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