Characterization and identification of wine yeasts with ability to use Acetic Acid

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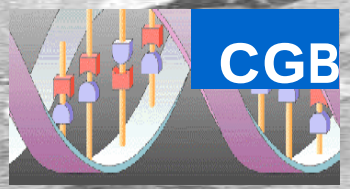


An enological problem

- **Acetic acid is the main component of volatile acidity, and critical for wine quality;**
- **Above a certain limit (0.8 g/l), acetic acid has a detrimental organoleptical effect (acid wine);**
- **This acid is mainly produced by bacterial spoilage and *Botrytis cinerea* infections of grapes; also formed by yeasts during alcoholic fermentation.**

Available solutions?

- **Distillation**
- **Stabilization followed by mixture with other wines**
- **Empirical enological practice called “remostagem”procedure**

The “remostagem”procedure

- **The acidic wine (1/3) is mixed with freshly crushed grapes or musts (2/3);**
- **The volatile acidity of this mixture should not exceed 0.6 g/l;**
- **Spontaneous fermentation (indigenous yeast species) reduce volatile acidity;**
- **The volatile acidity of the newly made wine rarely exceeds 0.3 g/l.**

(Ribéreau-Gayon *et al*., 2000)

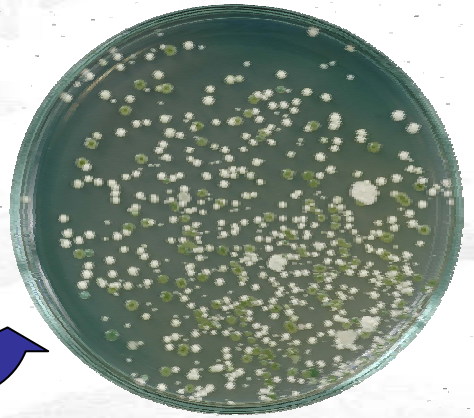
The aim of the study

- Isolate and characterize yeasts species able to reduce the acetic acid content of wines with high volatile acidity
- Develop a controlled biological deacidification procedure

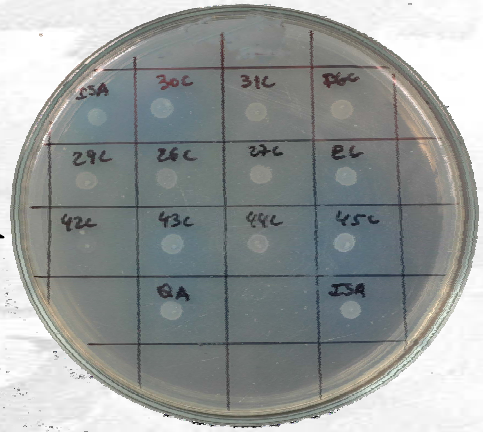
Strategy of yeast isolation and selection

“Remostagem” of a spoiled wine

Isolation of 135 isolates

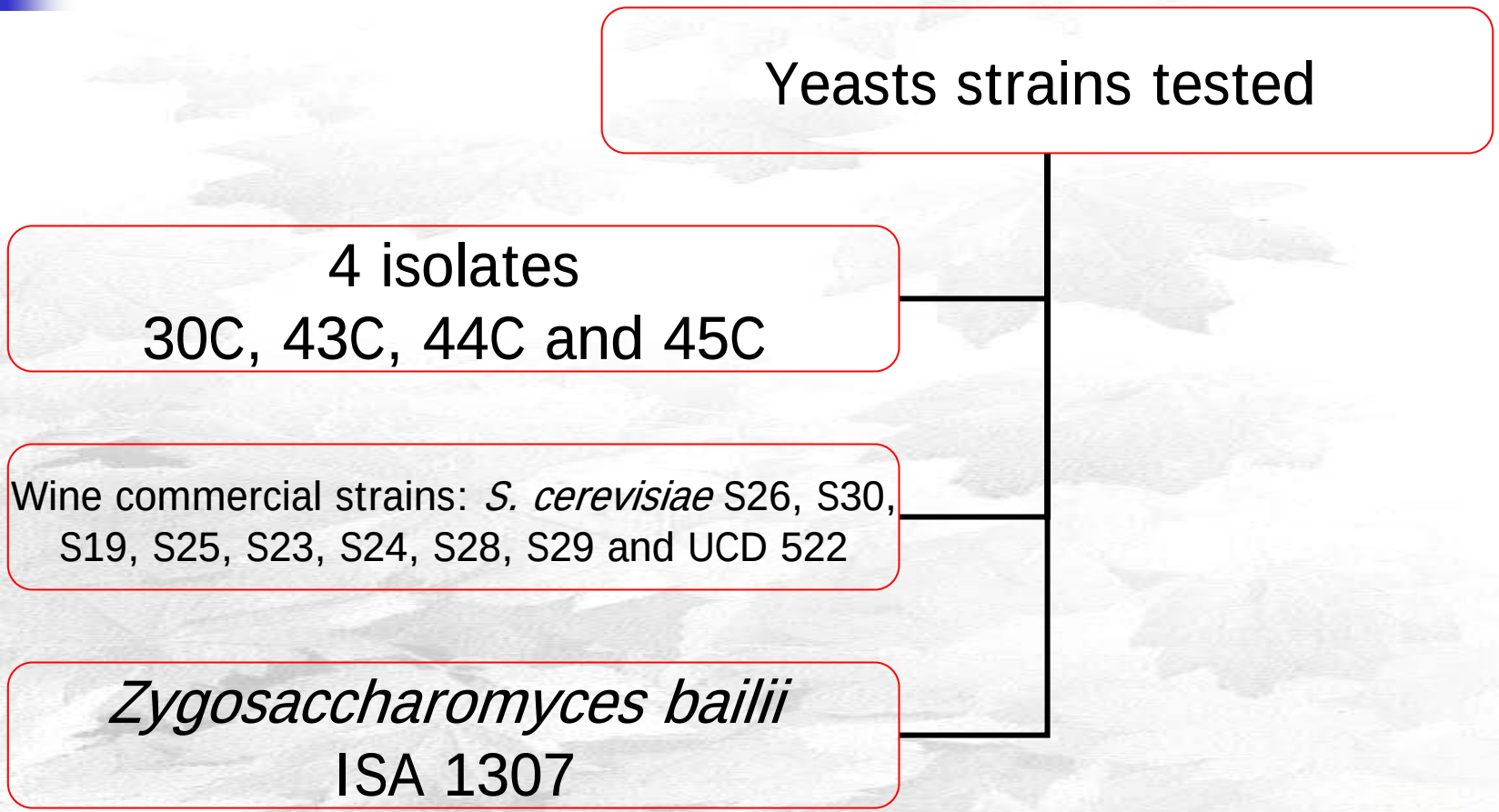


Screening of acetic acid utilization in a selective medium (Schuller, 1998)



Selected isolates
30C, 43C, 45C and 44C

Evaluation of acetic acid degradation



Evaluation of acetic acid degradation

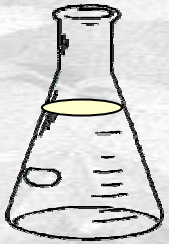
Growth conditions:

Minimal medium (MM) (Nicolau van Uden, 1967) containing acetic acid and glucose at 25ºC and pH 3.0

aerobic conditions

120 rpm

limited aerobic conditions



100 rpm

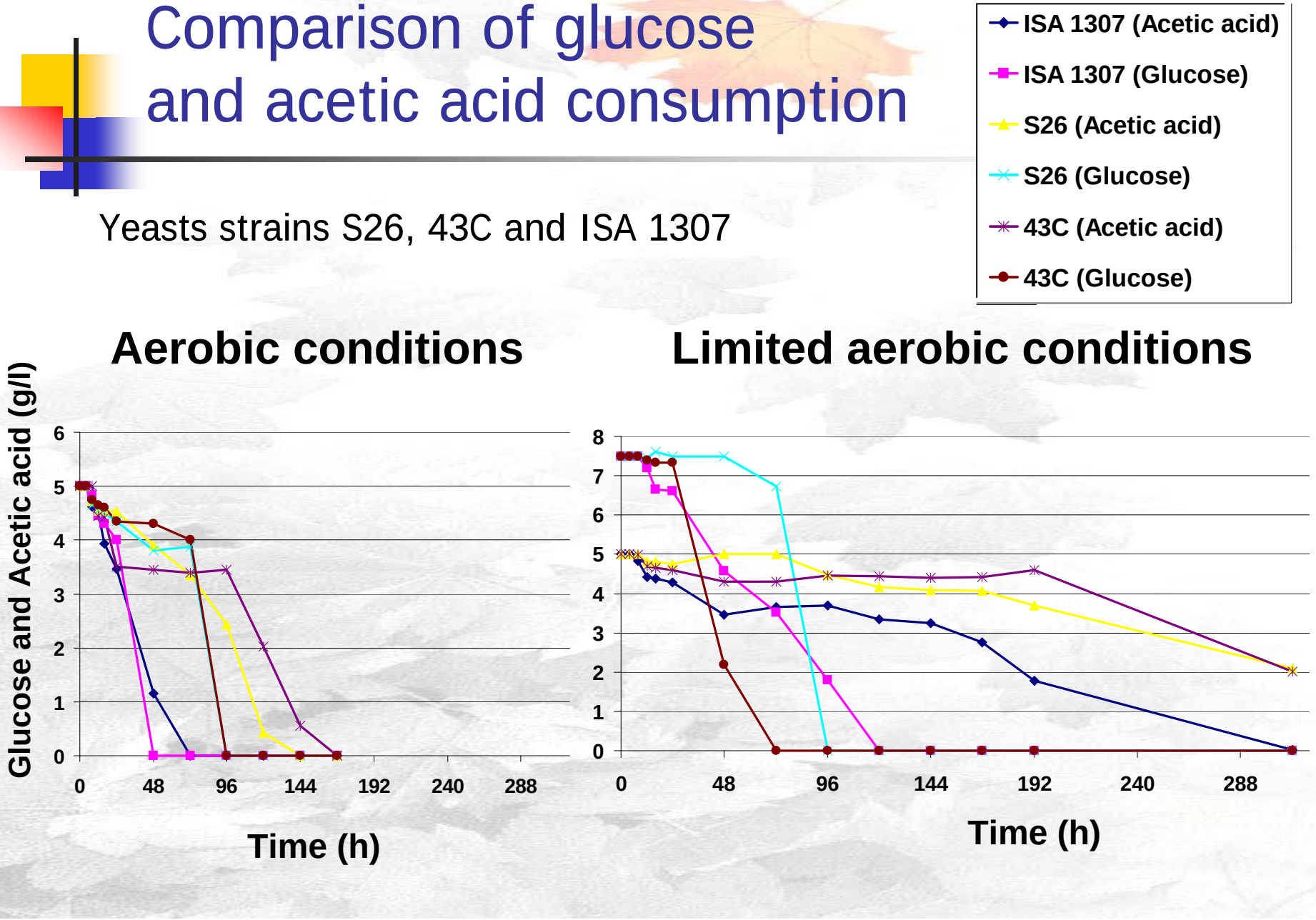
Aerobic conditions	**acetic acid (0.5% v/v) glucose (0.5% w/v)**	**Strains 30C, 43C, 44C, 45C, S26 and ISA 1307**
Limited aerobic conditions	**acetic acid (0.5% v/v) glucose (0.75% w/v)**	
	acetic acid (0.5% v/v) glucose (5% w/v)	
	acetic acid (0.25% v/v) glucose (0.75% w/v)	**Eight commercial strains**

Consumption of acetic acid, glucose and final pH values

	Aerobic conditions (216h)			Limited aerobic conditions (312 h)					
	acetic acid (0.5% v/v) glucose (0.5% w/v)			acetic acid (0.5% v/v) glucose (0.75% w/v)			acetic acid (0.5% v/v) glucose (5% w/v)		
Yeasts strains	Gluc. (g/l)	A. acid (g/l)	pH	Gluc. (g/l)	A. acid (g/l)	pH	Gluc. (g/l)	A. acid (g/l)	pH
ISA 1307	0	0 (72h)	2.60	0	0.02	2.61	0	1.92	2.58
S26	0	0 (144h)	2.65	0	2.09	2.86	0	4.41	2.70
43C	0	0 (168h)	2.72	0	2.02	2.78	0	4.77	2.65
45C	0	0 (168h)	2.71	0	4.01	2.81	0	4.71	2.58
30C	0	0 (192h)	2.75	0	4.40	2.82	0	4.90	2.60
44C	0	0 (216h)	2.73	0	3.99	2.83	15.11	3.59	2.60

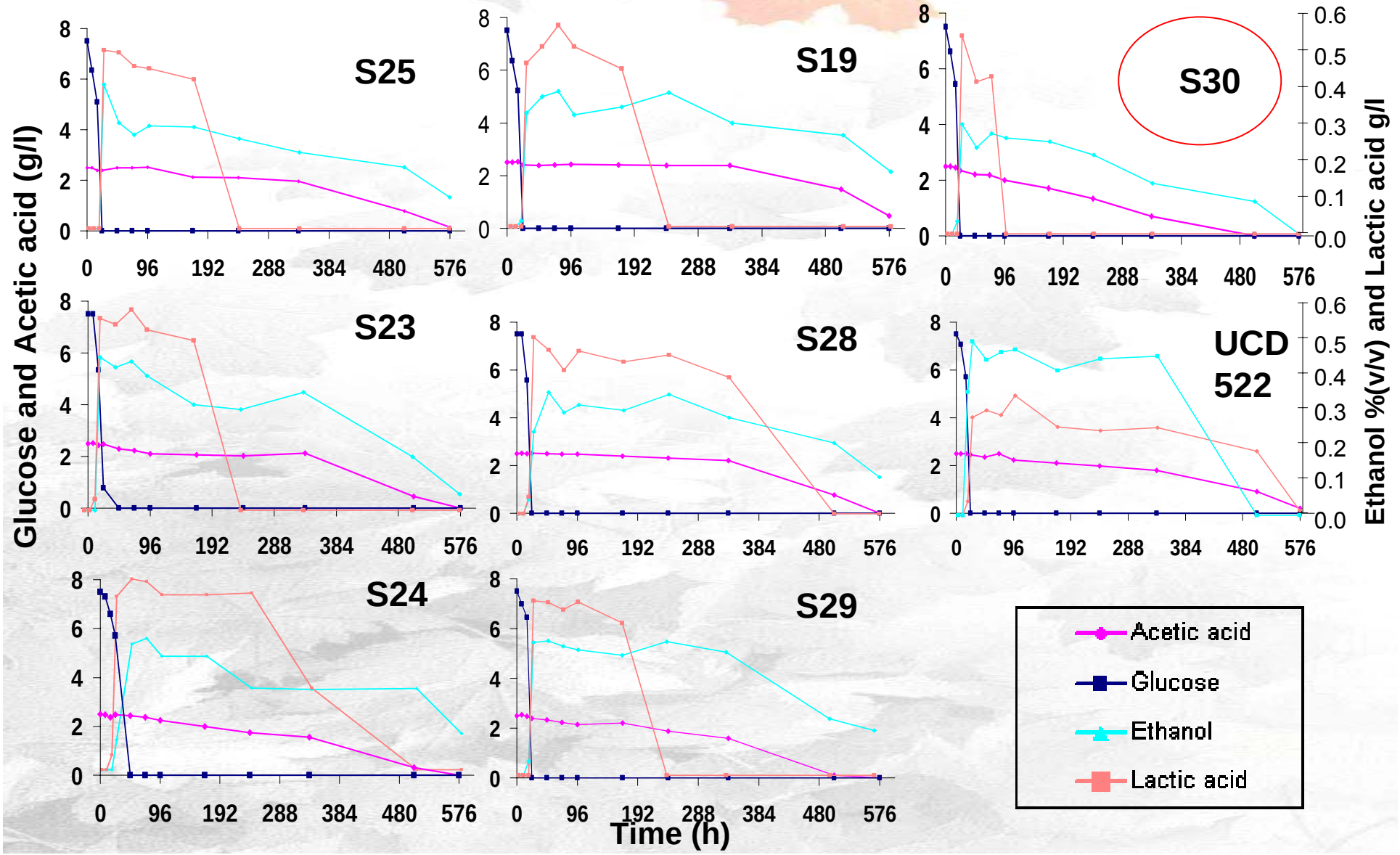
Comparison of glucose and acetic acid consumption

Yeasts strains S26, 43C and ISA 1307



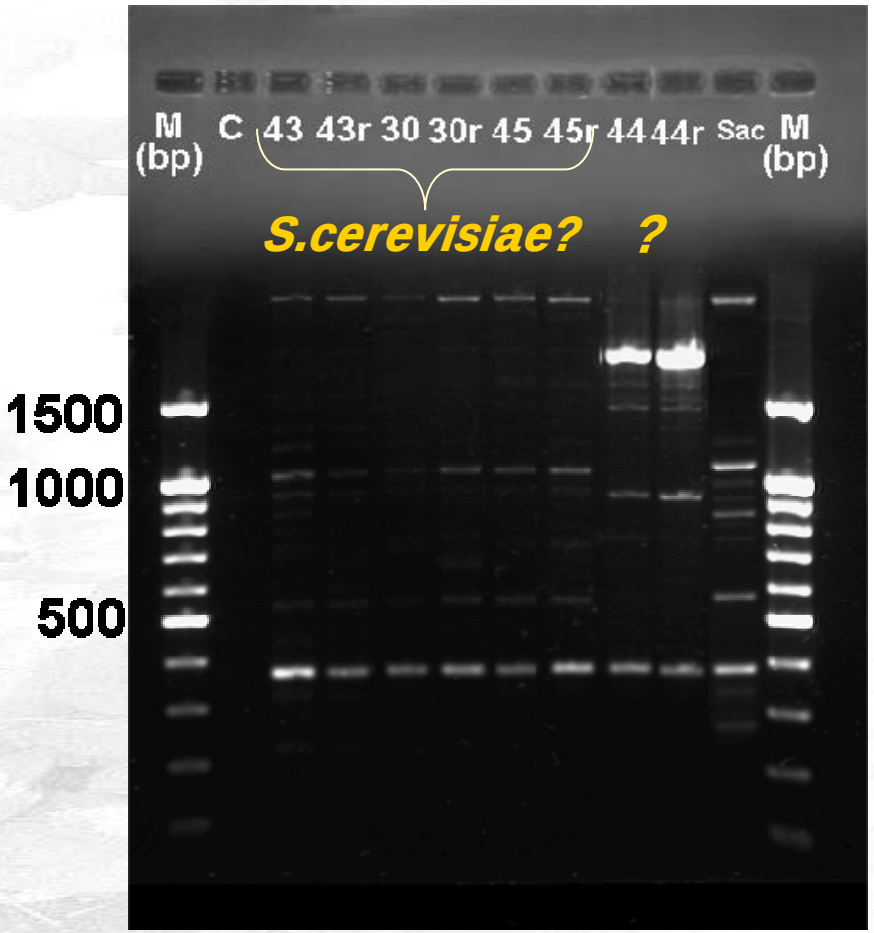
Comparison of glucose and acetic acid consumption, of eight commercial strains under limited aerobic conditions

MM + acetic acid 0.25% (v/v) and glucose 0.75% (w/v), pH 3.0 and 25ºC.



Molecular characterization of the isolated wine strains

- RAPD characterization with primer T3B
- Lane **Sac** is the strain *S. cerevisiae* PYCC 4072



Identification: D1 D2 region amplification and sequencing

D1 D2 variable domain at the 5' end of the 26S rDNA (nucleotides 63–642 for *Saccharomyces cerevisiae*) was amplified with primers NL-1 and NL-4 (O´Donnell, 1993).

GenBank BLASTN search

30C
43C } *S. cerevisiae* (99% - 100% identity)
45C

44C *Lachancea thermotolerans* NRRL Y-8284 (99 % identity)

Simulation assays of a "remostagem" process

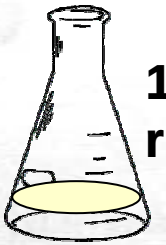
***Yeasts strains*:** 43C, 44C, 45C, S26, S30 and ISA 1307

Culture medium:
2/3 MM +
glucose (20% w/v)
1/3 acid white wine.

→ volatile acidity adjustment to 1.14 g/l acetic acid
pH 3.5
Initial ethanol concentration: 4% (v/v)

Pre-inoculum: 10 ml overnight culture

→ aerobic conditions

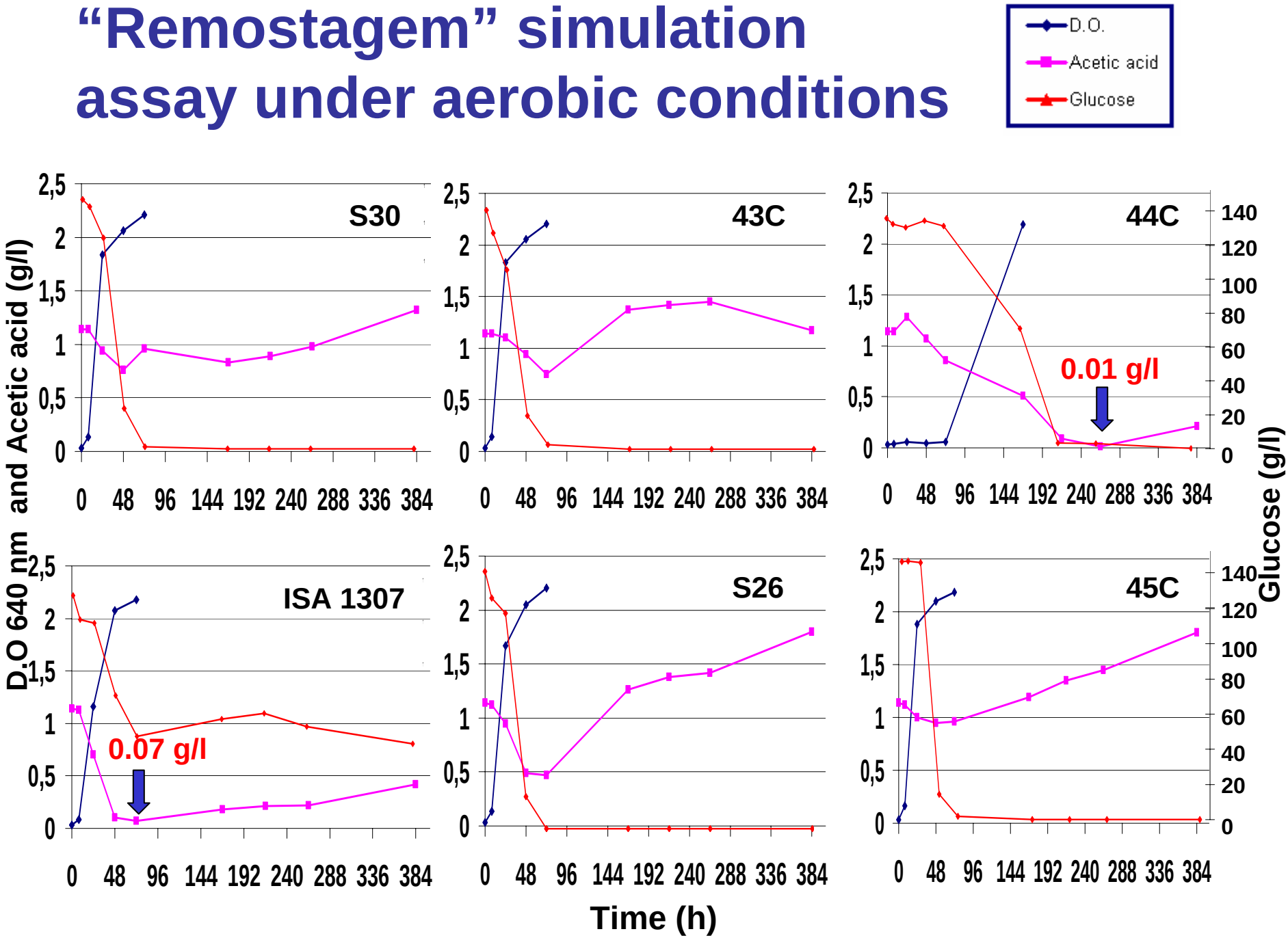


120 rpm

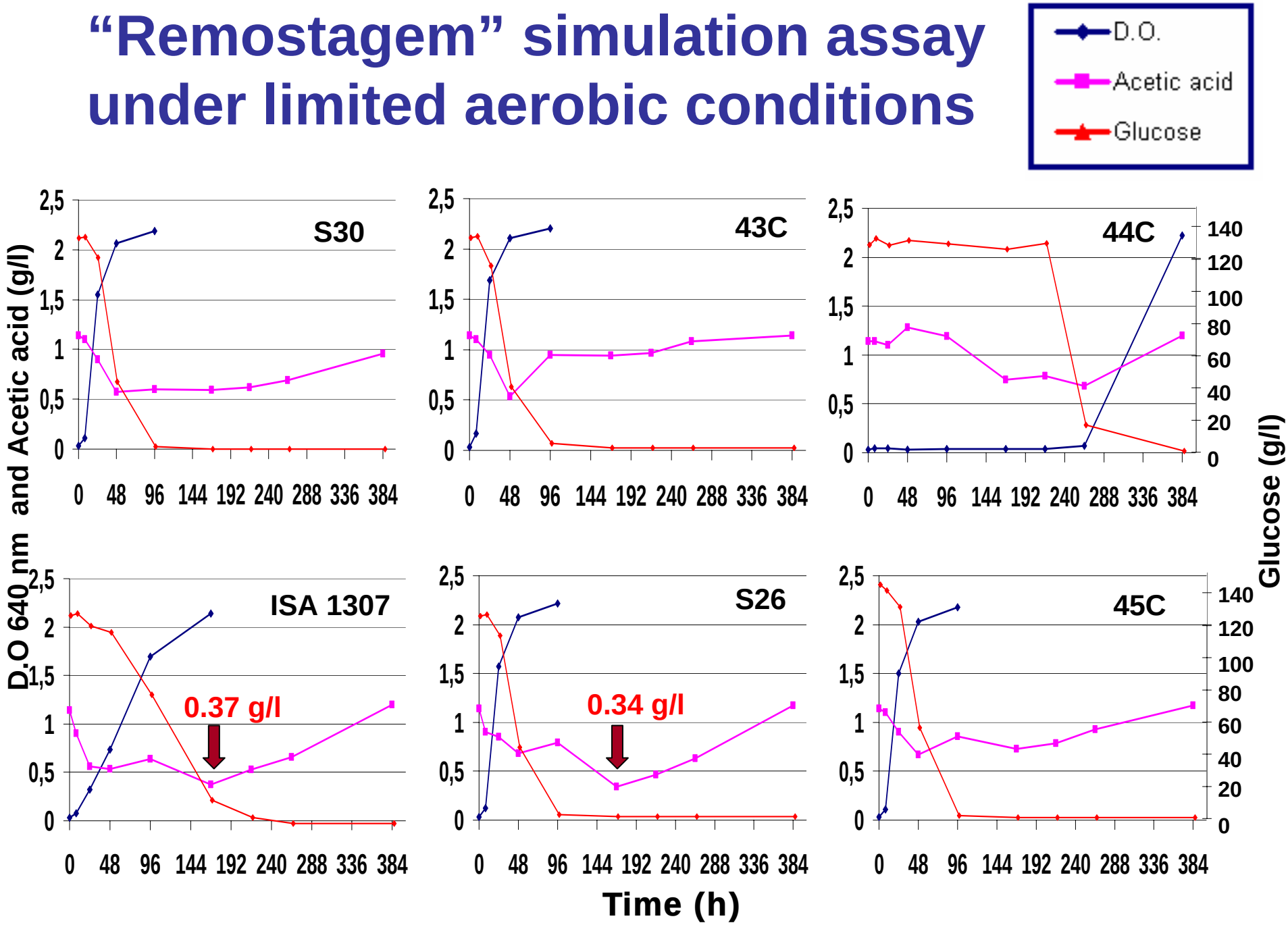
→ limited aerobic conditions

100 rpm

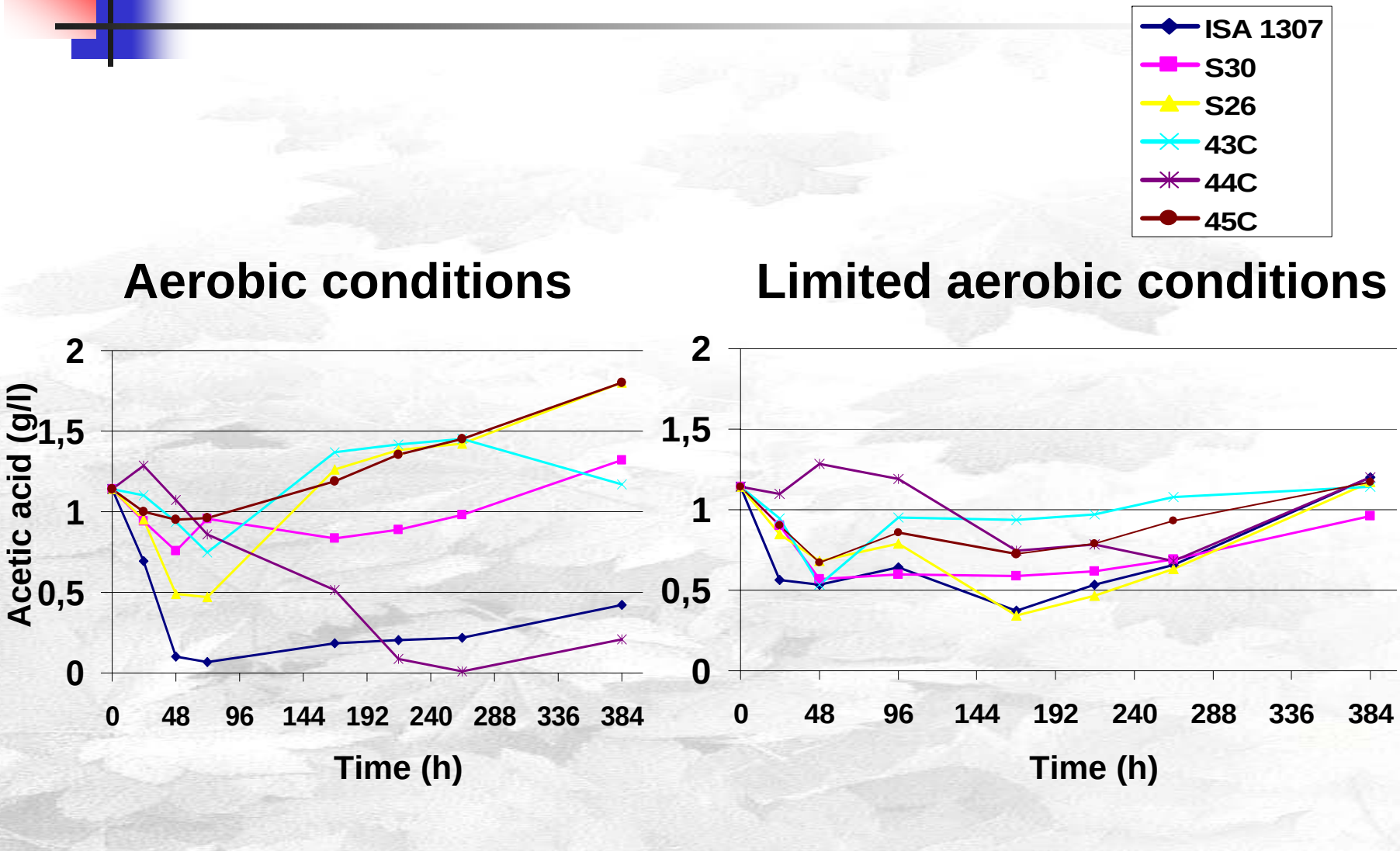
“Remostagem” simulation assay under aerobic conditions



“Remostagem” simulation assay under limited aerobic conditions



Comparison of acetic acid consumption during “remostagem” simulation assay under aerobic and limited aerobic conditions



Final remarks

- *L. thermotolerans* is able to utilize simultaneously glucose and acetic acid.
- A remostagem process revealed significant differences between the strains tested regarding acetic acid consumption:

Limited aerobic conditions (P≤0.05)
Z. bailii and S26
decrease acetic acid (<0.4 g/l)
display simultaneous consumption

Aerobic conditions (P≤0.001)
Z. bailii and 44C
exhausted acetic acid
display simultaneous consumption

- Under both conditions some strains started in a second phase to increase acetic acid concentration

Future perspectives

- Evaluate the capacity of *L. thermotolerans* 44C and *S. cerevisiae* S26 to perform biological deacidification of wines with excessive levels of acetic acid;
- Evaluate the fermentative profiles and the organoleptical properties of the wines deacidified by those strains;
- Scale-up of the optimized “remostagem” process.

Acknowledgements

Universidade de Trás-os-Montes e Alto Douro

Arlete Faia

Universidade do Minho

Manuela Côrte-Real

Dorit Schuller