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# A SYSTEM FOR LABORATORY FERMENTATIONS AND ITS APPLICATIONS

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A Dissertation Submitted to the Faculty of Science University of the W :watersrand, Johannesburg for the degree of Master of Science.

Johannesburg 1984.

ABSTRACT.

A laboratory system has been developed that gives fermentation patterns closely following those of brewery fermentations. Fermentations are conducted in 2 litre glass tubes, height 1,5 metres, inner diameter 47 mm. For convenience and compactness the fermentation tubes are designed to be displayed around the inside walls of transparent polyvinyl chloride modules, 8 tubes per module.

The laboratory fermentation system has been used to develop a test for predicting malt quality. Malt samples are mashed in the laboratory using a temperature programme similar to that used in the brewery. Worts resulting from the mash are fermented for 8 days. Results of these fermentations indicate the suitability of the worts for brewery fermentation.

The laboratory fermentation system has also been used to study yeast behaviour during fermentation. The parameters that have been investigated are: optimum aeration/oxygenation during propagation and fermentation; the effects of wort gravity/ composition of the propagation and fermentation medium; the effects of yeast conditioning; preliminary screening of alternative brewing yeast strains.

Declaration

I declare that the work forming the basis of this dissertation is my own work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Lynette Kruger.

day of 1984.

Dedicated to my Parents,

Hans and Maureen.

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PREFACE

This study was undertaken to develop a laboratory fermentation system that gave results very similar to those of brewery fermentations. Occasional problems encountered in certain of our breweries prompted the development of a system for predicting malt quality. Some of this work has been published in the M.B.A.A. Technical Quarterly 19 (1) 45-51 (1982).
The realisation that the knowledge of our brewing yeast strain Saccharomyces uvarum(x) was limited led to studies on the behaviour of strain (x) during fermentations.

I would like to acknowledge the technical assistance of Cathy Alcock in some of this work and the useful comments and discussions with David Ryder, Jim Murray, Barry Axcell and Eddie Gatner.

I also thank the Directors of South African Breweries Limited for permission to submit this dissertation.

# 1. INTRODUCTION

1.1. Laboratory fermentation tests have been used by many workers (12,22,29,39,49,66). The EBC Analytica Microbiologica (14) describes a system for the evaluation of brewing yeast strains. However, there is a certain lack of information regarding the similarity of the laboratory fermentation systems to brewery fermentations. Workers at the Kirin Brewery (24) studied 17 shapes and sizes of glass vessels for fermentation tests. They showed some reproducibility between fermentations in four of these tubes and brewery fermentations. One of these types is currently being used at Kirin for assessing the fermentability of malts.

Investigations were carried out to determine if a 1 litre fermentation tube, as used by the Kirin Brewery group, or a 2 litre fermentation tube (similar to that described by the E.B.C. Analytica Microbiologica) would be suitable for laboratory fermentation studies to reflect the process conditions at South African Breweries. The important considerations were reproducibility of results and the similarity between laboratory fermentations and plant fermentations.

The initial comparative studies between plant and laboratory fermentations were conducted in normal gravity (11,3°P) wort. However, as there is an increasing trend to brew at higher gravities further comparative studies were run in high gravity (14,1°P) wort. The advantages of brewing at high gravity are many-fold. (20,57,67,68.) The major advantages are savings in capacity and therefore in cost.

Therefore one of the aims of this project was to develop a system that gave good reproducibility of results and in which fermentations (for both normal and high gravity) closely followed comparable patterns in brewery fermentations.

1.2. Of increasing concern to the brewing industry is the problem of the value of standard malt analyses (7,8,9,15,34,45,46,47,64, 65). This concern reflects an upsurge of changes in the malting and brewing industries, e.g. increasing number of malting barley varieties, rapid malting methods and accelerated brewhouse and fermentation strategies.

This may be attributed to three considerations,
viz: a) the differing methods used by the three recommending bodies, ie. the Institute of Brewing (IOB), the European Brewing Convention (E.B.C.) and the American Society of Brewing Chemists (ASBC), which result in a certain lack of standardisation for judging brewing malts (7,8,64,65)
b) the interpretation of the various analytical data which may be subject to individual judgement and opinion (36), and most significantly,
c) the relevance of the data obtained from conventional recommended methods where individual malts, which may produce very similar results on analysis, may each behave quite differently in brewing (44,64,71).

Specific fermentation problems, ie, premature and/or heavy flocculation of our bottom fermenting yeast populations - resulting in 'hanging' or tailing fermentations - have occasionally been encountered at certain of our breweries. Investigations showed these problems to be associated with particular batches of malt. However, there were no indications from standard malt analyses to explain the reason(s) for such brewing yeast behaviour.

Flocculation of brewers yeast has been widely studied (4,18,40,41,55,59,60). In particular, the Japanese research group

at the Kirin Brewery Co. Ltd. have been engaged for a number of years in premature flocculation studies of bottom fermenting yeasts in primary fermentation (16,17,28,36,37,38,50,62) These studies concluded that a protein-linked carbohydrate fraction present in malt and wort was responsible for the premature flocculation phenomenon. As a result of this work Kirin now screen all malts, intended for use in their process, for premature flocculation inducer by means of a laboratory fermentation test.

The feasibility of using a laboratory fermentation system to predict malt quality for use in South African Breweries was investigated. The parameters investigated were: pitching procedure, mashing procedure and development of a grading system for malts. Experiments were designed to follow as closely as possible the systems used in the brewery. The relationship between an all malt laboratory fermentation test and brewery fermentation (incorporating adjuncts) was necessary to establish the limits for a malt grading system. The aim of this work was therefore to establish a malt grading system, related to fermentation performance that would be of practical use to the brewer.

1.3. Beer character and flavour is determined largely by the following factors:

a) Ingredients
b) Brewhouse procedures
c) Fermentation process
d) Cellaring procedures.

Of these factors the fermentation process is possibly the most important. The overall characteristics of a beer are ultimately determined during the fermentation stage. Fermentation is the process whereby wort is changed into beer by the acitivity of

yeast. The time required for wort to be fermented can vary widely and is influenced by temperature, wort composition, pitching rate, yeast strain and the type of plant. The time required to ferment worts of varied composition to a given degree depends on the extent of exponential growth reached in the early stages of fermentation. In 'sticking' or 'hanging' fermentations growth ceases too early. To get rapid and full attenuation it is necessary somehow to extend the growth phase (30,34,42,54,58).

Growth during fermentation can be regulated by either amino nitrogen or oxygen supply (30,31,34,58,[illegible]1,70). Oxygen, either prior to or during fermentation, is required by yeast cells almost exclusively for lipid synthesis, ie. sterols and unsaturated fatty acids (1,2,11,25,31,32,33,56,58,70).

In biosynthesis of yeast ster[illegible] ther[illegible] are several oxygen - requiring reactions. Cyclis[illegible] of squalene to yield the first sterol on the pathway, lanosterol, is one such reaction, while the other reactions involved include demethylation and desaturation reactions that take place during conversion of lanosterol to ergosterol. The requirement for molecular oxygen in sterol biosynthesis is greater than that for the synthesis of unsaturated fatty acids (3,56).

Different yeast strains vary in their requirement for oxygen (23,26,32). Four classes have been proposed by Kirsop (32) to classify yeasts according to their oxygen demand. Yeasts falling into classes 03 and 04 (high oxygen demand) are more susceptible to control by oxygen during fermentation than those belonging to classes 01 and 02 (low oxygen demand) (25,26,32).

There is little agreement on the optimum aeration/oxygenation regime during propagation (61), although various workers (31,32,58,68) have investigated methods and times of aeration prior to and during fermentation.

The major brewing yeast used at South African Breweries Saccharomyces uvarum (x) has a high oxygen demand and is classified as belonging to group 03. Oxygen therefore plays an important role both during propagation and fermentation of this yeast strain. The questions that need answering in these respects are:

a) Should one aerate or oxygenate Saccharamyces uvarum (x) during laboratory propagation for maximum benefit of both yeast crop and subsequent yeast performance?

b) What is the optimum method/dissolved oxygen level of aeration/oxygenation of a high gravity wort for Saccharomyces uvarum (x) to ferment in the desired manner?

In this study comparisons were made between continuous coarse aeration and oxygen supply, various aeration/oxygenation regimes being investigated to determine the most appropriate for our yeast and wort composition.

1.4. As mentioned earlier the time required for wort to be fermented can vary widely. When yeast is pitched into wort it is introduced into an extremely complex medium comprising simple sugars, dextrins, amino acids, peptides, proteins, vitamins, ions, nucleic acids and numerous other compounds. When conditions of temperature, pitching rate, yeast strain and oxygen are constant there are still marked variations in fermentation due to wort differences (34). Wort composition is thus a major contributing factor in fermentation. For example, the efficiency with which oxygen is utilised by yeast depends on the wort composition (21,32,70). High gravity adjunct worts do not ferment as well as all malt worts (29). In the light of these considerations the effect of various wort compositions/gravities of both propagation medium and fermentation medium on the performance of Saccharomyces uvarum (x) were investigated.

1.5. There is a lack of information on the effects of 'yeast conditioning' on fermentation performance. However, practical experience has shown that successive generations of yeast improve

with respect to fermentation performance. Experience has also shown that the first full brew following propagation is sluggish. The observation that the best method for laboratory propagation of yeast is the one that has the most passages of yeast through wort (3.3.2.) suggest that the acclimatisation of the yeast to wort (or 'conditioning' of the yeast) is important in determining the fermentative power of the yeast. Investigations were carried out to determine the effects of yeast 'conditioning' on Saccharomyces uvarum(x). Various propagation systems with differing numbers of passages through wort were undertaken in the laboratory.

The novel concept of 'pre-conditioning' a starting culture occured to the writer. This would involve making slope cultures on MYGP agar from the last stage of propagation of a 'conditioned' yeast. The standard method for making stock slope cultures involves using an overnight culture of yeast. If the 'conditioned' yeast maintains its beneficial character on solid media, then after a standard laboratory propagation it should display superior fermentation performance compared with a stock slope culture propagated in a similar manner. In this study this concept was explored.

1.6. Saccharomyces uvarum (x) is relatively sensitive with respect to its oxygen demand (Group 03) and also its response to worts of varying composition. As South African Breweries is using a larger percentage of locally produced malt for wort production and the possibilities for manipulation of wort composition are limited it appeared prudent to conduct preliminary screening of alternative brewing yeast strains. Maule (44) outlines some criteria for selection of a new yeast strain. These being:

1.) Rate of attenuation.
2.) Sedimentation.
3.) Yeast Growth
4.) Stability against mutation.
5.) Flavour.
6.) Special properties.

The objective of this study was to define a bottom fermenting brewing yeast strain with a much more robust physiological make-up than Saccharomyces uvarum (x). Two emminently desirable characteristics in an alternative yeast strain would be firstly the ability to ferment well in air saturated wort without creating an oxygen deficiency syndrome as in strain (x), and secondly the strain of choice would be relatively insensitive to changes in malt/wort composition (c/f strain (x)).

The microbiological culture collection at Central Laboratory contains a number of bottom fermenting brewing yeast strains of unknown potential. These were screened in the 2 litre fermentation tubes (2.3) for their ability to ferment at air saturation in wort comprising a high percentage (80%) of local malt.

## 2. MATERIALS AND METHODS.

2.1. Saccharomyces uvarum (x) is the major brewing strain used at South African Breweries.

Strain description:

Fermentation: glucose, galactose, sucrose, maltose and raffinose are rapidly fermented. The fermentation of melibiose is delayed. Lactose is not fermented.

Assimilation of carbon compounds: glucose, galactose, sucrose, maltose, trehalose, melibiose, raffinose, ethanol and DL lactic acid are utilized. L-sorbose, cellobiose, lactose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, glycerol, i-erythritol, ribitol, galactitol, D-glucitol, D-mannitol, alpha-methyl-D-glucoside, salicin, succinic acid, citric acid, i-inositol, potassium gluconate and methanol are not utilized.

Splitting of arbutin: absent.

Assimilation of nitrogen compounds: Neither potassium nitrate, sodium nitrite nor ethylamine hydrochloride are utilized.

Growth in vitamin medium: Absent.

Growth in 10% sodium chloride + 5% glucose in yeast nitrogen base: Absent.

Growth at 37°C: Absent.

Formation of amyloid material: Absent.

Splitting of fat: Absent.

Formation of pseudohyphae on corn meal agar: Absent.

Ascospore formation: Scant. Diploid cells are converted direct into asci;

1 - 2 spheroidal spores per ascus.

2.2. Worts A,B,C and a,b refer to different batches of standard production worts.

Worts A and B: original gravity 11,3°P
35% adjunct, maize grits
pH 5,2.

Wort C : original gravity 11,3°P
all malt wort
pH 5,2.

Worts a & b: original gravity 14,1°P
35% adjunct, maize grits.
pH 5,2.

2.3. Dimensions of Fermentation Vessels Investigated.

| | Kirin 1 litre Cylinders (23) | 2 litre Fermentation tubes |
|---|---|---|
| Overall length(mm) | 1040 | 1500 |
| Inner diameter of tube(mm) | 35 | 47 |
| Cone angle | - | 45° |

2.4. Design of a suitable System.

For convenience and compactness, the fermentation tubes were designed to be displayed around the inside walls of transparent polyvinyl chloride modules (inner diameter : 310 mm), 8 tubes per module.

Sampling was effected by means of a glass sample tap (2 mm bore) situated approximately midway up the fermentation tube. A 2 mm glass capillary tube extended into the centre of the tube for representative sampling.

A soft polyvinyl chloride gasket achieved an effective seal for the fermentation tube to protrude through the shell, at the sample

point and at the bottom of the tube. A glass tap (10 mm bore) was attached by means of P.V.C. tubing to the bottom of the fermentation tube for cropping yeast and emptying the tubes. A 13 mm glass ball, suspended from a length of nylon gut, was placed at the bottom of each tube to prevent the yeast from settling below the cooling water level (fig.1.a.)

For these studies, two fermentation modules were linked to one Lauda K4 refrigerated water bath to achieve attemperation (fig.1.b.)

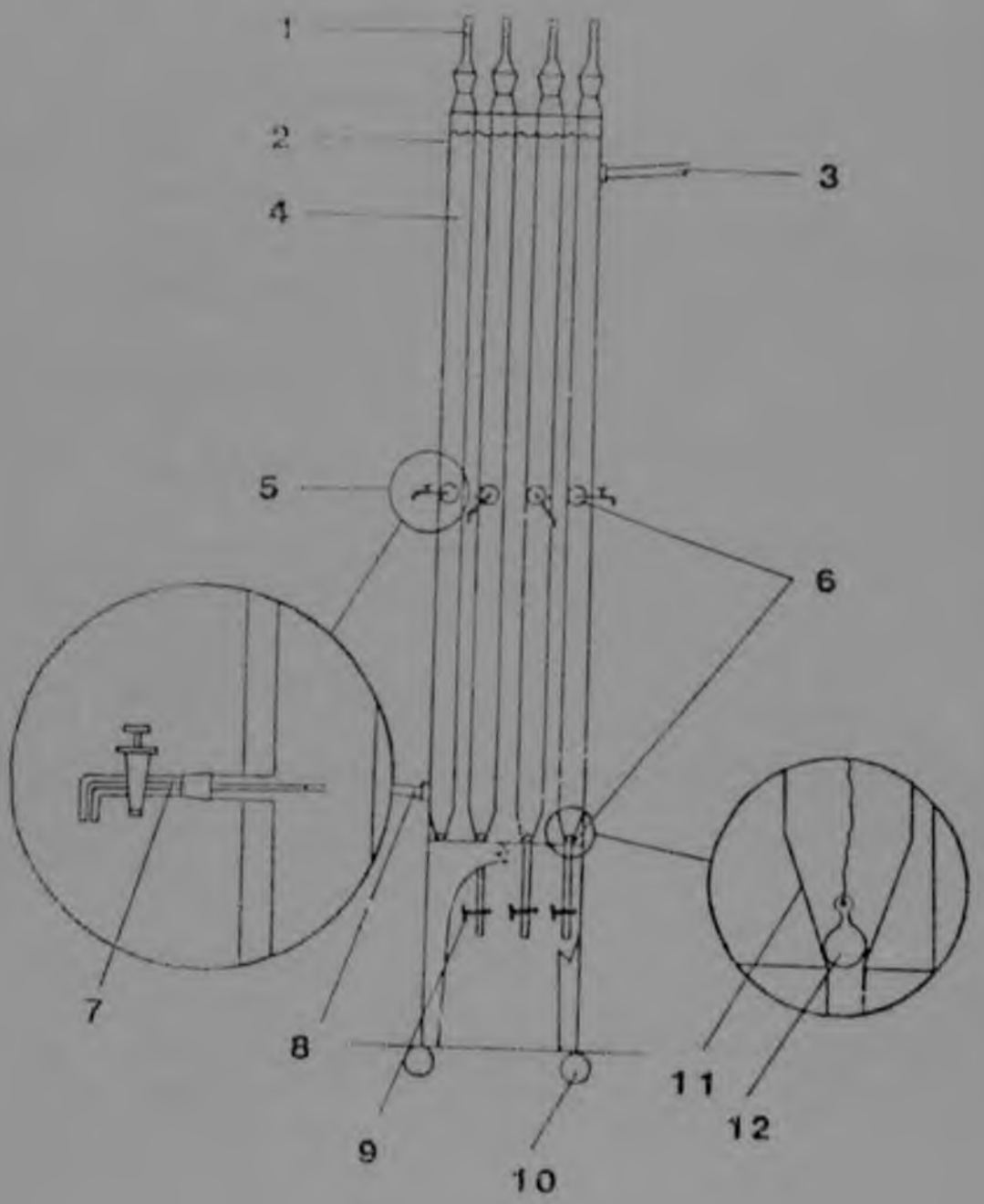
2.5. Mash Bath. (fig.2).

The stainless steel mash bath was locally built according to S.A.Breweries specifications. This included provision for 12 stainless steel mashing containers, each with a total working capacity of 2,9 litres of liquid. Stirring was effected by means of twin bladed impellers operating at a speed of 120 r.p.m.

The unit was operated by means of a Saftronics VM-ZK6 stator current controller. Temperature control was achieved by a Honeywell Versatrak programmer hooked to a Honeywell Versapack II temperature regulator. A consistent temperature rise of 1°C per minute was achievable.

2.6. Pitching Procedure

Unless otherwise stated all pitching of wort was carried out in the following manner: The consistency of the yeast was measured by method 2.10. The required volume of yeast was measured out and added to the wort. In all cases the volume of yeast was such to give a pitching rate of 6g/litre of pressed yeast. This corresponds to a pitching count of approx. 20 million cells per ml.


1
2
3
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10
11
12

Legend to Fig.1.

1. Cotton Wool Plug.

2. Hard transparent PVC shell housing eight fermentability tubes positioned around the periphery of the shell for easy observation. Diameter of shell : 31 cm.

3. Cooling water outlet.

4. Individual fermentability tube.
   Outer diameter : 50 mm
   Inner diameter : 47 mm
   Height of 2 litres wort in the tube : 1,17 meters,
   Overall height of tube : 1,5 metres.

5. Sampling tap (2 mm bore) 9 cm from base of tube.

6. Soft PVC seals

7. 2 mm capillary tube protruding into the centre of the fermentability tube for sampling.

8. Cooling water inlet.

9. Taps for cropping yeast or emptying tube. (10 mm bore)

10. Wheel

11. Cone angle 45°.

12. Glass ball to prevent yeast settling below the cooling water level.

Fig. 1.b) FERMENTATION MODULES LINKED TO COOLING UNIT



Fig. 2. MASH BATH



## 2.7. Yeast/Wort Aeration Procedures.

Various Yeast/Wort aeration procedures were used.

2.7.1. The wort/yeast mixture was shaken thirty five times in a stoppered flat bottomed five litre boiling flask to achieve air saturation of the wort. (approx. 8 p.p.m. dissolved oxygen.)

2.7.2. The wort/yeast mixture was shaken thirty five times in a stoppered flat-bottomed five litre boiling flask (approx. 8 p.p.m. dissolved oxygen), allowed to stand at room temperature for 1 hour and then shaken another thirty five times (approx. 6 p.p.m. dissolved oxygen).

2.7.3. The wort was oxygenated to a level of 14 p.p.m. dissolved oxygen using medical oxygen dispensed through a glass sinter.

2.7.4. The wort/yeast mixture was continuously oxygenated for 2 hours using medical oxygen dispensed through a glass sinter.

## 2.8. Propagation Procedures.

Various procedures were attempted.

2.8.1. A loopful of yeast was inoculated into 10 ml of wort and incubated overnight at 25°C. The wort/yeast mixture was then transferred into 200 ml of fresh wort and incubated overnight at 20°C with oxygenation via a sintered glass tube. The yeast/wort mixture was again transferred to 5000 ml fresh wort and incubated for 48 hours at 20°C with coarse oxygenation via a sintered glass tube. (Apparatus depicted in figure 3).

Fig. 3. APPARATUS USED FOR YEAST PROPAGATION (METHOD 2.8.1.)



Fig. 4. APPARATUS USED FOR YEAST PROPAGATION (METHOD 2.8.3)



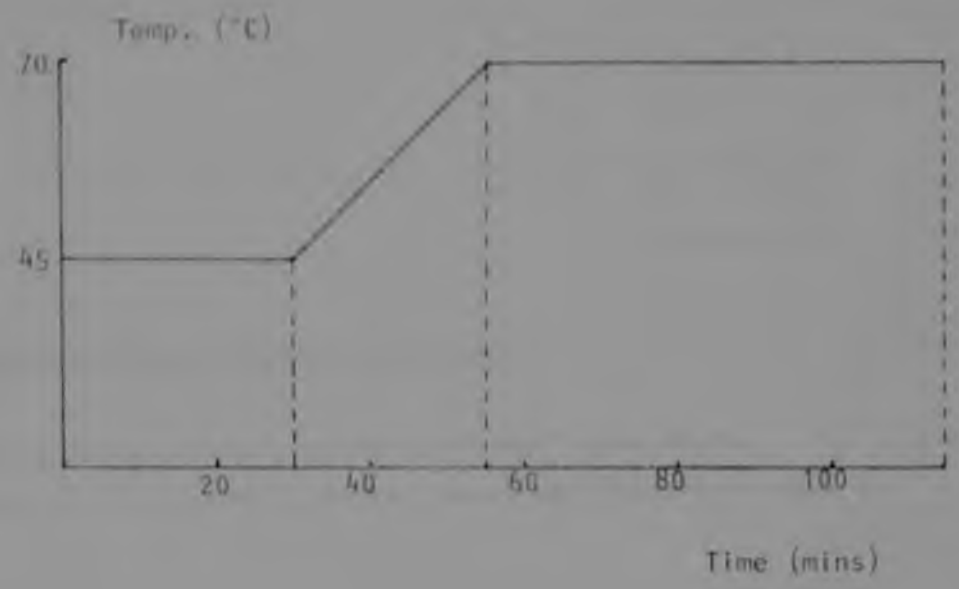
2.8.2. A loopful of yeast was inoculated into 10 ml of wort and incubated overnight at 25°C. The yeast/wort mixture was then topped up to 20 ml with fresh wort and incubated overnight at 25°C. The yeast/wort mixture was transferred to 200 ml of fresh wort and shaken for 3 days in an orbital incubator at 20°C.

2.8.3. A loopful of yeast was inoculated into 15 ml of wort and incubated overnight at 25°C. The yeast/wort mixture was transferred into 200 ml of wort and shaken for 3 days in an orbital incubator at 20°C. The yeast/wort mixture was then transferred into 5000 ml of fresh wort in a Cornelius Vessel (Illustrated in fig. 4) and incubated for 48 hours at 20°C with oxygenation via the outlet pipe of the vessel.

2.9. Mashing Procedures.

2.9.1. Mashing using the Congress Temperature Programme.

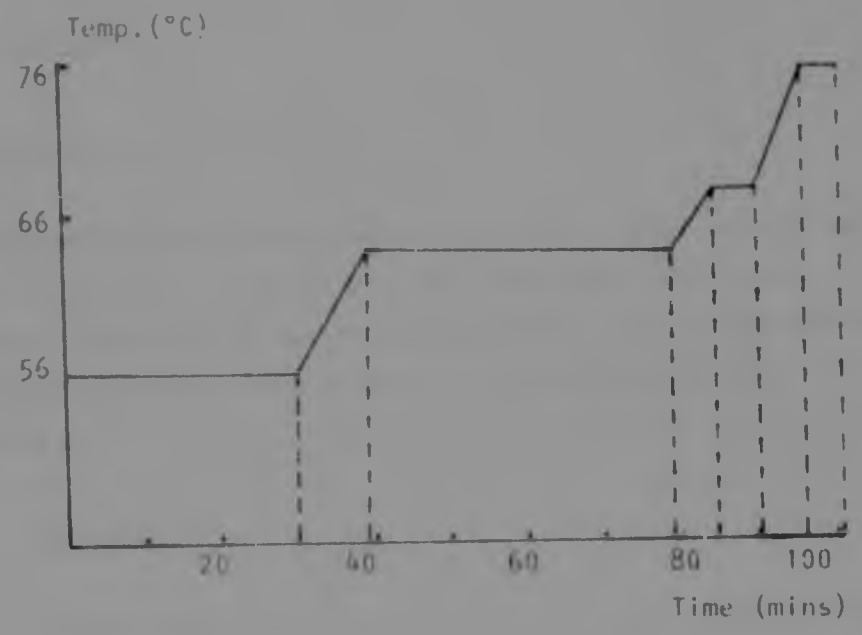
312g of malt sample was coarsely milled using a Casella mill and mashed into 1,9 litres of water according to the following programme:



The mash was mixed well and filtered through a mash filter cloth funnel. The filtrate was returned to the same funnel for a second filtration.
5,0 grams of hop pellets (Styrian) were added to 2,4 litres of sweet wort and the whole boiled for 60 minutes. The wort was then filtered hot through a fluted filter funnel (Whatman no.6, Schleicher & Schüll No. 597½) to remove spent hops and "hot break".
The flask containing the wort was then covered with aluminium foil and stored at 0°C until needed.

2.9.2. Mashing using the Brewery Temperature Programme.

The entire procedure was identical to method 2.9.1. except that the following mashing temperature programme was used.




2.10. Determination of Yeast Consistency.

The pitching yeast mixture was stirred until completely homogeneous. 10 ml of this mixture was then introduced into a

graduated centrifuge tube. The mixture was centrifuged at 3000 r.p.m. for 5 mins and the ratio of pressed yeast to liquid read off the centrifuge tube.

## 2.11. Determination of Viability according to the Methylene Blue Index.

The sample of yeast to be tested was diluted in saline to give a yeast count of approx. 1 x $10^7$ cells/ml. Equal amounts of the yeast suspension and 0,01% Methylene blue stain were mixed in a spot-plate. The mixture was allowed to stand for 1 minute prior to introduction into a haemacytometer counting chamber.
The ratio of colourless to blue cells was determined microscopically.

ie. $\frac{\text{number of colourless cells in a given area}}{\text{total number of cells in the same area}} \times 100$

= % viability.

## 2.12. Fermentation Procedures.

All fermentations were attemperated at 11°C. Yeast counts were measured twice daily until peak yeast count and once daily thereafter. Attenuation was measured daily. In certain instances pH and diacetyl values were measured on the last two days of fermentation.

### 2.12.1. Yeast Counts.

Samples for analysis were well mixed and diluted $^1/_{500}$ times using a Coulter Diluter II. The diluent used was Coulter Isoton + 0,002% EDTA. After dilution the samples were sonicated in an ultrasonic bath for 15 mins. The samples were gently mixed again and the counts read using a Coulter Counter Model D Industrial.

2.12.2. Attenuation

Fermenting wort samples were centrifuged at 3000 r.p.m. for 5 minutes to remove the yeast. The supernatant was filtered through Whatman no. 40 filter paper and the gravity measured using an Anton Paar Density Meter - Model DMA 55.

2.12.3. Diacetyl.

Samples for diacetyl analysis were membrane filtered (with a positive pressure) through a 0,45 um filter. Three ml aliquots of the filtrate were introduced into duplicate bottles and the bottles sealed.
Samples were oxidised in a water-bath at 60°C to achieve the complete conversion of precursor (alpha-acetolactate) to diacetyl. Samples were injected into a Carlo-Erba G.C. equipped with an electron capture detector. Injection was by automatic head space sampler. Injection volume was 0,7 ml. A 2 meter long glass capillary tube (O.D. 6 mm) packed with 10% carbowax 20 M on chromosorb WHP was used. The oven temperature was 80°C.

2.13. Limiting Attenuation Test.

200 ml of wort (14,1°P) was measured out into a sterile 500 ml conical flask fitted with a Bunsen valve. 15g of vacuum dried yeast was dispensed into a sterile sample bottle and a small amount of the test wort added. The yeast/wort mixture was shaken vigorously to bring the dried yeast into suspension, and added back to the remaining wort in the 500 ml flask.
The flasks were shaken in an orbital incubator attemperated to 20°C at a speed just sufficient to keep the yeast in suspension (a setting of 100 on the Gallenkamp Orbital Incubator) for 48 hours. The fermenting wort was centrifuged to remove the yeast. The

2.12.2. Attenuation

Fermenting wort samples were centrifuged at 3000 r.p.m. for 5 minutes to remove the yeast. The supernatant was filtered through Whatman no. 40 filter paper and the gravity measured using an Anton Paar Density Meter - Model DMA 55.

2.12.3. Diacetyl.

Samples for diacetyl analysis were membrane filtered (with a positive pressure) through a 0,45 um filter. Three ml aliquots of the filtrate were introduced into duplicate bottles and the bottles sealed.
Samples were oxidised in a water-bath at 60°C to achieve the complete conversion of precursor (alpha-acetolactate) to diacetyl. Samples were injected into a Carlo-Erba G.C. equipped with an electron capture detector. Injection was by automatic head space sampler. Injection volume was 0,7 ml. A 2 meter long glass capillary tube (O.D. 6 mm) packed with 10% carbowax 20 M on chromosorb WHP was used. The oven temperature was 80°C.

2.13. Limiting Attenuation Test.

200 ml of wort (14,1°P) was measured out into a sterile 500 ml conical flask fitted with a Bunsen valve. 15g of vacuum dried yeast was dispensed into a sterile sample bottle and a small amount of the test wort added. The yeast/wort mixture was shaken vigorously to bring the dried yeast into suspension, and added back to the remaining wort in the 500 ml flask.
The flasks were shaken in an orbital incubator attemperated to 20°C at a speed just sufficient to keep the yeast in suspension (a setting of 100 on the Gallenkamp Orbital Incubator) for 48 hours. The fermenting wort was centrifuged to remove the yeast. The

supernatant was filtered through Whatman No. 40 filter paper and the gravity of the filtrate measured.

L.A. = final gravity at 48 hours.

2.14. Statistics.

One way analysis of variance using the HP-67 ST1-06A programme was used to establish reproducibility of results obtained during fermentations and to calculate standard deviations.

Analysis of correlation coefficients and standard errors were performed using the HP-67 ST1-01A programme.

# 3. RESULTS

## 3.1. Development of a System for Laboratory Fermentations.

These investigations were carried out to develop a suitable system for laboratory fermentations.

### 3.1.1. Selection of a Suitable Fermentation Vessel

Worts A and B (collected ex paraflow from the brewery plant) and a Pauls & Sandars all malt wort (mashed in the laboratory according to method 2.9.1.) were pitched (2.6 & 2.7.1) with Saccharomyces uvarum (x, propagated according to method 2.8.1. Fermentations (2.12) were run concurrently in the 2 litre and one litre tubes described in section 2.3.

As can be seen from the results presented in fig. 5. fermentations in 1 litre tubes are not comparable to those in 2 litre tubes. The results also show that there is no reproducibility of results using duplicate 1 litre tubes. These experiments were repeated three times and similar trends were repeatedly obtained.

### 3.1.2. Reproducibility of Results in 2 litre Fermentation Tubes.

A series of experiments were run to determine the reproducibility of fermentation results using 2 litre fermentation tubes described in section 2.3.

Worts C and A (collected ex paraflow from a brewery) and different all malt worts (mashed in the laboratory according to method 2.9.1.) were pitched (methods 2.6 and

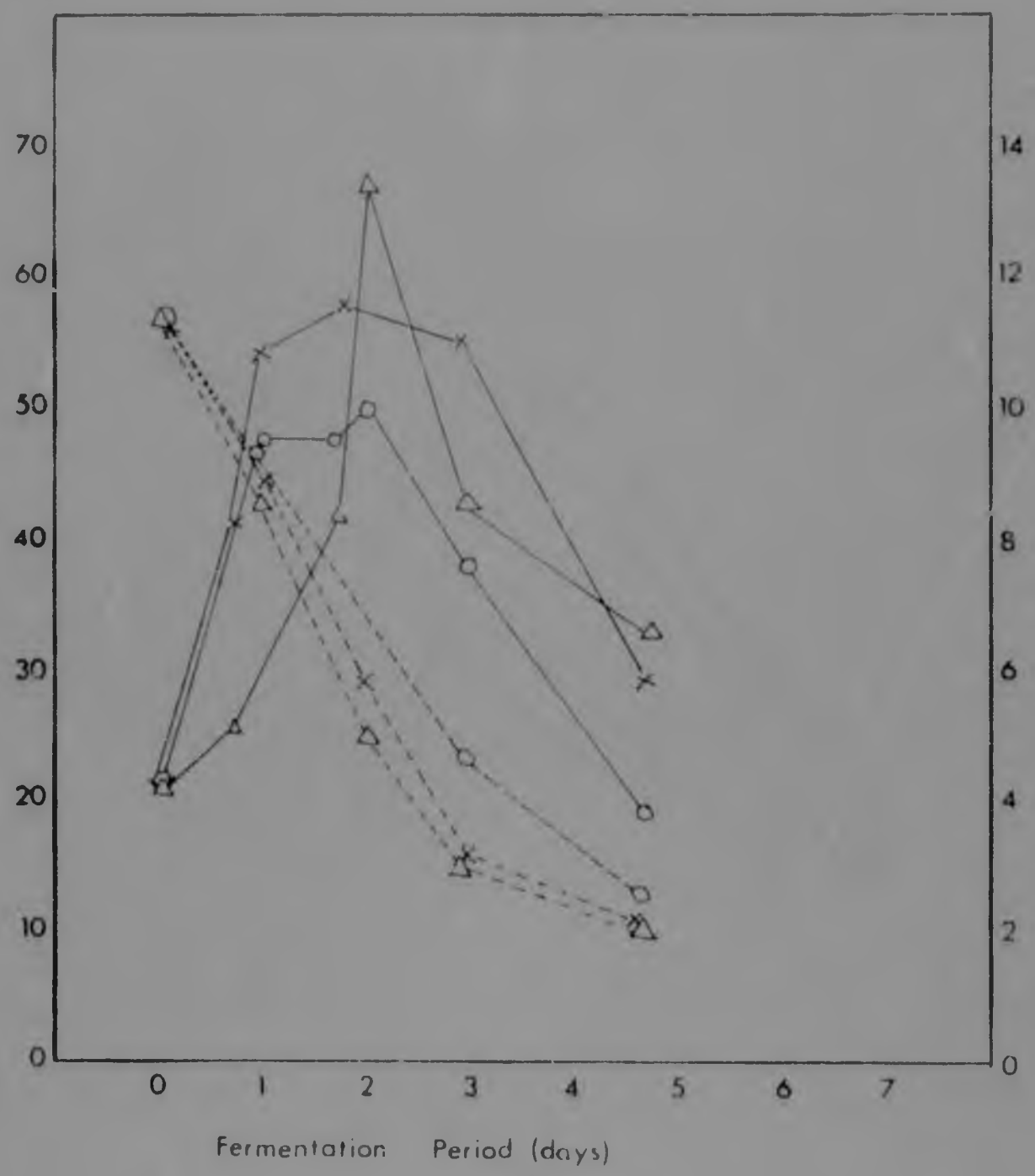
Fig. 5. COMPARISON BETWEEN KIRIN 1 LITRE TUBES AND 2 LITRE FERMENTATION TUBES.

Fig. 5. COMPARISON BETWEEN KIRIN 1 LITRE TUBES AND 2 LITRE FERMENTATION TUBES.

Wort A.

2 litre fermentation tube Δ——Δ

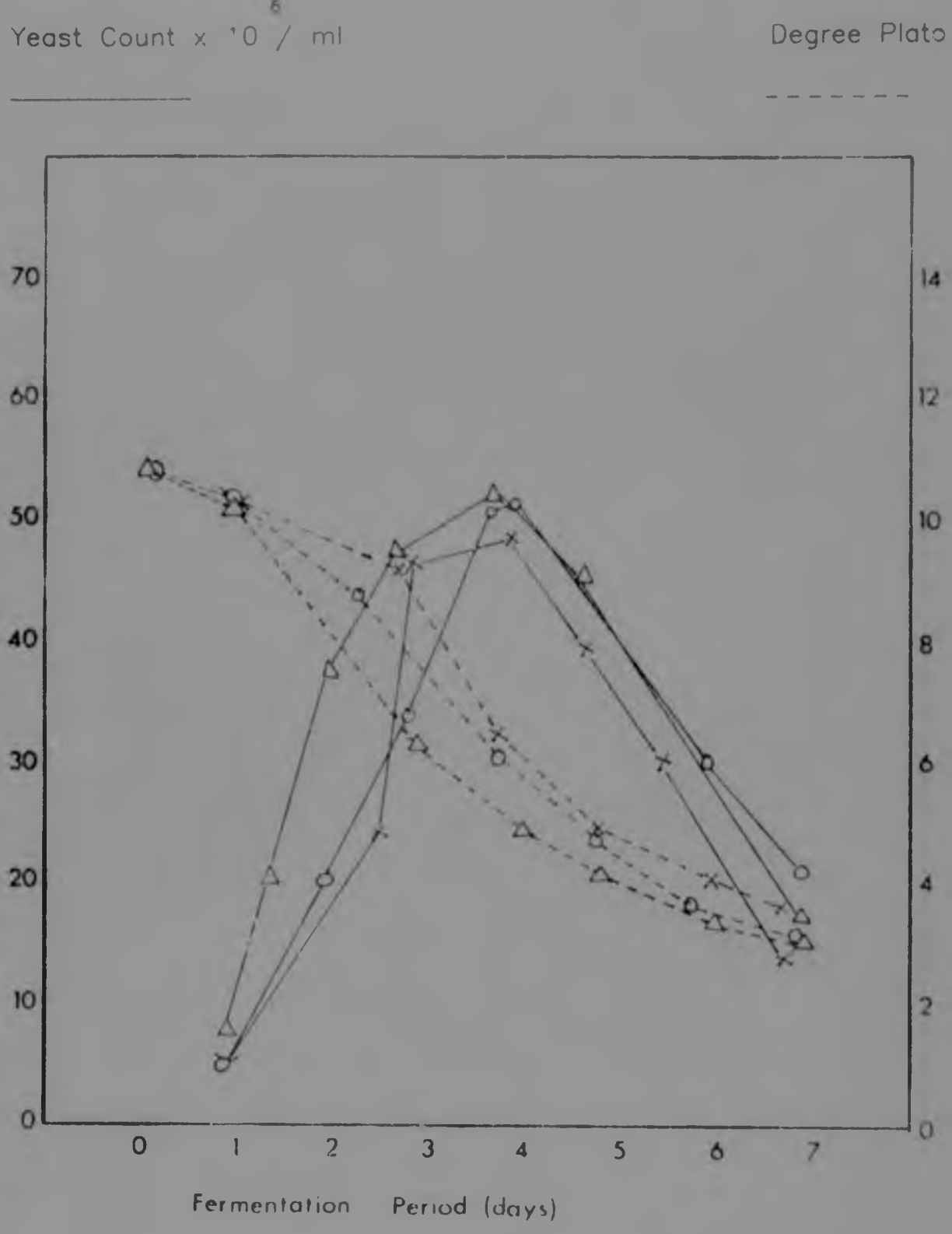
Kirin 1 litre fermentation tubes O——O x——x



Wort B.

2 litre fermentation tube Δ——Δ

Kirin 1 litre fermentation tubes ○——○ ×——×



Pauls and Sandars Malt Wort.

2 litre fermentation tube Δ——Δ

Kirin 1 litre fermentation tubes O——O X——X

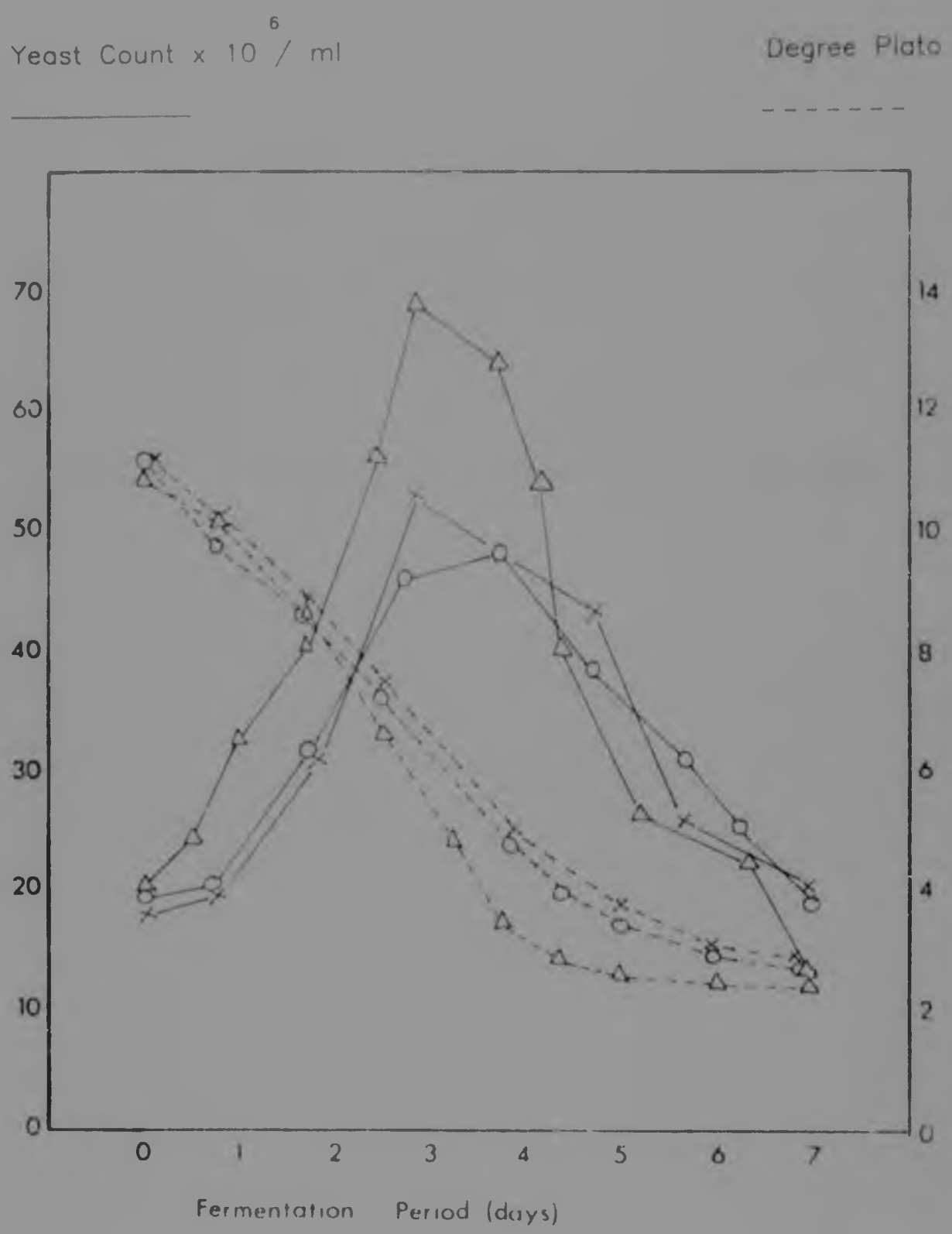
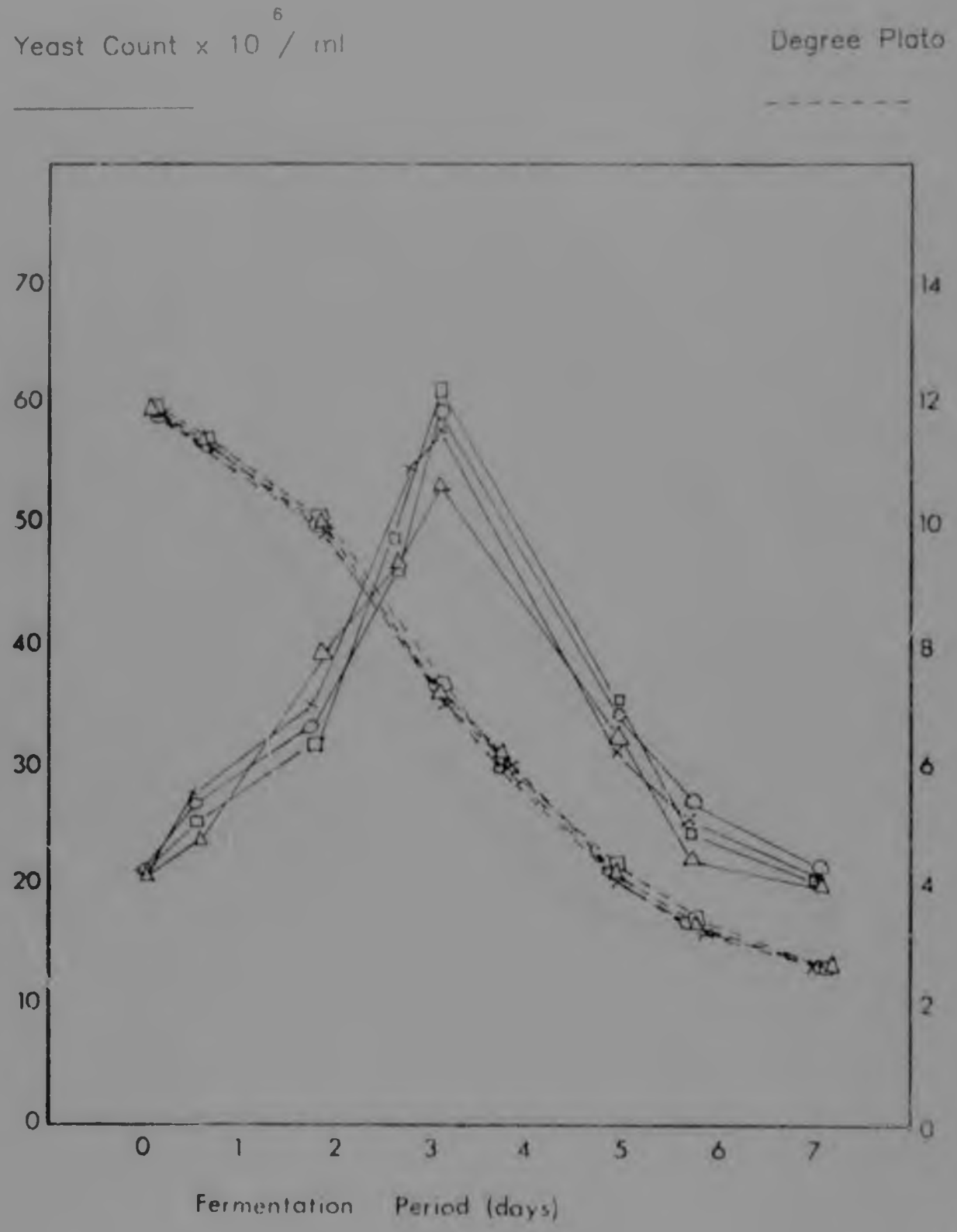


Figure 6. REPRODUCIBILITY OF FERMENTATIONS IN 2 LITRE TUBES.

Wort C (1)

Tube a) Δ——Δ  Tube b) ○——○

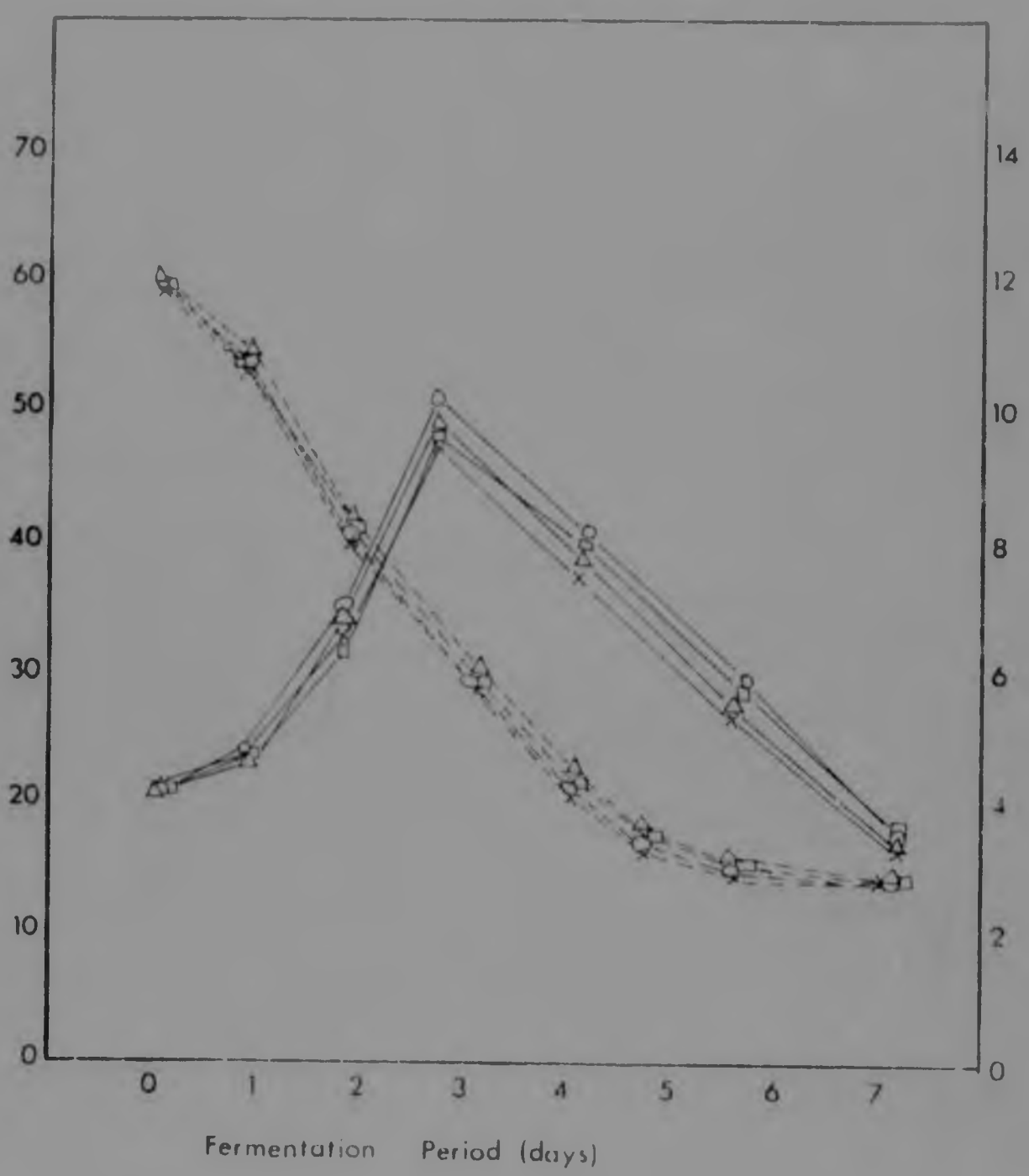
Tube c) ×——×  Tube d) □——□



Wort C (ii)

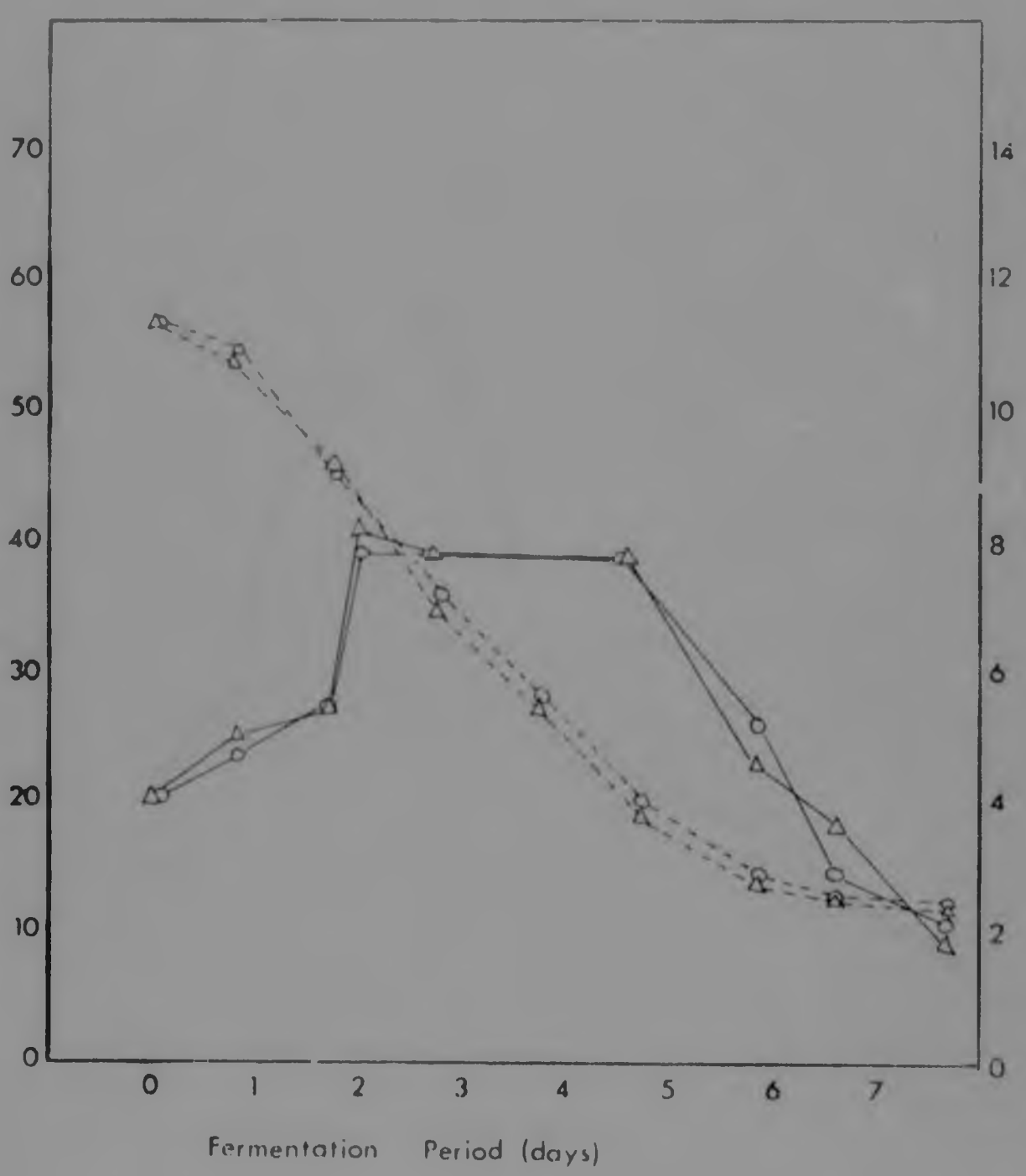
Tube a) Δ——Δ  Tube b) O——O

Tube c) ×——×  Tube d) □——□



Wort A (i)

Tube a) Δ——Δ
Tube b) O——O

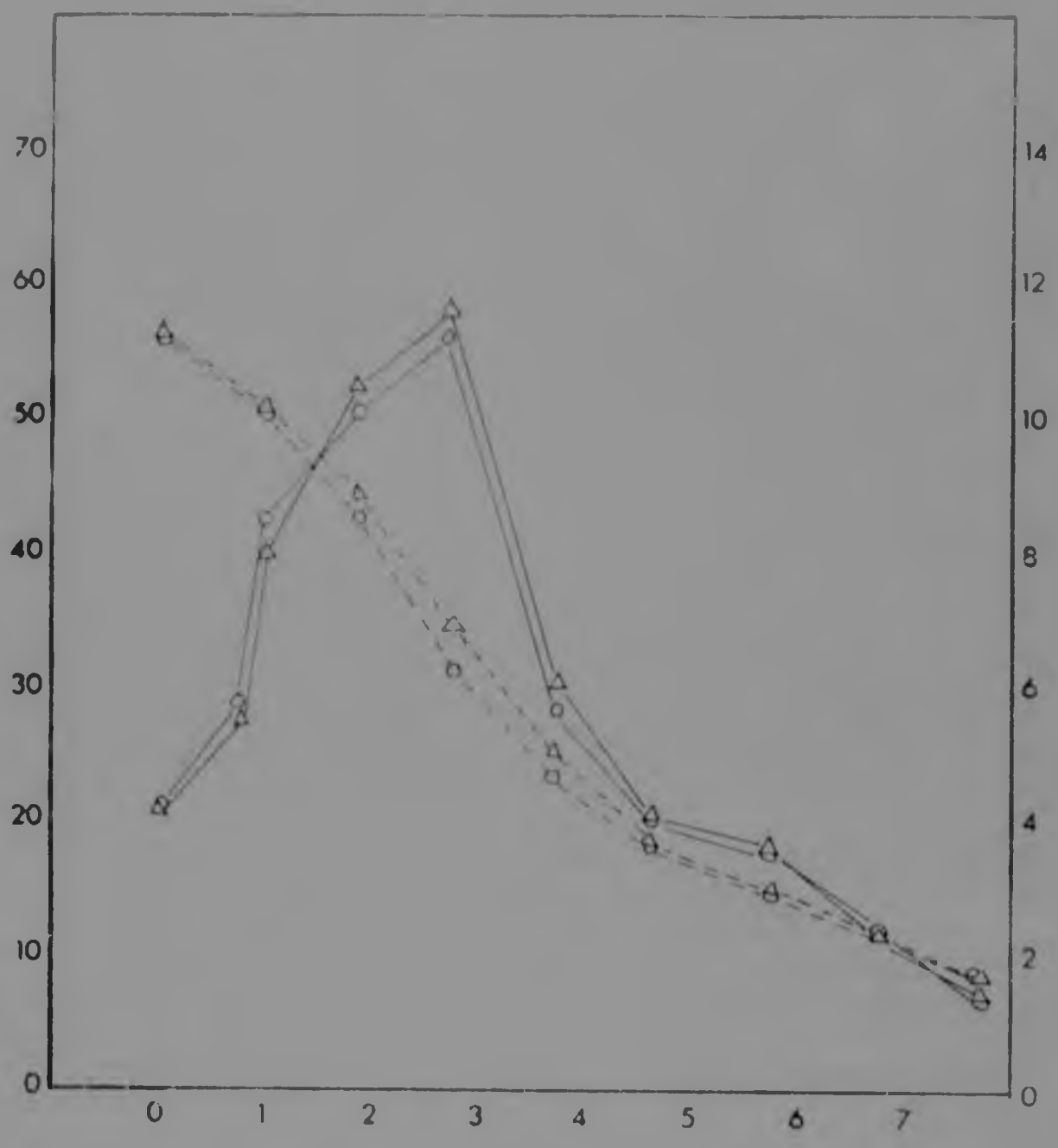


Wort A (1)

Tube a) Δ——Δ

Tube b) O——O

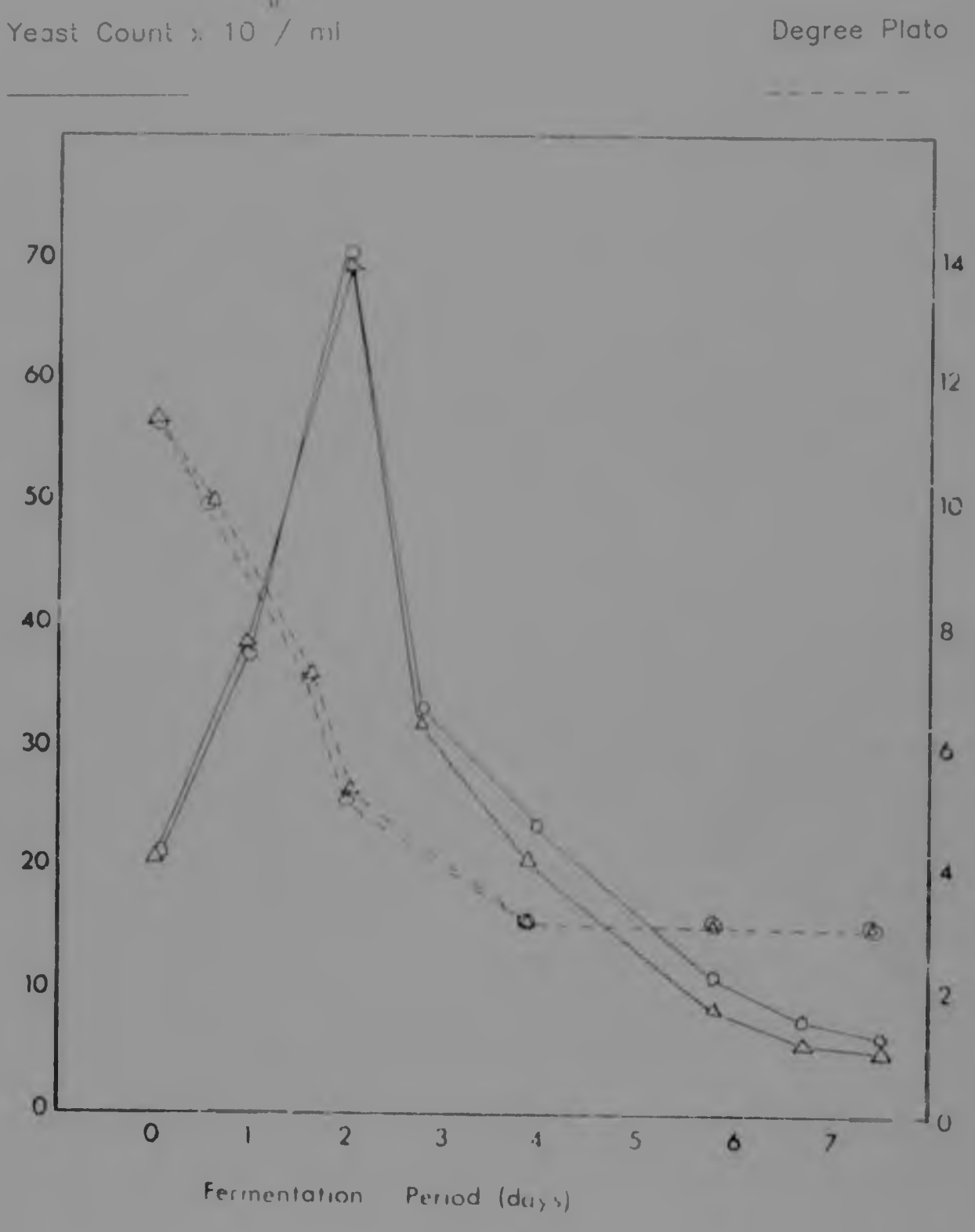
Yeast Count x $10^6$ / ml ———— Degree Plato - - - - - - -



Laboratory Mashed wort 1.

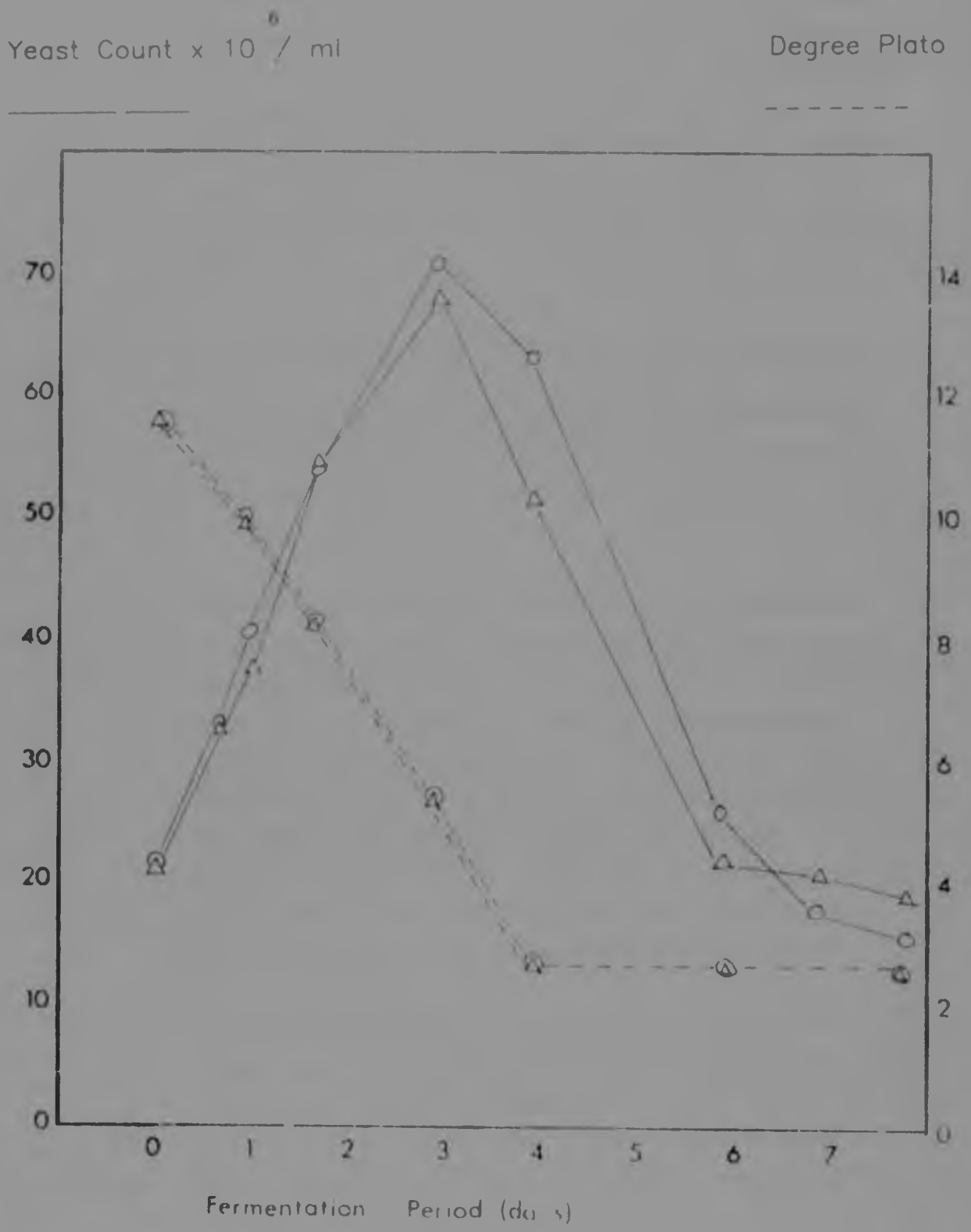
Tube a) Δ———Δ

Tube b) ○———○



Laboratory Mashed wort 2.

Tube a) Δ———Δ
Tube b) O———O



2.7.1) with Saccharomyces uvarum (x) propagated according to method 2.8.1. Experiments were run in duplicate and quadruplicate and each set of experiments repeated twice.

The results illustrated in fig.6 show that reproducibility was very good. The same results were obtained in repeat experiments. Analysis of variance showed that at the 99% confidence level the final gravity achieved was independent of the individual tests. Calculated from the mean standard deviations 3 standard deviations was ± 0,18°P for final gravity, ± 6,3 x $10^6$ cells/ml for peak yeast count and ± 3 hours for time of yeast peak.

3.1.3. Comparison between Plant and Laboratory Fermentations.

These investigations were carried out to establish the correlation between fermentations conducted in the 2 litre tubes described in section 2.3 and plant fermentations.

Fermenting wort samples were drawn ex the fermenting vessel in the plant. Fermentations (2.12) using these samples were then undertaken in the 2 litre fermentation tubes and the fermentation patterns compared against those of the respective brewery fermentations. For completeness various sizes of fermentation vessels were included in this study, ie. 330 hl and 2o40 hl cylindroconical vessels, and 1000 hl horizontal fermenters. Experiments were conducted using normal gravity (11,3°P) and high gravity (14,1°P) worts.

The results illustrated in tables 1 and 2 and .7 showed that fermentation velocities for each set of experiments were very similar.
Calculated from the 10 comparative studies in Table 1 (using normal gravity wort) the correlation coefficient

TABLE 1. Comparison between Plant and Laboratory Fermentations using Normal Gravity Worts.

| | °p @ 96 hrs. | | °p on day 8 | |
|---|---|---|---|---|
| | Plant | Laboratory | Plant | Laboratory |
| FV 487(330hl cylindro conical) | 3,9 | 4,1 | 2,3 | 2,3 |
| FV 403(330hl cylindro conical) | 3,0 | 3,1 | 2,3 | 2,3 |
| FV 123(2640hl cylindro conical) | 5,0 | 4,2 | 1,6 | 1,8 |
| FV 516(1000hl horizontal) | 2,4 | 2,8 | 2,1 | 2,0 |
| FV 124(2640hl cylindro-conical) | 2,8 | 2,9 | 2,0 | 2,0 |
| FV 417(330hl cylindro conical) | 2,2 | 2,4 | 2,0 | 2,0 |
| FV 418(330hl cylindro conical) | 2,2 | 2,4 | 2,0 | 2,1 |
| FV 454(1000hl horizontal) | 2,6 | 2,9 | 1,9 | 2,0 |
| FV 442(1000hl horizontal) | 2,0 | 2,3 | 1,8 | 1,9 |
| FV 507(1000hl horizontal) | 2,6 | 3,2 | 1,9 | 2,1 |

for °P on the 8th day between brewery and laboratory fermentations was 0,91 with a standard error of 0,05. The correlation coefficient for °P at 96 hrs was 0,94 with a standard error of 0,04. Analysis of results in Table 2 (high gravity wort) showed a correlation coefficient of 0,97 with a standard error of 0,03 for °P on the 8th day.

TABLE 2. Comparison between Plant and Laboratory Fermentations using High Gravity Worts.

RUN 1.

| Sample. | S.G. day 9(°P). | pH day 8 | Diacetyl (p.p.m.) | |
|---|---|---|---|---|
| | | | day 8 | day 9. |
| FV 420* | 3,6 | 4,36 | 0,22 | 0,18 |
| Tube 1* | 3,6 | 4,28 | 0,18 | 0,17 |
| Tube 2 | 3,4 | 4,29 | 0,19 | 0,16 |
| Tube 3 | 3,6 | 4,29 | 0,19 | 0,16 |
| Tube 4 | 3,6 | 4,32 | 0,19 | 0,18 |

RUN 2.

| Sample. | S.G. day 9(°P). | pH day 8 | Diacetyl (p.p.m.) | |
|---|---|---|---|---|
| | | | day 8 | day 9. |
| FV 421* | 2,6 | 4,34 | 0,20 | 0,17 |
| Tube 1* | 2,8 | 4,09 | 0,20 | 0,16 |
| Tube 2 | 2,6 | 4,00 | 0,17 | 0,16 |
| Tube 3 | 2,8 | 4,12 | 0,21 | 0,16 |
| Tube 4 | 2,8 | 4,10 | 0,20 | 0,18 |

RUN 3.

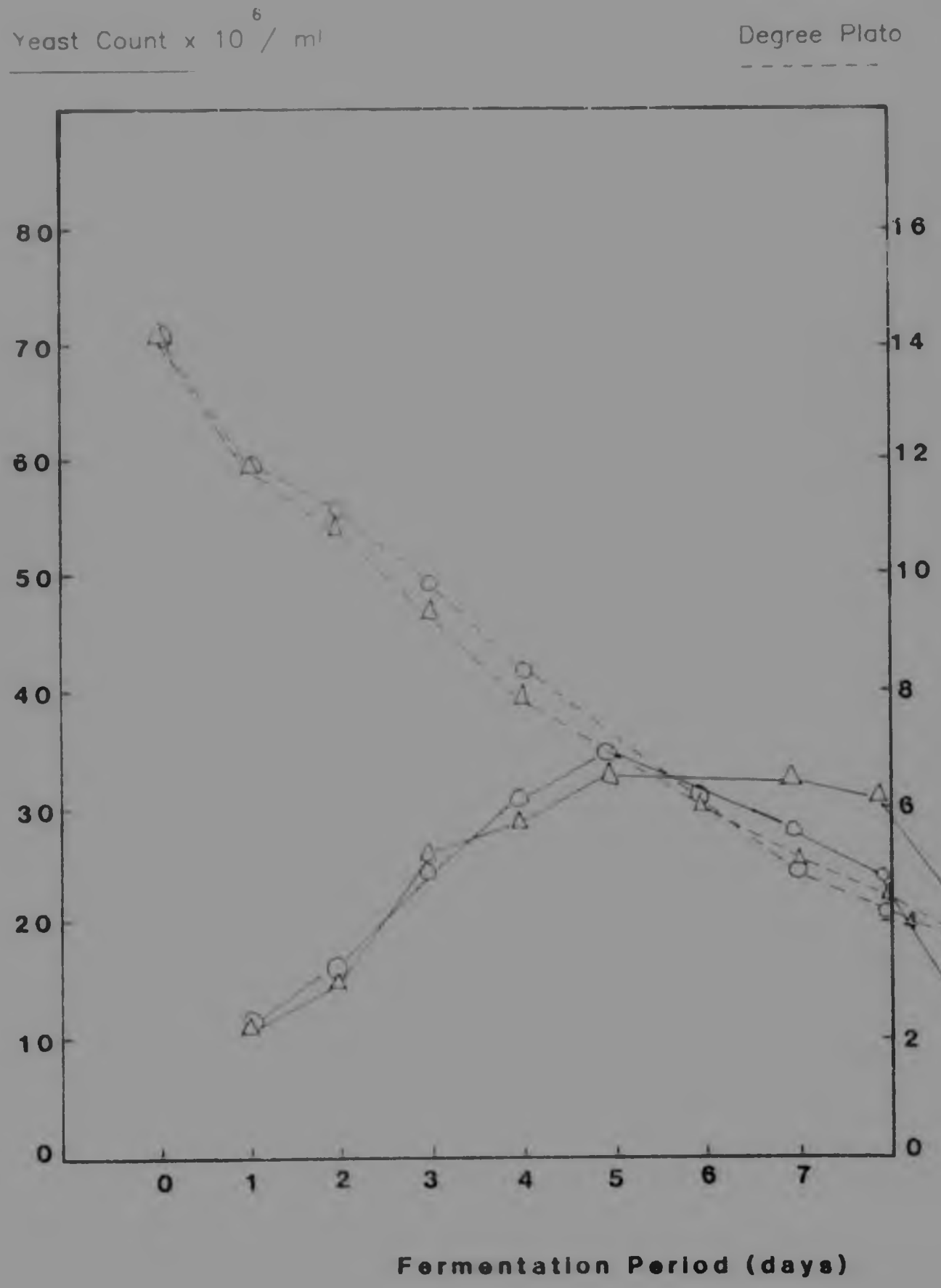
| Sample. | S.G. day 8(°P). | pH day 8 | Diacetyl (p.p.m.) | |
|---|---|---|---|---|
| | | | day 7 | day 8. |
| FV 481* | 2,4 | 4,23 | 0,18 | 0,14 |
| Tube 1* | 2,5 | 4,15 | 0,17 | 0,13 |
| Tube 2 | 2,4 | 4,22 | 0,19 | 0,15 |
| Tube 3 | 2,4 | 4,13 | 0,18 | 0,12 |
| Tube 4 | 2,4 | 4,12 | 0,16 | 0,13 |
| Tube 5 | 2,4 | 4,13 | 0,17 | 0,13 |

*Illustrated in Fig. 7.

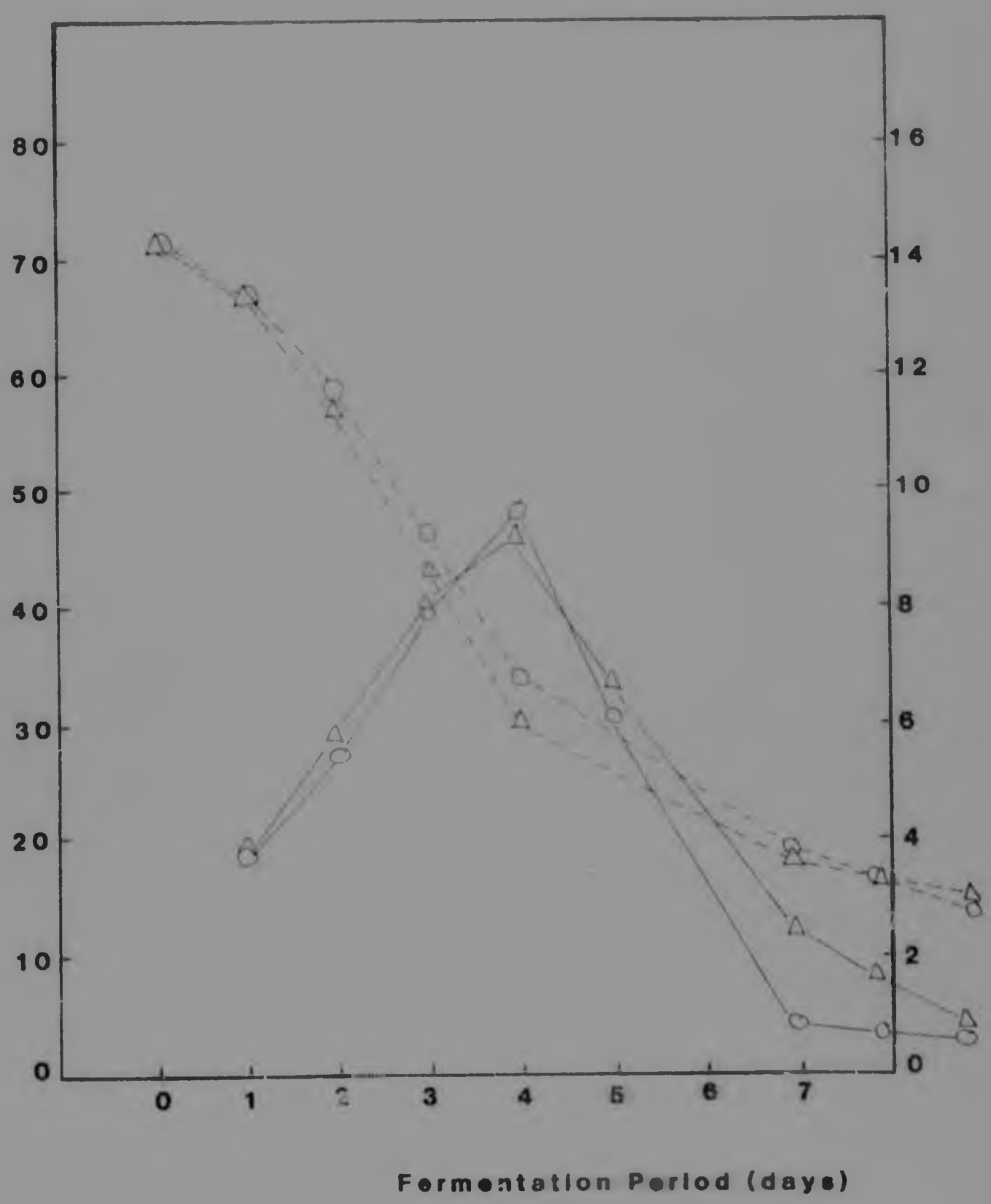
Fig. 7. COMPARISON BETWEEN PLANT AND LABORATORY FERMENTATIONS.

FV 420 ○——○

Sample of fermenting wor' ex FV 420 fermented in 2 litre fermentation tube. Δ——Δ

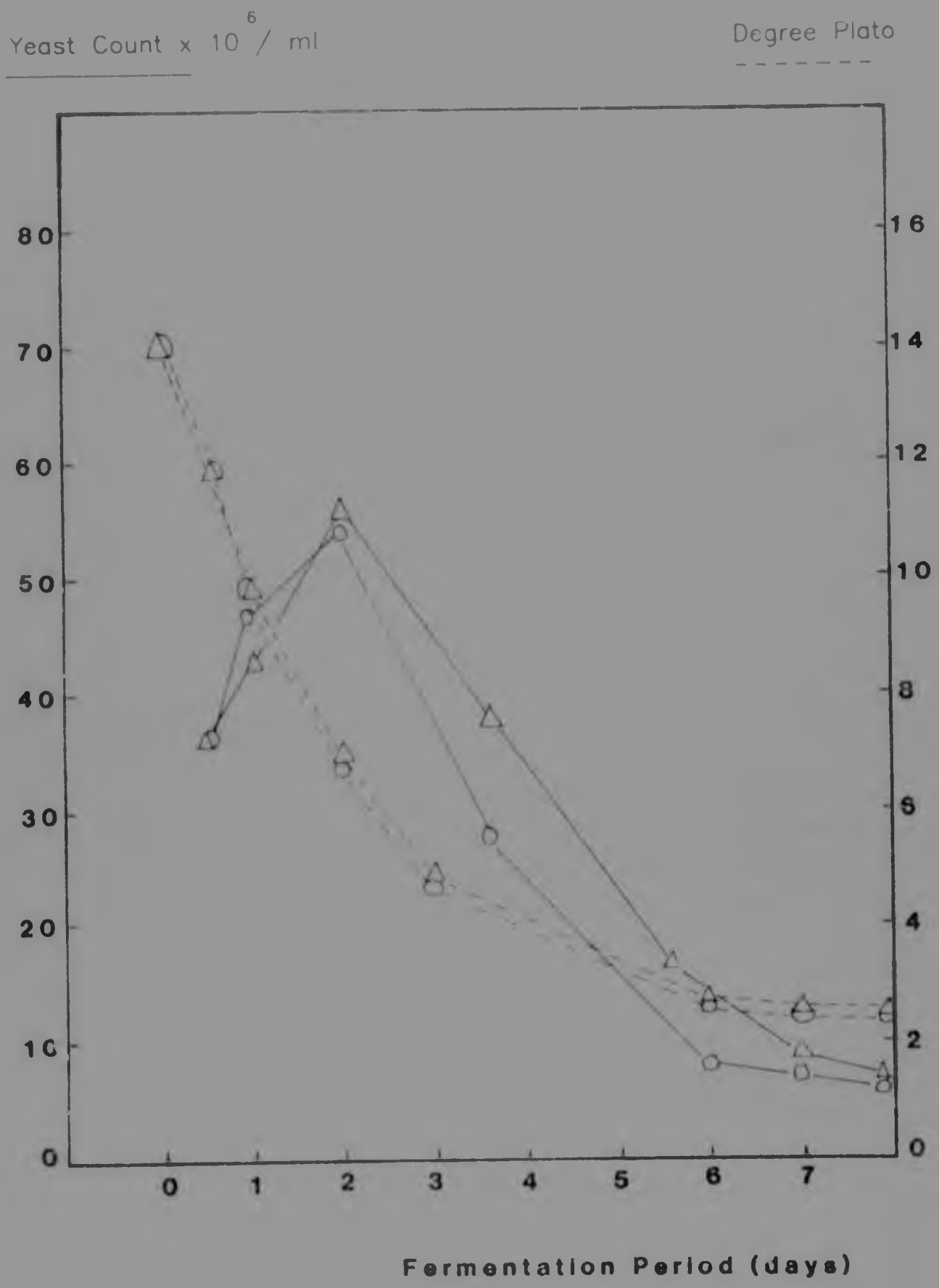


FV 421 O——O
Sample of fermenting wort ex FV 421 fermented in 2 litre fermentation tube. Δ——Δ



FV 481 O——O

Sample of fermenting wort ex FV 481 fermented in 2 litre fermentation tube. Δ——Δ



## 3.2. Predicting Malt Quality

Using the sophisticated system described in 2.4 and 2.5 investigations were carried out to determine if the laboratory fermentation system could be used to predict malt quality.

### 3.2.1. Pitching Procedure

The pitching procedure was designed to follow as closely as possible that used in the brewery. (methods 2.6. and 2.7.1.)

To standardise on the pitching yeast used, yeast was collected asceptically from the plant at the end of the first full brew fermentation after yeast propagation. The criteria for this yeast was a viability of 95% or greater (as measured by method 2.11) and the fermentation pattern good and down to 2,5°P or less prior to collection of the yeast.

Yeast was pitched into 2 litres of hopped wort adjusted to 11,3°P and allowed to stand for 2 hours prior to pouring into 2 litre fermentation tubes.

### 3.2.2. Mashing Procedure.

These investigations were carried out to determine what differences, if any, were reflected in the fermentability of a malt mashed according to the Congress temperature programme compared with the temperature programme used in the brewery for our major brand of beer.

Samples of malt were mashed according to methods 2.9.1 and 2.9.2. The resulting worts were pitched with Saccharomyces uvarum(x) according to methods 2.6 and 2.7.1. Fermentations (2.12) were conducted in 2 litre

fermentation tubes and the resulting patterns of respective fermentations compared with each other.

TABLE 3. Comparison between Congress Mashing Programme and Brewery Mashing Programme.

| Malt Sample. | $O_P$ @ 96 hours | | $O_P$ on da. 8 | |
|---|---|---|---|---|
| | Congress | Brewery | Congress | Brewery |
| Control* | 3,3 | 3,2 | 2,3 | 2,1 |
| No. 1. | 4,4 | 3,2 | 3,0 | 2,6 |
| No. 2. | 3,1 | 3,0 | 2,7 | 2,6 |
| No. 3. | 3,5 | 3,1 | 2,8 | 2,6 |
| Control | 3,6 | 2,8 | 2,4 | 2,1 |
| No. 4* | 3,4 | 2,9 | 2,7 | 2,4 |
| Control | 3,6 | 2,8 | 2,4 | 2,1 |
| No. 5. | 3,6 | 3,6 | 2,5 | 2,2 |
| No. 6. | 4,0 | 3,8 | 2,7 | 2,5 |
| No. 7. | 3,9 | 3,8 | 2,8 | 2,5 |
| No. 8. | 3,5 | 3,3 | 2,8 | 2,6 |
| No. 9. | 3,8 | 3,3 | 2,8 | 2,5 |
| Control | 4,6 | 3,8 | 2,4 | 2,2 |
| No. 10. | 4,0 | 3,8 | 2,4 | 2,2 |
| No. 11. | 3,6 | 3,6 | 2,8 | 2,8 |
| No. 12. | 4,4 | 4,3 | 2,6 | 2,4 |
| No. 13. | 4,2 | 4,2 | 2,8 | 2,6 |
| No. 14. | 4,3 | 4,2 | 3,0 | 2,6 |
| No. 15. | 4,6 | 4,4 | 2,8 | 2,5 |
| Control | 4,1 | 3,6 | 2,3 | 2,1 |
| No. 16. | 3,8 | 3,7 | 3,0 | 2,8 |
| No. 17* | 3,7 | 3,2 | 2,9 | 2,5 |
| No. 18. | 3,6 | 3,6 | 2,8 | 2,6 |
| No. 19. | 4,0 | 3,4 | 2,6 | 2,3 |
| No. 20. | 3,9 | 3,7 | 2,9 | 2,4 |

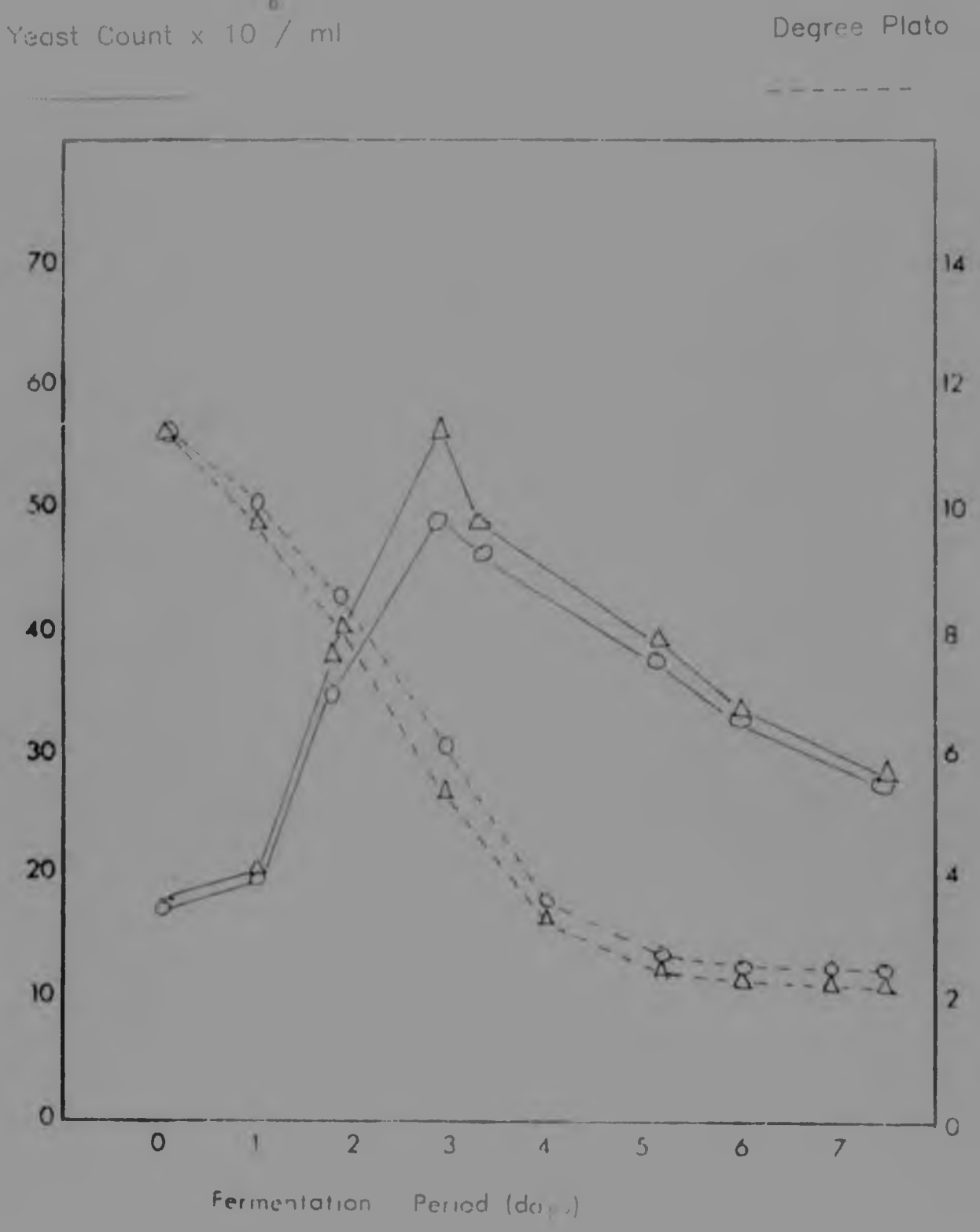
* Illustrated in Figure 8.

Fig. 8. COMPARISON BETWEEN CONGRESS MASHING PROGRAMME AND BREWERY MASHING PROGRAMME.

Control Malt.

Brewery T° Mash Δ ——— Δ

Congress T° Mash O ——— O



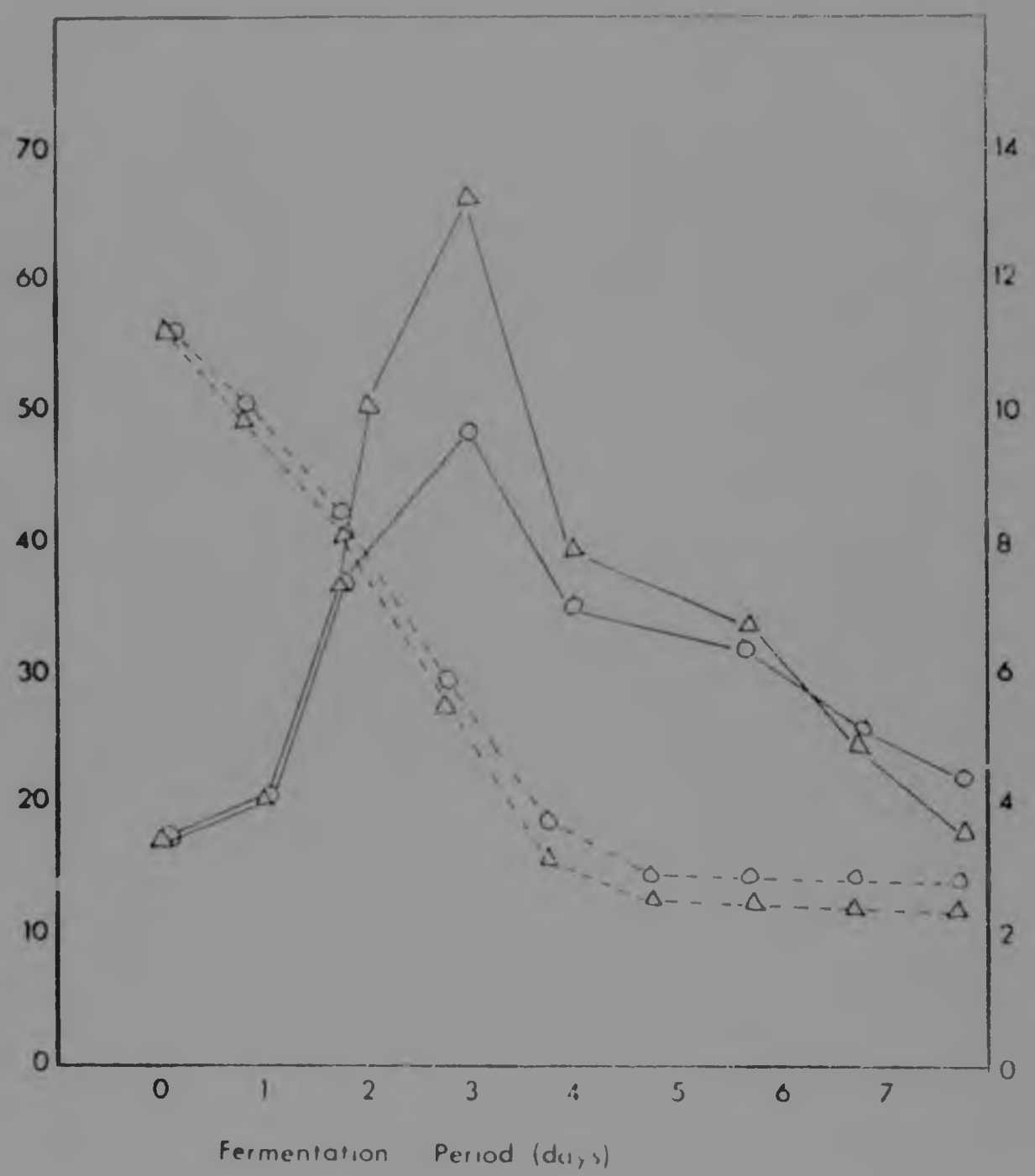
Malt Sample No 4

Brewery T° Mash Δ——

Congress T° Mash ○——

Yeast Count x 10 / ml

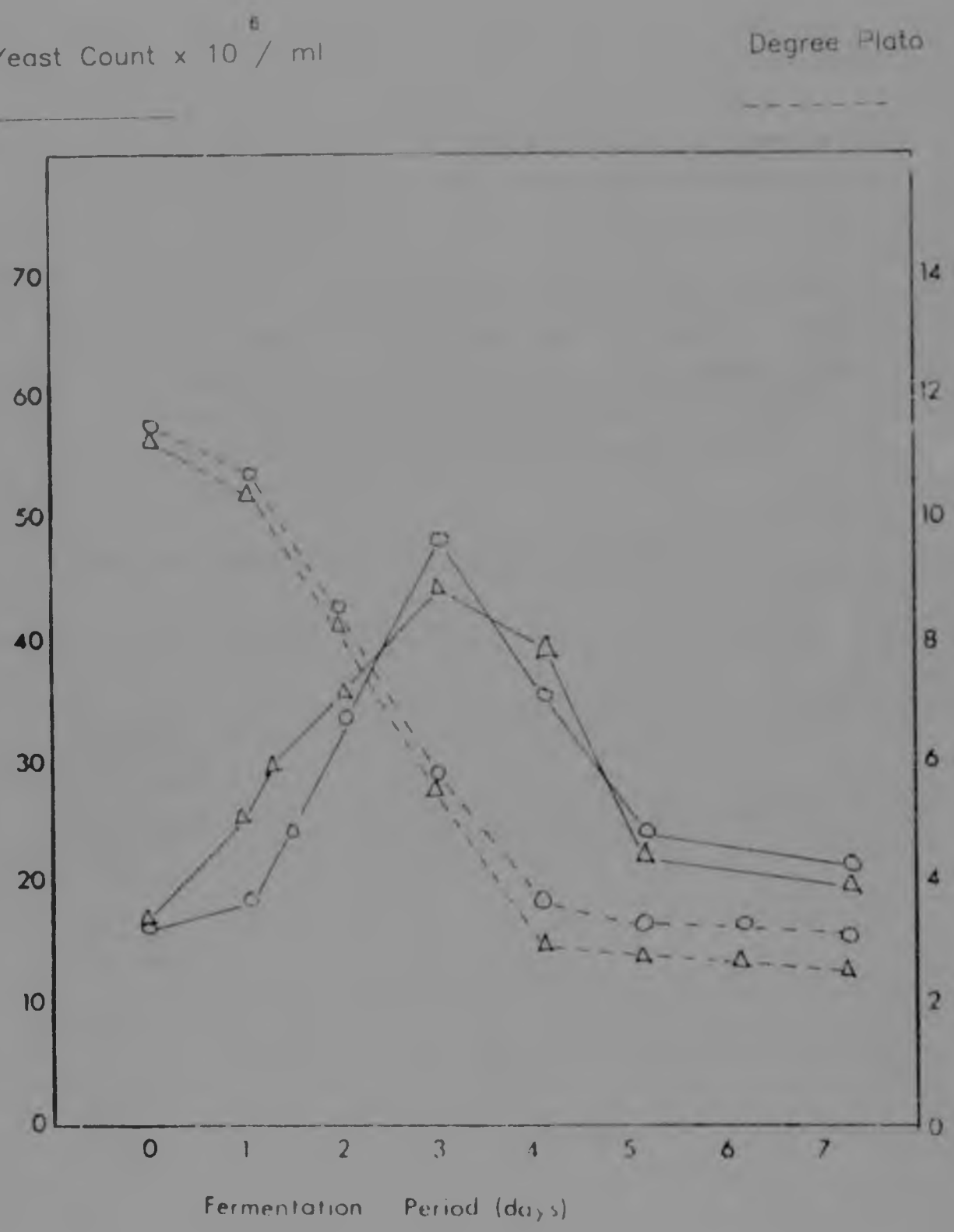
Degree Plato



Malt Sample No. 17

Brewery T° Mash Δ——Δ

Congress T° Mash O——O



From the results shown in Table 3 and fig. 8 it can be seen that fermentation patterns are improved using wort mashed according to method 2.9.2. c.f. method 2.9.1.

### 3.2.3. Fermentation Patterns of different Malts.

Samples of malt were received on a regular basis and mashed according to method 2.9.2. Resulting worts were pitched with Saccharomyces uvarum (x) according to methods 2.6 and 2.7.1. Fermentations were conducted in 2 litre fermentation tubes and the patterns monitored (method 2.12.)

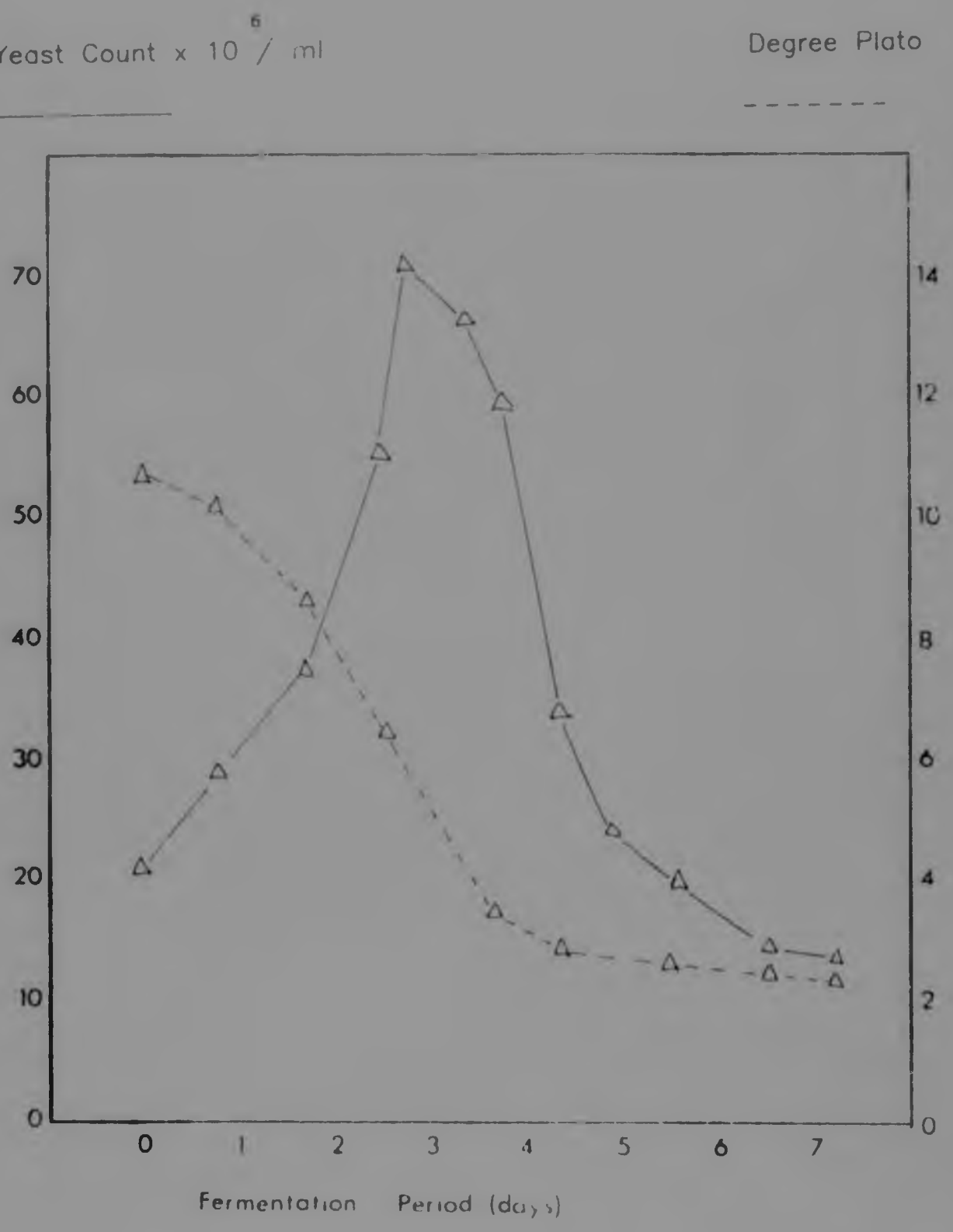
From a series of fermentations in the laboratory and in the plant a malt was chosen that fermented well both with respect to attenuation and yeast count pattern. This malt was set up as a control malt with each set of experiments. Over 50 malts were tested for their fermentation patterns.

TABLE 4. Fermentation Data of Different Malts.

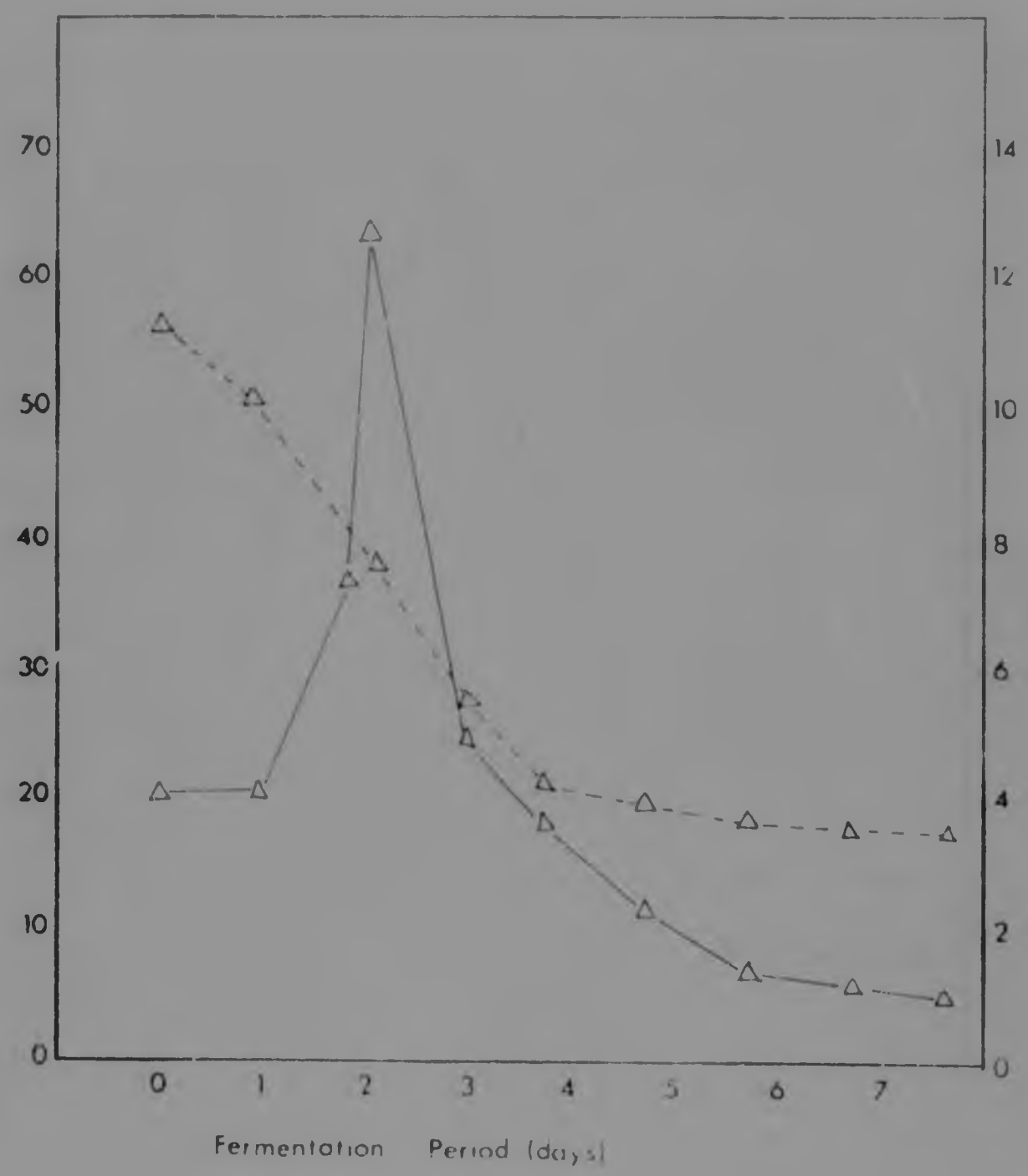
| | Malt Sample | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Pitching Yeast Count ($x10^6$/ml) | 21,0 | 20,1 | 19,9 | 25,0 | 20,5 | 16,4 | 18,8 | 17,7 | 16,1 | 18,5 | 16,9 |
| Peak Yeast Count ($x10^6$/ml) | 69,2 | 64,4 | 66,2 | 62,5 | 56,3 | 79,2 | 60,0 | 53,1 | 58,4 | 56,7 | 47,8 |
| Time of Peak(hrs) | 67 | 48 | 67 | 48 | 67 | 48 | 72 | 72 | 72 | 92 | 90 |
| S.G.96hrs. (°P) | 3,2 | 4,0 | 3,8 | 3,2 | 4,1 | 2,9 | 3,6 | 4,0 | 3,9 | 3,1 | 4,0 |
| S.G. day 8 (°P) | 2,1 | 3,6 | 2,8 | 2,9 | 3,2 | 2,7 | 2,8 | 2,3 | 3,0 | 2,1 | 3,2 |

Fig. 9. FERMENTATION PATTERNS OF DIFFERENT MALTS.

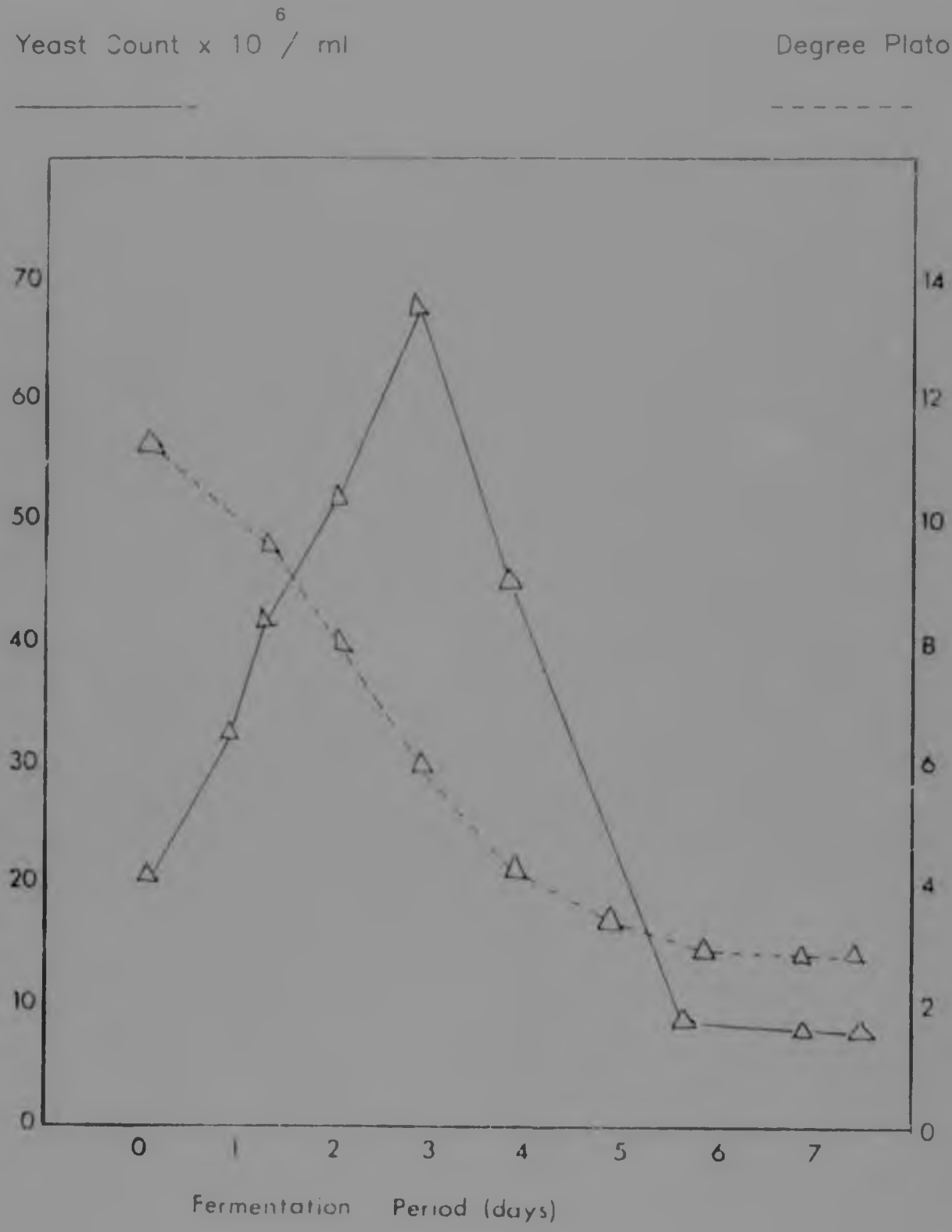
Control Malt



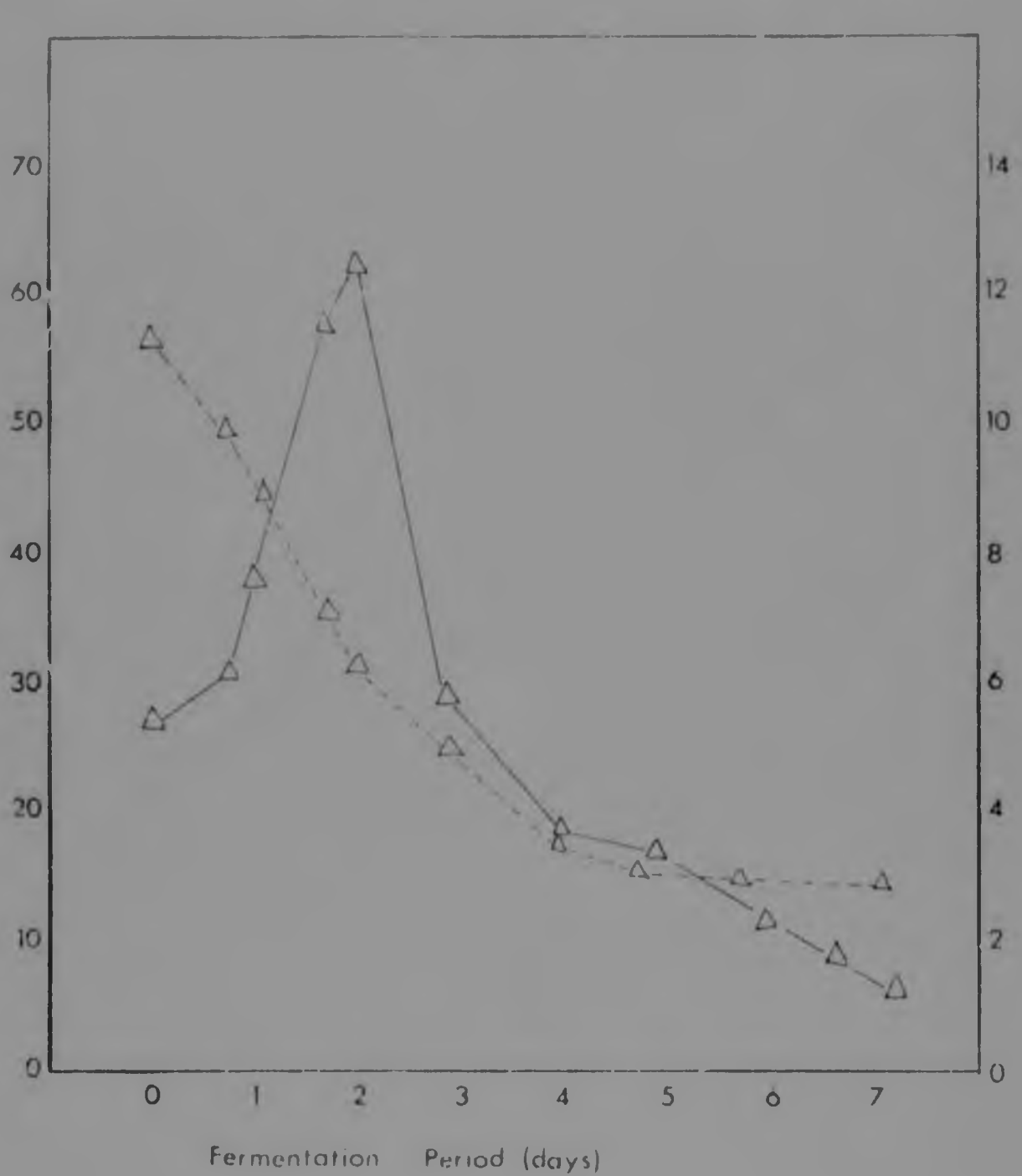
Malt Sample No. 1.



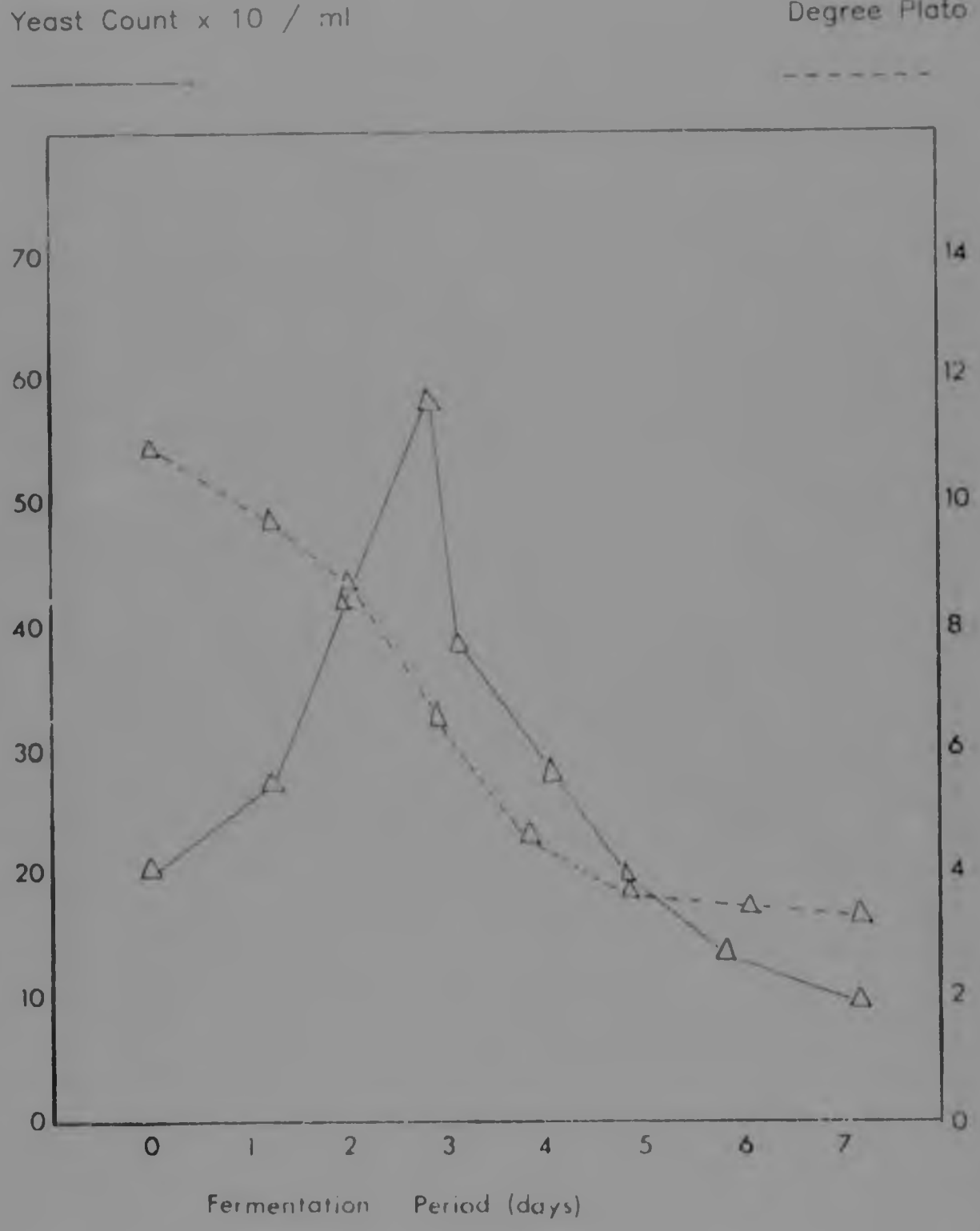
Malt Sample No. 2.



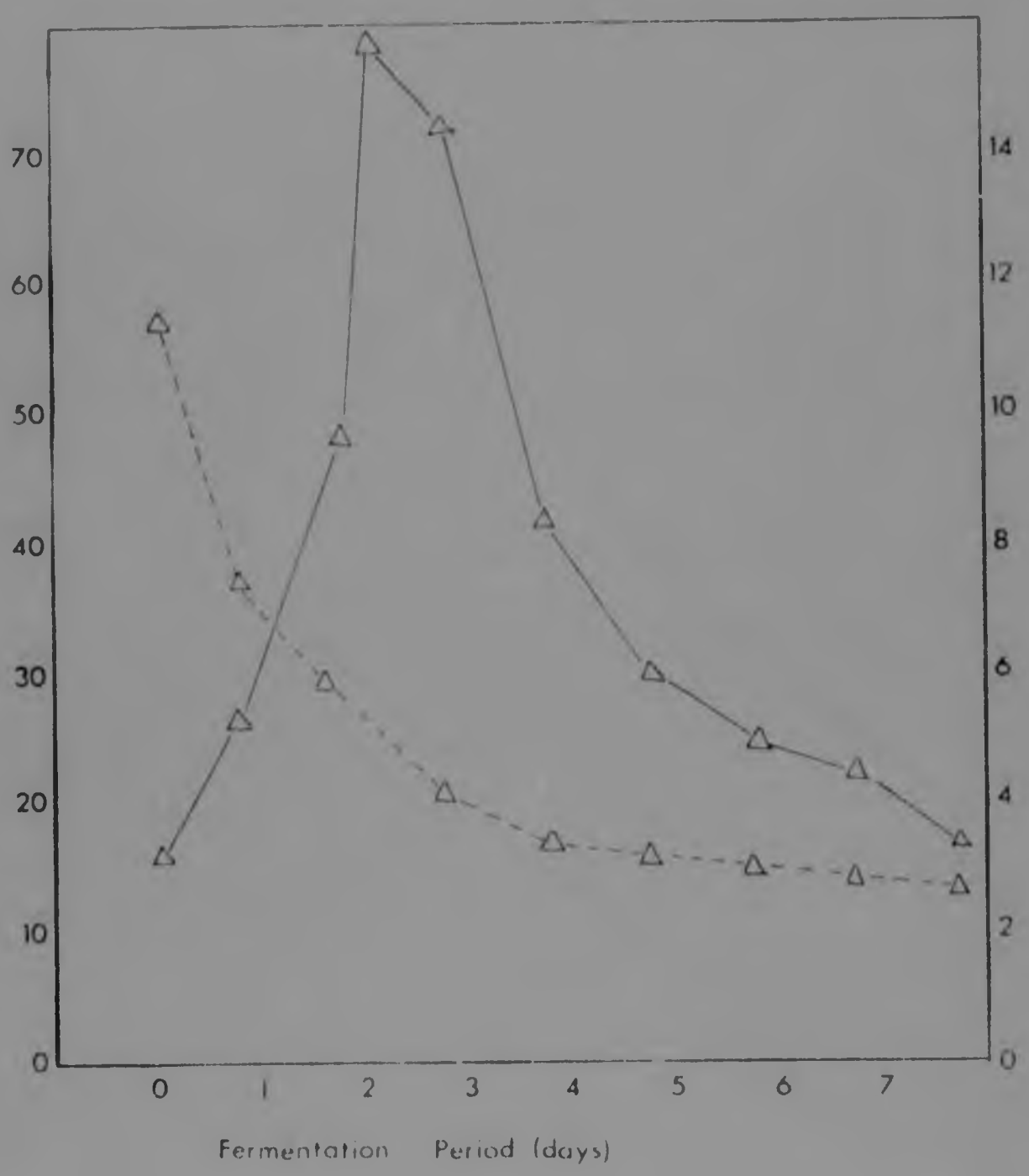
Malt Sample No. 3.



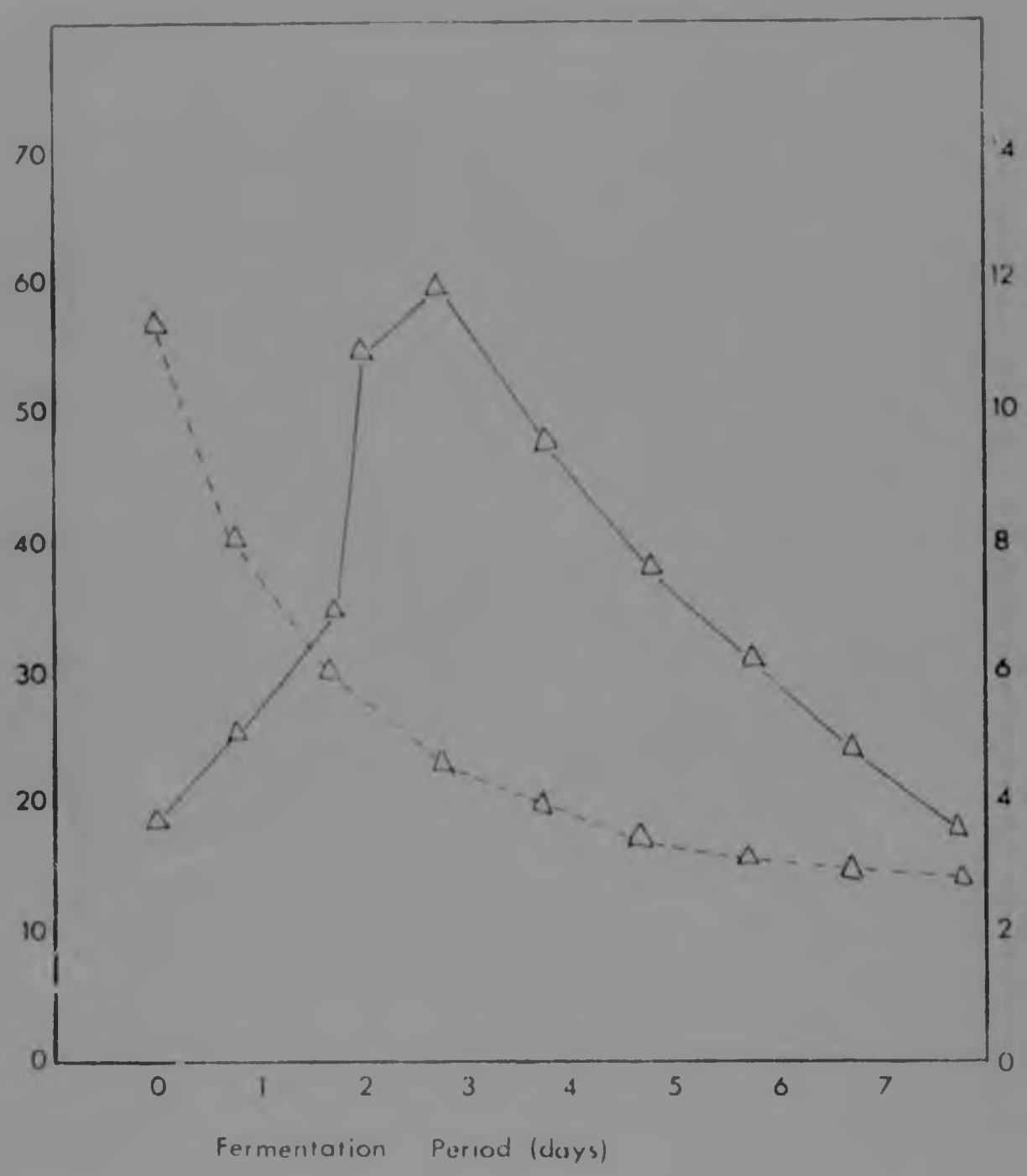
Malt Sample No. 4.



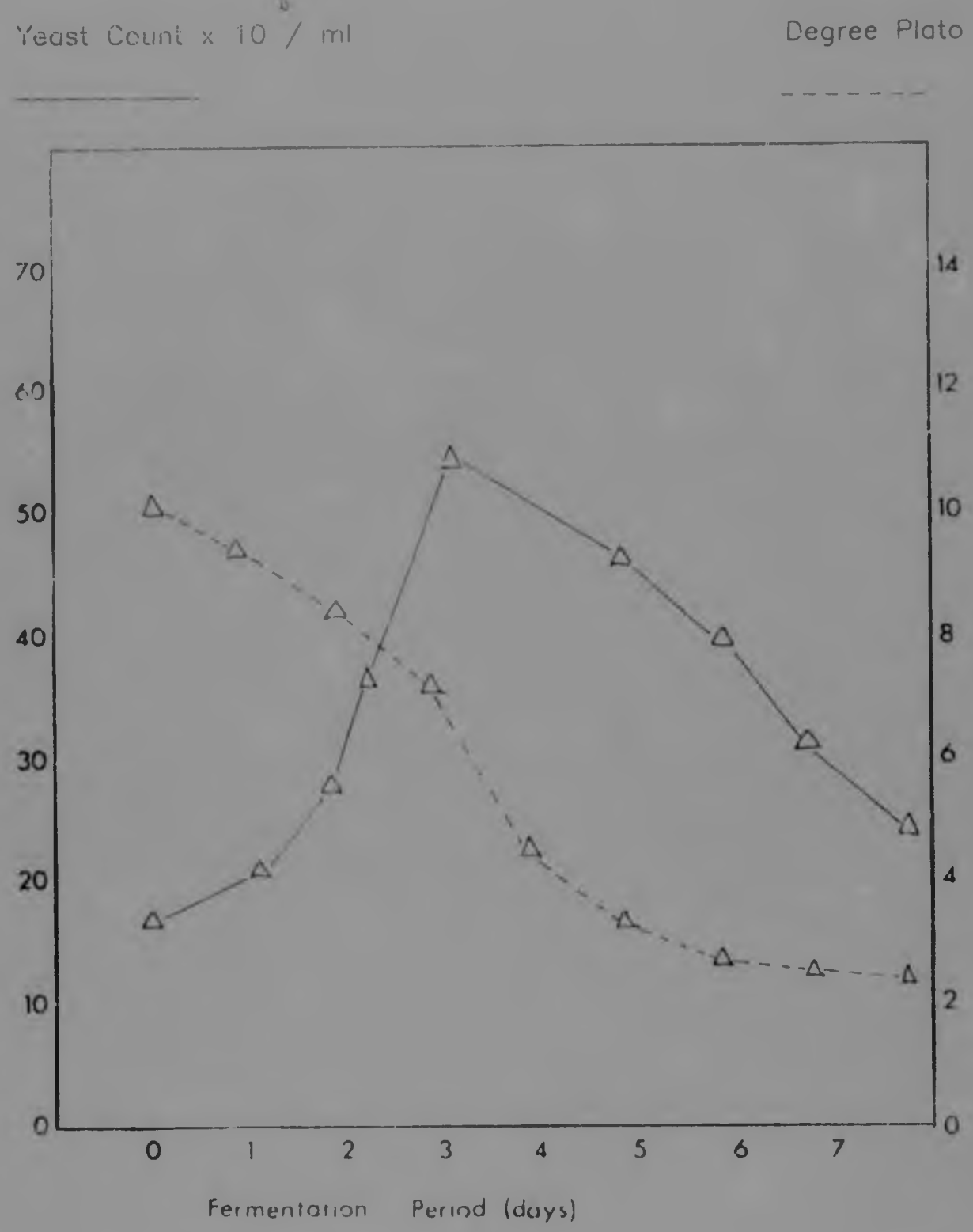
Malt Sample No. 5.



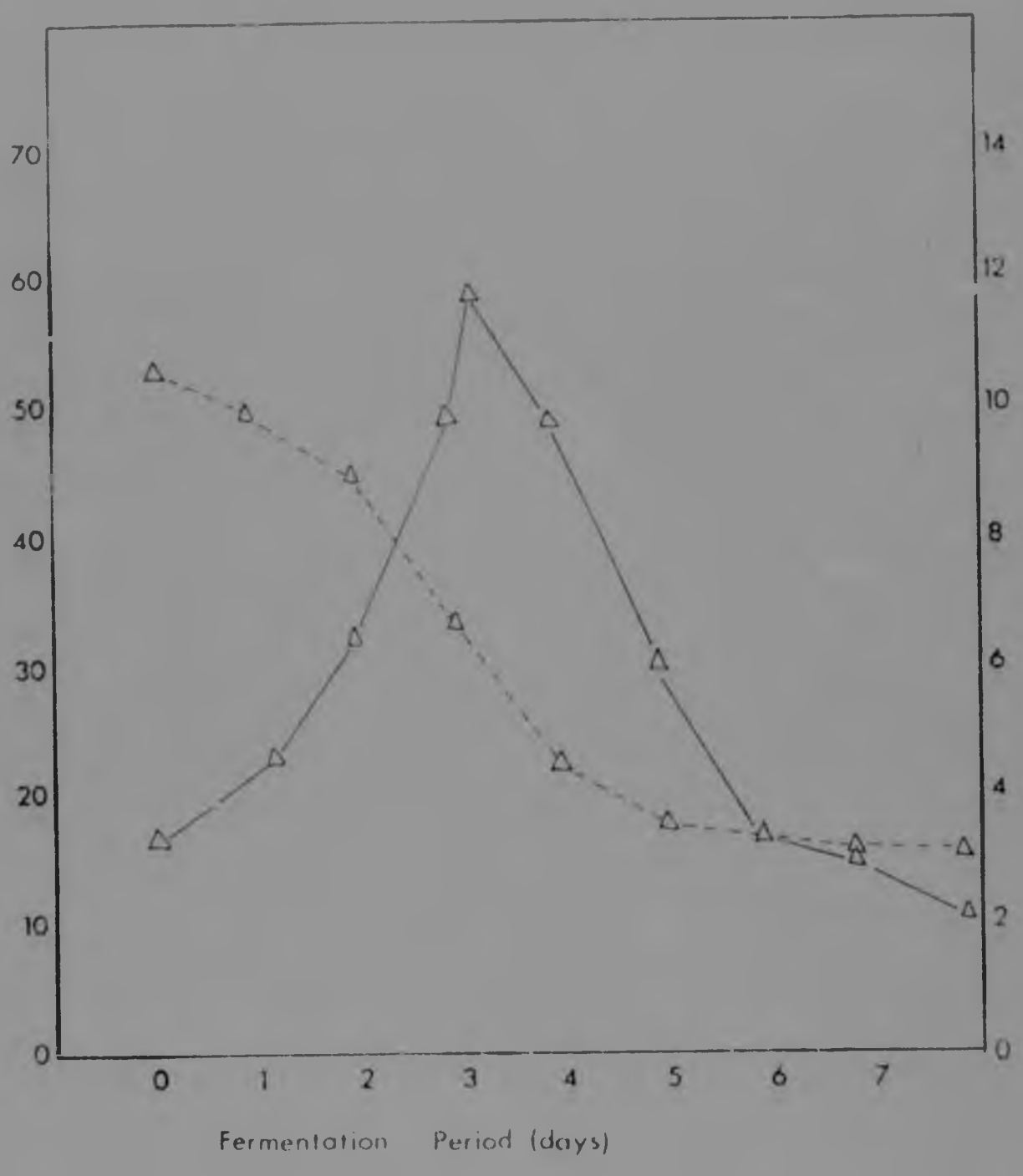
Malt Sample No. 6.



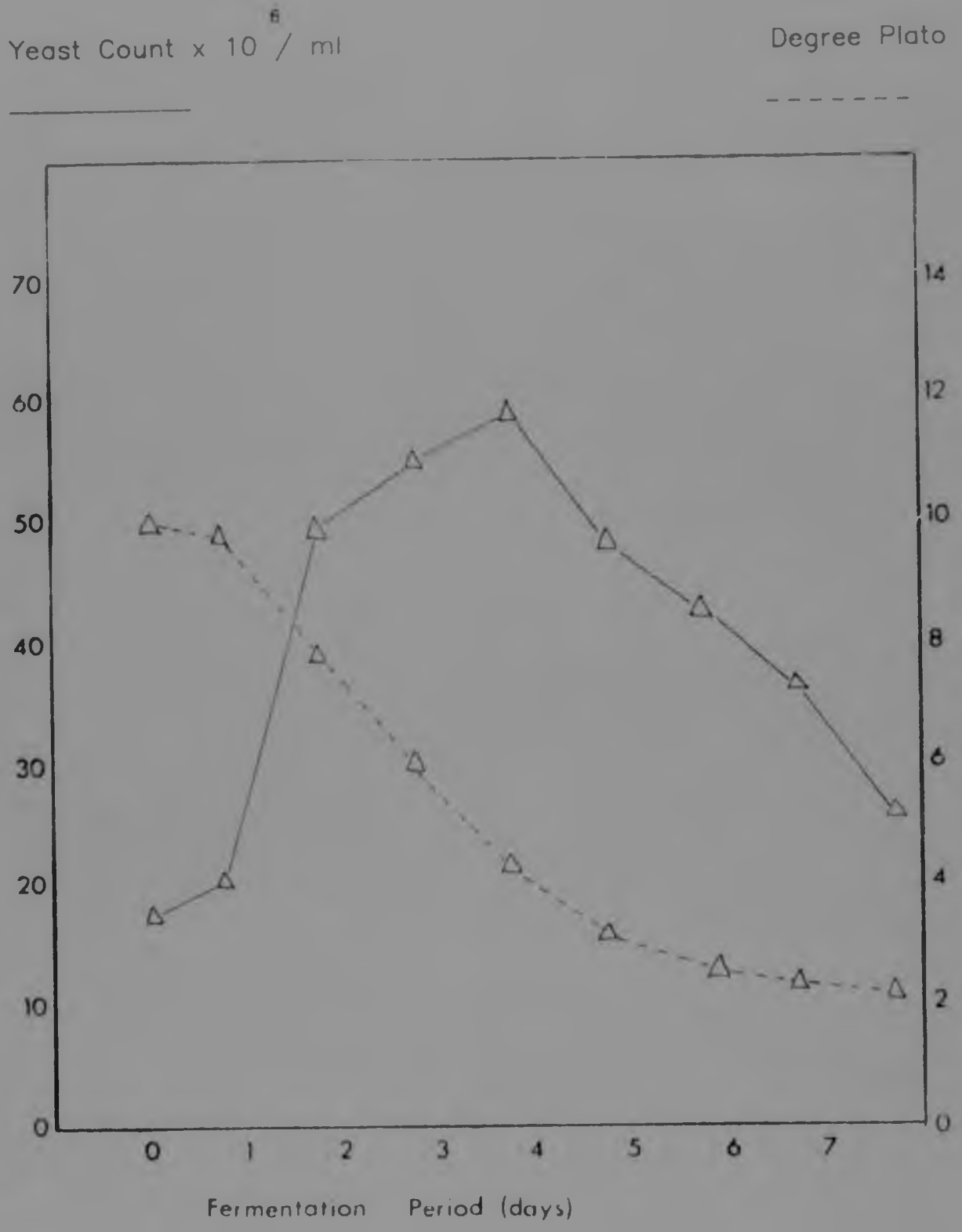
Malt Sample No. 7



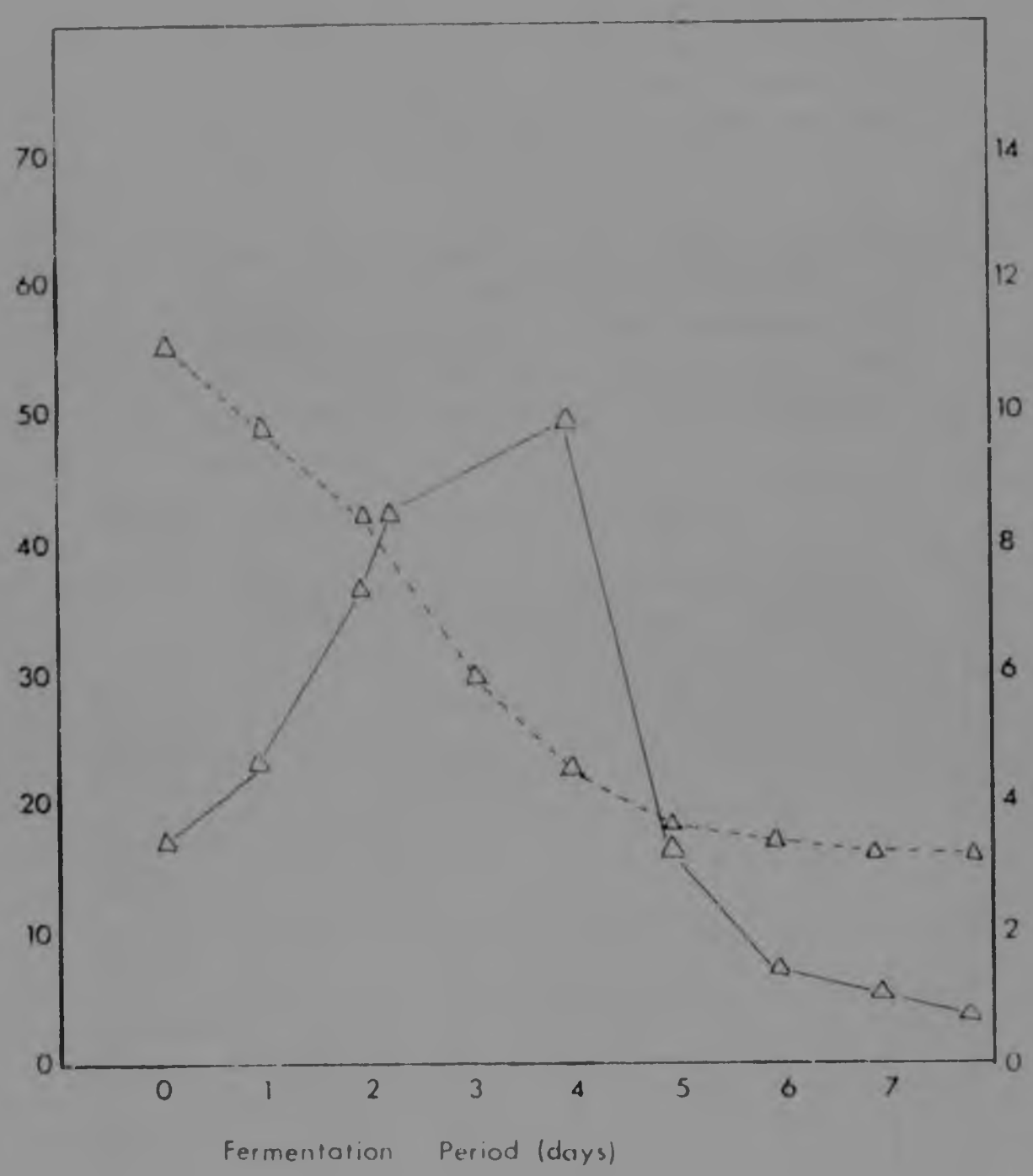
Malt Sample No. 8.



Malt Sample No. 9.



Malt Sample No. 10.



From the results illustrated in Table 4 and fig. 9. it can be seen that different malts can vary significantly in their fermentability. Experiments repeated three times showed the same results.

3.2.4. Setting the Limits for a Predictive Fermentation Test.

Various malts were selected based on their performance in the brewery plant. Malts were selected that fermented well in the plant and that gave no problems with respect to attenuation curve and yeast count pattern. Malts were also selected that created problems in the plant either with respect to yeast count pattern and/or attenuation curve.

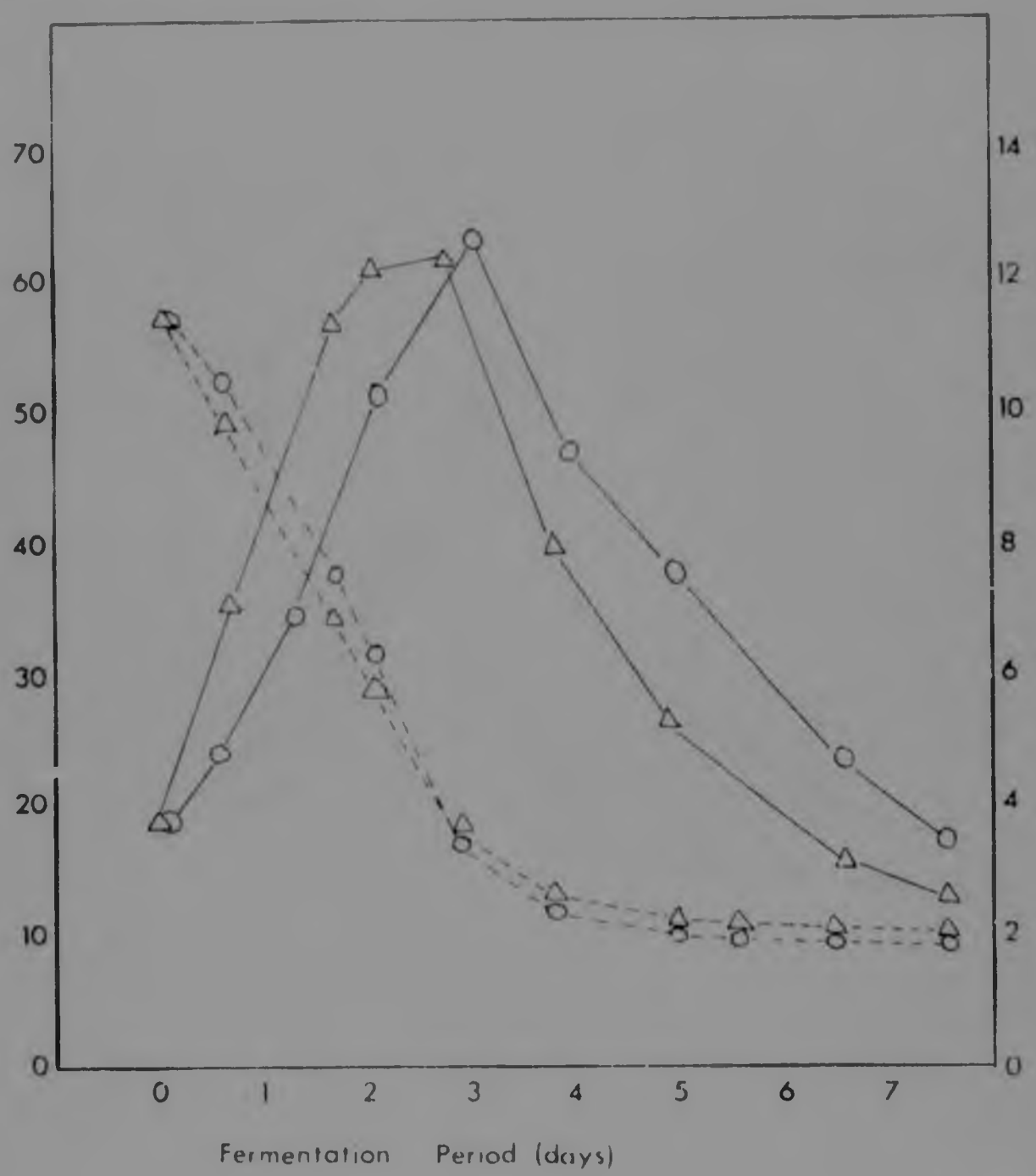
These malts were mashed in the laboratory according to method 2.9.2. The resulting worts were pitched with Saccharomyces uvarum(x) according to methods 2.6 and 2.7.1. Fermentations (2.12) were conducted in the 2 litre tubes described in 2.3. Twenty fermentations were run in total.

TABLE 5. Fermentation Data of Various Malts.

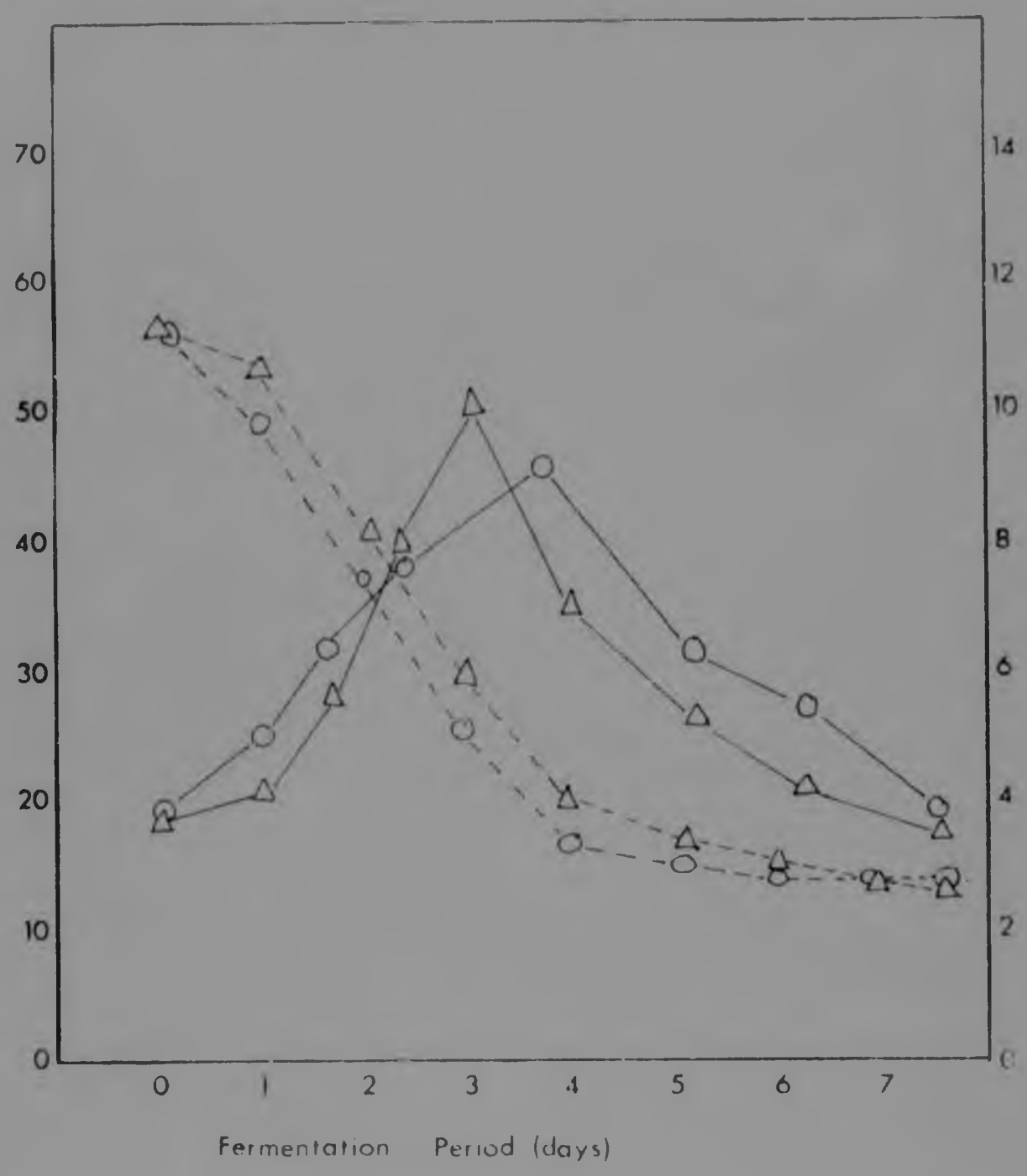
| | Malts Creating no Problem in the Brewery. | | Malts Creating certain Problems in the Brewery. | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Pitching Yeast Count ($x10^6$/ml) | 16,9 | 16,5 | 17,3 | 19,1 | 18,4 | 20,3 |
| Peak Yeast Count ($x10^6$/ml) | 60,1 | 62,8 | 50,5 | 45,6 | 55,3 | 50,4 |
| Time of Peak(hrs) | 67 | 72 | 72 | 90 | 67 | 48 |
| S.G.96hrs. (°P) | 2,5 | 2,3 | 3,5 | 3,0 | 3,8 | 4,2 |
| S.G. day 8 (°P) | 2,1 | 1,9 | 2,4 | 2,3 | 2,9 | 3,8 |

Fig. 10. FERMENTATION PATTERN: VARIOUS MALTS.

Malt 1 Δ——Δ
Malt 2 O——O



Malt 3 Δ——Δ
Malt 4 O——O



Malt 5 Δ——Δ
Malt 6 O——O

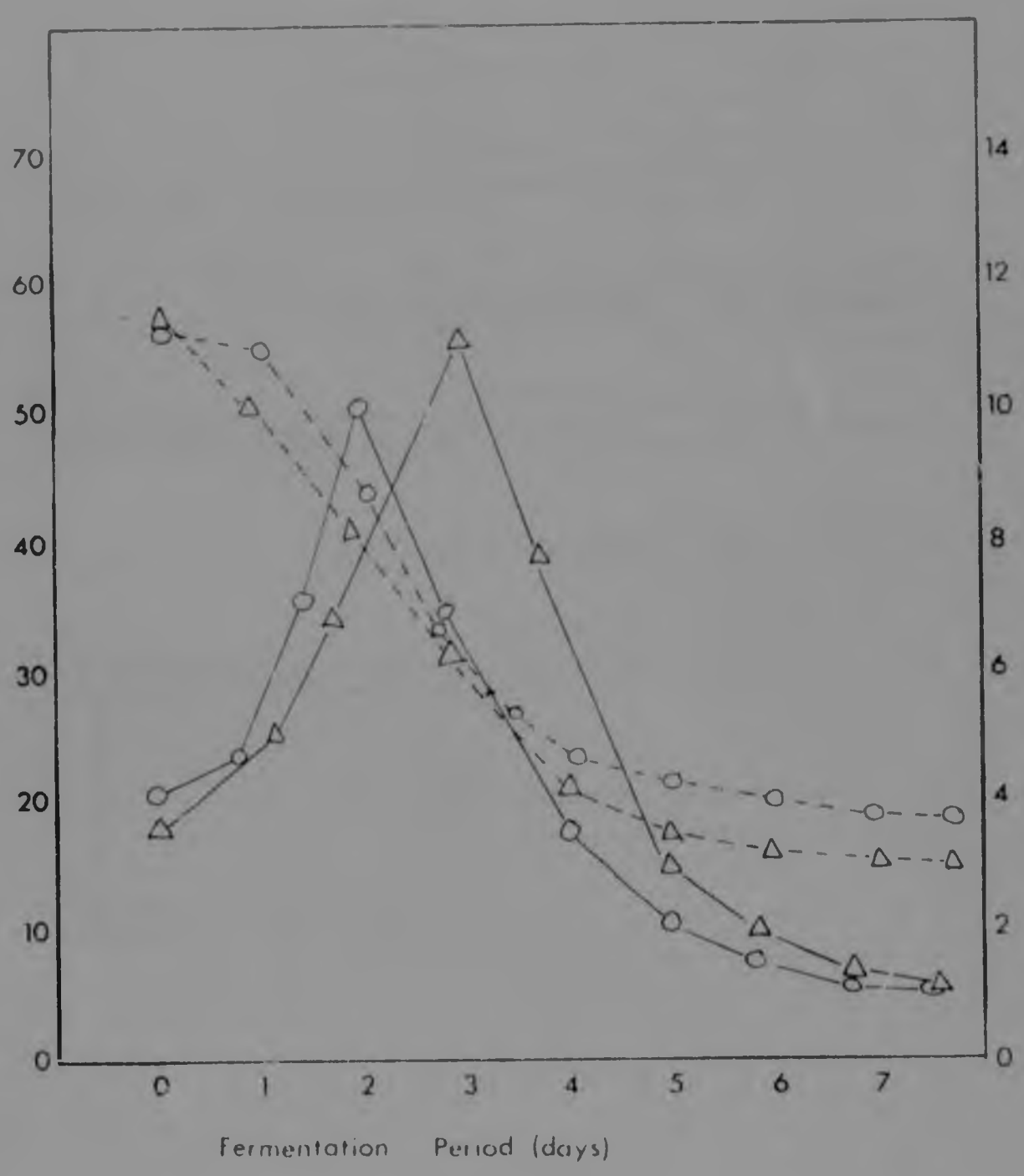
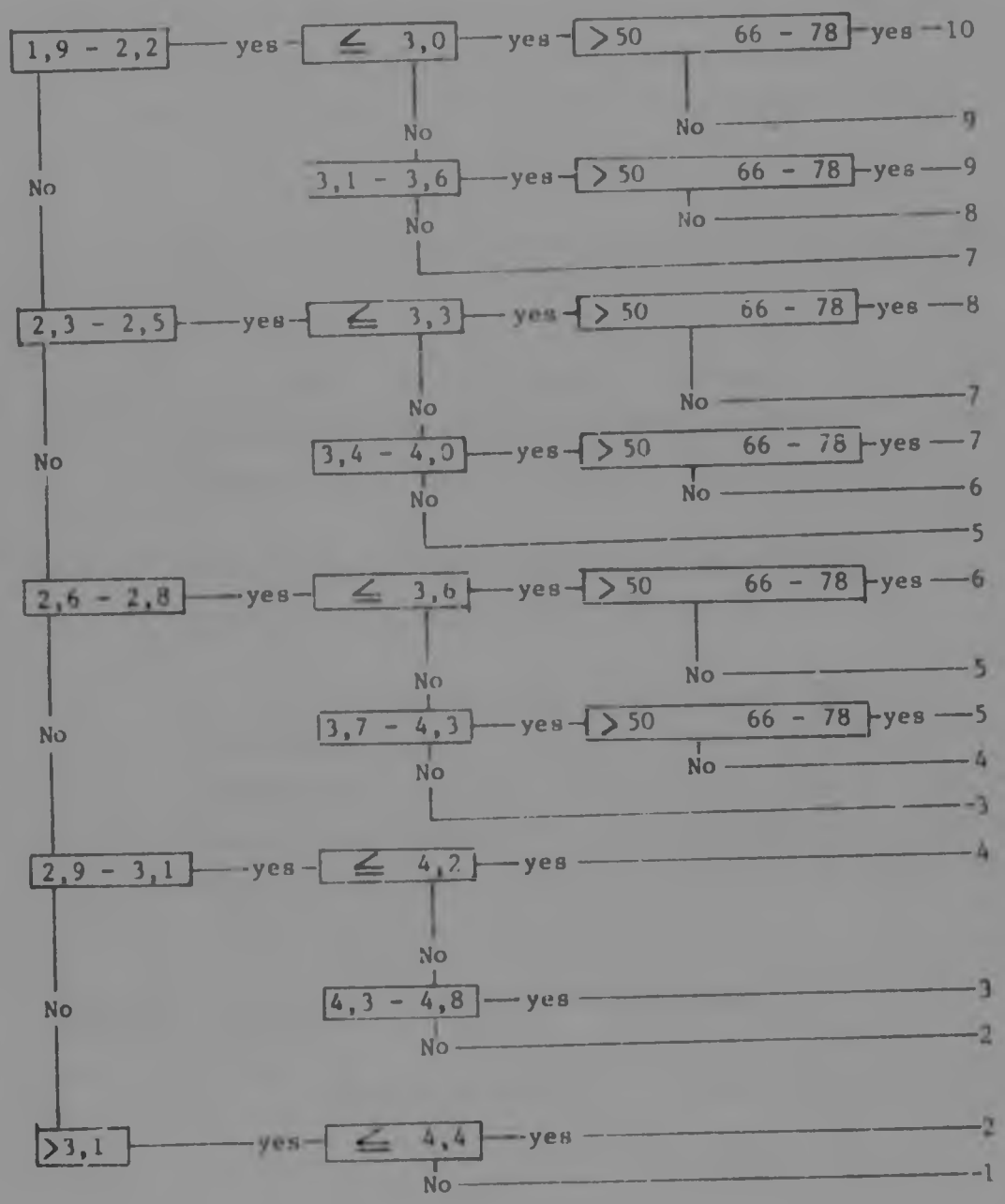


Fig.11.


MALT GRADING SYSTEM.
YEAST PEAK
Grade
°P on 8th day
°P @ 96 hrs
Count x 10⁶/ml & Time(hrs)
1,9 - 2,2
yes
≤ 3,0
yes
> 50
66 - 78
yes
10
No
9
No
3,1 - 3,6
yes
> 50
66 - 78
yes
9
No
8
No
7
2,3 - 2,5
yes
≤ 3,3
yes
> 50
66 - 78
yes
8
No
7
No
3,4 - 4,0
yes
> 50
66 - 78
yes
7
No
6
No
5
2,6 - 2,8
yes
≤ 3,6
yes
> 50
66 - 78
yes
6
No
5
No
3,7 - 4,3
yes
> 50
66 - 78
yes
5
No
4
No
3
2,9 - 3,1
yes
≤ 4,2
yes
4
No
4,3 - 4,8
yes
3
No
2
>3,1
yes
≤ 4,4
yes
2
No
1

Using the fermentation data given in Table 5 and fig. 10 and the fermentation data obtained from mashing and fermenting 12 other malts a 10 point malt grading system was constructed. (fig.11).

## 3.3. Yeast Research

The fermentation system described in 2.4 was used to investigate certain parameters of yeast behaviour. The parameters investigated could be of critical significance to the brewing process.

### 3.3.1. Relationship between Aeration and Fermentation Efficiency (F.E.)

As stated in the introduction controversy exists with reference to the oxygenation/aeration regime both during propagation and fermentation.

### 3.3.1a Medical Air vs Medical Oxygen During Laboratory Propagation of Saccharomyces uvarum (x).

A culture of Saccharomyces uvarum(x) was propagated according to method 2.8.1 in duplicate (A & B). In propagation A the 200 ml stage and the 5000 ml stage were aerated using medical air. In propagation B the 200 ml stage and the 5000 ml stage were oxygenated using medical oxygen.(Table 6).

TABLE 6. Propagation Data Using Air and Oxygen.

| | Aerated Propagation (A) | | Oxygenated Propagation (B) | |
|---|---|---|---|---|
| | Wort b | Wort a | Wort b | Wort a |
| Viability of Yeast (%) | 97 | 98 | 98 | 99 |
| S.G. (°P) | 10,4 | 10,42 | 9,39 | 9,97 |
| Yield (g) | 37,15 | 21,9 | 62,0 | 42,6 |

The yeast was cropped from the propagations and pitched in duplicate (according to methods 2.6 and 2.7.3) into wort b. This procedure was repeated and the yeast pitched into wort a.
Fermentations (2.12) were conducted in the 2 litre tubes. (2.3). (Table 7).

TABLE 7. Fermentation Data of Yeast Propagated with Aeration and Yeast Propagated with Oxygenation.

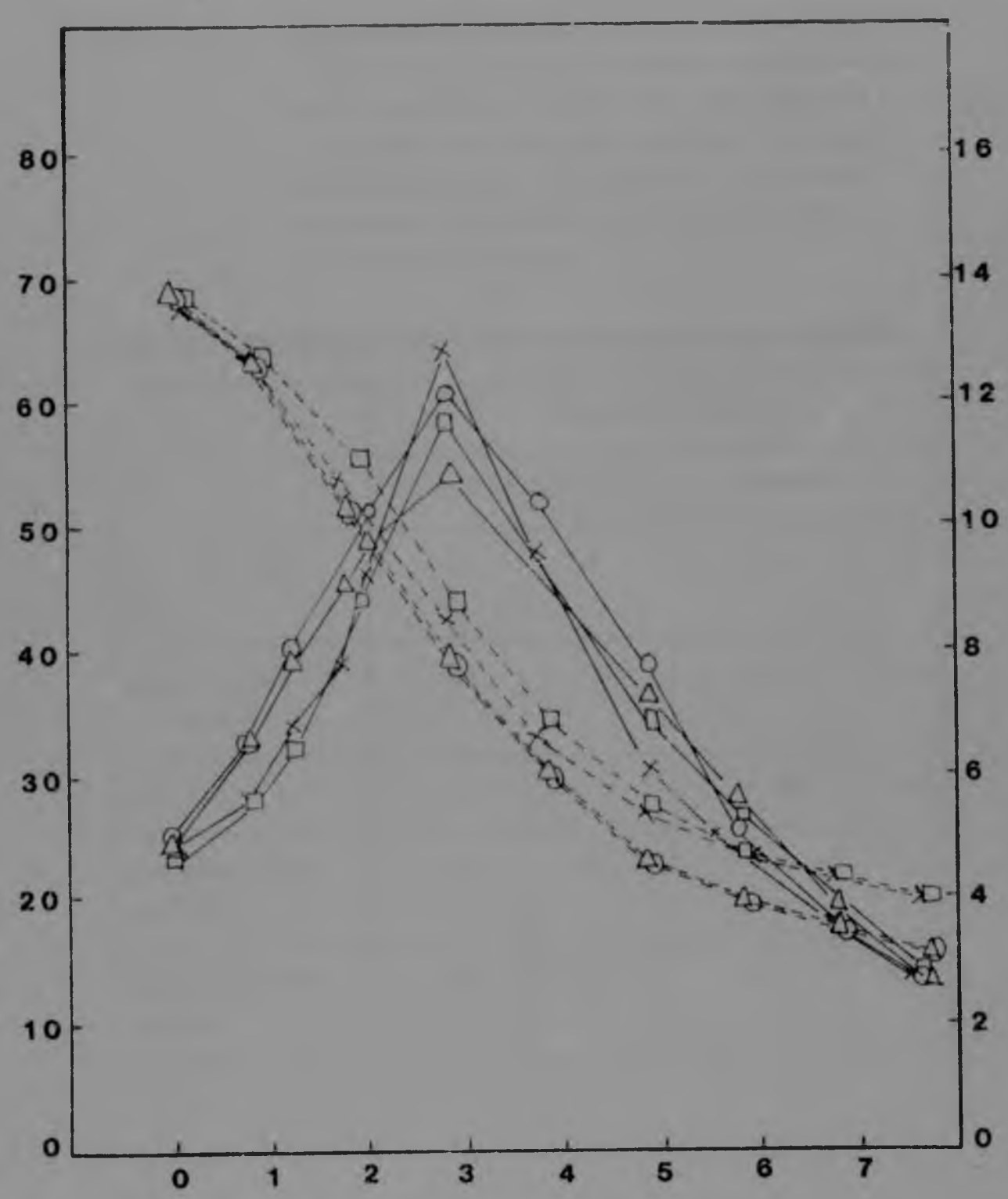
| | Using Yeast A | | | | Using Yeast B. | | | |
|---|---|---|---|---|---|---|---|---|
| | Wort a. | | Wort b. | | Wort a. | | Wort b. | |
| S.G. @ 187hrs. (°P) | 3,1 | 3,0 | 3,4 | 3,2 | 4,0 | 4,0 | 3,9 | 3,8 |
| Diacetyl d.8. (p.p.m.) | 0,18 | 0,18 | 0,16 | 0,16 | 0,14 | 0,14 | 0,15 | 0,14 |
| pH d.8 | 4,08 | 4,09 | 4,14 | 4,12 | 4,07 | 4,08 | 4,13 | 4,10 |
| Yeast Count d.8 ($x10^6$/ml) | 13,0 | 12,8 | 17,8 | 13,0 | 13,0 | 12,6 | 13,0 | 12,2 |
| Peak Yeast Count ($x10^6$/ml) | 55,0 | 68,0 | 58,0 | 52,9 | 59,0 | 65,0 | 62,1 | 60,4 |

Pitching of yeast was according to the standard method of 6g/l pressed using yeasts described in table 6.

Fig. 12. FERMENTATION PATTERNS OF YEAST PROPAGATED USING AERATION VS YEAST PROPAGATED USING OXYGENATION.

Yeast Aerated During Propagation (i) Δ—Δ (ii) ○—○

Yeast Oxygenated During Propagation (i) □—□ (ii) ×—×



3.3.1.b Optimising Aeration/Oxygenation for Saccharomyces Uvarum(x).

A series of experiments were conducted where different methods of aerating/oxygenating the wort and/or yeast and wort prior to or after pitching were used.

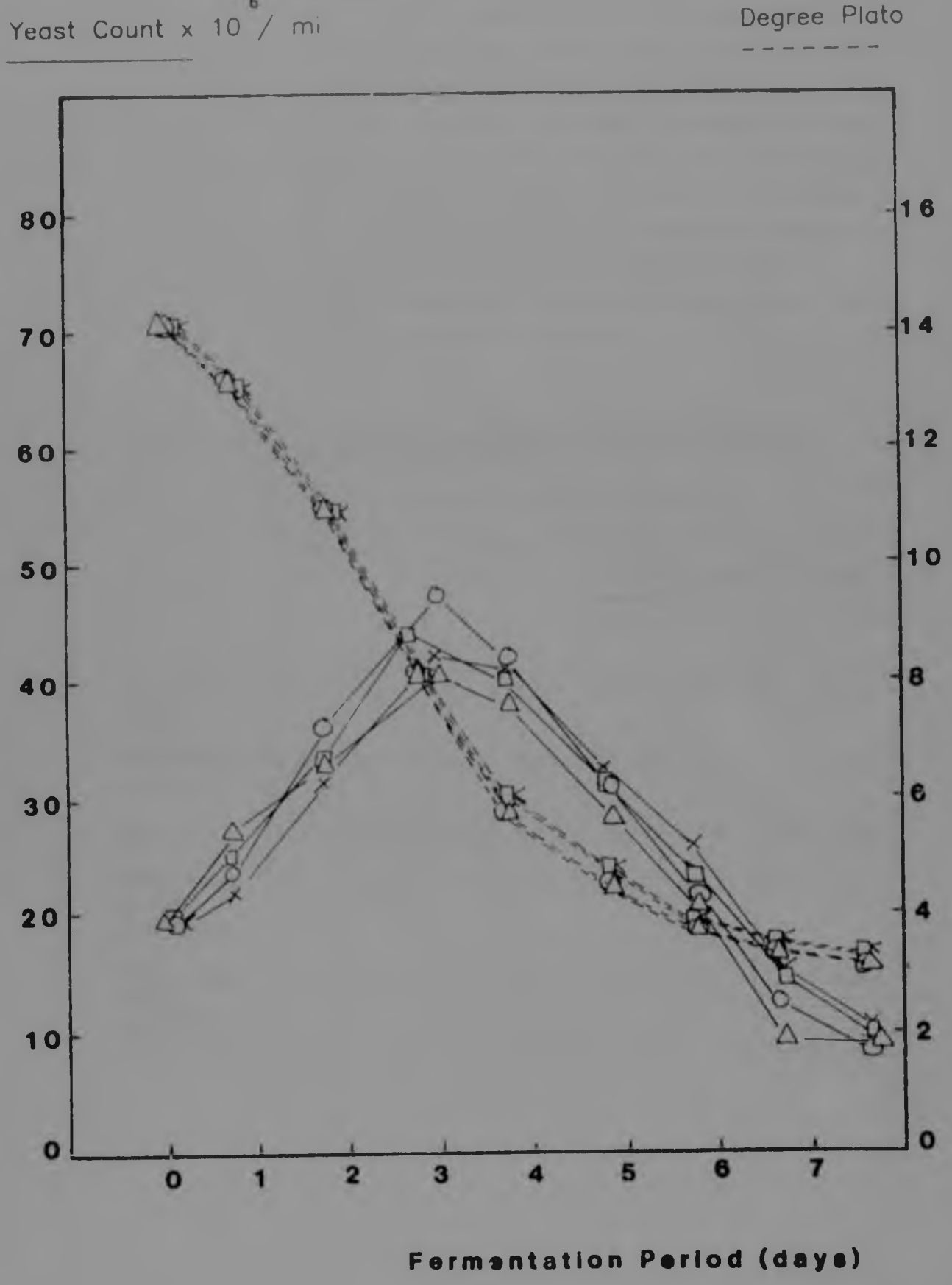
3.3.1.b(1) Saccharomyces uvarum(x) was collected ex yeast storage vessel (YSV) at a brewery and pitched into wort b according to method 2.6. The oxygenation procedures were according to methods 2.7.4 and 2.7.3 respectively. Fermentations (2.12) were conducted in the 2 litre fermentation tubes (2.3) in duplicate.(Table 8).

TABLE 8. Fermentation Data using different Oxygenation Regimes.

| | Oxygenation Method. | | | |
|---|---|---|---|---|
| | Method 2.7.4. | | Method 2.7.3. | |
| S.G. @ 233 hrs. (°P) | 2,8 | 2,8 | 2,9 | 2,9 |
| Diacetyl d.10. (p.p.m.) | 0,21 | 0,20 | 0,27 | 0,25 |
| pH d. 10. | 4,15 | 4,14 | 4,20 | 4,17 |
| Peak Yeast Count ($x10^6$/ml) | 40,1 | 49,4 | 45,1 | 43,9 |
| Yeast Count d.10 ($x10^6$/ml) | 4,1 | 4,1 | 5,2 | 4,0 |

Fig. 13. FERMENTATION PATTERNS OF YEASTS OXYGENATED ACCORDING TO METHODS 2.7.4 and 2.7.3.

Yeast pitched into wort with a D.O. 14 p.p.m.(i) □—□ (ii) ×—×
Yeast and wort aerated for 2 hours prior to pouring into 2 litre fermentation tubes.(i) △—△ (ii) ○—○



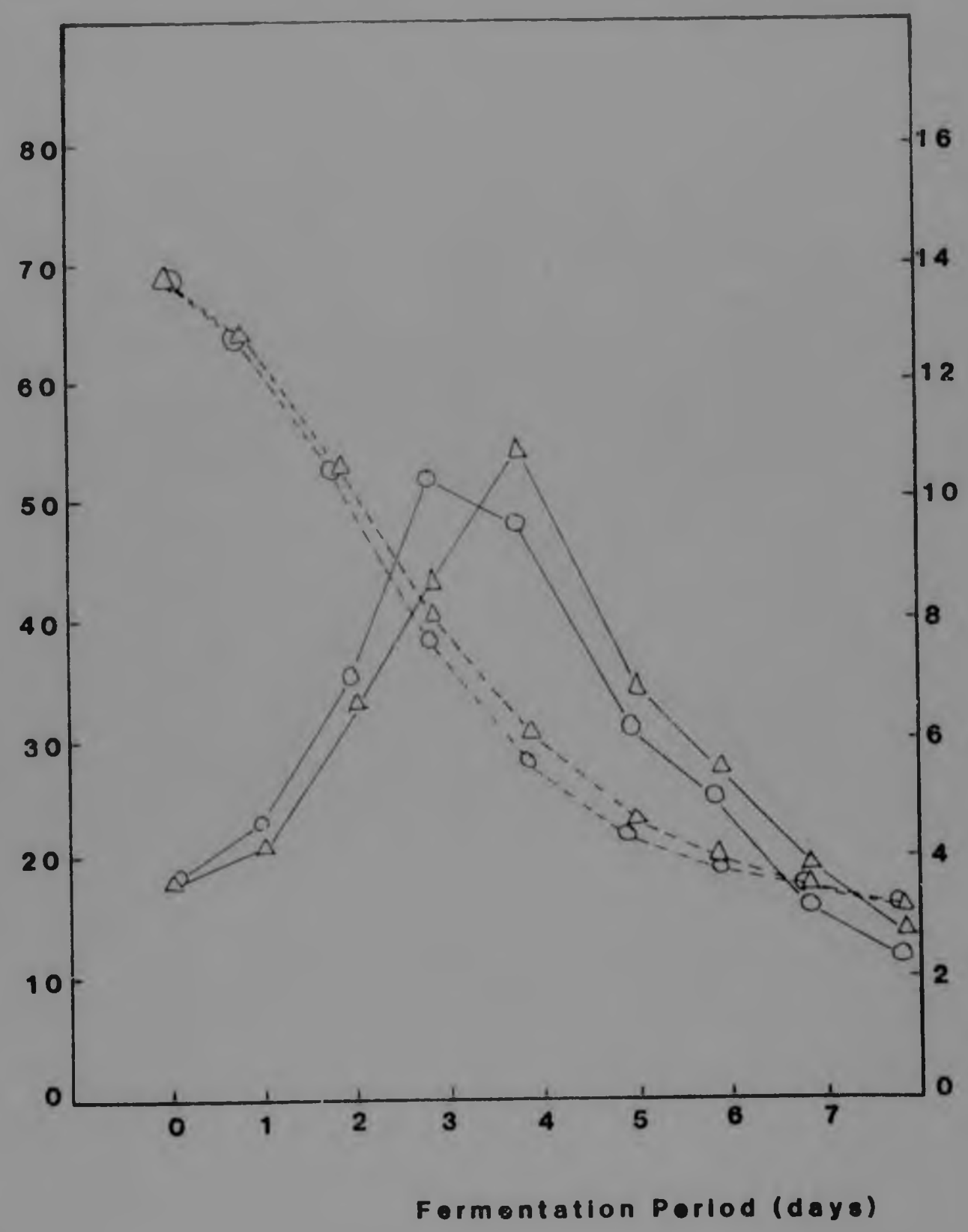
3.3.1.b(ii) Saccharomyces uvarum(x) was collected ex YSV and pitched into wort b according to method 2.6. The following aeration/oxygenation procedures were employed: according to the standard methods 2.7.3 and 2.7.2.; according to the standard method 2.7.3. except that 19hrs after pouring into the 2 litre tubes (2.3) the fermenting wort was collected from the bottom of the tubes and shaken 30 times prior to pouring back into the tubes ,ie rousing and aerating at 19hrs); according to the standard method 2.7.1 except that the fermenting wort was roused and aerated at 6 hrs and at 24 hrs. Fermentations (2.12) were conducted in the 2 litre tubes in duplicate.(Table 9).

TABLE 9. Fermentation Data of Various Aeration/Oxygenation Treatments of Yeast/Wort.

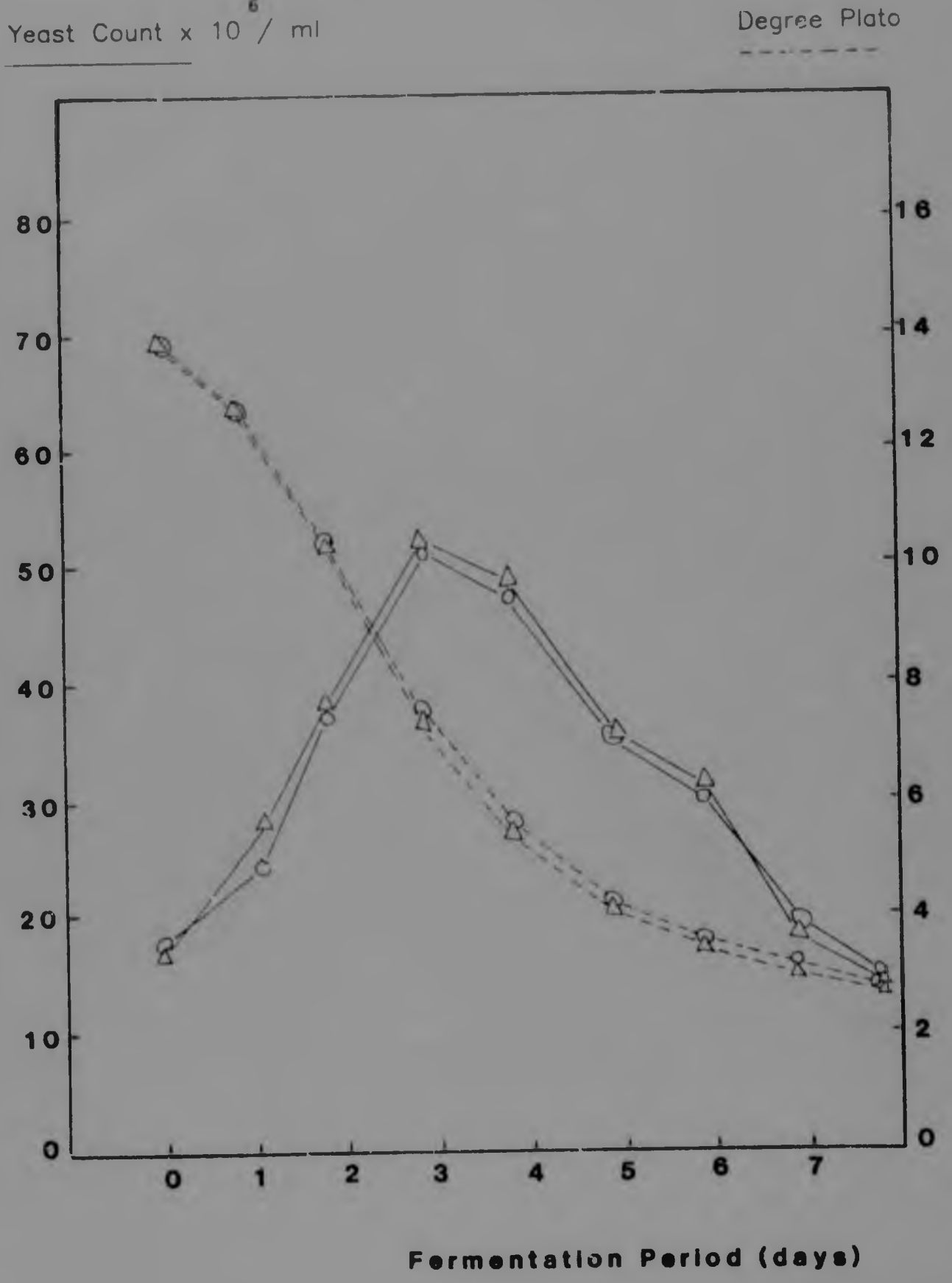
| | Aeration/Oxygenation Procedure. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Method 2.7.3. | | Method 2.7.2. | | Method 2.7.3. + rousing and aerating at 19 hrs. | | Method 2.7.1 + rousing and aerating at 6hrs 24 hrs. | |
| S.G. d.8 (°p) | 3,1 | 3,0 | 2,6 | 2,7 | 2,8 | 2,8 | 2,6 | 2,4 |
| Diacetyl d.8 (p.p.m.) | 0,21 | 0,25 | 0,17 | 0,17 | 0,15 | 0,19 | 0,14 | 0,13 |
| pH d.8. | 4,24 | 4,24 | 4,17 | 4,19 | 4,07 | 4,09 | 3,88 | 3,92 |
| Peak Yeast Count ($x10^6$/ml) | 51,3 | 52,5 | 52,0 | 51,2 | 44,5 | 45,2 | 63,2 | 62,3 |
| Final Yeast Count ($x10^6$/ml) | 13,7 | 11,8 | 13,1 | 14,1 | 9,6 | 9,0 | 9,3 | 9,6 |

Fig. 14. FERMENTATION PATTERNS OF VARIOUS AERATION/OXYGENATION TREATMENTS OF YEAST/WORT.

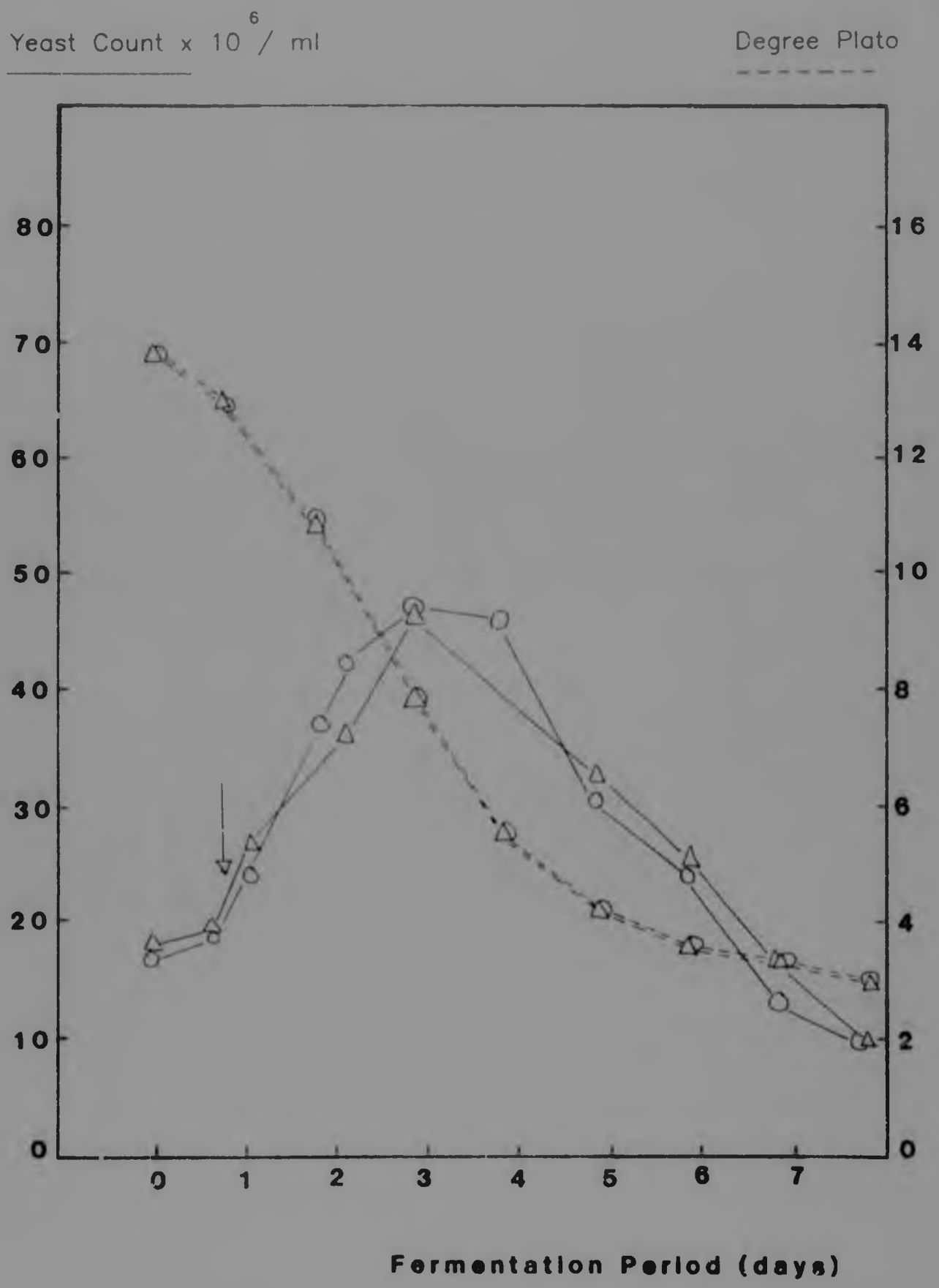
Yeast pitched into wort with a D.O. 14 p.p.m.(i) Δ—Δ (ii) O—O



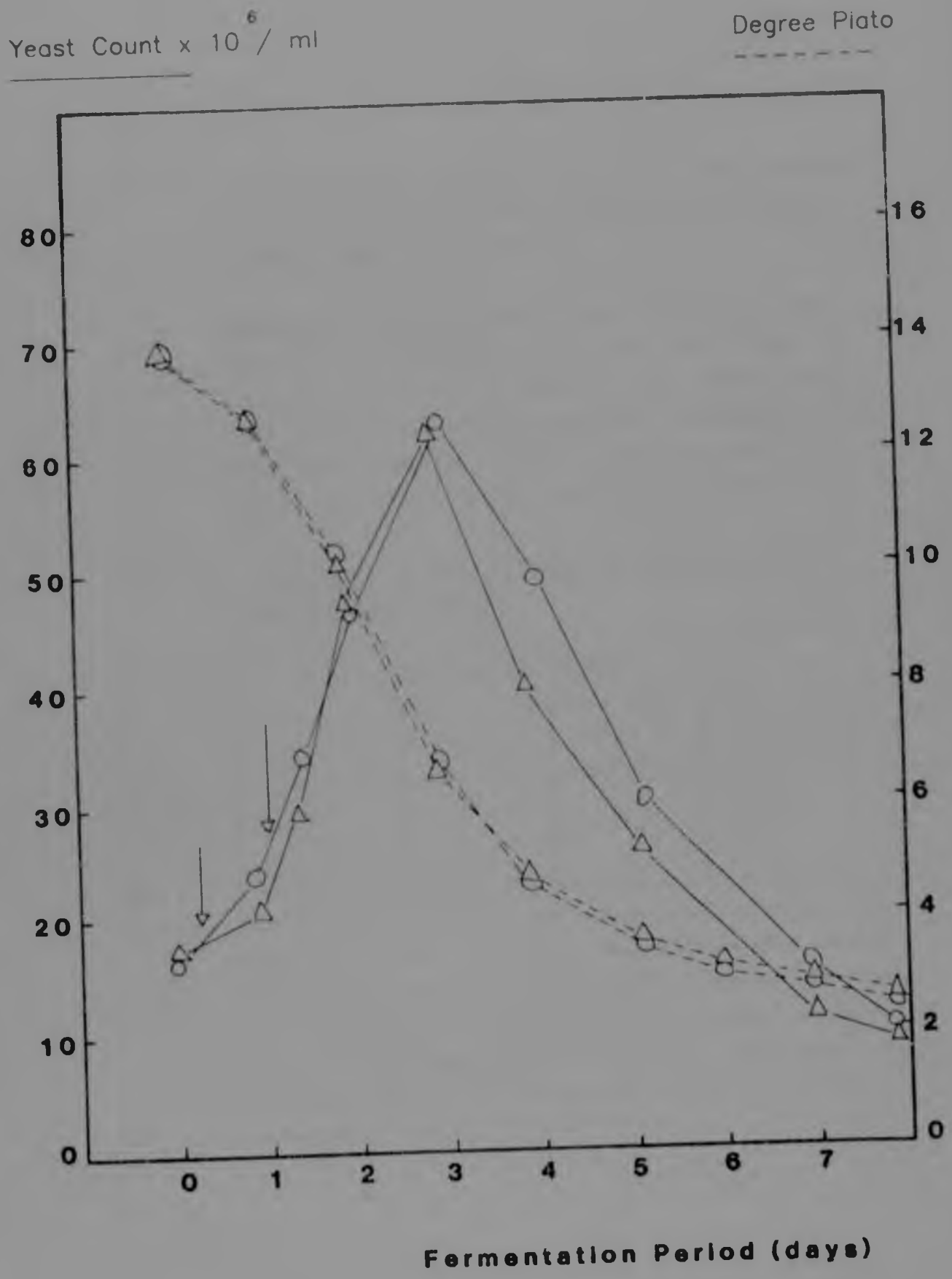
Yeast and wort shaken 30 times, allowed to stand for 1 hour and shaken again 30 times prior to pouring into 2 litre fermentation tubes. (i) Δ——Δ (ii) O——O



Yeast Pitched into wort with D.O. 14 p.p.m. Roused and aerated at 19 hrs (↓) (i) Δ—Δ (ii) O——O



Wort and Yeast shaken 30 times prior to pouring into 2 litre fermentation tubes. Roused and aerated at 6 hrs and 24 hrs (↓)
(i) Δ——Δ (ii) O——O



### 3.3.2. Effect of Wort Composition/Gravity of Propagation Medium and Fermentation Medium on Performance of Saccharomyces uvarum(x)

Various wort compositions, wort gravities and modes of propagation were investigated in the light of the fermentative power of the yeast variously generated.

### 3.3.2.a Effect of Wort Composition/Gravity of Propagation Medium on Yeast Crop and Subsequent Fermentation in worts of different Composition/Gravity.

Saccharomyces uvarum(x) was propagated according to method 2.8.1. Propagation medium was wort a, Wort A, laboratory mashed wort according to std. method 2.9.2 (wort D) and a laboratory mashed wort according to method 2.9.2 except that the original gravity was 14,1°P, ie. 600g of malt used (wort d), respectively.(Table 10).

TABLE 10. Yield(grms) of Yeast obtained by Propagation for 4 days in Various Worts.

| | PROPAGATION MEDIUM | | | |
|---|---|---|---|---|
| | Wort a | Wort A | Wort d | Wort D. |
| Run 1 | 36 | 49 | 123 | 118 |
| Run 2 | 45 | 55 | 114 | - |

Yeast crops from each of these propagations were pitched according to methods 2.6 and 2.7.2 into each of the above worts respectively. Fermentations (2.12) were conducted in the 2 litre fermentation tubes (2.3). Since the number of tubes was limiting, the experiment was run twice rather than in duplicate.

n all cases the yeast viabilities were 98% (according to method 2.11) (Table 11).

TABLE 11. Fermentation Data Using Yeast Propagated in Various Worts.

| Propagation Medium | Fermentation Medium | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wort a | | | Wort A | | | Wort d | | | Wort D. | | |
| | SG (°P | Diacetyl (ppm) | pH | SG (°P | Diacetyl (ppm) | pH | SG (°P | Diacetyl (ppm) | pH | SG (°P | Diacetyl (ppm) | pH |
| Run 1. | | | | | | | | | | | | |
| Wort a | 3,6 | 0,15 | 4,08 | 2,0 | 0,14 | 3,93 | 2,2 | 0,38 | 4,44 | - | - | - |
| Wort A | 3,6 | 0,14 | 4,02 | 2,3 | 0,12 | 3,93 | 2,2 | 0,39 | 4,44 | 2,2 | 0,27 | 4,28 |
| Wort d | 3,5 | 0,15 | 3,93 | 2,- | 0,18 | 3,91 | 2,2 | 0,32 | 4,40 | 2,2 | 0,22 | 4,30 |
| Wort D | 2,9 | 0,17 | 4,04 | 2,0 | 0,16 | 3,75 | 2,3 | 0,45 | 4,47 | 2,2 | 0,26 | 4,34 |
| Run 2. | | | | | | | | | | | | |
| Wort a | 5,0 | 0,18 | 4,10 | 3,4 | 0,13 | 4,02 | 2,6 | 0,39 | 4,31 | 2,1 | 0,20 | 4,18 |
| Wort A | 5,2 | 0,18 | 4,10 | 3,5 | 0,13 | 3,98 | 2,7 | 0,39 | 4,32 | 2,3 | 0,24 | 4,17 |
| Wort d | 5,2 | 0,20 | 4,11 | 3,4 | 0,17 | 3,97 | 2,8 | 0,47 | 4,33 | 2,3 | 0,34 | 4,20 |
| Wort D | 4,6 | 0,18 | 4,09 | 3,6 | 0,12 | 3,97 | 2,5 | 0,37 | 4,28 | 2,2 | 0,26 | 4,18 |

Pitching of yeast was according to the standard method of 6g/l pressed using yeasts described in table 10.

3.3.2.b Effect of Wort Composition/Gravity of Propagation Medium and Mode of Propagation on Subsequent Yeast Performance.

Saccharomyces uvarum(x) was propagated according to method 2.8.1 in wort d and wort a according to method 2.8.3. in worts d and a and according to method 2.8.2. in worts a,A,d and D.
The yeast crops from these propagations were pitched according to methods 2.6 and 2.7.2. into wort a in

duplicate. Fermentations (2.12) were carried out in the 2 litre fermentation tubes (2.3).
These experiments were repeated and similar trends were obtained.(Table 12).

TABLE 12. Fermentation Data of Yeasts Variously Propagated.

| | Mode of Propagation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Method 2.8.1 | | | | Method 2.8.3 | | | |
| | Wort d | | Wort a | | Wort d | | Wort a | |
| S.G.d.8. of fermentation (°P) | 3,6 | 3,5 | 4,1 | 4.1 | 2,7 | 2,6 | 3,1 | 3,2 |
| pH d.8 | 4,18 | 4,16 | 4,03 | 4,03 | 3,95 | 4,04 | 3,95 | 3,99 |
| Diacetyl d.8(ppm) | 0,20 | 0,20 | 0,12 | 0,12 | 0,17 | 0,16 | 0,13 | 0,13 |

| | Mode of Propagation Method 2.8.2. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wort D | | Wort d | | Wort A | | Wort a | |
| S.G.d.8. of fermentation (°P) | 4,3 | 4,0 | 4,2 | 4,0 | 4,8 | 4,5 | 4,7 | 4,7 |
| pH d.8 | 4,05 | 4,05 | 4,08 | 4,04 | 4,13 | 4,09 | 4,12 | 4,12 |
| Diacetyl d.8(ppm) | 0,11 | 0,11 | 0,10 | 0,10 | 0,10 | 0,10 | 0,10 | 0,10 |

Viewing all the experiments in section 3.3.2. it would appear that propagation and fermentation, although interlinked, can be viewed as two distinct operations.

3.3.3. Effects of "Yeast Conditioning"

As stated in the introduction "yeast conditioning" or acclimitisation of the yeast to its environment may play an important role in determining the fermentative power of the yeast.

3.3.3.a The Influence of the Contact Time and the No. of Passages through wort on the Fermentative Power of the Yeast.

The effect of yeast conditioning during propagation on subsequent fermentations was investigated.

3.3.3.a (i) Saccharomyces uvarum (x) was propagated according to the standard methods 2.8.1 and 2.8.3. as well as method 2.8.1, except that the 200 ml stage of propagation was incubated for 3 days shaking at 20°C instead of overnight with oxygenation. (Table 13). Propagation medium was wort a.

TABLE 13. Yeast Viability(%) and Final Wort S.G. of Various Propagations.

| | Mode of Propagation. | | |
|---|---|---|---|
| | Method 2.8.1. | Method 2.8.1. except 200ml shaken for 3 days @ 20°C. | Method 2.8.3. |
| S.G.(°P) | 9,86 | 9,57 | 5,96 |
| Viability | 98 | 98 | 99 |

Yeast cropped from the respective propagations was pitched according to methods 2.6 and 2.7.2 into wort a.

Fermentations (2.12) were run in duplicate. (Table 14).

TABLE 14. Fermentation Data of Yeasts Variously Propagated.

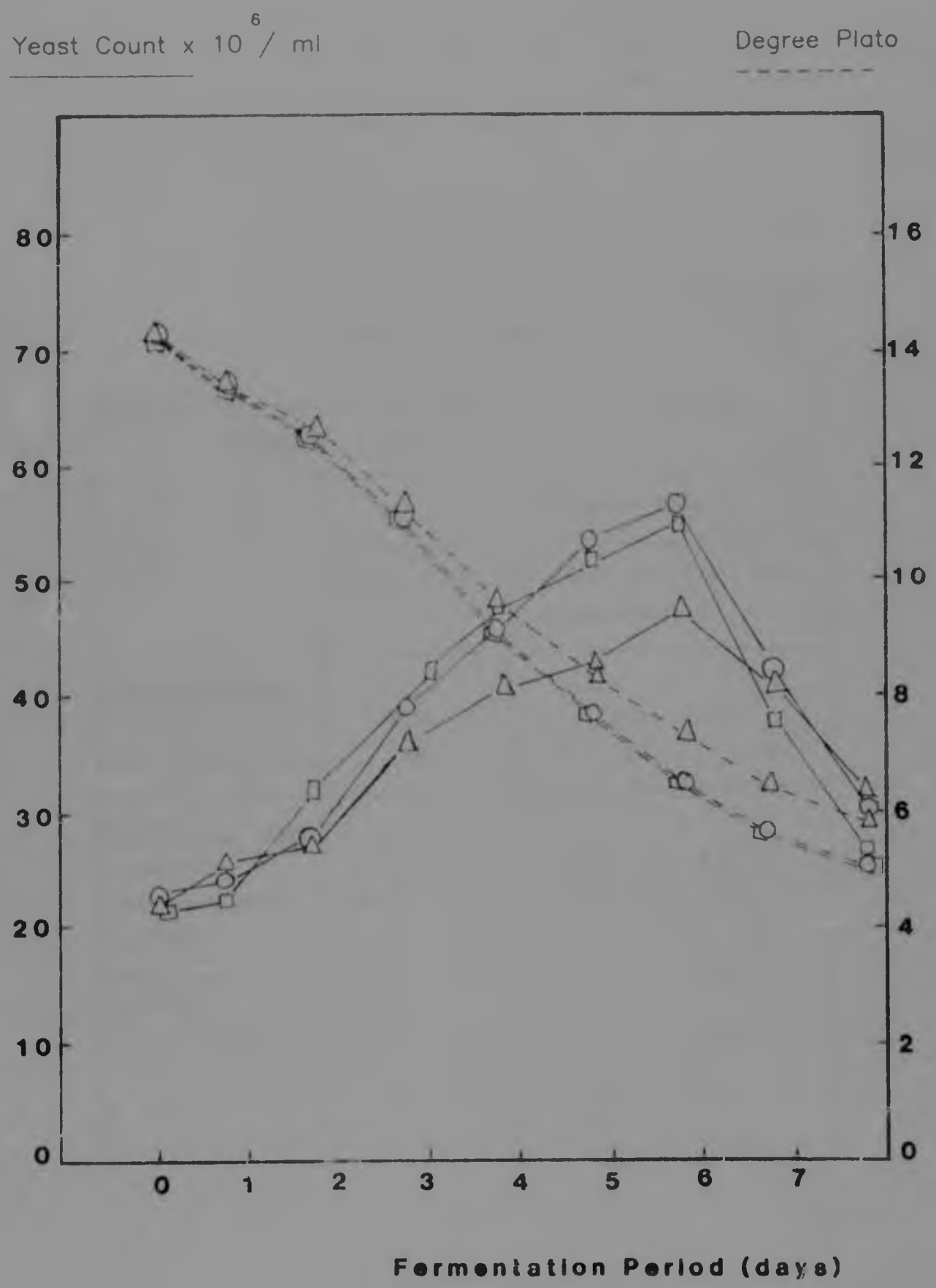
| | Pitching Yeast Propagation Mode. | | | | | |
|---|---|---|---|---|---|---|
| | Method 2.8.1. | | Method 2.8.1. except 200ml stage shaken for 3 days @ 20°C. | | Method 2.8.3. | |
| S.G.d.8 (°P) | 5,9 | 5,9 | 5,1 | 5,3 | 5,0 | 5,1 |
| Diacetyl (ppm)d.7 | 0,18 | 0,19 | 0,23 | 0,24 | 0,20 | 0,20 |
| d.8 | 0,17 | 0,18 | 0,20 | 0,21 | 0,1 | 0,18 |
| pH d.8. | 4,15 | 4,16 | 4,10 | 4,11 | 4,10 | 4,08 |

Pitching was by the standard method of 6g/l pressed using yeasts described in table 13.

3.3.3.a (ii) Saccharomyces uvarum (x) was propagated according to the standard method 2.8.1. Propagation was also done according to method 2.8.1 followed by five extra stages. These stages being: 20 ml inoculated into 200 ml → overnight incubation with oxygenation → 20ml into 800 ml → overnight incubation with oxygenation → 300 ml into 2100 ml → overnight

Fig. 15. FERMENTATION PATTERNS OF YEASTS VARIOUSLY PROPAGATED.

Yeast Propagated by Method: 2.8.1. Δ——Δ 2.8.1. except 200 ml stage shaken 3 days. ○ ——○ 2.8.3. □ ——□



incubation with oxygenation → 1 litre into 1 litre → overnight incubation with oxygenation → 1 litre into 3 litres → 72 hrs incubation with oxygenation. All incubations were at room temperature. These 5 extra stages simulated a plant propagation situation. Wort a was used for all propagation stages.

Yeasts were cropped from their respective propagations and pitched in duplicate into wort a according to methods 2.6 and 2.7.2. Fermentations (2.12) were run in duplicate.(Table 15). Repeat experiments gave similar results.

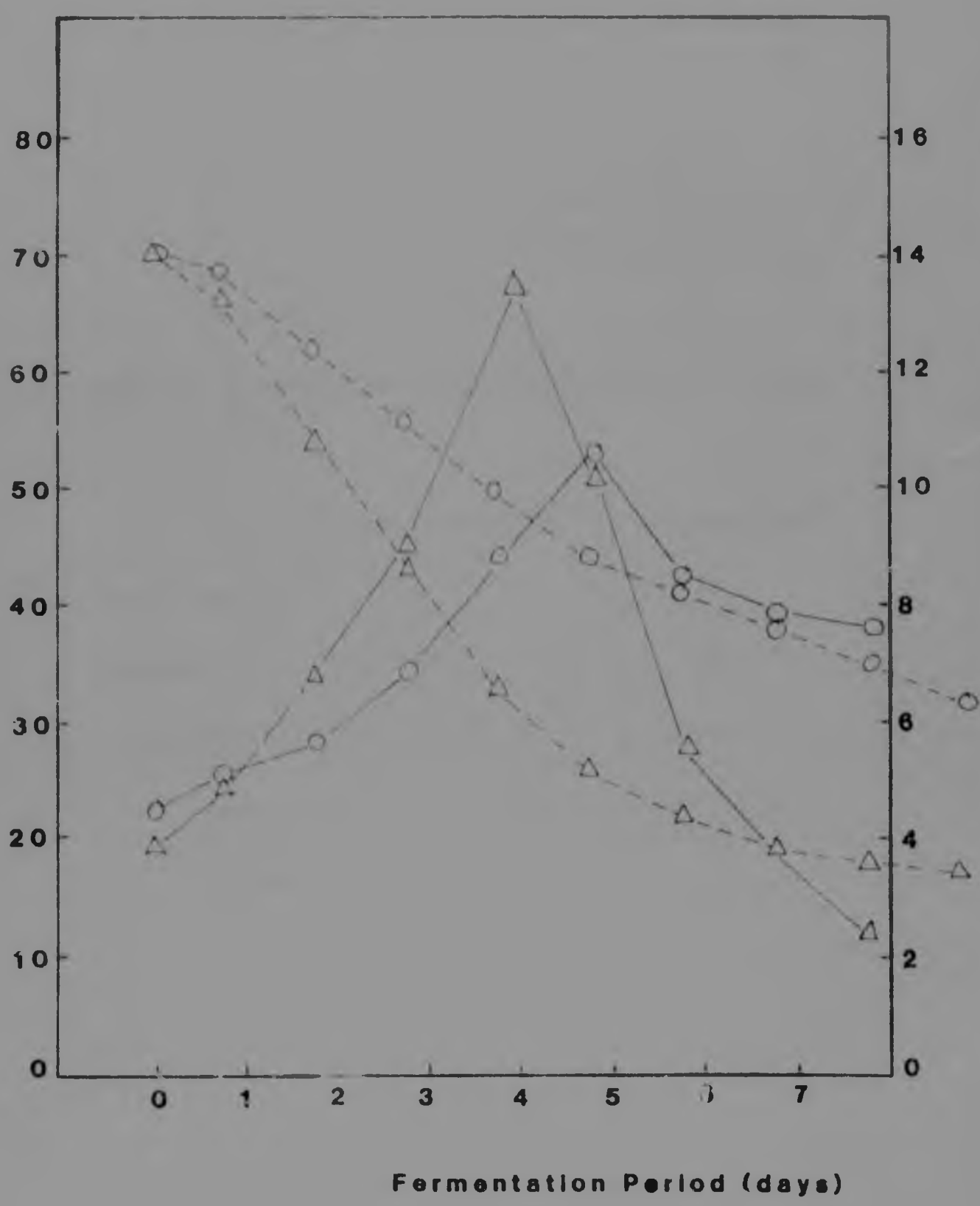
TABLE 15. Propagation and Fermentation Data of Variously Propagated Yeasts.

| | Propagation Mode | | | |
|---|---|---|---|---|
| | Method 2.8.1. | | Method 2.8.1 and 5 steps of conditioning | |
| **Propagation Data.** | | | | |
| S.G. (°P) | 9,41 | | 9,27 | |
| Yeast Viability(%) | 99 | | 98 | |
| **Fermentation Data** | | | | |
| S.G.d.9(°P) | 6,3 | 6,7 | 3,4 | 3,3 |
| Peak Yeast Count ($x10^6$/ml) | 44,8 | 53,6 | 68,7 | 70,7 |
| Yeast Count d.8 ($x10^6$/ml) | 39,3 | 39,0 | 11,9 | 11,7 |
| Diacetyl(ppm)d.7 | 0,49 | 0,53 | 0,17 | 0,20 |
| d.8 | 0,41 | 0,34 | 0,14 | 0,16 |
| pH d.8. | 4,19 | 4,19 | 3,98 | 3,97 |

Fig. 16. FERMENTATION PATTERNS OF 'CONDITIONED' YEAST VS 'UNCONDITIONED' YEAST.

'Conditioned' Yeast Culture Δ——Δ

'Unconditioned' Yeast Culture O——O



3.3.3.b Maintaining Beneficial Effects of Conditioning on Solid Media.

The concept of 'pre-conditioning' a slope culture was investigated ie. making slope cultures from the last stage of propagation of a "conditioned" yeast.

Saccharomyces uvarum(x) was propagated according to method 2.8.1 and then taken through an extra 5 stages of propagation (simulating plant propagation.) Slope cultures were made from the last stage of this propagation on MYGP agar. Wort a was used for propagation. One of the 'conditioned' slope cultures was propagated alongside a stock slope culture according to the standard method 2.8.1. Wort a was used for both propagations. (Table 16).

TABLE 16. Propagation Data using a "Conditioned" and a 'Stock' Slope.

| | "Conditioned" Slope | "Stock" Slope |
|---|---|---|
| Yield of Yeast(g) | 36 | 36 |
| Viability(%) | 99 | 98 |
| Y.Count $x10^6$/ml | 3,4 | 3,4 |

Yeast cropped from the respective propagations was pitched in duplicate according to methods 2.6 and 2.7.2 into wort a.

Fermentations (2.12) were conducted in the 2 litre tubes (2.3.) (Table 17).

TABLE 17. Fermentation Data Using Yeast Propagated from a 'Conditioned' Slope and a 'Stock' Slope.

| Sample | | S.G.(°P) d.8 | Y.Count ($x10^6$/ml) d.8 | pH d.8 | Diacetyl (ppm) d.8 | Peak Y.C. ($x10^6$/ml) |
|---|---|---|---|---|---|---|
| "Conditioned" Slope | 1 | 3,6 | 23 | 4,10 | 0,17 | 56,9 |
| | 2 | 3,6 | 17,2 | 4.04 | 0,16 | 56,0 |
| "Stock" Slope | 1 | 5,3 | 32,3 | 4,19 | 0,15 | 36,0 |
| | 2 | 5,2 | 30,3 | 4,19 | 0,16 | 37,5 |

From the results presented in Table 17 and Fig.17 it would appear that one can 'pre-condition' a slope culture so that after standard laboratory propagation it ferments better than a 'stock' slope culture.

### 3.3.4. Preliminary Screening of Alternative Strains of Brewing Yeasts.

As stated in the introduction it would be desirable to define a bottom fermenting yeast strain with a more robust physiological make-up than the strain Saccharomyces uvarum(x)

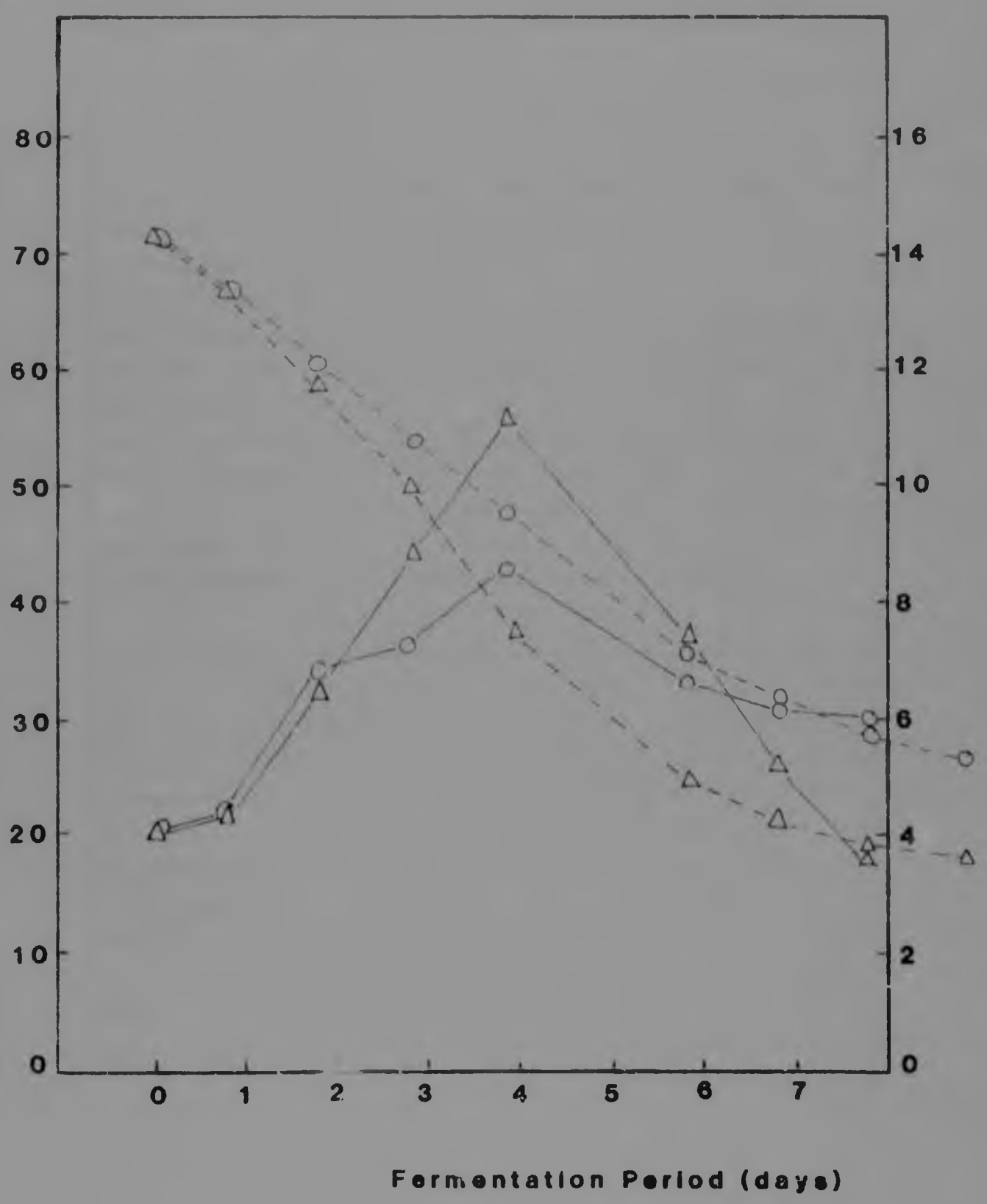
The brewing yeast strains held in the culture collection at Central Laboratory were propagated in wort a according to method 2.8.2.
Saccharomyces uvarum(x) was propagated in the same manner and used as a control yeast.

The end stage 250 ml of propagation was pitched into 1750 ml of wort a and aerated according to method 2.7.1.

Fig. 17. FERMENTATION PATTERNS OF YEAST PROPAGATED FROM a 'CONDITIONED' AND a 'STOCK' SLOPE.

Yeast Propagated from a 'Conditioned' Slope Δ——Δ

Yeast Propagated from an 'Unconditioned' Slope O——O



Fermentations (2.12) and a Limiting Attenuation Test (2.13) were conducted on all yeasts.

As this was a preliminary screen all fermentations were run singly. (Tables 18 & 19).

TABLE 18. Fermentation Data of Various Brewing Yeast Strains.

| | | YEAST STRAIN | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | (X) | MB1 | MB2 | MB3 | MB4 | MB5 | MB6 |
| Pitching Yeast Count($x10^6$cells/ml) | | 21,6 | 25,0 | 18,4 | 24,3 | 21,6 | 23,5 | 34,5 |
| Peak Yeast Count ($x10^6$cells/ml) | | 58 | 44,1 | 33,0 | 47,2 | 51,5 | 50,2 | 70,2 |
| Time of peak(hrs) | | 68 | 49 | 49 | 68 | 68 | 72 | 72 |
| Yeast Count ($x10^6$cells/ml) | d8 | 5,6 | 4,3 | 2,2 | 3,9 | 4,5 | 4,7 | 7,9 |
| | d9 | 4,2 | 3,2 | 1,5 | 3,0 | 3,5 | 3,2 | 5,2 |
| | d10 | 3,5 | 2,5 | 1,4 | 2,4 | 2,8 | 3,0 | 3,7 |
| S.G. (°P) | d8 | 4,2 | 5,5 | 4,5 | 3,0 | 4,1 | 4,2 | 3,3 |
| | d9 | 3,8 | 5,3 | 4,4 | 2,9 | 3,7 | 3,9 | 3,1 |
| | d10 | 3,6 | 4,9 | 4,2 | 2,7 | 3,5 | 3,7 | 2,9 |
| pH | d8 | 3,81 | 4,04 | 3,87 | 3,86 | 3,89 | 3,98 | 3,90 |
| Diacetyl(ppm.) | d8 | 0,12 | 0,14 | 0,12 | 0,12 | 0,13 | 0,135 | 0,14 |
| P.G./L.A. | d10 | 1,3 | 2,6 | 1,9 | 0,4 | 1,2 | 1,4 | 0,6 |
| Ranked | | 4 | 6 | 5 | 1 | 3 | 4 | 2 |

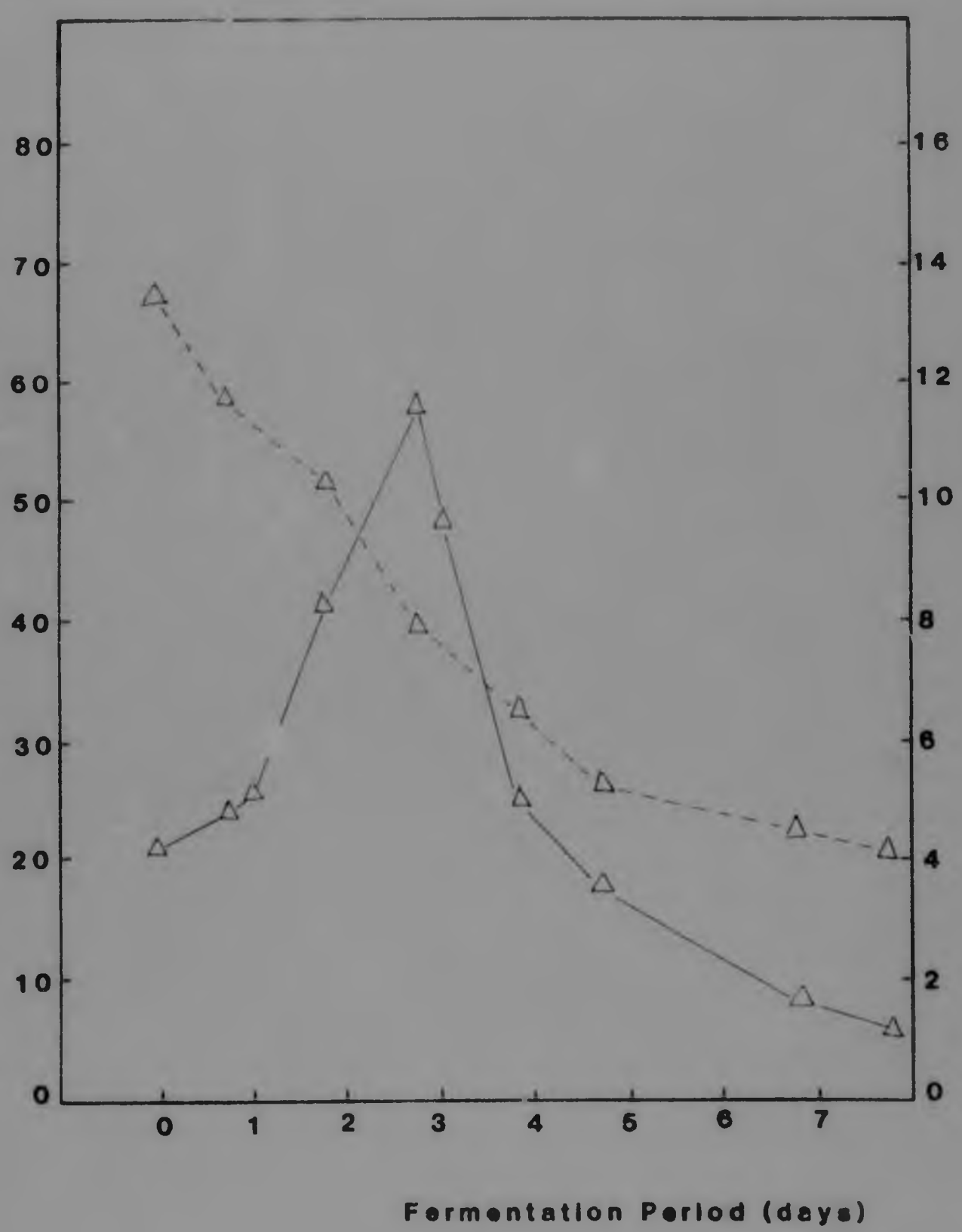
TABLE 19 Fermentation Data of Various Brewing Yeast Strains.

| | | YEAST STRAIN | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | (X) | MB3 | MB6 | MB7 | MB8 | MB9 | MB10 |
| Pitching Yeast Count($x10^6$cells/ml) | | 20,5 | 18,2 | 20,0 | 14,6 | 17,0 | 16,2 | 19,5 |
| Peak Yeast Count ($x10^6$cells/ml) | | 44,1 | 48,8 | 68,7 | - | 42,0 | 43,8 | 54,7 |
| Time of peak(hrs) | | 91 | 91 | 114 | - | 114 | 114 | 114 |
| Yeast Count ($x10^6$cells/ml) | d8 | 6,4 | 3,4 | 29,2 | 16,7 | 27,5 | 33,6 | 51,5 |
| | d9 | 4,1 | 2,7 | 19,0 | 14,8 | 26,5 | 29,3 | 41,9 |
| | d10 | 3,4 | 2,3 | 15,6 | 14,9 | 28,6 | 28,5 | 23,5 |
| S.G. (°P) | d8 | 4,4 | 3,2 | 2,6 | 12,4 | 2,2 | 2,2 | 3,8 |
| | d9 | 4,0 | 3,0 | 2,2 | 12,3 | 2,0 | 2,0 | 3,8 |
| | d10 | 3,8 | 2,9 | 2,2 | 12,3 | 2,0 | 2,0 | 3,8 |
| pH | d7 | 3,96 | 3,96 | 4,05 | 4,93 | 3,84 | 3,86 | 4,10 |
| Diacetyl(ppm.) | d9 | 0,09 | 0,14 | 0,20 | 0,15 | 0,09 | 0,10 | 0,30 |
| P.G./L.A. | d10 | 1,8 | 0,9 | 0,2 | 10,3 | 0 | 0 | 1,8 |
| Ranked | | 4 | 3 | 2 | 6 | 1 | 1 | 5 |

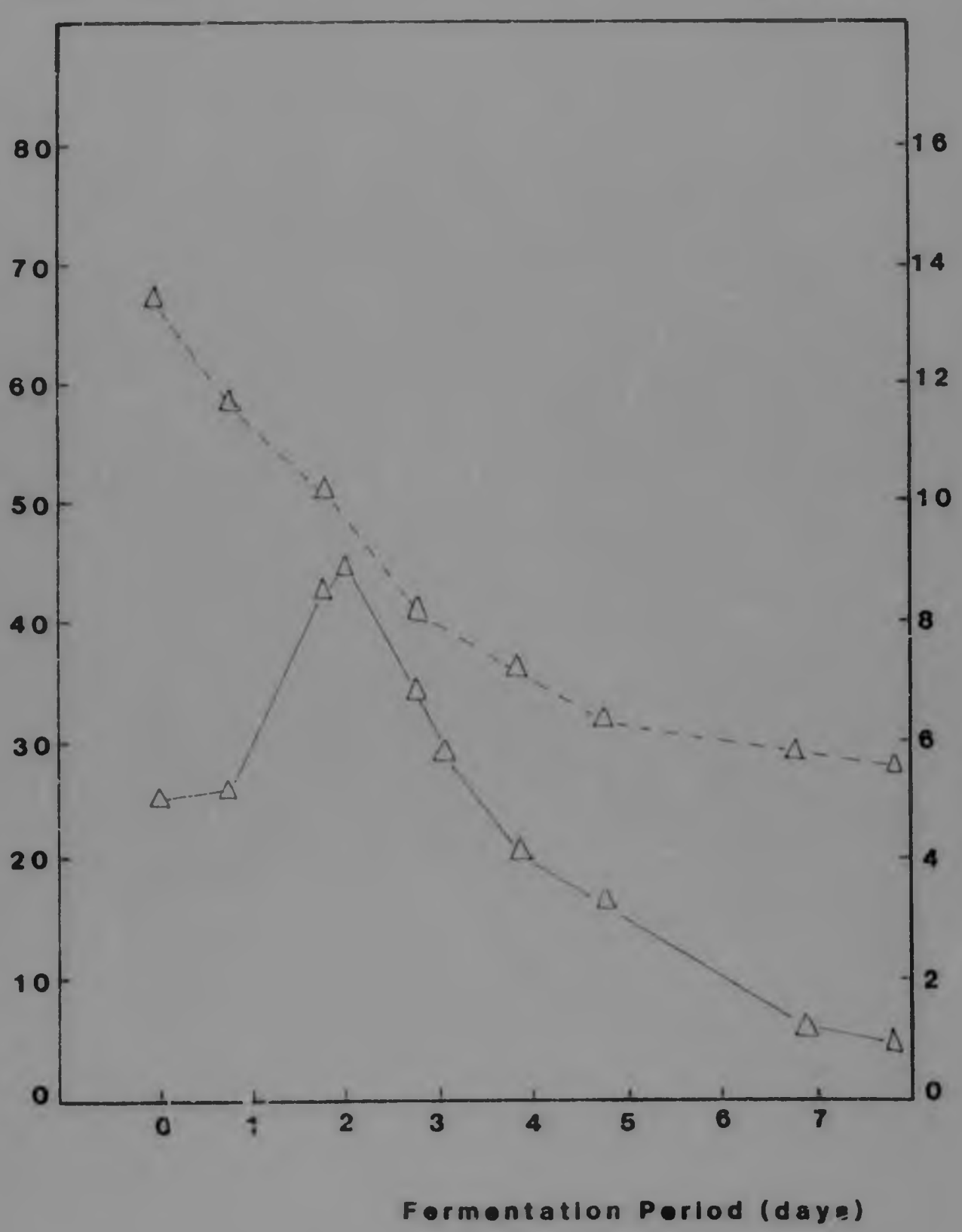
This preliminary screen showed that strains MB-3, MB-6, MB-8 and MB-9 would be suitable candidates for more extensive testing.

Fig. 18. FERMENTATION PATTERNS OF VARIOUS BREWING YEAST STRAINS.

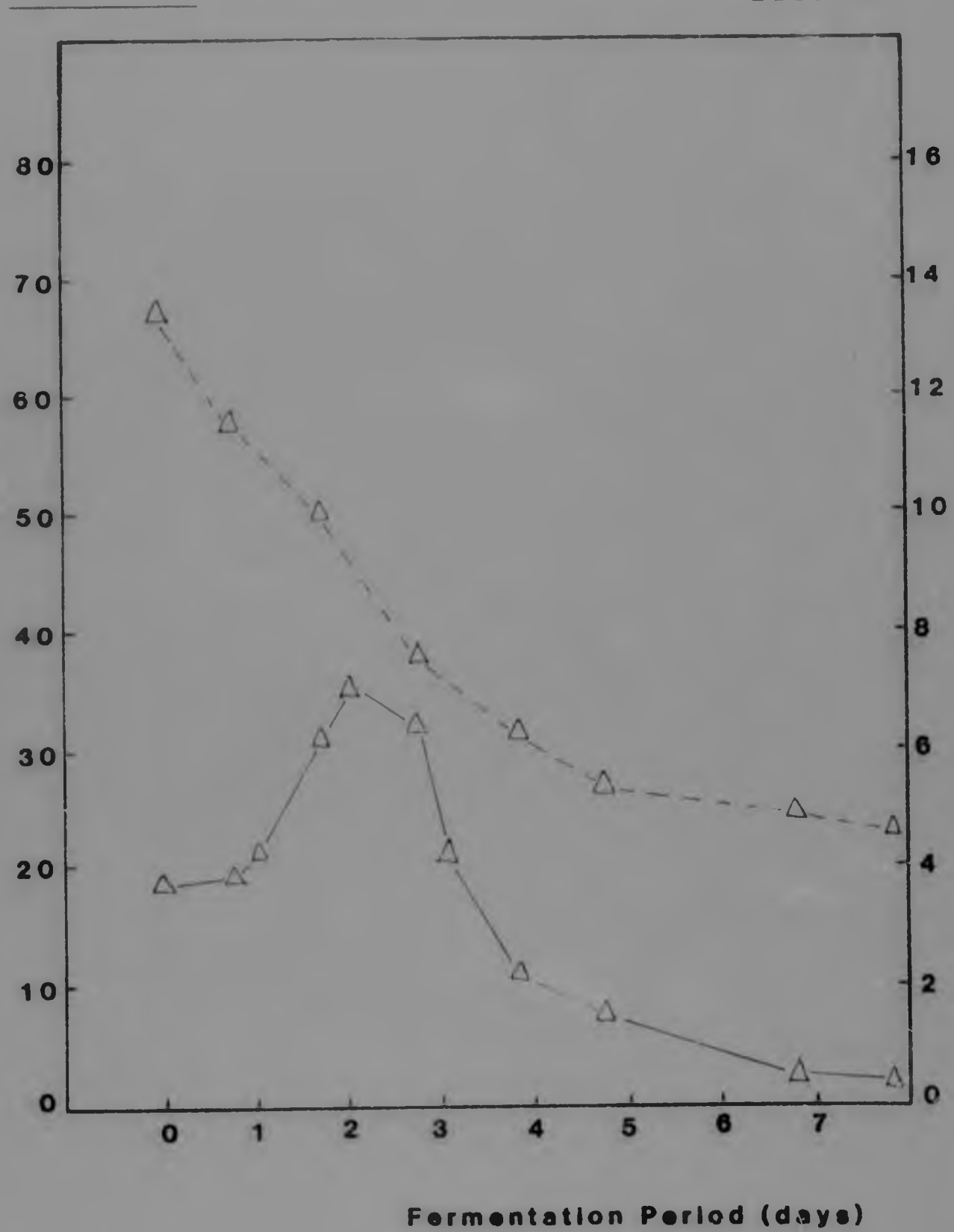
Yeast Strain Saccharomyces uvarum (x)



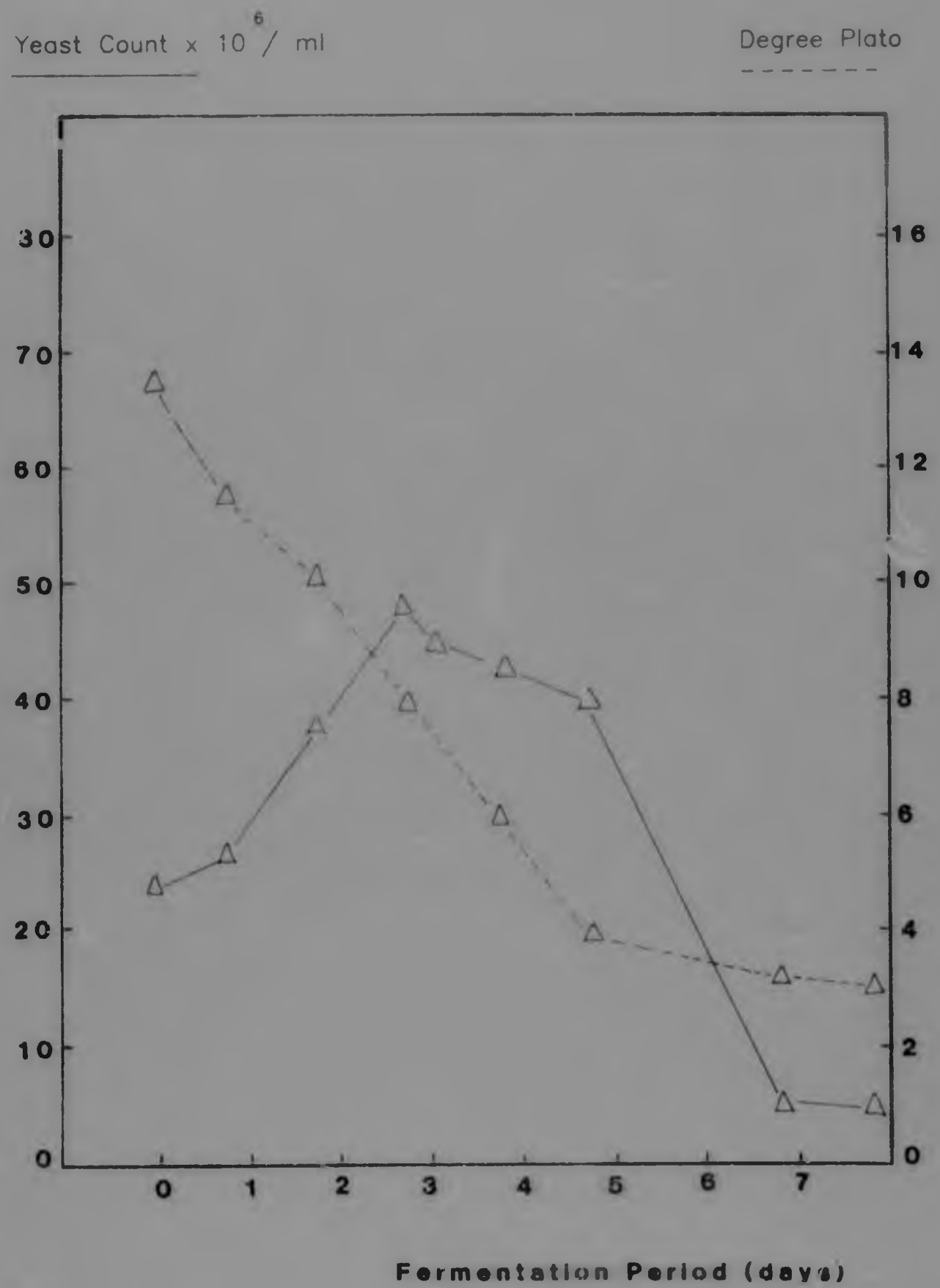
Yeast Strain MB 1



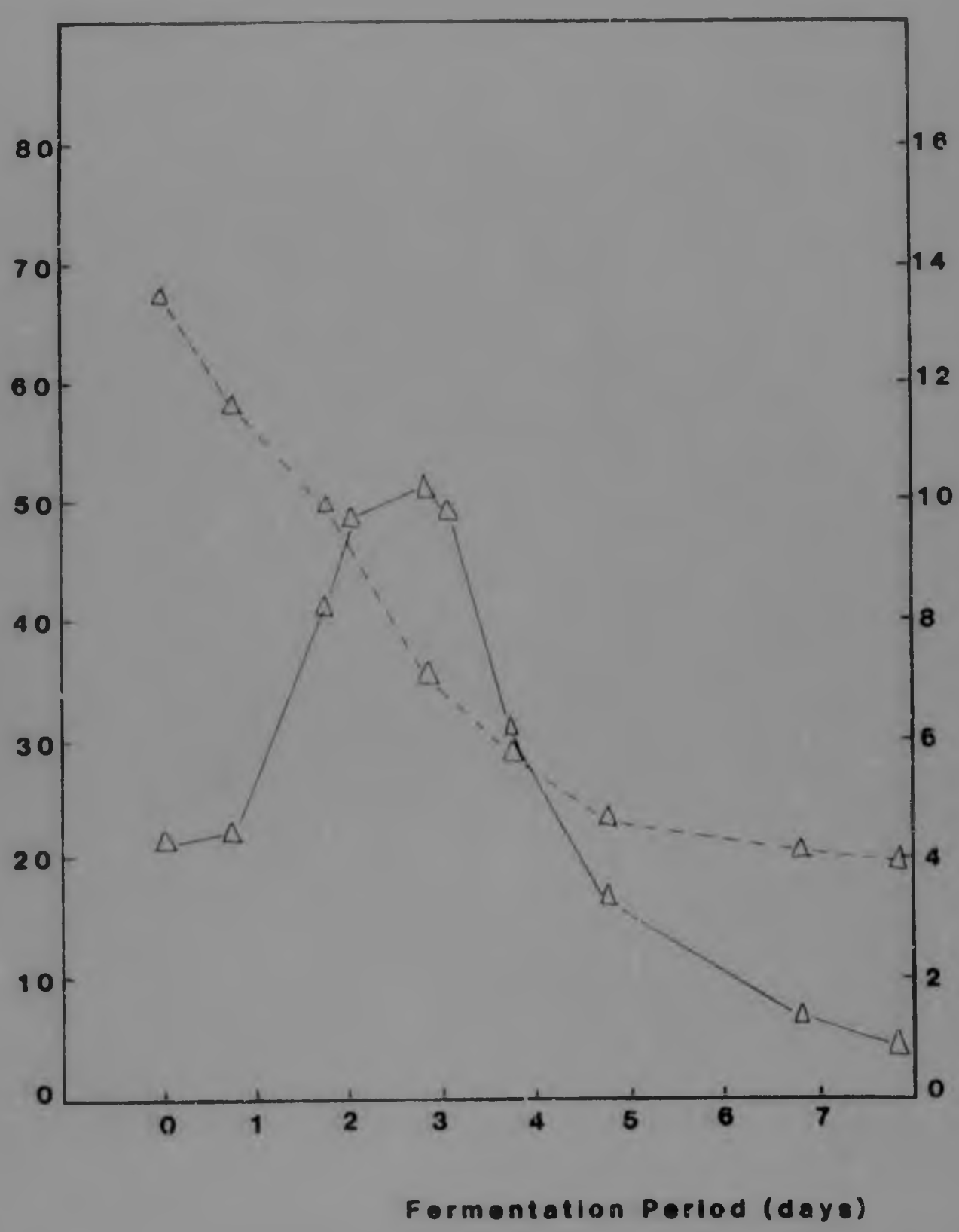
Yeast Strain MB 2



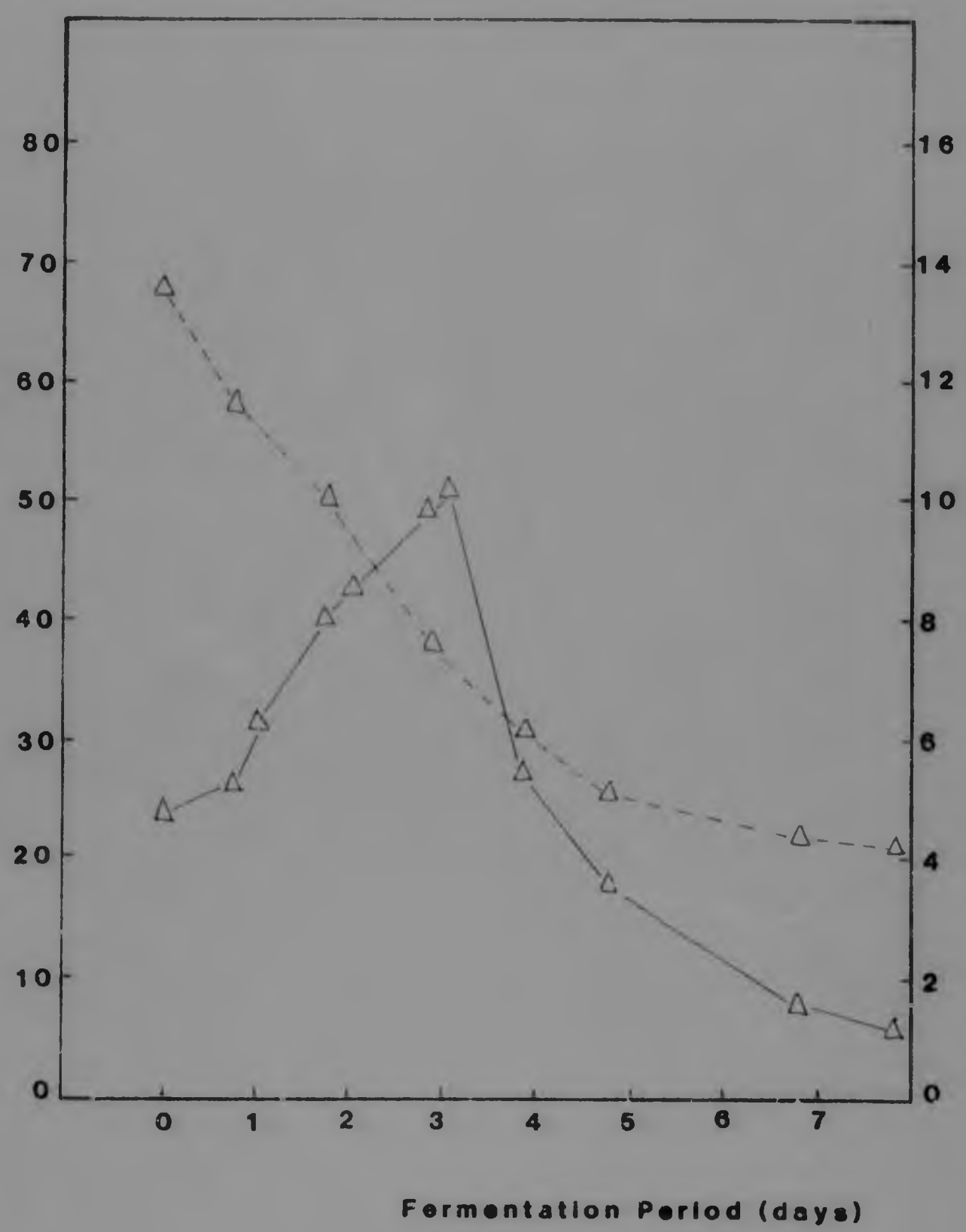
Yeast Strain MB 3



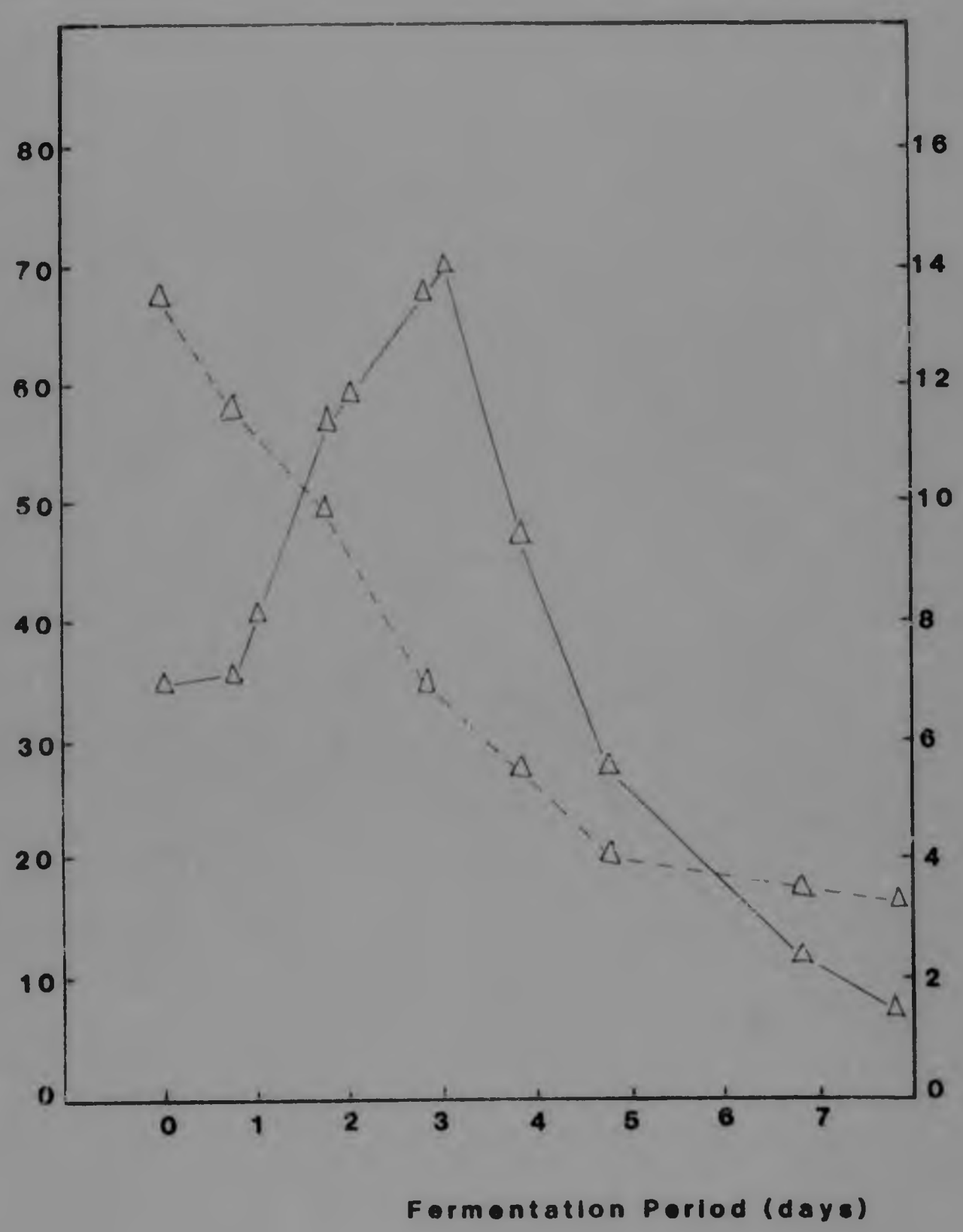
Yeast Strain MB 4



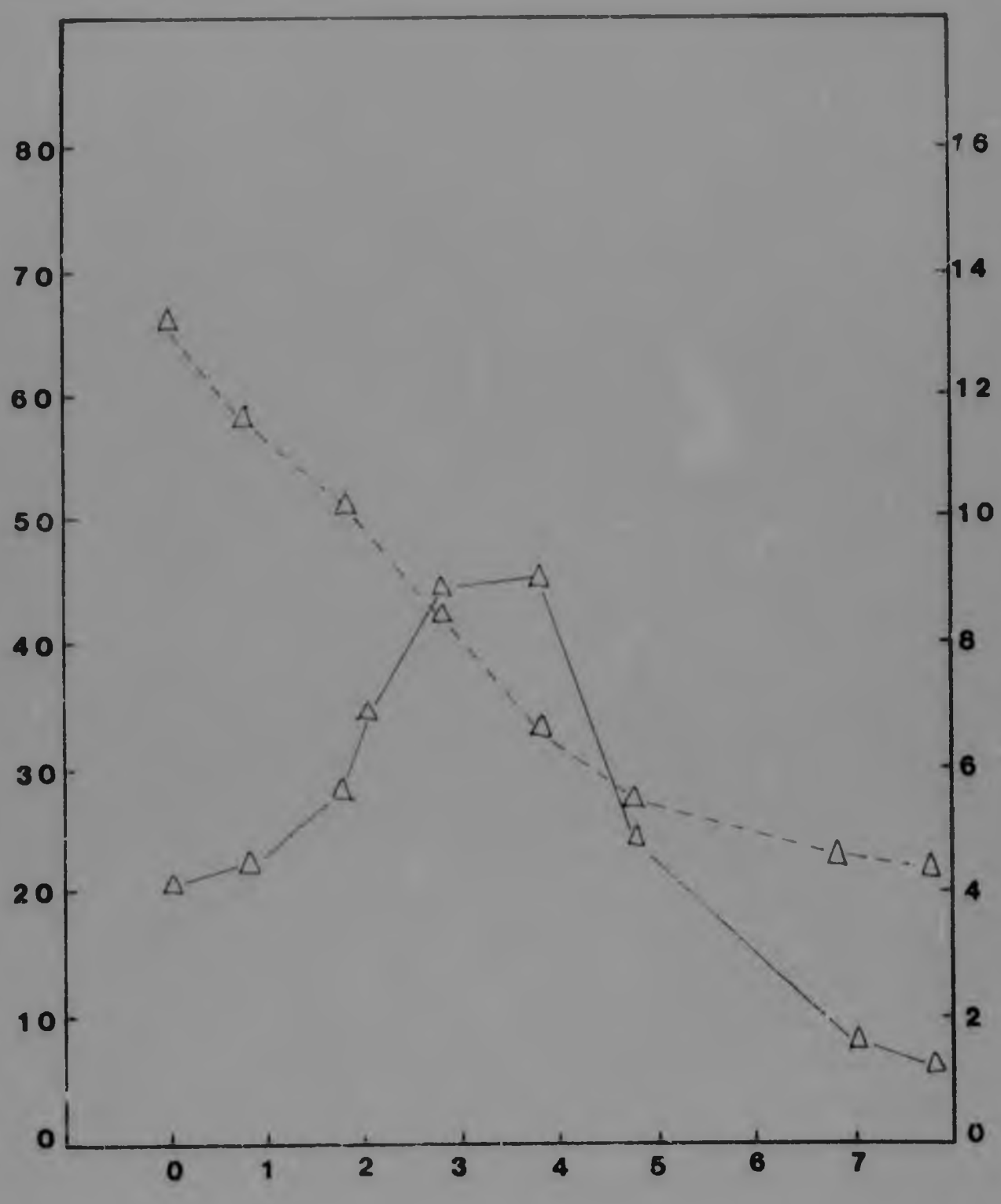
Yeast Strain MB 5



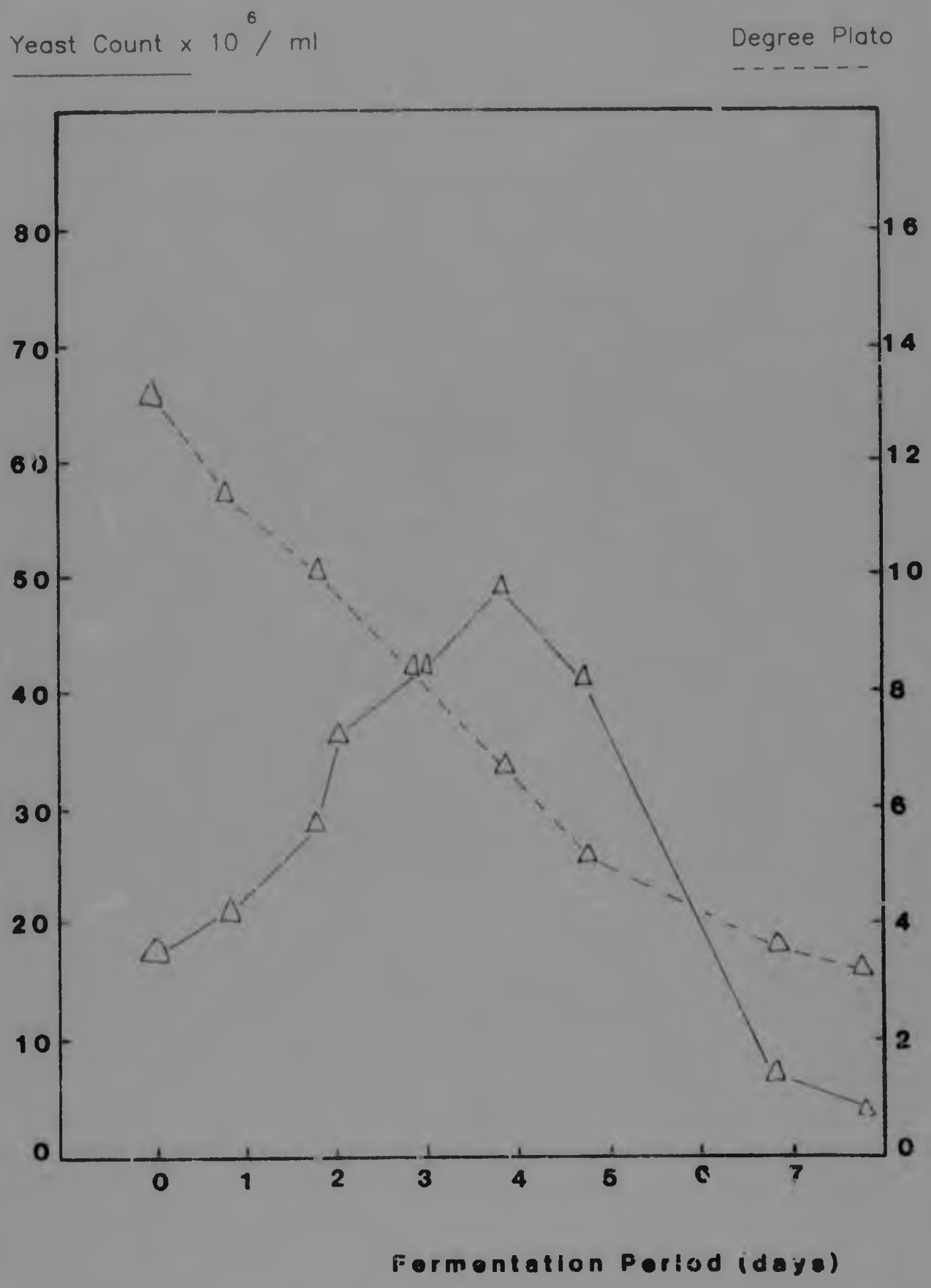
Yeast Strain MB 6



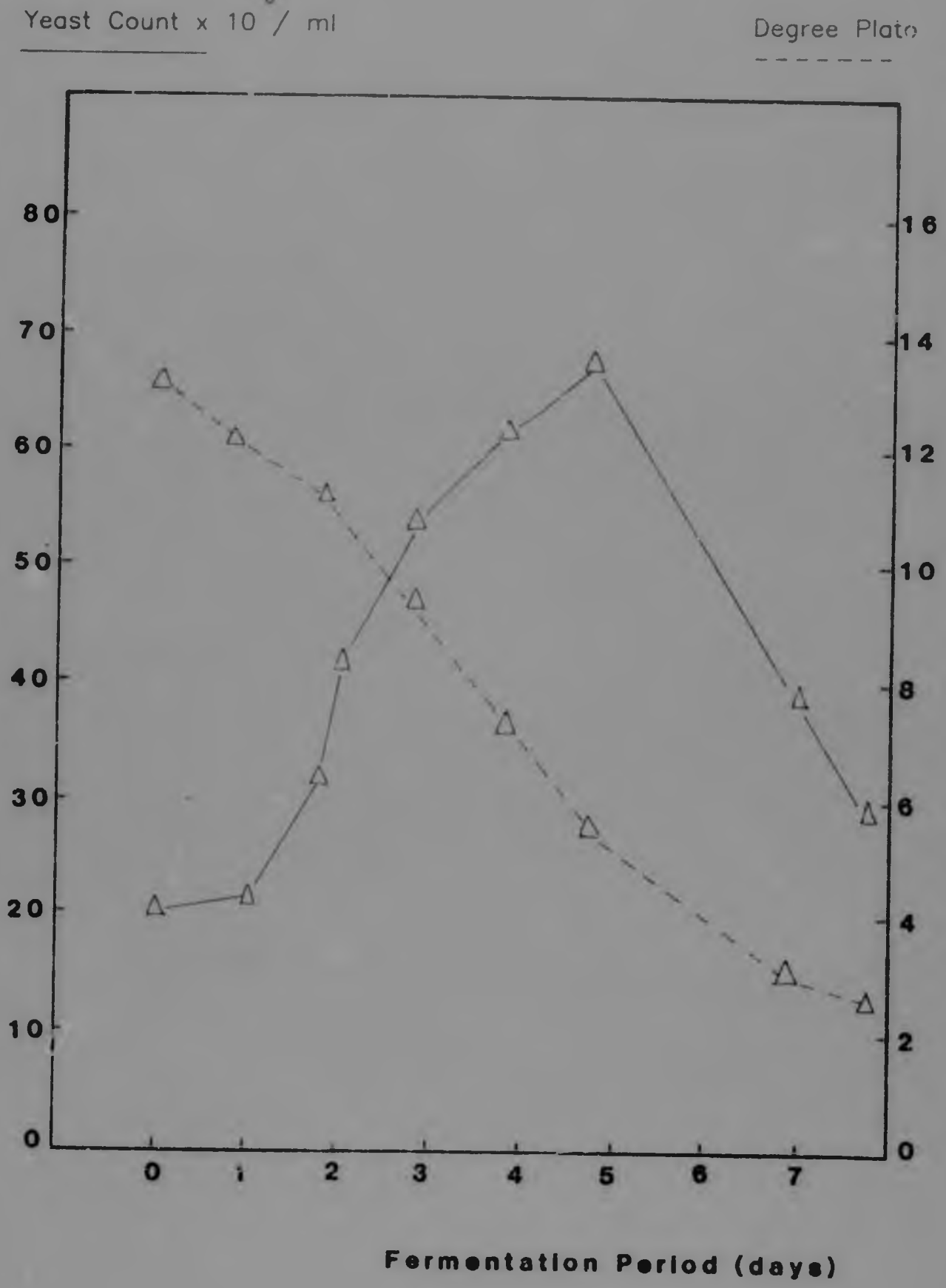
Yeast Strain Saccharomyces uvarum (x)



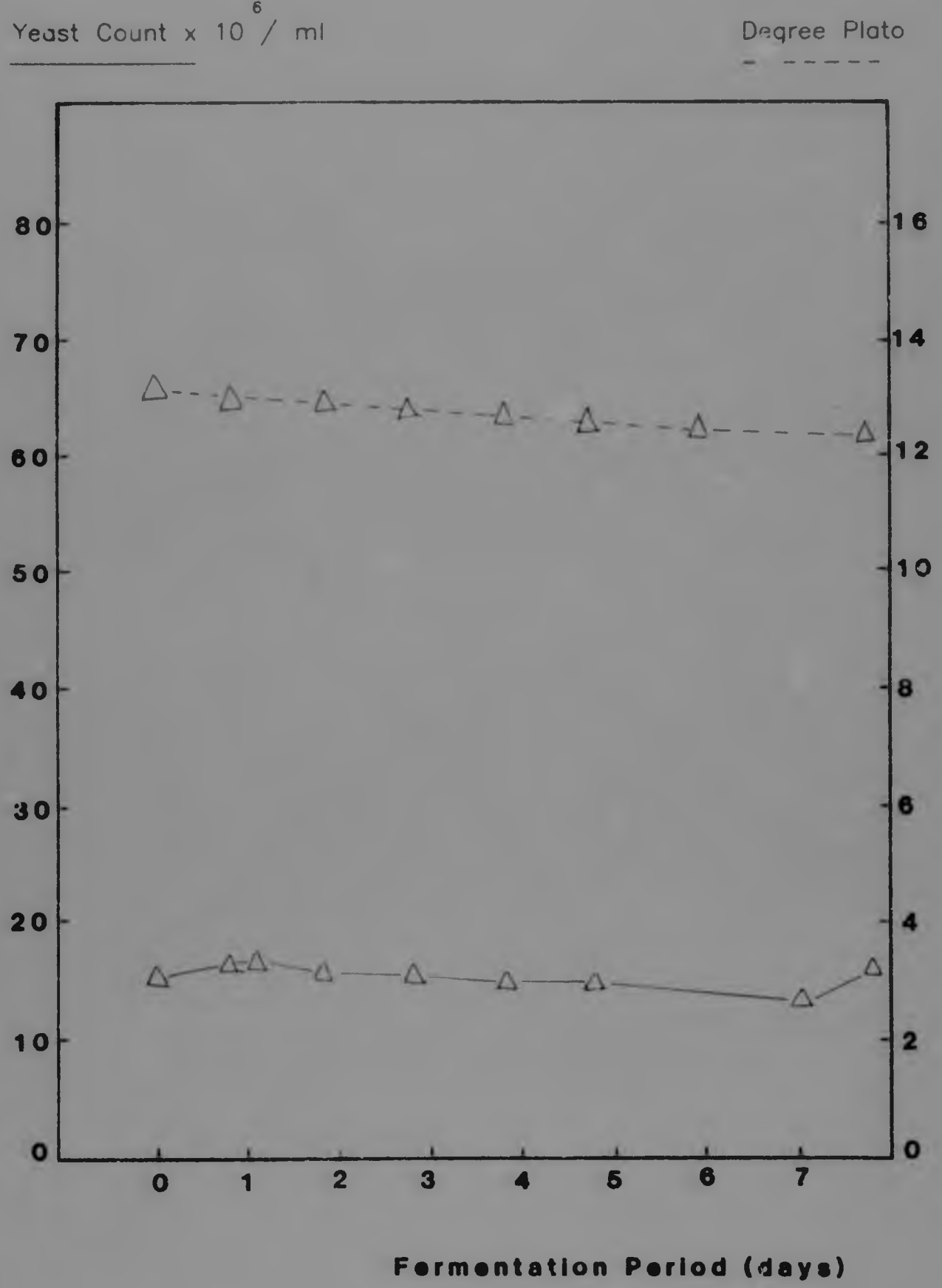
Yeast Strain MB 3



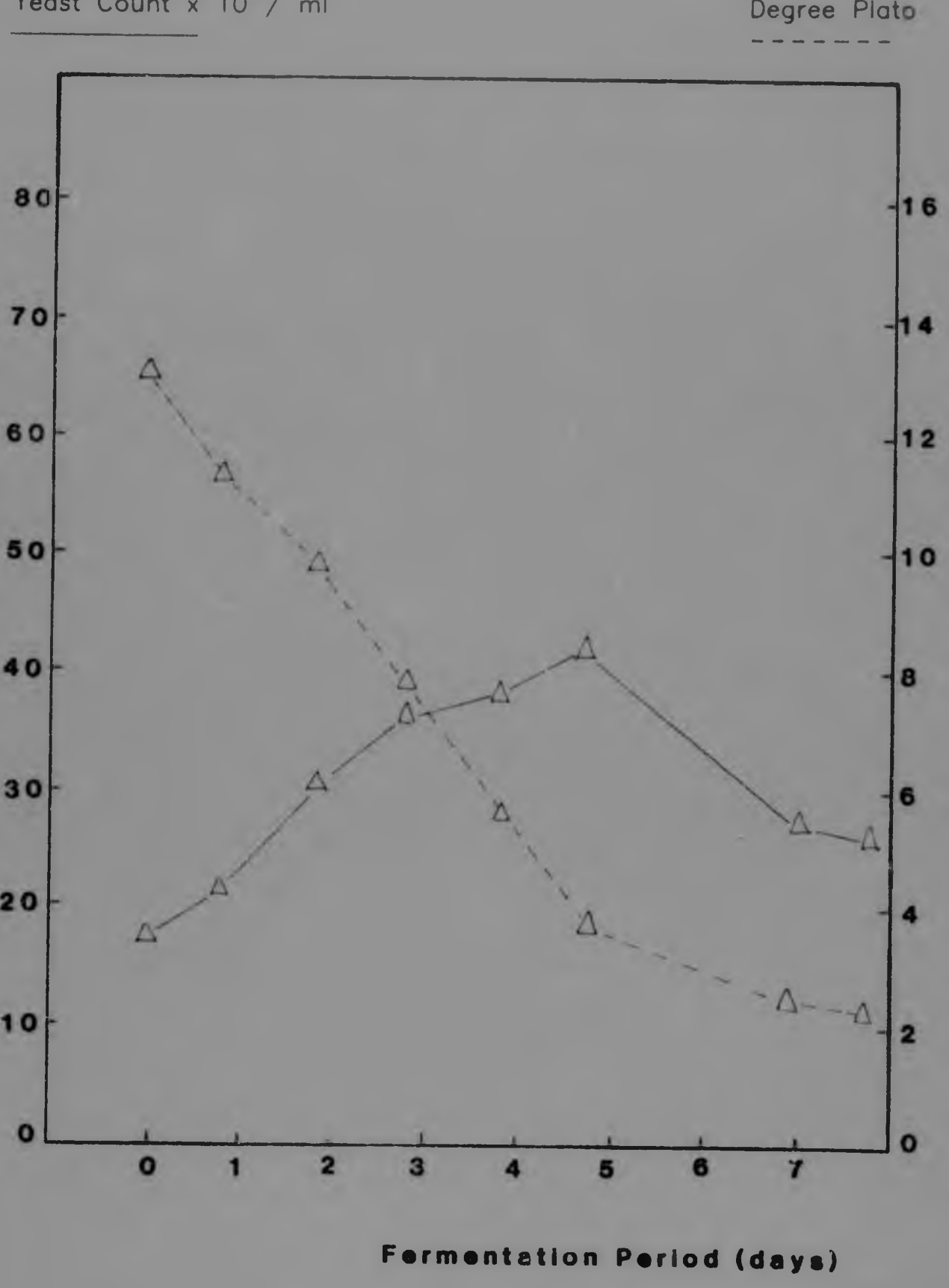
Yeast Strain MB



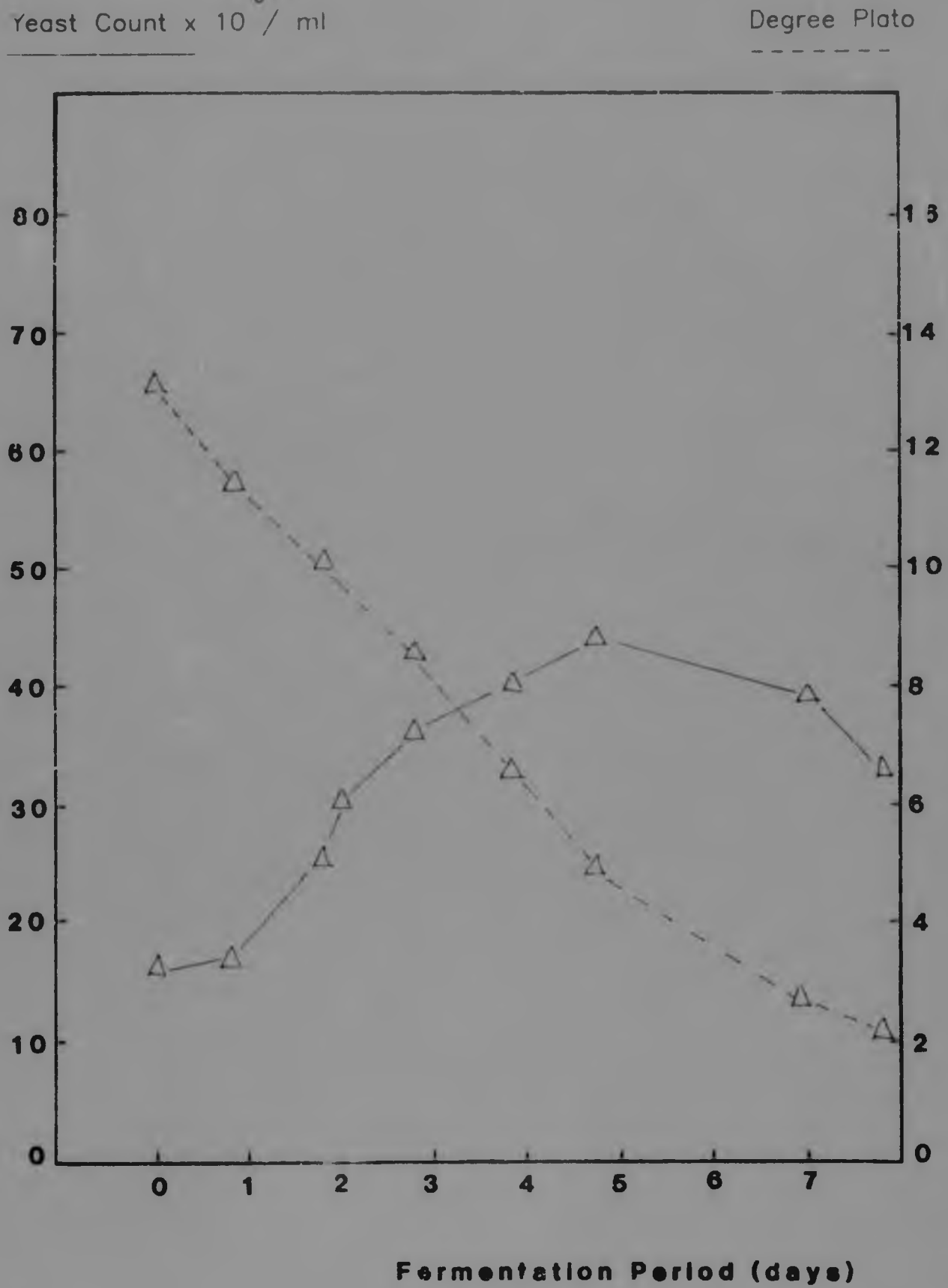
Yeast Strain MB 7



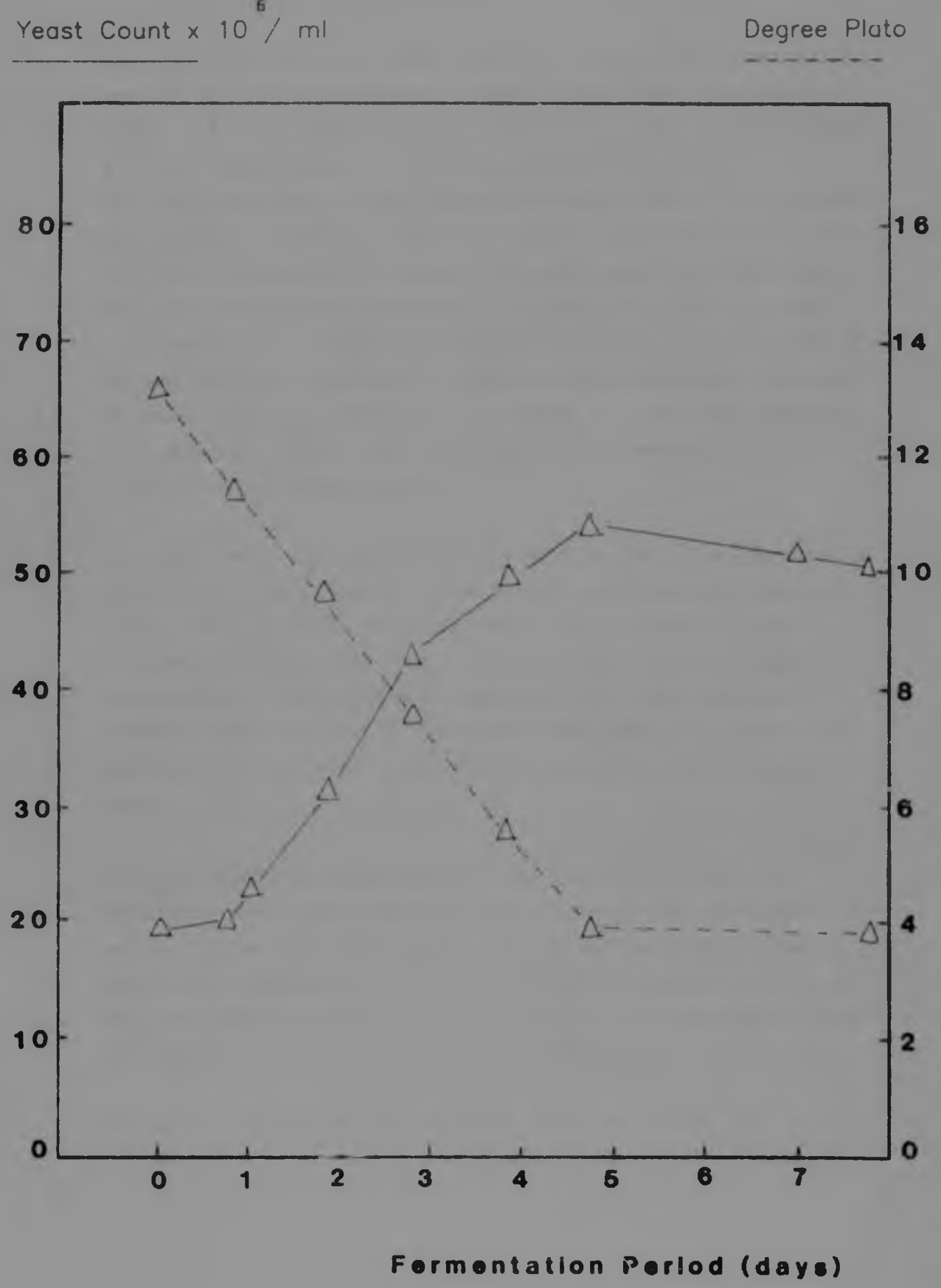
Yeast Strain MB 8



Yeast Strain MB 9



Yeast Strain MB 10



## 4. CONCLUSIONS AND DISCUSSION.

4.1. In selecting a suitable vessel for conducting fermentation studies two configuration and capacity tubes have been studied; namely a 1 litre tube as used by the Kirin Brewery group (24) and a 2 litre tube similar to that described by the E.B.C. Microbiologica (14). The investigations outlined in 3.1.1. showed that results in 1 litre tubes and 2 litre tubes did not correlate very well (illustrated in fig.5) Possibly more important was the fact that duplicate experiments in 1 litre tubes did not yield consistent data. Another consideration was the initial volume of fermenting liquor required to enable daily sampling for analysis of yeast counts and gravities. A volume of 1 litre was found to be inadequate. It was therefore decided to investigate the 2 litre tubes in greater detail.

In establishing the reproducibility of data from four experiments in 2 litre tubes (figure 6), analysis of variance has shown that at the 99% confidence level the final gravity achieved was independent of the individual tests, ie. results were highly reproducible. Using the mean standard deviations obtained 3 standard deviations was ± 0,18°P for final gravity, ± 6,3 x $10^6$ cells/ml for peak yeast count and ± 3 hours for time of yeast peak.

For all subsequent experiments it has been assumed that if duplicate results are within 3 standard deviations the results are valid. It has also been assumed that if differences in results obtained in experiments are greater than ± 3 standard deviations that the differences are significant and due some factor other than experimental technique or tube differences.

An important aspect of this project was to establish the

correlation between plant and 2 litre laboratory fermentations. From the results illustrated in Fig.7. it was evident that fermentation velocities and °P on the 8th day of fermentation were very similar. Only small differences in gravity during fermentation were apparent and results at the end of fermentation were within 0,2°P. Calculated from the 10 comparative studies, using normal gravity (11,3°P) wort, illustrated in Table 1 the correlation coefficient for °P on the 8th day between brewery and laboratory fermentations was 0,91 with a standard error of 0,05. The correlation coefficient for °P at 96 hours was 0,94 with a standard error of 0.04.
Analysis of the data in table 2 (high gravity wort) showed the correlation coefficient for °P on the 8th day between laboratory and brewery fermentations to be 0,97 with a standard error of 0,03.
Using the t distribution test these correlations were shown to be highly significant.

A laboratory fermentation system has therefore been developed that gives reproducible results and that shows good correlation with brewery fermentations. This system is felt to be superior to that described by Kirin (24) in that reproducibility of results under the laboratory conditions are improved and correlation with plant fermentations has been proven. In no other system has a statistical correlation been shown.

The system has been designed to be compact, allow easy observation of the flocculation characteristics of the yeast and convenient for sampling, cleaning and repair.

4.2. One application of the sophisticated laboratory fermentation system developed would be to predict malt quality as it relates to fermentation performance of Saccharomyces uvarum(x).
The investigations outlined in 3.2 were designed to assess the feasibility of using the laboratory fermentation system for this purpose. In all investigations it was attempted to follow as closely as possible the practises of the brewery plant.

The laboratory mashing procedure (Congress Mash) recommended by the governing brewing bodies does not closely follow the practical brewery situation. The experiments outlined in 3.2.2. were designed to assess the differences in worts resulting from a Congress Mashing temperature programme as compared with those resulting from a Brewery mashing temperature programme. Attenuation curves were consistently lower using Brewery temperature mashed worts than Congress temperature mashed worts (Table 3, Figure 8). Although these results were consistent, the differences obtained were not significant with reference to the limits outlined in 4.1. However, as a Brewery temperature mashing programme approaches what happens in practise, it was decided to use this temperature programme for all subsequent experiments.

The different malts mashed and fermented as described in 3.2.3. and illustrated in fig. 9. showed that malts can vary significantly in their fermentability. The variability in °P at the end of fermentation, time of yeast peak and peak yeast count was greater than 3 standard deviations for these parameters. These differences were not reflected in standard malt analyses.

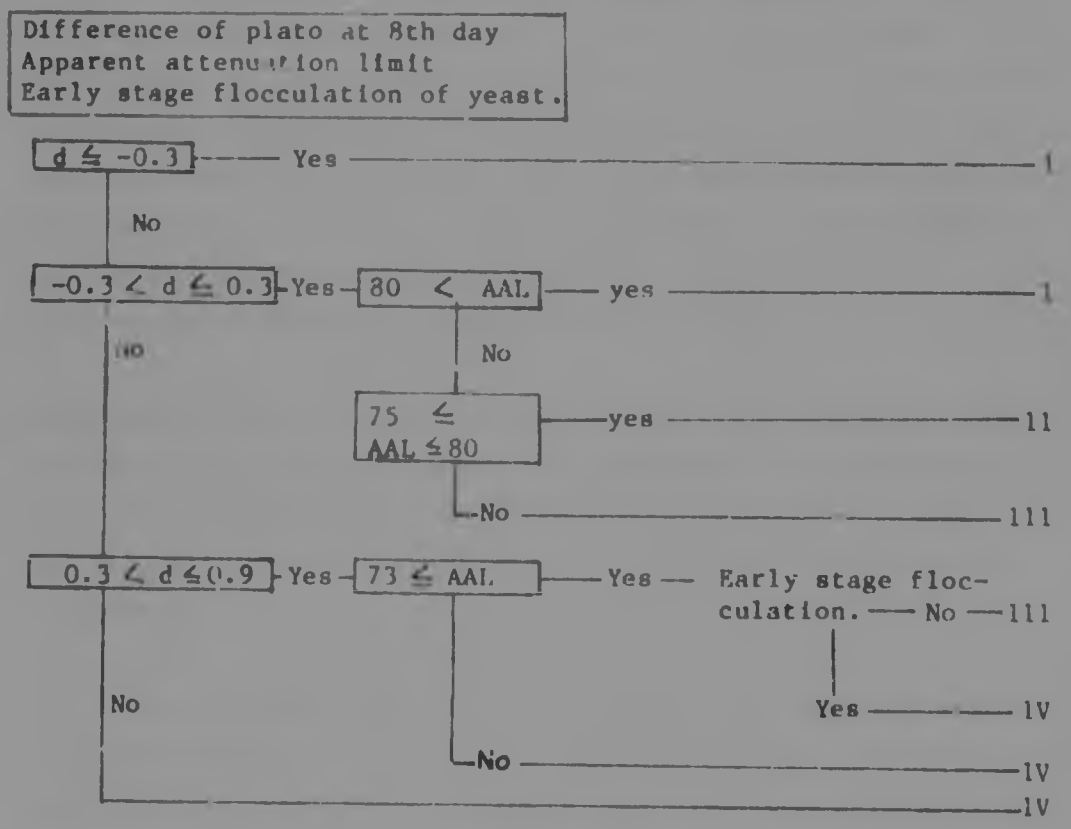
Knowing that different malt samples vary in their fermentability it was necessary to establish the limits for a malt grading system. Various malts of known behaviour in the brewery were mashed and fermented in the laboratory. From the results illustrated in table 5 and fig. 10 and the fermentation data obtained from 12 other malts a 10 point grading system was constructed (fig. 11).
This grading system differs significantly from that used by the Kirin Brewery Group. The Kirin estimation of fermentability is depicted in the following figure: (Personal communication).

KIRIN ESTIMATION OF FERMENTABILITY

| Fermentability | Difference of plato at 8th day. | apparent attenuation limit (AAL) | Early stage flocculation of yeast. |
|---|---|---|---|
| I good | $d \leq -0.3$ | | |
| | $-0.3 < d \leq 0.3$ | $80 < AAL$ | |
| II normal | $-0.3 < d \leq 0.3$ | $75 \leq AAL \leq 80$ | |
| III slightly bad | $-0.3 < d \leq 0.3$ | $AAL < 75$ | |
| | $0.3 < d \leq 0.9$ | $73 \leq AAL$ | not recognized |
| IV bad | $0.3 < d \leq 0.9$ | $73 \leq AAL$ | recognized |
| | $0.3 < d \leq 0.9$ | $AAL < 73$ | |
| | $0.9 < d$ | | |

d = (sample malt-control malt)

Flow chart of fermentability estimation




The important difference between the 10 point system developed and

the 4 category system used by Kirin is that the 10 point system is more comprehensive and sophisticated. The Kirin system does not take into account yeast growth duri.g fermentation. This is a very important factor as not only does the extent of yeast growth affect attenuation, but it also plays an important role in determining the flavour of the final product. With respect to the time of yeast flocculation the Kirin system only recognises this phenomenon if the malt falls into category 111. No provision is made for late flocculation of the yeast. This can be important, not only in terms of flavour profile but also from the practical point of cropping yeast for subsequent fermentations. Another important aspect of fermentation is the rate of attenuation. Experience has shown that this impinges on flavour as well as being indicative of yeast performance. The Kirin system takes no cognisance of this parameter. In the 10 point system the limits set for °P at 96 hours were designed to include the desired rate of fermentation. The malt is penalised if the rate of attenuation is too slow.

The Kirin test uses the apparent attenuation limit (AAL) as part of its screen. This test is very similar to the Limiting Attenuation (LA) test described in method 2.13. It is felt that as this test forms part of the standard malt analyses and a malt sample is penalised if the L.A. is out of specification, this is a duplication of information that is unnecessary. More important are the parameters specifically related to fermentation performance as measured by the 10 point system.

The fermentation system developed has therefore been successfully used to predict malt quality. The fermentability test is now in routine use at South African Breweries as an additional malt specification which gives information related to fermentation performance.

4.3. Another application of the laboratory fermentation system developed would be in the field of yeast research. In any brewing process it is important to optimise yeast handling practises in order to obtain consistent fermentations.

As mentioned in the introduction there is little agreement on the optimum aeration/oxygenation regime during propagation (61). The regime would also vary markedly depending on the yeast strain used and its particular oxygen demand.

The experiments detailed in 3.3.1a were designed to ascertain whether aeration or oxygenation of yeast Saccharomyces uvarum(x) during propagation is desirable.

The results in Tables 6 and 7 and fig. 12 showed that:

1) With respect to the viability of the pitching yeast and subsequent yeast count patterns during fermentation there was no significant difference between yeast aerated and oxygenated during propagation.
2) The yield of yeast from an oxygenated propagation was almost twice that of an aerated propagation.
3) Yeast aerated during propagation attenuated better during subsequent fermentation than yeast oxygenated during propagation.
4) Diacetyl values on day 8 of fermentation were marginally better using yeast oxygenated during propagation compared with aerated yeast.

Although it is important to generate yeast of good fermentative power during propagation, the major objective of yeast propagation is to generate yeast bulk. It was therefore decided that oxygenation of yeast during propagation was preferable to aeration even though the fermentative power of aerated yeast was marginally better than that of yeast propagated with oxygen.

Another important parameter was to establish the optimum aeration/oxygenation regime for fermentation of Saccharomyces uvarum(x)(section 3.3.1b)

Jakobsen(25) found the optimum aeration time for pitching yeast (with a high oxygen demand) to be 2 hours. However, from the results in table 8 and fig 13 there would appear to be no advantage in aerating wort plus yeast for 2 hours prior to pouring into 2 litre tubes compared with the procedure of pitching yeast into wort with a dissolved oxygen content of 14 ppm when working with the yeast Saccharomyces uvarum(x). Similar fermentation

patterns and diacetyl values were achieved by either method of aeration/oxygenation of the wort. However, it is also clear that successive contacts of the yeast/wort mixture with air, either by simple shaking of pitched wort or by rousing the fermentations during the early stages, elicits superior diacetyl removal and wort attenuation as compared with fermentations of yeast pitched into wort with a dissolved oxygen content of 14 ppm. (Table 9, Figure 14). These findings agree with the statement by Kirsop(32) that yeasts with a high oxygen demand can be stimulated by additional aeration during fermentation.

The effect of the wort composition / gravity of the propagation medium and fermentation medium on the performance of Saccharomyces uvarum (x) were also investigated (3.3.2.) Various modes of propagation were also studied.

The best yeast crop was obtained by propagating in high gravity all malt wort (Table 10). Viabilities of yeasts were not affected by the propagation medium. The propagation medium had little influence on fermentation performance except when the yeast was pitched into a high gravity adjunct wort where propagation in normal gravity all malt wort appeared beneficial with respect to attenuation of the wort.

Considering fermentation performance with respect to wort composition and gravity of the fermentation medium, it would appear from table 11 that whilst both parameters exert a controlling influence on the course of fermentation, the effects of wort composition are more pronounced. Yeast performance in normal gravity was superior to high gravity wort. However, more striking was the improved fermentation efficiency in an all malt wort compared with an adjunct wort. This supports the work done by Kirsop and Harding on all malt and adjunct worts (21,30). (The high diacetyl levels on day 8 of fermentation in all malt worts are reflections of the higher free amino nitrogen content when compared with adjunct worts. This is also reflected in the higher

pH's on day 8 when compared with adjunct worts). Possibilities to offset the decreased fermentation efficiency in adjunct worts may be to increase the oxygen during fermentation and/or to add amino nitrogen (serine) to the worts (21,27,65).

The effects of various wort compositions / gravity of propagation medium and modes of propagation on the subsequent fermentative power of the yeast generated when pitched into high gravity adjunct wort, were then investigated (3.3.2). The wort composition of the propagation medium exerted an influence on subsequent fermentation performance, an all malt wort being a superior medium for propagation compared with an adjunct wort (Table 2). However, the more significant finding was that mode of propagation plays an important role in the subsequent fermentative power of yeast.

The best method of propagation for laboratory purposes is according to the following scheme: loopful of yeast inoculated into 15 ml wort, incubated at 25°C overnight; transferred to 200 ml wort, incubated at 20°C, shaking for 3 days; transferred to 5000 ml wort in a Cornelius Vessel, incubated at 20°C with coarse oxygenation for 48 hours (method 2.8.3). This is the best method of propagation irrespective of whether an all malt or adjunct wort is used as the propagation medium.
Considering the various modes of propagation investigated, either having less passages through wort or a shorter contact time with the wort than method 2.8.3. it would appear that yeast 'conditioning' plays an important role in propagation. This conditioning could be the result of the number of passages through wort, the contact time of the yeast with the wort, or both.

Although Maule (43) has suggested that the increment ratio during propagation should not be greater than 1:5, the purpose of this is mainly to combat infection. There is little published data on the effects of yeast conditioning during propagation and its importance. The effects of yeast 'conditioning' during propagation were thus investigated, the results revealing that the

contact time of the yeast with its propagation medium was important (Table 13,14, Fig. 15). Using the same number of propagation steps but increasing the contact time of the 200 ml stage when propagating according to method 2.8.1. the fermentation performance of the yeast generated was significantly improved compared with the yeast generated by the standard method of propagation (2.8.1.). The fermentation efficiency was improved to the extent of being equivalent to that of yeast generated by method 2.8.3.

As outlined in 3.3.3.a(ii) the effects of the number of passages through wort during propagation was investigated. The results illustrated in table 15 and fig. 16 showed these effects to be even more dramatic than the contact time during propagation. There was approximately a 3°P improvement in final gravity of fermentation using a yeast propagated by eight passages through wort (method 2.8.1 + 5 propagation steps) compared with yeast propagated by three passages through wort (standard method 2.8.1).

From these results it is evident that yeast 'conditioning' does play an important role in propagation, both with respect to contact time and number of passages through the propagation medium. This could considerably influence the time required for the first full brew fermentation in a brewery to reach PG-LA and therefore impact on capacity.

Having established the importance of yeast conditioning, the experiments detailed in 3.3.3.b. were designed to investigate the novel concept of preparing slope cultures from the last stage of a propagation involving eight passages through wort, ie. preparing a 'conditioned' slope culture. Stock slope cultures in the laboratory are prepared using overnight cultures of Saccharomyces uvarum(x). The results illustrated (table 17 and fig.17) that 'pre-conditioning' a slope culture was beneficial, a 'conditioned' slope culture when propagated by the standard method (2.8.1) displayed superior fermentation performance compared with a stock slope culture propagated in a similar manner. This is an

important finding as one can prepare a battery of 'conditioned' slope cultures from one propagation involving eight passages through wort. Using these slopes as starting cultures for laboratory propagation, three passages th ough wort (method 2.8.1) are then adequate to produce a yeast of good fermentative power. This is in contrast to using stock slope cultures, where for each propagation undertaken, eight passages through wort would be necessary to produce a yeast of equivalent fermentative power. Therefore, having a supply of 'conditioned' slope cultures available, one can alleviate the time consuming exercise of an extra five passages through wort during propagation when the need arises for a yeast culture.

The experiments outlined in 3.3.4. have shown that the fermentation system provides a very useful technique for screening of large numbers of brewing yeast strains for likely candidates to replace Saccharomyces uvarum(x) as the major brewing yeast at South African Breweries. From the fermentation patterns illustrated in fig. 18 it is apparent that different brewing strains react very differently to the conditions of wort composition, temperature and pitching rate employed at South African Breweries. Some strains were not significantly different to the strain Saccharomyces uvarum(x), one strain (MB 7) did not ferment at all and strains MB 3, MB 6, MB 8 and MB 9 displayed significant improvement with respect to attenuation curve. These 4 strains would be likely candidates for more extensive testing to select an alternative brewing yeast strain.

This test therefore provides an excellent technique for preliminary screening of promising new yeast strains.

In summary a sophisticated fermentation system has been developed that gives reproducible results and shows good correlation to brewery fermentations. This system has successfully been used to predict malt quality as it relates to fermentation performance. The system has also been used to conduct extensive studies on yeast and has led to the following recommendations for yeast

propagation and handling.

During laboratory propagation Saccharomyces uvarum(x) should be continuously coarsely oxygenated. The culture should be 'conditioned' either by using a sufficient number of passages through wort or by using a 'conditioned' slope as a starter culure. The best medium for propagation is an all malt wort.

To achieve satisfactory fermentation, successive contacts of the yeast/ wort mixture with air are preferable to pitching the yeast into wort with a dissolved oxygen content of 14 ppm. An all malt wort is a superior fermentation medium compared with an adjunct wort. This could possibly be offset by increasing the oxygen during fermentation and/or adding amino nitrogen (serine) to an adjunct wort.

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