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The Functional Relationship Between Baker's Yeast Intracellular Lysine and Aeration Rate and Sodium Chloride

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ABSTRACT

The results of this study confirm the enhanced production of L-lysine by *Saccharomyces cerevisiae* during the fermentation of glucose at a 0.6*M* concentration of NaCl previously observed (*8*). Changes in NaCl concentration above or below 0.6*M* caused a drop in the maximum production of lysine. Fermentations run at 1.0, 1.4, and 1.8 VVM aeration rates and at 0.0, 0.3, 0.6, 0.9, and 1.2*M* NaCl concentrations gave the highest intracellular lysine yield at 0.6*M* and 1.4 VVM.

Index Entries: Baker's yeast, effects of lysine, aeration rate, and NaCl on growth rate; yeast, effects of lysine, aeration rate, and NaCl on Baker's yeast; lysine, effect on growth rate of Baker's yeast; aeration rate, effect on growth rate of Baker's yeast; sodium chloride, effect on growth rate of Baker's yeast; *Saccharomyces cerevisiae*, production of L-lysine from; glucose, production of lysine by fermentation of.

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INTRODUCTION

During a study of the metabolic pathway of lysine synthesis by *Saccharomyces cerevisiae*, Jensen and Shu (3) observed that the aeration rate of the fermentation greatly affected the lysine content of the yeast cells. The highest and lowest aeration rates they used produced cells with relatively low lysine levels, whereas intermediate rates yielded the highest lysine content. The aeration rates used by Jensen and Shu cannot be determined from their paper, although the authors stated that the airflow usually employed was 1.0 volume of air per volume of growth medium per minute, i.e., 1.0 VVM.

In 1981 Tanner et al. (*8*) reported that, when a culture of Baker's yeast (*S. cerevisiae*) producing ethanol in a glucose (5% wt/vol) culture medium was supplemented with 0.6*M* sodium chloride and was moderately aerated (1.4 VVM), the maximum concentration of intracellular L-lysine formed per gram of yeast cell mass was approximately four times as large as in the absence of such exogenously added NaCl under the same conditions. Negligible lysine enhancement was observed when the culture was not aerated, even in the presence of NaCl (*9*).

Since 0.6*M* is the salt level found in most sea waters, this observation suggests that marine or brackish water may prove useful in the production of byproduct lysine-enriched yeast food for human or animal consumption, along with ethanol, the primary fermentation product, widely used for chemical feed stock or fuel.

This is a report of the study of the effect of variations in the aeration rate upon the yield of L-lysine in *S. cerevisiae* growing in a fermentation mash containing 10% (wt/vol) glucose and 0.0–1.2*M* NaCl.

MATERIALS AND METHODS

Fermentation Organism

The fermentation organism was *S. cerevisiae* purchased at a local grocery in the form of Fleischmann's brand active dry yeast.

Growth Medium

The culture medium was the Synthetic Medium C of Maxon and Johnson (M.-J. medium) (*5*) supplemented with 0.0–1.2*M* NaCl. The glucose concentration was 10% wt/vol.

Fermentation Setup

Fermentation was initiated by inoculating an 800 mL volume of sterile M. J. medium with 2 g dry yeast. The fermentations were carried out in stirred and aerated fermentors, which were 1.0 L beakers (tall-type) fitted with a sterilizable elastomer headplate. The fermentor contents

were stirred by means of a magnetic stirring bar 1 in. in size and revolving at 400 rpm.

The headplate permitted the entry to the fermenting mash of accessories for the following controls:

1. The airflow into the mash through the air inlet was intermittently adjusted to the desired aeration rate by means of an in-line rotameter. This was necessary since the volume of the growth medium decreased with each sampling. (Note: The O_2 was monitored by means of a Beckman Dissolved Oxygen (D. O.) Analyzer, Model 777. For all runs, the dissolved oxygen of the fermenting mash dropped from 5.0 to 7.0 ppm at the beginning of the fermentation to ca. 1.0 ppm within 20 min.)
2. The pH of the fermentation was controlled at pH 5.0 by means of a pH probe connected to a Fisher Model 36 Automatic Titrimeter charged with a neutralizing solution of 10% NH_4OH.
3. The temperature of the fermentation was controlled at 32°C by immersion of the fermentor in a constant temperature water bath.
4. The composition of the fermenting mash was partially controlled by the installation of an ice-cooled water condenser on the fermentor effluent air port. The condenser captured most of the moisture and ethanol in the evolved gas mixture and returned them to the fermentor.

When necessary to maintain conditions of practical sterility, the pH and O_2 probes and the thermometer were sanitized with absolute ethanol. All other equipment and media were sterilized in a steam pressure sterilizer at 120–125°C for 15 min.

Sampling Protocol

At 0 h, as soon as the yeast cells were well mixed with the growth medium, i.e., when clumps of yeast cells were no longer seen floating on the surface of the growth medium, 5.0 mL samples of the inoculated growth medium were removed by means of a measuring pipet with a large orifice. The samples were handled as follows:

1. Sample No. 1 was pipetted into a test tube capped with foil, and frozen for storage. Just prior to the lysine analysis, this sample was thawed and the sample test tube was held in a boiling water bath for 20 min, to extract the lysine in the amino acid pool of the yeast cells. After cooling, the sample was centrifuged to remove cells and debris. The supernatant was used for the assay of total (extracellular plus intracellular) L-lysine.
2. Sample No. 2 was centrifuged immediately. The supernatant liquid was transferred to a test tube capped with foil and frozen for storage. The pellet was discarded. The supernatant was

thawed prior to the lysine assay and used to determine the concentration of extracellular L-lysine, i.e., lysine released into the growth medium by the yeast cells.

At the same time the 5.0 mL samples were collected, a 1.0 mL sample of inoculated growth medium was taken for determination of yeast cell mass concentration.

Sampling was repeated, in general, every hour for the first 4 h, then at 6, 8, 10, 12, and 24 h.

Determination of Yeast Cell Mass Concentrations

A standard curve for yeast cell mass, g/L vs absorbance (optical density at 595 nm) was prepared by suspending weighed amounts of dried yeast in 25 mL volumes of water to give suspensions in the range 0.0–10.0 g/L. The absorbance was measured in a Fisher Electrophotometer II spectrophotometer at 595 nm. Deionized water was used as the blank.

The absorbance of the inoculated growth medium or an appropriate dilution of the fermenting mash was read on the spectrophotometer and was interpolated on the standard curve to determine dry yeast cell mass.

Assay of L-Lysine Concentration

A 2.0 or 3.0 mL volume of the total lysine or extracellular lysine sample was analyzed for L-lysine by the microbiological method described in the Difco Manual (*1*). The assay bacterium was *Pediococcus acidilactici* (formerly *P. cerevisiae* P-60) (NRRL B-1116). The endpoint was measured at 650 nm absorbance. The Difco method was modified by adding to the lysine assay medium used in the standard curve tubes the same concentration of NaCl as contained in the fermentation mash being analyzed. It was also necessary to increase the incubation period beyond the standard time of 20 h to produce adequate growth of the organism in the presence of high NaCl.

RESULTS

At 1.4 VVM

The concentrations of intracellular and extracellular L-lysine produced during the fermentation of glucose by *S. cerevisiae* at aeration rate 1.4 VVM and varying levels of NaCl are summarized in Figs. 1 and 2. At 0.0*M* NaCl, the maximum intracellular lysine of 2.2 mg/g dry weight was produced at 12 h. Following the peak, the concentration of intracellular lysine slowly diminished, presumably via yeast metabolism. During the first few hours a small amount of extracellular lysine was observed. This was depleted by 8 h.

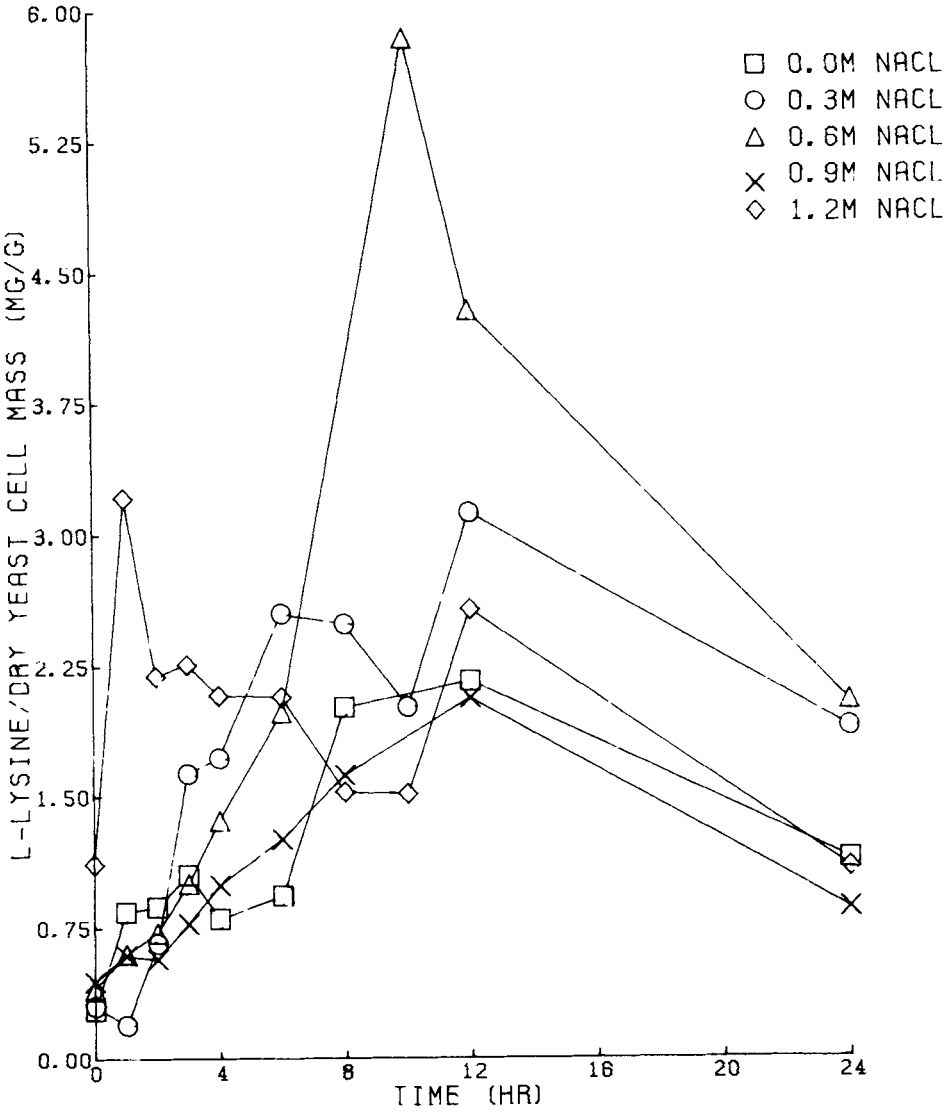

Fig. 1. Production of intracellular L-lysine by *S. cerevisiae* during fermentation of 10% glucose at 1.4 VVM and 0.0–1.2*M* NaCl.

When the NaCl content was increased to 0.3*M*, the concentrations of lysine synthesized were increased slightly over those given at 0.0*M* NaCl. The maximum intracellular lysine was 3.1 mg/g dry cell mass at 12 h. Again, after the peak concentration, the level of intracellular lysine slowly dropped. During the early hours of fermentation there was a significant level of extracellular lysine that then slowly fell.

When the NaCl level was raised to 0.6*M*, the production of intracellular lysine was dramatically increased, with a maximum of 5.8 mg/g recorded at 10 h. Very little extracellular lysine was released.

An increase in the concentration of NaCl to 0.9*M* caused a sharp drop in the amount of intracellular lysine produced. The peak was only

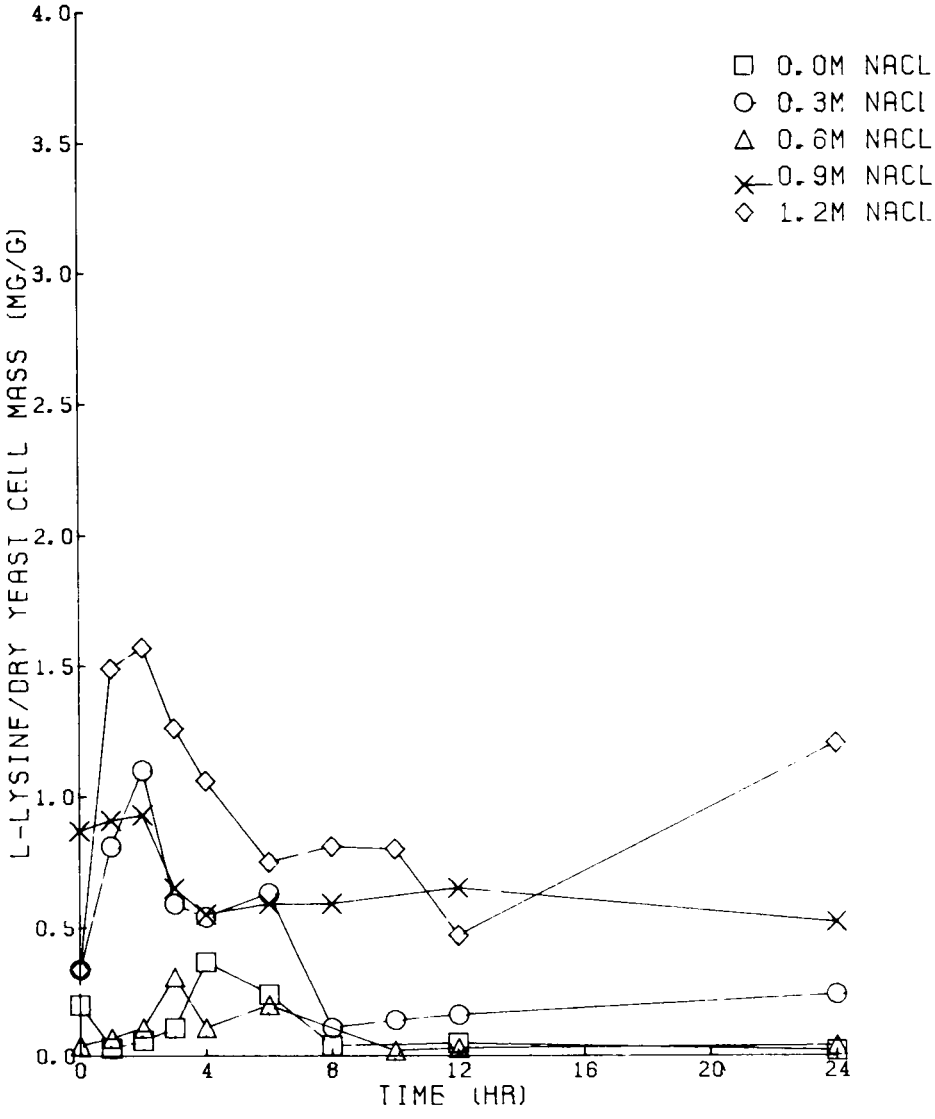

Fig. 2. Production of extracellular L-lysine by *S. cerevisiae* during fermentation of 10% glucose at 1.4 VVM and 0.0–1.2*M* NaCl.

2.1 mg/g at 12 h. On the other hand, the amount of extracellular lysine observed was much larger than at lower NaCl levels.

A further increase in NaCl to 1.2*M* caused an interesting change in pattern. There was an unusually high level of intracellular lysine at 1 h. This was followed by a drop, then a rise to a secondary peak of 2.6 mg/g at 12 h, an incubation time that showed the highest lysine levels in the other NaCl concentrations. The production of extracellular lysine significantly exceeded that at the other NaCl levels and was still high at 24 h.

The growth of the yeast cells was inhibited as the concentration of NaCl increased. In a 24-h period, the maximum dry yeast cell mass for a

0.0*M* NaCl run was 12.2 g/L, compared to 4.6 g/L at 1.2*M* NaCl (see Table 2). Thus the high salt concentration retarded cell growth by nearly 3 times over the no salt fermentation.

It might appear that the higher yield of lysine per gram of yeast cells at 0.6*M* NaCl is caused by the lowered cell reproduction caused by the high NaCl content. This hypothesis is eliminated by the observation that at 10 hr the total lysine content for the 0.6*M* NaCl is almost 40 mg/L fermentation mash, while the lysine content for the 0.0*M* NaCl is just over 20 mg/L (interpolated at 10 h). Thus, the lysine content for the 0.6*M* NaCl fermentation is nearly twice that of the no salt batch, despite having a smaller yeast cell mass. This caused an increase of nearly three times in the intracellular lysine in mg/g cells.

At 1.0 VVM

When the aeration rate was reduced to 1.0 VVM (Fig. 3), intracellular lysine production was lowered, except for 0.9*M* NaCl (see Tables 1 and 2). The values for the two different 0.9*M* NaCl runs were averaged to give the curve shown in Fig. 3. The maximum production of intracellular lysine occurred at 8–12 h in the presence of 0.6 and 0.9*M* NaCl and at 24 h at 0.3*M* NaCl. At the latter concentration of salt, the lysine level was apparently still rising at 24 h.

The extracellular lysine curves in Fig. 4 show the same general pattern as at 1.4 VVM, i.e., significant production of lysine in the early hours, followed by a slow reduction during the rest of the run. The highest level of extracellular lysine was given at 1.2*M* NaCl, as was the case at 1.4 VVM. AT 24 h the extracellular lysine at 1.2*M* NaCl was higher than at the other NaCl concentrations, as it was at 1.4 VVM.

At 1.8 VVM

When the aeration rate was increased from 1.4 to 1.8 VVM, the maximum intracellular lysine production (Fig. 5) for the various salt levels was again reduced from that at 1.4 VVM, except for 0.9*M* NaCl (see Tables 1 and 2). The highest lysine content at this aeration rate occurred at 0.6*M* NaCl.

The general pattern of extracellular lysine production at all NaCl levels at 1.8 VVM was the same as for the other two aeration rates (Fig. 6). At 1.8 VVM the maximum production of extracellular lysine came at 0.6*M* NaCl, since the extracellular lysine at 1.2*M* NaCl was unable to be determined. This was caused by a darkening of the lysine samples as they were boiled (*see* Sampling protocol). This "browning" effect affected the photometric scan and made a quantitative determination impossible.

Comparison of 1.0, 1.4, 1.8 VVM

By comparing Figs. 1–6, several general conclusions can be made. For intracellular lysine, the peak is reached between 8 and 12 h, with the

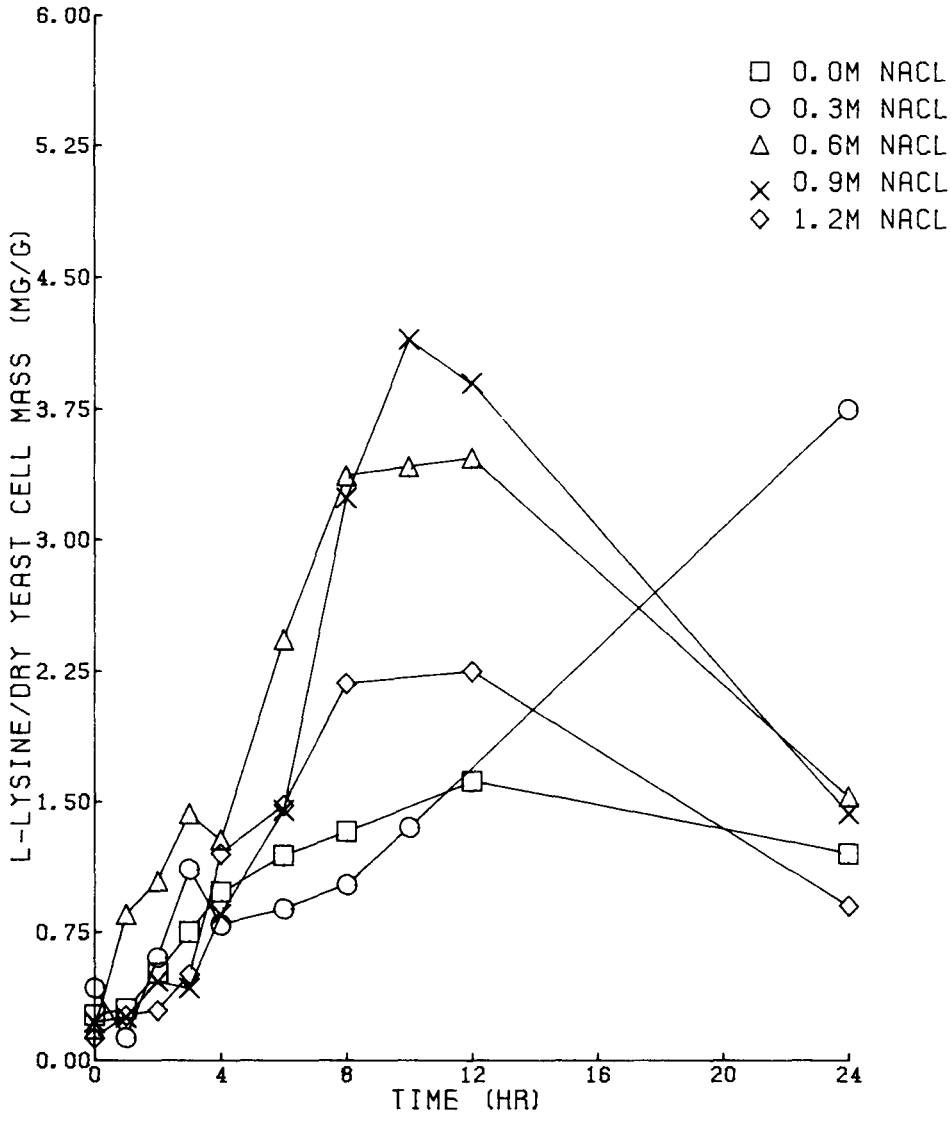

Fig. 3. Production of intracellular L-lysine by *S. cerevisiae* during fermentation of 10% glucose at 1.0 VVM and 0.0–1.2*M* NaCl.

maximum point at 0.6*M* NaCl (with the exception of 1.0 VVM, where the maximum point is at 0.9*M* NaCl). The extracellular lysine starts out (relatively) high and steadily drops as the fermentation proceeds. Extracellular lysine is a maximum for 1.2*M* NaCl (except at 1.8 VVM, where the absence of data for 1.2*M* NaCl for extracellular lysine makes it impossible to determine). Higher levels of NaCl tend to inhibit the growth of the yeast cells during the fermentation at all aeration rates. Despite this lack of growth, the cells produce more intracellular lysine than without any salt. An overall summary of the data in Fig. 7 clearly shows that the maximum point for intracellular lysine production in yeast cells occurs at 0.6*M* NaCl and 1.4 VVM air.

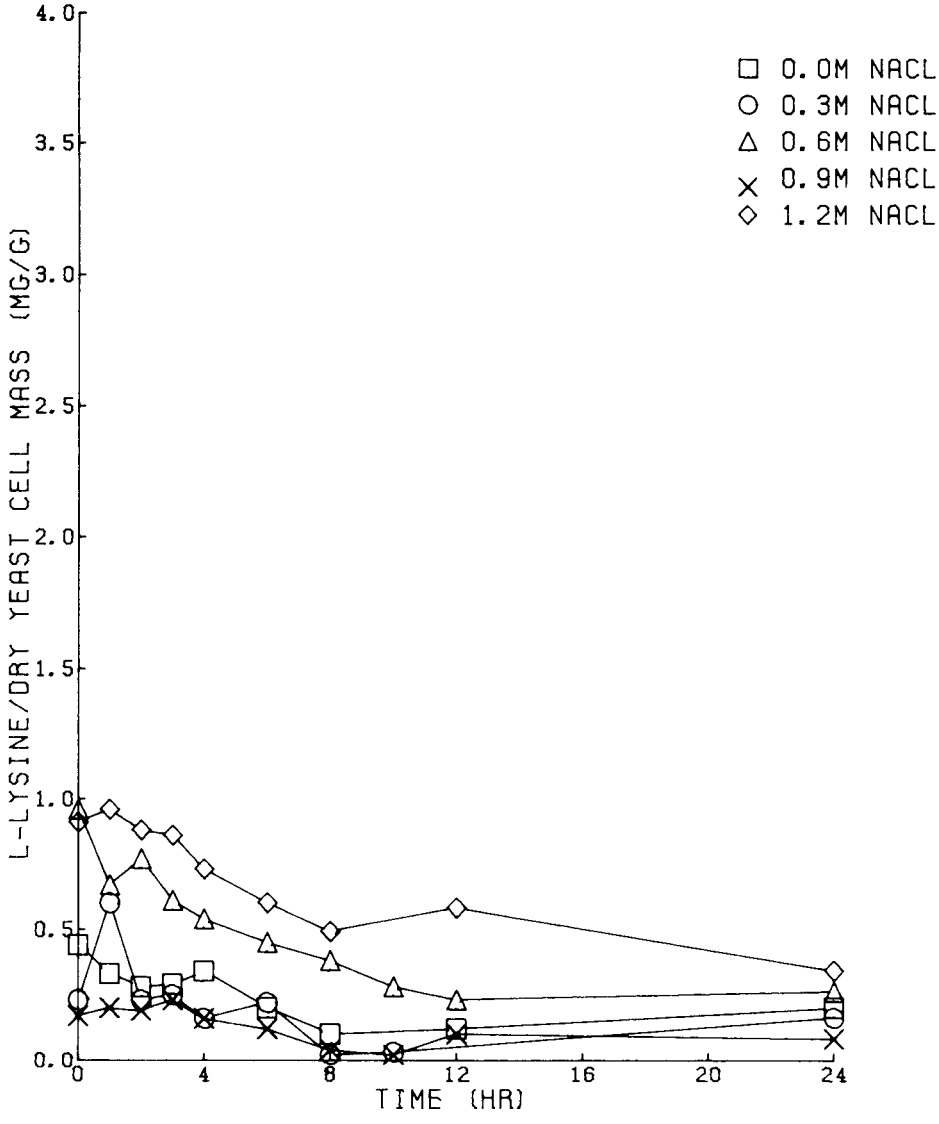

Fig. 4. Production of extracellular L-lysine by *S. cerevisiae* during fermentation of 10% glucose at 1.0 VVM and 0.0–1.2*M* NaCl.

Lag Times in Growth

Ross and Morris (*7*) and Norkranz (*6*) have shown that increasing the NaCl content of the growth medium proportionately lengthens the lag period of the yeast growth curve in liquid culture. Wei et al. (*9*) reported similar inhibition in unaerated semisolid cultures without pH control. It can be seen in Fig. 8 that the lag phase-lengthening effect of salt was confirmed, but that the aeration rate markedly influenced the effect exerted by NaCl.

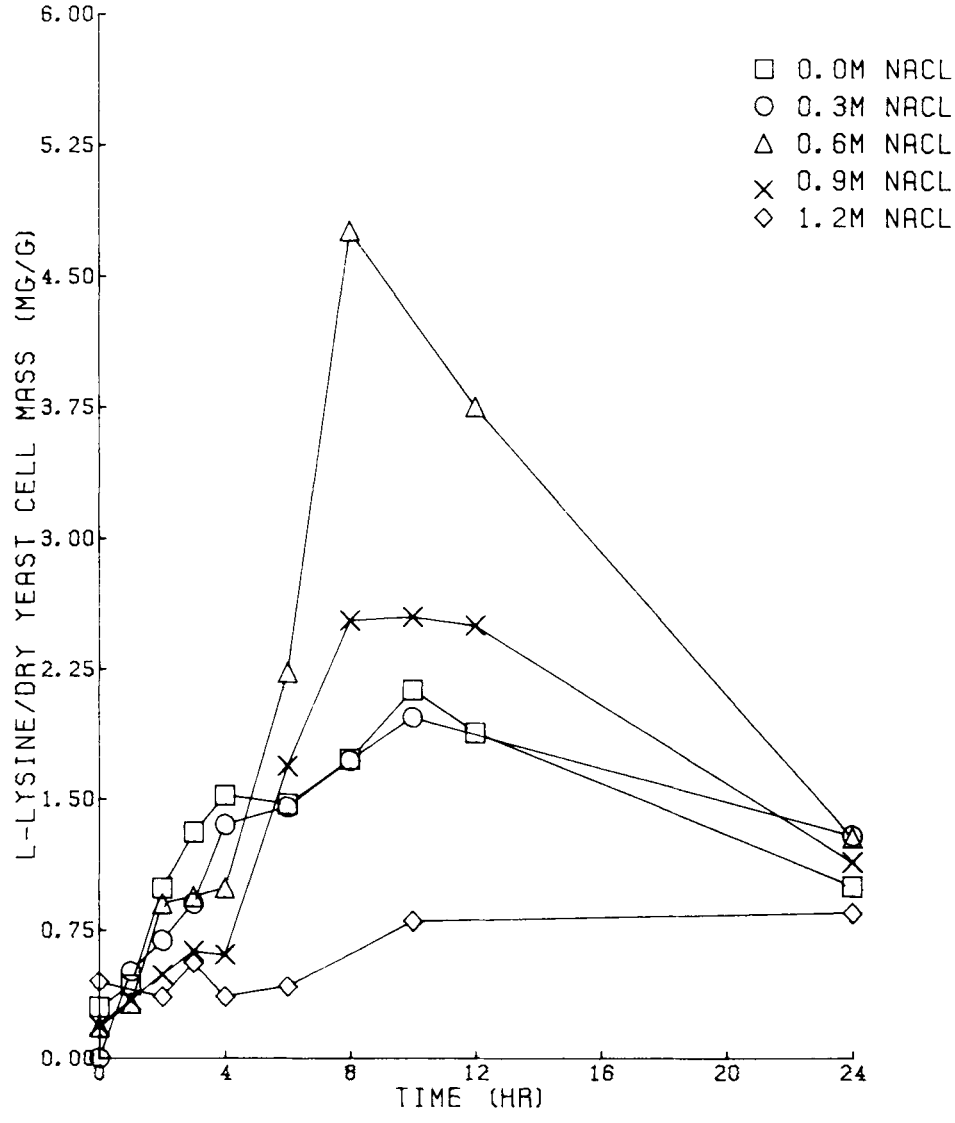

Fig. 5. Production of intracellular L-lysine by *S. cerevisiae* during fermentation of 10% glucose at 1.8 VVM and 0.0–1.2*M* NaCl.

Determination of the length of the lag phase of the growth curve is carried out to help elucidate the behavior of yeast cells during fermentation. The lag period might be defined as the time necesssary for cells to adapt to a changed environment. It is postulated that the lag phase would be shortened by favorable conditions, such as high aeration rates and low NaCl concentrations.

The calculation of lag time is demonstrated in Fig. 9. The straight line *ab* is drawn parallel to the *x*-axis at the initial cell concentration, i.e., the amount of dry yeast initially weighed and added to the growth medium, about 2.5 g/L yeast cell mass. A second straight line *cd* parallel to the *x*-axis is drawn through the lowest yeast cell concentration actually

observed during the first few hours of fermentation, in this case 2.3 g/L. The line *cd* is needed only when the lowest observed cell concentration differs significantly from the initial inoculum. The line *ef* represents the average of the two values, e.g., 2.4 g/L.

Next, the line *gh* is drawn representing the early growth curve developed by a linear least-squares fit of the growth data. The growth curve oscillates about the least-squares line. Finally, the line *ij* is drawn parallel to the least-squares line and tangent to the trough of the second oscillation at about the 4-h point. This tangent is extended to intersect line *ef* at point *k*, which locates the lag time.

Lag times shown by varying growth conditions are plotted in Fig. 8. Although the values in Fig. 8 are no more accurate than 25%, the paths of the curves confirm the supposition previously made that lag time is decreased by lowering the NaCl concentration and by increasing the aeration rate.

Oscillations

The related phenomena of biological rhythms and physiobiological oscillations have been of great interest in recent years. For example, Kraepelin (*4*) described the oscillations shown in populations of starving *S. cerevisiae*. The curve in Fig. 9 is representative of the oscillations in yeast cell mass concentration observed in most of the fermentations in the present study. In general, the period of the oscillations lengthened as the fermentation proceeded, while the amplitude decreased during the first few hours and increased later.

TABLE 1
The Effect of Aeration Rate on the Highest Concentration of Intracellular Lysine, mg/g Dry Yeast Cell Mass, at 8–12 h Fermentation Time and at Various Concentrations of NaCl[a]

	Aeration rate, VVM		
NaCl, *M*	1.0	1.4	1.8
0.0	1.5[b]	2.2	2.1
0.3	1.4[c]	3.1	2.0
0.6	3.7	5.8	4.8
0.9	3.7	2.1	2.6
	4.8[d]		
1.2	2.3	2.6[e]	0.8

[a]In most runs the maximum production was recorded during this incubation time.
[b]Milligrams of intracellular lysine/g dry yeast cells.
[c]Actually the maximum was 3.8 mg/g at 24 h.
[d]Two runs were made.
[e]Actually the maximum was 3.2 mg/g at 1 h.

TABLE 2
Lysine Production at Various NaCl Concentrations Mg L-Lysine/g Dry Yeast Cells

1.0 VVM Air

Ferm. time (hr)	0.0*M* NaCl			0.3*M* NaCl			0.6*M* NaCl			0.9*M* NaCl			1.2*M* NaCl		
	Yeast cells (g/l)	Intra-cellular	Extra-cellular	Yeast cells (g/l)	Intra-cellular	Extra-cellular	Yeast cells (g/l)	Intra-cellular	Extra-cellular	Yeast cells (g/l)	Intra-cellular	Extra-cellular	Yeast cells (g/l)	Intra-cellular	Extra-cellular
0	2.98	0.26	0.44	2.60	0.42	0.23	2.80	0.18	0.96	2.84 2.64[a]	0.07 0.37	0.07 0.27	2.30	0.13	1.91
1	3.61	0.30	0.33	2.82	0.13	0.60	2.82	0.85	0.67	2.80 2.74	0.11 0.40	0.07 0.33	2.70	0.26	0.96
2	3.36	0.51	0.28	3.40	0.60	0.23	2.98	1.04	0.77	2.90 2.60	0.42 0.50	0.10 0.27	2.74	0.29	0.88
3	3.76	0.75	0.29	3.60	1.11	0.25	3.44	1.43	0.61	3.60 2.84	0.47 0.37	0.17 0.28	2.80	0.50	0.86
4	4.08	0.98	0.34	4.40	0.79	0.16	4.28	1.28	0.54	3.72 3.40	0.62 1.14	0.11 0.24	3.00	1.20	0.73
6	5.60	1.19	0.20	5.08	0.88	0.22	3.96	2.43	0.45	4.00 4.08	1.17 1.91	0.13 0.12	3.32	1.48	0.60
8	7.36	1.33	0.10	6.16	1.02	0.02	4.48	3.73	0.38	4.12 3.92	2.84 3.67	0.05 0.03	3.48	2.18	0.49
10	—	—	—	7.84	1.35	0.03	5.40	3.42	0.28	4.28 4.04	4.75 3.57	0.02 0.02	—	—	—
12	9.92	1.62	0.12	—	—	—	5.68	3.47	0.23	4.44 4.16	4.46 3.39	0.09 0.10	3.64	2.25	0.58
24	10.40	1.20	0.20	5.60	3.75	0.16	7.44	1.51	0.26	5.52 5.16	1.43 1.43	0.09 0.08	4.64	0.93	0.34
0	3.40	0.28	0.20	2.35	0.30	0.34	2.50	0.40	0.04	2.52	0.44	0.87	1.45	1.11	0.34
1	3.32	0.84	0.03	2.30	0.19	0.81	2.74	0.60	0.07	2.74	0.59	0.91	1.68	3.21	1.49

1.4 VVM AIR																
2	3.40	0.87	0.06	2.45	0.66	1.10	3.72	0.72	0.11	2.80	0.57	0.93	2.15	2.19	1.57	
3	3.80	1.05	0.11	2.70	1.63	0.59	3.60	1.00	0.31	3.52	0.77	0.65	2.30	2.26	1.26	
4	4.60	0.80	0.37	3.10	1.72	0.54	3.65	1.36	0.11	3.64	0.99	0.55	2.63	2.08	1.06	
6	7.36	0.93	0.24	4.45	2.55	0.63	5.00	1.98	0.20	3.70	1.25	0.59	2.80	2.07	0.75	
8	8.40	2.01	0.04	6.15	2.49	0.11	—	—	—	3.76	1.62	0.59	2.72	1.52	0.81	
10	—	—	—	7.17	2.01	0.14	6.48	5.84	0.02	—	—	—	2.62	1.51	0.80	
12	10.56	2.16	0.05	4.48	3.12	0.16	7.84	4.28	0.03	3.84	2.06	0.65	2.40	2.57	0.47	
24	12.24	1.13	0.02	5.31	1.89	0.24	7.60	2.04	0.04	4.60	0.85	0.52	2.68	1.08	1.20	
1.8 VVM AIR																
0	2.70	0.30	0.33	2.80	0.00	0.50	2.86	0.18	1.01	2.62	0.19	0.53	2.46	0.45	—[b]	
1	2.86	0.43	0.28	2.94	0.51	0.20	2.80	0.32	1.00	2.90	0.34	0.45	2.88	—	—	
2	3.64	0.99	0.30	3.48	0.69	0.17	3.20	0.90	0.91	2.86	0.49	0.35	2.80	0.36	—	
3	3.90	1.31	0.43	3.76	0.90	0.27	3.28	0.94	1.01	3.00	0.63	0.27	2.86	0.56	—	
4	4.60	1.52	0.46	4.36	1.35	0.23	3.72	0.99	0.89	2.94	0.61	0.34	3.06	0.36	—	
6	7.36	1.47	0.15	6.00	1.45	0.22	4.72	2.23	0.59	3.60	1.69	0.17	3.10	0.42	—	
8	11.52	1.73	0.08	8.72	1.72	0.03	5.44	4.76	0.39	3.72	2.53	0.16	3.40	—	—	
10	9.20	2.13	0.13	9.68	1.97	0.04	—	—	—	4.12	2.55	0.17	3.76	0.80	—	
12	9.68	1.88	0.10	—	—	—	8.00	3.75	0.18	4.28	2.50	0.14	—	—	—	
24	10.96	1.10	0.15	9.76	1.29	0.10	8.24	1.28	0.32	5.08	1.14	0.14	3.86	0.85	—	

[b] Two runs were made
"Browning" effect made results impossible to determine

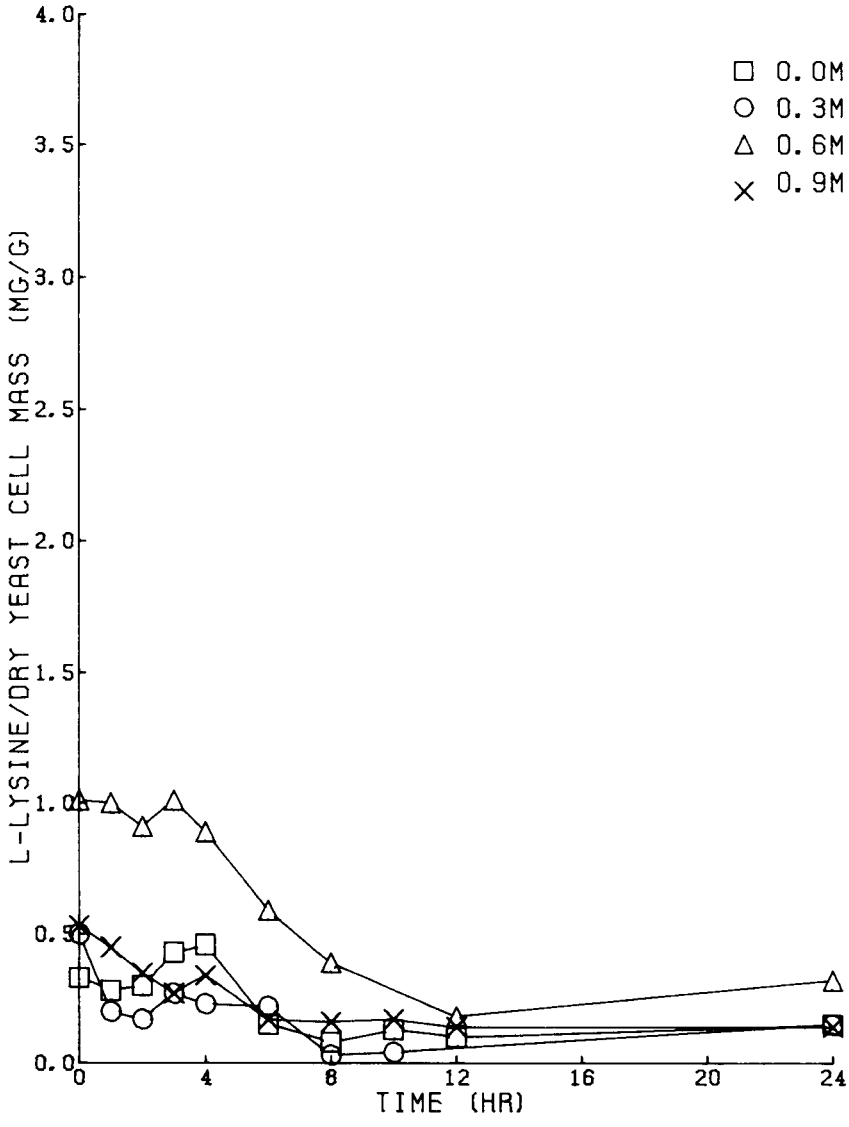

Fig. 6. Production of extracellular L-lysine by *S. cerevisiae* during fermentation of 10% glucose at 1.8 VVM and 0.0–0.9*M* NaCl.

DISCUSSION

The results of the search for the local optimum in the lysine per cell response surface as a function of NaCl concentration and aeration rate are summarized in Fig. 7. With an approximate variance in the data for lysine/cell mass of +/− 1.0 mg/g, it appears that the optimum is at 0.6*M* NaCl (roughly the concentration of NaCl in sea water). The optimum aeration rate then lies in the range 1.4–1.8 VVM.

Eroshin et al. (2) used a simple quadratic expansion with two independent variables to describe the yield coefficient of Baker's yeast under

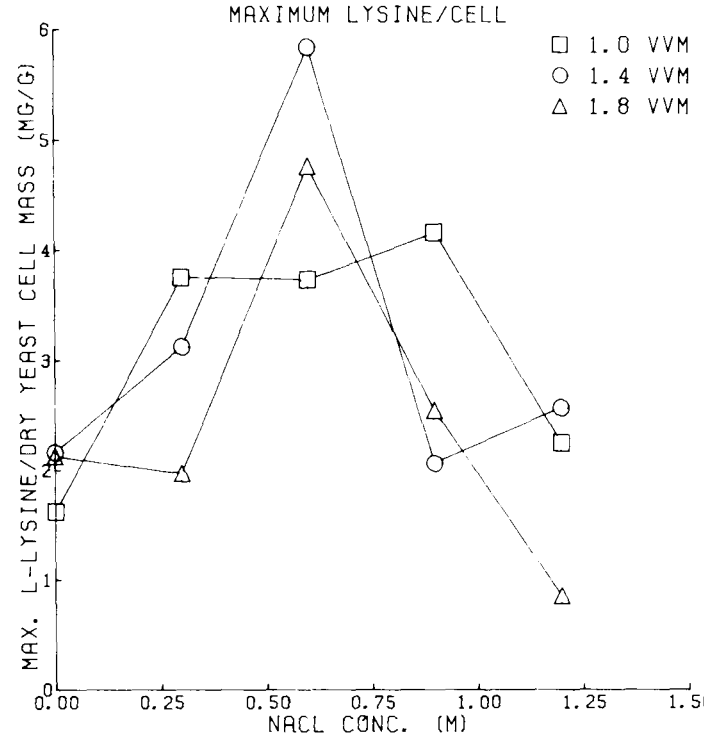

Fig. 7. The maximum concentration of intracellular L-lysine per gram of cell mass produced by varying the NaCl concentration from 0.0–1.2*M* and the aeration rate from 1.0 to 1.8 VVM.

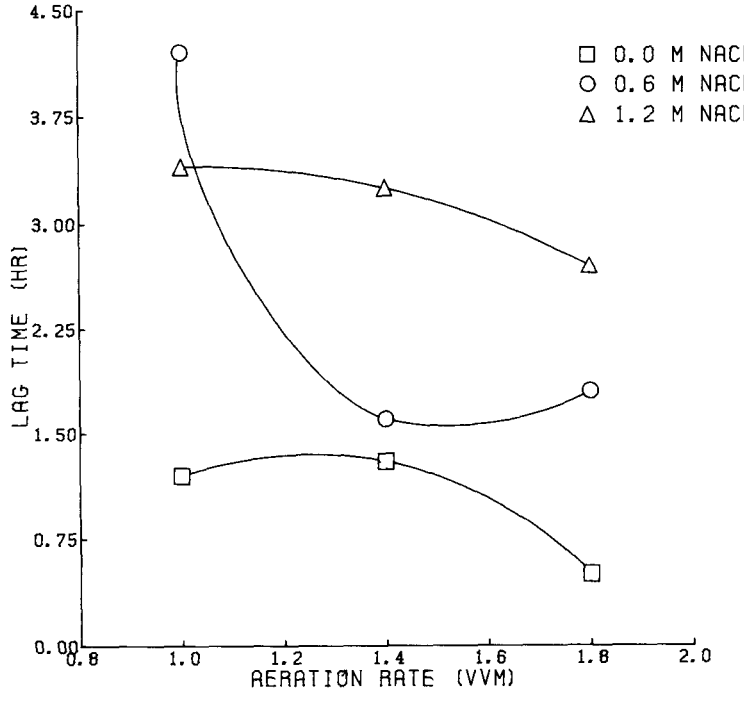

Fig. 8. Effect of aeration rate on the length of the lag phase of the yeast growth curve in the presence of varying concentrations of NaCl.

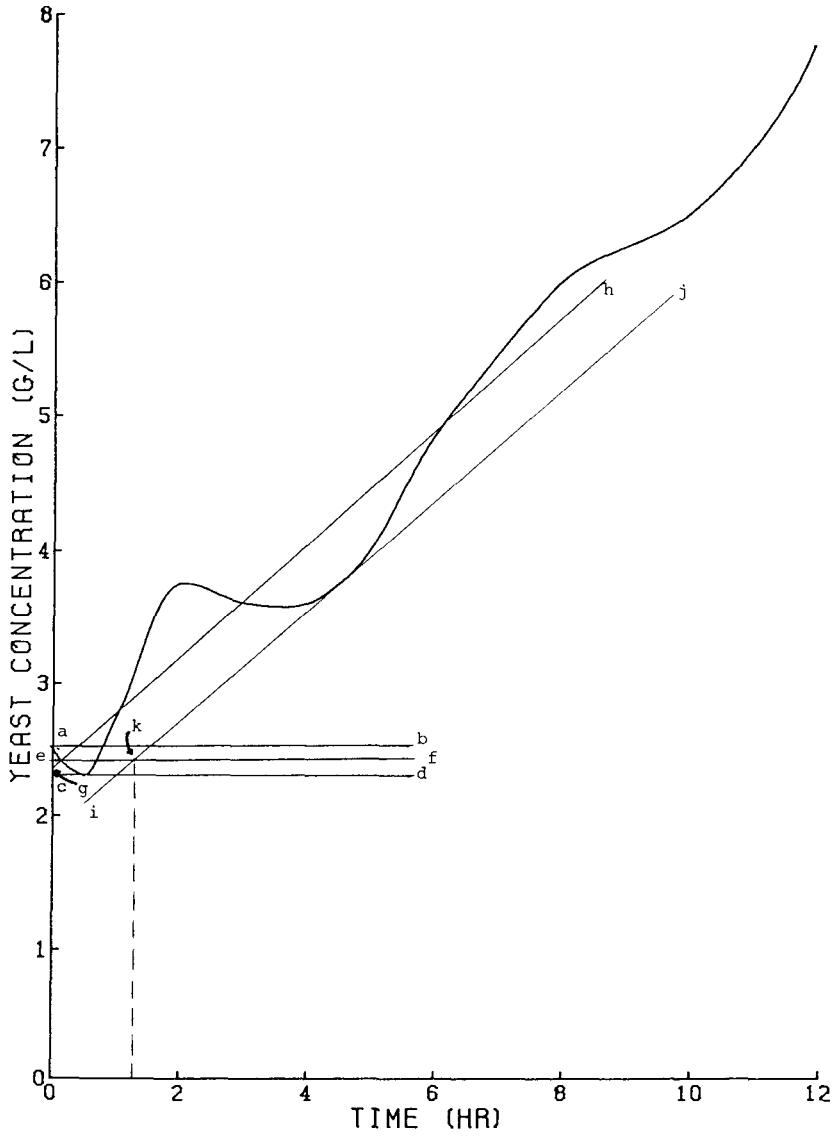

Fig. 9. Oscillation in yeast cell mass in the early growth period of yeast fermentation of 10% glucose. Example of methods of calculation of lag time: Line *ab*, original inoculum of yeast cells, g/L; Line *cd*, minimum observed concentration of yeast cells, g/L; Line *ef*, average of original inoculum and minimum observed concentration of yeast cells, g/L; Line *gh*, base line of growth oscillations; Line *ij*, line parallel to oscillations base line and tangent to trough of oscillation.

the influence of temperature and pH. Following this procedure, a quadratic relationship for the maximum specific lysine content as a function of salt concentration and aeration rate was determined.

The basic form of the regression equation used was:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1x_2 + a_4x_1^2 + a_5x_2^2 \quad (1)$$

where y = maximum specific lysine yield in mg/g, x_1 = aeration rate in VVM, and x_2 = concentration of NaCl in mol/L.

A regression fit using the packaged program MINITAB on the DEC-10 digital computer gave the model:

$$y = -3.74 + 8.32x_1 + 9.47x_2 + 1.66x_1x_2 - 2.93x_1^2 - 5.82x_2^2 \quad (2)$$

over the range:

$$1.0 \leq x_1 \leq 1.8 \text{ VVM}$$

$$0.0 \leq x_2 \leq 1.2M \text{ NaCl}$$

The standard deviation of y about the regression line was 1.06 mg lysine/g yeast cells. An analysis of the T-ratios of the coefficients confirmed that the salt concentration had a greater impact than the aeration rate in yield.

In an effort to simplify Eq. (1), forms with fewer coefficients were also fitted to the data. The equation with the highest degree of confidence was:

$$y = 1.85 + 9.19x_2 - 1.50x_1x_2 - 5.82x_2^2 \quad (3)$$

This model gave the smallest standard deviation of any form tried, 0.995 mg lysine/g yeast cells. In addition, of all curve fits, the T-ratios for the coefficients in Eq. (3) gave the highest confidence levels.

Using Eq. (3) in the noted range of the variables, the relative importance of the two variables was indicated. The "salt terms" all had much more influence than the aeration terms.

When the cross product term, x_1x_2, of Eq. (3) was omitted, the equation did not change significantly:

$$y = 1.82 + 7.30x_2 - 5.95x_2^2 \quad (4)$$

The standard deviation of y about the regression line was 1.04 mg lysine/g yeast cells.

Further analysis shows that Eq. (4) gives a parabolic curve with the maximum inflection point at 0.61M NaCl, which was essentially the maximum found experimentally (*see* Table 1).

Although no aeration term is explicitly present in Eq. (4), the aeration rate is embedded in the constant, 1.82. A minimum aeration rate is required, otherwise there is a negligible response in lysine production, as shown by Jensen and Shu (*3*).

By taking the partial derivative of y with respect to x_2 [in Eq. (3)], while holding x_1 constant, a similar analysis can be performed, showing that for the aeration rates of 1.0, 1.4, and 1.8 VVM, the maximum peaks of lysine production occur at 0.66, 0.61, and 0.57M NaCl. These results indicated the nature of the polynomial fit in order to describe the experimental data.

To measure the relative importance of the polynomial coefficients, a closer look at the T-ratios was taken. For Eq. (2), only the two "salt terms" showed high T-ratios—about 3.0. The other four terms had T-ratios under 1.0. In Eq. (3), the air-salt cross product term had a T-ratio

of 1.50 while the T-ratios of the other terms were about 3.5. For Eq. (4), the removal of the cross product term produced T-ratios for all terms of about 3.33. The high values for the T-ratios for every term in Eq. (4) indicate that each of the three terms is important.

In summary, in the range of interest, the effect of NaCl upon the maximum specific lysine yield of Baker's yeast was of much greater magnitude than that of the aeration rate in batch fermentation. Results for two independent variables and a six constant equation are essentially equivalent to results given by the truncated three constant equation using one independent variable. The latter only served to indicate more strongly the effect of salt concentration over aeration rate. It should be emphasized, however, that some air is needed for the lysine effect to be observed. For a moderate range of aeration rates, the point of maximum specific lysine production occurred at about 0.6M NaCl, the approximate concentration of seawater.

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