Stabilized Chlorine Dioxide in Fuel Ethanol Fermentation: Efficacy, Mechanisms and Residuals

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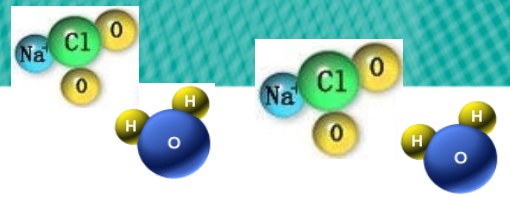
Derrick Okull, PhD
Technical Service Manager
Dupont Nutrition and Biosciences

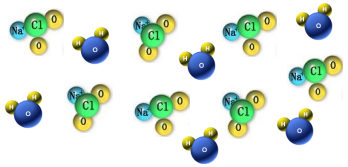


Stabilized Chlorine Dioxide (ClO_2) – What is it?

- **NOT** chlorine/bleach
- Buffered sodium chlorite solution, antibiotic-free aqueous solution
- Historically used in food processing/water treatment industries for contamination control/sanitation
- **14yr history in fuel ethanol** – GRAS status, with expert concurrence, for use as a processing aid in potable alcohol and fuel ethanol production with resulting distillers' grains approved for use in animal feed (cattle, swine, poultry).
- Controls lactic and acetic acid producing bacterial contamination
- Suitable for ethanol production from sugar and/or grain substrates
- Generated, concentrated solutions traditionally used in paper bleaching

Property	Value/Description	
Crystallization Point	-12°C	10°F
Freezing Point	-18°C	0°F
Boiling Point	106°C	223°F
pH	9.5 to 9.8	
Density (g/mL) @ 20 °C	1.18 to 1.21	
Color	Clear light yellow solution	
Odor	Faint chlorine-like smell	

Stabilized Chlorine Dioxide – how it works



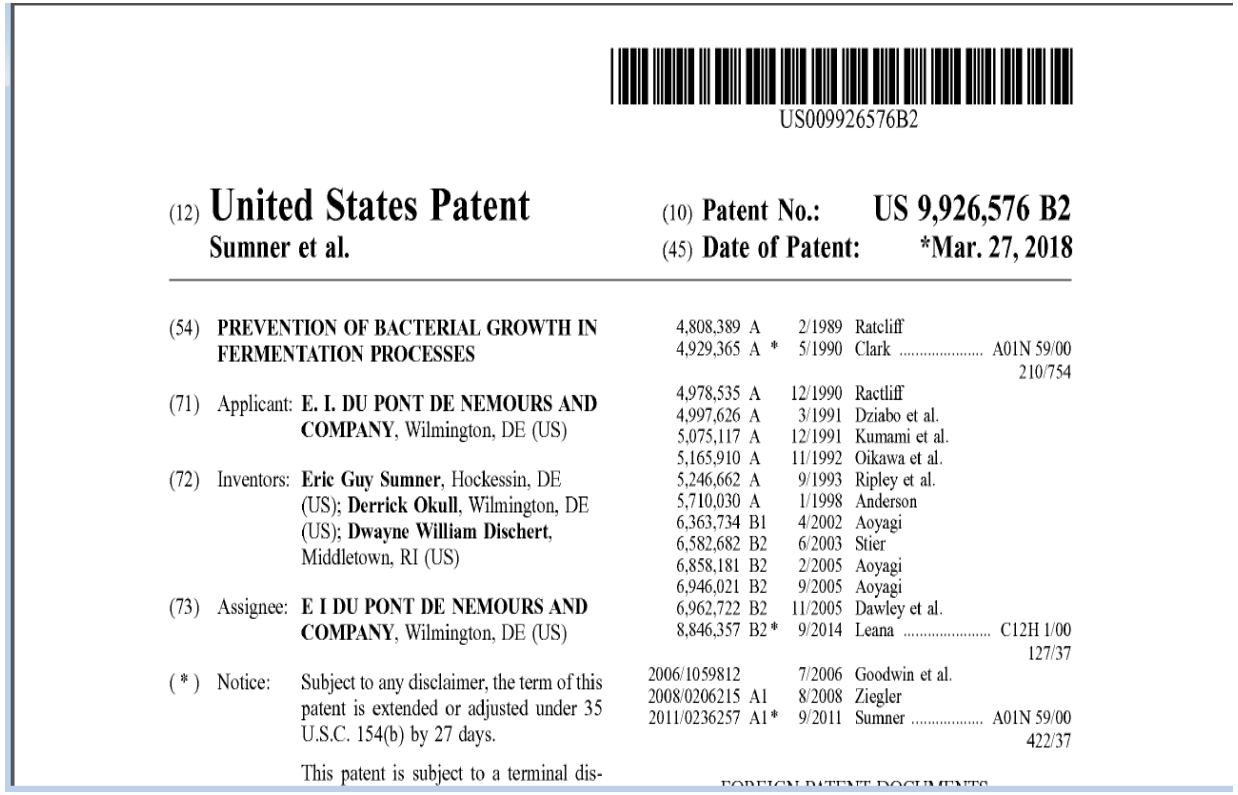
- A solution of 15 – 25 % Sodium Chlorite
- Chlorine dioxide is formed under acidic pH conditions
- When applied correctly, inactivates bacteria without harming yeast
- Activity is pH dependent – Need pH < 5.4 to be effective

Chlorite + Acid ⟷ Chlorous acid + Chlorine dioxide

$ClO_2^- + H^+ \longleftrightarrow HClO_2 + ClO_2$

Chlorite + Low pH mash/other substrate

Low pH/Acidic conditions drive conversion of Chlorite into more active form to kill bacteria on contact



US009926576B2

(12) **United States Patent**
Sumner et al.

(10) **Patent No.: US 9,926,576 B2**
(45) **Date of Patent: *Mar. 27, 2018**

(54) **PREVENTION OF BACTERIAL GROWTH IN FERMENTATION PROCESSES**

(71) Applicant: **E. I. DU PONT DE NEMOURS AND COMPANY**, Wilmington, DE (US)

(72) Inventors: **Eric Guy Sumner**, Hockessin, DE (US); **Derrick Okull**, Wilmington, DE (US); **Dwayne William Dischert**, Middletown, RI (US)

(73) Assignee: **E I DU PONT DE NEMOURS AND COMPANY**, Wilmington, DE (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 27 days.

This patent is subject to a terminal dis-

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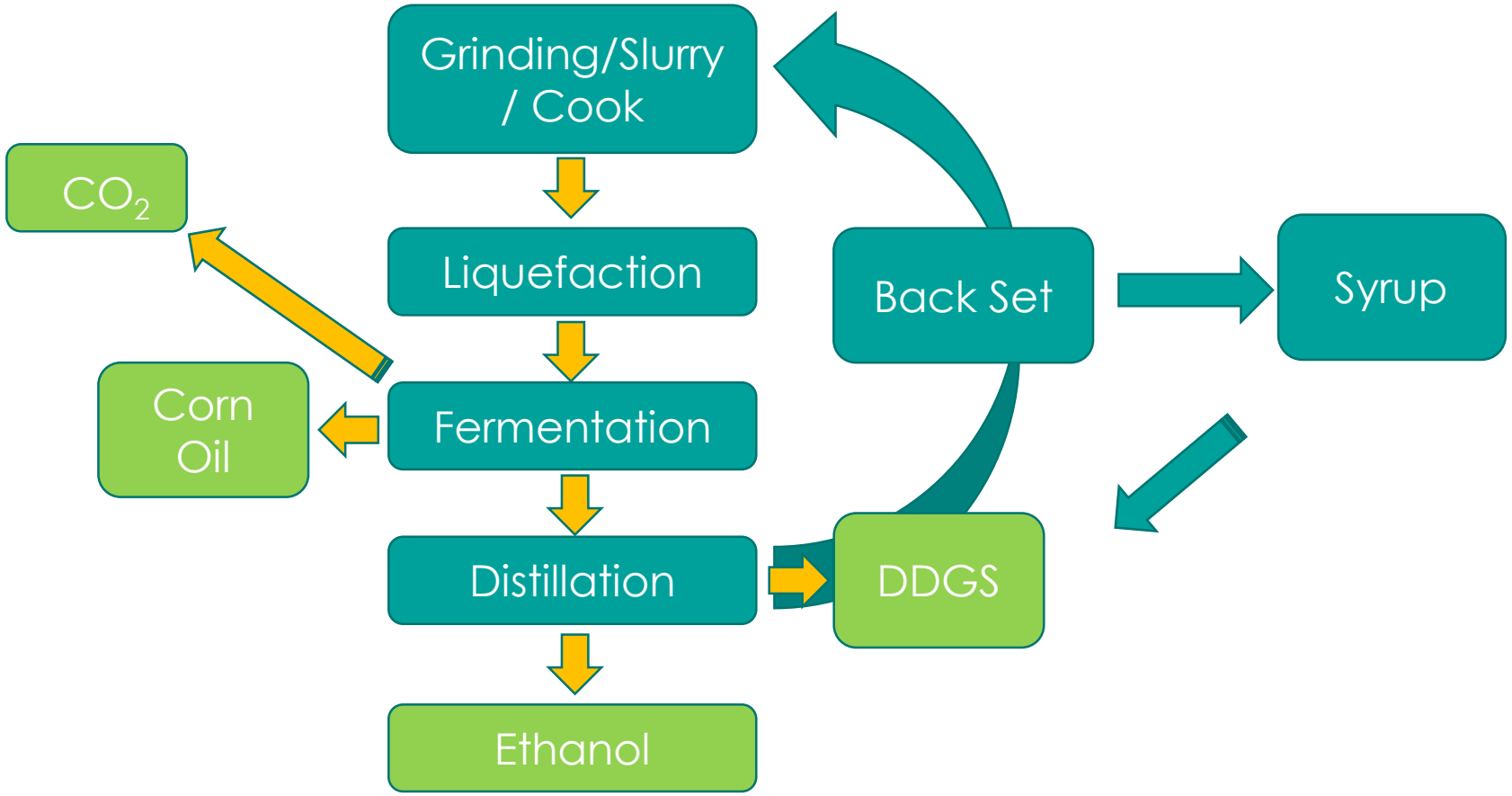
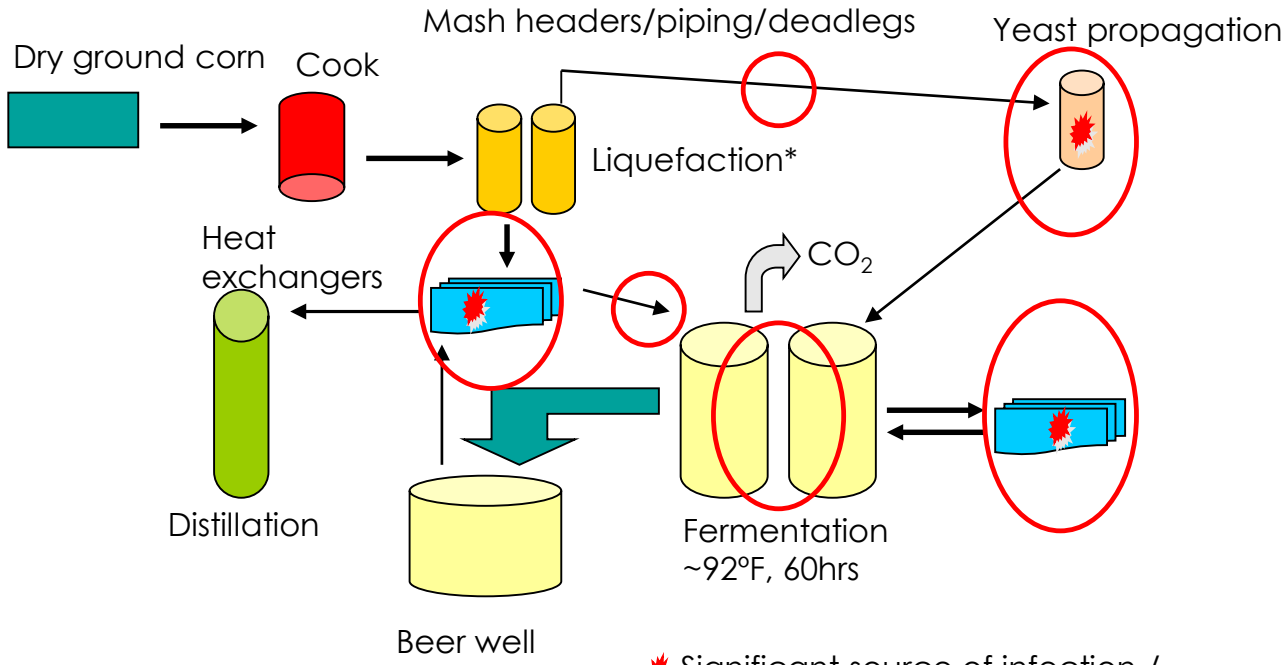
Bacterial Contamination in the Ethanol Process:

A Primer

ETHANOL PRODUCTION - DRY GRIND PROCESS OVERVIEW



WHERE DOES CONTAMINATION COME FROM?

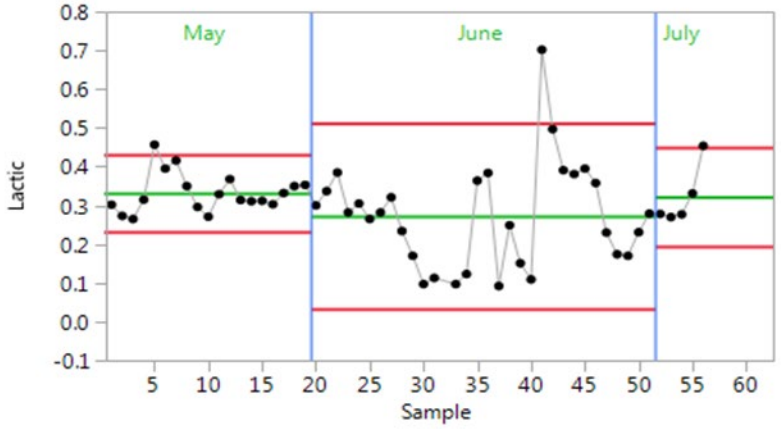


Significant source of infection / re-contamination

- Conducive temperature – 88 – 92°F
- pH – 4.9 – 5.6
- Food source – sugar, proteins, and trace/micronutrients
- Moisture – mash
- Protection - biofilms

Microbiological Sampling of Various Fuel Ethanol Operations	
Location	Typical Level of Lactic Acid Bacteria (CFU/g)
Slurry Tank	100
Liquefaction Tank	100
Pre-heat exchanger	100
Post-heat exchanger	10000000
Early Fermentation (<15 h)	10000
Mid Fermentation (15-30 h)	1000
Late Fermentation (>30h)	1000
Yeast Propagation	100

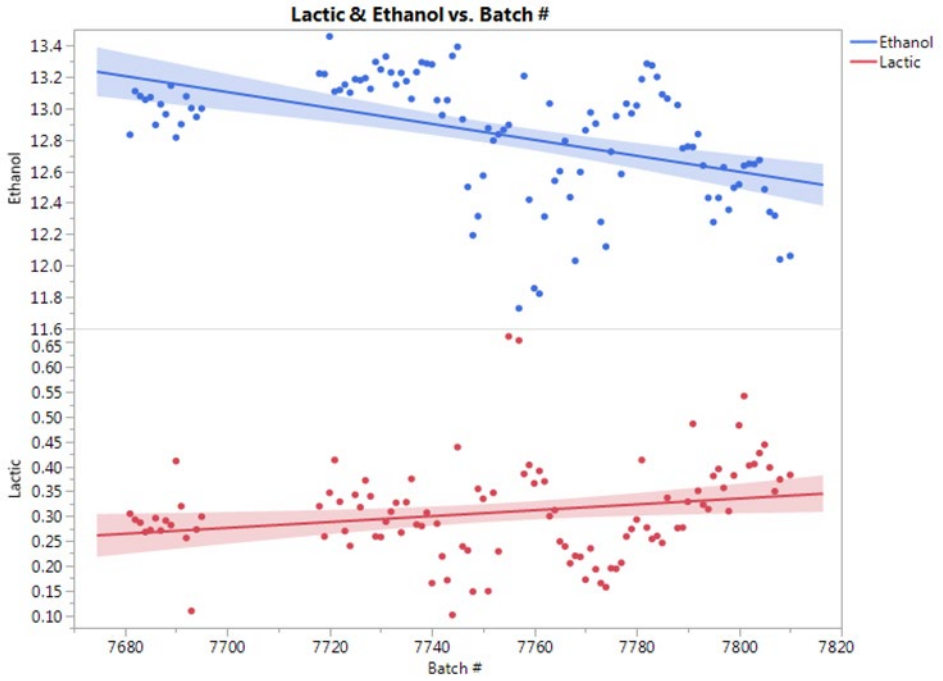
Drop lactic acid in a contaminated process



WHY DOES BACTERIA CAUSE LOSSES?

- Competition with inoculant yeast for nutrients
- Increased production of organic acids (lactic and acetic) by contaminant bacteria instead of ethanol
- Inhibition of yeast
- Recycle through backset can also compound inhibition
- Downtime, cleaning costs from fouling as a result of unfermented sugar going into distillation

Decline in Ethanol titer from a contaminated process



Controlling Contamination

Process Controls

CIP/ Sanitation
Temperature
High Solids
High Yeast inoculum
pH Control/ Acids

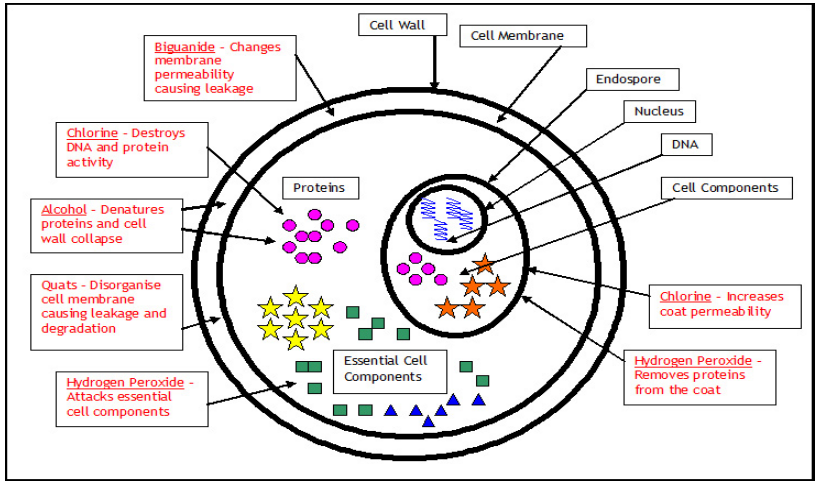
Antimicrobials

Hop Acids
Oxidatives
eg ClO_2, H_2O_2,Iodine
HOCl
Quaternary Compounds
Glutaraldehyde
Bacterial Peptides

Antibiotics

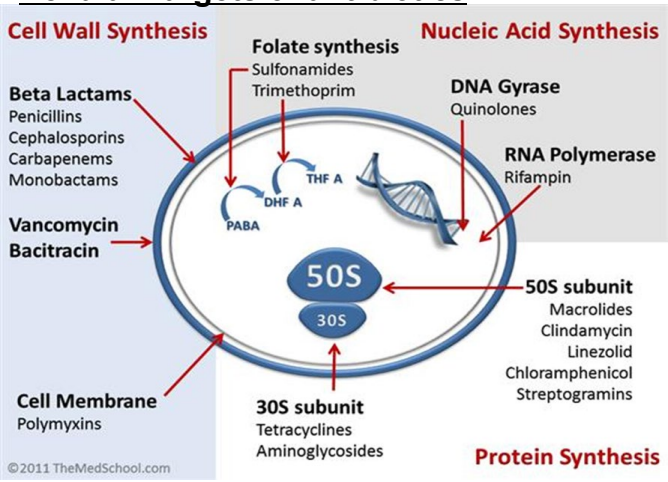
Virginiamycin (Lactrol™)
Monensin
Erythromycin
Penicillin
Combinations

Cellular Targets of some common disinfectants



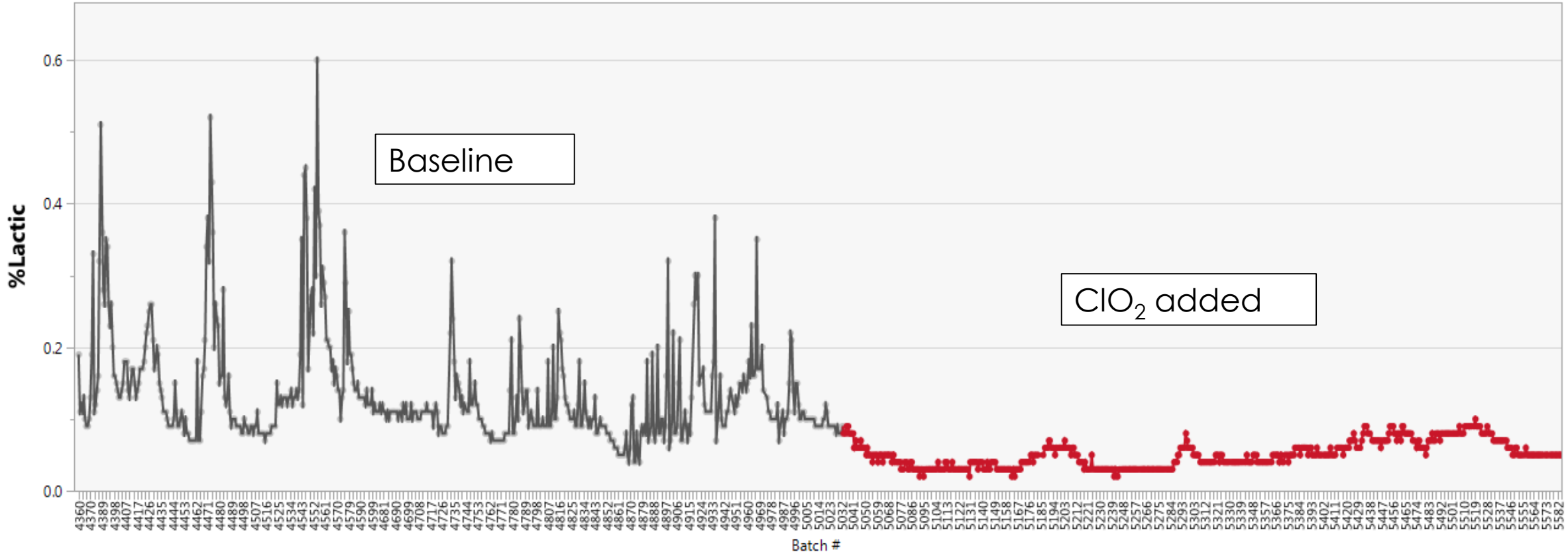
http://www.connect2cleanrooms.com/news-article/Rotational-Cleaning

Cellular Targets of antibiotics



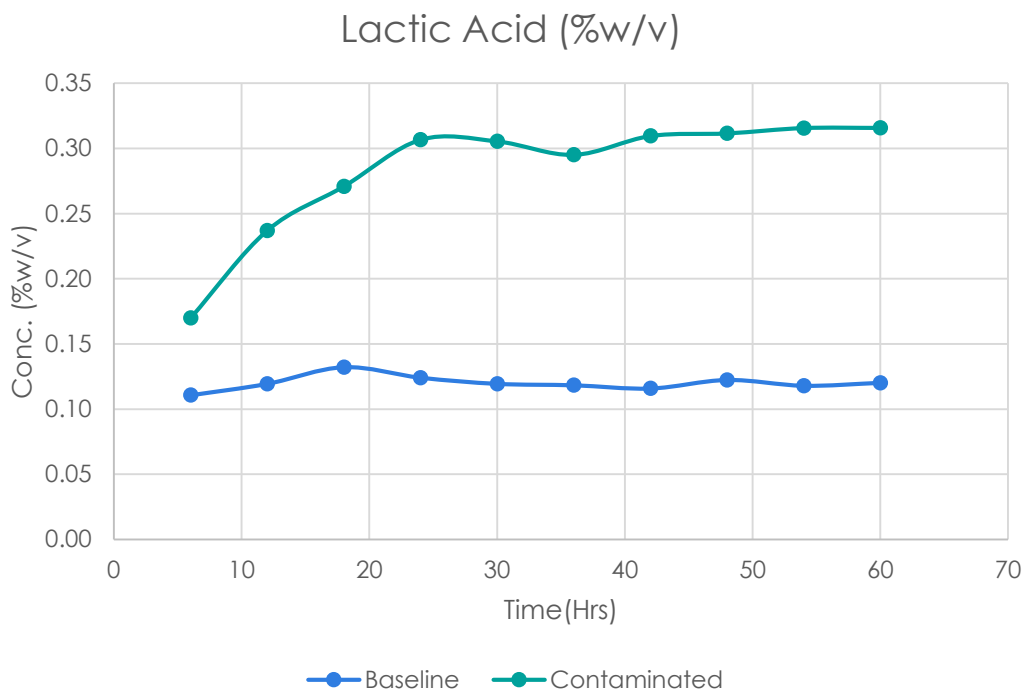
Example Plant Data

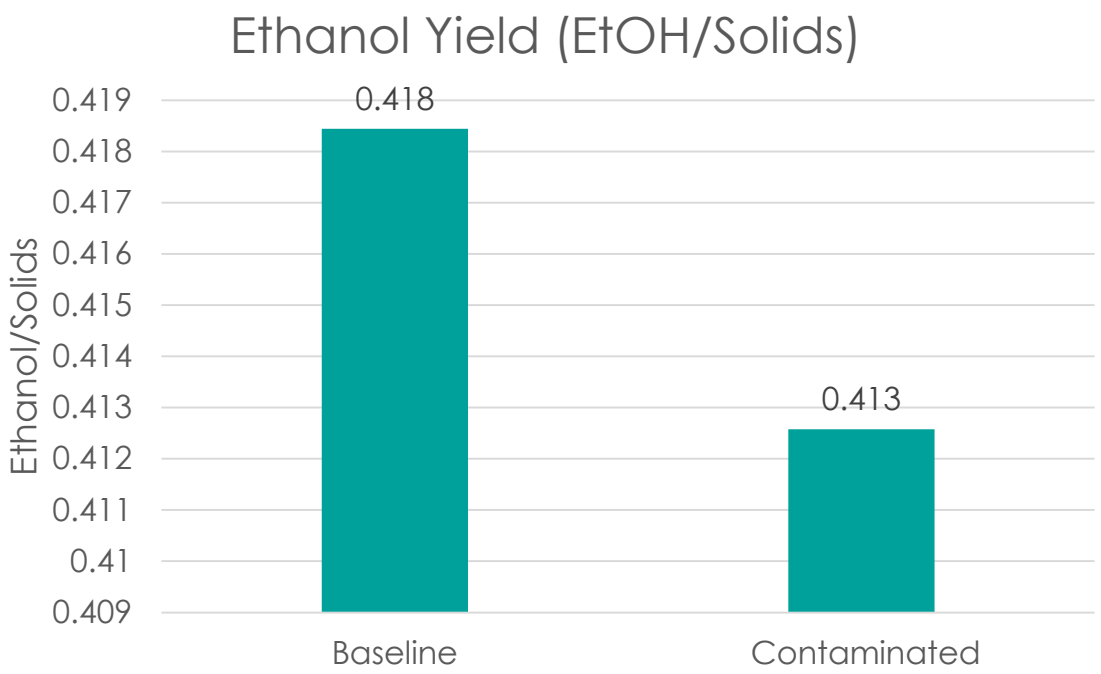
Lactic acid levels in a process before and after addition of ClO_2



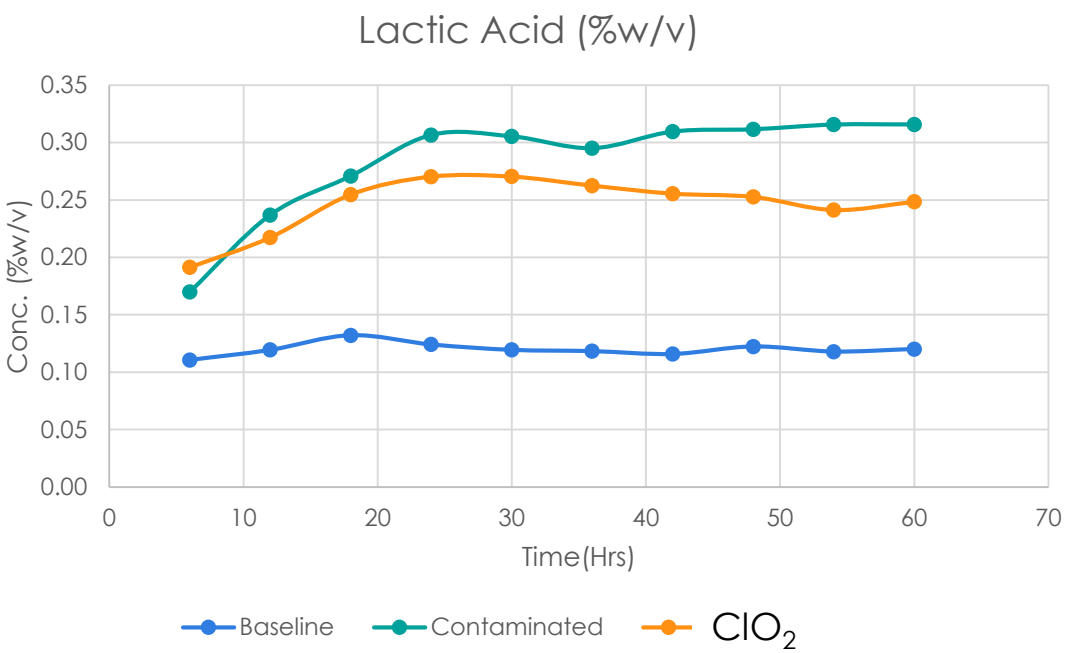
ClO_2 Plant Performance

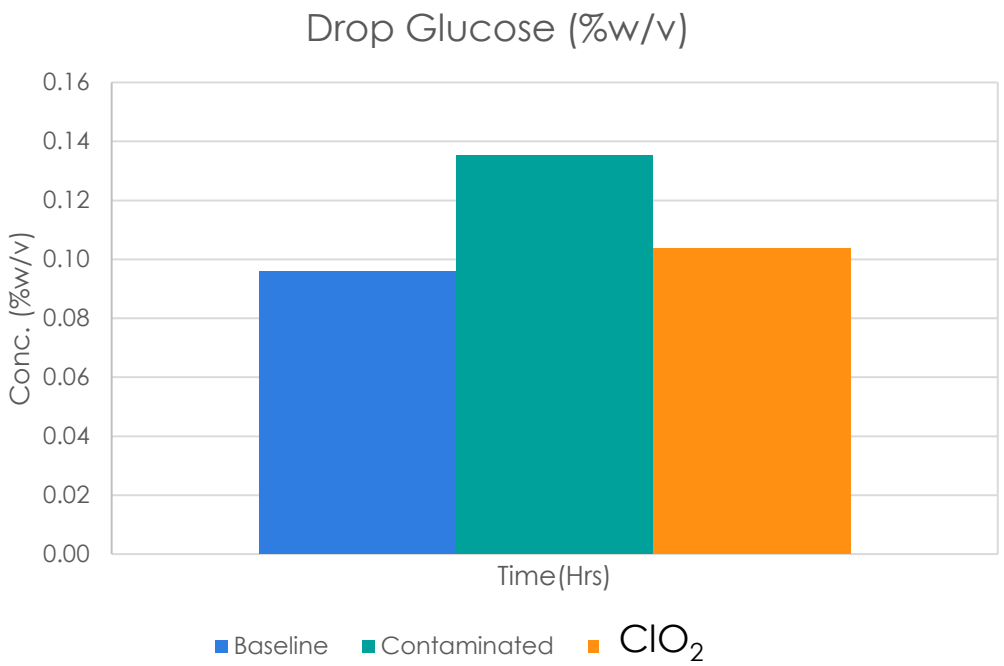
ClO_2 was trialed in conjunction with antibiotics at a 100 MGPY Dry Grind Ethanol Plant experiencing an active infection resulting in elevated lactic acid levels, high drop sugars, and decreased ethanol yields.





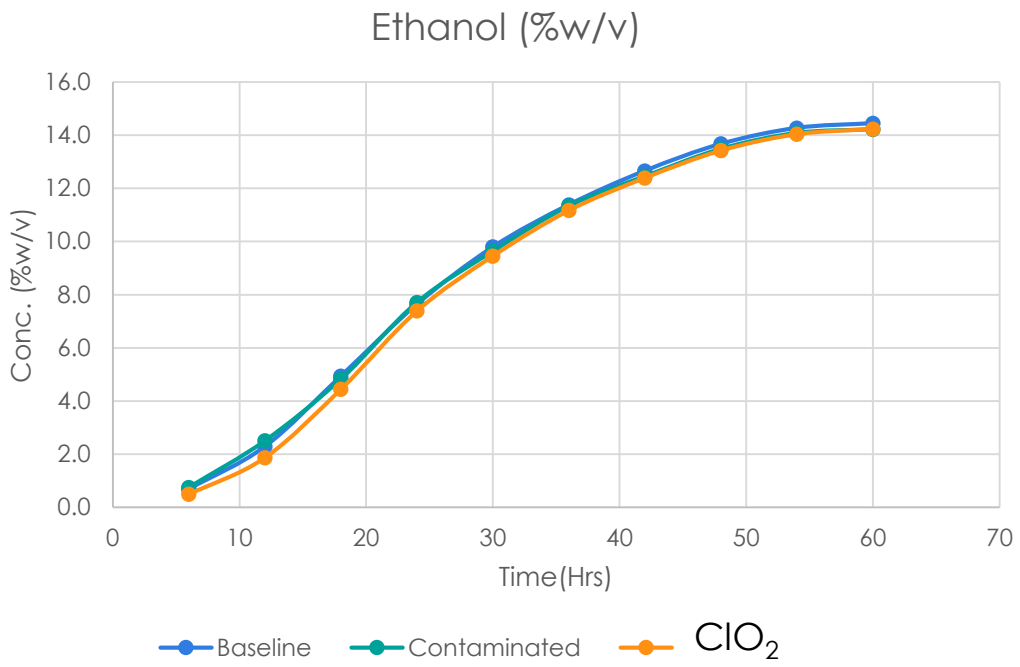
ClO_2 Performance During Active Contamination

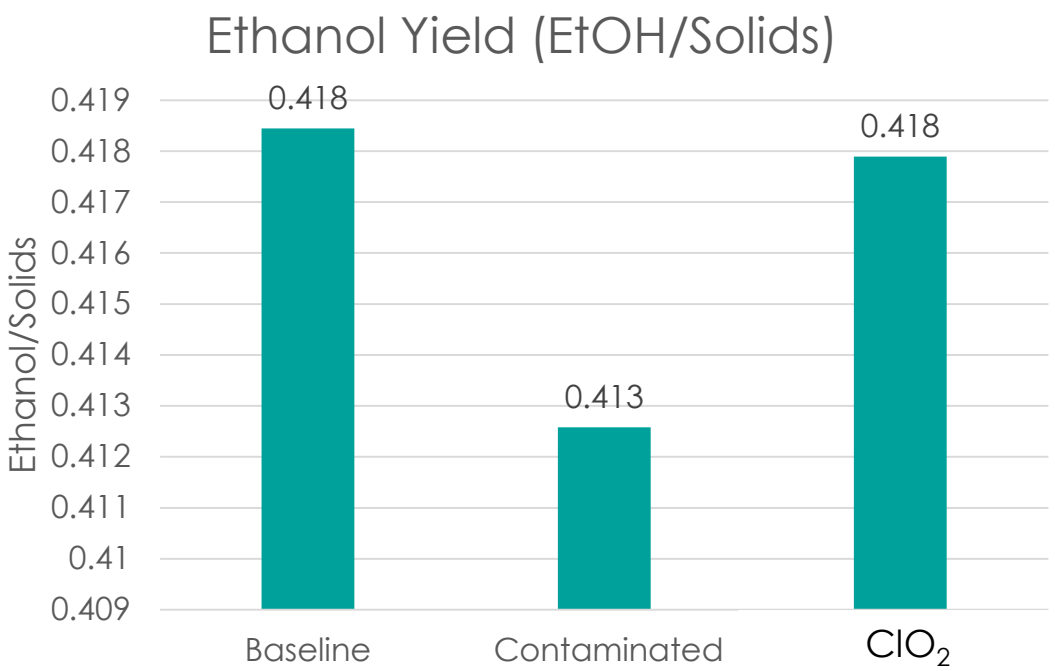




Dosing with ClO_2 helped to reduce the contamination in the system and move the plant back towards baseline profiles than treatment with antibiotics alone.

ClO_2 Performance During Active Contamination





Dosing with ClO_2 helped to decrease lactic acid concentration in the system and move yields and sugar data back to baseline.

Product Trail

Engineering controls in place to limit exposure potential to workers

Sodium Chlorite ($NaClO_2$)

H_2O

Other Ingredients

Blending Process

Blending Process at Toll Manufacturing facility

Stable Product

Packaging/Storage/ Transportation

Transport to customer by tote

Customer must receive guidance on recommended material safe handling, PPE and storage requirements

Customer storage

Possible Dosing Points

Customer Process

Grain Feedstock (Corn/Wheat)

Milling

Liquefaction

Yeast Propagation

Fermentation

CO_2

Atm.

Distillation

Dehydration

Solids Separation

Evaporation

DDGS

Distillers Corn Oil

Transfer to Animal Feed producer

Blended Animal Feed

Ethanol

Ethanol to Market

EtOH-blended gasoline

Proven safe (GRAS status) and approved for use in production of potable alcohol and fuel ethanol production with co-products intended for use in animal feed.

https://www.agweb.com/mobile/article/garage_sale_on_distillers_grain_products_naa_university_news_release/

Residuals in DDGS

	Vol	ClO_2 dose (ppm)	Vol of FXL (gal)	Wgt (kg)	$NaClO_2$ (kg)
Preferm vol	180000	1000	180	720	159
Ferm 1	387000	1000	387	1548	341
Total working vol	1211000	468.2	567	2268	499

Assumptions:

- Continuous fermentation – vessels in series
- Dosing in the first two vessels in the train at up to 1000ppm (0.1% of working volume of dosed vessels)
- At drop, dilution has occurred across the system – total working volume includes all vessels in the train
- Stabilized ClO_2 contains 15-22% sodium chlorite ($NaClO_2$)
- At this dose, the system will have an estimated 499kg of chlorite
- End products of reaction in acidic environment are NaCl (salt) and $NaClO_3$ (chlorate)at about a 2:1 ratio, with the chlorate getting further reduced to NaCl

Table 5.1. Chemical composition of corn grain and co-products from fuel-ethanol production (as-fed basis)

Item	Corn Grain[a]	Corn DDGS[b]	Corn HP-DDG[c]	Corn Germ[c]
	%			
Dry matter	89.0	89.0	91.7	91.1
ME_n[d] (kcal/kg)	3,350	2,770[e]	–	–
TME_n[f] (kcal/kg)	3,470	2,851	2,682	3,881
Crude protein	8.5	26.5	39.6	14.9
Ether extract	3.8	10.1	3.6	15.8
Linoleic acid	2.2	–	–	–
Crude fiber	2.2	7.0	7.5	5.1
Neutral detergent fiber	9.63	32.22[g]	22.20	21.10
Acid detergent fiber	2.83	11.90[g]	11.20	7.50
Calcium	0.02	0.07	0.02	0.02
Phosphorus, total	0.28	0.77	0.44	1.35
Phosphorus, non-phytate	0.08	–	–	–
Phosphorus, available	–	0.48	0.26[h]	0.34[h]
Sodium	0.02	0.20	0.13	0.01
Chloride	0.04	–	–	–
Potassium	0.30	0.85	0.43	1.48
Sulfur	0.08	0.84[i]	0.81	0.19
Arginine, total	0.38	1.09	1.41	1.13
Histidine, total	0.23	0.68	1.08	0.42
Isoleucine, total	0.29	0.96	1.35	0.44
Leucine, total	1.00	3.00	5.09	1.04
Lysine, total	0.26	0.73	1.12	0.79
Methionine, total	0.18	0.50	0.93	0.25
Cystine, total	0.18	0.54	1.32	0.36
Methionine + cystine, total	0.36	1.04	2.25	0.61
Phenylalanine, total	0.38	1.31	2.15	0.60
Threonine, total	0.29	0.96	1.53	0.57
Tryptophan, total	0.06	0.21	0.33	0.19
Valine, total	0.40	1.30	1.93	0.67

Note: Because of an appreciable variation in nutrient and energy contents (discussed in the text), diet formulation should be performed with nutrient and energy values specific to the particular co-

- Typical Sodium in DDGS ~ (2000 ppm) 0.2%
- Potential from high dose ClO_2 – **155 ppm (0.0155%)**

[b] Data from Waldroup et al. (2007) except as noted.

https://www.card.iastate.edu/products/books/distillers_grains/pdfs/chapter5.pdf (K. Bregendhal, Chapter 5)

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Thank you!