EFFECT OF PRE-CULTURE CONDITIONS ON THE SENSITIVITY OF YEASTS TO THE GLUCOSE EFFECT

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Abstract

In an experiment to determine the effect of pre-culture conditions on the sensitivity of yeasts to the glucose effect, the yeast species *Saccharomyces uvarum* (*carlsbergensis*) K19, was used. This species is from the collection of the Industrial Microbiology Laboratory of ENSAIA-INPL, Nancy France. The yeast was initially cultured on industrial wort with constant aeration throughout the culture period in the first instance and without aeration throughout the culture period in the second instance, creating aerobic and anaerobic conditions.The yeasts from the two culture different media were harvested and placed in synthetic media containing known sugar concentrations of glucose and maltose as substrates, and fermentations were carried out at 30°C. Cells were harvested during growth and the specific rate of fermentation (fermentation intensity) measured, using the Warburg respirometer. Irrespective of initial and main culture media used, yeasts from anaerobic preculture media had highly significantly lower ($P<0.05$) fermentation intensities than those cultivated initially under aerobic conditions. Fermentation intensity on glucose after preculture on glucose is significantly lower ($P<0.05$) for yeasts grown under anaerobic conditions when compared to those with access to oxygen. Oxygen limitation during preculture growth of the yeasts in either glucose or maltose media depressed fermentation on glucose or maltose thereafter.

Introduction

In nature, carbon and nitrogen sources always occur in diverse and complex forms. Yeast is able to use a wide variety of compounds as carbon and nitrogen sources (Magasanik, et al; 2002). One criterion considered in selecting industrial strains for brewing, baking, wine and the distilling industries is the capability to rapidly and completely utilize the fermentable carbohydrates available (Meneses, F.J., et al 2002, Stewart,G.G et al, 1998).Brewer's wort is a typical example of a nutritional environment containing the sugars sucrose, fructose, glucose, maltose and maltotriose, nitrogen and other non-sugar and non-nitrogenous compounds(Stewart et al, 1998). In order to select the best options from the diversity of available carbon and nitrogen sources, yeasts have developed molecular mechanisms of sensing and regulation which include induction and repression of key systems(Maganasik,et al,2002,Hofman-Bang,1999,Thevelen,J.M, 1994 and Maganasik, et al ,2005). *Saccharomyces cerevisiae* and many other types of yeasts may thrive on a variety of carbon sources, but glucose and fructose are the preferred sources. When one of these sugars is present in the culture medium, the enzymes required for the utilization of the alternative carbon sources are synthesized at low rates or are not

synthesized at all. Sugar catabolite repression (Gancedo, J.M, 1998) ensures an ordered sequence of sugar utilization, and during fermentation, brewing strains utilize sucrose, glucose, maltose and maltotriose in this approximate sequence with some degree of overlap (Stewart, et al 1998). However, altered patterns of sugar utilization amongst brewing, wine, baking and distilling strains have been reported (Menese et al, 2002; Berthals,et al 2004).

Carbon catabolite repression or simply, catabolite repression is an important part of global control system of various bacteria and micro-organisms (Deutscher, 2008). Catabolite repression allows these micro-organisms to adapt quickly to a rapidly metabolisable carbon and energy source first (Madigan *et.al.*2009). This is usually achieved through inhibition of synthesis of certain enzymes involved in catabolism of carbon sources other than the easily metabolisable carbon source. Catabolite repression was first shown to be initiated by glucose and therefore is sometimes referred to as the glucose effect (Deutscher, 2008). Yeasts adapted to the fermentation of maltose are sensitive to catabolite repression by glucose (Masschelein,et al 1963).Glucose repression in the yeast *Saccharomyces cerevisiae* has been studied severally, indicating a complex regulatory system involving different metabolic pathways. (Westergaard *et. al.* 2007)

Materials and Methods

The yeast strain used, *S.uvarum* K19 is from the collection of "Laboratoire de Microbiologie Industrielle de L'ENASIA, Nancy France".
Preculture preparations were carried out following the methods described by Amata,et al 1990, which are a modification of those described by Ahvenainen(1982) and Marc(1982). This method may be described as a progressive fermentation of increasing quantities of wort. The volume of the medium being increased at each step, until enough yeast in optimal physiological state is produced. The starting volume was 20ml and the final volume was 2000ml. This procedure was to ensure enough yeast culture for the experiments. The temperature was maintained at 30°C throughout this period using a temperature controlled water-bath. For aerobic preculture growth, constant aeration was carried out. For anaerobic preculture growth, the process was conducted without aeration. Yeasts from the two different culture media were then added to synthetic media of known glucose and maltose concentrations.

Fermentations: Fermentations were carried out at 30°C using glucose and maltose as substrates. The synthetic media had the following composition: Ammonium sulphate $[(NH_4)SO_4]$ 2.6gl^{-1}, Potassium dihydrogen phosphate $[KH_2PO_4]$ 1.02gl^{-1}, Sodium hydrogen phosphate $[Na_2HPO_4.12H_2O]$ 1.23gl^{-1} and yeast extract 1gl^{-1}. The pH of the synthetic media was adjusted to pH 4.5. Initial glucose/maltose concentrations were 8gl^{-1}. Cells from the two different preculture media were added to the synthetic media, and fermentations carried with constant agitation at 250rpm. To ensure uniformity, cellular concentration at

time of addition was adjusted to $0.65x10^7$ cells/ml, using standard turbidimetric methods, relating optical density measurements on a digital colorimeter, Chemtrix type 24, to dry weight of cells. The optical density measurements were carried out at wave lengths of 660nm. Cells were harvested with an ultra-centrifuge, Beckman: LS-508, during the exponential growth phase (stage at which fermentation activities are maximal) and washed three times in a buffer solution of calcium chloride ($CaCl_2.5H_2O$:$1.47gl^{-1}$)

Manometric Techniques: Fermentation intensities (specific rate of fermentation) of the harvested cells from the glucose and maltose media were determined by manometric techniques, using the Warburg apparatus (Umbreit, et al 1964).

The reaction medium for manometric measurements had the following composition.

Glucose/Maltose	$20gl^{-1}$
KH_2PO_4	$8.84gl^{-1}$
$Na_2HPO_4.12H_2O$	$0.595gl^{-1}$
$CaCl_2.5H_2O$	$0.417gl^{-1}$

The cells were harvested from the synthetique medium, centrifuged, then washed three times and then re-dissolved in a calcium chloride ($1.47gl^{-1}$) buffer solution. The pH of the medium was adjusted with a digital pH meter to pH 4.5 using a 1M HCL solution. Cellular concentration was adjusted to between 1000 - 3000µg of wet yeast per ml, using standard turbidimetric methods already described. Each reaction flask contained 2ml of reactants, i.e. 1ml of reaction medium and 1ml of yeast cells dissolved in the buffer solution. The trials were conducted at 28°C under nitrogen gas (anaerobic conditions). Fermentation Intensity values were expressed as $X\mu lCO_2.h^{-1}.mg^{-1}$ (volume of CO_2 produced per h per mg wet yeast).

Experimental Design and Data Analysis: The experiment was a 2x2x2 factorial experiment with two levels of preculture media (glucose and maltose), two levels of main culture (glucose and maltose) and two levels of oxygen application (aerobic and anaerobic conditions). Data generated were analyzed using the SPSS computer software. Significantly different means were separated using the Duncan's multiple range test procedure of the same computer software. Orthogonal contrasts to determine differences between different factor combinations of interest were also carried out.

Results and Discussion

*Fermentation Intensity as affected by oxygen limitation during preculture growth.*Fermentation intensity on glucose after initial growth on glucose with yeasts from aerobic preculture growth, was $230\mu lCO_2.h^{-1}.mg^{-1}$, while fermentation intensity on glucose after initial growth on glucose with yeasts from anaerobic preculture growth, was $120\mu lCO_2.h^{-1}.mg^{-1}$. Fermentation intensity

values on maltose after initial growth on glucose were $40\mu lCO_2h^{-1}.mg^{-1}$ for yeasts from aerobic preculture growth, and $5\mu lCO_2h^{-1}.mg^{-1}$ for yeasts from anaerobic preculture growth. With maltose as initial substrate, fermentation intensity values on maltose were $240\mu lCO_2.h^{-1}.mg^{-1}$ for aerobic preculture growth and $150\mu lCO_2.h^{-1}.mg^{-1}$ for anaerobic preculture growth, while the values for fermentation intensity on glucose were $140\mu lCO_2h^{-1}.mg^{-1}$ for yeasts from aerobic preculture growth and $90\mu lCO_2h^{-1}.mg^{-1}$ for yeasts from anaerobic preculture growth (Table1).

Table 1: Fermentation Intensity as Affected by Oxygen Limitation During Preculture growth

		Aerobic preculture growth	Anaerobic preculture growth
Initial growth on glucose	F.I. on glucose	230	120
	F.I. on maltose	40	5
Initial growth on maltose	F.I. on glucose	140	90
	F.I. on Maltose	240	150

Effect of oxygen limitation during preculture growth on percentage decrease in fermentation intensity

Oxygen limitation during initial preculture growth on glucose resulted in a 47.8% decrease in fermentation intensity on glucose while decrease in fermentation intensity on maltose was 87.5%. Oxygen limitation during initial culture growth on maltose resulted in a 35.7% decrease in fermentation intensity on glucose while decrease in fermentation intensity on maltose 37.5 %(Table 2).

Table 2: Effect of Oxygen Limitation During Preculture Growth on Percentage Decrease in Fermentation Intensity

		Oxygen	No Oxygen	% Decrease In F.I.
Initial Preculture Growth on glucose	Fermentation Intensity on glucose	230	120	47.8
	Fermentation Intensity on maltose	40	5	87.5
Initial Preculture Growth on maltose	Fermentation Intensity on glucose	140	90	35.7
	Fermentation Intensity on maltose	240	150	37.5

Table 3 shows the acclimation of the yeast strain to substrate change as affected by oxygen limitation during preculture growth.

Table 3: ***Effect of Oxygen Limitation During Preculture Growth on Acclimation to Substrate*** **Change.**

		Native Substrate (F.I.)	Non-native Substrate (F.I.)	%Decrease in Fermentation intensity
Initial growth on glucose	Aerobic conditions	230	140	39.1
	Anaerobic conditions	120	90	25.0
Initial growth on maltose	Aerobic Conditions	240	40	83.3
	Anaerobic conditions	150	5	96.7

Under aerobic conditions, with glucose as initial substrate, percentage decrease in fermentation intensity was 39.1% as compared to 25% under anaerobic conditions. With maltose as initial substrate, percentage decrease in fermentation intensity under aerobic conditions was 83.3%, while under anaerobic conditions it was 96.7%.

Tables 4 and 5 show the effects of substrate change (catabolite repression) on percentage decrease in fermentation intensity.

Table 4: ***Percentage Decrease in Fermentation Intensity As Affected By Substrate Change (Aerobic Metabolism).***

Substrate	Glucose Culture	Maltose Culture	Maltose culture efficiency relative to Glucose culture
Native	230	240	104.3%
Non-Native	40	140	350%
Catabolite Repression	82.6%	41.7%	

Table 5: ***Percentage Decrease in Fermentation Intensity as Affected by Substrate Change (Anaerobic Metabolism)***

Substrate	Glucose culture	Maltose culture	Maltose culture efficiency relative to glucose culture
Native	120	150	125%

Non-native	5	90	1800%
Catabolite Repression	95.8%	40%	

Catabolite repression is 82.6% under aerobic conditions with glucose as the culture substrate, and 41.7% with maltose as the culture substrate. Under anaerobic conditions, catabolite repression is 95.8% with glucose as culture medium and 40% with maltose as culture medium.

Results obtained were subjected to statistical analyses to determine significant differences between the means.

Fermentation intensity on glucose after initial growth on glucose was significantly different ($P<0.005$) with yeasts from aerobic preculture growth when compared to yeasts from anaerobic preculture growth. Fermentation intensity on maltose after initial growth on maltose was also significantly different ($P<0.05$) with yeasts from aerobic preculture growth when compared to yeasts from anaerobic preculture growth (Table 6).

Table 6: Fermentation Intensity As Affected by Oxygen Limitation During Preculture Growth

		Oxygen Limitation	
Preculture medium	Main culture medium	Aerobic	Anaerobic
Glucose	Glucose	230.50 ± 0.96^{b}	119.70 ± 1.05^{e}
	Maltose	40.30 ± 1.56^{g}	5.50 ± 0.96^{h}
Maltose	Glucose	139.50 ± 0.96^{d}	89.90 ± 1.10^{f}
	Maltose	239.40 ± 1.01^{a}	149.40 ± 1.00^{c}

*Mean ± Standard error.
Means with different superscripts differ significantly ($P<0.05$)

The contrast coefficients for the various treatment combinations were analyzed and the results of the analyses are presented in Table 7.

Table 7: Contrast Coefficients for the Various Treatment Combinations of Interest

Treatments								
Contrast Coef	**GGAE**	**GGAN**	**GMAE**	**GMAN**	**MGAE**	**MGAN**	**MMAE**	**MMAN**
1	- 1	-1	-1	-1	1	1	1	1
2	1	1	-1	-1	1	1	-1	-1
3	1	-1	1	-1	1	-1	1	-1
4	1	1	-1	-1	0	0	0	0
5	0	0	0	0	1	1	-1	-1
6	0	0	1	1	-1	-1	0	0
7	0	0	1	-1	0	0	0	0
8	0	0	0	0	1	-1	0	0
9	1	-1	1	-1	0	0	0	0
10	0	0	0	0	1	-1	1	-1

*Treatment code	Preculture medium	Culture medium	Oxygen status
GGAE	Glucose	Glucose	Aerobic
GGAN	Glucose	Glucose	Anaerobic
GMAE	Glucose	Maltose	Aerobic
GMAN	Glucose	Maltose	Anaerobic
MGAE	Maltose	Glucose	Aerobic
MGAN	Maltose	Glucose	Anaerobic
MMAE	Maltose	Maltose	Aerobic
MMAN	Maltose	Maltose	Anaerobic

The contrast tests of the various treatment combinations of interests are presented in table 8.

Table 8: ***Contrast Tests Of The Various Treatment Combinations Of Interest***

Contrast	Value of contrast	t-test value	Significance
1	222.20	71.906	**
2	145.00	46.924	**
3	285.20	92.294	**
4	304.40	139.310	**

5	-159.40	-72.950	**
6	-183.60	-84.026	**
7	34.80	22.523	**
8	49.60	32.102	**
9	145.60	66.635	**
10	139.60	63.889	**

** = Highly significant (P<0.01)
1 = Glucose as preculture vs. maltose as preculture
2 = Glucose as culture medium vs. maltose as culture medium
3 = Aerobic vs. anaerobic
4 = Glucose as preculture and culture vs. glucose as preculture and maltose as culture
5 =Maltose as preculture and glucose as culture vs. maltose as preculture and maltose as culture
6 = Maltose as culture medium vs. glucose as culture medium
7 = Glucose as preculture and maltose as culture in an aerobic condition vs. glucose as preculture, maltose as culture under anaerobic conditions
8 =Maltose as preculture and glucose as culture under aerobic conditions vs. maltose as preculture, glucose as culture medium under anaerobic conditions
9 = Glucose as culture medium under aerobic conditions vs. maltose as culture medium under anaerobic conditions
10 = maltose as preculture medium under aerobic conditions vs. maltose as preculture medium under anaerobic conditions

Results indicate highly significant (P<0.01) differences between the treatment combinations of interest.

Conclusion

Under aerobic conditions, maltose culture had slightly significantly higher (P<0.05) fermentation intensity on its native substrate than glucose on its native substrate, and significantly (P<0.05) much higher fermentation intensity on its non- native substrate than glucose on its non-native substrate. Glucose can be metabolized well even without acclimation under aerobic conditions.

Under anaerobic conditions, maltose culture had significantly (P<0.05) much higher fermentation intensity on both native and non-native substrates and showed higher catabolite repression by glucose metabolism, 87.5% as compared to 47.8%. Both glucose and maltose cultures were however significantly (P<0.05) affected by their non-native substrates under anaerobic conditions. Catabolite repression is much higher when glucose is the culture substrate under aerobic and anaerobic conditions. With anaerobic conditions the repression is near total (87.5% decrease in fermentation intensity).

Maltose culture efficiency relative to glucose culture is significantly higher ($P<0.05$) for non-native substrates under aerobic and anaerobic conditions.

References

Amata,I.A. and P.Germain (1990) J.Inst.Brew.96:131-134

Ahvenainen, J. (1982) J.Inst.Brew.88:367-370

Berry, D.R. and C.Brown. (1987) Yeast Biotechnology, Allen and MP; Umwin, Boston, Massachusetts.

Berthals,N.J., Cordeo Otero,R.R., Baner,F.F.,Thevelen,J.M. and pretorious,I.S. FEMS,yeast research, 2004,4: 683-689.

Deutscher, J. (2008) Current opinion in microbiology.11 (2):87-93. Doi:10.1016/j.mib 200F.02.007.

Duncan,D.B.(1955).Multiple ranges and multiple F-tests biometrics 11:1-42

Entian,K.D. and H.J.Schulter.(1986) Microbiol. Sc.3: 366-371.

Gancedo,J.M. and C. Gancedo, In F.K.Zimmermann and K.D.Entian eds.(1997) Mol. Microbiol.6:5-21

Gancedo,J.M (1998). Mol. Biol. Rev. 62(2):334-361.

Hofman-Bang,J.,(1999) Molecular Biotechnology12: 35-73.

Madigan,M.S., J.M.Martinko, P.V.Dunlap and D.P.Clark.(2009). Brocks biology of microorganisims.12th ed. San Francisco,CA: Pearson/Benjamin Cummings.

Masschelein,CH.A.,H.Jenardy,C.L.Ramos-Juenehomme,J.E. Haboucha and A.Devreux(1963), characterisation biochimique et biophysique de quelques souches de levures de brasserie.Proceedings E.B.C: 381-399.

Magasanik,B. and C.A.Kaiser. (2002), 290:1-18.

Magasanik,B.(2005) Proceedings of the national academy of sciences,USA, 102: 16537-16538

Menese,F.J.,P.P.Henschkle and V. Jiranak(2002) J.Inst .Brew.108: 310-321.

Marc,I.(1982) Modélisation de la fermentation de la biérè dans un réacteur cylindroconique.Thésè de Docteur- Ingénieur: 68-81.

Ramos-Jeunehomme,C., J.Haboucha, A.Devreux and Masschelein,CH.A.(1973). Proceedings E.B.C:111-123.

Raines,M.(1994).Wort Aeration booklet; Brewer's resources, Camarillo, California.

Stewart,G.G. and I.Russell. (1998). Brewing science and technology Series 111. The institute of brewing London.

Thevelen,J.M.(1994) Yeasts, 10:1753-1790.

Trumbly, R.J. (1992). Mol.Microbiol, 6:5-21

Umverit,W.W., R.H.Burris, J.F. Stauffer. (1964). In "Manometric Techniques" Burgess Publishing Company 4th Edition.

Westergaard,S.L., A.P. Oliviera,Bro.C., L.Olsson and J.Nielsen (2007) Biotech. Bioeng. 96:134-145.