BIOCHEMICAL UTILIZATION OF WASTE SULFITE LIQUOR: MIXED SUGAR FERMENTATION

by

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ABSTRACT

In order to follow the course of fermentation of the sugar portion of the WSL an accurate quantitative TLC technique was developed by employing a buffered silica gel layer and using an acetone - butanol solvent mixture. Quantitative densitometric evaluation followed a ceric acid visualization step with an analysis error of ± 4% .

A study of the effect of temperature, stirring and bacto concentration showed that the best operating conditions were 36°C, 700 r.p.m and 13.3 g/ℓ respectively. The effect of these variables on the concentration of dissolved oxygen, yeast growth, alcohol-production, and Mannose, Dextrose and Galactose consumption are reported, along with a kinetic study of the process.

The results fitted the published Kono and Asai model. A calculation of product synthesis was also developed.

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I. INTRODUCTION

Waste sulfite liquor (WSL) from the pulp and paper industry may be used in the manufacture of many valuable by products, instead of being burned or discarded into rivers and lakes, with the advantage of maintaining the ecological balance and preserving the environment. The need for some method of utilization of the WSL resulting from the production of high yield pulp is more urgent as a consequence of the increase in the number of sulfite pulp mills switching to the high yield process in order to survive (1). Past experience has demonstrated that high yield pulp may be used in most products. The major advantage of the high yield process is the lowering of the BOD from 500 to 600 Kg/ton to approximately 250 Kg/ton. This improvement by itself will not allow plants to meet projected government standards, but is prompting research toward economical waste treatment or recovery systems.

WSL contains mainly lignosulfonates and polysaccharides. There are several schemes to utilize the WSL in the production of alcohol (2, 3, 4, 5, 6), and yeasts (3, 5, 6, 7, 8) in addition to other valuable chemicals such as Furfural (2), Acetic Acid (2), Fumaric Acid (9) and crystalline sugars (10). These schemes were based on the treatment of the WSL as it is; that is without prior treatment.

In order to expedite research on WSL, and to benefit from their respective experience, the research group on pulp and paper at the University of Quebec, Three Rivers, (UQTR) and the Department of Chemical Engineering at the University of Ottawa have undertaken a joint project.

This joint project includes two major parts, separation of the lignosulfonates and polysaccharides, and fermentation of the sugars. The first part of the project will be studied at the University of Quebec, while the second part will be studied at the University of Ottawa.

a) Separation of lignosulfonates and polysaccharides

The two main components are separated by solvent extraction of the sugars contained in a concentrated WSL. Untreated WSL is about 10% solids. This solids concentration is increased to 50-75% by evaporation of water. The concentrated WSL is then contacted with an alcohol and two phases are formed. The alcohol phase is rich in sugars and the water phase rich in lignosulfonates (11, 12). The process works with methanol as well as water-insoluble alcohols due to the high concentration of soluble solids. Before fermentation of sugars, the alcohol must be removed and replaced with water.

The separation of carbohydrates and lignosulfonates can also be done by the ion exchange process (13). A study on the industrial feasibility of this process for the treatment of high yield WSL is being conducted (16).

b) Fermentation of the sugars

The research work at the University of Ottawa is divided into steps leading to the design of a continuous fermentation process with a maximum output of yeasts cells as a source of protein and the lowest possible sugar content in the discarded effluent.

A previous study (15) was done in order to check the equipment and analysis and to set the variables. It was based on the fermentation of single sugars, using the sugars found in the WSL, on a batch basis. The sugar portion of the WSL contains mainly Hexoses and Pentoses in the ratio of 75:25%. Attention was given to the Hexoses since the pentoses are very difficult to ferment (55) by the yeast. The Hexoses consist mainly of Mannose, Dextrose and Galactose in the proportion of 63:24:13.

The objectives of this work were:

1. To develop an analysis for individual sugars in the mixed sugar solution in order to follow the course of fermentation.
2. To study the effect of mixing, temperature and nutrient concentration on the yeast growth and product (alcohol) formation.
3. To study the kinetics of this fermentation, and to model the batch fermentation in order to set conditions for the continuous fermentation of the treated WSL.

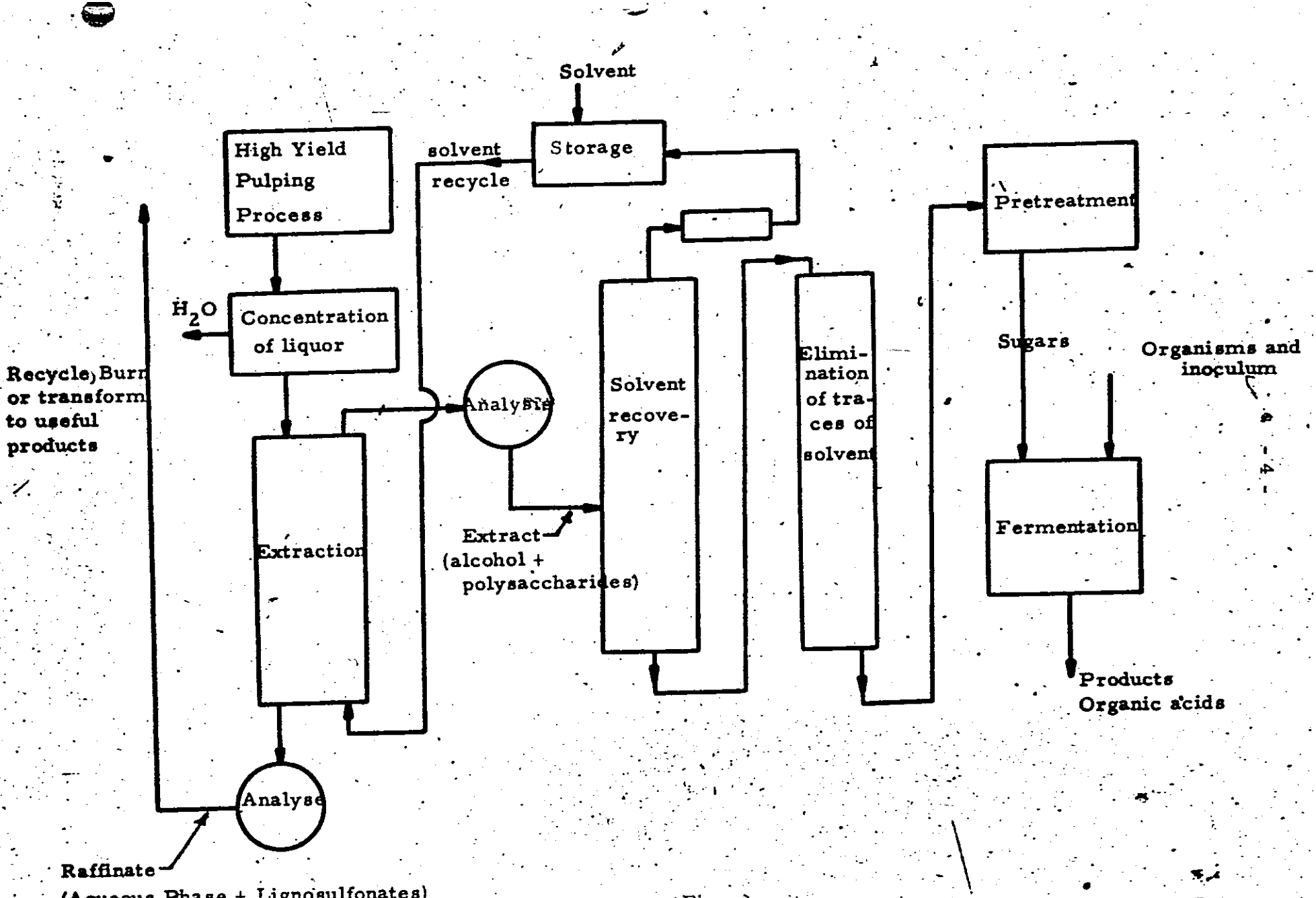

Fig. 1

Recovery System for Waste Liquors from High Yield Pulping Process

II. LITERATURE SURVEY AND THEORY

II.A. Analysis Methods of Mixed sugar Solution

The mixed sugar solution to be analysed consisted of D-Mannose, D-Glucose and D-Galactose. The initial proportion of each was 63:24:13 respectively in a 33.3 g/l total concentration. The published methods for the analysis of such type of solution of very closely related sugars were few. They include Paper, Liquid, Gas, and Thin-layer Chromatography.

Paper chromatography is the oldest and most intensively investigated method of them all. Although Paper chromatography (17, 18, 19) was studied and used in the pulp and paper laboratories for many years it is now being replaced by other methods because it is time consuming and involves a complicated technique which may result in considerable error.

The initial requirement for gas chromatography is conversion of the sugars to volatile derivatives, because the sugars cannot be separated as such. Many of the sugar derivatives which fulfil the requirement of gas chromatographic analysis (20) are not rapidly prepared. MacMillan (20) and co-workers have demonstrated the rapid and quantitative preparation of the trimethylsilyl ethers of carbohydrates. Complete analysis of sugar samples using this method may take as long as two days. The major disadvantages of this method are the difficulty of achieving complete resolution of the peaks corresponding to the sugars (21), the error related to the degradation of the sugars and the interference of small components in

the gas chromatographic analysis.

Another gas-liquid chromatography technique has been proposed for the quantiative analysis of sugars (22). In this method the sugars are hydrolyzed with sulfuric acid using a two step technique. A portion of the hydrolysate is neutralized and the sugars are reduced to the alditols with sodium borohydride. The alditols are acetylated with acetic anhydride and the alditol acetates are then precipitated in ice water and extracted with methylene chloride for injection into the gas chromatograph.

Thin-layer chromatography (TLC), as we know it today, was first described by Stahl (23) in 1958. In 1961, the use of TLC for the separation of carbohydrates was first reported (24). Since that time, TLC has been used extensively for the qualitative as well as quantitative (25) analysis of carbohydrate. The technique is discussed in detail in the following sections.

TLC has the following advantages over other chromatographic techniques for mixed sugar solutions:

a) Analysis is rapid so that complete analysis may be obtained within a reasonable period of time, usually in a few hours, compared to a few days in paper chromatography.

b) It is useful for either sugars or sugar derivatives so that the errors involved in the preparation of sugar derivatives, as for gas-liquid chromatography, is minimized.

c) It gives excellent separation and easily interpreted results.

d) It allows a greater variety of detecting reagents or conditions (Conditions that would destroy paper for example).

II. B. Quantitative Thin-Layer Chromatography

II. B. 1. Mechanism

In the TLC separation, a sugar mixture is made to move with the help of a suitable migrating solvent through a thin layer of sorbent material which has been applied to an appropriate support (23).

The separation depends on the molecular size and the polarity of both the chemical mixture and the migrating solvent (26). The material being separated proportions itself between the solvent and the active sites of the solid adsorbent particles. The polarity of the components, established by virtue of the type and number of functional groups in the molecule, determines what this proportion will be. Polar solvents cause the greatest movement of the sample over the sorbent.

The following list shows a group of solvents arranged in order of polarity (26).

Solvent	Dielectric constant
n-Hexane	1.9 (20°C)
carbon tetrachoride	2.2 (20°)
Benzene	2.3 (20°C)
Trichloro ethylene	3.4 (16°C)
Chloroform	4.8 (20°C)
Ethyl acetate	6.0 (25°C)

n-butanol	17.0 (25°C)
Acetone	21.0 (25°C)
Ethanol	24.0 (25°C)
Water	80.0 (20°C)

The mechanism of separation is usually one of adsorption, partition, or reversed phase partition, and in actual practice a combination of several mechanisms may be involved. By proper selection of sorbent, solvent, and developing conditions, a chosen mechanism can be made to predominate.

The most common operating mechanism in TLC is adsorption, which is useful for the separation of lipophilic organic compounds of medium or law polarity. Silica gel and aluminum oxide are the sorbent materials most often selected for adsorption chromatography.

Partion TLC is valuable for the separation of water-soluble, inorganic compounds and quite polar organic compounds. In the past, paper was preferred for this type of chromatography. Now, cellulose in the form of crystalline powder or ground is being used, although unactivated silica gel, carrying an absorbed phase of water, can be used. In partition TLC, as in adsorption chromatography, a proportion is established between the developing solvents and, in this case, the absorbed stationary liquid phase. Here, much like in separatory funnel extraction, solubility (or the so-called partition coefficient) of molecules being separated, determines this proportion (26).

Reversed-phase partition TLC utilizes a very non-polar stationary phase and a polar mobile phase. Silica gel and cellulose plates may be treated with solutions of lipophilic materials prior to development in order to apply the desired stationary phase. Although used less frequently than other TLC mechanisms, reversed-phase partition TLC is particularly efficient in resolving closely related lipophilic materials (e.g., a homologous series of fatty acids differing only in carbon chain lengths).

Consideration of these mechanisms is necessary in selecting migrating solvents, pretreatment reagents, and developing conditions if the full potential of TLC is to be reached (23).

II. B. 2. The R_F Value

The value computed by dividing the distance the component travels (measured from spot centre), by the distance the solvent front travels is called the R_F value (26, 27).

$$R_F = \frac{\text{distance of centre of spot from origin}}{\text{distance of solvent front from origin}}$$

These values in TLC depend on a number of factors, all of which must be controlled if reproducibility of results is desired. The following are the most important factors (23):

1. Quality of adsorbent
2. Thickness of adsorbent layer
3. Degree of activity of adsorbent

4. Quality and nature of solvents used
5. Degree of chamber saturation
6. Temperature
7. Running distance
8. Technique of development (ascending, descending, or horizontal development)
9. Amount of sample
10. Impurities present in sample mixtures

Careful standardization of developing conditions are still the only way toward a good separation.

II. B. 3. The Preparation and buffering of the thin-layer

Several methods have been reported (23, 24, 25, 28) for the preparation of the adsorbent layer. A slurry of the adsorbent, e. g. Silica gel G, according to Stahl (23), 30 g of adsorbent in 60 ml water is applied to 5 plates (20 x 20 cm) by means of a Desage applicator to give a layer of 250 μ thick. The plates are dried at 110°C for 30 min. prior to use. This method is a standard (26) and most of the manufacturers of fabricated and professionally coated plates follow this recommended method by Stahl.

There are several other methods for the preparation of plates for specific purposes with other adsorbents and using buffer solutions instead of water (23, 24) as 0.02M sodium acetate solution, 0.02M boric acid, pH5 phosphate buffer and some other solutions.

The adsorbant layer may also be sprayed. As reported by R.E. Wing (24). Silica Gel (20 g in 50 ml water) was sprayed using a round-bottomed flask air sprayer.

Commercially prepared plates contain binders which make the plates hard and safe for shipping (24), but the separations are affected so that use of these plates requires different irrigant mixtures than do laboratory prepared plates.

It is necessary to activate the plates before use since room humidity has an influence on the activity of the gel. The plates are exposed to temperature of 100-130°C for a period of time. Then stored in a desiccator. The time and temperature of heating determines the activity of the layer. A shorter time or lower temperature will decrease the adsorption activity.

The buffered layer must be stable in the chosen medium. The pH value of the buffer solution must not change during the separation and the buffer concentration in the layer should remain constant.

Due to excessive heating of the layer the concentration of non-volatile buffers rises. Unequal evaporation of components of a volatile buffer can lead to changes in pH.

II. B. 4. Sample Application

The spotting of the sample can be done with a fine

capillary tube or by micropipette (23, 24, 25, 26, 28) or by the use of new automatic spotters (29) manufactured recently by the leading chromatographic suppliers to control the amount of sample and the size of spot by using air jets. Controlled spot size will eliminate the difference in initial diameters of spots which effects the lateral diffusion of the migrated spot. This precluded measuring the density of the standards and samples for quantitative purposes. When the precision of the fiber optic reflectance scanner was checked (29), it was found better than ± 1%. But when standards of the same concentrations were spotted without the use of the spotter, chromatographed, and scanned, the precision was no better than ± 25%. For silica gel layers the optimum quantity of sugar for application is 5-50 μg per spot, whereas for other absorbents this may be in the amount of 0.5 - 25 μg per spot (23).

Buffering the layer increases the capacity of the layer to be spotted with more sample (23), but on the other hand increasing the amount of sugar per spot decreases the quality of separation and increases the time required for complete resolution.

II. B. 5. Resolution

The early types of TLC developing (Resolving) chambers resembled those used in Paper Chromatography and their shape was modified. Newly acquired data then led to the use of special narrow chambers. A better overall picture is obtained when the types of chamber so far used are classified according to the nature of the development.

1. Chambers for ascending development
2. Chambers for descending development
3. Chambers for horizontal development
4. Chambers for thin-layer electrophoresis

Where possible, glass is preferable and only occasionally the expansive stainless V4A-Steel is used. Plastic chambers may be used with aqueous and mild solvents.

Adsorption - TLC has created a series of special problems which have been only gradually recognised and solved. First, Stahl (23) drew attention to the importance of the degree of saturation of the atmosphere in the chamber and showed that it was necessary to adhere to certain conditions here also. He also introduced the ratio "evaporation surface area; chamber volume" as a charactaristic. This ratio is 1:20 in the usual rectangular chamber but only 1:0.1 to 0.5 for narrow chambers. He attributed particular significance to chamber saturation after it had been possible to eliminate in this way the so called edge effect. The "edge effect", noted by Demole (23) occurs especially when mixtures of solvents are used which differ considerably in polarity, vapour density and density. The substances (spots) near the edge of a thin layer migrate further than the same spots in the centre. The effect is due to inadequate chamber saturation. The plate divides the chamber in two. The developing solvent evaporates more rapidly at the edge of the side carring the layer, in order to saturate the rear of the chamber as well. Stahl (23) therefore suggested chromatography in rectangular tanks with chamber saturation.

Solvent selection of TLC follows different rules. The relative solubility of the compounds in two liquid phases must be considered. The analyst has the option of varying the make-up of one or both of these phases. When the sample being separated is very polar, the mobile solvent systems listed below are generally useful (26):

1. Butyl alcohol/acetic acid/H_2O (4:1:5)
2. Iso-Propyl alcohol/Ammonia/H_2O (9:1:2)
3. Formamide/chloroform/benzene (varing ratios)

When separating organic soluble compounds of medium polarity, a stationary phase of formamide, propylene glycol, or lower alcohol in low-boiling solvent may be applied.

For nonpolar compounds to be separated, a stationary phase of silicone oil, paraffin or undecane is applied. The migrating solvents used are very polar such as (acetonitrile/acetic acid/water) (26).

There are several techniques to develop spotted plates. Continuous development involves the passage of solvent through the sorbent from the bottom of the plate to a region where it is allowed to evaporate. This technique is particularly advantageous with nonpolar solvents for the separation of a mixture whose components show only small differences in R_F values. In this method, the developing chamber must be modified to allow for evaporation at the upper end.

In stepwise development several different solvents are used in succession, passing through the solvent in the same direction with intermediate drying of the sheet.

Multiple development is a special case of the stepwise technique where the same (rather than different) solvent is used several times. Again the plate should be dried between successive migrations.

These development techniques further separate spots having low and quite similar R_F values. Two or three passes will often separate components that are indistinguishable in single migration.

Two dimensional development involves much the same principle as stepwise development except that the plate is rotated 90° before performing second migration.

Thin-layer electrophoresis (26) may be carried out using cellulose plates and conventional aqueous electrolytes. In this procedure, components of the mixture under study are driven by an electrical potential. This voltage is applied across a sheet which has first been impregnated with the electrolyte solution. Individual components moves at a rate which is dependent on the type and amount of electrical charge on the molecule as well as its configuration.

II. B. 6. Visualization

A chromatogram can be evaluated only when all the substances separated can be identified. Should the substance be colourless one of the following visualization technique may be used (23, 24, 25, 26):

1. Spraying with materials to form colored compounds.

2. Fluorencence technique
3. The use of Iodine vapours
4. Autoradiography
5. Bioautography

1. Spraying with materials to form colored compounds

Spraying with a reagent solution is the most widely used technique for visualisation of colourless substances on chromatograms. A uniform and finely atomised spray is necessary to colour evenly. It is essential to have a compressed inert gas sprayer. (23).

Many reagents are quoted for individual compound classes to enable the analyst, through judicious choice, to classify a compound in a different class with more certainty. A warning must be given, however, against over estimating the specificity of the reagent concerned. Thus reducing compounds such as ascorbic acid react as well as amino acids with ninhydrin. Unambiguous characterisation is therefore possible only by using several different reagents.

The use of a non-specific destructive (23, 24) reagent such as sulfuric acid in conjunction with inorganic layers permits the detection of sugars which can not be visualized by other methods. The air-dried plate is sprayed with concentrated sulfuric acid. (5-50% acid in alcohol or water) and heated up to 100-150°C for 5-10 min. to char the organic substances. The anisaldehyde-sulfunic acid of Stahl (23) is sensitive to (0.05 µg of sugar) and gives characteristic colour for each sugar.

Several other visualization methods have been used which give colour reactions with carbohydrates and their derivatives (24); the following are some of them:

(1) Aniline diphenylamine
(2) Naphthoresorcinol + sulfuric acid
(3) Naphthoresorcinol + phosphoric acid
(4) Silver nitrate + base
(5) Urea + phosphoric acid
(6) Ceric sulfate + sulfuric acid + water
(7) Sodium nitroprusside + sodium periodate

2. Fluorescence technique

Numerous substances absorb U. V. light of a particular wave length. Some of them then emit it as visible light. This phenomenon, termed fluorescence (23) is a first-rate, very sensitive means of identifying small amounts of substances on chromatograms. If the substances themselves are not fluorescent, they can often be converted into flurescent derivatives or decomposition products.

Even non-fluorescent compounds which however clearly absorb in U. V. region, can be detected in U. V. light. Thus if a fluorescent layer such as silica gel GF_{254} is exposed to short wave light (254 nm), all substances which absorb in this region stand out distinctly as dark zones on the green fluorescing layer as back ground (23).

3. Iodine Vapours

Preferential adsorption of iodine vapour makes many

substances visible. A few crystals in the bottom of a closed container are all that are needed (26).

4. Autoradiography

Visualization of spots containing radioactively tagged material is accomplished by placing the migrated, dried plate in direct contact with X-ray film. The time required will be governed by the amount of activity and the type of film chosen. The following listing may be used as a guide in selecting the proper type of film (26):

a) Kodak Royal Blue Medical X-ray film Highest speed
b) Kodak Single Coated Medical X-ray film- blue sensative-used with anthracene-impregnated plate.

5. Bioautography

Biological procedures can be utilised in order to detect substances which have a particular physiological activity (23). Zones having bioactivity often promote or inhibit bacterial growth. The culture solutions may be placed directly on the chromatogram during this process (26).

II. B. 7. Densitometric Determination

Densitometric determination is a new contribution to this field of chromatography which allows quantitative measurement of the migrated components on the chromatogram. Two modes of operation are possible (30):

VISIBLE MODE:

The visible mode utilizes the principle of diffuse reflectance. Two beams of light are carried by means of fiber optic bundles to the TLC plate perpendicular to its surface. During scanning, one of these beams (the reading head) illuminates the area spotted while the other (the reference head) is directed over an area free from spots. The reflected light is carried back to cadmium sulfide cells by other fiber optic bundles, where the reflected light intensities of the reading head and reference head are compared. The difference in the reflected signals is converted to electrical output A selection of filters is provided to vary the illumination source frequency so that it may be matched to the colour produced by the sample or staining system (30).

U. V. MODE:

The U. V. mode of operation utilizes fluorescence and fluorescence quenching techniques. The instrument is equipped with both long and short wave fluorescent tubes. After a TLC plate containing U. V. indicating phosphorous has been spotted and prepared, it should be covered with Quartz Cover Plate. At that point the plates assembly should be turned over so that the assembly has the quartz plate on the bottom an TLC plate upside down on the top. This assembly should be placed on the Densitometer plate carriage. When the proper UV wave range is selected the Densitometer may then be operated normally. The U. V. light will pass through the glass plate, irradiate the sample plate, and since the resulting fluorescence will be

visible light move, the reading head S will again carry the light intensities to the cadmium sulfide cells where the compared difference will be converted into electrical signals.

The output signals created by the scanner is forward to chart recorder. The peaks recorded can be analysed and quantitative data may be obtained.

II. C. Fermentation of Mixed Sugar Solution

II. C. 1. Introduction

When microorganisms are grown in a medium containing two or more sugars the growth curve may exhibit more than one growth phase, sometimes separated by a distinct lag period (31, 32). This phenomenon, referred to as "diauxie" growth, was reported by Baidya et al (33) and Harte and Webb (34). Since that time the adaption of microorganisms to growth on various sugars has been investigated in great detail (33). In particular the information which has been obtained from these studies has contributed greatly towards the present views on the mechanisms controlling enzyme synthesis (33).

Harte and Webb (34) classified the sugars into two groups:

a) Those which, if mixed with glucose, show no diauxie growth.

b) Those which, if mixed with glucose, show diauxie growth and do not show it if mixed with each other.

There are certain minor exceptions to this classification but in general it can be said that an organism normally contains "constitutive" enzyme able to metabolize group A sugars, and that it can metabolize group B sugars only if grown under conditions fostering the induction of adaptive enzymes. Recently the generalized term "adaptive" has tended to be displaced by the terminology induced enzyme synthesis, discussed in terms of specific enzymes. A few systems, i.e. organism

in mixed sugar, have come in for detailed investigation (34), but not the particular one under study in this thesis. By analogy to other published systems, it can be assumed that all the enzymes necessary for one or other of the known pathways are available constitutively inside the cell and that the diauxic lag must be associated with the induction (production) of other enzymes. This might, for example, be maltase (α-glucosidase) to hydrolyze the disaccharide and/or a cell-membrane permease system to allow sugar penetration inside the cell (34).

Wang and Humphrey (31) studied the fermentation of mixed sugar solutions. They reported that when a microorganism is cultivated on two different energy sources, one can generally expect that the microorganism will metabolize the most favorable source first, then the second best, and so on. This is, they call it, a "catabolite repression". They claimed that the mechanism is as follows: as the microorganism attacks and consumes the more favorable carbon source, the waste products of this degradative metabolism (or catabolism) prevent or inhibit the microorganism from synthesizing those enzymes that can attack the less favorable carbon source.

Aeration is an important factor to be considered in aerobic fermentation. Oura (35) studied the effects of the intensity of aeration on the growth of baker's yeast. He kept all the environmental factors constant, except the intensity of aeration. From the data he obtained it was possible to calculate the energetics of the yeast growth. If the energy requirements for cell maintenance, sugar uptake, and for the synthesis of

materials within the cell by the yeast were calculated, a view of the energy required for the formation of the cell structure was produced. Oura indicated that the major part (75 - 85%) of the total energy requirement was used for the development of cellular organization rather than for doing biosynthetic work. He found that energy needs of the cells grown under the more aerobic conditions were higher than those of the more simply structured anaerobic cells.

The role of nitrogen and phosphorus was studied by Simek et al (36). It was established that the growth rate and the protein content of the yeasts was affected by the ratio of N/P. They conclude that the necessary nitrogen content in culture medium is 0.1% or 0.5% (as ammonium sulfate) and that the necessary phosphous content is 0.025%. Also, they indicated that the most favorable ratio of nitrogen to phosphous nutrients added to culture medium is 4:1. They concluded that it was not advisable to increase the nitrogen and phosphorus concentration of the culture medium beyond the minimum because this neither enhances the growth capacity of yeast, nor gives any significant increase of yield.

Einsele et al (37) investigated the effect of mixing in hydrocarbon fermentation. They concluded that with an improved mixing effect it was possible to increase the productivity by 25%. They found that, due to the build up of flocs, the influence of mixing may have a profound effect on the observed growth kinetics. It was also known that at higher cell concentrations in complete mixing may result in oxygen limitation.

II. C. 2. Fermentation of Waste Sulfite Liquors

The quantity of wood sugars contained in the annual production of spent sulfite liquor exceeds 1 million tons (38) on a world-wide basis. Although this quantity is only a fraction of the potential which could be derived from the wood industries, it nevertheless represents a substantial tonnage which has the definite advantage of being collected at and available to central processing plants. This availability in quantity is a first step along the road to economic utilization.

Utilization of the carbohydrates of WSL has traditionally been centered on fermentation methods (46). One advantage of fermentation is that the sugars are converted to products which are more easily recovered. Some of the products which can be produced from WSL by fermentation are lactic acid (38), fumaric acid (9), acetone, butanol, butyric acid, propionic acid (38), acetic acid, furfural (2), methane (38) and edible mushrooms (40). However, the only commercial products are ethyl alcohol (2, 3, 4) and Yeasts (3, 4, 7, 8).

Kalguzhnyi and Ivanyukovick (7) cultivated feed yeast in WSL with a significant excess of nitrogen. Avela et al (2) fermented the WSL to yield a major product of alcohol and minor products of furfural and acetic acid. Helena Karezewska (3) fermented various concentrations of the WSL. She enriched the liquor with nitrogen and phosphorous acid subjected it to alcoholic fermentation for 48 hrs. by strains of saccharomyces cerevisiae Ma, Schizosaccharomyces pombe nr 2, and Candida tropicalis P6. Fermentation was carried out at 34-38°, pH

5.8 - 6.8 with continuous aeration. Alcohol production was 32.6 ml/100 g of sugars. Helena Karczewka in another publication (8) reported an alcohol production as high as 68.2 ml per 100 gr. of fermenting sugars using selected cultures of the yeast Schizosaccharomyces Pombe. Raitseva (4) fermented WSL with 41-60 ml alcohol per 100 gr of fermentable sugars.

Many workers have concentrated on the production of yeasts from WSL which may be considered a promising aspect as a source of unicell protein. Helena Karczewska (3) studied the production of fodder yeast from WSL using the yeast Candida tropicalis P6 strain at 34-38°C and pH of 5.8-6.8 with continous aeration. For optimum yield of yeast it was recommended not to increase the WSL concentration above 50% of its initial concentration. Considerable work (41) was reported concerning the utilization of WSL in the production of Torula yeast in Germany during the second World War. Major producers of yeast from WSL currently are France, Italy, Japan, Roumania, Sweden, U.S.A. and U.S.S.R. (41). Apparently Canada does not produce any at present but has potential of upwards of 500,000 tons. The major disadvantage of this yeast is the cost of production (41) and contamination with lignins whicharenot degraded by the yeast.

II. C. 3. Parameters affecting the fermentation process

Fermentation, as in any other process, occurs in time; independent of it but dependent on conditions affecting its course (42).

From the multiple conditions associated with fermentation it is possible to single out those which are statistically significant and those which are less significant, and, thus, from the point of view of a simplified consideration of the course of fermentation may be neglected. To study the course of fermentation on the basis of mathematical statistical elaboration three statistically significant parameters have been chosen which affect the whole process:

1) concentration of biomass, m
2) concentration of limiting substrate, s
3) concentration of product, p

The course of the fermentation process can be described in a multidimensional space whose axes are the parameters characterizing the state of the process. This space is termed by J. Hroncek (42) the "Fermentation Field" and the process course in this field can be described by a space curve. Using the alcoholic fermentation as a model, the course of this process can be depicted in a three dimensional space having the biomass concentration, m, concentration of the limiting substrate (sugar), s, and concentration of the inhibiting product (ethylalcohol), p, axes. Each point of the spatial curve representing the course of the fermentation is determined by three victors; dm/dt (rate of biomass formation) ds/dt (rate of change of substrate concentration), and dp/dt (rate of product formation). Another vector, termed the "control vector" (42), of the fermentation process, can be added which changes the course of the fermentation field. The introduction of the idea of "the fermentation field" may be regarded as the most important step in studies involving living mass.

II. C. 4. The Fermentation Kinetics

The kinetics of yeast growth were investigated by Anderson (43) and found to follow a pattern similar to that of adsorption and catalysis in that a large number of parameters, each with saturation effects, is involved. After an initiation period in which the yeast becomes accustomed to a new environment, the growth rate is first order in yeast concentration and also depends on the concentrations of sugar, oxygen, available nitrogen, minerals, vitamins and hydrogen ion, and on the temperature. The rate equation as a product of terms for each of the vital substances,

$$\frac{dC_y}{dt} = k c_y \left[\frac{b_s C_s}{1 + b_s C_s}\right] \left[\frac{b_o C_o}{1 + b_o C_o}\right] \cdots \cdots \left[\frac{b_n C_n}{1 + b_n C_n}\right] \qquad (1)$$

where C_y is the yeast cell volume per unit volume of solution, k is a rate constant, C_s is the sugar concentration, b_s is a constant for sugar, and so forth for oxygen (o), nitrogen (n) and others. It is assumed that other variables such as temperature and p H are constant. For an excess of any of the nutrients the product bC becomes large compared to unity and the term in brackets approaches unity.

In the experiment considered in this work all nutrient substances except sugar are supplied in large excess so that

the rate expression becomes:

$$\frac{dC_y}{dt} = kC_y \frac{b_s C_s}{1 + b_s C_s} \qquad (2)$$

Since yeast growth occurs both by the growth of individual cells and by cell division with further growth and division of new cells, a rate equation for the number of cells per unit volume may differ slightly from that for the volume of yeast per unit volume.

A material balance for the sugar may be used to relate sugar and yeast concentrations.

$$R\ (C_y - C_y^o) = C_s^o - C_s \qquad (3)$$

where $1/R$ is the yeast cell volume which results from the utilization of a unit amount of sugar and the superscript o indicate an initial value . Combining with equation (2) to eliminate c_s yields,

$$\frac{dC_y}{dt} = k C_y \left[\frac{b_s (C_s^o - R(C_y - C_y^o))}{1 + b_s (C_s^o - R(C_y - C_y^o))} \right] \qquad (4)$$

which may be integrated for the initial condition, $y = y^\circ$ at $t = o$, to give,

$$C_y = C_y^o e^{kt} \left[\frac{C_y^o}{C_y} \left[1 - \frac{R}{C_s^\circ} (C_y - C_y^o) \right] \right]^{\frac{1}{b_s (C_s^\circ + R C_y^\circ)}} \quad (5)$$

The growth curve of equation (5) will not include the induction period and transition at the start of growth nor the loss of cell volume after the sugar has been consumed.

A similar model of the kinetics of growth of the yeast saccharomyces cerevisiae was developed by Peringer et al (44). It expressed analytically the dependence of maximum specific growth rate and growth yield upon dissolved oxygen tension and defined the relationship between these two parameters.

Yarovenko and Nakhmanovich (45) studied the fermentation of carbohydrate by the yeast saccharomyces cerevisiae. They reported that the amount of alcohol which is produced by 1 g biomass per hour in individual fermenters with different fermentation time are (C_{a_1}, C_{a_2}, C_{a_3}) and may be obtained from the following relations,

$$C_{a_1} = (D P_1)/C_{y_1} \quad (6a)$$

$$C_{a_2} = D (P_2 - P_1)/C_{y_2} \quad (6b)$$

$$C_{a_3} = D(P_3 - P_2)/C_{y_3} \qquad (6c)$$

where P_1, P_2, P_3 are alcohol content in the corresponding fermenter in ml/l, C_{y_1}, C_{y_2}, C_{y_3} = concentration of yeast biomass in g/l and D = dilution rate (hr^{-1}). The k values which characterized the activity of yeast are highest in the main fermentor (1.6 - 2.7) and then they decrease.

Kono and Asai (1) studied the kinetics of fermentation process and derived a model mathematically which resulted in a triangle - like graph. This model is developed from the two general equations of production rate,

$$\frac{dC_y}{dt} = k_y C_y \qquad (7)$$

$$\frac{dC_a}{dt} = k_{a_1} C_y + k_{a_2} (1 - \varphi) C_y \qquad (8)$$

where C_y is the cell concentration, C_a, is the product concentration, k_{a_1}, k_{a_2}, are production rate constants and ky is the growth rate constant. φ represent an apparent coefficient of growth activity and its value depends on the phase of fermentation i.e., for the Induction phase $\varphi = o$, for the transient phase $\varphi = \varphi$, for the exponential (46) growth phase $\varphi = 1$ and for declining growth phase $\varphi = \frac{C_{yc}}{C_{ym} - C_{yc}} \times \frac{C_{ym} - C_y}{C_y}$ where C_{yc} is

the critical cell concentration, or cell concentration at the boundary of an exponential growth phase and declining growth phase. C_{ym} is the maximum cell concentration predicted by the theoretical procedure. When the values of $\frac{dC_y}{dt}$ and $\frac{dC_a}{dt}$ are plotted against C_y, a triangle-like graph should be obtained. It should be noted that this model requires the fitting of the data in the mentioned four regions each by a different fitting equation depending on the nature of the curve to get a straight line fitting. From this graph, the slope of the lines can be evaluated which correspond to k_{a_1} and k_{a_2}. Kono and Asai classified the fermentation process according to k_{a_1} and k_{a_2} as follows:

TABLE 1 Kono and Asai classification

k_{a_1}	k_{a_2}	description
+	+	Product formation associated with growth and non growth
+	o	Product formation associated with growth
o	+	Product formation associated with nongrowth
+	-	Product formation associated with growth and decreased with non growth

III. APPARATUS AND EXPERIMENTAL PROCEDURE

III. A. Apparatus

The steam autoclave used for sterilization was a New Brunswick Scientific (NBS) model AE 15-10, supplied with pressure relief valve. The autoclave was modified by adding an extra heating coil and designing a cooling jacket to allow for fast heating-up and cooling down. This modification was done by adding a 1000 watt coil to the 1500 watt coil originally present and a valve for removal of the water inside the autoclave 120 feet of cooling tubes were wrapped around the outer surface area. Time for heating up to the required temperature was reduced from 50 min. to 20 min. and cooling down time was as short as 7 min. compared with 90 min. or even more. The fermentor used was a NBS microferm bench fermentor model MF-114 with a 5 liter fermentation vessel. A detailed drawing of the fermentation vessel is shown in Figure 2. Agitation was achieved by a single impellor of 6 flat straight blades located above the air sparger. The impellor is 5 cm in diameter and located 7 cm from the bottom. A four-tube baffle assembly served as heat exchanger to control the temperature inside the vessel. The fermentor was equipped with a temperature control unit with a built-in strip-chart temperature recorder, an air flow control rotameter, an agitator r. p. m. controller, an air sterilizers and an automatic foam and level controller.

Continuous recording and control of pH was achieved to within 0. 1 pH unit automatically with an NBS modular pH controller model pH-22. Two persitaltic addition pumps added acid or base on demand. Steam sterilizable pH electrodes with stainless steel immersion holders were mounted in threaded fittings in the fermentor

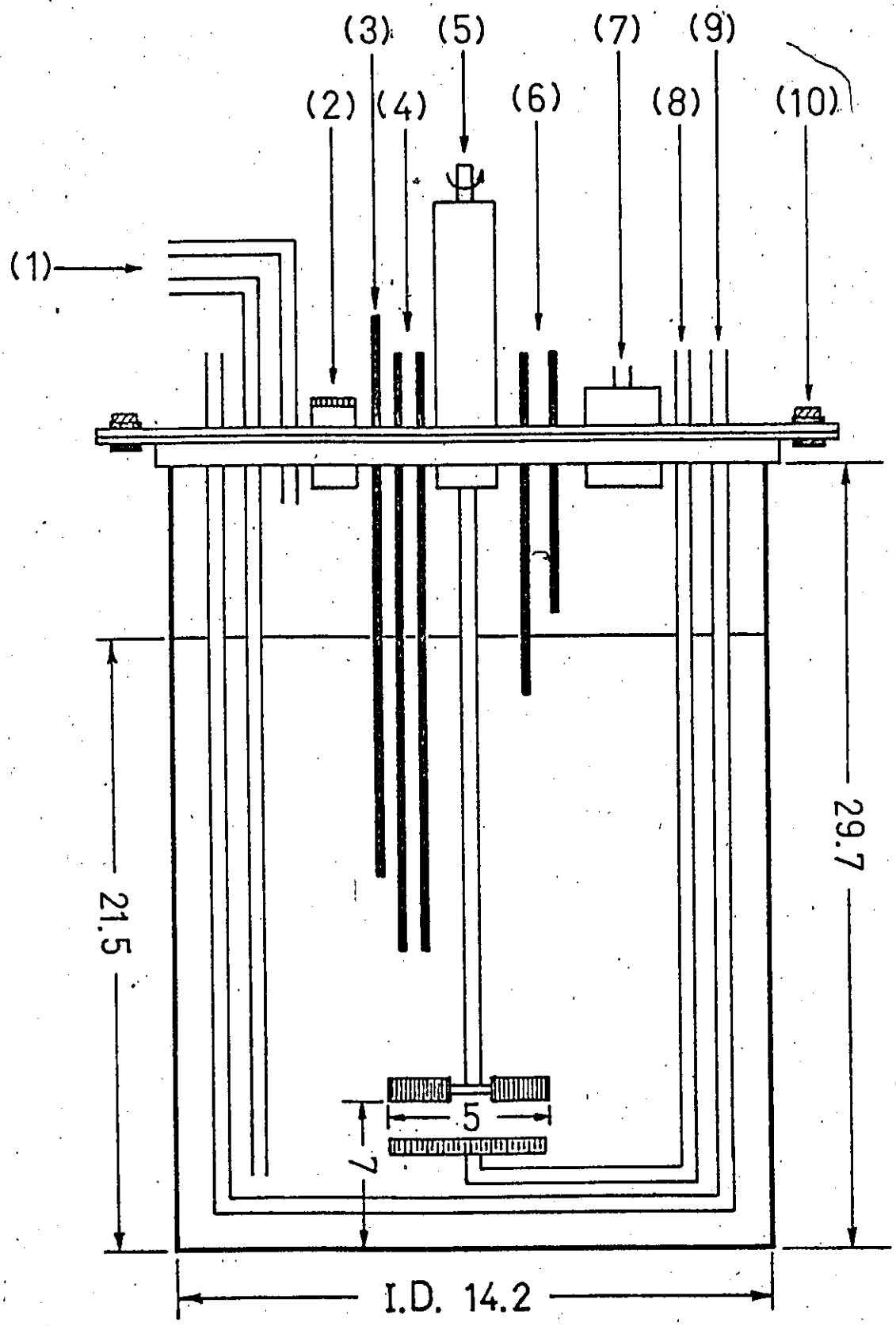

Fig. 2 The fermentation Vessel

The fermentation vessel component :

(1) Inoculator - sampler tubes

(2) Injection syptum

(3) D.O. probe

(4) pH electrodes

(5) Variable speed drive and impellor shaft

(6) Level - Foam control probes

(7) Effluent air condenser

(8) Air tube and sparger

(9) Temperature control buffles

(10) Vessel top plate screw

headplate and connected to the pH instruments.

An NBS model D. O. -50 analyzer was used together with an NBS steam-sterilizable electrochemical membrane-type oxygen electrode model M1016-0201.

An NBS inoculator-sampler, model S20, was used. It consisted of a removable glasscollected tube and a built-in air filter chamber. Inlet and Outlet lines, connected to a sterilizable plastic syringe pump, were used for pumping liquid (or yeast culture) into and out of the fermentor. Figures 3 and 4 shows the fermentor in details connected to its accessories (the pH controller, the D.O analyzer and the inoculator sampler).

The centrifuge was an International Equipment Co. model HN-S with a diameter of 15 cm and a maximum speed of 3200 rpm.

The gas chromatograph used for ethanol determination was supplied by Fisher Scientific Co. Model Fisher Series 2400, with hydrogen flame ionization detector and automatic temperature programmer. A 10 ft. long x 1/4 inch diameter column was used supplied by Chromatographic Specialties, Brockville, Ontario, and packed with chromasorb-W (30-60 mesh) coated with carbowax 20 M.

The TLC densitometer used was supplied by Kontes Glass Co., Vineland, New Jersey. The chromaflex K-495000 model was equipped with a built-in short and long wave U. V. light source for fluorescence measurements, and had as well the filtered visible-light reflectance modes. Scanning could be done automatically in four rates, 2 cm, 4 cm, 6 cm and 10 cm per minute. The output was connected to a Fisher recorder model Recordal series 5000, with range 0.001-10 mv. Figure 5 illustrates the densitometer used and the recorder.

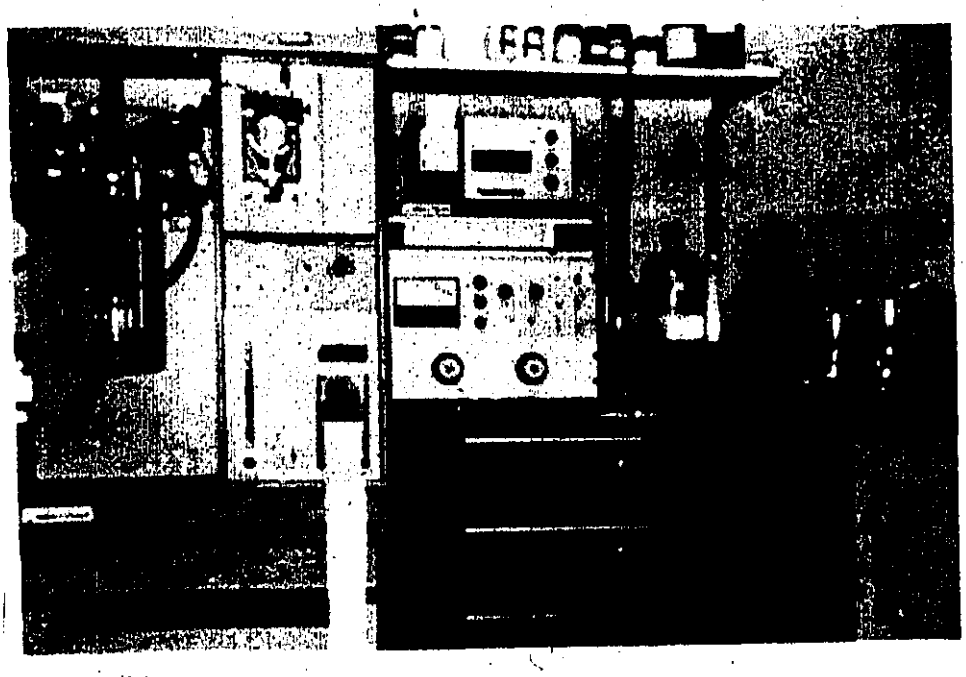

Fig. 3 Fermentor assembly (the N.B.S. Fermentor, pH controller and D.O. Analyzer)

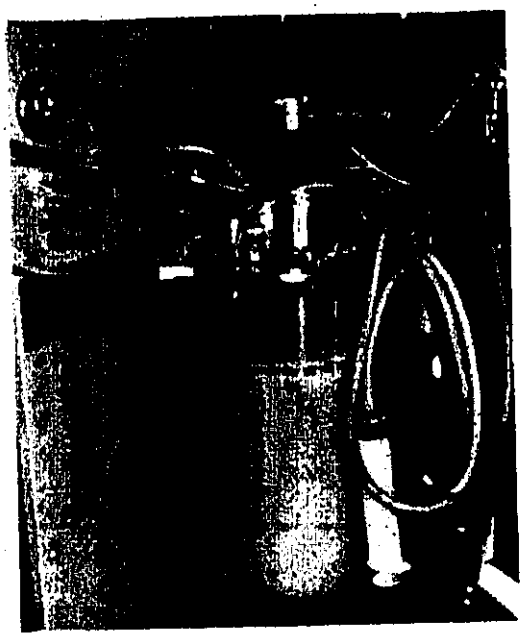

Fig. 4 The fermentation vessel connected with the fermentor and the inoculator-sampler.

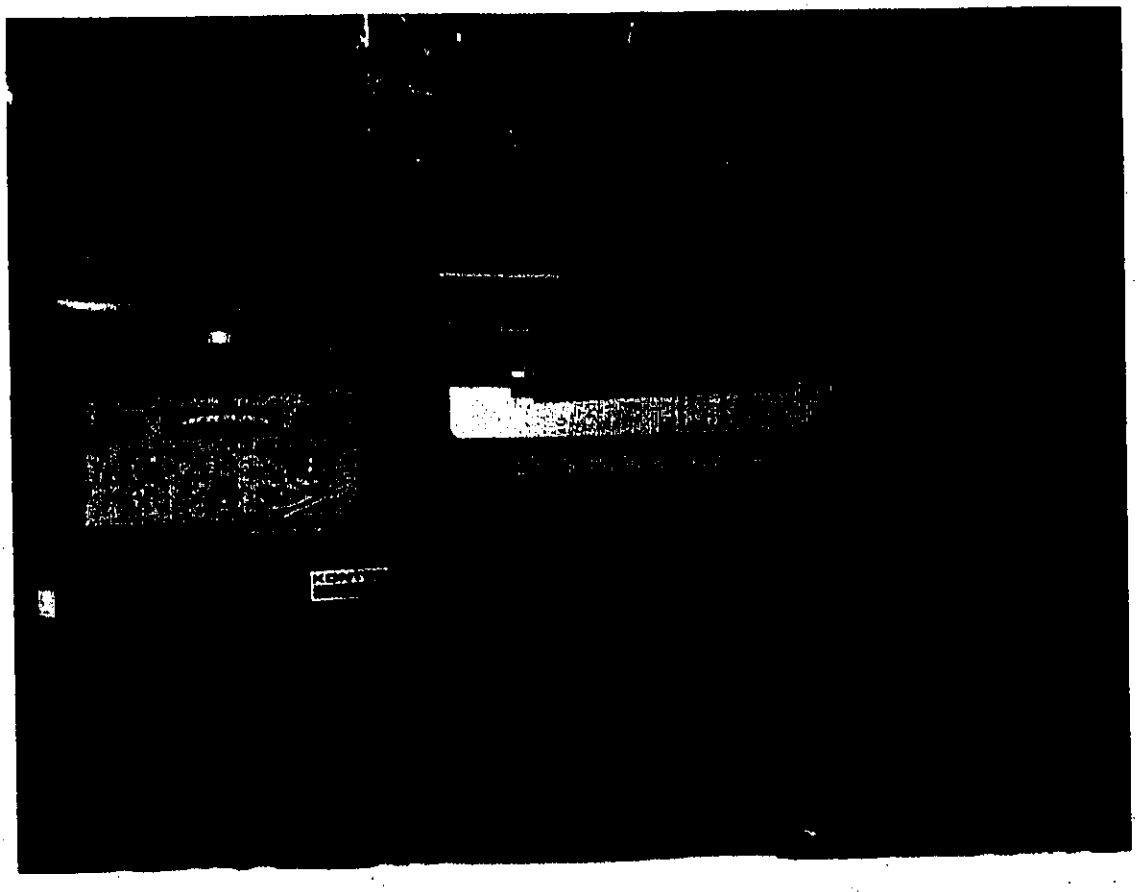

Fig. 5 Kontes TLC densitometer connected to Fisher Scientific recorder

The other apparatus used consisted of a Kontes K-416330 chromaflex Spotter with six spotting positions complete with an air flow regulator (Kontes 2 PA-Air) which allowed the regulation of air from 0.1-1 l/min. Silica gel TLC plates supplied by Fisher Scientific Co., precoated with silica gel GF, thickness 250 microns, size 20 x 20 cm catalog No. 6-601 A were used. Two Gelman developing chromatography chambers model 51325-1, supplied by Fisher Co. were used, as well as a Hamilton chromatographic syringe, 10 µl capacity #701. The sprayer used was supplied by Fisher Co. (Sparatool Aerosol model cataloge number 15-233).

III. B. Materials

- D(+) Mannose, reagent grade, Fisher Scientific M-121-75767.
- D-Glucose, (Dextrose), Anhydrous, granular reagent grade, J. T. Baker chemical Co., #1916.
- D(+) Galactose, Auhydrous, powder, reagent grade, J. T. Baker Chemical Co., M675.
- Bakers Yeast, Saccharomyces cervisiae, supplied by Morrison Lamoth Bread Company, Ottawa.
- Yeast Nitrogen base, Dehydrated, (Bacto), Difco Laboratories, Detroit, Michigan, (Composition is listed in the appendex).
- Acetone, certified A. C. S., Spectrophometry grade, Fisher Scientific A-19.
- 1-butanol, certified, 99 mol%, Fisher Scientific A384.
- Ethyl Alcohol, Certified, 99%, Fisher Scientific.
- Sulphuric Acid, Reagent A. C. S., Allied Chemicals Canada Ltd.
- Ammonium hydroxide, Reagent, A. C. S. Specification, the McArthur Chemical Co., Canada.
- Acid Hydrochloric, Reagent, conforms to U. S. P. and A. C. S. Specification, the McArthur Chemical Co.
- Ceric sulphate, powder, BDH Chemicals Toronto, Canada.
- Phosphate-Citrate Buffer Solution pH 4.00 $\pm$ .02 at 25°C, Fisher Scientific Company, SO-P-82.
- Antifoam B (silicon emulsion), J. T. Baker Chemicals Co., B531.
- Sodium Bisulfite, Granular, reagent grade, A. C. S. Specifications, J. T. Baker Chemicals Co., 3556.

III. C. Experimental Procedure

Figure 6 outlinesthe experimental procedure flow diagram. The following one detailed description of each step.

III. C. 1. Medium Preparation

The substrate was made to resemble exactly the composition of the sugar portion of the WSL. A 100 g of total sugar was dissolved in 2. 980 l of distilled water in the fermentor vessel. The sugar solution was composed of 63% Mannose 24% Dextrose and 13% Galactose. The yeast nitrogen base (Bacto) was then added in amounts varing from 10-40 grams depending on the operating conditions. A 20 ml portion of distilled water was placed in a separate bottle to be sterilized and used for the yeast suspension and inoculation. The fermentor head plate was screwed tightly to the vessel which wastransferredto the sterilization unit.

III. C. 2. Sterilization

The fermentation vessel containing the sugar and Bacto solution along with the fermentor air filters, sampling device and the 20 ml distilled water bottle were placed in the autoclave for sterilization. This was accomplished by heating up to 90°C, holding at this temperature for 5 minutes, and then cooling down. This procedure was recommended by Anderson (43). He argued that since the yeast is fairly resistant to disease, complete sterilization is unnecessary, the holding time at 90°C being necessary only to kill off wild yeast cells.

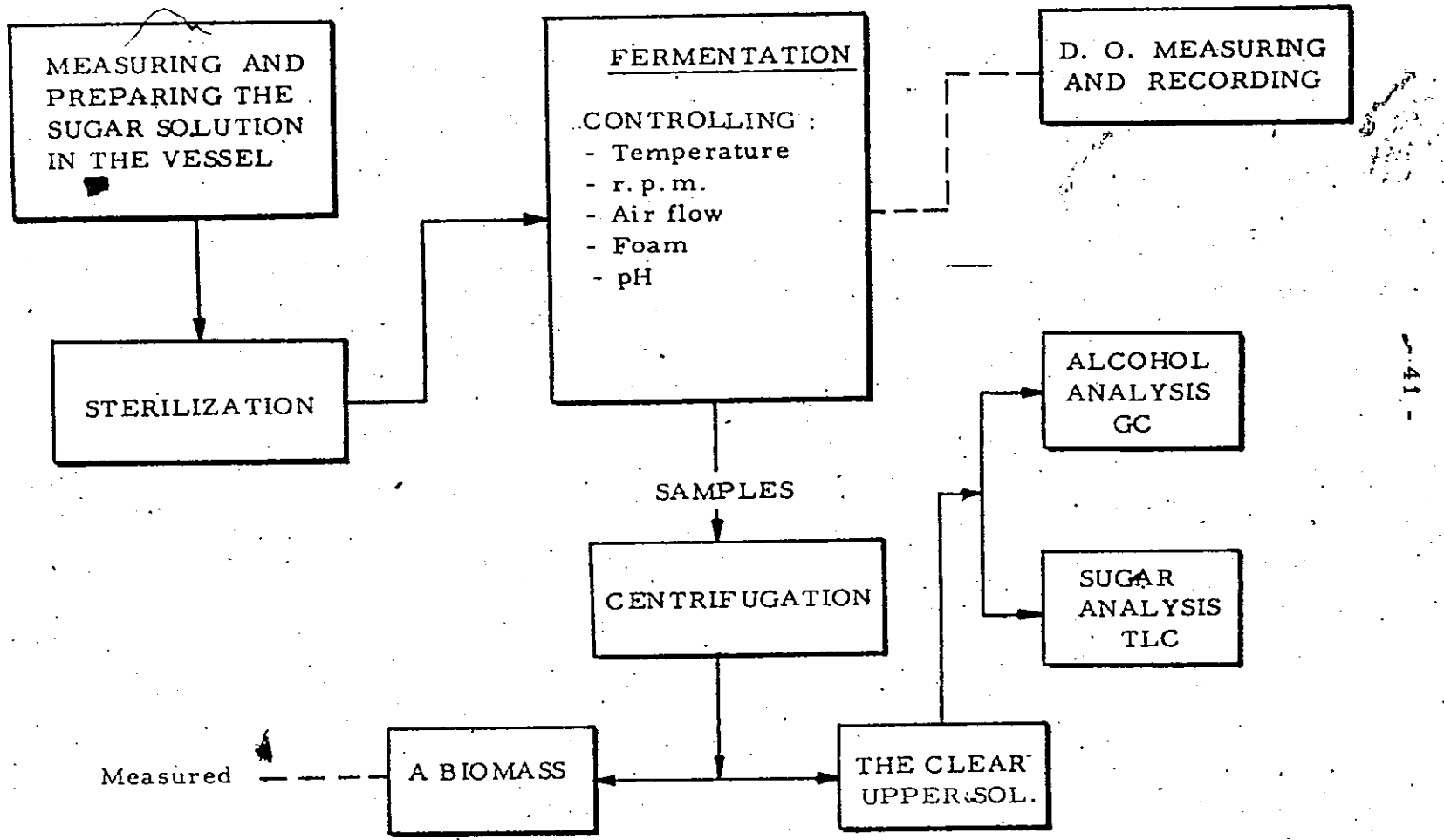

Fig. 6 The Experimental procedure flow diagram

III. C. 3. Conditioning and Yeast Inoculation

After sterilization the fermentation vessel was allowed to cool down to the desired temperature. This step was speeded up by connecting the vessel with the fermentor temperature control unit and starting the agitation and air flow. The pH control unit was connected to the vessel and drops of acid or base were allowed to flow into the vessel to reach the desired pH level (pH 5.00).

After reaching the designed levels of temperature, pH, r.p.m. and air flow, four grams of fresh yeast cells was suspended in 20 ml sterilized distilled water and introduced into the fermentor medium through the inoculation-sampling tube. The tube was flushed several times through the injector to insure complete transfer of the yeast inoculum to the fermentation vessel.

Allowing 3-5 minutes for homogeneous distribution of the yeast cells, the time was recorded (as zero time or the start) and a sample was taken. This sample (#1) was considered the standard. At the same time, the D. O. analyzer was calibrated and the range was set to 100 assuming that at this moment the medium had the maximum level of dissolved oxygen.

III. C. 4. Sampling

Samples were taken with the sampling device every one and half hours. The fermentor vessel top plate had two stainless steel sampling pipes one of them terminating near the bottom of the vessel, and the other (see Figure 6)

above the solution level inside the vessel. The sampling device was connected to these pipes. Samples were transferred into the sampling collecting tube by creating a vaccum using a 50 ml plastic syringe. The sampling tube was flushed by applying pressure using the same syringe. Representative samples were assured by recirculating three tube volumes (about 10-15 ml each) before taking a sample, eliminating the effect of dead volume in the sample tube.

III. C. 5. The Temperature and pH Control

The fermentation temperature was maintained constant during the run to within $\pm$ .25°C. A four-tube baffle assembly served as a heat exchanger using hot or cold water. The individual baffles had a width of 1.8 cm, a length of 29 cm and a thickness of 3 mm. Temperature was detected by a thermocouple and recorded by a built-in strip-chart temperature recorder. Adjusting the flow rate of cooling/heating water resulted in good control.

The pH was set to 5.00 in all runs according to the results obtained by DeKee (15). Addition of 2N HCl or 4N NH_4OH was made automatically by the pH controller. The volume of acid and base added was recorded each hour and a half.

III. C. 6. Dissolved Oxygen measurement

The D. O. analyzer was connected to the D.O. probe immersed in the fermentation vessel.

Although a linear relationship between the D. O. concentration and the potential difference was expected, several problems affecting the sensitivity and durability of this sensor were observed. The boiling and loss of the electrolyte filling the electrode annular space during sterilization appear to be the major problem. A change of the electrochemical potential by time was observed. However the electrode performed satisfactory for a period up to two months with 35 consecutive sterilizations.

III. C. 7. Yeast Growth Determination

Samples of 10 ml each were placed in graduated centrifuge tubes and centrifuged at 3000 r. p. m. to produce a compact yeast cell mass at the bottom of the tubes. Cell volume was recorded as the volume of the yeast cell mass produced after 7 min. of centrifugation. Since the yeast may continue to produce carbon dioxide which may swell the volume of the yeast biomass, readings of cell volume were taken immediately after centrifugation (43).

The supernatant liquid was collected for alcohol and sugar analysis.

III. C. 8. Alcohol Analysis

A gas chromatography technique was used to determine the amount of alcohol present in the samples. A Fisher Scientific gas chromatograph with a Hydrogen Flame Ionization detector was used. A 5 µl sample was injected using a Hamilton

micro-syring #701 (of maximum capacity 10 µl) mounted on an automatic injector (Repro-jector supplied by SHANDON). The column, described earlier, was maintained at initial temperature of 180°C with an upperlimit of 182°C, the detector at 250°C and the injector at 200°C. The carrier gas used was nitrogen flow at a rate of 35 cc/min. at 65 p.s.i.g.; the hydrogen flow rate was 35 cc/min. at 10 p.s.i.g., while the air flow rate was 120 cc/min. at 35 p.s.i.g.

The Attenuator was kept at 64 and Range at 10^{-10}. The recorder span was 2 mv and the chart speed was 0.25 in/min.

III. C. 9. Sugar Analysis

Quantitative TLC analysis was performed on 20 x 20 cm glass plates precoated with Silica Gel GF. 254 (layer thickness 250 microns). The plates were activated and buffered before use. The plate was kept for one hour in the oven at 110°C; then sprayed by 0.1 M Sodium bisulfate solution and dried at 110°C for 10 min. The plate is then sprayed with the citrate-phosphate buffer (pH 4) and kept in the oven at 110°C for 30 min. Then transferred to a dessicator till use.

The clear liquid from the centrifuged samples was used for alcohol and sugar analysis. A 5 µl sample was spotted using a Hamilton syringe and the Kontes Chromaflex spotter. Four samples were spotted on each plate plus a standard sample (spotted in the middle).

To get the desired spot of controlled diameter and density, the sample was taken using the syringe which was then inserted

in the glass tube of the chromaflex spotter. This supported the syringe and directed the needle to the surface of the plate through the guide holes of the manifold. The manifold had six spotting positions, and each position consisted of a guide hole for the spotting needle and 4 holes spaced evenly around 360° for directing the inert gas (Nitrogen) uniformly around the needle and onto the surface of the thin-layer plate. The velocity of the gas flow through the manifold(0.5 l/min.) regulated the size of the origin spot. The distance from the manifold to the plate surface was regulated by a slide guide and lock nuts. This distance was 6 mm in all the runs. In order to have the spots at a certain reproducible distance from the bottom of the plate, the plate was placed in the apparatus so that the corner of the top edge lined up with the 30 mm mark on the side scale. With the plate in position and constant gas flow the syringe was introduced in its guide so that the needle just touched the plate surface, the syringe plunger was then pushed down slowly at a constant speed and the solution was spotted onto the thin layer plate in a uniform manner.

The plate is developed at ~25°C with Acetone: n-butanol: water mixture (53:40: 7). The development took place in a saturated Gelman chromatographic chamber. The distance run was 15 cm and the elution time was 55 min. After drying under a stream of air a second development was repeated with the same conditions to ensure best separation.

The plates weredried in a stream of air, then sprayed with an acid solution of 1 g. ceric sulfate dissolved in 100 ml of 10% sulfuric acid solution in water. The plate was then kept

at 110°C for 15 min. It was important to use uniform spray for reproducible results.

Quantitative measurements were made by the Kontes densitometer, scanning the plate horizontally across columns, that is, all mannose spots were scanned on one pass, all Dextrose were scanned on the next, and so on. The densitometer operated on the visible light reflectance mode. Scanning speed was 10 cm/min. and scanning attenuator was 200. The densitometer was connected to Fisher recorder series 5000 with a sensitivity of 0.01 mv and chart speed of 5 inch/min. Peak height was analysed and compared to calibration curves shown in Figure 35. A photographic filter photar, ND. 6, series 4 was used on the light source of the densitometer.

IV. RESULTS AND DISCUSSION

IV.A. The Analytical Challenge

The main objective of this work was to study, on a batch basis, the fermentation of a sugar solution similar in composition to the sugar portion of the WSL.

The major problem in this work was the development of a rapid, easy to perform and economical method for the analysis of the sugar mixture in the fermentation medium. This step was essential in order to follow the course of fermentation.

As mentioned in section II.A., TLC seemed to be the only solution to a simple, rapid and sensitive method for the quantitative analysis of low molecular weight sugars and their derivatives. In the few years since the first published work (1961), the TLC method has been adopted for a variety of analytical problems. Only a few literature reports, however, were available on TLC of pure sugars. Therefore intensive work was done to study this new method and the factors controlling the separation and quantitative evaluation of sugars.

Initially, it was necessary to acquire the technique of TLC using standard procedures and equipment. The use of the Kodak Chromat/0/Screen 60 Analysis kit for sugars was helpful to understand the nature and acquire the skill of TLC.

All of the sugars, D-Mannose, D-Glucose and D-Galactose, have the same molecular weight and the same number of -OH groups. And since the separation on the thin-layer depends on the variation in molecular size and the polarity of these sugars, it was expected

that separation would be difficult. Consideration was first given to the already published procedures. Several mixtures of solvents were used as listed in table 2. Also several layers were employed such as Silica Gel G, Silica Gel GF, Cellulose MN (23,24,25, 47) and a buffered layer of these substances with various buffer solutions such as 0.02 M sodium acetate, 0.02 and 0.1 N boric acid, 0.02 M sodium borate, sodium phosphate buffer of pH 5, 0.1 M sodium bisulfite (23)

TABLE 2 Solvents tried in TLC of a mixture of Mannose Dextrose and Galectose

	Mixture	Composition V/V	Ref.
1.	Ethyl acetate - 65% Isopropanol	65 + 35	(51)
2.	Methanol - Chloroform - acetone - conc. ammonium hydroxide	42 + 16.5 + 25 + 16.5	(51)
3.	1 - butanol - acetic acid - ether - water	9 + 6 + 3 + 1	(11)
4.	Ethyl acetate - Acetic acid - Methanol - water	12 + 3 + 3 + 2	(11)
5.	1 - Propanol - water	17 + 3	(11)
6.	Acetone - Methanol	9 + 4	(11)
7.	Ethyl acetate - Methanol - water	37 + 40 + 23	(12)
8.	Ethyl acetate - Pyridine - water	40 + 20 + 40 100 + 35 + 25	(51) (12)
9.	Chloroform - acetone - 95% ethanol	38 + 38 + 24	(51)
10.	Chloroform - methanol	90 + 10	(51)
11.	Chloroform - 96% ethanol	5 + 4	(12)
12.	n-butanol - acetone - water	4 + 5 + 1	(52)
13.	n-butanol - acetic acid - water	4 + 1 + 4	(52)
14.	phenol - water	3 + 1	(52)

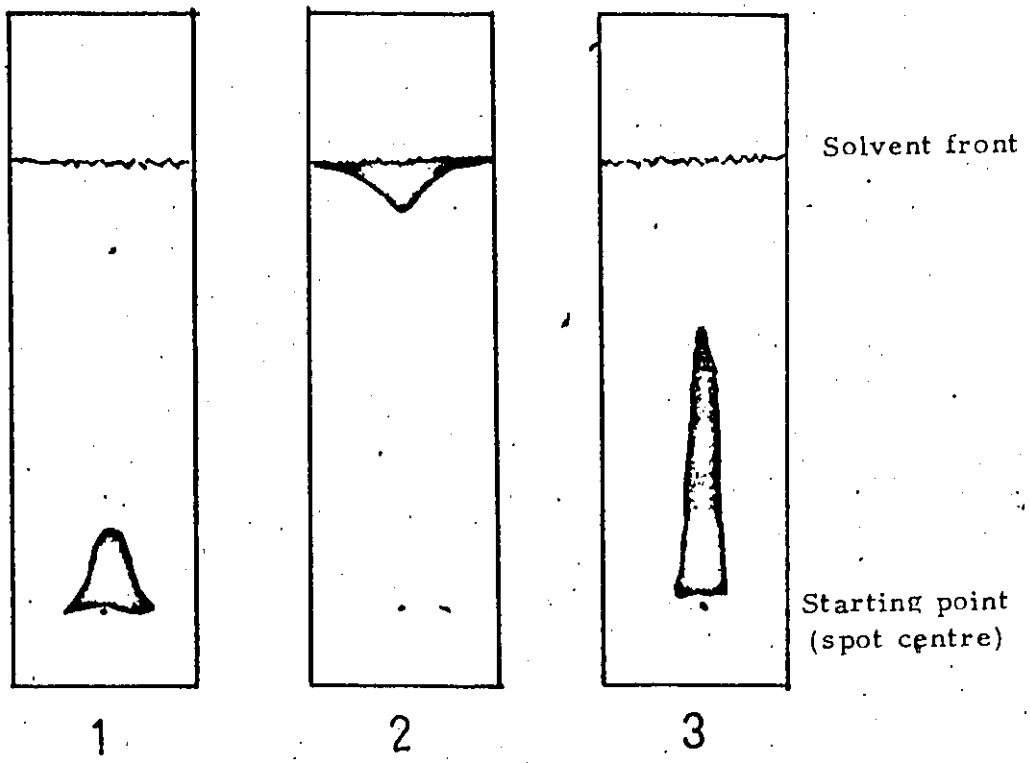

Figure (7-a): Troubles associated with early trails using the solvents mentioned in table 2.

1. no migration & decentralization in Δ shape at the starting point.
2. Migration without separation and decentralization at the solvent front.
3. Oblique formation without separation.

None of the tabelled solvents gave satisfactory separation on either buffered or unbuffered layers using the published buffer solutions mentioned above. Results obtained are indicated in figure (7-a). Also, attempts with solutions of modified composition were unsuccessful. It is believed that one of the reasons why these solvents did not gave satisfactory separation is due to the nature of the thin layer. Plates used in published literature were prepared in the laboratory with certain of their specifications slightly different from the commercially prepared plates we used; such as the use of organic or inorganic binder, or the thickness of the layer.

Starting from this point, trials to get a rather more selective layer were done. After several trials, it was found that buffering the layer twice with different buffer solutions (0.1 M sodium bisulfate and pH 4 citrate-phosphate buffer) improved the selectivity of the layer for the migration of the mentioned sugars. These buffer electrolytes act in the inter-molecular spaces in the thin layer. They cause a selective behaviour toward the migration, by diffusion, of the spotted mixture of sugar by decreasing the mobility of sugar molecules with different values depending on the shape and polarity of these molecules. The position of -OH groups and the configuration of -H and -OH groups of the mentioned sugars are the reasons behind their different migration mobility on such buffered layer. It should be mentioned that the diffusion of the spotted sugar mixture occur only in one forced direction, upward. This is due to the movement of the solvent in that direction by capillary forces. The moment the solvent front reaches a relatively high level and the solvent rising velocity slows down, diffusion will occur in all directions. Development or resolution of spotted plates should be done with a reasonable solvent front rising

velocity to ensure no decentralization of the spots.

Based on the above mentioned facts and experience acquired though the trails of different solvent mixtures, a mixture of solvent was formulated of Acetone: n-butanol : water (53 : 40 : 7) . This solvent mixture was found to have, after trial and error with different proportions, the best selectivity and a reasonably fast movement. The liquid front reached the desired height (15 cm) in 55 min.

It was also found that a double resolution for the same plate ensured complete separation. It should be mentioned here that the buffering and resolution steps are unique and this thesis may be considered the first publication of such methods in the field of TLC.

Selection of the visualization solution for the sugars was based on the nature of the thin layer and the binder used. Since an inorganic layer and binder were used a mixture of sulphuric acid and ceric sulfate in water was employed to char the organic sugar spots. Good results were obtained from this type of visualization since it produced dark spots on a white background.

Figure (7 -b) is a reproduction of one of the early plates obtained by the use of the developed method. Individual sugar samples were as a mixture of sugars. Migrated spots were eluted to different levels. They are of circular shape with a slightly larger diameter than the original spot. A good separation for the component sugars of the mixture spot was achieved. For each migrated spot the distance ($\frac{a}{b}$) is the R_f value. The R_f values for D-mannose, D-glucose and D-galactose were 0.59, 0.50 and 0.41 respectively and if D-glucose R_f is taken to be 100, the following are the

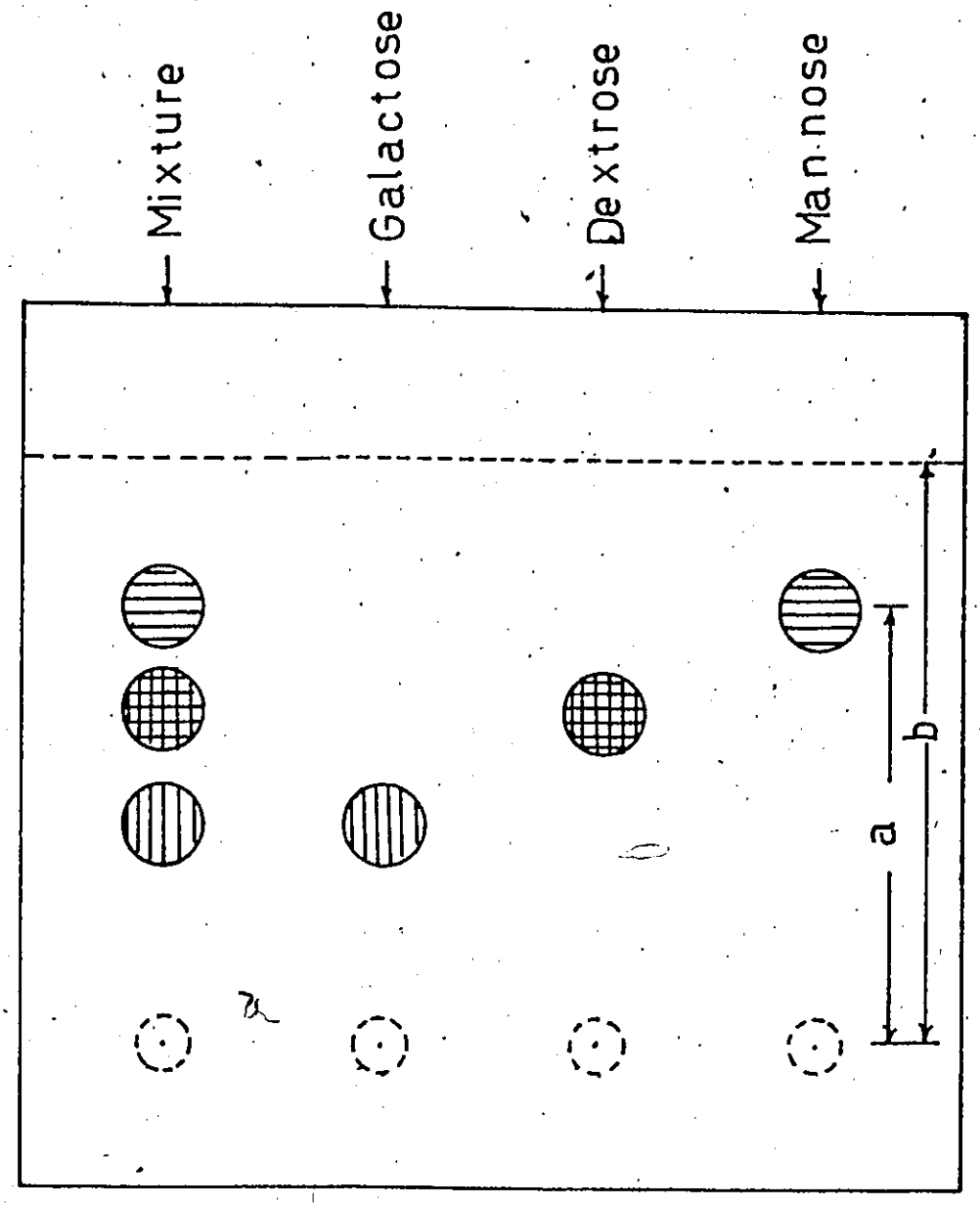

Figure (7-b) a sketch of an early resolved plate using the developed method

R_f values using the developed method.

Sugar	R_f
D + Glucose	100
D + galactose	82.8
D + Mannose	117.2

Samples of mixtures of known concentration were spotted, resolved and visualized by the above method, and calibration curves were then made upon scanning of these plates. Figure 35 in the appendices section shows the calibration curves of the three sugars involved.

Plates for the analysis of actual runs were prepared by the same technique. Furthermore, a standard sample was spotted in the middle point for each analysis to check the analysis accuracy. This sample was the first sample taken at the start of fermentation immediately after the injection of the yeast cells. A sketch of the first prepared plate for run No 17 is shown in figure (7-c).

The TLC plate after visualization was mounted in the densitometer and scanned using the technique described in the experimental section. As mentioned scanning could be carried out horizontally or vertically, i.e, by scanning all the mannose spots in one pass, or scanning the migrated spots of the three sugars in one vertical pass. For most of the experimental work the horizontal scanning was used. It should be mentioned here that the calibration curves given in the appendices (figure 35) were developed by horizontal scanning. Vertical scanning was used to check results in the early stages of

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Run No 17(i)
Samples No.
2, 3, 1, 4, 5.

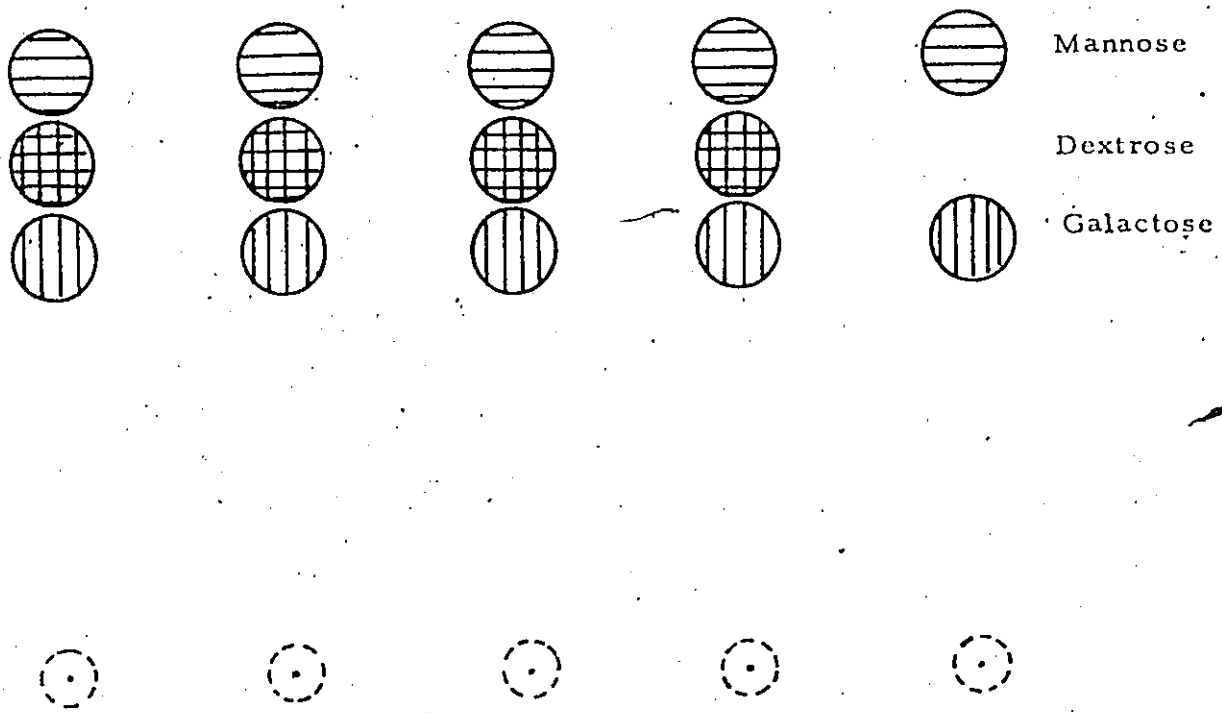

Fig. 7. A sketch for a TLC plate

this work. The Peak height was the parameter used since peaks had a sharp triangle shape. Figure (7-d) shows a xerox copy of the scans obtained.

The following table shows the values of Mannose obtained by scanning the first plate prepared for run No 17 and the corresponding g/ℓ values using the calibration curves (xerox copy of this plate in figure (7-c) and figure 35 shows the calibration curves used).

Peak (sample) number	2	3	1	4	5
Peak height, cm	10.0	9.5	10.5	8.5	2.1
Mannose value, g/ℓ	19.9	18.8	20.8	16.8	4.1

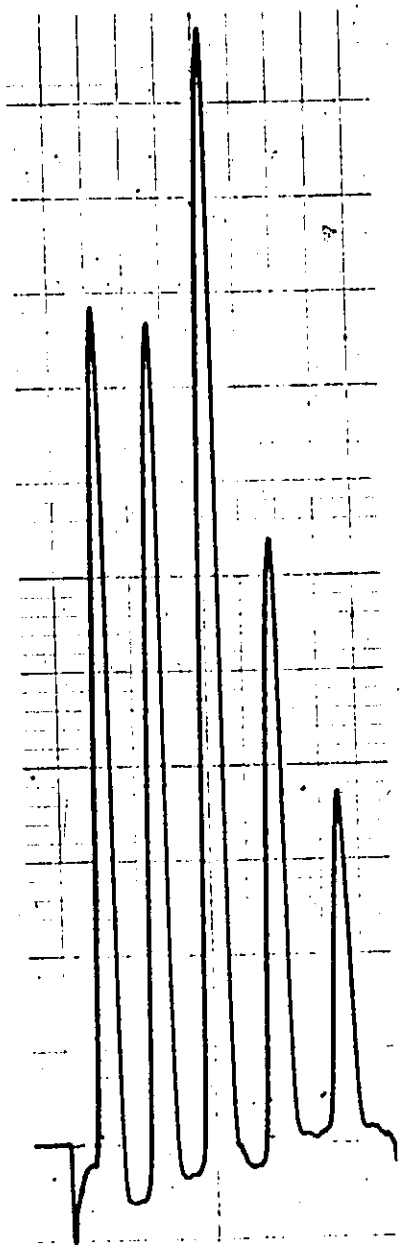

Figure (7-d): Xerox copy of the peaks obtained by horizontal scanning of galactose

IV.B. The Mixed Sugar Fermentation Studies

The study of the fermentation of the synthetic sugar mixture solution resembling the composition of the sugar portion of WSL was based on the work done and results of the first step of research undertaken by the University of Ottawa. That step was studied by Dekee (15) and results of fermentation of single sugars were presented. Fermentation of the mixed sugar solution was performed by the procedure given in section II.C. and results recorded in section VIII.C.

Twenty runs were performed, each run requiring 16 - 18 hours. The first ten runs were to establish the feasibility of the fermentation and to test the equipment, method of analysis, and control. A run was also made to ensure that the accuracy of analysis was identical to that of the previous research work by Dekee's (15) This run gave similar results with respect to yeast, alcohol and sugar (single) analysis. Data obtained are listed together with Dekee data using the conventional analytical methods in section VIII.A. in the Appendices.

In the second set of runs, the objective was to study the effect of temperature, stirring and bacto concentration on the dissolved oxygen; yeast growth; alcohol production, total sugar consumption and Mannose, Dextrose and Galctose consumption. Collected data are tabulated in section VIII.C.

In order to study these variables and their effect knowledge should be developed on the nature of the fermentation process of the mentioned mixed sugar solution using the Saccharomyces cerevisiae yeast. A model run was selected and is discussed in the next section.

IV.B.1. The Model Run

Run number 14 was selected as a model for application of kinetic models, product synthesis calculation and discussion of results. The studies applied to this run may be applied to the other runs as well.

The operating conditions were as follows :- [illegible]

Fermenting solution volume		[illegible]
Total sugar present - (Mixed)		[illegible] rose, [illegible] galactose)
Microorganism used	:	Baker Yeast [illegible] 4 g)
Air flow rate	:	4000 cc/min.
Air Pressure	:	14.7 p.s.i.a.
Agitator speed	:	700 R.P.M.
PH	:	5
Temperature	:	32^{o}C
Yeast nitrogen base added	:	40 g

Air was flowing throughout the run

Dissolved Oxygen was recorded automatically

Acid and base consumption recorded.

Table of results appear in section VIII.C. (Table 11)

Figure 8 shows the yeast growth curve. The lag phase appears to be the period of the first hour of fermentation. The concentration may be constant, but this does not mean that the cells are quiescent or dormant ; on the contrary, during this stage the individual cells increase in activity beyond their normal status (49). Physiologically they are very active and are metabolising and there is a slight swelling of the cell size.

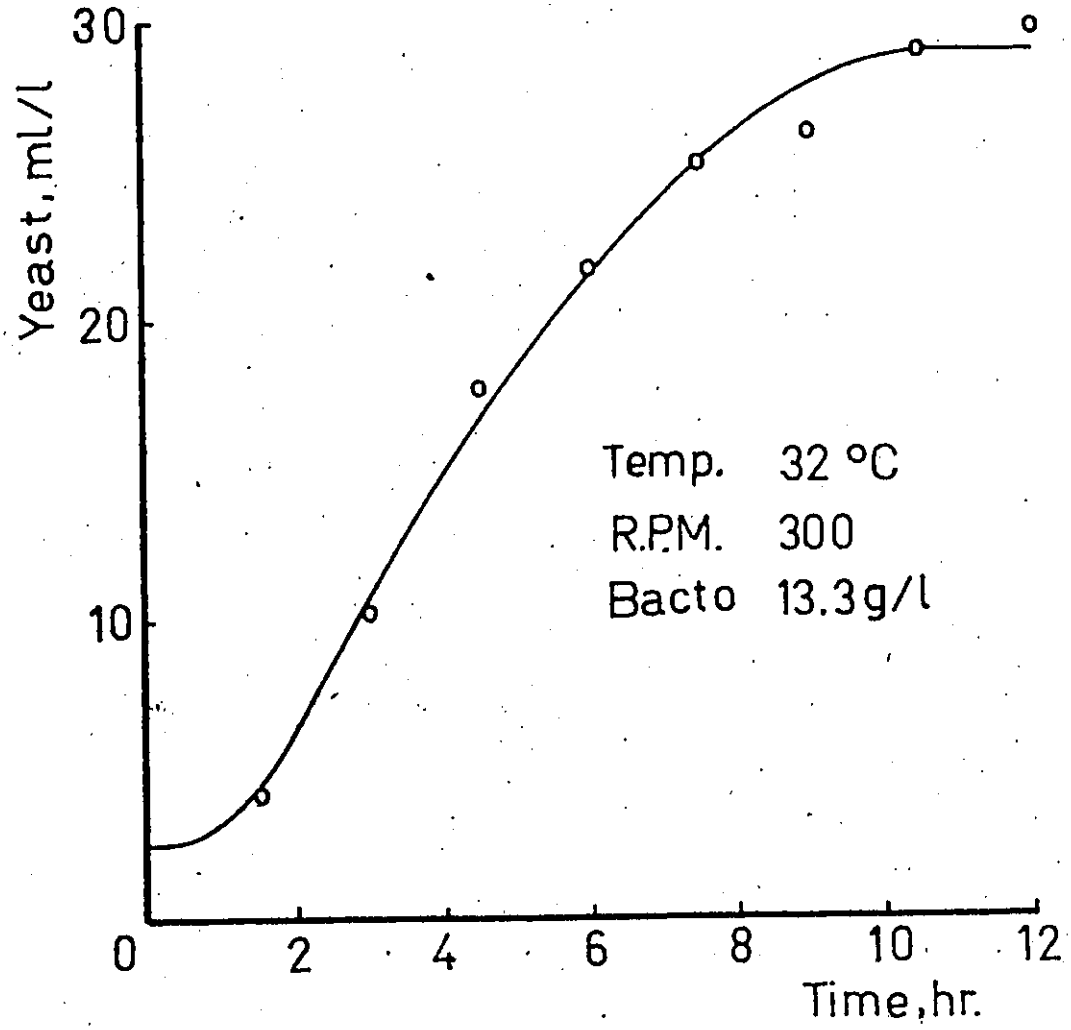

Fig. 8 The Model Run, Yeast Growth Curve

These two facts are noticed from fig 8 where a slight increase in yeast volume was noticed (see also the table of results) which is due to the increase in the cell volume and not to a new population. Also Fig 10 shows that sugar has been consumed from the very start of the fermentation which proves the activity of the yeast cells. The lag period may also be explained from the enzyme formation point of view. The yeast in this new environment may be deficient in enzymes and co-enzymes.

Following this lag period, a one Logarithmic phase is noticed for this type of mixed sugar fermentation. According to the Harte and Webb (34) classification mentioned in section II.C.1, the fermentation of these sugars present in the sugar portion of WSL do not show a diauxie growth. Also the Baker's yeast used contains the necessary enzymes to hydrolyze the three sugars simultaneously.

The stationary phase of cell growth appears after 9 hours. This can be attributed to a variety of circumstances particularly the exhaustion of sugars. As noticed from Fig. 10 both the Dextrose and Mannose are fully consumed before 9 hours.

Fig. 9 shows the alcohol production curve and the dissolved oxygen curve. The highest level of alcohol production is noticed after about 7 1/2 hours while at that time the dissolved oxygen approaches the zero level. This may be explained by the fact that the production of alcohol is favoured by the anaerobic conditions. Curve 1 represents the actual amount of alcohol produced. This curve is calculated by adding the measured amount of alcohol in the fermentation vessel to the amount of

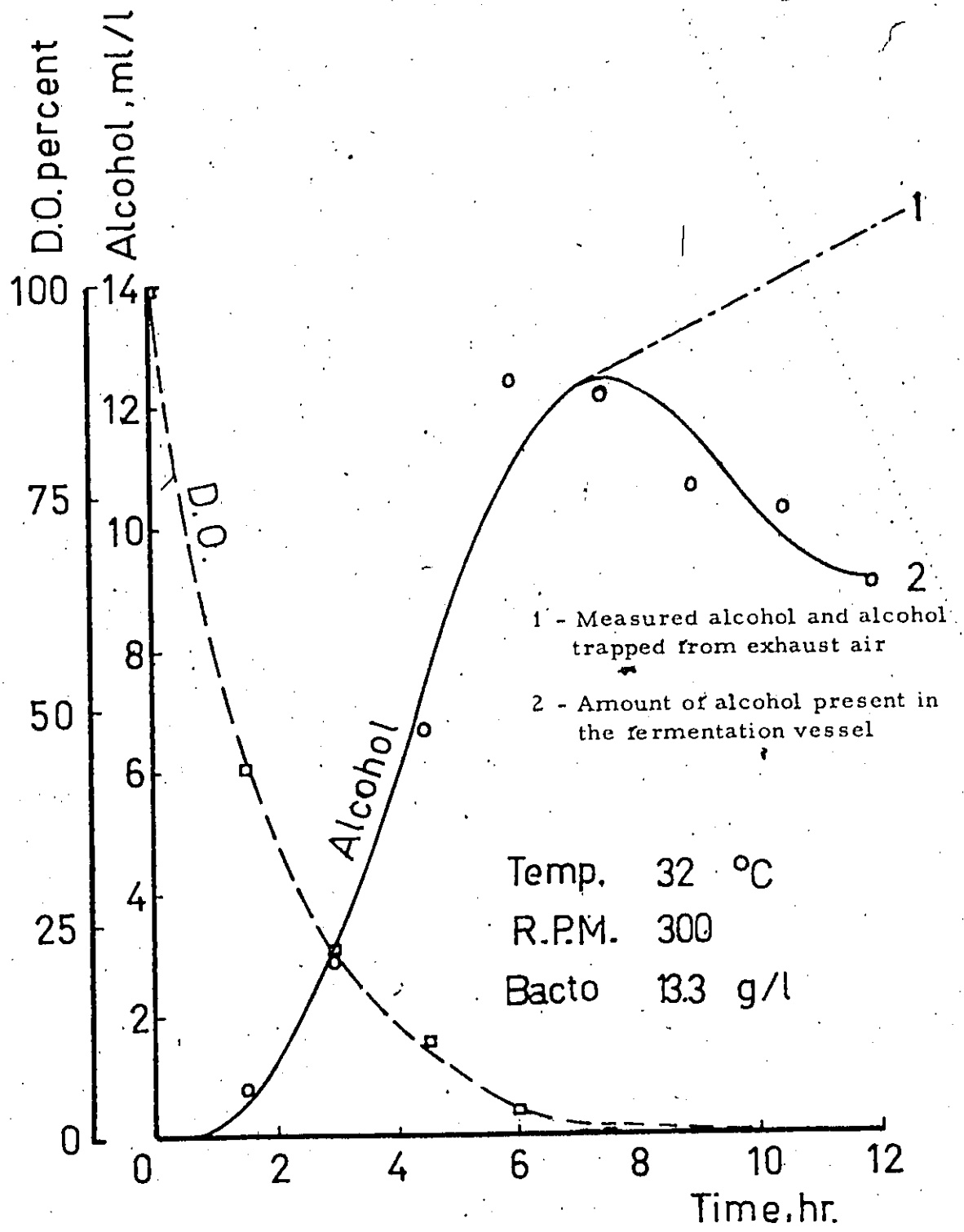

Figure 9

The Model Run,
Alcohol production curve and
The Dissolved Oxygen curve

alcohol escaping with the exhaust air which was trapped in cold water traps and detected by GC. Curve 2 represents the amount of alcohol present in the fermentation vessel only. The drop in the amount of alcohol present was due to loss of alcohol carried away by the air.

Fig. 10 shows the sugar consumption curves. Galactose disappears after 12 hrs and seems to be hard to digest. Dextrose is obviously an easy sugar to be consumed by the yeast cells, while Mannose may be considered the most favorable source of energy. The expectation of Wang and Humphrey (31) of the "Catabolite repression" (section II.C.1) for mixed sugar fermentation seems to be untrue. From Fig. 10 one can generally judge that the consumption of all the three sugars, Mannose, Dextrose and Galactose, begins simultaneously from the start of fermentation. The only difference that could be reported, is the rate of consumption. Mannose has the highest rate, followed by Dextrose and then Galactose which has a rather slow rate of consumption. For example for the period of 3.0 hours to 6.0 hour from the start of fermentation (which is considered in the Log phase of yeast growth), the rate of consumption of Mannose was 3.47 g/ℓ.hr for Dextrose 1.83 g/ℓ.hr and for Galactose only 0.44 g/ℓ. hr.

The dissolved oxygen concentration throughout the run was measured by employing the D.O. automatic analyzer and D.O. electrode. Since we had a constant flow rate of air bubbling inside the fermentation vessel, it was observed that the dissolved oxygen concentration was related to the yeast cell volume. Alcohol production was at the maximum value when the dissolved

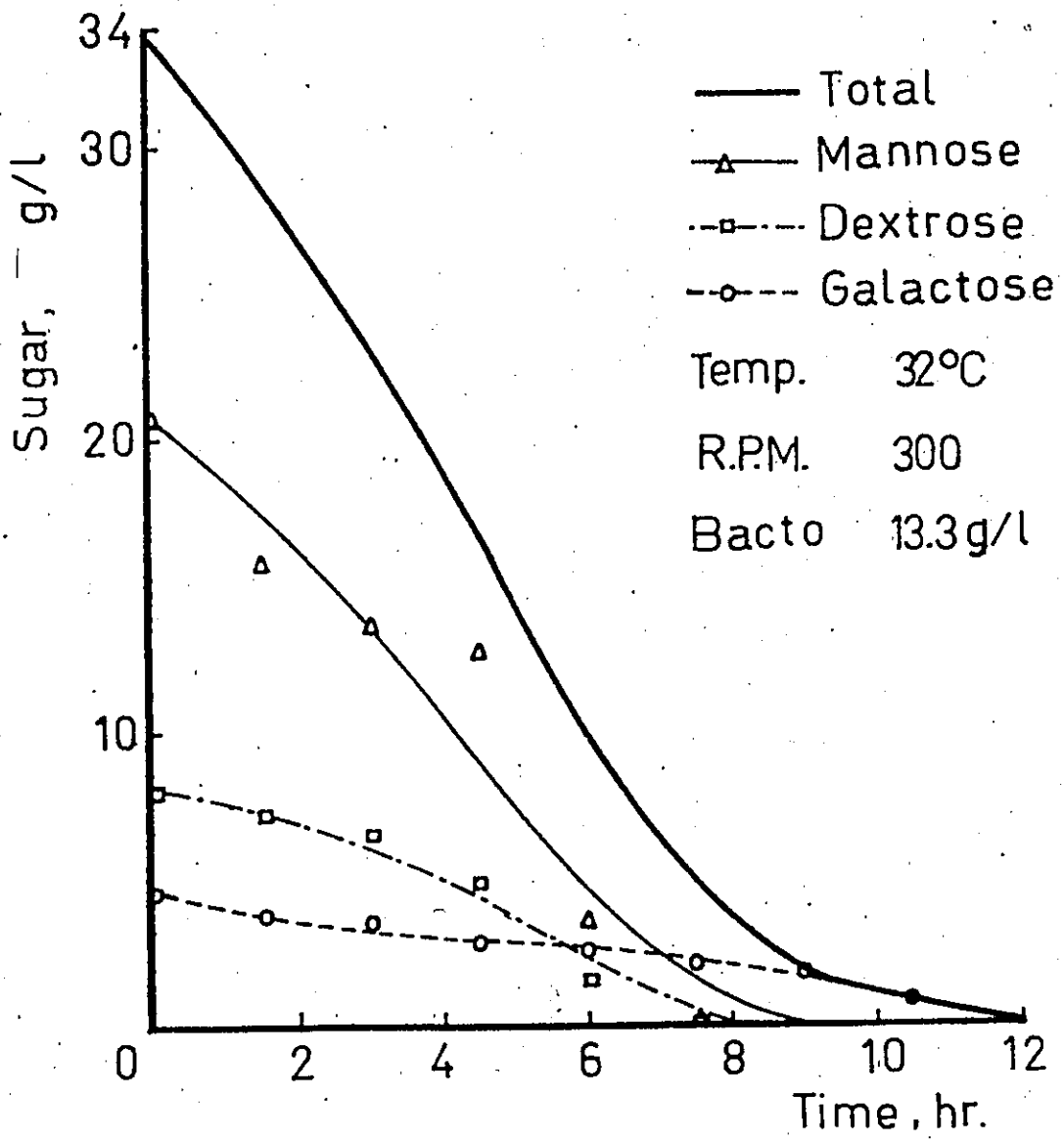

Fig. 10 The Model Run, Sugar Consumption Curves

oxygen level was near zero. A higher oxygen level could only be possible if there was continuous removal of yeast cells from the reaction medium, so that the production of alcohol could be avoided and conditions could still favour the yeast growth (48). Fig 10a shows the model run curves.

IV.B.2. Product synthesis Calculations

When the mixed sugar solution of Mannose, Dextrose and Galactose is fermented under the previously mentioned conditions using the yeast saccharnomyces cerevisiae, the following products may be expected

1. Alcohol, in the form of ethanol, measured by GC. is produced.
2. Protein, in the form of yeast cell growth, measured by volume ratio is produced.
3. Energy is used in the biological processes.

All the mentioned substrate sugars are represented by the formula $C_6 H_{12} O_6$, therefore, in alcohol fermentation the following equation represents the sugar consumption :

$$C_6 H_{12} O_6 \longrightarrow 2\ CO_2 + 2\ CH_3 CH_2 OH \qquad (9)$$

i.e., there is a factor of sugar to alcohol production equal to

$$F_1 = \frac{\text{sugar}}{\text{alcohol}} = \frac{180}{2 \times 46} = 1.956 \ \frac{\text{g. sugar}}{\text{g. alcohol}}$$

Similarly there are other factors for the transformation of the sugar substrate into yeast cells F_2 ($\frac{\text{g. sugar}}{\text{g. yeast}}$) and for the production of energy F_3 ($\frac{\text{g. sugar}}{\text{hr.g. yeast}}$) where sugar is converted to CO_2 and water.

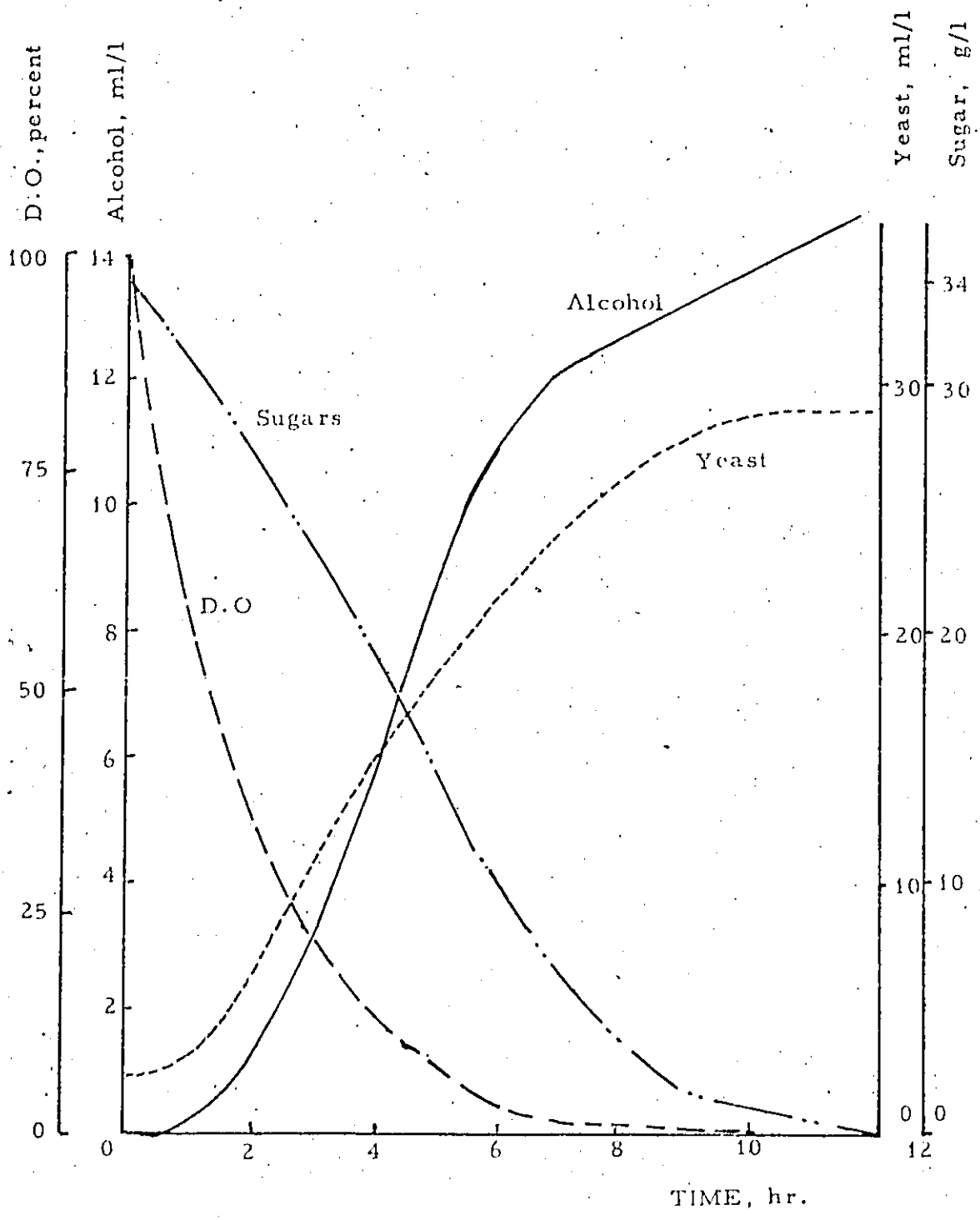

Fig (10-a). Model run curves

Using these factors, F_1, F_2 and F_3, one may calculate the percent of sugar used to produce either alcohol, yeast or energy. If C_a is the concentration of alcohol at any time in g. / ℓ , C_s is the concentration of sugars in g / ℓ , C_{s_o} is the starting concentration of sugars, C_y is the concentration of yeast cells in g / ℓ and C_{y_o} is the concentration of yeast cells at the start of fermentation, then

$$\% \text{ sugar used to make alcohol} = \frac{C_a \times F_1}{C_{s_o}} \times 100 \quad (10)$$

$$\% \text{ sugar used to make yeast} = \frac{(C_y - C_{y_o}) F_2}{C_{s_o}} \times 100 \quad (11)$$

$$\% \text{ sugar burned directly to } CO_2 = \frac{C_y \times F_3}{C_{s_o}} \times 100 \times t \quad (12)$$

If the amount of sugar burned for energy is considered very small and neglected to simplify the calculation, we end up with two factors; F_1 the factor of sugar consumed to make alcohol and, F_2 the factor for yeast.

Consider the data in the model run when 79% of the sugar present was consumed, corresponding to the sample taken after 6 hours (sample No 5, i.e: $\frac{C_{s_o} - C}{C_{s_o}} \times 100 = 79\ \%$) . At that time the alcohol produced was 12.5 mℓ / ℓ. Therefore the toal amount of sugar used to make alcohol after 6 hrs was:

$$\frac{\text{Alcohol concentration x density x } F_1}{\text{Initial sugar concentration}} \times 100 =$$

$$= \frac{12.5 \times 0.79 \times 1.956}{33.3} \times 100 = 58.0\ \%$$

Therefore, the amount of sugar used to make yeast after 6 hrs of fermentation = 79 - 58.0 = 21 % of original sugar added.

The amount of yeast present after 6 hours was obtained using table 3 in order to get the value of C_y

TABLE 3

Calculation of C_y

Time of sample (hrs)	$\frac{C_{s_o} - C_s}{C_{s_o}} \times 100$	Vol of yeast $m\ell / \ell$	C_y (=Vol of yeast x 0.53)* g/ℓ
0	0	2.5	1.33
1.5	20.12	3.0	2.13
3	27.32	8.0	5.39
4.5	32.13	10.0	9.60
6	78.97	15.0	11.73
7.5	93.39	20.0	13.33
9	93.99	22.0	13.86
10.5	97.59	26.0	15.46
12	100	30.0	16.00

* Note for conversion factor for $m\ell/\ell$ to g/ℓ yeast

$2.5\ m\ell/\ell \equiv \frac{4}{3}$ g/ℓ yeast

$x\ m\ell/\ell \equiv \frac{4}{3} \times \frac{1}{2.5} \times x$ g/ℓ = 0.53 x g/ℓ

where 4 g of yeast were added at the start of the run to the run to the 3 litre fermentation vessel.

at this point (after 6 hrs) C_y = 11.73

Therefore, to calculate F_2 :

if 21 % of the sugar gives $\frac{C_y - C_{y_o}}{C_{s_o}} \times F_2$

$$21 = \frac{11.73 - 1.33}{33.3} \times F_2 \times 100$$

$$\therefore F_2 = 0.67 \text{ g sugar/g yeast.}$$

Using the factors, F_1 and F_2, to calculate the per cent sugar used to make yeast and alcohol at the end of fermentation

$\therefore$ The % sugar used to make yeast

$$= \frac{16 - 1.33}{33.3} \times 0.67 \times 100 = 29.5 \%$$

and The % sugar used to make alcohol

$$= 100 - 29.5 = 70.5 \%$$

(corresponding to 15.0 mℓ/ℓ alcohol while experimental results show a production of 14.9 mℓ/ℓ by adding the amount of alcohol present in the reactor to that trapped in cold water trapes). The following curves were calculated as in Table 4 and Table 5 .

TABLE 4

Alcohol production

% Sugar consumed (Total) $\frac{C_{s_o} - C_s}{C_{s_o}} \times 100$	% of total sugars used to make alcohol. $\frac{C_a \times 1.956 \times .79}{C_{s_o}} \times 100$
20.10	3.75
27.3	13.0
79.0	58.0
100.0	70.5

TABLE 5

Yeast Production

% Sugar consumed (Total) $\frac{C_{s_o} - C_s}{C_{s_o}} \times 100$	% Sugar used to make yeast $\frac{C_y - C_{y_o}}{C_{s_o}} \times 0.67 \times 100$
27.3	8.0
32.1	16.6
79.0	20.9
93.39	24.1
100.0	29.5

By plotting these values, Fig.11 is produced which represents the amount of sugar used for synthesis of each product.

Note that the sum of the sugar plus yeast contributions should equal the % sugar consumed, and only do so at 79 and 100% sugar consumed, the points used to obtain the parameters F_1 and F_2. In Fig.11 the alcohol curve intersects the horizontal axis and at this abscissa value the yeast curve should break in slope, and pass through the origin. Unfortunately data in this region is insufficiently accurate to demonstrate this.

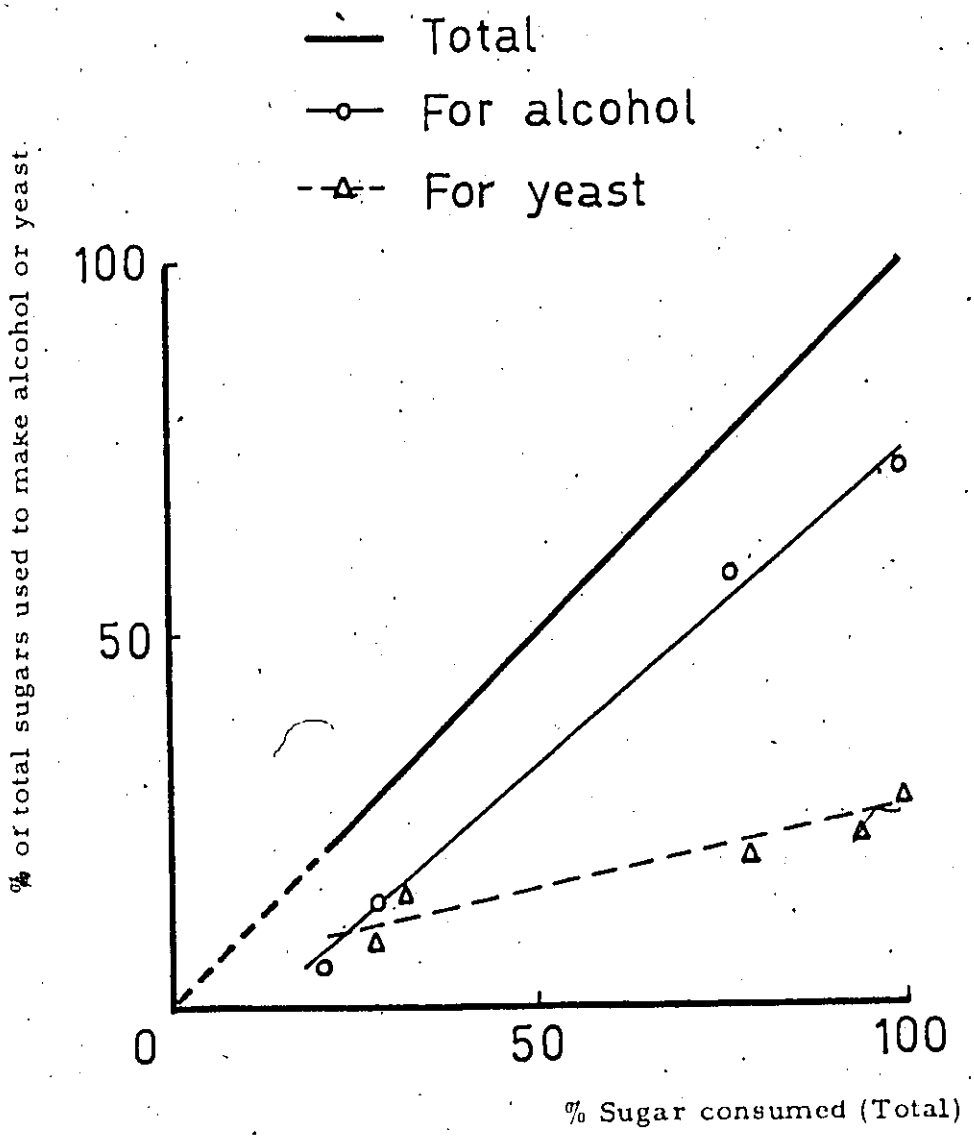

Fig. 11 Fermentation Product Synthesis

IV.B.3. Kinetic Analysis

As mentioned in section II.C.4. fermentation kinetics have been studied by many workers. Among the well established published works, the study of Kono and Asai (46) was selected to be tested by the data obtained.

In this section the numerical fitting of our data to the published model is presented. The following are the follow-up of the same calculation procedure given by Kono and Asai.

To convert the experimental data from $m\ell/\ell$ to g/ℓ the volumetric concentration was multiplied by 0.533 for the yeast and 0.79 for the alcohol.

Table 6 converts the results of the model run from $m\ell/\ell$ to g/ℓ.

TABLE 6

Conversion of Run No 14 results to g/ℓ

Time (hr.)	Yeast		Ethanol	
	($m\ell/\ell$)	(g/ℓ)	($m\ell/\ell$)	(g/ℓ)
0	2.5	1.333	0	0
1.5	4	2.133	0.8	0.64
3.0	10	5.333	2.8	0.24
4.5	18	9.600	6.6	5.28
6.0	22	11.733	12.5	10.0
7.5	25	13.333	12.1	9.68
9.0	26	13.866	10.6	8.48
10.5	29	15.466	10.4	8.32
12.0	30	16.00	9.0	7.2

The next step was to divide the yeast growth curve and alcohol production curve into four regions corresponding to the phases of induction (I) transient (II) exponential (III) and declining growth (IV) and to fit every region of this curve by the equations recommended by Kono and Asai.

The drawing in Fig. 12 is a schematic representation of the relationships between the cell concentration C_y and growth and production rates $\frac{dC_y}{dt}$ and $\frac{dC_a}{dt}$ in the mentioned four regions. The slopes of the lines $\overline{OP}$, $\overline{OQ}$ and $\overline{OR}$ represent the values of k_y, k_{a_1} and k_{a_2}.

From Figure 12, the following results were obtained :

$k_y = \overline{OP} = 0.425 \quad hr^{-1}$

$k_{a_1} = \overline{OQ} = 0.400 \quad hr^{-1}$

$k_{a_2} = \overline{OR} = 0 \quad hr^{-1}$

Also, $C_{y,c} = 7 \quad g/\ell$

$C_{a,c} = 4.2 \quad g/\ell$

$t_c = 3.6 \quad hr.$

$C_{y,c}$ is the critical yeast concentration and $C_{a,c}$ is the critical alcohol concentration. These are calculated using the equations derived by Kono and Asai and these values used to determine the t_c, the critical time, which in turn will locate the boundary line between zone III and zone IV and, thus, point P and Q are determined.

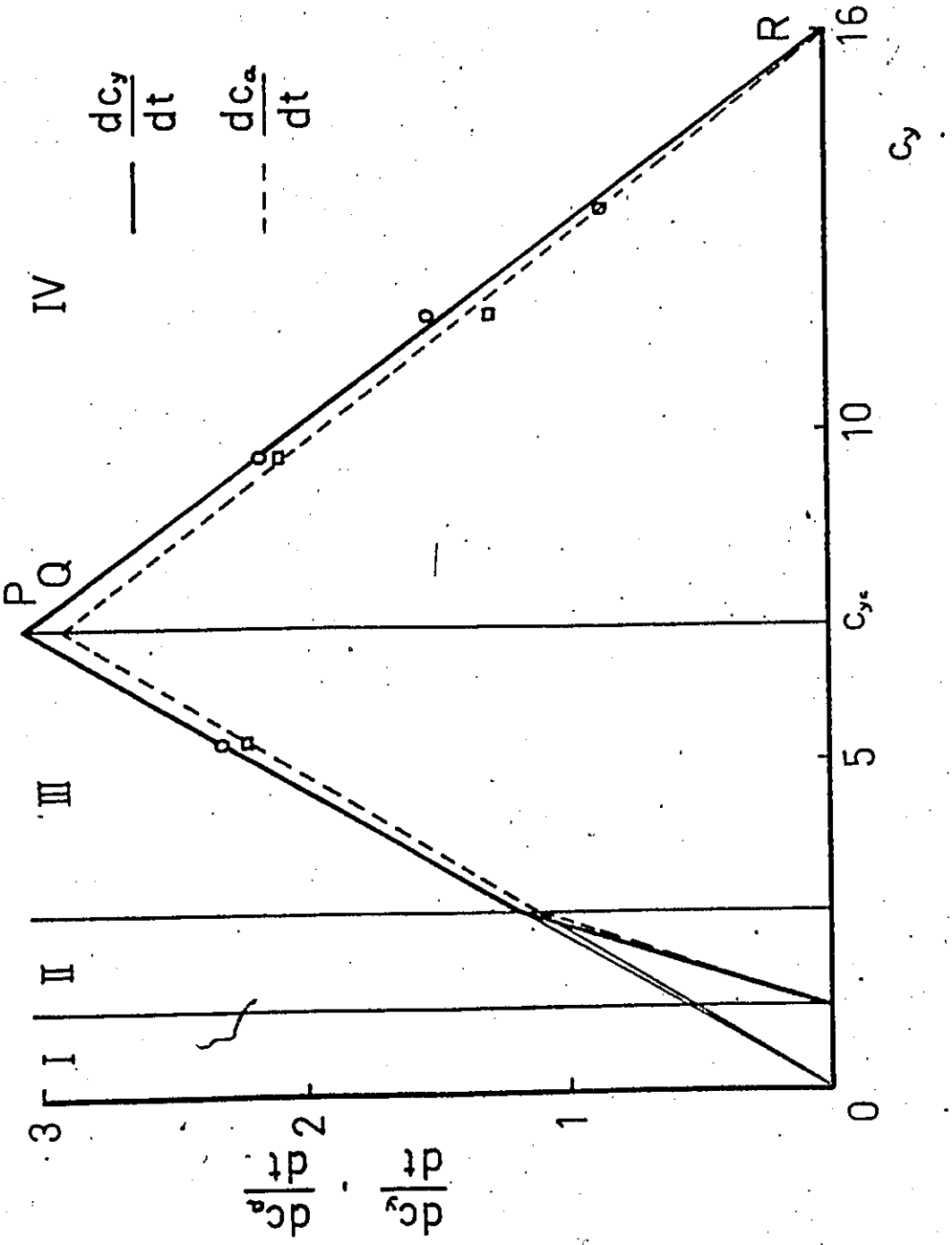

Fig. 12 Kono and Asai Model

Such type of fermentation processes may be classified, according to Kono and Asai classification as indicated in table 1 section II.C.4., as a fermentation process where the product formation is associated with growth. The conclusion is true for most of the runs since most of them were stopped before any decline in the yeast population. In the other few runs where a slight decline in the yeast volume was noticed there was alcohol formation. It is fair to mention that the yeast cell volume is not an accurate measure to the yeast cell population especially at the end of the run where the source of food is limited and there are chances that the cell dimensions are altered or reduced and consequently the cell volume. Also, facilities were not available to judge whether there was budding (yeast division) or not.

Consequently, the data obtained fitting to Kono and Asai model was satisfactory.

IV:B.4. The effect of Temperature

A temperature range of 28° C - 40°C was studied and results were collected for the effect of temperature on seven items as follows :

1. The dissolved oxygen in the fermentation vessel
2. The yeast growth
3. The alcohol production
4. The Total sugar consumption
5. Mannose consumption
6. Dextrose consumption
7. Galactose consumption

Fig. 13 shows the concentration of dissolved oxygen (D.O.) analyzer. As mentioned before, the D.O. content may be considered related to the population of the yeast cells. A sharp decrease in the D.O. content indicates a rapid growth of yeast which consumes this D.O. The curves in Fig. 13 shows that 36°C is the temperature where the population build-up is most rapid .

On the other hand temperature may affect the value of the mass-transfer coefficient. Aiba (51) gave the equation

$$\frac{K_{LA_1}(T_1)}{K_{LA_2}(T_2)} = \frac{\bar{T}_1 \mu_2}{\bar{T}_2 \mu_1} \qquad (13)$$

where

T is the temperature in °C

$\bar{T}$ is the temperature absolute °K

μ is the liquid viscosity .

So, one may conclude that the higher the temperature the better the aeration. This conclusion is agreeable with the curves given when considering the yeast growth curves, plotted in Fig. 14, as well. This may be due to increase in the mass-transfer coefficient of oxygen which will result in an increase in the specific rate at which cells respire through their outer membrane and thus the oxygen uptake rate increases. From the data obtained, one may conclude that oxygen utilization is both growth and cell-concentration related and, thus, depends largely on the factors that control those two parameters.

Temperature was found to affect the yeast growth rate or in other words the generation time is strongly dependent upon

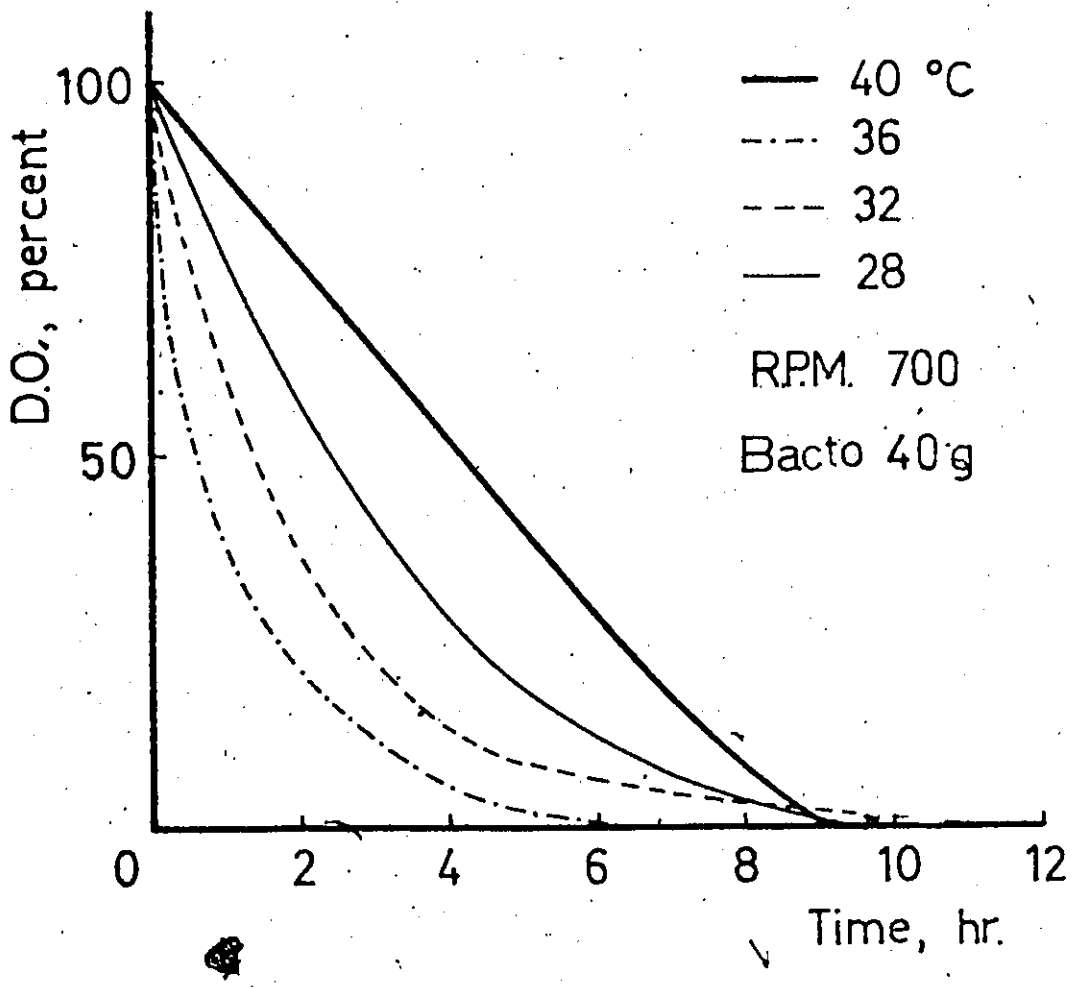

Fig. 13 The effect of temperature on the dessolved oxygen in the fermentation vessel

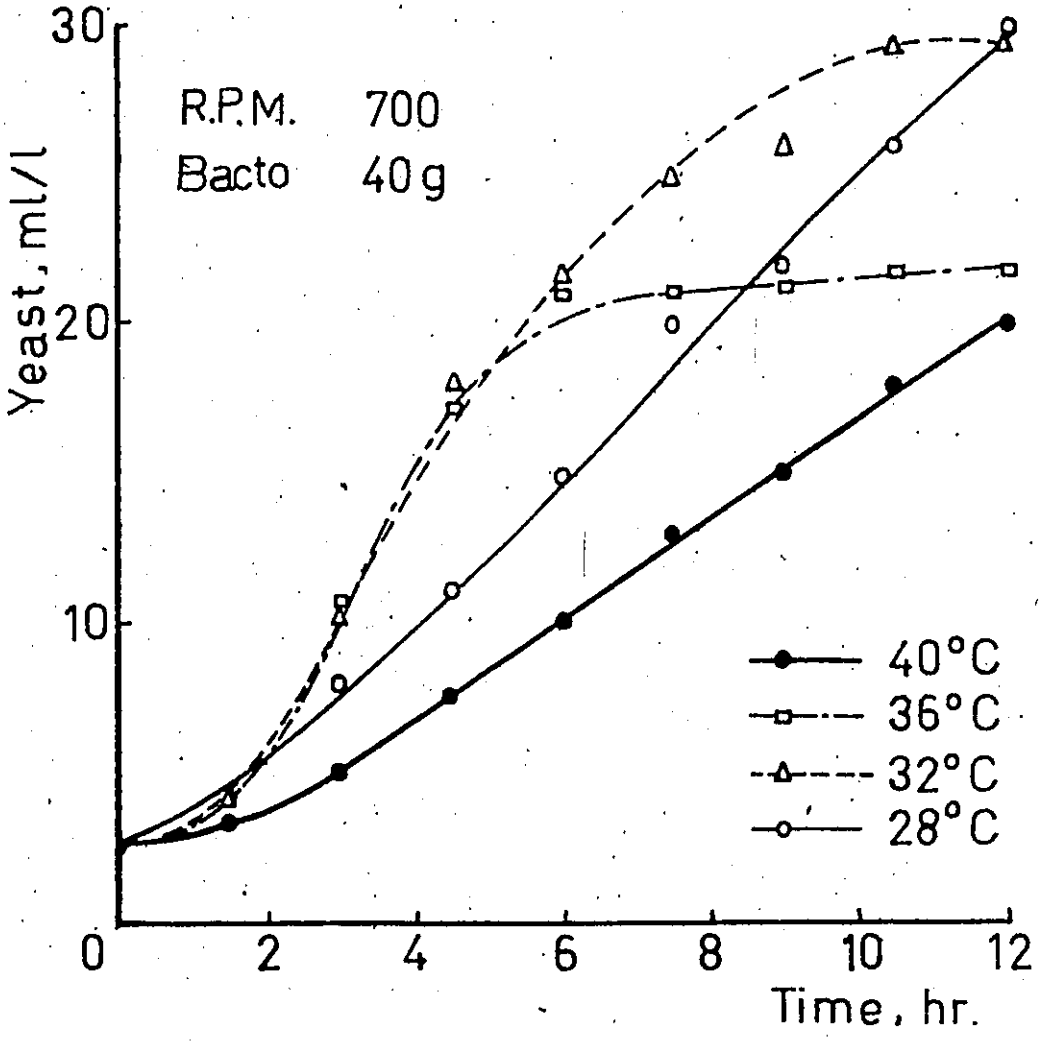

Fig. 14 The effect of temperature on the yeast growth

temperature. Optimal growth requires a certain specific range that is from 32°C to 36°C. At a higher temperature a decrease in both the rate and the ultimate cell volume was observed. At lower temperatures only the rate of growth was affected.

Alcohol, as the product of fermentation, is produced by the yeast population under aerobic and anaerobic conditions. It is expected, therefore, that the amount of alcohol produced will depend mainly on conditions favouring the yeast growth since at higher densities of yeast population we have both a large number of cells to produce alcohol and favourable anaerobic conditions due to the decrease of the amount of oxygen per cell. Both 32°C and 36°C were ideal as illustrated in Fig. 15.

Fig. 16 shows that total sugars are consumed at the highest rate when the temperature is around 36°C. It could be that at this temperature there is a production of a large number of enzymes by the yeast cells which result in a rapid consumption of the sugar substrate (51). 36°C is obviously the optimum operating condition for fast consumption of sugars.

The results obtained for Mannose consumption resemble closely those of the total sugar since Mannose consisted of 62% of the sugar diet. 36 % is the favorable one for the Mannose consumption as illustrated in Fig. 17.

As indicated in Fig. 18, there is really a considerable difference between the rate of Dextrose consumption at 36°C and that at the other temperatures and 36°C is definitely favoured. (The rate is higher during the first 4 hours by about 23% of that rate of 32°C which is considered the second best temperature).

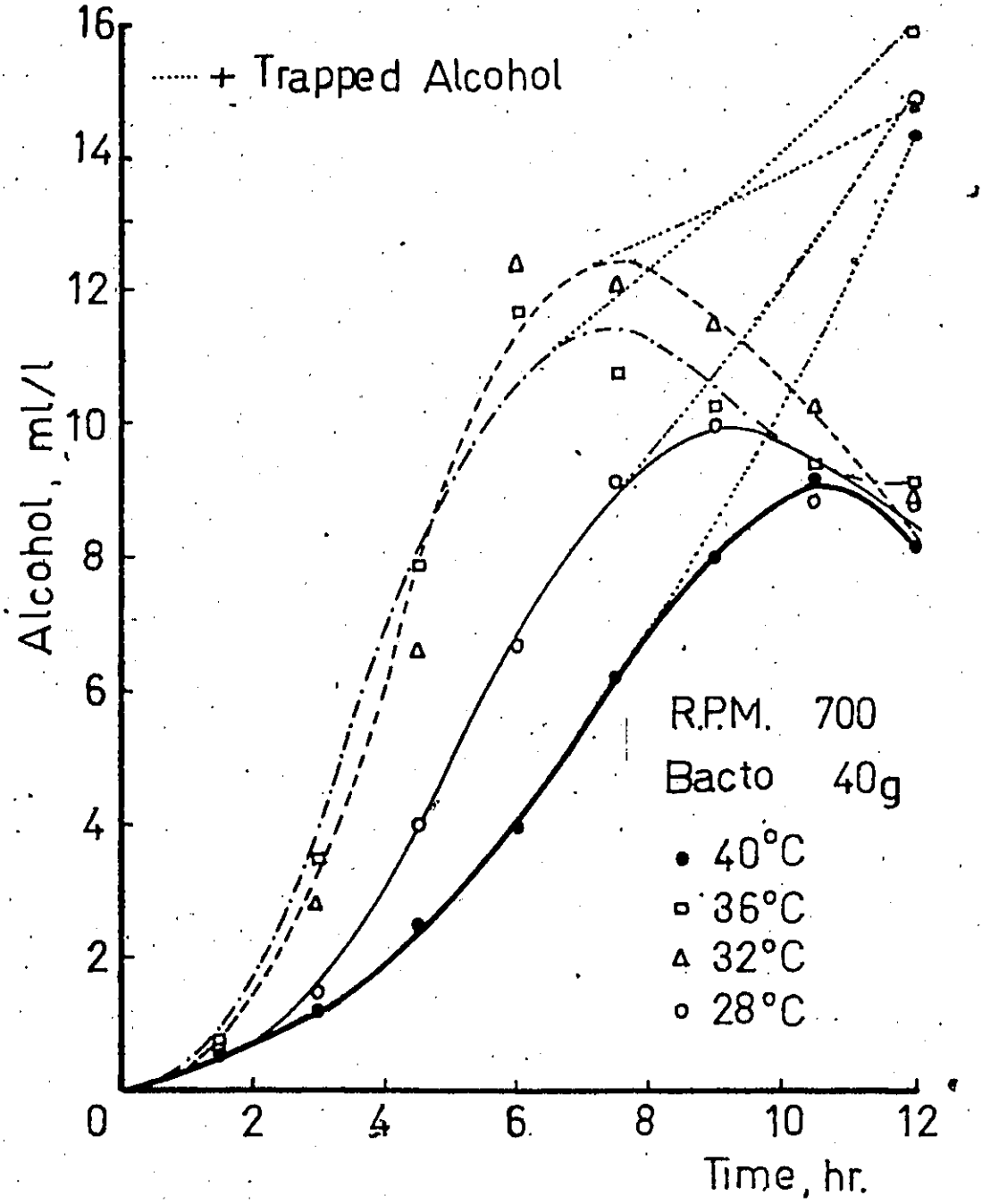

Fig. 15 The effect of temperature on alcohol production

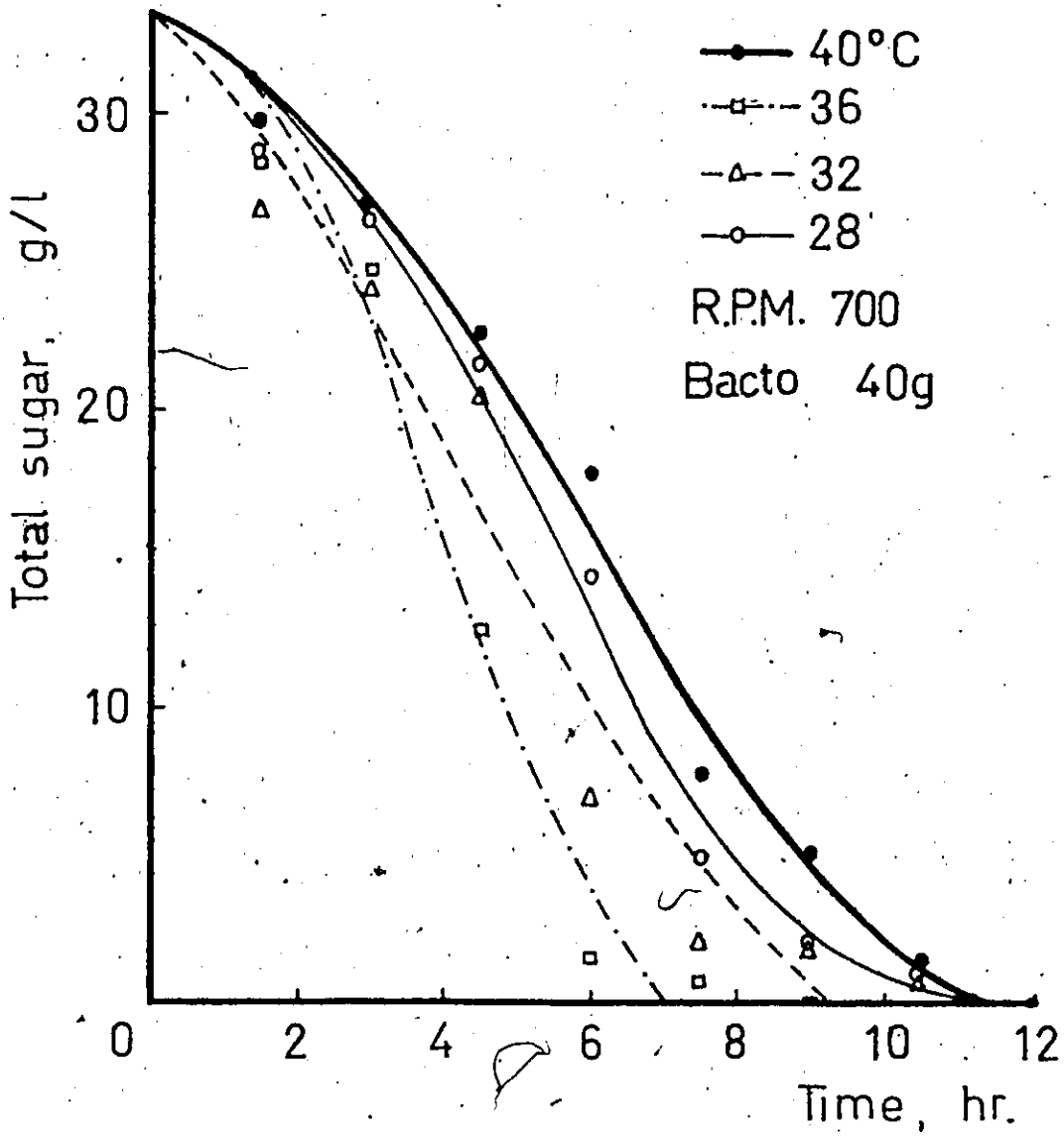

Fig. 16 The effect of temperature on the consumption of sugars (total)

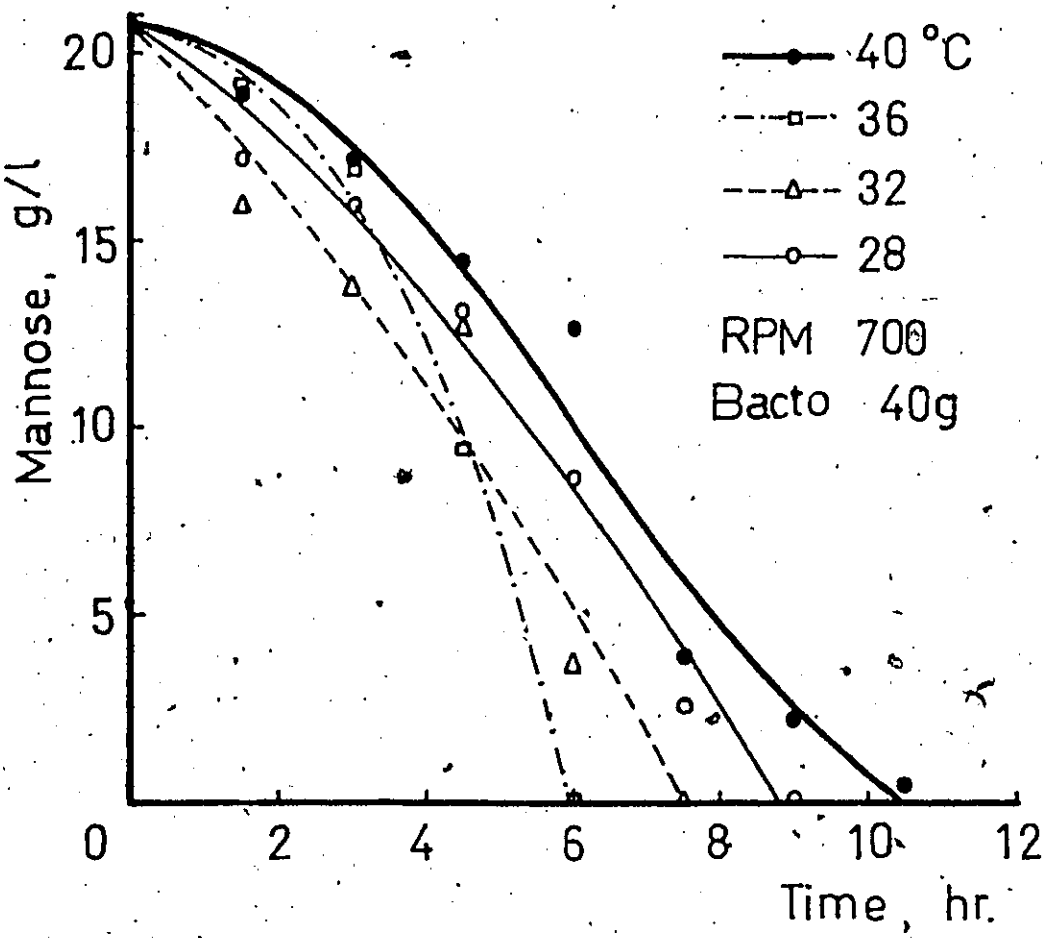

Fig. 17 The effect of temperature on the consumption of Mannose

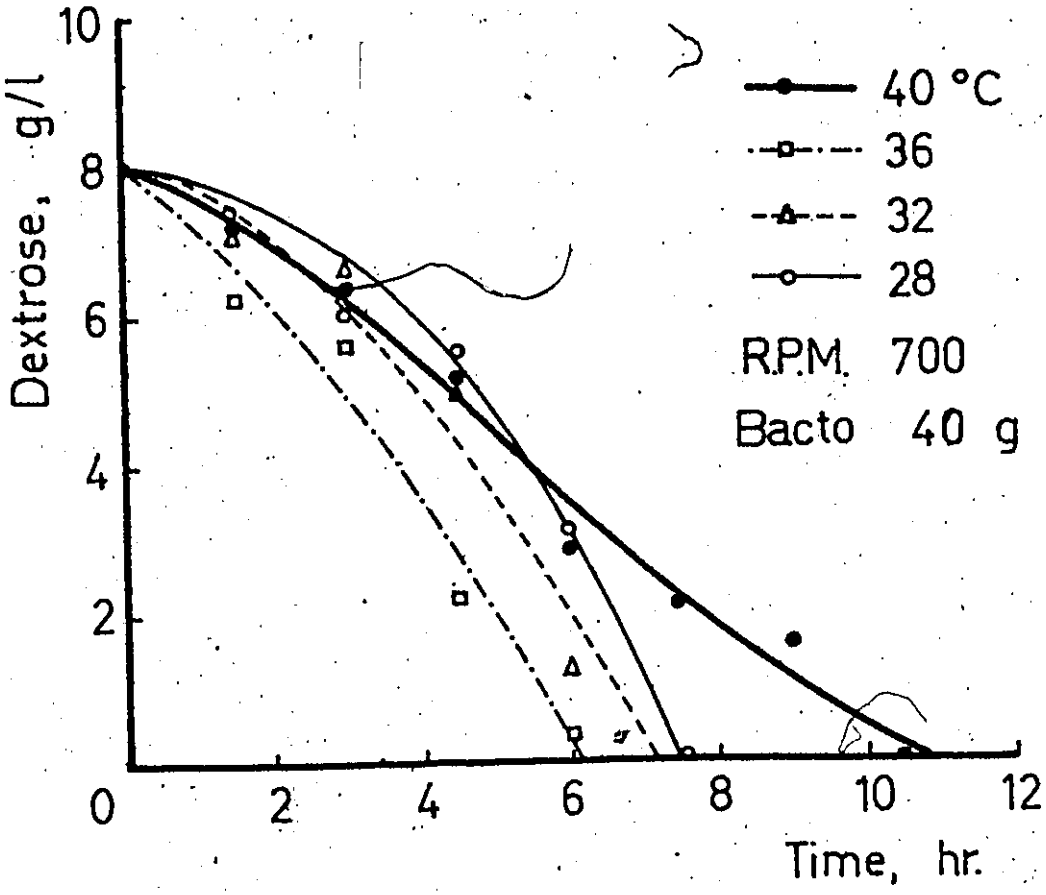

Fig. 18 The effect of temperature on the consumption of Dextrose

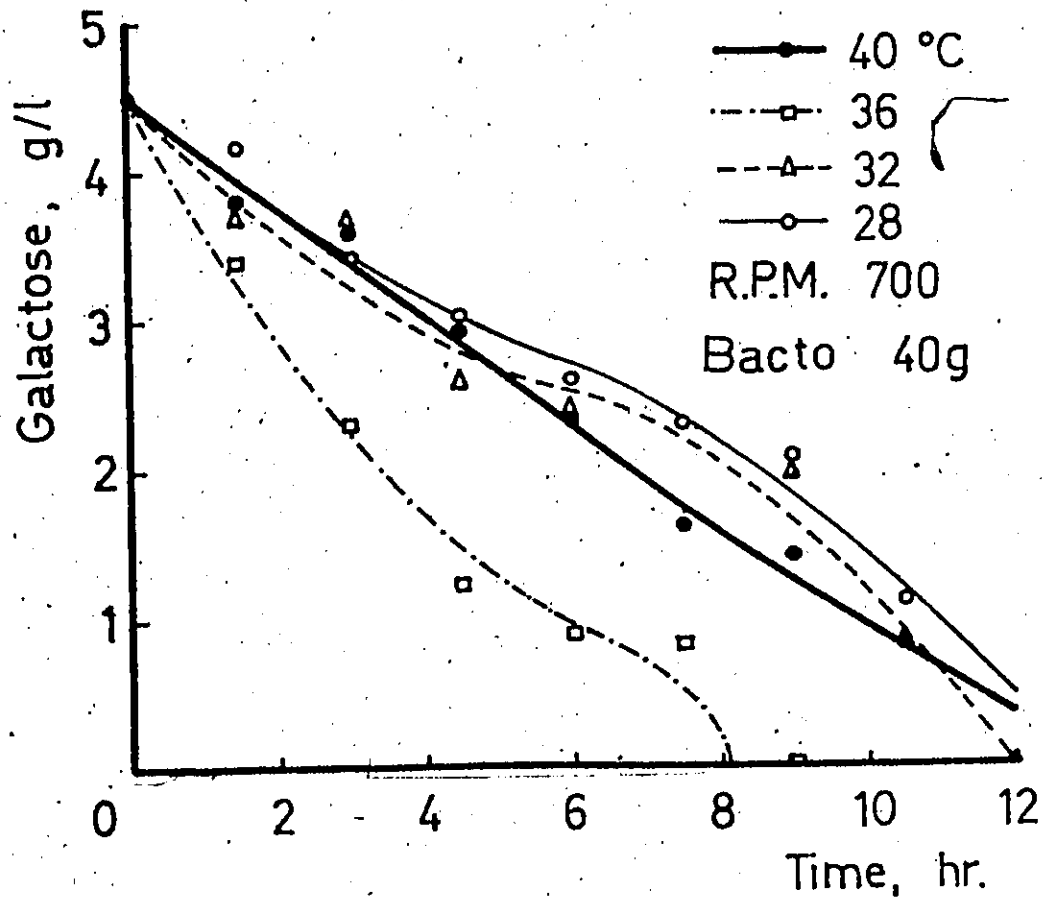

Fig. 19 The effect of temperature on the consumption of Galactose

Galactose may be considered the most difficult sugar, in this group, to be digested by the yeast. However, only at 36°C and 32°C we were able to reach the zero level within the fermentation period which lasted for 12 hours. Galactose was totaly consumed after 8 hours at 36°C and after 12 hours at 32°C. This means that we have to use a temperature close to these values for complete removal of this sugar from the sugar portion of the waste sulfite liquor.

IV.B.5. The effect of stirring

The stirring effect on the fermentation process under study is indicated in Figures 20 - 26. The stirring was performed at the 700, 500, 300 and 100 r.p.m levels. The effect of mixing on, the D.O., yeast growth, alcohol production, total sugar consumption, Mannose consumption, Dextrose consumption and Galactose consumption is reported.

An increase in mixing decreases the air-bubble size and increases the mass-transfer coefficient. This improves the availability of oxygen for the yeast cells and hence, increases the rate of growth. This conclusion is in agreement with the results in Figures 20 and 21. Alcohol productions as mentioned in the previous section, is affected by the yeast population. The mixing power has a little effect on the consumption of total sugar as well as individual component sugars. It is obvious from Figures 23, 24, 25 and 26 that mixing has a negligible effect on sugar consumption.

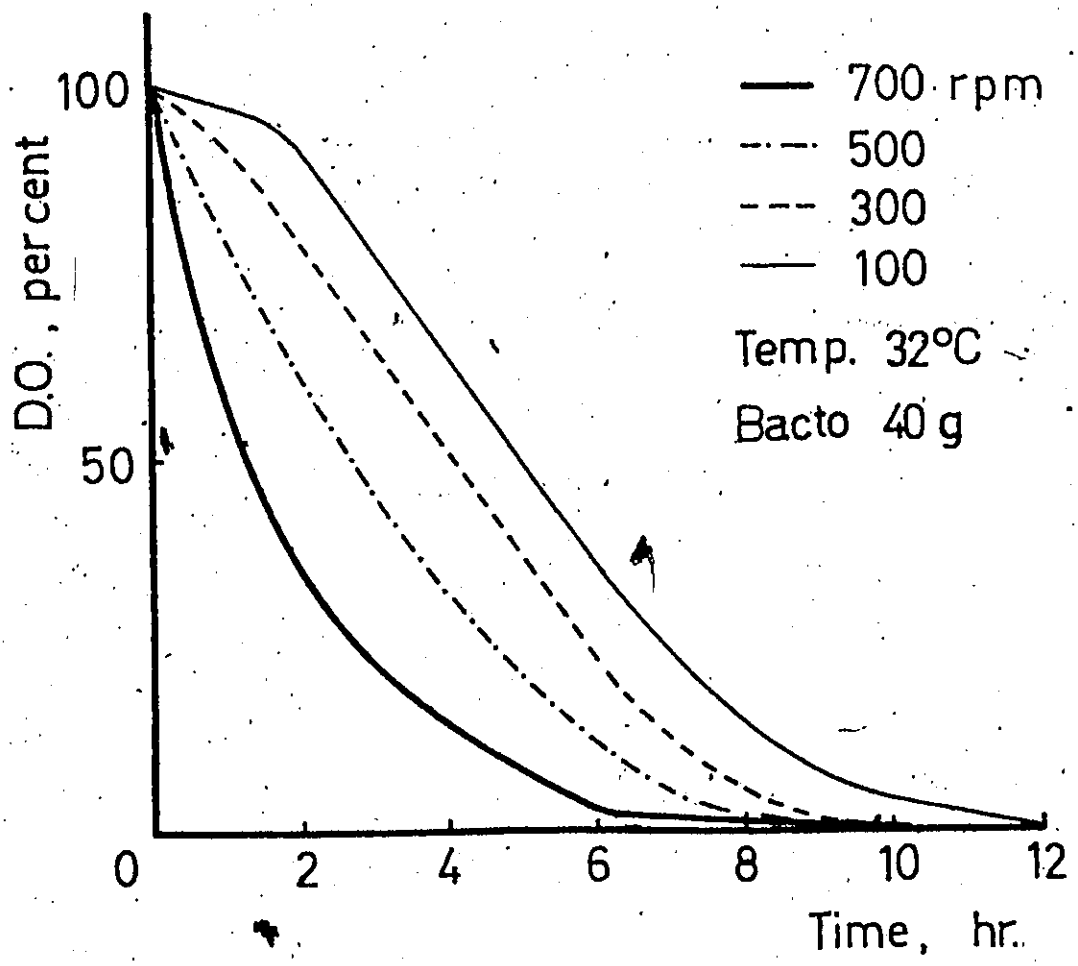

Fig. 20 Effect of mixing on D.O. percent

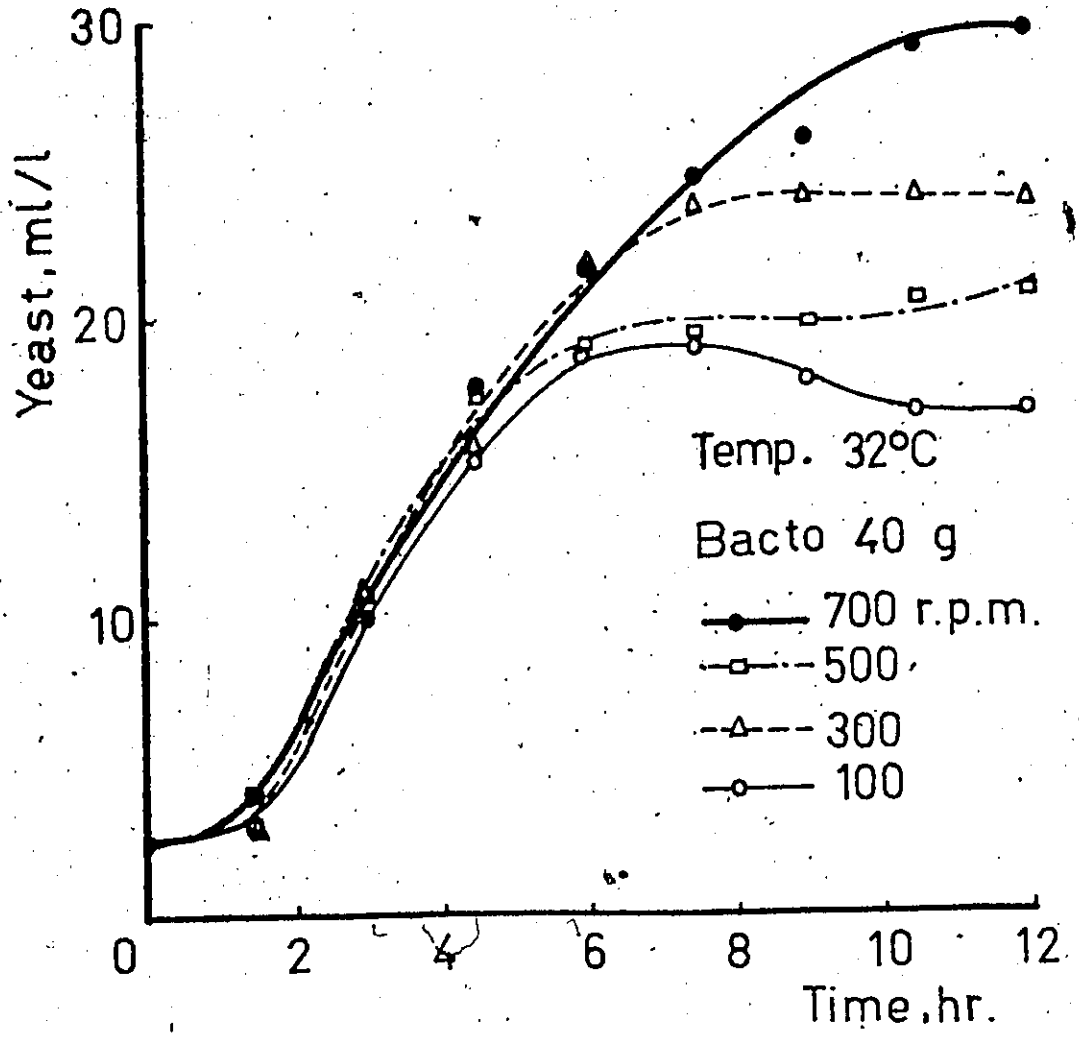

Fig. 21 Effect of mixing on yeast growth

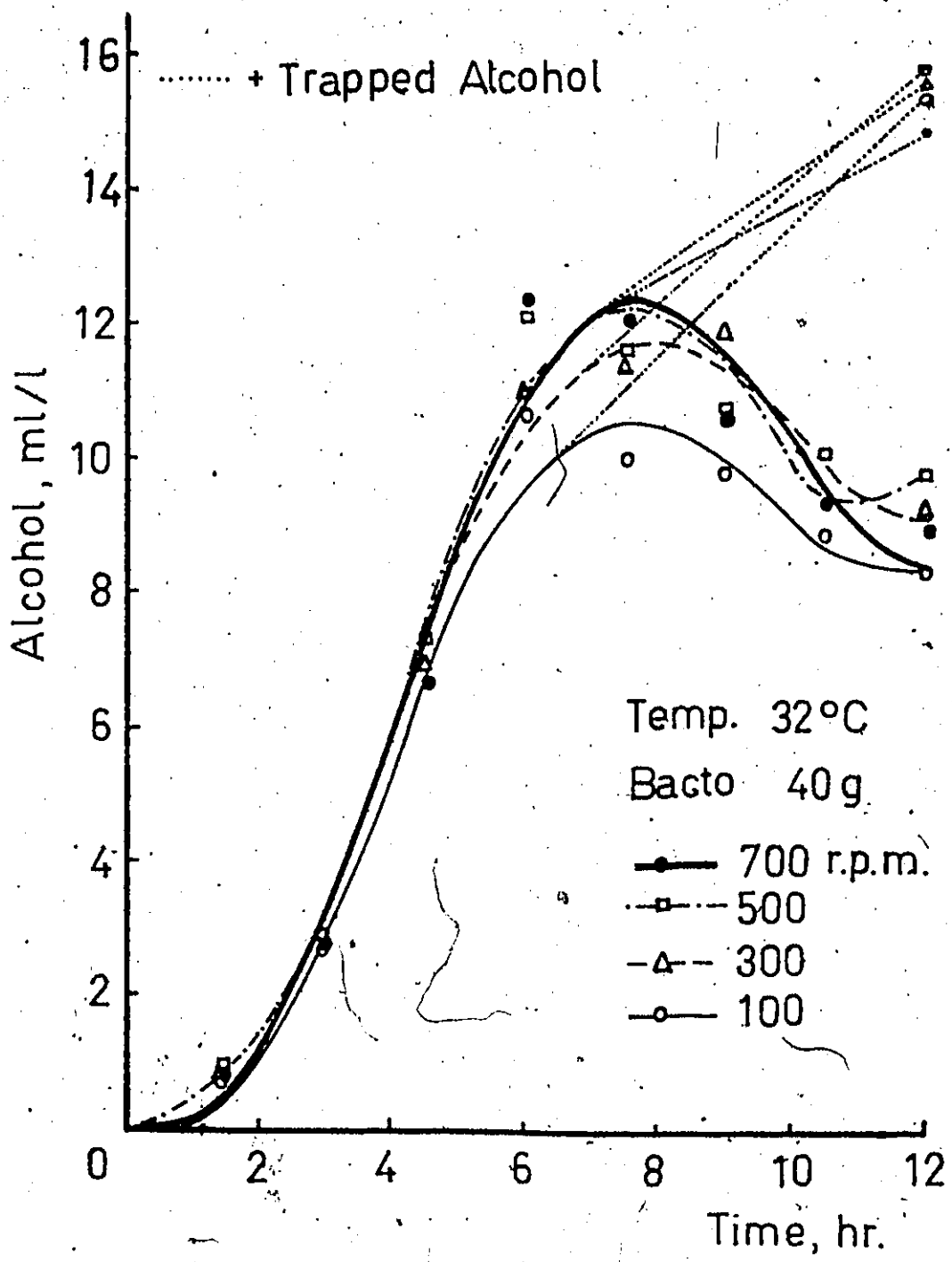

Fig. 22 Effect of mixing on alcohol production

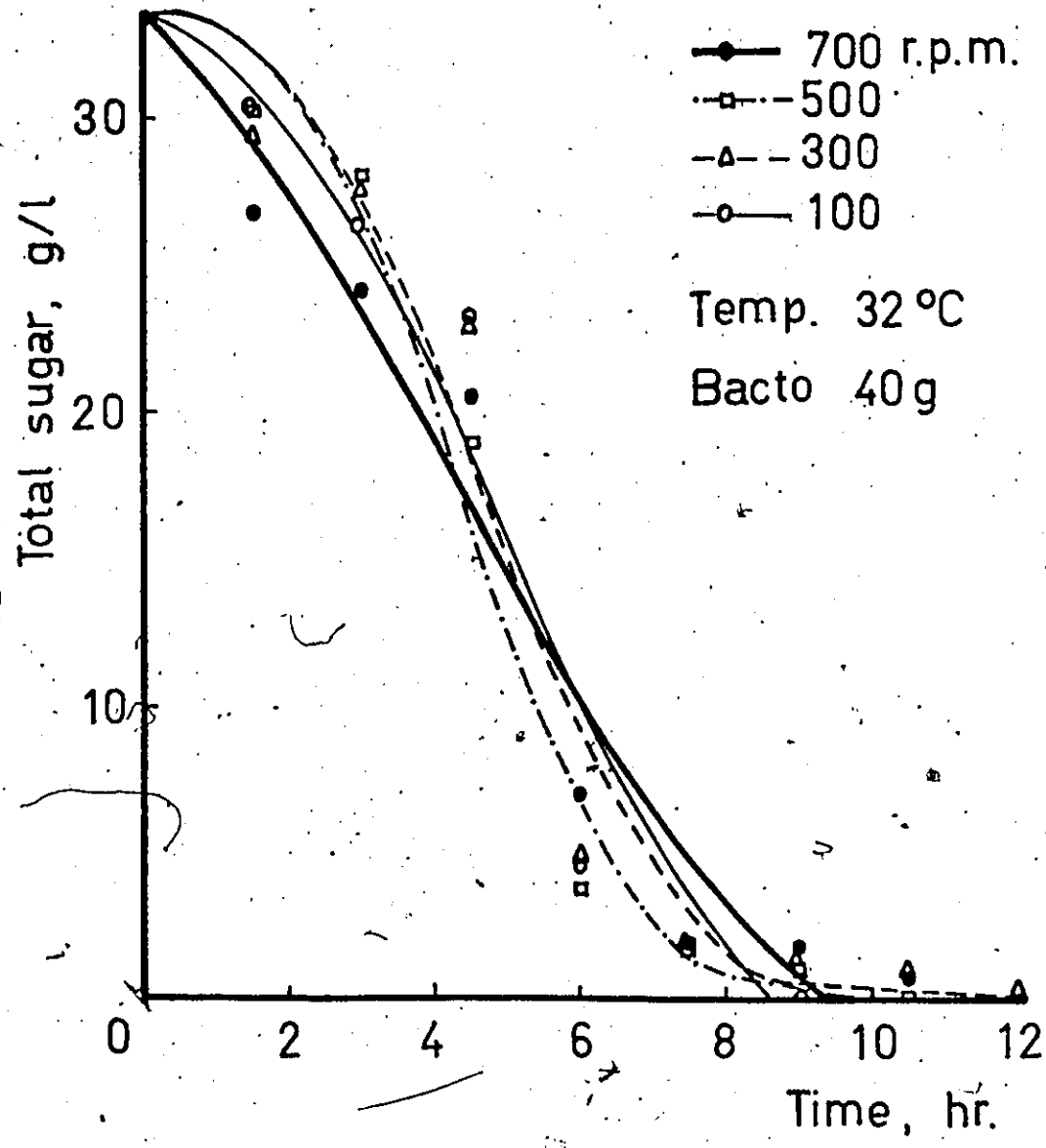

Fig. 23 Effect of mixing on total sugar consumption

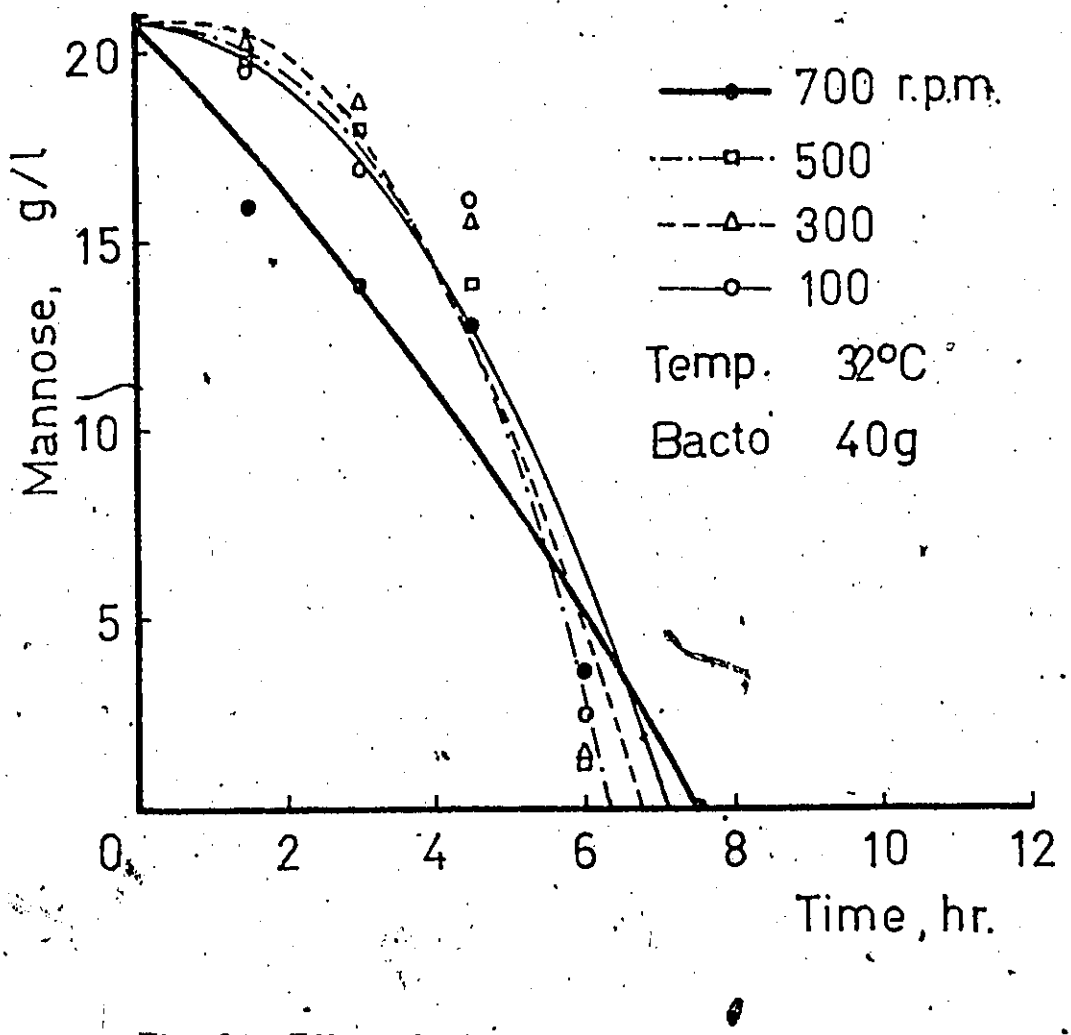

Fig. 24 Effect of mixing on Mannose consumption

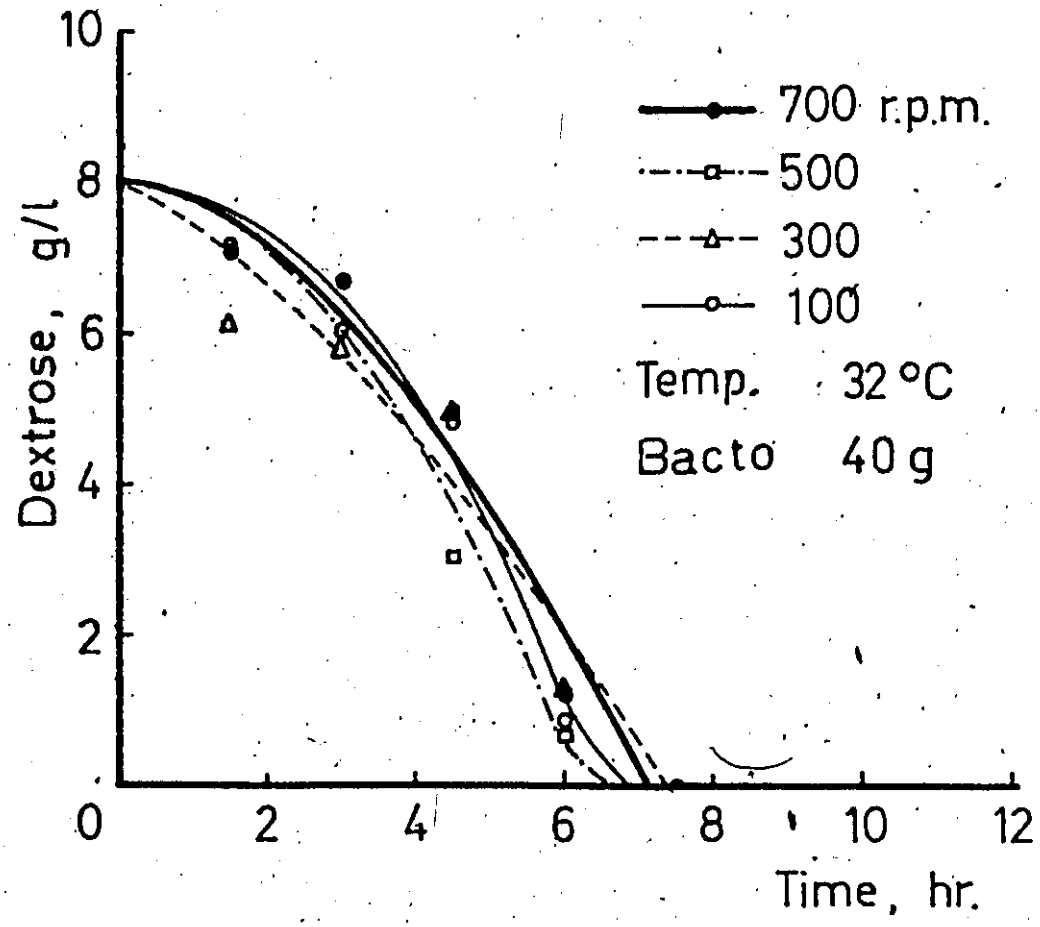

Fig. 25 Effect of mixing on Dextrose consumption

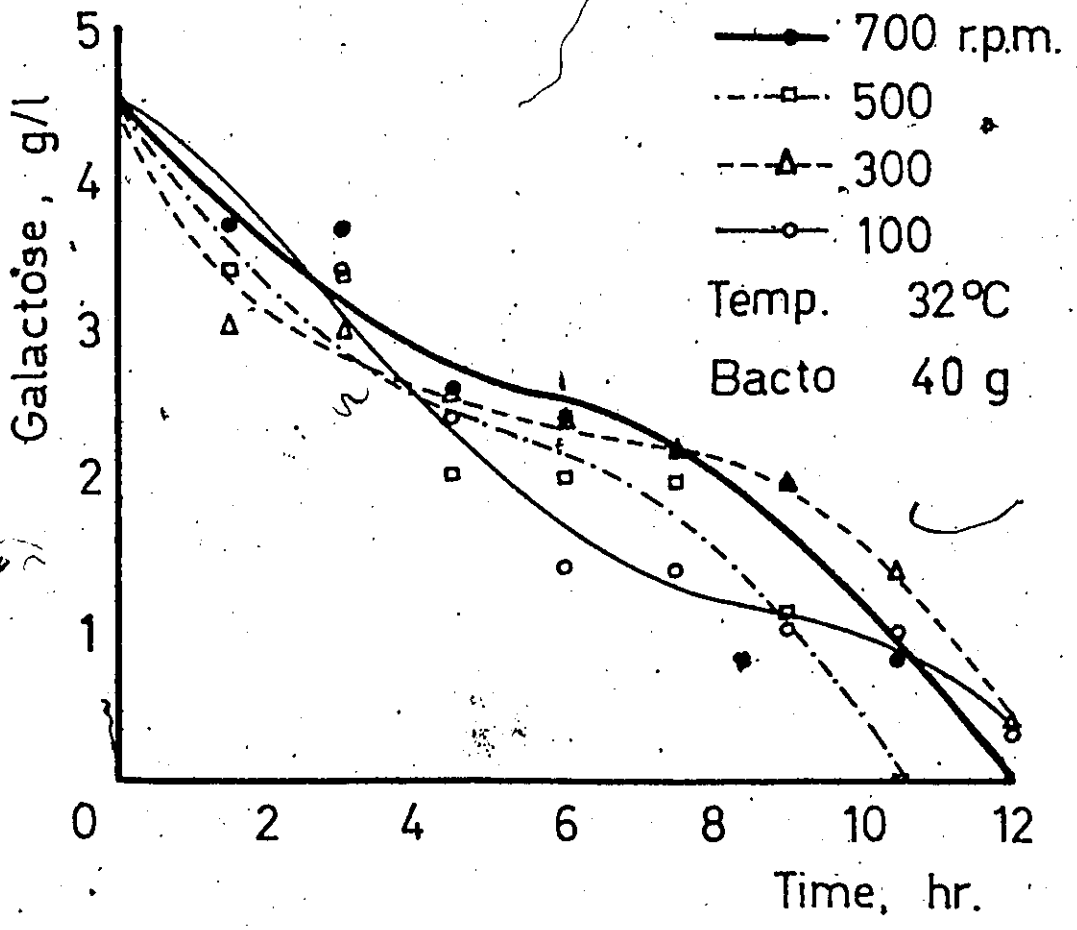

Fig. 26 Effect of mixing on Galactose consumption

IV. B.6. The effect of Bacto concentration

The yeast nitrogen base (Bacto) is a vital nutrient for the growth of yeast. It is clear from Figures 27 and 28 that the yeast growth is greatly affected by the Bacto concentration. Acceptable results were obtained when using 40 and 20 g per batch of Bacto. The Bacto composition is given in section VIII.E.

Figure 27 shows that when using the highest level of Bacto concentration (40 g per batch) the D.O. in the fermentation vessel was decreased to nearly zero after 10 hours due to a fast build-up of yeast population. On the other hand upon using a lower level of Bacto concentration such as 5 or 10 g per batch, the D.O. was never reduced to zero. This may have also contributed to a weak and small population of yeast cells. A 25 g of D.O. were present at the end of the run in the second case.

These results agree with the yeast growth results presented in Figure 28. A large difference is noticed according to the amount of Bacto added.

It is possible that the amount of Bacto added affected the biological pathway. This could affect both the product formation and the rate of consumption of the component sugars. Figure 29 shows that alcohol formation at the lowest level of Bacto added, 5 g, was equal to that at 20 g which leads to the conclusion that changing the amount of Bacto may change the pathway and lead to such results.

The total sugar consumption was slightly affected by changing the Bacto concentration from 40 to 10 g/3ℓ, but going down to 5 g resulted in a remarkably slower rate of total sugar consumption. This is also the case with Mannose and Dextrose consumption as indicated in Figures 31 and 32.

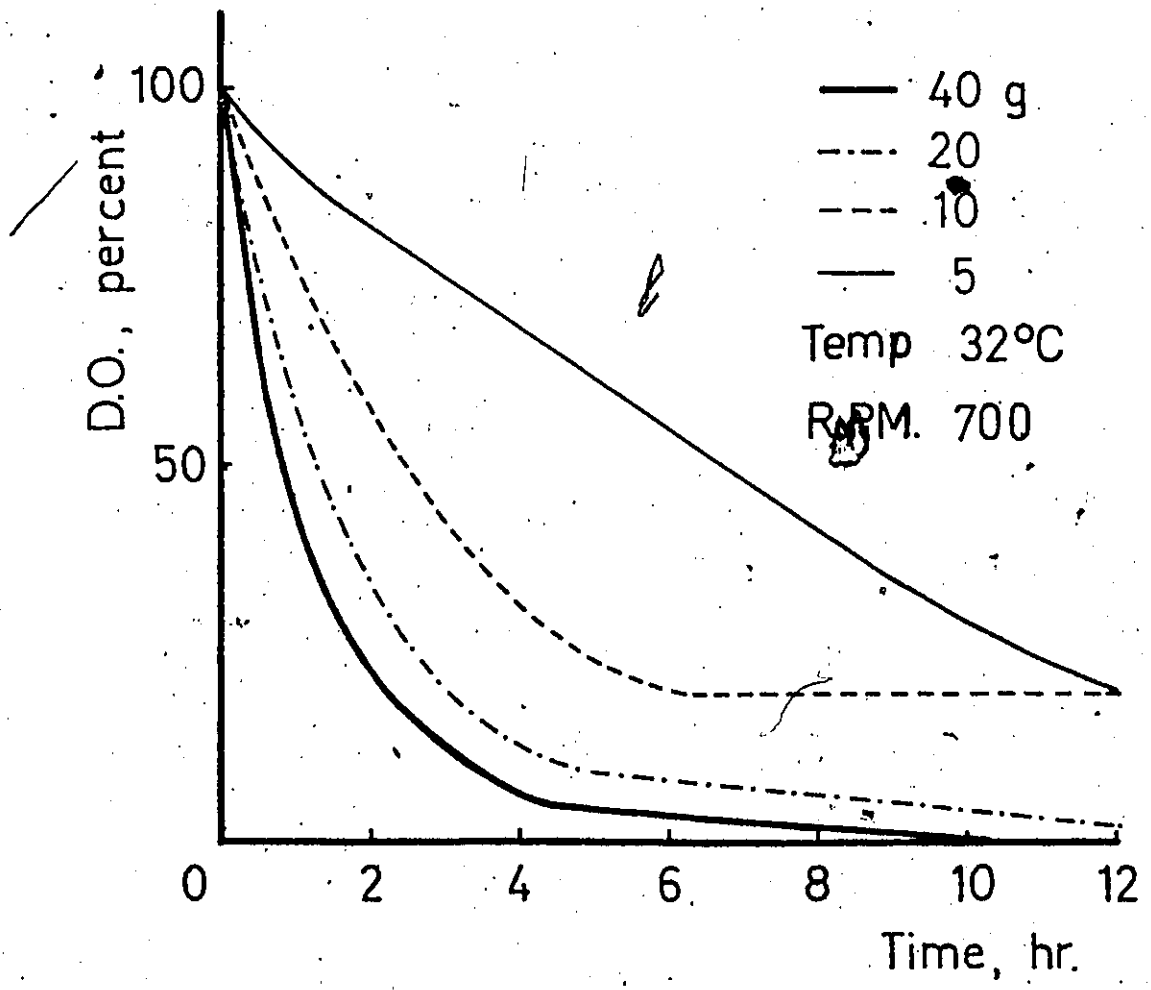

Fig. 27 Effect of Bacto conc. on D.O. %

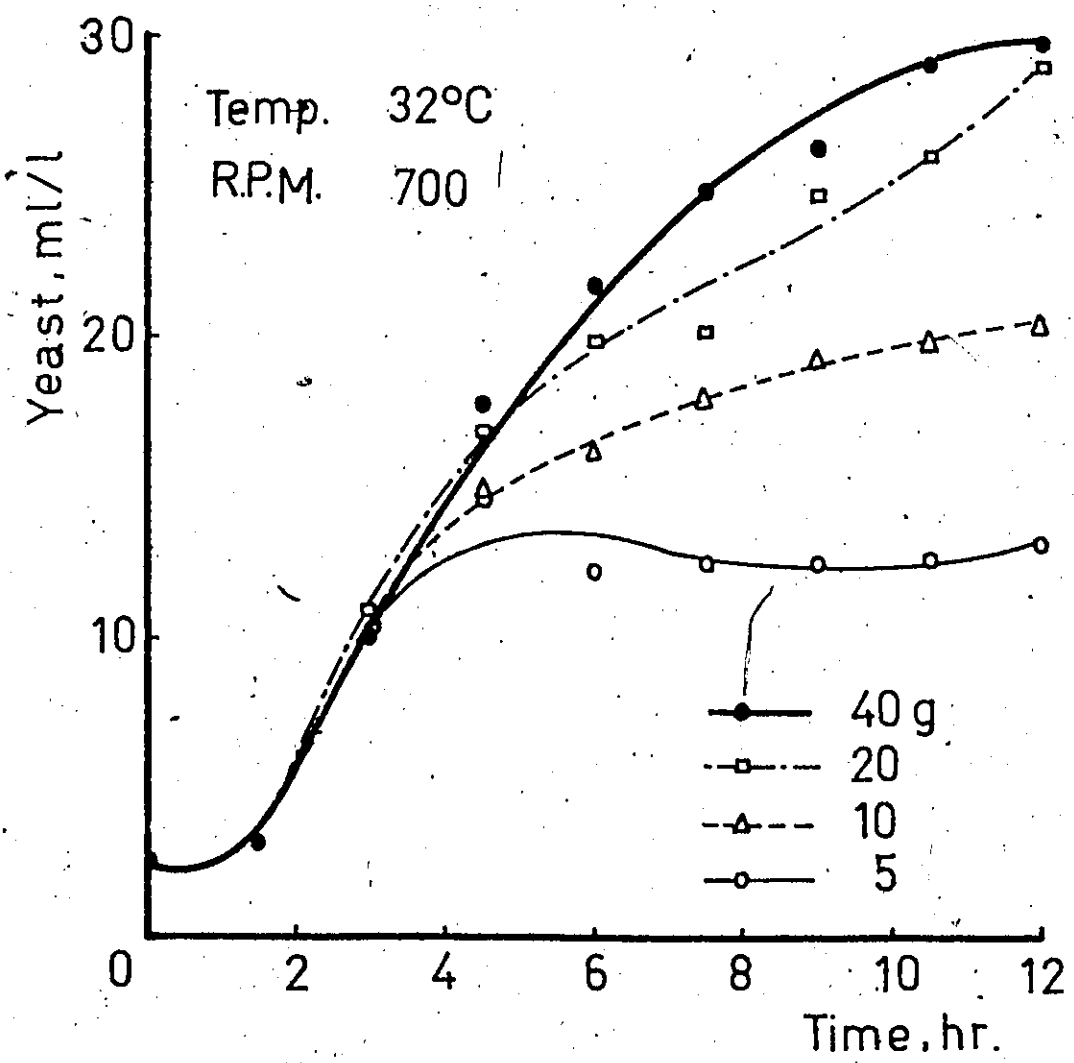

Fig. 28 Effect of Bacto conc. on yeast growth

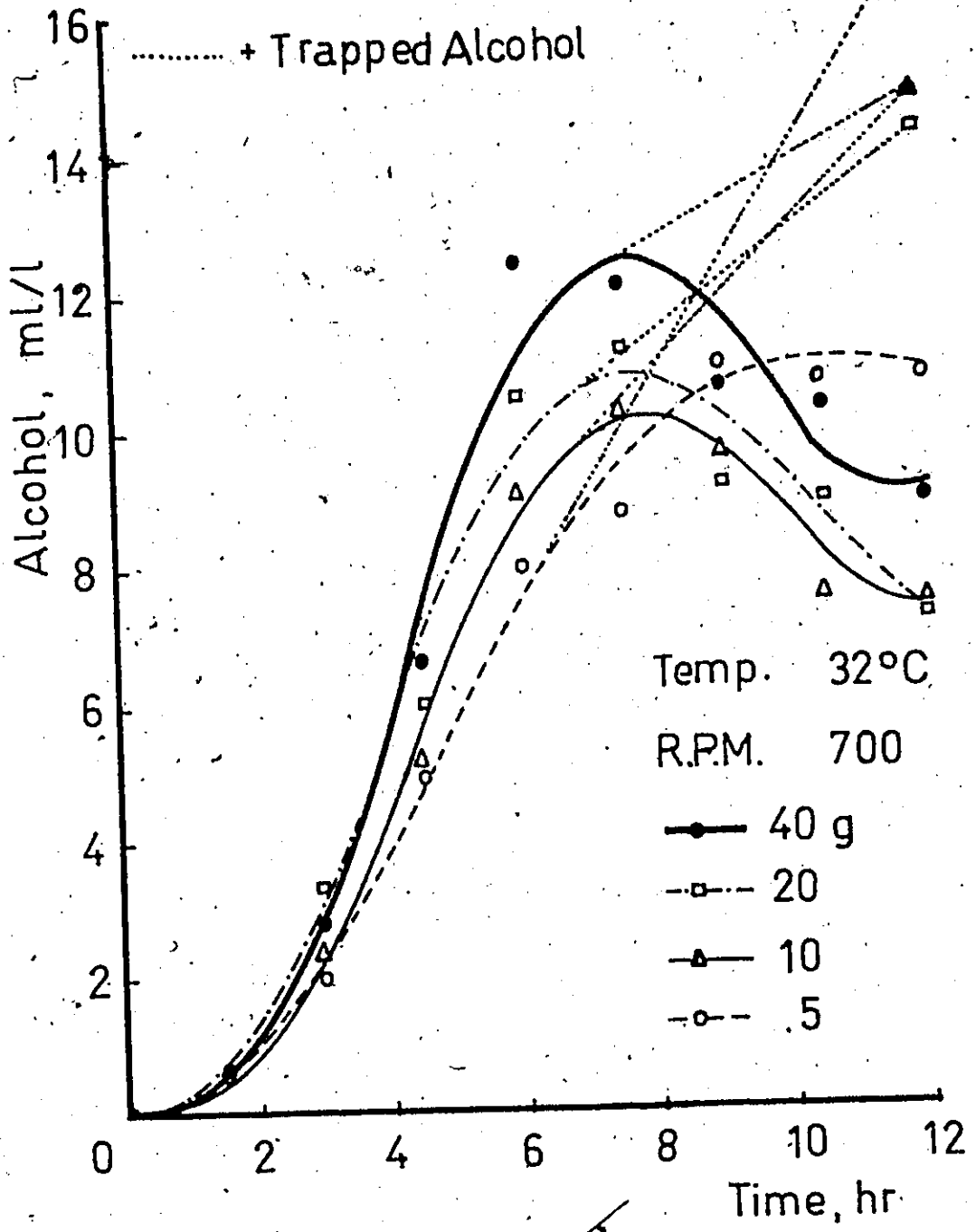

Fig. 29 Effect of Bacto conc. on alcohol production

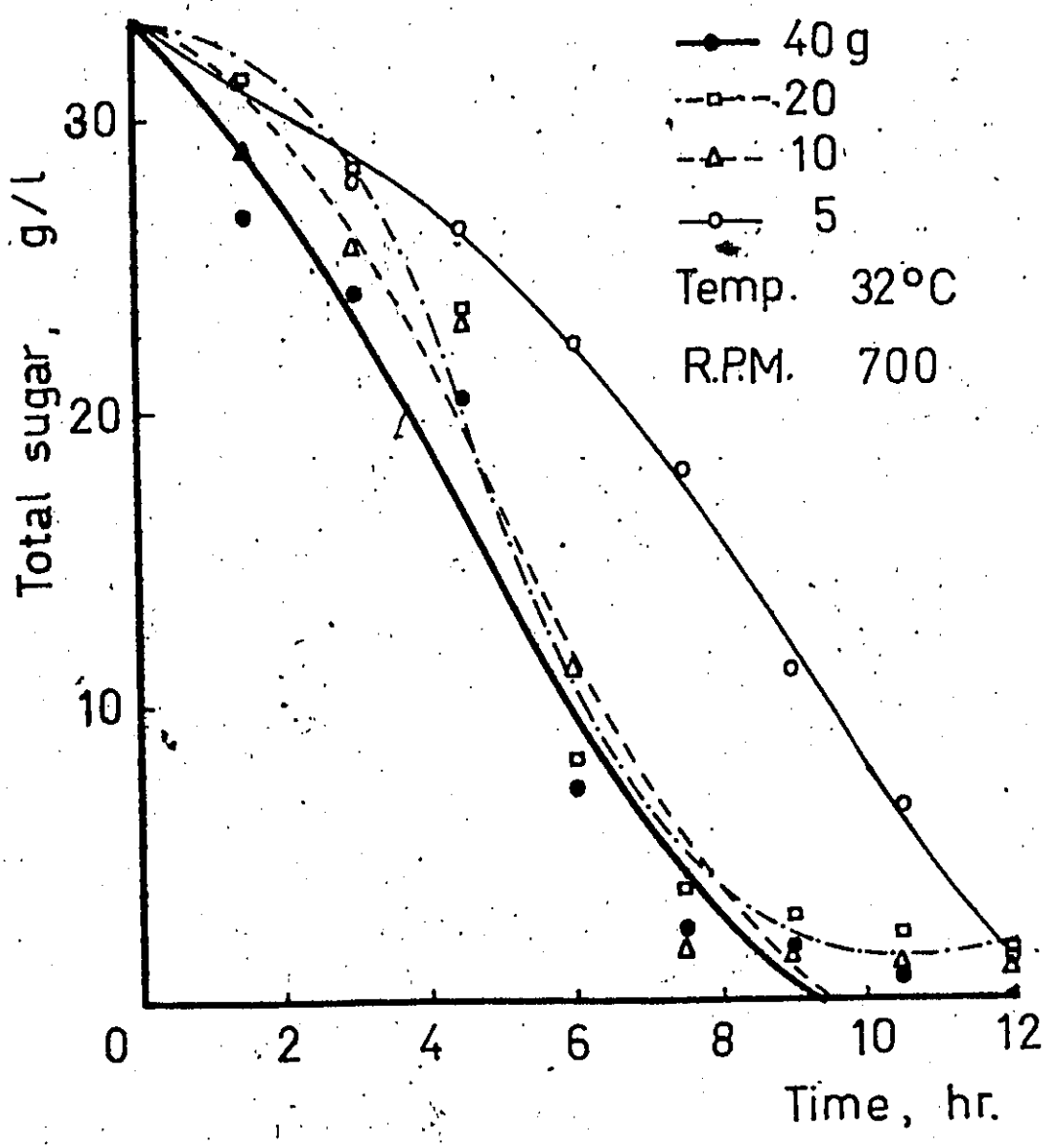

Fig. 30. Effect of Bacto conc. on total sugar consumption

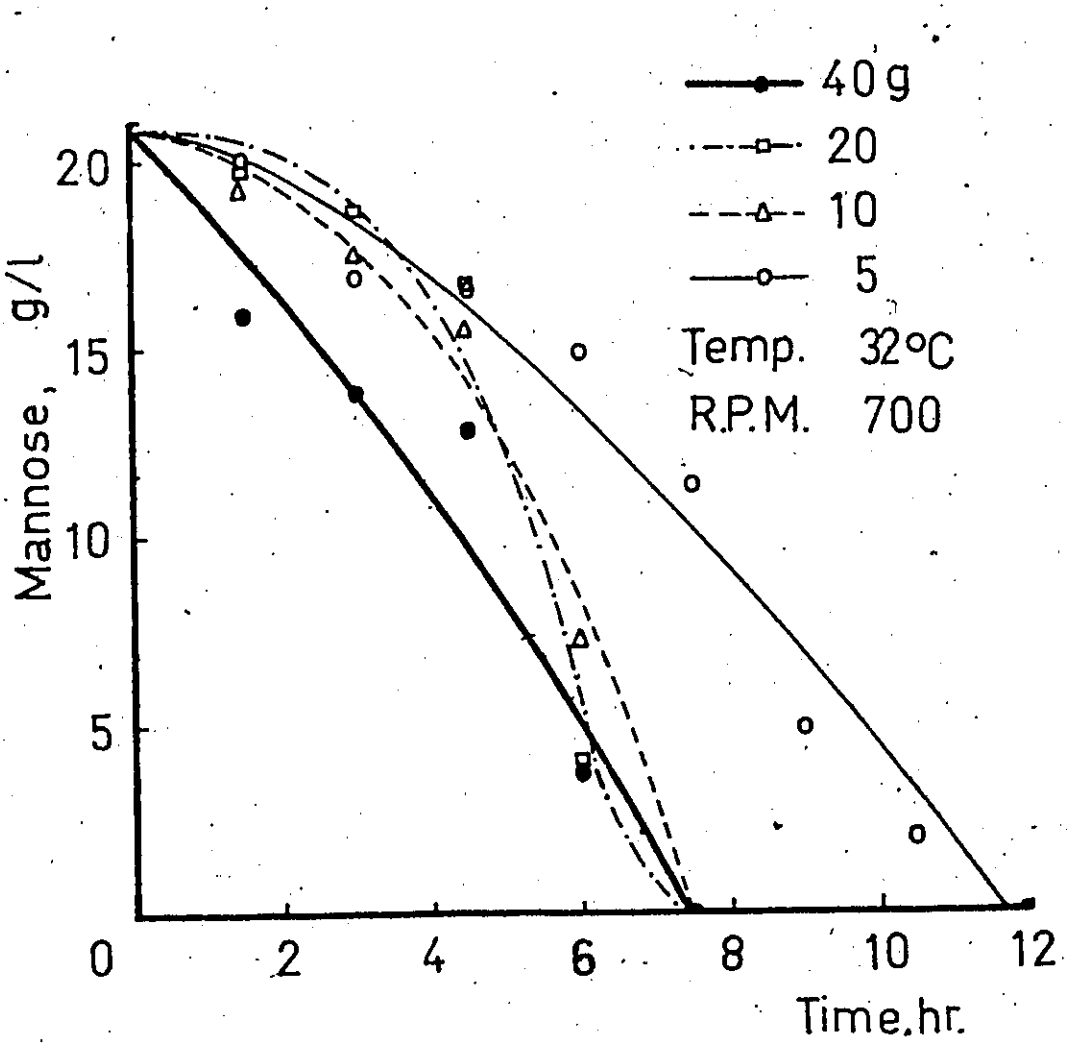

Fig. 31 Effect of bacto conc. on Mannose consumption

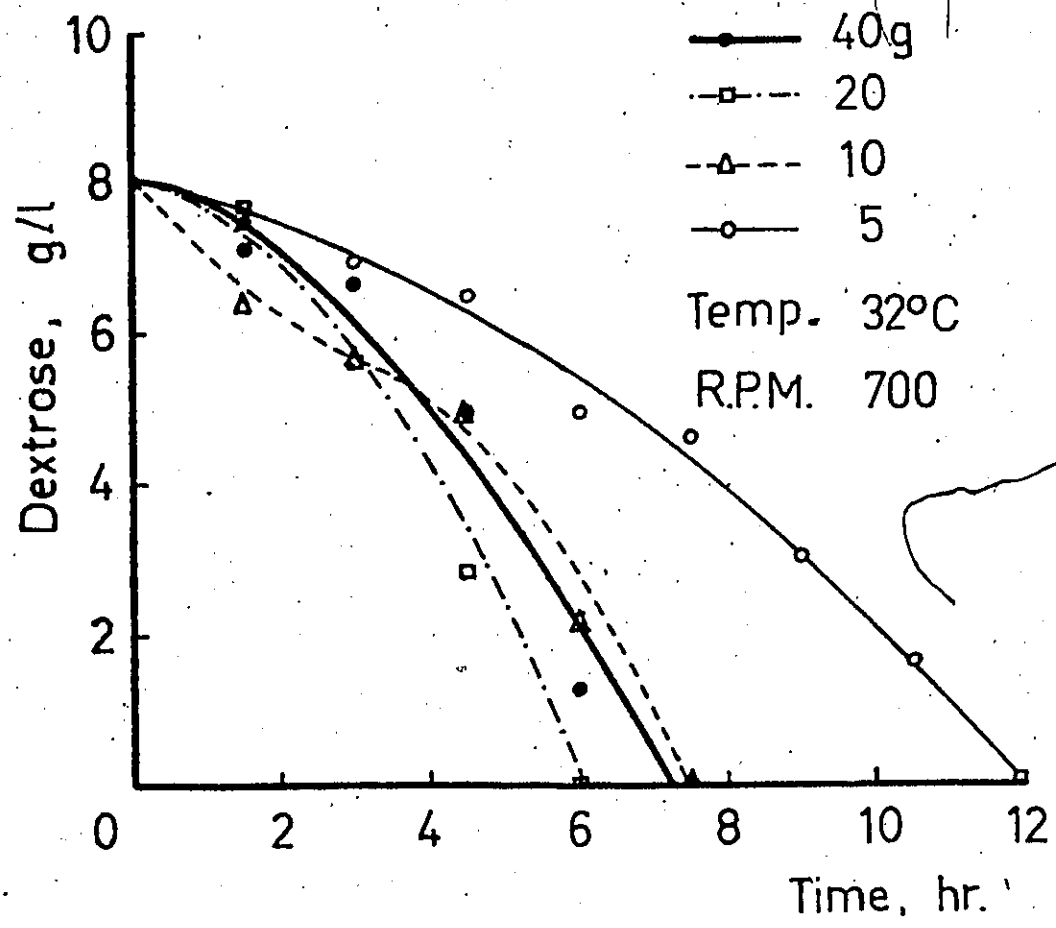

Fig. 32 Effect of Bacto conc. on Dextrose consumption

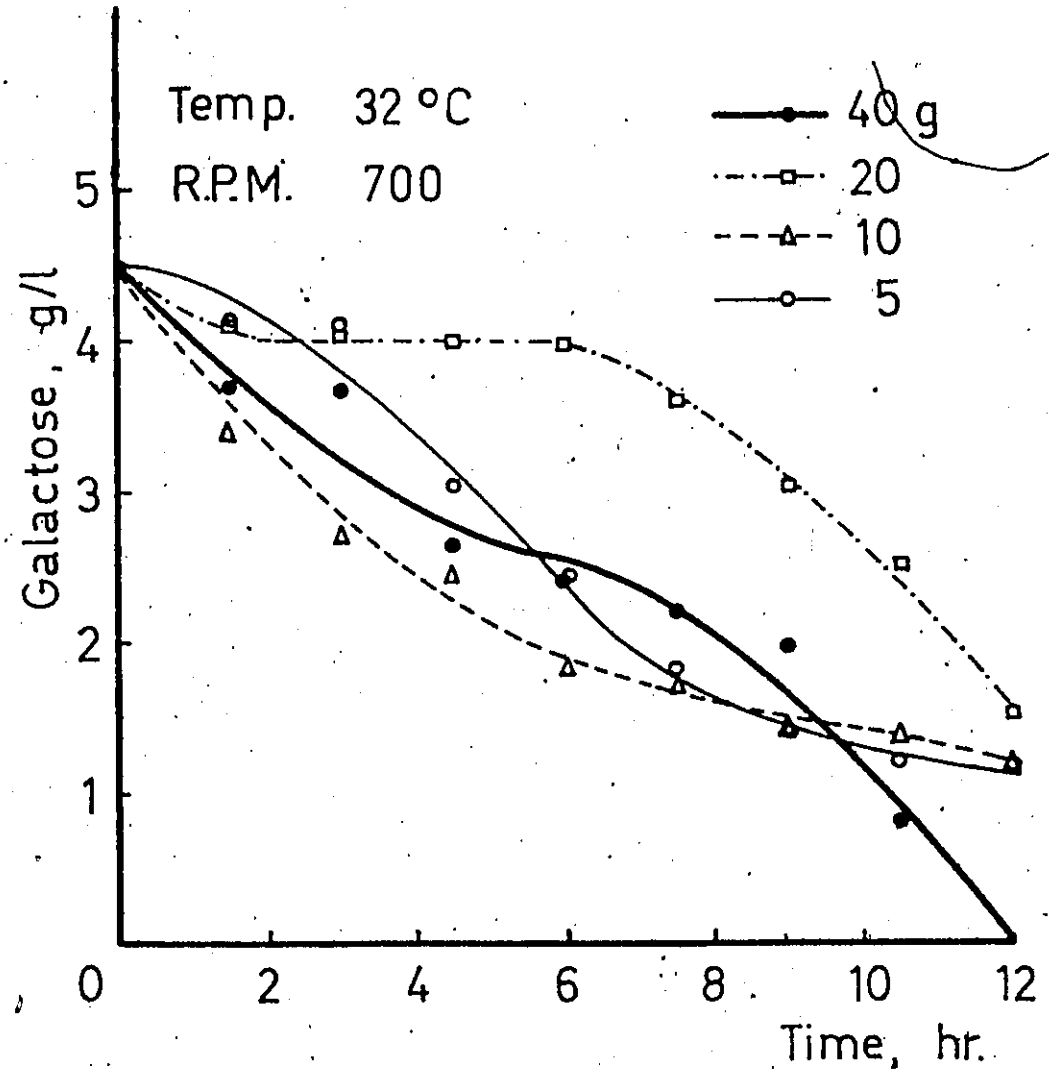

Fig. 33 Effect of Bacto conc. on Galactose consumption

For Galactose different results were obtained when raising the Bacto concentration. One can conclude from Figure 33 that in order to consume the Galactose content of the mixed sugar solution a high level of Bacto concentration should be provided.

V. CONCLUSIONS

Thin-layer chromatography is a good quantitative method for the analysis of the mixed sugar portion of the waste sulfite liquor. Buffering the thin-layer is the key to obtaining complete separation of D-Mannose, D-Glucose and D-Galactose. The best developing solution consisted of an acetone-n-butanol-water mixture in the proportion of (53 : 40: 7). Quantitative densitometric evaluation was possible following a visualization step by charing with a ceric acid solution.

The best conditions to ferment the synthetic mixed sugar solution of the sugar portion of the WSL using Saccharomyces Cerevisiae yeast at pH 5 were :

Temperature	36°C
r.p.m	700
Bacto concentration	13.3 g / ℓ

Collected results show neither diauxie growth nor catabolite repression. The data fit the published Kono and Asai kinetic model.

Calculation of the product synthesis shows that only 29.5 % of the sugar mixture was used to make yeast while 70.5 % was used to make alcohol.

The effect of temperature, stirring and Bacto concentration was studied and their effect on the level of dissolved oxygen, yeast growth, alcohol production, total sugar consumption, Mannose consumption, Dextrose consumption and Galactose consumption was discussed.

VI. NOMENCLATURE

b	Constant, defined by equation (1) page 27
C	Concentration, g/ℓ
D	Dilution rate, hr^{-1}
F_1	Factor for the transformation of sugar substrate into alcohol, g. sugar/g. alcohol
F_2	Factor for the transformation of sugar substrate into yeast cells, g. sugar/g. yeast
F_3	Factor for the utilization of sugar substrate for cell energy, g. sugar/hr/g. yeast
k	Biochemical reaction rate constant, defined by equation (1) page 27
K_{LA}	Mass transfer coefficient, moles/hr. cm^2
P	Product concentration, mℓ/ℓ
R_f	Defined on page 9.

t	Time, hr
T	Temperature, ^{o}C
$\overline{T}$	Temperature, ^{o}K
ϕ	Apparent coefficient of growth activity, defined by equation (8) page 30
μ	Liquid viscosity, cp

Subscripts :

a	Alcohol
c	Critical
L	Liquid
s	Sugar
y	Yeast
o	Initial state

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VIII . APPENDICES

VIII. A. The Junction Run* Results :

Operating Conditions:

Volume of sugar solution	=	3 litres
Temperature	=	32°C
Stirring	=	700 r.p.m.
pH	=	5
Bacto	=	40 g / batch
Air flow rate	=	7000 cc/min at 70° F and 1 Atm.
Air pressure	=	14.7 p.s.i.a.
Amount of sugar	=	100 g Dextrose
Amount of yeast	=	4 g

Air was flowing for the first 4 1/2 hours and then was turned off for the rest of the run. pH was controlled automatically using a 2 N HCl and 4 N NH_4 OH solutions. The yeast used was the same used by Dekee (15) and was kept in the refrigerator for a period of five months.

* Comparison with previous work of Mr D. Dekee (15).

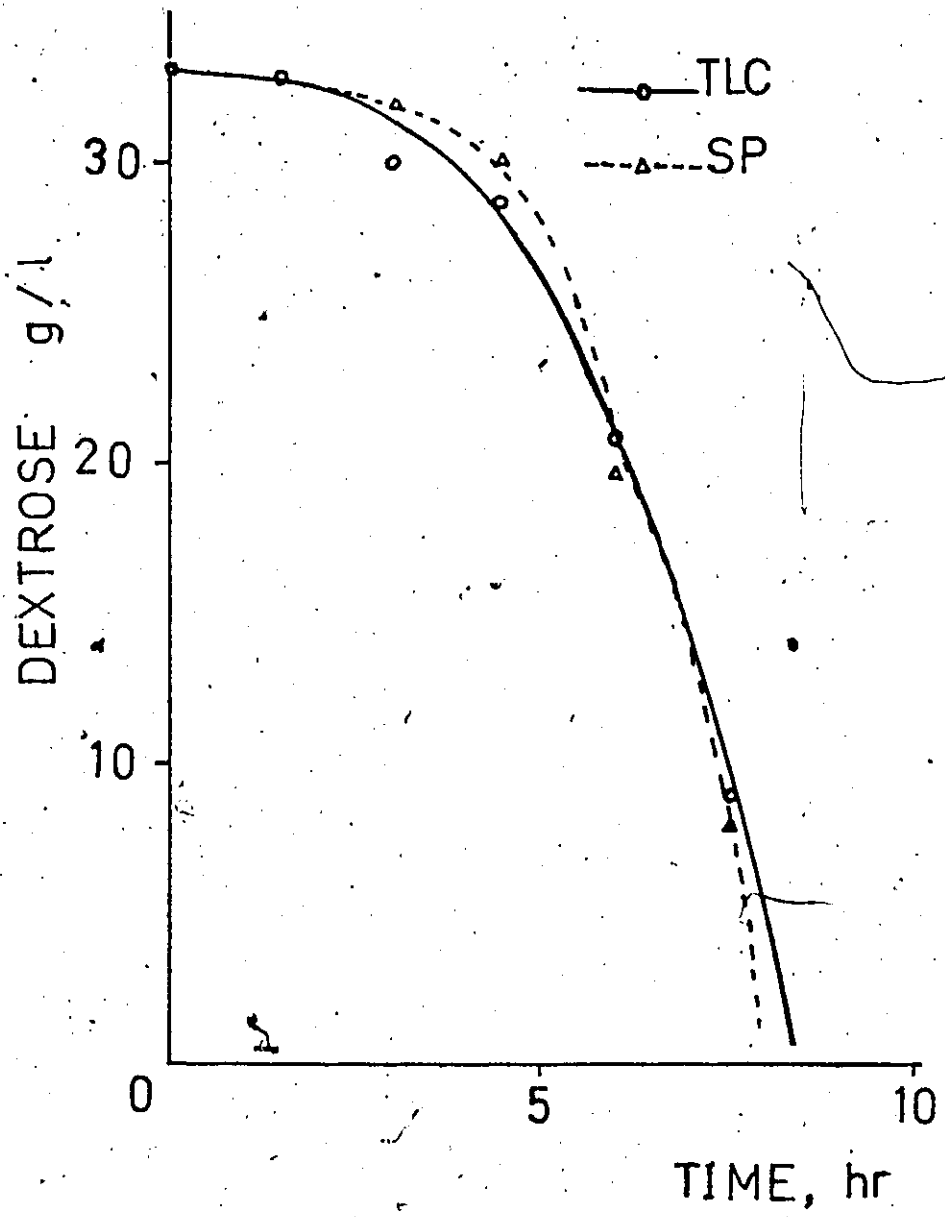

Figure 34: The Junction Run Curves for Sugar Analysis

TABLE 7

Results of the junction run

Same No	Time (hr)	yeast (mℓ/ℓ)	Alcohol (mℓ/ℓ)	Sugar g/ℓ	
				TLC	SP
1	0	3.5	0	33.3	33.3
2	1.5	4.0	0	33	33
3	3.0	8.0	0.1	30	32
4	4.5	12.0	3.2	28.8	30.1
5	6.0	14.0	8.6	21.0	19.9
6	7.5	17.0	14.8	9.0	8.2
7	9.0	18.0	16.8	0	0

VIII. B. Computer program used for curve fitting

VIII. C. Tables of Results

In the forthcoming pages, the results of the different runs of the mixed sugar fermentation (runs 11-20) will be given. The runs were conducted under different conditions of temperature, r. p. m. and Bacto concentration; while the other operating conditions were kept constant as follows:

Volume of mixed sugar solution	=	3 litres
Air flow rate	=	4000 cc/min.
Air pressure	=	14. 7 p. s. i. a.
pH	=	5
Amount of mixed sugar	=	100 g
Yeast	=	4 g
Acid	=	2 N HCl
Base	=	4 N NH_4OH

Air was flowing all the time and dissolved oxygen was recorded automatically on graph paper.

The following abreviation were used:

M for Mannose

D for Dextrose

G for Galactose

TABLE 8

Run No. 11 Temp. = 40°C R.P.M. = 700 Bacto conc. = 40 g/31

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0	2.5	0	0	0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.5	0.8	3.7	18.8	7.2	3.8	28.9	
3	3.0	5.0	1.2	1.0	6.8	17.0	6.4	3.6	27.0	
4	4.5	7.5	2.5	1.0	11.6	14.5	5.2	2.9	22.6	
5	6.0	10.0	3.95	1.0	17.6	12.8	2.8	2.3	17.9	
6	7.5	13.0	6.2	1.0	22.3	3.8	2.1	1.6	7.5	
7	9.0	15.0	8	1.05	26.2	2.1	1.6	1.4	5.1	
8	10.5	18.0	9.2	1.6	31.0	0.5	0.0	0.8	1.3	
9	12.0	20.0	8.2	8.8	31.0	0.0	0.0	—	—	6.2

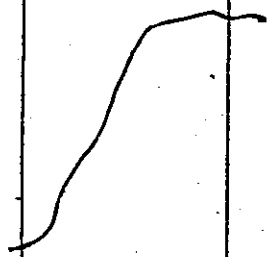

TABLE 9

Run No. 12 Temp. = 36°C R. P. M. = 700 Bacto Conc. = 40 g/31

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.8	1.8	3.7	19.2	6.2	3.4	28.8	
3	3.0	10.0	3.5	1.8	9.5	16.8	5.7	2.3	24.8	
4	4.5	17.0	7.9	1.8	20.5	9.4	2.1	1.2	12.7	
5	6.0	21.0	11.8	1.8	31.1	0.0	0.4	0.85	1.25	
6	7.5	21.0	10.7	1.8	33.95	0.0	0.0	0.85	0.85	
7	9.0	21.0	10.2	5.7	34.85	0.0	0.0	0.0	0.0	
8	10.5	22.0	9.5	8.8	34.85	0.0	0.0	0.0	0.0	
9	12.0	22.0	9.2	—		—	—	—	—	6.8

TABLE 10

Run No. 13 Temp. - 28°C R. P. M. = 700 Bacto conc. = 40 g/31

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.6	0.6	4.9	17.1	7.4	4.2	28.7	
3	3.0	8.0	1.4	11.0	11.0	16.0	7.0	3.4	26.4	
4	4.5	10.0	4.0	14.2	17.0	13.2	5.5	3.0	21.7	
5	6.0	15.0	6.6	15.8	25.5	8.8	3.0	2.6	14.4	
6	7.5	20.0	9.2	17.0	34.0	2.5	0.0	2.3	4.8	
7	9.0	22.0	10.0	17.7	35.9	0.0	0.0	2.1	2.1	
8	10.5	26.0	8.8	18.7	39.5	0.0	0.0	1.1	1.1	
9	12.0	30.0	8.8	19.1	42.6	0.0	0.0	—	—	6.2

TABLE II

Run No. 14 Temp. = 32°C R. P. M. = 700 Bacto conc. = 40 g/l

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	4.0	0.8	7.7	5.6	15.8	7.1	3.7	26.6	
3	3.0	10.0	2.8	12.0	11.9	13.8	6.7	3.7	24.2	
4	4.5	18.0	6.6	14.3	19.8	13.0	5.0	2.6	20.6	
5	6.0	22.0	12.5	15.0	29.4	3.4	1.2	2.4	7.0	
6	7.5	25.0	12.1	17.2	31.9	0.0	0.0	2.2	20.2	
7	9.0	26.0	10.6	20.8	35.0	0.0	0.0	2.0	20.0	
8	10.5	29.0	10.4	24.0	40.5	0.0	0.0	0.8	0.8	
9	12.0	30.0	9.0	25.3	43.5	0.0	0.0	0.0	0.0	5.9

TABLE 12

Run No. 15 Temp. = 32°C R. P. M. = 300 Bacto conc. = 40 g/31

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.8	22.0	14.6	20.2	6.1	3.0	29.3	
3	3.0	11.0	3.0	39.0	28.0	18.8	5.8	3.0	27.6	
4	4.5	15.5	7.0	54.0	42.0	15.6	5.0	2.6	23.2	
5	6.0	22.0	11.0	63.9	54.2	1.2	1.3	2.4	4.9	
6	7.5	24.0	11.4	65.4	55.5	0.0	0.0	2.0	2.2	
7	9.0	24.0	12.0	66.2	56.2	0.0	0.0	1.4	2.0	
8	10.5	24.0	9.4	66.6	56.6	0.0	0.0	0.4	1.4	
9	12.0	24.0	9.4	67.2	57.1	0.0	0.0	0.0	0.4	6.4

TABLE 13

Run No. 16 Temp. = 32°C R. P. M. = 500 Bacto conc. = 40 g/3l

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	4.0	1.0	11.0	7.3	19.8	7.0	3.4	30.2	
3	3.0	11.0	2.9	13.4	12.6	17.9	6.7	3.4	28.0	
4	4.5	17.5	7.3	15.8	21.5	13.8	3.0	2.0	18.8	
5	6.0	19.0	12.2	17.2	29.9	1.0	0.6	2.0	3.6	
6	7.5	19.5	11.6	17.9	31.2	0.0	0.0	2.0	2.0	
7	9.0	20.0	10.8	18.3	32.55	0.0	0.0	1.1	1.1	
8	10.5	21.0	10.2	19.0	35.4	0.0	0.0	0.0	0.0	
9	12.0	21.0	9.8	19.9	37.5	0.0	0.0	0.0	0.0	6.0

TABLE 14

Run No. 17 Temp. = 32°C R. P. M. = 700 Bacto conc. = 20 g/31

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.8	13.5	9.3	19.9	7.7	4.1	31.7	
3	3.0	11.0	3.4	22.2	17.9	18.8	5.6	4.0	28.4	
4	4.5	17.0	6.0	26.5	26.5	16.8	2.8	4.0	23.6	
5	6.0	20.0	10.6	35.5	39.1	4.1	0.0	4.0	8.1	
6	7.5	24.0	11.2	36.7	40.8	0.0	0.0	3.6	3.6	
7	9.0	25.0	9.2	37.0	42.5	0.0	0.0	3.0	3.0	
8	10.5	26.0	9.0	38.3	44.2	0.0	0.0	2.3	2.3	
9	12.0	29.0	7.2	40.6	47.5	0.0	0.0	1.5	1.5	7.2

TABLE 15

Run No. 18 Temp. = 32°C R. P. M. = 700 Bacto conc. = 5 g/3l

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.6	18.8	12.8	20.0	7.4	4.1	31.5	
3	3.0	10.0	2.0	33.0	24.0	16.8	6.9	4.1	27.8	
4	4.5	15.0	4.9	45.6	31.1	16.8	6.5	3.0	26.3	
5	6.0	12.0	8.0	59.2	46.0	15.0	4.9	2.4	22.3	
6	7.5	12.5	8.8	65.3	51.7	11.5	4.7	1.8	18.0	
7	9.0	12.5	11.0	65.4	53.1	6.7	3.0	1.4	11.1	
8	10.5	12.5	10.8	65.4	53.8	3.8	1.6	1.2	6.6	
9	12.0	13.0	10.8	65.5	54.5	0.0	0.0	1.2	1.2	6.2

TABLE 16

Run No. 19 Temp. = 32°C R. P. M. = 700 Bacto conc. = 10 g/31

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.8	17.5	11.0	19.3	6.4	3.4	29.4	
3	3.0	10.0	2.4	25.0	18.0	17.5	5.7	2.7	25.7	
4	4.5	15.0	5.2	35.1	28.7	15.6	5.0	2.5	23.1	
5	6.0	16.0	9.2	42.0	37.7	7.3	2.2	1.8	11.3	
6	7.5	18.0	10.4	45.1	42.5	0.6	0.0	1.7	1.7	
7	9.0	19.5	9.8	46.3	43.7	0.0	0.0	1.4	1.4	
8	10.5	20.0	7.6	47.6	45.2	0.0	0.0	1.4	1.4	
9	12	20.5	7.6	48.4	46.4	0.0	0.0	1.2	1.2	7.5

TABLE 17

Run No. 20 Temp. = 32°C R. P. M. = 100 Bacto conc. = 40 g/3l

Sample No.	Time (hr)	Yeast (ml/l)	Alcohol (ml/l)	Acid (ml)	Base (ml)	Sugar (g/l)				Alcohol Trapped (ml/l)
						M	D	G	Total	
1	0.0	2.5	0.0	0.0	0.0	20.8	8.0	4.5	33.3	
2	1.5	3.0	0.6	24.0	14.0	19.5	7.1	3.7	30.3	
3	3.0	10.0	2.6	50.1	32.6	16.8	6.0	3.4	26.2	
4	4.5	15.0	6.6	63.0	46.5	16.2	4.8	2.4	23.4	
5	6.0	19.0	10.7	77.3	59.7	2.3	0.8	1.4	4.5	
6	7.5	19.0	10.0	84.4	63.9	0.0	0.0	1.4	1.4	
7	9.0	18.0	9.8	98.4	71.8	0.0	0.0	1.0	1.0	
8	10.5	17.0	8.9	125.6	89.0	0.0	0.0	1.0	1.0	
9	12	17.0	8.4	142.1	97.8	0.0	0.0	0.3	0.3	7.2

VIII. D. Calibration Curve for sugar analysis.

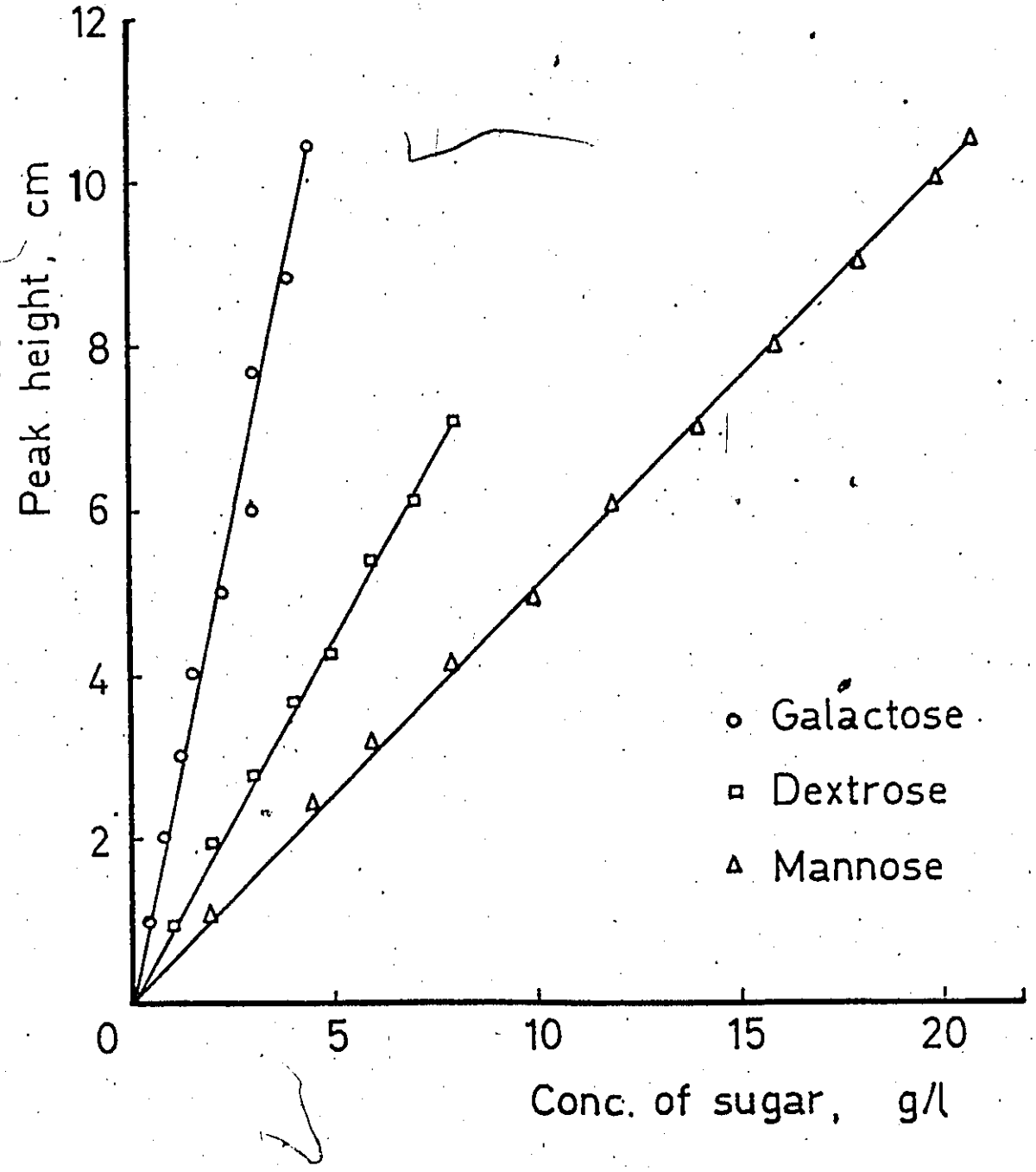

Fig. 35 Calibration curve of sugar analysis by TLC

VIII . E. . Composition of Bacto used

100 g. of Yeast Nitrogen Base Contain:

Copper sulphate	589 mcg
Biotin	.29 mcg
Folic acid	29 mcg
Boric acid	7.365 mg
Potassium iodide	1.473 mg
Ferric chloride	2.946 mg
Manganese sulfate	5.892 mg
Sodium molybdate	2.946 mg
Zinc sulfate	5.892 mg
Calcium pantothenate	5.892 mg
Inositol	29.460 mg
Niacin	5.892 mg
p-Aminobenzoic acid	2.946 mg
Pyocydoxine Hydrochloride	5.892 mg
Riboflavin	2.946 mg
Thiamine Hydrochloride	5.892 mg
L-Histidine Monohydrochloride	147.318 mg
DL - Methionine	294.636 mg
DL - Tryptophane	294.636 mg
Magnesium sulfate	7.365 g
Sodium chloride	1.473 g
Calcium chloride	1.473 g
Ammonium sulfate	73.650 g
Monopotassium phosphate	14.732 g

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