WASHING RECOVERED YEAST WITH CHLORINE DIOXIDE

George Agius Andrew Gould

MBAA Technical Session 2014

Sealed Air Re-imagine™

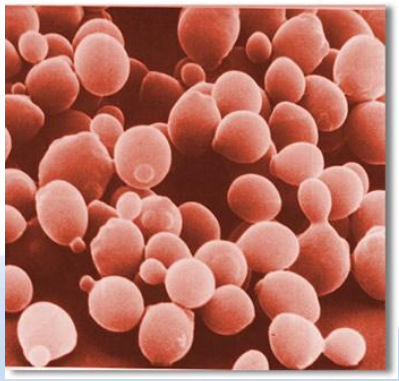
SLEEMAN EST 1834 BREWERIES LTD.

Acknowledgements

- Thanks to
  - Sleeman Brewery, Guelph Ontario for the use of their laboratories to carry out this work.
  - Jonathan Crawshaw, Sleeman Brewery, Guelph, Canada, for facilitating this work.
  - Bart Schuurman Hess, Sealedair, Food Care, Oakville, Ontario, Canada for his enthusiasm for the work, and bringing us all together.

Yeast Washing

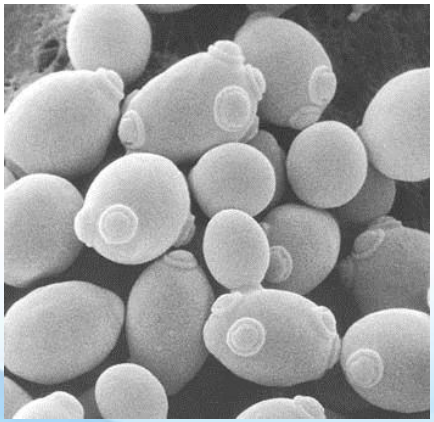
Pitching yeast is reported to act as a reservoir for low levels of bacterial contamination



Briggs 2004

Yeast Washing

Need to determine if the brewery is going to adopt yeast washing as a part of its brewing strategy



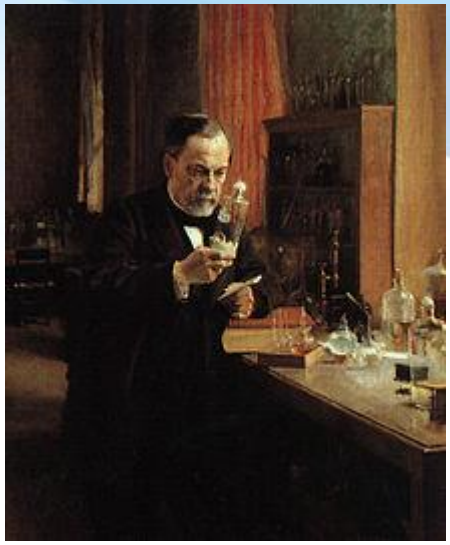
Yeast Washing

- The strategies:

  a. Wash all yeast
  b. Wash only when problems are evident
  c. Not wash at all

- We will be addressing the first two strategies

Yeast Washing Background



- Yeast “purification” with Louis Pasteur
  - Around 1876
  - Found by lowering pH of yeast, the accompanying bacteria declined in number
  - It is reported - He grew the yeast in acidified cane sugar for two generations

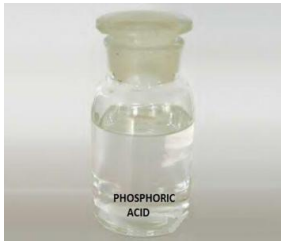
Hind 1937

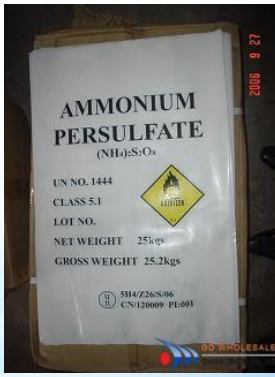
Yeast Washing Background

- Following Pasteur, others used an acidified wort process – serial tanks involved
- Using 0.1% Tartaric acid
- pH reduced to approximately 3.9
- Reduction in yeast infection reported

Yeast Washing Background

- Yeast washing has evolved into the following methods:
  1. Distilled or sterile water wash
  2. Acid wash
  3. Acid wash with ammonium persulfate
  4. (Antibiotics?)





Yeast Washing Background

- Antibiotics?
  - There use was proposed in the late 1940s and early 1950s
    - Tyrothricin and Polymyxin B
  - It was quick realized that to use antibiotics was irresponsible and the use of antibiotics was never done.

- Nisin?

  - A small polypeptide was used and accepted by the dairy industry
    - Research showed it as having limited use in the brewing industry and it has never found favour

Briggs 2004

Yeast Washing Background

- Yeast washing has evolved into the following methods:

1. Distilled or sterile water wash
2. Acid wash
3. Acid wash with ammonium persulfate

Yeast Washing Using Sterile Water

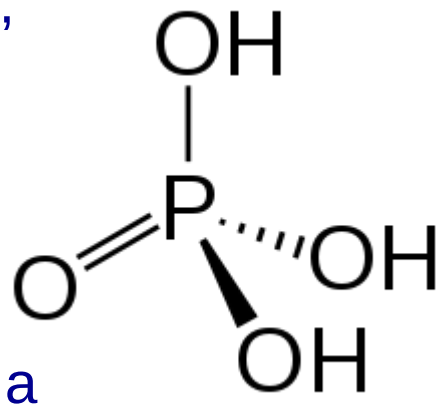
- Large volumes of sterile cold hard water
- Mixed with yeast slurry
- Yeast is allowed to settle out
- Water is decanted off the yeast
  - The theory is the bacteria and dead cells will be removed with the water – dilute out the bacteria and dead cells
- This is repeated 2 or 3 times in the process

Hardwick 1995

Acid Washing Yeast

- The most common method
- Many acids are reported to be used: phosphoric, citric, tartaric, sulphuric

Most common acid is food grade phosphoric acid



- Yeast slurry is acidified with dilute acid (10%) to a pH of 2.3 ± 0.1
- The acidified slurry is slowly mixed for approximately 2 hours and kept at refrigeration temperatures of 4^{o}C

McCabe 1999

Acid Washing Yeast

- Criteria for acid washing yeast:
  - Use food grade acid
  - Wash yeast as a beer or water slurry
  - Chill yeast and acid to less than 5^{o}C
  - Stir slowly and constantly
  - Stir throughout the washing process
  - Maintain temperature at less than 5^{o}C
  - Monitor the pH
  - Do not wash more than two hours
  - Pitch yeast immediately after washing

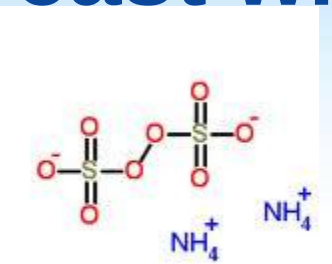
McCabe 1999

Acid Washing Yeast

- Effectiveness of Acid washing:
  - Reported that aerobic contaminants are removed
  - Reported that the anaerobic beer spoilage organisms are more resistant
  - Reported that wild yeast are unaffected

Goldammer, 2008

Acid Washing Yeast with Ammonium Persulphate

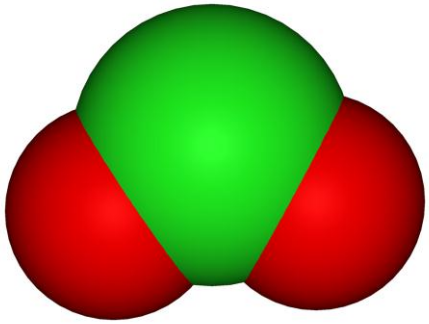


- More effective than acid washing
- 0.75% Ammonium Persulphate added to the acid washing process
- Contact time is a maximum of one hour prior to pitching
- Reported to be harder on the pitching yeast

Next steps

- The three strategies are used but have been found to not be totally effective against the anaerobic beer spoilage bacteria
- The work was to investigate a new yeast washing protocol:

Chlorine Dioxide



Why Chlorine dioxide?

- Chlorine Dioxide is a strong but selective oxidizing agent
- Chlorine dioxide is effective over a wide pH range (2-10)
- Does not react with poly phenols (tannins), that can leave a taste in the parts per trillion
- Does not produce halogenated methane (CHX_3)

Masschelein J.W.

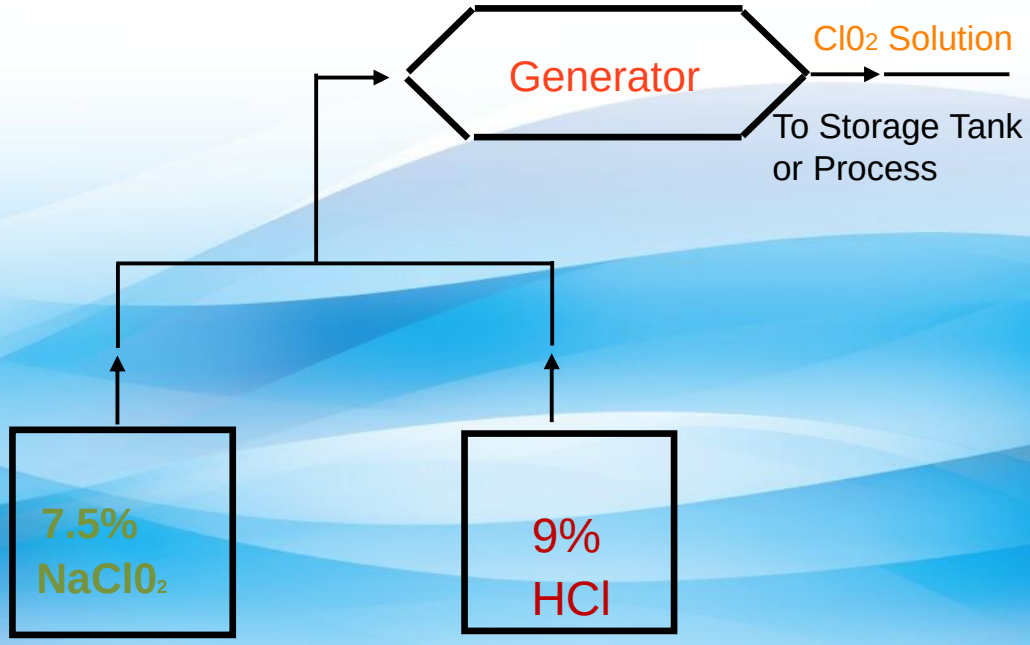
Disadvantages of chlorine dioxide

- Cannot be shipped or stored for long periods and has to be generated on site
- Generation requires mixing a strong acid with a solution of sodium chlorite in the right proportion and for the requisite time or some well known variation
- Requires specialized equipment for safe generation
- Requires careful handling as chlorine dioxi gas can escape form solution and easily exceed safety limits in the air

ACID CHLORITE REACTION

- Two pumps mix solutions of sodium chlorite and a strong acid -- hydrochloric acid.
- Reaction occurs in a chamber typically 10 minutes residence time
- Reaction is stoichiometric and 99% complete if the chemicals are in right concentration.

Schematic Acid/Chlorite Generation of ClO_2



$$5\ NaClO_2 + 4\ HCl \longrightarrow 4\ ClO_2 + 5\ NaCl + 2H_2O$$

Will chlorine dioxide eliminate bacteria without damaging yeast?

- Not much reported in the literature
- Paper tried using activated chlorine dioxide solution mixture at pH 3 using a nominal 50ppm chlorine dioxide
  Johnson D., (1998)
- Mode of generation used did not specify time and used a weak acid with chlorite
- Produces indeterminate solution mixture where precise concentration of chlorine dioxide is not known
- Need to start with known concentration of chlorine dioxide for control

The test procedure used to challenge the bacteria infecting the yeast is outlined in **Figure1:**

1. Lager yeast (*Saccharomyces carlsbergensis*) slurry sample was obtained from an actual pitching tanks in a brewery.

2. 5.0ml of lactobacillus sp inoculum added to the yeast.

3. A sample of the 2000ppm solution of chlorine dioxide from a Prominent Generator was measured for its chlorine dioxide content.

4. A calculated volume of the nominal 2000ppm chlorine dioxide solution is added to 100.0 ml of the inoculated yeast to obtain a desired initial concentration e.g. 100 ppm. The concentration of the 2000ppm +/- 100ppm chlorine dioxide solution was checked using the Hach DPD method kit for chlorine dioxide #58700-51.

5. Time is started

6. Samples of the yeast are plated using a Mann-Rogosa-Sharpe (MRS) medium for anaerobes and incubated at 28^{o}C. Samples of the yeast were also plated on Universal Beer Agar (UBA), and incubated at 28^{o}C . The aerobic bacteria were counted after 3days and the anaerobic after 5 days.

7. The viability of the yeast was determined using 0.2% Eosin Y stain [*McCaig,R*].

1. Lager yeast (*Saccharomyces carlsbergensis*) slurry sample was obtained from an actual pitching tanks in a brewery.

1

H Yeast 500 mL

2

2. 5.0ml of lactobacillus spa inoculum added to the yeast.

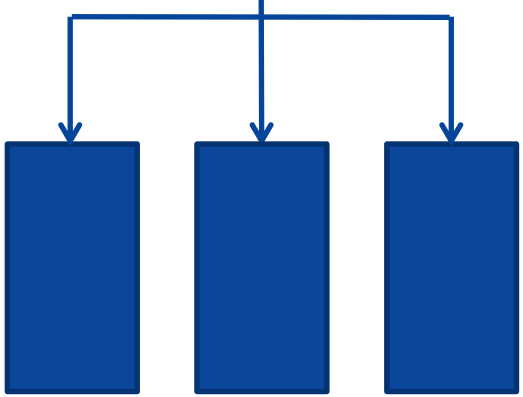
Lactobacillus sp 5 mL

0.5 ml

100 ml in each

3

3 Divided into 3 X100ml samples.



4

Chlorine Dioxide 2000 ppm

4. A calculated volume of 2000ppm ClO2 added to each yeast vial to achieve desired concentration. (5 mins apart) Mixed gently. Time started

100 ppm (5.3 mL) **175 ppm (11.1 mL)** **250 ppm (25 mL)**

5

Contact time	Chlorine Dioxide Concentration ppm			Viability	pH
	100	175	250		
0					
15					
30					
45					
60					
120					

5. Samples from the different treatments were plated at 15 minutes intervals.

Plated on MRS and UBA using S/T technique using
1/10 dilution
15 - 20 9mL dilution blanks
15 - 20 MRS/UBA plates

TABLE 1 – RESULTS FROM EXPERIMENT 1

- Table 1 shows the results for the first experimental attempt
- In this experiment the chlorine dioxide starting concentrations tested were 25ppm and 100ppm
- Table 1 top window shows the initial anaerobe and aerobe bacterial concentrations and the initial yeast viability
- The lower window shows the variation of the bacterial concentration with time after exposure to the chlorine dioxide
- The subsequent slides provide comments on the results in Table 1

TABLE 1

Starting Conditions	Aerobic [UBA(C)]	Anaerobic [MRS(C)]	Viability (%)
Yeast as collected (Lager CY-3) Tank 204	0	0	85
Bacteria mixture	TNTC	TNTC	
Yeast plus bacteria	TNTC	TNTC	

Contact time (min)	Concentration of chlorine dioxide (ppm)				Viability (%)	
	25		100		25ppm	100ppm
	Aer	Anaer	Aer	Anaer		
0	TNTC	TNTC	TNTC	TNTC	82	82
15	TNTC	TNTC	0	500	74	74
30	TNTC	TNTC	0	250	74	72
45	TNTC	TNTC	0	75	75	77
60	TNTC	TNTC	0	45	79	71
90	TNTC	TNTC	0	0	68	74

TABLE 1

Starting Conditions
Yeast as collected (Lager CY-3 204
Bacteria mixture
Yeast plus bacteria

Chlorine dioxide at 100ppm can eliminate both the anaerobic and aerobic bacteria.

The anaerobic bacterial load was TNTC, which is not a typical situation, and it took around 90mins to reduce the anaerobic bacteria to zero, and 15mins to eliminate the aerobic bacteria.

Contact time (min)	Concentration of chlorine dioxide (ppm)					y (%)
	25		100			100ppm
	Aer	Anaer	Aer	Anaer		
0	TNTC	TNTC	TNTC	TNTC		82
15	TNTC	TNTC	0	500	74	74
30	TNTC	TNTC	0	250	74	72
45	TNTC	TNTC	0	75	75	77
60	TNTC	TNTC	0	45	79	71
90	TNTC	TNTC	0	0	68	74

TABLE 1

Starting Conditions	[MRS(C)]	Viability (%)
Yeast as collected (Lager CY-3) Tank 204		85
Bacteria mixture	TC	
Yeast plus bacteria	TC	

Yeast viability was reduced from 80% to 74% in the first 15 minutes.

Yeast viability did not decrease any further after the first 15minutes

Contact time (min)	Concentration of ch				Viability (%)	
	25		100		25ppm	100ppm
	Aer	Anaer	Aer	Anaer		
0	TNTC	TNTC	TNTC	TNTC	82	82
15	TNTC	TNTC	0	500	74	74
30	TNTC	TNTC	0	250	74	72
45	TNTC	TNTC	0	75	75	77
60	TNTC	TNTC	0	45	79	71
90	TNTC	TNTC	0	0	68	74

TABLE 2 – RESULTS FROM EXPERIMENT 2

- Table 2 shows the results for the second experimental attempt
- In this experiment the chlorine dioxide starting concentrations tested were 100ppm and 250ppm
- Table 2 top window shows the initial anaerobe and aerobe bacterial concentrations and the initial yeast viability
- The lower window shows the variation of the bacterial concentration with time after exposure to the chlorine dioxide
- The subsequent slides provide comments on the results in Table 2

TABLE 2

Starting Conditions	Aerobic [UBA(C)]	Anaerobic [MRS(C)]	Viability (%)
Yeast as collected (Lager H) Tank 202	0	0	93
Bacteria mixture	TNTC	TNTC	
Yeast plus bacteria	TNTC	115	

Contact time (min)	Concentration of chlorine dioxide (ppm)				Viability (%)		pH	
	100		250		100	250	100	250
	Aer	Anaer	Aer	Anaer				
0	TNTC	115	TNTC	115	93	93	4.47	4.47
15	0	0	0	0	80	80		
30	0	0	0	0	75	78		
45	0	0	0	0	83	80		
60	0	0	0	0	80	78		
90	0	0	0	0	80	78		
120	0	0	0	0	84	75	3.31	2.37

TABLE 2

Starting Conditions	Aerobic [UBA(C)]	Anaerobic [MRS
Yeast as collected (Lager H) Tank 202	0	0
Bacteria mixture	TNTC	TNTC
Yeast plus bacteria	TNTC	115

Contact time (min)	Concentration of chlorine dioxide (ppm)				Viab	(%)		
	100		250			250	100	250
	Aer	Anaer	Aer	Anaer				
0	TNTC	115	TNTC	115	93	93	4.47	4.47
15	0	0	0	0	80	80		
30	0	0	0	0	75	78		
45	0	0	0	0	83	80		
60	0	0	0	0	80	78		
90	0	0	0	0	80	78		
120	0	0	0	0	84	75	3.31	2.37

The experiment was repeated under a more realistic initial bacterial load in experiment 2.

Both aerobic and anaerobic bacteria were eliminated after the first 15mins at 100ppm

TABL

	Anaerobic [MRS(C)]	Viability (%)
Yea	0	93
	TNTC	
	115	

Yeast viability was reduced from 93% to 80% in the first 15 minutes.

Yeast viability did not decrease any further after the first 15minutes.

Chlorine dioxide presumably eliminates any weak yeast cells. Any remaining yeast cells resist being killed by further exposure to chlorine dioxide.

C				naer	Viability (%)		pH	
					100	250	100	250
0	TNTC	115	TNTC	115	93	93	4.47	4.47
15	0	0	0	0	80	80		
30	0	0	0	0	75	78		
45	0	0	0	0	83	80		
60	0	0	0	0	80	78		
90	0	0	0	0	80	78		
120	0	0	0	0	84	75	3.31	2.37

Conclusions

- Chlorine dioxide at 100ppm appears to be specific in eliminating anaerobic and aerobic bacteria from yeast
- The viability of the yeast does not continue to decrease with time after a small drop in the first 15 minutes. This indicates that the yeast can survive the action of chlorine dioxide
- The process of washing recovered yeast with chlorine dioxide takes considerable less time, 15 - 30 minutes, than the classical acid wash or acid-persulfate wash at 2 or more hours
- More work is required to determine the minimum effective concentration of chlorine dioxide between 25ppm and 100ppm
- Based on these observations the washing of yeast with chlorine dioxide merits further investigation to work out the minimum concentration of chlorine dioxide and the details of carrying it out in *a practical and safe manner*

References

- [1] Priest F. G. and Stewart G., Handbook of Brewing (2006) 2nd ed., pp. 318-320. CRC Press Taylor and Francis Group
- [2] Bah, S. and Mckeen W. E., (1965) Beer spoilage bacteria and their control with phosphoric acid – ammonium persulphate wash. Canadian Journal of Microbiology, 11(2):309-318
- [3] Masschelein, W. J. (1979) In: Chlorine Dioxide: Chemistry and Environmental Impact of Oxychlorine Compounds pp.153-163. Ann Arbor science Publishers, Ann Arbor, MI.
- [4] Johnson D., (1998). Coming Clean: A new method of washing yeast with chlorine dioxide, The New Brewer, Sept/Oct.
- [5] McCaig, R. (1990), Evaluation of the fluorescent dye 1-anilino-8-naphthalene sulfonic acid for yeast viability determination. J. Am. Soc. Brew. Chem. 48 pp.22-25

References continued

- [6] Hind, H. L., (1937) Brewing Science and Practice, Volume 2, Brewing Process. 4th Ed. Chapman & Hall. London. pp. 803-4.
- [7] Goldammer, T., (2008) The Brewer's Handbook. Apex Publishers. pp. 82-4
- [8] Briggs, D. E., Boulton, C. A, et al, (2004) Brewing Science and Practice, Woodhead Publishing Limited. pp. 36-7.
- [9] Hardwick, W. A., (1995) Handbook of Brewing. Marcel Dekker Inc. pp. 193-4
- [10] McCabe, J. T., et al. (1999) The Practical Brewer 3rd Ed., Master Brewers of the Americas. pp. 292-3.

THANK YOU

Sealed Air
Re-imagine™

SLEEMAN
EST. 1834
BREWERIES LTD.