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Optimizing Wine Yeast Biomass Production: Effect of Culture Medium Composition in *Saccharomyces cerevisiae* for Scaling-Up Bioreactor Systems

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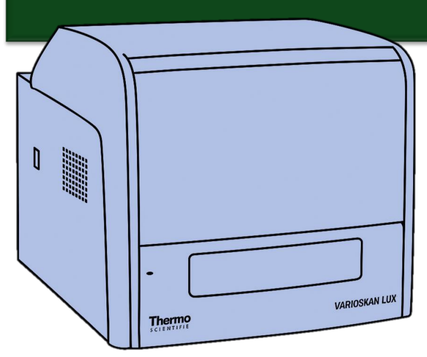
INTRODUCTION & AIM

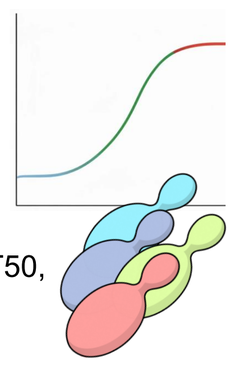
Large-scale biomass production of *Saccharomyces cerevisiae* is essential for oenological and industrial applications. The efficiency and cost-effectiveness of biomass cultivation depends significantly on the composition of the culture medium. Generally ~~using~~ beet or sugarcane molasses are used as substrate [1] for biomass production. Molasses are a particularly interesting substrate for yeast growth due to their reduced cost and high sugar concentration (65%–75% sucrose), although they should be supplemented with a nitrogen source and vitamins for optimal growth [1].

Depending on the fermentation phase a different source of nitrogen is used by yeasts to grow, such as ammonium salts and vitamins. Yeast extract and peptone are used as nitrogen sources in laboratory-scale media but their costs are prohibitive for industrial scale production of yeasts in cellars using bioreactors [2], so other common nitrogen sources are needed.

The aim is to study the potential of different nitrogen sources for yeast growth in different culture media for four different commercial *S. cerevisiae* strains.

METHODS





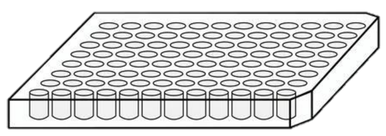
S. cerevisiae (VIN13, NT50, NT116 ELEGANCE)

96-well plate microfermentations

20 h at 24ºC, x5 replicates

no oxigenation or extra feeding agitation

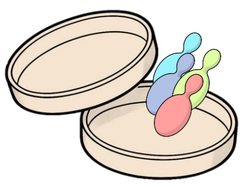
growth monitored by OD_{600nm} each 30 min



Culture media composition:

- YPD (20 g/L D-glucose + 20 g/L peptone + 10 g/L yeast extract)
- Commercial medium - (88 g/L commercial dry medium)
- Medium 1 - (110 g/L sucrose + 3 g/L diammonium phosphate (DAP) + 2 g/L Malic acid)
- Medium 2 - (110 g/L sucrose + 7,5 g/L $(NH4)_2SO_4$ + 3,5 g/L KH_2PO_4 + 0,75 g/L $MgSO_4$ + 3 g/L DAP)
- Medium 3 - (110 g/L sucrose + 20 g/L Actimax Plus (Agrovin) + 2 g/L Malic acid)
- Medium 4 - (110 g/L sucrose + 10 g/L Actimax Plus + 1,5 g/L DAP)
- Medium 5 - (110 g/L sucrose + 20 g/L Actimax Vit (Agrovin))
- Medium 6 - (110 g/L sucrose + 10 g/L Actimax Vit + 1,5 g/L DAP)

At day 7 of fermentation, viable population (cfu/mL) was determined (YPDA plates, 28ºC, 4 days)



CONCLUSIONS

- Similar growth curves for each medium were achieved by all commercial *S. cerevisiae* strains evaluated.
- Culture medium 2 and 5 were discarded by their higher costs respect to the commercial medium.
- Culture medium 3 did not produce satisfactory yeast growth, despite having a composition similar to the commercial medium, as well as medium 1 and 4.
- The culture medium 6, which contains sucrose and Actimax Vit and DAP, achieved higher yeast growth yields than the commercial medium and YPD at 7 days of fermentation and represented a more cost-efficient and promising alternative for biomass production, 0,168 € less per liter.

RESULTS & DISCUSSION

Table 1. Chemical general parameters of culture media evaluated. YAN stands for Yeast Assimilable Nitrogen. n.d. stands for Not detected.

Liquid culture media	Brix	pH	YAN (mg/l)	Malic acid (g/L)
YPD	2,0	7,0	2954,0	n.d.
Comercial medium	10,9	5,5	3090,5	2,7
Culture medium 1	14,9	5,0	796,6	1,9
Culture medium 2	17,2	6,3	2392,6	n.d.
Culture medium 3	16,8	7,2	2727,4	2,9
Culture medium 4	11,9	7,1	417,7	n.d.
Culture medium 5	11,2	6,4	76,3	n.d.
Culture medium 6	11,1	7,0	249,6	n.d.

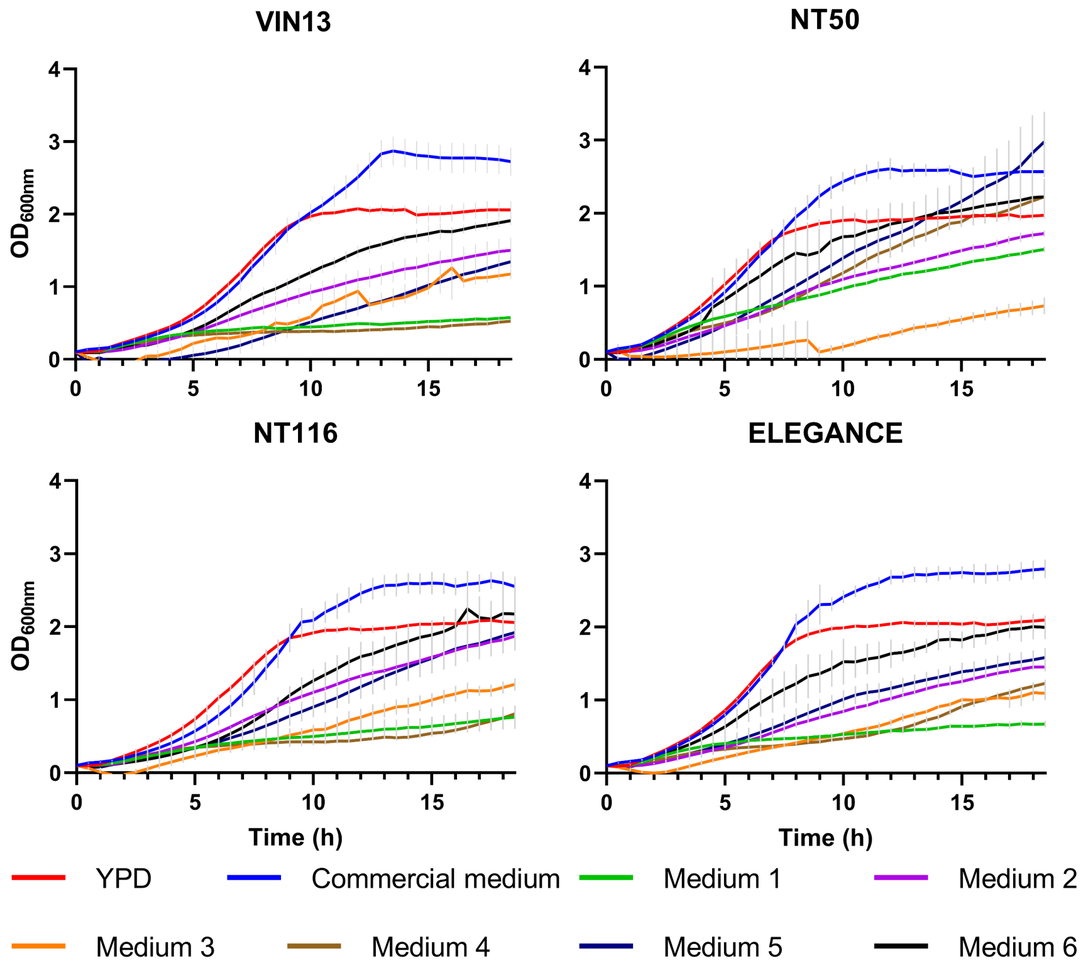


Figure 1. Growth curves of 4 different commercial *S. cerevisiae* yeasts (VIN13, NT50, NT116 and ELEGANCE) evaluated in the different culture media. Data is represented by the mean of 5 replicates, and error bars represent standard deviation.

Table 2. Costs of all culture media evaluated by liter of product.

Liquid culture medium	Total (€/L)
YPD	5,5740
Comercial medium	0,4444
Culture medium 1	0,1408
Culture medium 2	0,4628
Culture medium 3	0,3826
Culture medium 4	0,2239
Culture medium 5	0,4486
Culture medium 6	0,2758

Table 3. Viable cells (cfu/mL) from 5 replicates at day 7 of fermentation.

	Viable yeast (cfu/mL)			
Yeast strain	**VIN13**	**NT50**	**NT116**	**Elegance**
YPD	9,90E+07	4,90E+07	1,25E+08	1,28E+08
Comercial medium	1,53E+08	1,06E+08	1,56E+08	1,56E+08
Culture medium 1	1,00E+06	1,00E+06	1,00E+06	1,00E+06
Culture medium 2	4,00E+07	1,90E+07	7,60E+07	3,10E+07
Culture medium 3	7,30E+07	4,90E+07	6,00E+07	6,30E+07
Culture medium 4	2,29E+08	1,74E+08	8,50E+07	1,03E+08
Culture medium 5	1,84E+08	1,26E+08	8,10E+08	7,26E+08
Culture medium 6	3,65E+08	5,24E+08	7,66E+08	3,43E+08

REFERENCES

[1] Pérez-Torrado, R., Bruno-Bárcena, J. M., and Matallana, E. (2005). Monitoring stress-related genes during the process of biomass propagation of *Saccharomyces cerevisiae* strains used for wine making. Appl. Environ. Microbiol. 71, 6831–6837. doi: 10.1128/AEM.71.11.6831-6837.2005.

[2] A. Tropea, D. Wilson, N. Cicero, A.G. Potortì, G.L. La Torre, G. Dugo, D. Richardson, K.W. Waldron, Development of minimal fermentation media supplementation for ethanol production using two *Saccharomyces cerevisiae* strains, Nat. Prod. Res. 30 (2015) 1009–1016. doi: 10.1080/14786419.2015.1095748.