

### The problem

*Fusarium* moulds attack barley and other grains already on the field and they can further expand during (wrong) storage, steeping and germination.

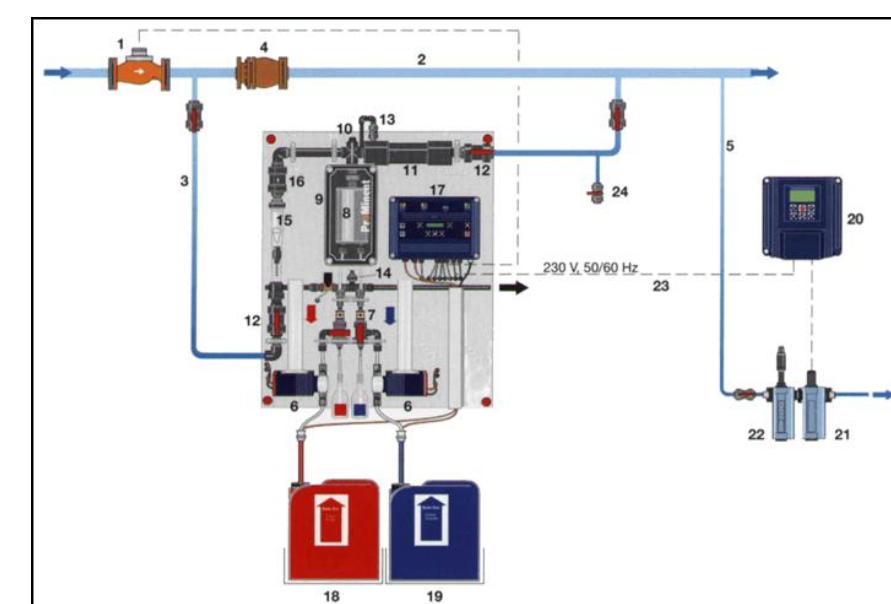


- During barley germination: can disturb the process by taking nutrition away from the germ and Mycotoxins produced by *Fusarium* are toxic for the germ.
- During brewing (fermentation): Mycotoxins produced by *Fusarium* are toxic for the yeast leading to fermentation failures.
- Impact on the quality
  - Malt:
    - Source of exogenic (external) enzymes causing undesired changes.
    - Formation of so called red grains.
  - Beer:
    - Deviations in taste and color.
    - Proteins produced by *Fusarium* contribute to gushing.
    - Some mycotoxins produced by *Fusarium* are cancerogenic.

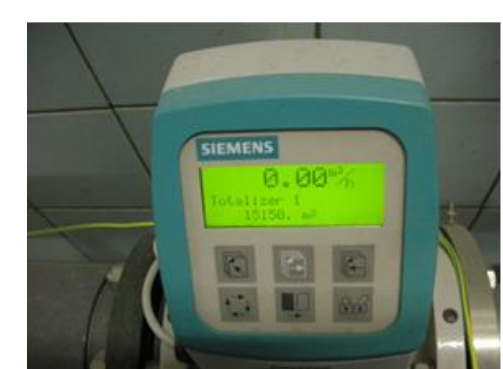
### The concept

#### Push back *Fusarium* by Chlorine Dioxide (ClO<sub>2</sub>).

- Water treatment with ClO<sub>2</sub> during steeping and / or germination.
- Project of Sealed Air and Research Institute for Brewing and Malting in Prague
- Field trials conducted on a malting site in Czech republic
- Under normal conditions ClO<sub>2</sub> is a gas; most commonly produced on-site by mutual reaction of hydrochloric acid and sodium chlorite using special generators:



- ProMinent generator with output 2kg/h.
- ClO<sub>2</sub> dosing controlled by flow meter.
- Connected with the system via telemetry



### Key Performance Indicators (KPI's)

- Microbiological analysis of barley and malt (Counts of *Fusarium* colonies)
- Number of red grains (determined during industrial trials only)
- Wort analysis
  - Content of *Fusarium*-specific toxins (DON, DON 3 glykosid)
  - Residual ClO<sub>2</sub> in the green malt

### Laboratory and pilot plant tests

Addition of 10 ppm ClO<sub>2</sub> into steeping water

Concept confirmation:

- a) 1 g barley in test tube
  - Reduction of *Fusarium* colonies
  - Reduction of toxins
  - No ClO<sub>2</sub> residues in the malt
  - No deviations in wort quality
- b) Micro malting pilot plant (4 x 0,5 kg barley in 11 L water)



Table 1: *Fusarium* counts on barley (average from duplicates) during steeping treatment with tap water (blank) or chlorine dioxide.

Step	Blank (log CFU/g)	ClO <sub>2</sub> (log CFU/g)	Log reduction vs. Blank	Reduction vs. Blank (%)
start (prior to 1 steeping)	4.88	--	--	--
after 1st steeping (16 hours)	5.99	--	--	--
after 2nd steeping (4 hours)	4.84	3.66	1.18	93.4
after 2nd aeration (20 hours)	4.80	3.64	1.16	93.1
after 3 <sup>rd</sup> steeping	4.62	3.50	1.12	92.6

### Field trials (Steeping step)

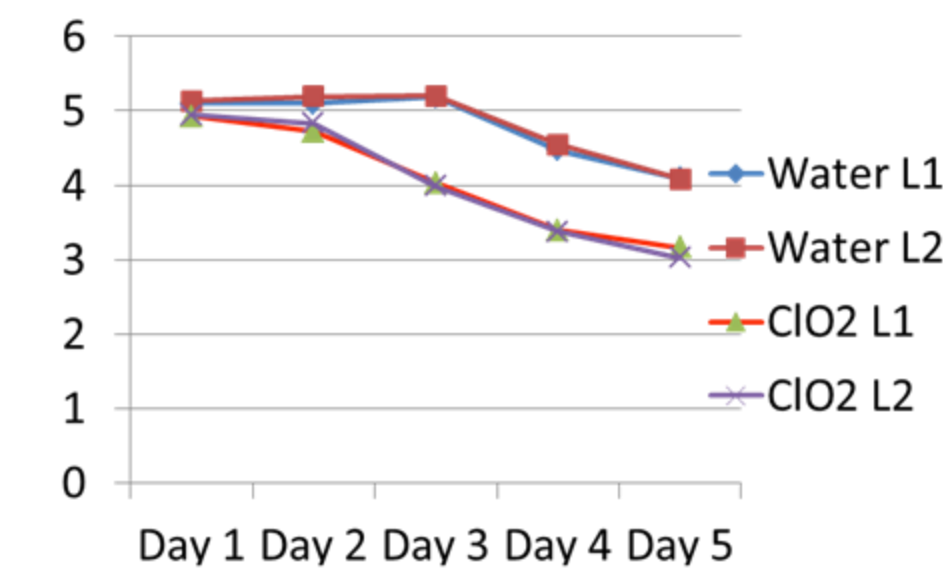
- 56MT barley and 56m<sup>3</sup> water treated with ~10 ppm ClO<sub>2</sub> in a steeping tank
- Key difference compare to pilot plant: **The ratio steeping water with ClO<sub>2</sub> / barley was 1:1 while in the pilot plant it was 5.5:1.**
- The ratio of ClO<sub>2</sub> and barley is very important and it did not correspond with the conditions in the pilot plant. An increase of volume of water in the steeping tank is not possible, hence has to increase the concentration of ClO<sub>2</sub>.

*Fusarium* was reduced after steeping by (log 1-2) but increased growth during germination was observed (not shown).



### Field trials (Germination step)

- To get closer to micro plant conditions.
- To reduce *Fusarium* growth during germination.
- To reduce total cost of water treatment with ClO<sub>2</sub> compared to a treatment via steeping tank
- Use concentration: 20 ppm ClO<sub>2</sub>



Comparison of *Fusarium* sp. counts with and without ClO<sub>2</sub> on lines (y-axis is showing log cfu/g barley). Counts are averages of 5 individual samples from different place in germination boxes (standard variation was less than 5%).

Number of red grains in the samples of ready barley (200 g). (Average reduction by 62%)

Batch No.	ClO <sub>2</sub> treatment	Number of red grains
C12 L1 211012	No	35
C12 L2 211012	No	36
C12 L1 221012	Yes	16
C12 L2 221012	Yes	11

Comparison of gushing potential by pieces sprayed with and without ClO<sub>2</sub> (Average Reduction by 68%)

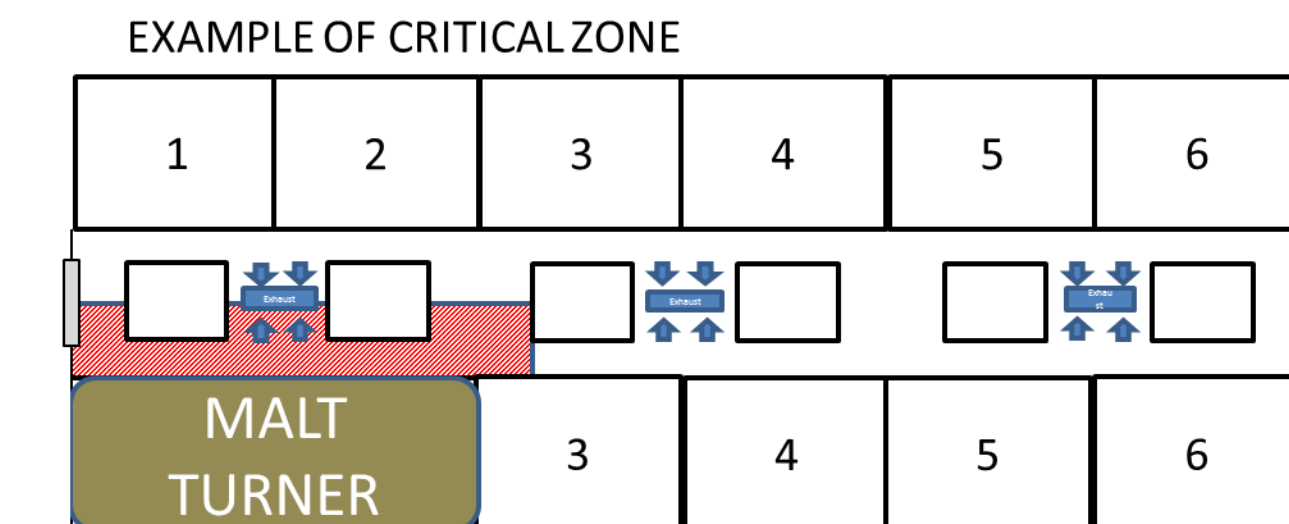
Batch No.	ClO <sub>2</sub> treatment	Gushing (ml)
C12 L1 211012	No	24
C12 L2 211012	No	26
C12 L1 221012	Yes	11
C12 L2 221012	Yes	5

Wort Analysis: Shows no deviation from normal values

Sample code	Sample No.			
	C12 L1 211012	C12 L2 211012	C12 L1 221012	C12 L2 221012
Type of water spraying	No	No	Yes	Yes
Moisture content of malt (%)	3,6	4,5	4,2	4,1
Extract of malt (%)	79,9	79,8	80,1	80,0
Mash method according to Hartong and Kretschmer VZ 45 °C (%)	47,1	45,7	47,5	48,2
Kolbach index (%)	40,8	40,9	41,7	41,2
Diastatic power (jWK)	294	368	377	381
Final attenuation of laboratory wort from malt (%)	79,2	79,5	79,6	79,8
Friability (%)	100	98	99	98
β - Glucan content of malt (mg/l)	25	30	31	21
Protein content of malt (%)	12,6	12,8	12,8	12,9
Total nitrogen of malt (%)	2,01	2,04	2,05	2,07
Soluble nitrogen of malt (mg/l)	819	835	857	852
Soluble nitrogen of malt (%)	0,82	0,83	0,86	0,85
Saccharide extract of malt (%)	74,8	74,6	74,7	74,7

### Operators Safety Studies (Germination zone)

- Chlorine Dioxide concentration in the air was measured with GasAlert Extreme GAXT-V-DL (BW Technologies)



- Maximum ClO<sub>2</sub> level in air directly at nozzles was 0.9 ppm, but in general it drops down rapidly with distance as well as with time (after switching the water spray off):
  - 30sec after finishing the spray the concentration dropped to 0.06 ppm (measure at nozzle outlet)
  - 60 sec after finishing the spray < 0.03 ppm (measure at nozzle outlet)
- Safety rules were set for operators working in germination area during ClO<sub>2</sub> application.

Move the malt turner in the right position. Switch on malt turning and water spraying. Go to the nearest air exhaust area where the ClO<sub>2</sub> concentration is already below hygienic limit. Follow the operation from there.

Put on gas mask if you have to go to the turner while it is running or to switch off water supply after the operation is finished.

Wait 2 minutes until ClO<sub>2</sub> concentration drops below hygienic limit and finish the turning process (equalizing of the pile).

IMPORTANT: If the turning runs simultaneously on both lines (L1 and L2) use gas mask all the time while staying in the germination department. Same rule applies for using hose for water spraying.

A gas mask is also required when a worker is present at the boxes that are being sprayed. Specifically, a gas mask is required when a worker is present:

### Conclusions

- The presented concept was proven as a contributor to *Fusarium*, gushing and red grain reduction.
- It does not, however, solve the issues completely.
- No impact on the quality of the produced malt was found (no residues of ClO<sub>2</sub> and/or by-products).
- No deviations in the key parameters of the wort.
- ClO<sub>2</sub> approved as an auxiliary material (processing aid EC 1333/2008) by CZ for this process.

- The following limitations must be always taken into consideration:

- Material compatibility.
- Processes type of grains.
- Worker's safety.
- Compatibility with local legislation.
- Compatibility with end-customer requirements (e.g. no usage of auxiliary materials).