IDENTIFICATION AND ELIMINATION OF GERMINATION INHIBITORS FOR THE REUSE OF STEEP-OUT WATERS

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SCOPE

- INTRODUCTION
- LITERATURE SEARCH
  - GERMINATION INHIBITORS
  - WASTEWATER TREATMENT
- EXPERIMENTAL PROTOCOL
- RESULTS
  - STEEP-OUT (Steep Effluent) WATER COMPOSITION
  - STEEP-OUT WATER RECYCLING TESTS
  - PILOT SCALE TREATMENTS
  - CHARACTERISATION OF INHIBITION
- CONCLUSIONS AND PERSPECTIVES

INTRODUCTION

- Lack of water in the world
- Need for a better management of water resources
- Malt Industries affected by this question
  - France = 1st malt exporting country
  - French and British malting industries: 22 Mm^3/year of wastewater.

Main Malting process steps: **Steeping** Ÿ Germination Ÿ Kilning

Most important water consumption and effluent generation stage

OBJECTIVES

Å Minimisation of water consumption and wastewater reject, through water reuse :

- Investigations on steep-out water composition
- Study of the germination inhibition phenomenon
- Set up of a treatment process

GERMINATION INHIBITION

- Phenolic compounds inhibit ATP synthesis ⇒ slower cellular activities
- Tannins: complex with gibberellins ⇒ loss of gibberellin activity
- Short chain fatty acids
- Mycotoxins and pesticides (glyphosate) are germination inhibitors.
- Husks: contain inhibiting substances
- Glucose: affects osmotic pressure decreasing water uptake- similar role for glycerol and salts (increase ionic strength)
- Microflora: compete with grain tissues for oxygen
- Hydrogen cyanides, ammonia, ethylene, mustard oils, organic acids, unsaturated lactones, aldehydes, essential oils (antibiotic properties, permeability decrease) alkaloids
- Other parameters known to be responsible for dormancy: light, UV, temperature, storage conditions.

GERMINATION INHIBITION

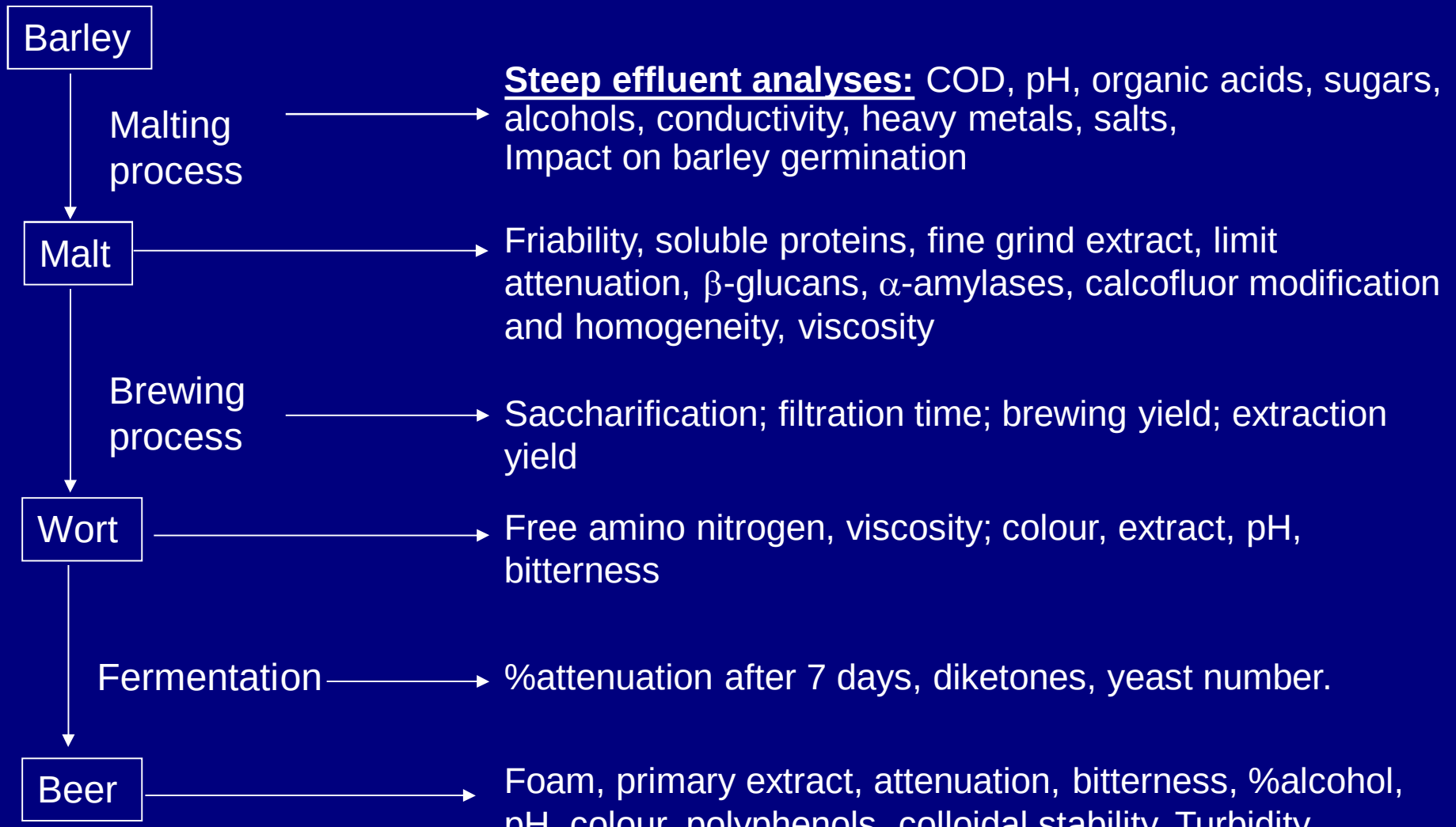
IN BRIEF:

- Many substances and phenomena inhibit seed germination.
  - Mechanisms involved are not easily explained.
  - Mechanisms and the molecular identity non-specific
- Major compounds already identified in barley steep ï out water:
  - pesticides, mycotoxins, phenolic compounds, microflora, organic acids, sugars, metals, anions and cations
  - Inhibition essentially in outer grain layers; chemical rather than physical.
- Project
  - study water composition to characterise inhibition;
  - develop an appropriate system to eliminate it.

WASTEWATER TREATMENT

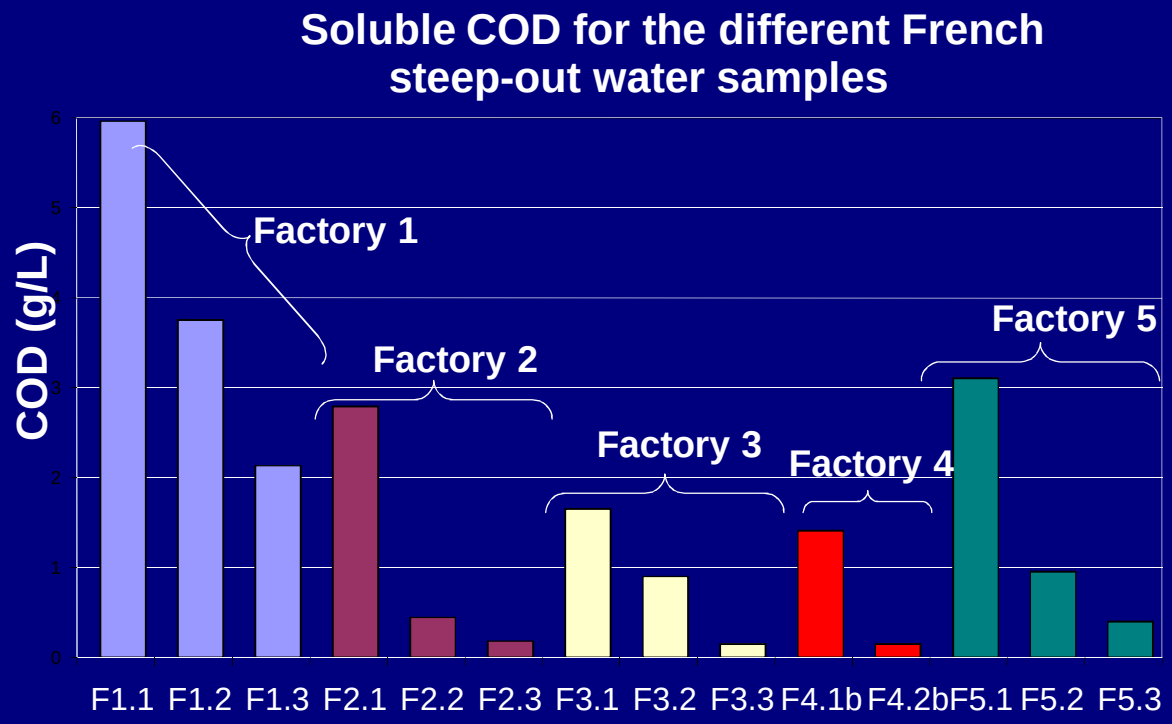
- **PHYSICO-CHEMICAL TREATMENTS**
  - Coagulation ï Flocculation; Sedimentation; Membrane filtration.
- **BIOLOGICAL TREATMENT**
  - Biodegradation of organic substances (sugars, organic acids, proteins)
- **MEMBRANE BIOREACTOR TREATMENT**
  - Biological treatment + membrane filtration; Compact system; Higher biomass level
  - MF, UF, NF, RO (high cost), according to water quality to reach.
- **OTHER TREATMENTS**
  - Activated carbon: Low cost; Colour removal; Adsorption of heavy metals.
- European Directive (98/83/CE): different potability parameters to control.
- Schildbach (2005): 44% recycling quota of MBR-treated water.

EXPERIMENTAL PROTOCOL



CHARACTERISATION OF STEEP-OUT WATERS

- Industrial steep-out waters:
  - Different process parameters;
  - Several barley cultivars.
- Water analyses: COD, conductivity, pH, microorganisms, organic substances (sugars, alcohols, organic acids)



Impact of these waters on barley germination: Conventional Germination tests.

CHARACTERISATION OF STEEP-OUT WATERS

Inhibition rates *(I.R)* calculated compared with reference (distilled water):

Different durations (24h, 48h, 72h) and 2 water volumes (4ml and 8 ml):

$$IR_{24h} = \frac{N_{(24h,\ ref)} - N_{(24h,\ sample)}}{N_{(24h,\ ref)}} \times 100$$

4ml tests

		IR_{24h}	IR_{48h}	IR_{72h}	IR on Germination index
Unfiltered	**min**	**4%**	**3%**	**1%**	**2%**
	max	**36%**	**12%**	**7%**	**23%**
Filtered	**min**	**0%**	**0%**	**0%**	**0%**
	max	**17%**	**9%**	**7%**	**16%**

8ml tests

		IR_{24h}	IR_{48h}	IR_{72h}	IR on Germination index
Unfiltered	**min**	**3%**	**7%**	**5%**	**0%**
	max	**71%**	**54%**	**48%**	**30%**

QUANTIFICATION OF INHIBITION

REPEATABILITY TEST

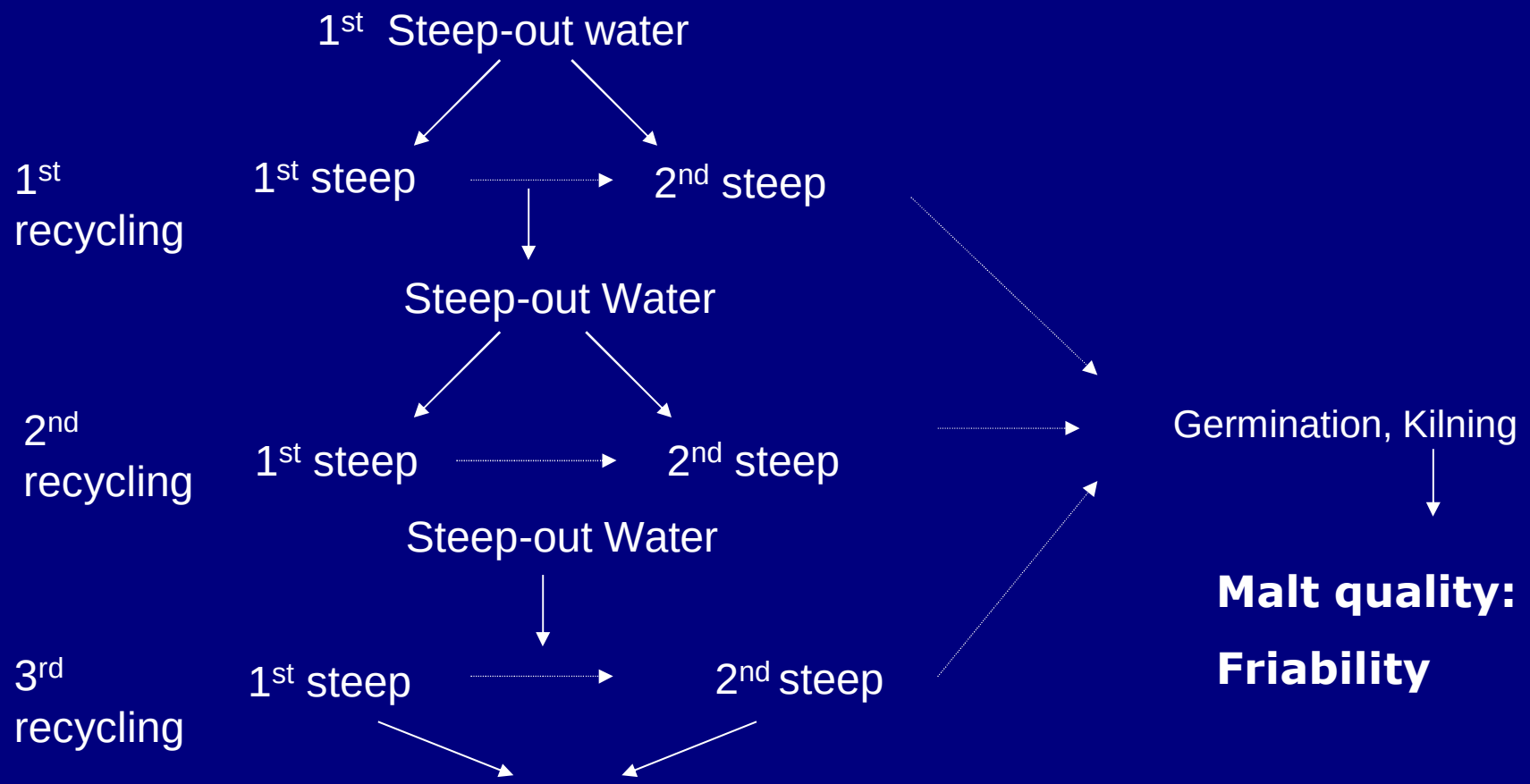
10 simultaneous germination tests are carried out in 8ml distilled water. Germinated seeds counts after 24h.

Sample	1	2	3	4	5	6	7	8	9	10
% germination	94	92	94	92	91	94	91	93	92	94
Mean: 92.7			SD: 1.25				VC: 1.35%			

10 simultaneous germination tests were carried out in 8ml of **IFBM steep-out water**. Germinated seeds counts after 24h.

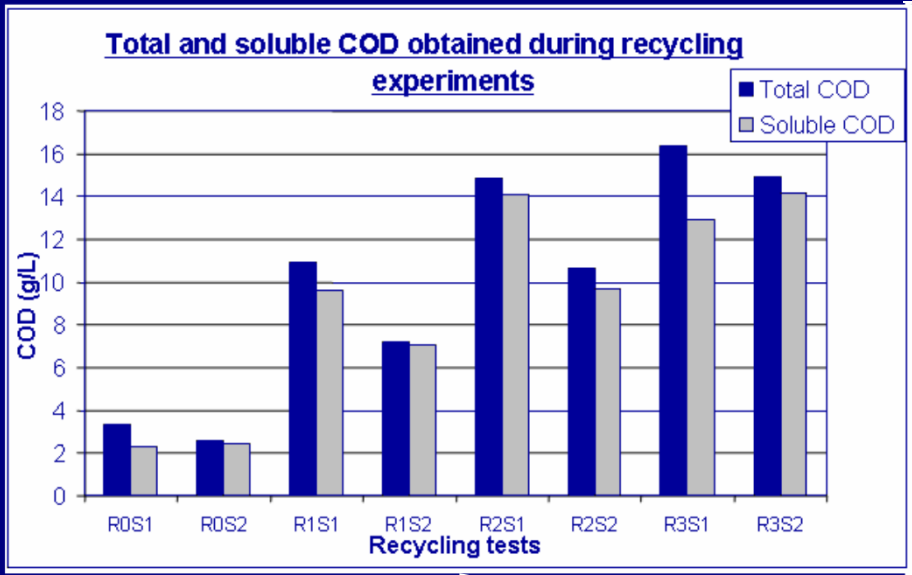
Sample	1	2	3	4	5	6	7	8	9	10
% germination	52	53	54	51	52	51	53	54	52	52
Mean: 52.4			SD: 1.02				VC: 1.95%			

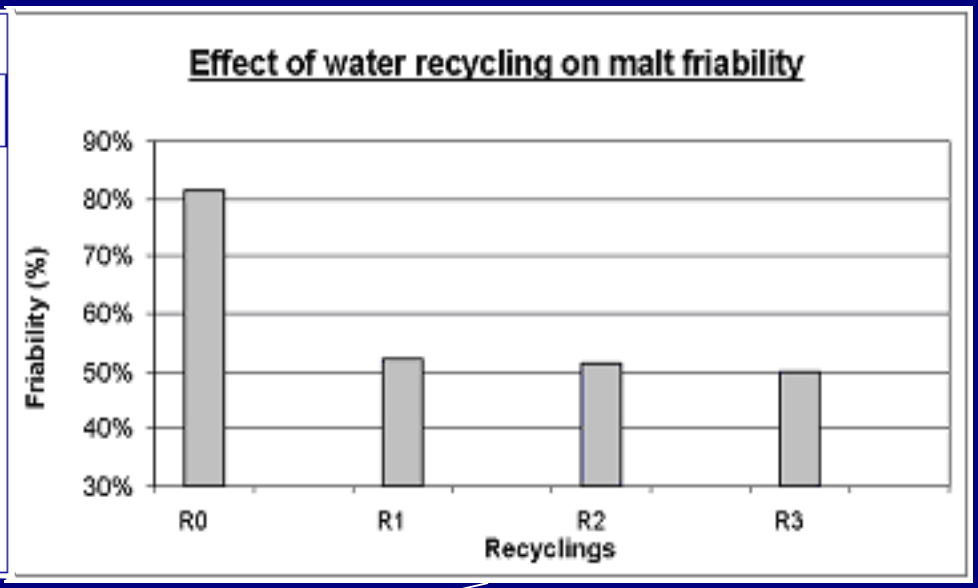
RECYCLING STRATEGY



Water analyses:
Total and soluble COD; bacteria and yeast contents; Inhibitory potential.

RECYCLING TESTS





Degradation of the quality of water used for steeping

⇒ Germination delay

⇒ Negative impact on malt quality.

🔔 Germination delay cannot be made up for after steeping.

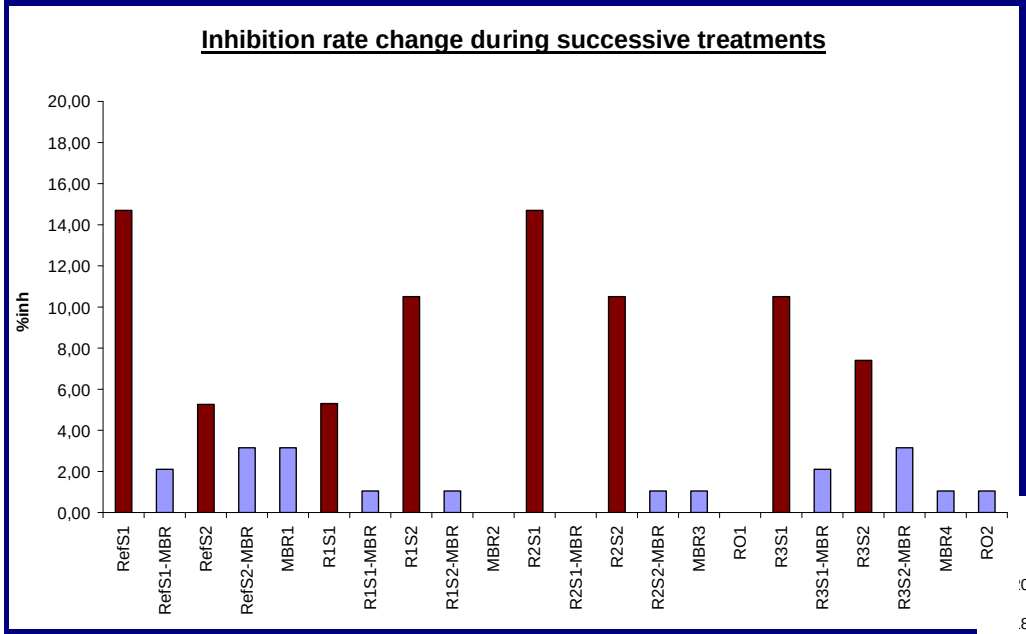
🔔 Presence of an inhibition due to steep-out water

PRESENTATION OF THE PILOT SCALE TREATMENT SYSTEMS

	Sys1	Sys2
Membranes	Submerged	External
Sludge concentration (g/L)	6-7	6-10
Sludge Volume (m3)	3	2
Temperature (°C)	24-26	24-38
Draining	No	Yes
Nutrient addition	Yes	No
membrane pore size (cut-off)	0,1-0,2 µm	0,02 µm
Membranes cleaning	At the end of trials	After each trial

1 reference test	1 reference test
2 MBR recycling tests	2 MBR recycling tests
2 MBR+RO recycling tests	2 MBR+RO recycling tests
	1 MBR+GAC recycling test

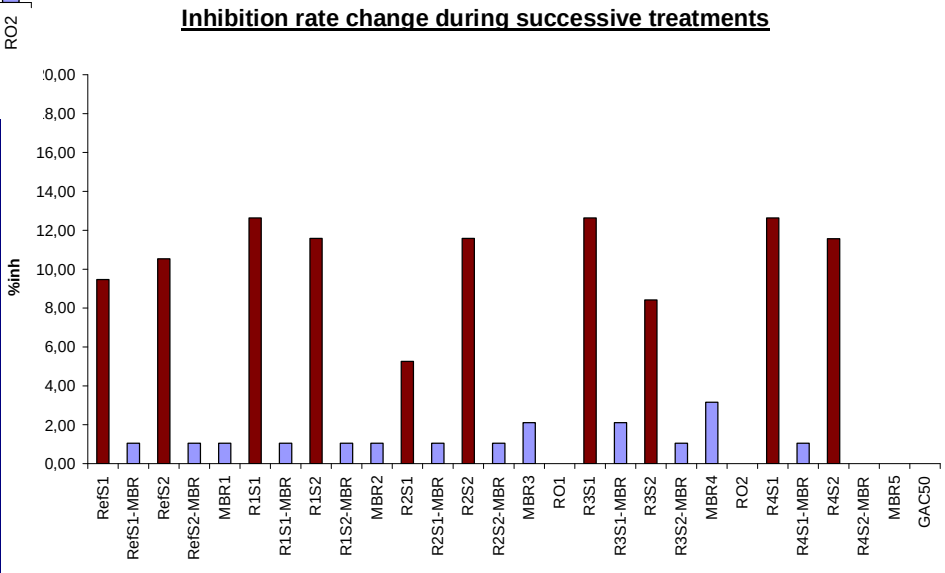
INHIBITION REMOVAL



Steep-out waters
Treated waters

Sys2 external UF membranes

Sys1 submerged MF membranes



WATER QUALITY

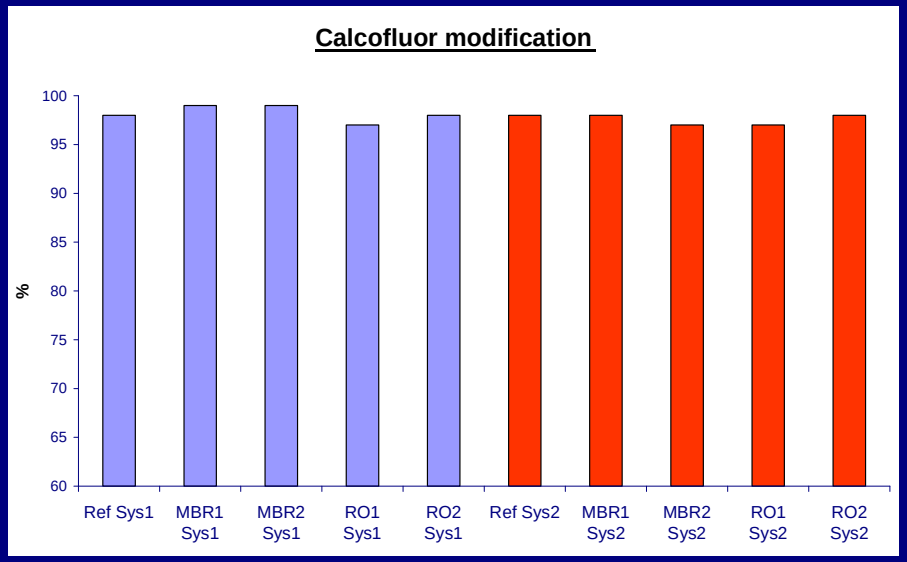
- **Conductivity**: < authorized limit (1 mS/cm) only with **GAC** and **RO**.
- **COD**: < authorised limit only with **GAC** and **RO** (30 mg/L).
- **pH**: authorised limits respected, except for **GAC (pH > 9).**
- **Phosphorus**: Only **RO** purifies to authorised limits (0.4mg/L).
- **Heavy metals**: In MBR treated waters: Legal limits achieved for all metals, except **Fe and Mn**; In **RO** and **GAC** treated waters: all elements are < legal limits.

- **There was minimal difference between the two systems for MBR treated water quality;**
- **These differences may be due to the membrane pore size difference and the functioning conditions.**

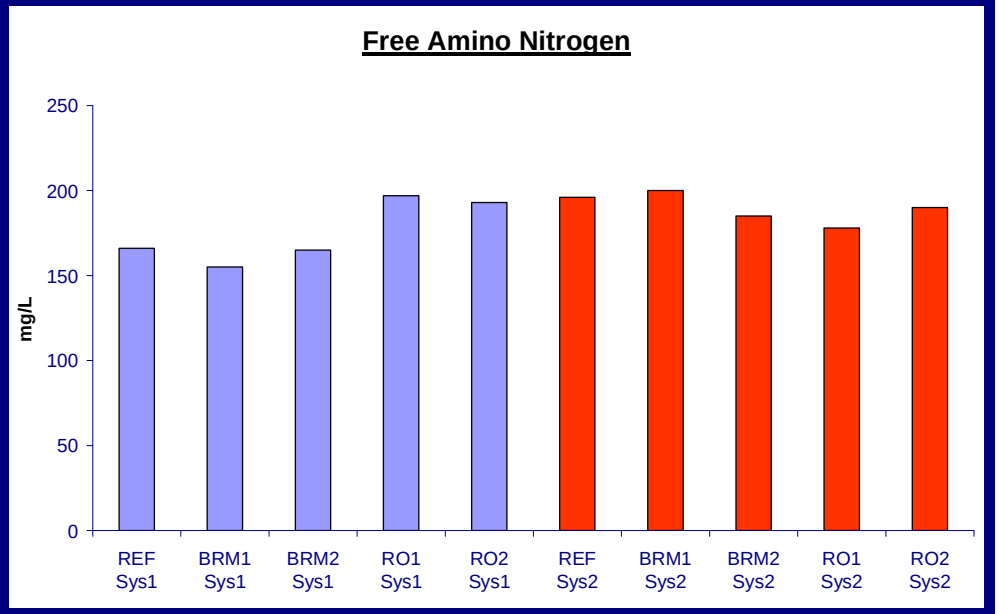
ELIMINATION OF PESTICIDES AND MYCOTOXINS

- Pilot malting with barley naturally contaminated with T2-HT2 toxins;
- Pesticides addition in the steep effluent water (pesticides found in the characterisation of steep effluent);
  - Study of the elimination of pesticides with the *Sys1* MBR:
    - Chlormequat chloride: 80% of elimination in treated water;
    - Azoxystrobin: 50,5% of elimination in treated water;
    - Fenpropimorph: 99,5% of elimination in treated water;
  - Mycotoxins elimination with *Sys1*:
    - 98% of elimination for T2 toxin;
    - 37% of elimination for HT2 toxin.

MALT AND WORT QUALITIES



Sys1 **Sys2**



BREWING AND FERMENTATION PROCESS

	Ref Sys2	MBR2 -Sys1	RO2 - Sys1	MBR2 - Sys2	RO2 - Sys2
Brewing					
Saccharification (min)	***15***	***15***	***15***	***15***	***15***
Time for wort filtration + washings (min)	***155***	***155***	***155***	***157***	***153***
Time for filtration of 450 kg (min)	***42***	***41***	***42***	***42***	***40***
Brewing yield (%)	***73,8***	***71,8***	***72,9***	***72,5***	***71,4***
Extraction yield (%)	***88,3***	***86,5***	***87,6***	***86,6***	***85,5***
Fermentation					
% attenuation after 7 days	***92,3***	***93,2***	***94,1***	***96,1***	***95,6***

Å BEER QUALITY

foam, alcohol, colour, bitterness, clarity, pH, colloidal stability.

⇒ **Malts obtained with different treated waters led to Beers of comparable qualities.**

INDUSTRIAL VALIDATION

- Water treatment results (MBR + RO)
- Malting trials with treated waters (30 tonne batch size)
- Brewing and fermentation trials

Were arried out using the same tretament methodology as pilot scale trials, with external membrane bioreactor

CONCLUSION: Results comparable with pilot scale trials

CHARACTERISATION OF INHIBITION

- EFFECT OF CULTIVARS
  - IFBM steeping process
  - Different Spring barley cultivars
  - Inhibition tests carried out with the resulting steep-out waters

	Inhibition rates	
cultivar	**Unfiltered**	**Filtered**
V1	**25%**	**6%**
V2	**52%**	**17%**
V3	**50%**	**8%**
V4	**56%**	**25%**
V5	**49%**	**25%**
V6	**52%**	**15%**
V7	**46%**	**13%**

Different grain compositions ⇒ Different inhibitory potentials.

CHARACTERISATION OF INHIBITION

Å FRACTIONATION TESTS

Objective: Study the impact of separate molecular fractions on barley germination.

	10 KDa	3 KDa	1 KDa	500 Da
Steep-out water	40%	29%	34%	30%
Filtrate	14%	11%	19%	2.7%
« washed » retentate	20%	10%	28%	38%

Colourless fraction

Inhibition rates calculated for each fraction

⇒ **Coloured molecules** of apparent molecular weight **> 500 Da** are implicated in the inhibition phenomenon.

CHARACTERISATION OF INHIBITION

Å INVOLVEMENT OF HUSKS

Barley husks contain important amounts of phenolic compounds.

Barley cultivar	Steep-out water	% germinated grains	% inhibition
Reference test with *Scarlett* cultivar	Distilled water	93	-
Reference test with *Naked barley* cultivar	Distilled water	94	-
Scarlett barley cultivar	*Scarlett* Steep-out water	41	55
	Naked barley Steep-out water	44	52
Naked barley cultivar	*Scarlett* Steep-out water	89	5
	Naked barley Steep-out water	90	4

Inhibition acts on barley grain husks

CONCLUSIONS

- Germination inhibitors were characterised.
- Efficient elimination of inhibition through treating steep-out water
- Production of potable water only possible through coupling the MBR treatment system with reverse osmosis
- Recycling of treated water into the malting process produced satisfactory malt, wort and beer physico-chemical and sensory analyses