RHEOLOGICAL PROPERTIES OF YEAST CELL WALLS

by

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ABSTRACT

Chemical analysis, rheological measurements and hydrodynamic studies have been employed to characterize structural and morphological properties of yeast cells and cell wall components.

Saccharomyces cerevisiae A364A and its morphological mutant JD7 were selected for this study. The mutant exhibits dimorphic phenotypes, i.e. mycelium-like and yeast-like, depends on the nutritional status of the medium. The cell wall components, which consist primarily of glucan and alkali insoluble glycogen, were prepared by heating cells in alkali. Microscopic examination showed that the alkali insoluble residues remain in the shape of whole cells.

The carbohydrate compositions of various morphological form of yeast were also investigated. The glucan content of mycelium-like cells of JD7 is higher than that of yeast-like form, whereas the mannan content is higher in strain A364A.

Rheological measurements indicated that branched and elongated cells of Saccharomyces cerevisiae JD7 exhibit a much higher viscosity than spherical cells of A364A and have a lower critical concentration, while the yeast-like cells of JD7 have moderate viscosities with a critical concentration inbetween the other two morphological types.

In comparing the viscosity profiles of cell wall components and those of whole cells, we found that the cell wall components exhibit a viscosity 3 to 4 times higher than that of the whole cells. This suggests that the number of cell units in the suspension has a dicisive effect on the viscosity.

Yield stress of certain types of cell wall components was extremely high and comparable to other hydrocolloidal polysaccharides, such as carboxymethyl cellulose.

Hydrodynamic volumes of these materials estimated by sedimentation studies were high and suggesting that a large amount of water is associated with the cell wall aggregates during sedimentation. The water may either be occluded within the sac-like cell wall components or be entrapped between the loosely packed settling aggregates. The thickening effect, the high yield stress as well as the large hydrodynamic volume suggest a possible use of yeast cell wall as a food thickening agent.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

For sucessful Single Cell Protein (SCP) applications in food, the variable of economy, technology, nutrition and safety must all be considered. However, many requirements for SCP applications to food are sometimes incompatible with the technological constraints of low cost production. The objective of the research program at M.I.T. dealing with this problem is to use powerful genetic techniques to alter the size, shape, and other properties of yeast cells in order that both the production process and the functionality of the SCP and its components could be improved on. Two-stage-fermentation processes using conditional mutants are being investigated. In the first stage, the organisms of interest are to be propagated in the most economical manner possible and then transfered to a second stage fermentation reactor where desirable phenotypic properties may be expressed. A novel morphological mutant of Saccharomyces cerevisiae isolated by DeAngelo (1978) is one such potential candidate for this application. This dimorphic strain, Saccharomyces cerevisiae JD7, is a conditional cell division cycle mutant, its phenotype is controlled by an unknown nutritional factor.

The objective of this thesis is to characterize, compare, and analyze the chemical and structural properties of some morphological types of yeast cells and yeast cell wall components. Saccharomyces cerevisiae A364A and its morphological mutant, JD7, are employed in this study. The potential applications of some yeast cell walls as thickening agent for food systems were also investigated.

LITERATURE SURVEY

This literature survey collects background informations relevent to this thesis which includes the following:

1. Applications of yeast and yeast fractions.
2. Applications of morphological mutants in Single Cell Protein production.
3. Rheological properties of yeast suspensions.
4. Chemical and structural properties of yeast cell wall.
5. Use of cell walls as a food thickening agent.

Applications of Yeast and Yeast fractions

Yeast throughout history, have been used to leaven bread and to brew ale, beer and wines. In the 1930's, residual yeast from brewing operations and yeast produced as baker's yeast were dried and used in foods, as a source of vitamins, minerals, proteins and unknown nutrients. As a source of protein, dried food yeast contains approximately 33 to 40% true protein on a weight basis. As such dried yeast has been used at low levels in bread, cereals, peanut butter

mixtures and other food proteins mainly as vitamin supplements.

Yeast fractions have also been used as a source of nutrients. Autolyzed yeasts or yeast cells disrupted by mechanical or chemical means are sources of such fractions. In these cases, the protein may have been recovered from the disintergrated yeast cells by alkaline extraction or solubilized by autolysis due to proteolytic enzymes present in yeast.

In addition, yeast proteins may be used as a flavoring or functional ingredient. Autolyzed or hydrolyzed yeast extract has been used as a flavoring agent to give meaty or nutty flavor in food. Often yeast extract has a dark brown color and for this reason it is also used as a coloring agent. Yeast proteins may also be used as functional ingredient such as surface active agents (Rha, 1975).

Production of Single Cell Protein (SCP) of this sort is one of the most promising new sources of protein that can be produced independently of tranditional agricultural processes and environmental constraints. The specter of a world food and protein shortage, the urgent need for energy and environmental conservation and reevaluation of the effeciency associated with food production have led to a continuing interest in the possibility of utilizing Single Cell Protein as a source of food protein. An additional advan-

tage is that Single Cell Protein from microbial sources may result as a by-product from processes being developed to produce chemicals and/or chemical feedstocks from a variety of substrate, e.g. cellulose, n-paraffin ,etc..

Application of Conditional Mutants in Single Cell Protein Production

Selection of morphological mutants has been found to be useful in increasing product formation (Stanffer and Backus,1974; Spizek, 1965; Blumauerova, 1969) in some fermentations and in optimizing operation processes (Wiekerham, Kurtzman and Herman, 1970). A two stage fermentation employing temperature sensitive morphological mutants has been suggested by Miyasaka and co-workers (1978,1980) to decrease the recovery costs in Single-Cell-Protein production. In the first stage of this process, SCP organisms are propagated under optimal conditions of production and then the organisms are transferred to a second stage reactor where desirable phenotypic properties can be expressed. Signals for expression of phenotypic properties can be extrinsic parameters, such as temperature or level of oxygen or carbon dioxide, or intrinsic parameters, such as a specific nutrients requirement. Saccharomyces cerevisiae strains, 4471, 374, and 377, harboring cell division cycle (cdc) mutations, were investigated for this purpose, all strains exhibited significant morphological changes when the growth temperature was shifted from a permissive (25 or 30 C) to a non-permissive temperature (37 ^{0}C) due to their

inability to complete the budding process with the consequence that the cells enlarged and formed aggregates.

Rheological Properties of Yeast Cell Suspensions

Rheological properites, i.e. viscosity, yield stress, shear dependency, etc., of microbial suspensions have been recognized as factors influencing parameters such as mass transfer, momentum transport and heat transfer in various types of fermentation operations and these transport phenomena in turn directly affect the ability to maintain a controlled homogeneous environment of the production organisms. Furthermore, from the technical point of view, application of rheology in microbial processes has special significances, because:

1. Rheological measurements can provide gross structural characterization which is not possible in complex systems using only biochemical analyses or microscopic examinations.

2. Some types of rheological measurements are relatively simple and rapid.

3. Once a correct structural model is developed based on rheological properties, rheological measurements may be adapted directly to on-line process monitoring for control strategies.

Unfortunately, the rheological properties of yeast suspension has received very little attention in recent reviews on the rheological properties of microbial suspensions (Blanch et al, 1976; Charles, 1978; Metz, 1978). In other microbial systems, e.g. mycelial broth, cell morphology, cell concentration, intercellular interactions and rate of shear are claimed to be four major factors influencing viscosity of microbial suspensions.

effects of volume concentration on the rheological behavior of microbial suspensions

The Einstein equation:

$$\eta_S = \eta_O (1 + 2.5 \phi_S)$$

where η_S =viscosity of suspension, η_O =viscosity of suspending medium, ϕ_S =volume fraction of spherical particles or cells is applicable to Newtonian, rigid spheres suspensions and it becomes less applicable when concentration of suspensions increases and the particles deviate more from ideal rigid sphere.

Deindoerfer and West (1960) reported that at low values of fractional volume viscosity could be correlated by Einstein's equation (1906), whereas the equation of Vand (1948):

$$\eta_S = \eta_O \cdot (1 + 2.5\phi_S + 7.25 \phi_S^2)$$

may be applied to suspensions at higher volume fractions (>0.04).

Many theoretical and experimental studies have been made to investigate the validity of the extended Einstein's equations, i.e.,

$$\eta_S = \eta_O (1 + a\phi_S + b\phi_S^2 + c\phi_S^3 + \ldots)$$

The specific structure of these equations as well as the value of the parameters were variant among published results (Saito, 1950; Koga,1959; Aiba et al, 1962). The discrepency in the published results may in part be due to the difficulties in determination of the volume fraction of microbial cells (Reuß, 1979) and the different methods for viscometric measurements employed. The higher terms in these types of equations bring into account interactions due to collisions between the particles in the absence of flocculation (Metz, 1978). However, these types of relations are not completely satisfactory over a wide range of cell concentration (Lee et al.,1969; Rao and Hang 1975; Shimmons et al., 1970). The influence of particle diameter is neglected, this can cause large errors.

Mooney equation (1950):

$$\ln\eta_r = \frac{a \cdot \phi_S}{(1 - \phi_S / \phi_{max})} \qquad (\eta_r = \eta_S/\eta_O)$$

where ϕ_S =volume fraction

a = Einstein-constant

ϕ_{max} =maximum packing density

for $\phi_s < 0.2$, a =2.5

is one of the frequently used semi-empirical relations, that contain parameters which are a function of particle diameter.

effects of shear rate on the flow behavior of microbial suspensions

To quantatively express flow behavior of fluid the Power Law equation,

$$\tau = \tau_0 + K\dot{\gamma}^n$$

is in general the most satisfactory. The equation relates the shear stress (τ), yield stress (τ_0), consistency index (K), rate of shear ($\dot{\gamma}$) and flow behavior index (n). It can represent Newtonian, Bingham plastic, Pseudoplastic and shear thickening properties depending on the values of constants (Charm, 1963; Rha, 1975).

For dilute suspensions of spherical cells, ideal flow behavior with no yield stress and a flow behavior index of unity is usually observed. In such a case, the flow characteristics of the system can be completely represented by single paramenter, the 'Newtonian viscosity'.

Concentrated microbial systems and mycelial suspensions generally exhibited pronounced non-Newtonian rheological characterics. The existing models for filamentous mould

suspensions includes the following (Blanch and Bhavaraju, 1976):

(1) Bingham model (Solomons and Weston, 1961; Deindoerfer and West, 1960)

$$\tau = \tau_0 + \eta\dot{\gamma}$$

In this model, the flow behavior of mould suspension is described as Bingham plastic. This means that this model includes the yield stress which has to be exceeded before the fluid will flow while the flow behavior at high shear stresses is Newtonian.

(2) Pseudoplastic model (Deindoerfer, 1960)

$$\tau = K\dot{\gamma}^n + \tau_0$$

where $n<1$

This model implies that the rate of shear increases more than in proportion to the shear stress. Shear dependency of this sort indicates that continueous broken down or rearrangement of the structure, resulting in less resistance to flow (Rha, 1978).

(3) Casson model (Bongenaar et al., 1973; Reol et al., 1974)

$$\sqrt{\tau} = \sqrt{\tau_0} + K_c\sqrt{\dot{\gamma}} \quad ; \; K_c = \text{Casson Viscosity}$$

This model is originally derived by Casson (1959) for pigment-oil suspensions has been applied successfully to a large variety of suspensions (Kooyman, 1971; Charm, 1963).

Because all these models do not follow Newton's Law, i.e. the rate of shear does not increase in proportion to the shear stress. It is more convienent to deal with an apparent viscosity which is defined as the ratio of the shear stress to the rate of shear at a given value of shear rate. The apparent viscosity is especially useful when it is determined at the pertinent shear rate for a practiclal purpose (Rha, 1978).

effects of morphology on the rheological behavior of microbial suspensions

It is well known from the literature that the shape of the particles has a profound effect on the viscosity of a suspension (Sherman, 1960). Carilli et al. (1961) have shown quantitatively that cellular morphology influences the viscosity of mould suspensions. For *Aspergillus niger*, three morphological types were compared: large pellets, small pellets and a filamentous suspension. A difference in viscosity by a factor of three was reported between the filamentous suspension and the large pellets. The viscosity of the small pellet in suspensions was found to be inbetween the filamentous and large pellets.

Reols et al (1974) developed a mathmatical model, based of Casson model, for the rheological behavior of filamentous mould suspensions. They treated the filamentous mycelial particles like polymer molecules and assumed that each particles was coiled to give a spherical shape. An additional

assumption was that the spherical coils aggregate into linear agglomerates. Their assumptions allowed the Casson model to be applied. The workers employed the 'excluded volume' concept to establish a relation between the total volume fraction of the mycelial particles and the particle morphology, i.e.,

$$\phi_s = \sqrt{\delta_1} \cdot Cm$$

where ϕ_s =volume fraction of mycelial particles as spheres. δ_1 =morphology factor dependent on the length-diameter ratio of mycelial particles. Cm =biomass concentration. Both Kc and τ_o are then a function of the volume fraction of the mycelial particle (ϕ_s) and thus of biomass concentration and a morphology factor. A final equation including a factor for the influence of morphology of mould and the influence of the concentration of biomass was then deduced:

$$\sqrt{\tau} = \sqrt{\delta} \cdot Cm\{1 + f(Cm)\sqrt{\dot{\gamma}}\}$$

where $f(Cm) = \eta_o^{\frac{1}{2}}\{\frac{1}{Cm\sqrt{\delta}} + C_1\}$

δ =morphology factor =$f(\delta_1)$

Cm =biomass concentration

η_o =viscosity of suspending medium

C_1 =constant

τ =shear stress

$\dot{\gamma}$ =shear rate

The major objections against this theoretical model are (Metz, 1978):

1. Network interactions between the branched hyphae should not be considered as negligible.

2. The assumption that the hyphae are coiled into spheres is conflict with many microscopic examinations.

3. The assumption of Casson that coils arrange in the form of rouleaux with an orientation is questionable.

4. The randomizing effect of Brownian motion essential for the validity of polymer rheological theories is absent in mould.

Metz (1978) in his review on the rheology of mould suspensions concluded that the theoretical model of Reols et al has too many objections against its assumptions to be a useful basis for further work, however, the introduction of a morphological factor is of great value.

In Reuβ et al.'s studies (1979) on the viscosity of yeast suspensions, experimental results were correlated by an equation of the form:

$$\eta_s/\eta_o = 1/(1 - (h_s \cdot \phi_s)^a)$$

where parameter 'a' depends on the morphology of the yeast ,while h_g is a function of the osmotic pressure. The equation suggests that as h_s approaches a value of one, the viscosity approaches infinity. Thus, $1/h_s$ can be interpretated as maximum packing volume of suspensions. The increase in h_s at high values of the osmotic pressure indicates that the volume fraction for close packing is decreasing.

effects of cellular interactions on the rheological behavior of microbial suspensions

At high concentrations, partical interaction takes place in suspensions and further increases in cell concentration causes an increase in the rate of change in the apparent viscosity and yield stress. The concentration of suspension at which the viscosity of the suspension begins to approach infinity, namely critical concentration, is function of the size, shape and surface nature of the particle in the suspensions.

The interaction between suspending particles in a suspension may be the result of (Rha, 1978):

1. Physical entanglement of molecules or particles caused by large size and high degree of branching or irregular shape.

2. Network formation caused by ionic, co-valent, or secondary intermolecular or interparticle interactions.

3. Crowding as a results of the elimination of solvents.

One of the most distinctive properties of mycelial suspensions is the entanglement of hyphae. Visco-elastic properties often play a role in these suspensions at every low shear rates, while at sufficiently high shear rates the system flow like a Newtonian fluid. A theoretical model has been proposed by Metz (1978) to explained the non-Newtonian behavior of mold suspensions based on the intercellular interactions. In this model, the existence of a network structure composed of aggregates is assumed. Upon shearing, the aggregates break into small fragments. At high shear rates, the aggregates are assumed to be broken down completely, i.e., there is no continuous structural network. Flocculation is still taking place but the shear stresses are pulling the cluster apart as soon as they are formed. The viscoelastic behavior of mould suspensions at very low shear rates can then be explained as a consequence of the network structure.

Chemicl and Structural Properties of Yeast Cell Wall

Properties of yeast cell walls such as their mechanical strength, morphological features and biological activities are undoubtly based on their particular chemical composition. Bartricki-Garcia (1968) pointed out that a close correlation exists between the taxonomic classification and fungal cell wall composition among fungi. Polysaccharide, which represents 80-90% of the dry matter of yeast cell wall, are composed of amino sugars, hexoses, hexuronic acids, methyl pentose, and pentoses (Bartnicki-Garcia, 1970). Glucose and N-acetyl-D-glucosamine (GlcNAc) usually represent the chemical elements of skeletal wall polysaccharideds, such as chitin and glucans. The other sugars are present mainly in the form of various homo- and heteropolysaccharides, often in chemical complexes with protein.

Three major types of polysaccharides that constitute the yeast cell wall are (Farkas, 1979):

1. Glucan: The generic name 'glucan' covers a large group of D-glucose polymers differing both in type and in relative proportions of individual glycosidic bonds. The most abundant glucans of yeast cell walls are those with the β-configuration (Manner and Masson, 1969, 1973). Most yeast cell wall glucans contain mixed β-1,3 and β-1,6 glycosidic bonds, which constitue the skeletal microfibrillar portions of the walls.

2. Mannan: Yeast mannan is a polymer composed of protein and two carbohydrate moieties differing in their structure ane mode of attachment to the peptide (figure 1). The polysaccharide moiety is represented by an α-1,6-linked polymannose backbone to which short chains of mannosyl units linked together by α-1,2- and α-1,3-glycosidic bonds are attached predominantly by means of an α-1,2 bond. The polysaccharide moiety is linked via a diacetylchitobiose bridge by an N-glycosidic bond to an asparaginyl residue in the protien part of the molecule (Nakajima and Ballou, 1974). The second carbohydrate moiety of yeast mannan consists of short mannooliglsaccharides containing both α-1,2- and α-1,3-glycosidic bonds attached at their reducing ends by an O-glycosidic linkage to serine or threonine residues or both in the protein (Ballou, 1976; Cabib, 1975).

3. Chitin: Chitin, a β-1,4 polymer of GlcNAc is exclusively located in yeast bud scar (Cabib and Bowers, 1971, 1975).

Based on availble chemical, biochemical, immunochemical, and cytological evidences, Lampen (1968) suggested a structural model for the cell wall of Saccharomyces cerevisiae (figure 2). In his model, the innermost microfibrillar

layer, composed of insoluble β-glucan, is linked via protein to the outer wall layer, composed of manno-protein molecules mutually linked by phosphodiester bridges between their polysaccharide moeties. The phosphodiester cross-linking is supposed to form a physical barrier that holds within the wall structure the extracellular mannoprotein enzyme invertase, acid phosphotase, and others. The periplasmic space of yeasts has been also reported to contain a large amount of glycogen (Gunja-Smith,1977,1974).

The different polysaccharides, due to their distinctive physiochemical properties, fulfill specific function in the cell walls. The crystalline polysaccharides chitin and β-glucan are the components responsible for the mechanical strength of the wall, while the amorphous homo- and heteropolysaccharides, often in association with proteins, play the role of cementing susbstances and constitute the carbohydrate moieties of extracellular enzymes and cell wall antigens (Ballou,1974; Raschke, 1974; Gander, 1974; Lampen,1968).

Application of Cell Walls as Food Thickening Agents

Many hydrocolloids are widely used in foods based on their unique textural, structural and functional characteristics, e.g. they provide stabilization for emulsions, suspensions, and foams and general thickening properties. Most of these materials, which are sometimes classified as gums,

are derived from natural sources, although some are chemical modified to achieve desired characteristics. Many stabilizers and thickeners are polysaccharides, such as gum arabic, guar gum, carboxymethyl cellulose, carrageenan, agar, starch and pectin. Some specific functions of hydrocolloids in food include improvement and stabilization of texture, inhibition of crystallization (sugar, ice), stabilization of emulsions and foams, improvement of icing on baked goods, and encapsulation of flavors. Hydrocolloids are generally used at concentration of about 2% or less in food because many exhibit limited dispersability, and the desired functionality is provided at these levels. The effeciency of hydrocolloids in many applications is directly dependent of their ability to increase viscosity.

Use of plant cell wall as food thickening agent has first been suggested by Holmes et al. (1978). Rheological properties of parenchyma cell wall , recovered from pressed cranberries are studied by Holmes et al. (1978, 1977) in detail. The workers concluded that suspensions of parenchyma cells have certain advantages over other food thickeners due to their high water occluding capacity and large yield stresses at low cell wall material concentrations (1-2%).

Sucher et al (1975) proposed that insoluble residues from yeast protein extraction processes, which consists of a mixture of whole yeast cell walls, fragments of yeast cell walls, and some proteinaceous material, have interesting

functional characteristics that include holding water and imparting thickening properties in aqueous food system. In addition, they discovered that the addition of yeast glucan to liquid food systems, in the proper proportions, gives the food product a fat-like mouthfeel even when these food contains little or no fat. This is very useful in formulating low calorie products, such as salad dressing, ice cream, puddings, sour cream, based dip, and other low calorie foods.

MATERIALS AND METHODS

Yeast Strains

Saccharomyces cerevisiae A364A and its morphological mutant, strain JD7, were employed in this study. These two stains possess similar genotypes (a, ade 1, ade 2, his 7, lys 2, tyr 1, and gal 1), while JD7 is a cell division cycle mutant, whose cellular division is blocked at some stage prior to nuclear division in the cell devision cycle. This mutant was orginally isolated and characterized by DeAngelo (1978).

Growth Media

Growth medium employed were a complex medium, YPD, and a chemically defined medium, SDC. Yeast strains have been maintained on YPDA agar slants at 4 °C. The compositions of media are as shown in table 1.

Yeast Fermentation

The fermentations were carried out in fermentor of 7.2 liter total volume (New Brunswick Scientific, New Brunswick, NJ). The working volume was 5 liter and air flow rate was 1 vvm (volume of air per liter per minute). The cultures were stirred at 500 rpm with double turbine impeller.

TABLE 1

Composition of Media

Component	Amount (%)
YPD	
Dextrose (Bacto)	2.0
Peptone (Bacto)	2.0
Yeast Extract (Bacto)	1.0
*pH adjusted to 5.5.	
SDC	
Dextrose (Bacto)	2.0
Yeast Nitrogen Base W/O amino acids	0.67
Adenine Sulfite	0.002
Histidine	0.002
Uracil	0.002
Tyrosine	0.003
Lysine	0.003
YPDA	
Dextrose (Bacto)	2.0
Peptone (Bacto)	2.0
Yeast Extract (Bacto)	1.0
Adenine Sulfite	0.003
Agar (Bacto)	2.0

Temperature control was set at 30^{0}C, pH was maintained automatically at pH 5.5 by additionng of 1N NaOH. To prevent foam formation, small quantities of 5 % solution of Antifoam FG-10 (Dow and Corning, Midland, MI) were added as required. Portions of a glucose solution were added by batch feeding to avoid Crabtree effect (Schaffeld,1980). Growth was followed by Klett-Summerson colorimeter at a wave length of 660nm.

Recovery and Preparation of Cell Fractions

To collect and isolate cell wall fractions, the scheme as is outlined in figure 3 was followed. For 'hot alkaline treatment', washed cells were suspended in 1N NaOH at concentrations of 15-20 mg/ml, heat for 10,20 or 30 minutes at 100 ^{0}C in a water bath. An equal volume of cold buffer was then added to stop the reaction. Supernantant and residues were separated by centrifugation. The residues were washed once with 0.1N NaOH and twice with distilled water.

Heating cell suspensions in alkali results in disruption of cell wall and cell membrane structures, such that macromolecules within the cells are able to diffuse into the extracting solution, and also the alkali may solubilize protein within the cellular structure.

Dry weight measurement

Millipore filters (Type GS 0.22 μm, Millipore Corp., Bedford, MA) were employed in this assay, 2 ml or 5 ml portions of cell suspensions were filtered through preweighted filters. The yeast cakes were dried at 60 ^{0}C and weighed. Dry weight concentration of the samples are expressed as gm of dry mass/liter of suspension.

Chemical Analysis

protein assay

Protein contents of freeze dried samples of yeast cells and cell wall fractions were assayed by the Lowry method (Herbert et al, 1971): a 0.5 ml sample containing 0.5 to 1.0 mg of dried mass was mixed with 0.5 ml of 1N NaOH. The suspension is heated for 15 mins. at 100^{0}C and allowed to cool. 2.5 ml of reagent A (*) was added and the sample is hold at room temperature for 20 minutes. 0.5 ml of 1N Folin-Phenol reagent was added and rapidly mixed. The mixture is allowed to stand at room temperature for another 30 minutes.. Absorbance at 660nm was read with a Gilford Spectrophotometer. Bovine serum albumin was used for preparation of the standard curve.

(*) Reagent A was prepared immediately before use by mixing a,b,c, in the ratio of 100:0.5:0.5.

(a)4% Na_2CO_4 in 0.1N NaOH

(b)2% $CuSO_4.5H_2O$ in distilled water

(c)4% NaK tartarate in distilled water

carbohydrate analysis

Fractions of carbohydrate were first extracted step by step following the procedures shown in fig. 4. Fractionated carbohydrates were then assayed by the Phenol-sulfuric acid method (Trevelyan and Harrison, 1956).

Measurements of Physical Properties

apparent viscosity

Concentrated yeast cell suspensions and cell wall suspensions were prepared in phosphate buffer (pH 7), and a series dilutions were made from this concentrate with phosphate buffer. Each sample was placed in a 25^{0}C water bath and allowed to reach thermal equilibrium. Viscosity was measured at 25 ^{0}C with Brookfield Rotational Viscometer (model LVY,RVT, Brookfield Engineering Laboratories, Stoughton, MA). Readings were taken for every revolution until the indicator attained a constant value. Viscometer rotational rate was starting with lowest rotational speed and progress to the highest speed (for LVT 0.5-100rpm, RVT 0.3-60rpm). The shear stress (τ) and apparent viscosity (η) were calculated from the following equation (Brookfield Engineering Lab.)

$$\eta = \frac{\tau}{\dot{\gamma}}$$

$$\text{shear rate} \quad \dot{\gamma} = \frac{2\omega r_2^2 r_1^2}{r_i^2 (r_2^2 - r_1^2)}$$

$$\text{shear stress} = \frac{A(FST/100)}{2\ L \cdot r_1^2}$$

where r_1 = radius of spindle=1.2576cm.

r_2 = radius of container=1.3811cm.

w = angular velocity of the measuring spindle.

N = revolution per second.

L = length of spindle=9.2398cm.

FST = full-scale torque

LVT:637.7 dyne/cm.

RVT:7187.0 dyne/cm.

A = dial reading.

yield stress

Yield stress of each sample was obtained by applying Casson equation(Casson,1959) which is:

$$\sqrt{\tau} = \sqrt{\tau_0} + Kc\sqrt{\dot{\gamma}}$$

τ_0 = yield stress

Kc = constant

By ploting $\sqrt{\tau}$ versus $\sqrt{\dot{\gamma}}$ yield stress is calculated as the square of Y intercept.

Sedimentation properties

settling rate

10 ml of dilute suspensions (0.2-0.5 weight %) was placed in a 10 ml graduated cylinder, covered with paraffin film and allowed to set for 1 hour at room temperature (22-25^{0}C). The suspensions were then inverted 10 times and mixed well, the height of the interface between the settling material and supernatant were recorded as a function of time. Settling rate was measured by ploting the height percentage versus time and calculated from the slope (Michaels,1962;

Callaja et al, 1977). The height percentage is the ratio of the height of interface to the height of suspension.

hydrodynamic volume

The cell wall fractions were assumed to sediment as aggregates composed of cell clumps with occluded and associated water. The hydrodynamic volume, which is defined as the ratio of aggregate volume to the volume of solid material, and the mean aggregate diameter are related to sedimentation velocity by employing modified Stokes' law equation of Michaels and Bolger (1962):

$$Q_o = \frac{g\,(\rho_a - \rho_w)\,\overline{d_a}^2}{18\,\mu_w}(1 - \phi_a)^{4.65}$$

where Q_o = initial settling rate

g = gravitational acceleration

ρ_a = cellular aggregate density

ρ_w = fluid density

$\overline{d_a}$ = mean aggregate dismeter

μ_w = fluid viscosity

ϕ_a = aggregate volume fraction

Rearrange equation to:

$$Q_o^{\frac{1}{4.65}} = V_{sa}^{\frac{1}{4.65}}(1 - C_{a/cwm}\cdot\phi_{cws})$$

where V_{sa}= Stokes' settling velocity for a single aggregate and $C_{a/cwm}=\phi_a/\phi_{cws}$ And then:

$$V_{sa} = \frac{g\ (\rho_{cws} - \rho_w)\ \overline{d_a}^2}{18\mu_w\ C_{a/cwm}}$$

ρ_{cws} =Density of cell wall material.

If one plots $Q^{\frac{1}{4.65}}$ against the corresponding values of volume fraction (ϕ_{cws}) a straight line should result. From the intercept of the abscissa, $C_{a/cwm}$ can be determined and from the ordinate intercept, $\overline{d_a}$ can be calculated. The density of cell wall suspension and wall materials were determined by pyconometer method.

Microscopy

Light microscopy: Whole cells and cell walls were observed and photographed with a phase constrast light microscope at the magnification of 625 or 1000.

Scanning electron microscopy: Samples were dehydrated through increasing concentration of ethyl alcohol solutions. The dried samples were mounted on polished stubs and coated with gold by a spatter coater (model No.12121, SPI, Westchester,PA). Pictures were taken with a Scanning electron microscope (Cambridge Strescan, Cambridge, England.).

RESULTS AND DISCUSSIONS

Morphology

The strain, Saccharomyces cerevisiae JD7, previously isolated by DeAngelo (1978) from a mutagenized culture of S. cerevisiae A364A, has been characterized as a cell division cycle mutant whose division is blocked after nuclear migration to the bud neck. Morphological properties of the yeast cells as observed with both light and scanning microscope are described in Table 2.

TABLE 2

Morphology of Yeast Cells on Different Growth Media

Strain	Growth Medium-SDC	Growth Medium-YPD
Saccharomyces cerevisiae A364A	spherical and yeast-like, diameter=5μ	same as left
Saccharomyces cerevisiae JD7	yeast-like, elongated aggregated.	mycelium-like, 3-5 times longer than the normal cells, branched, entangled and aggregated.

Figure 5 shows that the three dimensional structure of strain JD7 in the aggregated mycelium-like state consists of branches of undivided yeast cells. With a fluorescent nuclear staining technique, DeAngelo(1978) characterized the mycelium like cells of JD7 as branches of undivided yeast cells containing only one nucleus between the first and second cell. The nutritional signal for dimorphism of JD7 remains unknown. The morphology of cell wall residues collected after hot alkaline treatment, corresponded exactly to those of the whole cells from which they are derived, except that they appeared darker in the phase contract microscope and no cytoplasmic materials could be detected. Yeast cells and cell walls of different morphologies are shown for comparism in figures 7 through 12. Shown in figure 6, is the scanning electron microscopic picture of the JD7 mycelium-like cell wall material. The cell wall material was observed to be collapsed and flat, this suggesting that the cell wall residues are empty sacs composed mainly of cell wall polysaccharides.

Chemical Composition

Data obtained as the chemical composition of yeast whole cell is summarized in table 3.

The protein and total carbohydrate content of these two strains were similar. Glycogen and trehalose contents varied between the two strains and the carbohydrate contents of

TABLE 3

Chemical Composition of Yeast Cells

(%)	JD7-YPD*	JD7-SDC	A364A-YPD	A364A-SDC
Trehalose	4.5±0.4	3.2±0.8	10.1±0.6	5
Alkali-soluble Glycogen	5.4±0.7	6.4±1.1	5.7±0.2	9
Total Glycogen	17.3	18.2	13.8	20.3
Mannan	12.8±0.1	11.6±0.4	16.3±0.4	12.1
Glucan	14.6±0.9	13.8±o.3	12.1±0.5	11.5
Total Carbo-hydrate	50	43	49	41
Protein	54	45	52	43

* Strain-Medium

both strains were found to be dependent upon growth conditions. Of perhaps more significance, are the polysaccharide contents of the cell walls and their dependence on morphology. By comparing the glucan and mannan contents in these yeast samples, no firm conclusion on the relation of composition and the morphology can be drawn, although the glucan contents of mycelium-like forms seemed to be higher than that of the yeast-like forms, and the mannan content was higher in the parent strain A364A. These differences may be due to the changes in the structural build-up within the yeast cell wall through mutation.

Detailed structural analysis of yeast polysaccharides can be done by various analytical techniques, such as selective alkaline degradation, selective acid hydrolysis, selective acetolysis, enzymatic degradation, NMR spectroscopy, immunological methods, etc.(Ballou, 1976). However, none of these chemical methods is useful in quantatively characterizing the bulk structural properties, which are considered to be more relevant for some applications.

The protein and mannan contents of the yeast cells grown in chemically defined medium, SDC, were lower than those of cells grown in rich medium. This may be a result of an amino-acid limitation in the defined medium.

Chemical analysis data of cell wall residues after hot alkaline treatment is shown in Table 4.

The percentage of recovery, i.e. the ratio of the dry weight of cell wall residue to the dry weight of whole cells before treatment ranged from 33 to 39%. All the cell wall residue samples were found to be high in carbohydrate, mainly glucan and glycogen. Protein and mannan were not detected in these residues.

TABLE 4

Chemical Composition of Yeast Cell Wall

Composition(%)	JD7-YPD*	JD7-SDC	A364A-YPD
Trehalose	-	-	-
Alkali-soluble Glycogen	0.7	1.4	1.6
Acid-soluble Glycogen	39.8	31.2	37.9
Total Glycogen	40.5	35.6	38.3
Mannan	ND	ND	ND
Glucan	39.5	35.9	38.3
Total carbohydrate	78	75	80
Protein	ND	ND	ND

ND - not detected
*strain-medium

Rheological Properties of Yeast Suspensions

viscosity profiles

To compare the gross structural properties of yeast suspensions, viscometric measurement were done as described in Materials and Methods. In figures 13-15, the relations between viscosity and shear rate at different dry weights and morphologies are summarized. In all the cases, a shear-thinning effect was observed. That is, as the shear rate increases, the viscosity decreases and approaches a constant apparent viscosity. It was also noted that, the more concentrated yeast suspensions require a higher shear rate to reach a constant value. This phenomenon can be explained as perhaps being due to the existence of a cellular network structure in the suspensions, which is in a dynamic state,

continueously being built-up and brokendown. As the shear rate increases the network structure is completely destroyed and the system then behaves as a Newtonian fluid.

The viscosity-dryweight relationships for spherical cells of S. cerevisiae strain A364A, yeast-like and mycelium-like cells of S. cerevisiae strain JD7 are presented in Figure 16. All these cells were collected at stationary growth phase when the distinctive morphologies were fully expressed. The viscosity of whole cell suspension was found to be a function of the dry weight concentration and morphology of yeast cells. The spherical cells of control strain S. cerevisiae A364A had little contribution to the viscosity of suspension up to a concentration as high as 70 gm/l. Suspensions of mycelium-like cells, on the other hand, exhibited much higher viscosities than the others. The viscosity of mycelium-like cells suspension begins to increase drastically with increasing dry weight concentraiton at a concentration of 25 g/l. Suspensions of flocculated and elongated cells of JD7 grown in SDC had moderate viscosities, with a critical concentration (the concentration at which the viscosity begins to approach infinty) in between the other two extremes. The considerably larger size and the extensive intercellular interaction are the most important features responsible for higher viscosities. The relationships of apparent viscosity and shear rate of wall materials after hot alkaline treatment are shown in

figures 17-19. For all the morphologies, the viscosity of wall material was much higher than that of whole cells. The viscosity of cell wall materials was also found to be dependent upon the concentration of cell walls and the morphology of the intact cells. Presented in figure 20 is a summary of the viscosity profiles of whole cells and cell walls. Also noteworthy is the observation that the viscosity of the mycelium-like cell wall suspensions was extremely high, with a critical concentration as low as 10 gm/l. If the dry weight of cell wall material is converted into the weight of the whole cells from which they were derived, a profile is obtained as shown in the dotted line in figures 21-23. The viscosity-concentration relationship can thus be compared on the basis of the number or volume of cell mass in the suspension, assuming equal volumes of whole cells and cell wall material. It was interesting to find that the viscosity profiles of cell wall material after conversion to the original dry weights are very similar to that of whole cell. This suggested that it is the cell unit concentration that determines the viscosity of cell suspension. The dramatic thickening effect of mycelium-like cell wall material and the simplicity in its chemical composition suggest its possible use as a food thickening agent.

yield stress

To compare the thickening properties with other commerical food thickeners, the yield stress of the mycelium-like cells and cell walls were estimated by the Casson equation and the results are shown figure 24 as a function of dry mass concentration. The mycelium-like cell wall residues exhibit a high yield stress when compared to that of whole cells and the value is comparable to that of carboxymethyl cellulose. Yield stress is generally employed to approximate the stress required to start a flow. A high value of yield stress suggests the ability of these materials to stabilize fluids.

hydrodynamic volume

To study the thickening mechanism of yeast cell wall residues, the hydrodynamic volume (Ratio of aggregate volume to wall material volume) was estimated by employing a modified Stokes' law (Michaels and Bolger, 1962). The sedimentation data for yeast-like and mycelium-like JD7 cell walls were shown in figures 25 & 26, respectively. The initial settling rates (Q_o) were estimated from the slope of the linear regions of these curves. A plot of $Q_o^{\frac{1}{4.65}}$ versus the corresponding value of volume fraction resulted in straight lines (figures 27 and 28). From the slopes and abscissa intercepts of these lines, hydridynamic volume ($C_{a/cwm}$) and mean aggregate diameter ($\overline{d_a}$) were derived.

A summary of results are in Table 5.

The derived mean aggregate diameters were close to that actually observed for cell wall materials under the microscope. The large values for hydrodynamic volume indicated that one volume of the cell wall material was capable of holding a large amount of fluid during sedimentation. The fluid may either be occluded in the cytoplasmic cavities or entrapped between the loosely packed settling aggregates.

TABLE 5

Hydronamic Volume and Mean Aggregate Diameter of Yeast Cell Walls

Morphology	$\overline{d_a}(\mu)$	$C_{a/cwm}$
Mycelium-like JD7	167	139
Yeast-like JD7	113	98

*$\overline{d_a}$ = mean aggregate diameter
$C_{a/cwm}$ = hydrodynamic volume

The thickening effect of the resulting cell wall materials can thus be explained in part as due to their large water holding capacity.

CONCLUSIONS

1. Saccharomyces cerevisiae strain JD7 has similar chemical composition as its parent strain, Saccharomyces cerevisiae A364A, although their cellular morphologies were appearently different.

2. The distinct morphology of the mycelium-like strain JD7 is most likely due to changes in the chemical structure of the yeast cell wall polysaccharides, especially glucan.

3. Yeast cell wall fractions isolated by hot alkaline treatment from either strain were high in carbohydrate, mainly glucan and alkali-insoluble glycogen. The cell wall fractions maintained the same shapes as the orginal cells, but contain no protein or cytoplasmic material and mannan.

4. The viscosity profile of yeast suspensions mainly depends on the morphology of the cells. Larger and more branched cells, i.e. S. cerevisiae JD7 in the mycelium-like form results in suspensions with higher viscosity than others. Volume fraction is one of the controlling parameters. However, interactions between cells are believed to

play an important role in creating high viscosity of cell suspensions.

5. The viscosity of cell wall fractions is extremely high when compared to whole cells at the same dry weight concentration. The critical concentration of the cell wall fraction of mycelium-like JD7 is as low as 10 gm/l (1%). The high viscosity and yield stress in such yeast cell wall preparation suggest the possibility of employing this material as a food thickening agent.

6. By series of observations conducted in this investigation, it was found that the following factors are responsible for the rheological properties of yeast cells and cell-wall preparations:

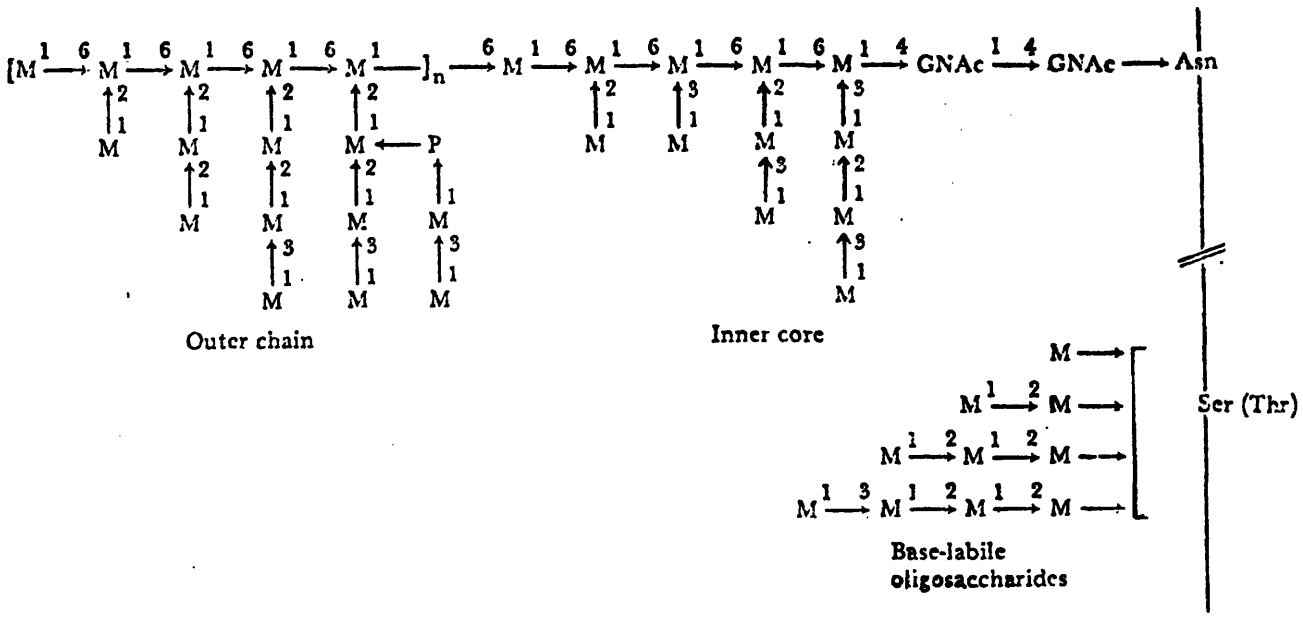
   a) The three dimensional structure of yeast glucan, which is responsible for retaining the shape of the cells.

   b) The volume occupied by the hydrated material. The larger the hydrated volume the higher the viscosity.

   c) The surface properties of yeast cells. Surface properties result in various interrelationships between single units of cells. Cell aggregation and floccuation may take place depending

on a given surface property and consequently results in a distinctive rheological behavior of the yeast preparation.

d) The ability to immobilized water, either by occluding water in sac-like structures of the yeast cell wall or entrapping it in between the loosely packed cellular aggregates.



M: Mannose

GNAc : N-Acetyl-Glucosamine

Detailed structure of the mannan from S. cerevisiae showing the base-labile oliglsaccharides arrached to hydroxyamino acids, the inner core and the outer chain.
All anomeric linkages have the α-configuration except for the trisaccharide unit: βMan(1→4)βGNAc(1→4) GNAc linked to asparagine. (From Ballou, 1976)

Figure 1: Chemical Structure of Yeast Mannan

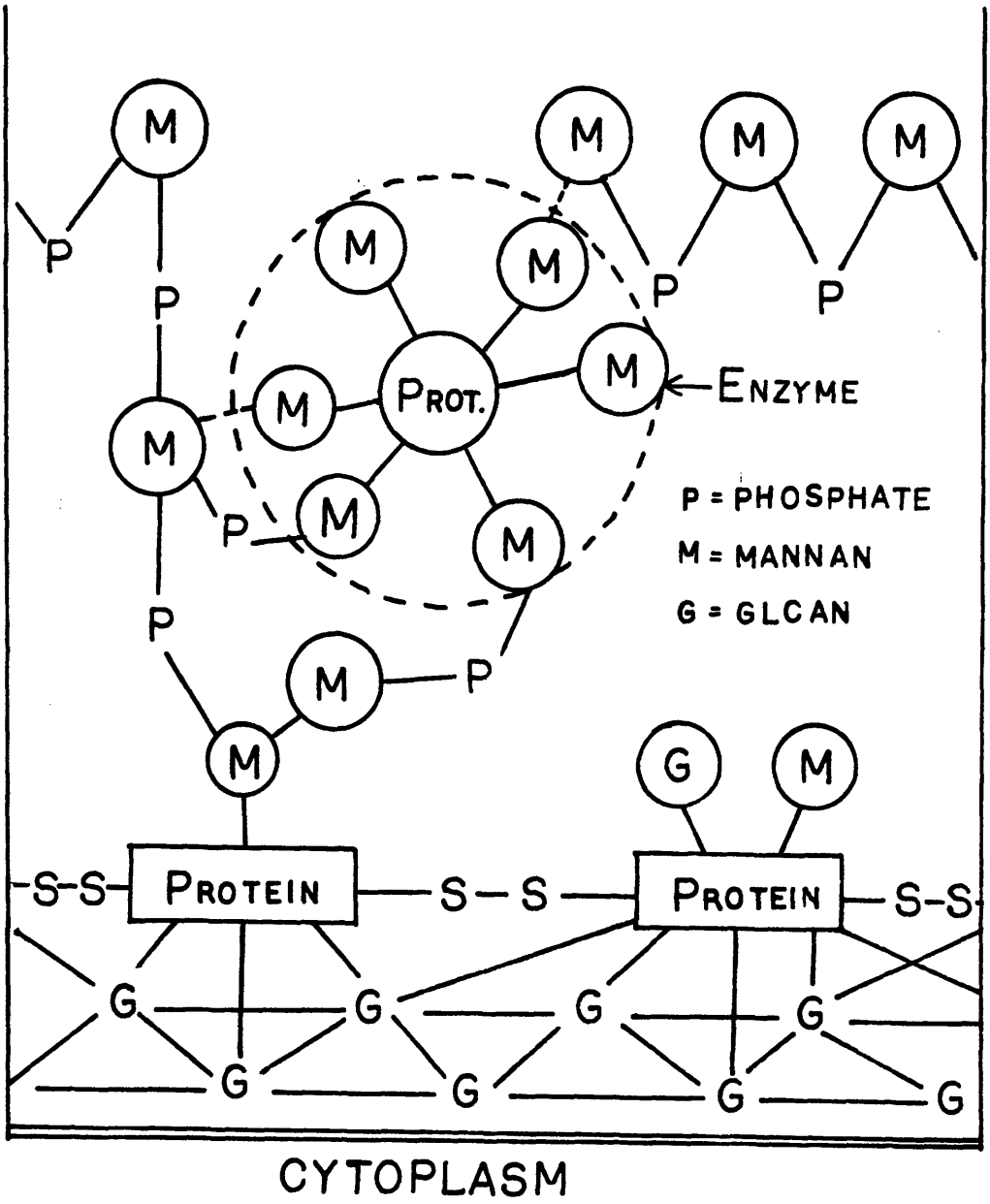


Figure 2: Schematic Structure of Yeast Cell Wall

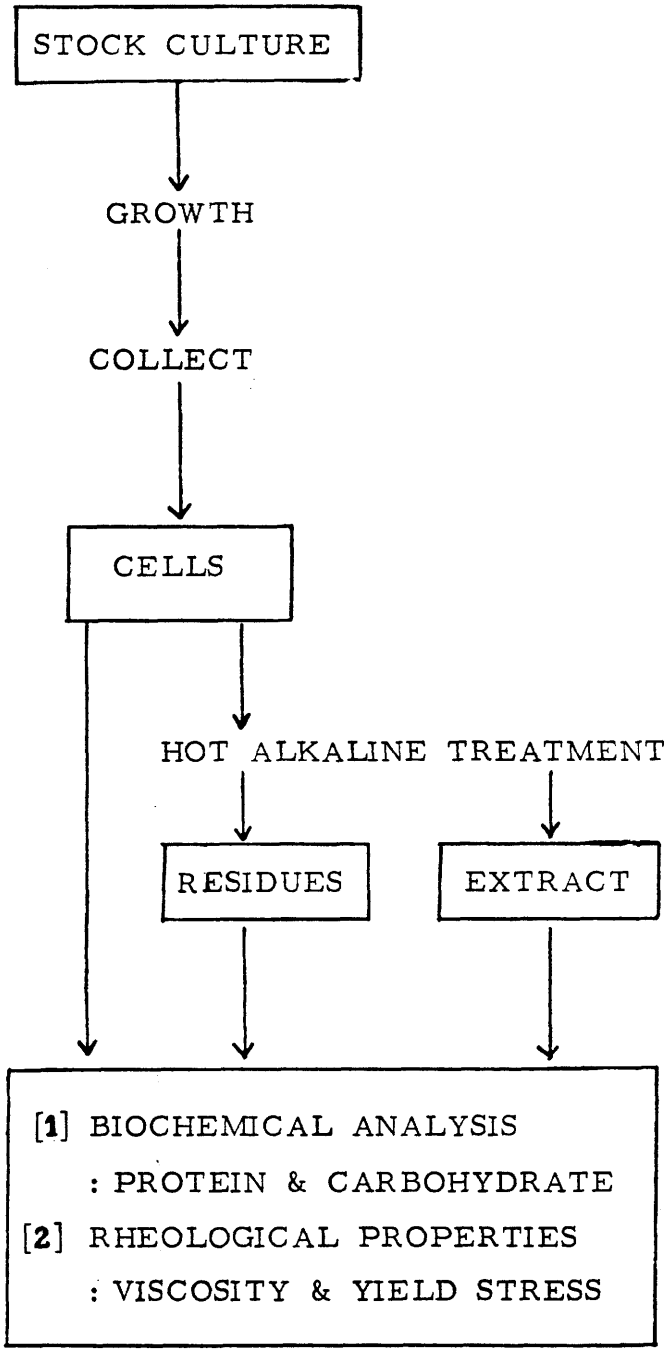


Figure 3: Flow Sheet for Experimental Procedures

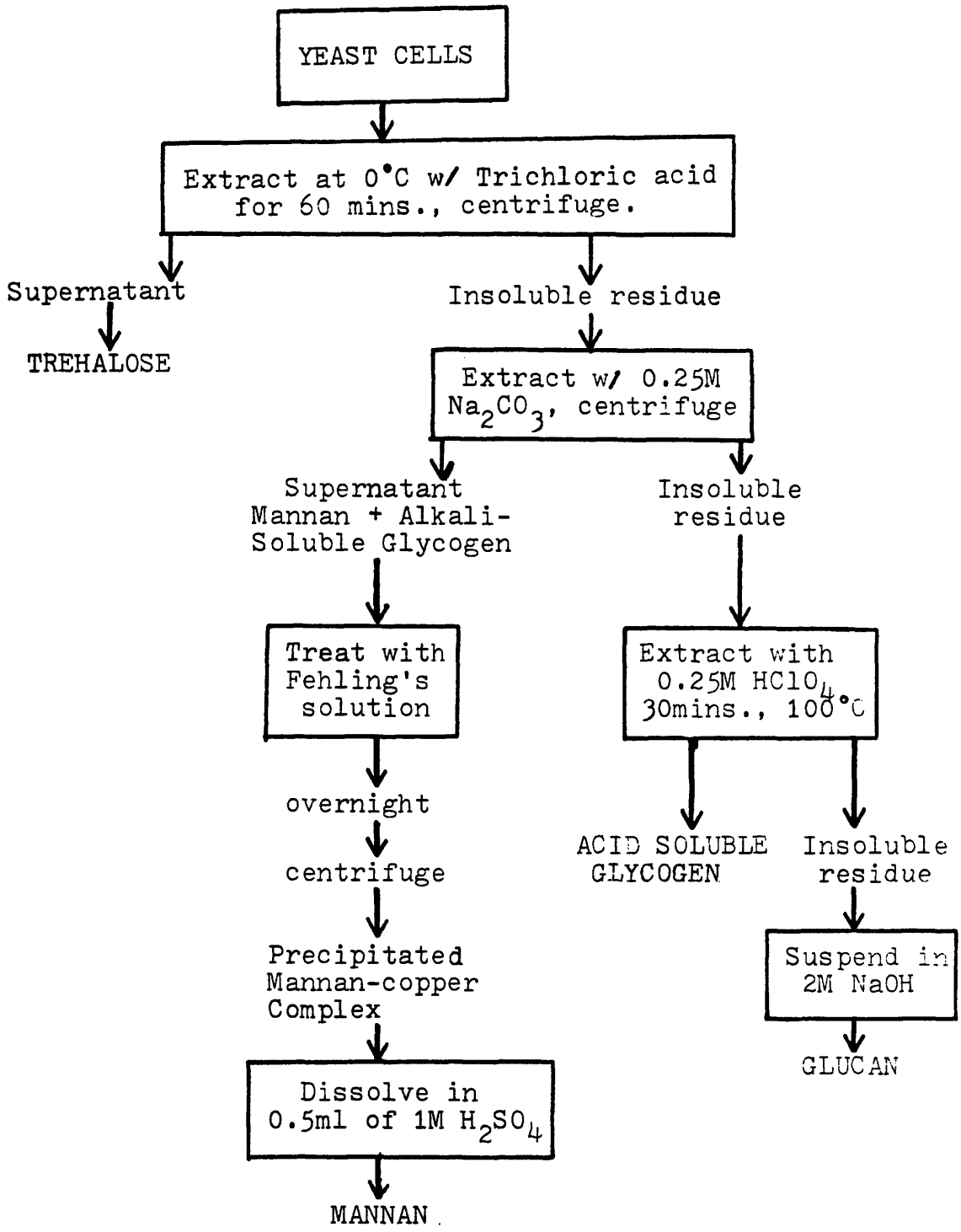


Figure 4: Extraction of Carbohydrate from Yeast Cells

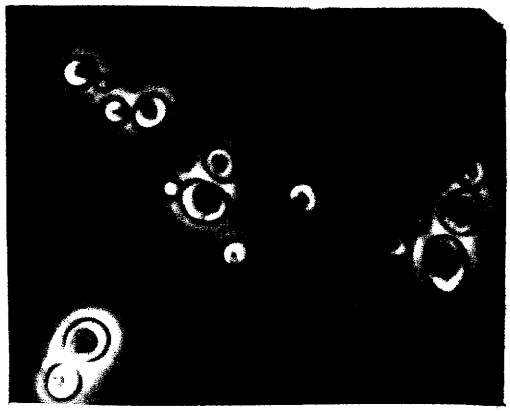


Fig.7. Morphology of S.cerevisiae A364A(x1000)

Fig.8. Cell wall residues of S. cerevisiae A364A(x1000)

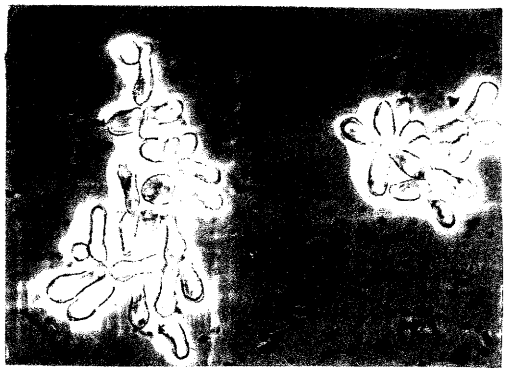


Fig.9. Morphology of S. cerevisiae JD7(x625) grown in SDC.

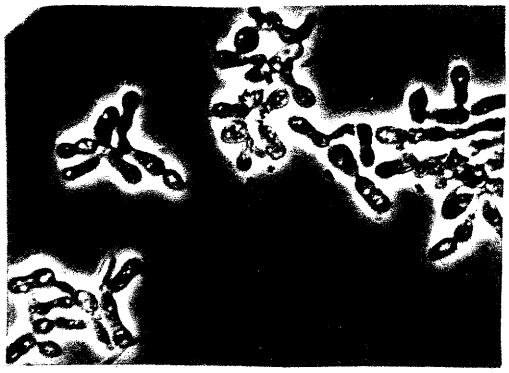


Fig.10.Cell wall residues of S. cerevisiae JD7(x625) grown in SDC.

Fig.11. Morphology of S. cerevisiae JD7(x625) grown in YPD.

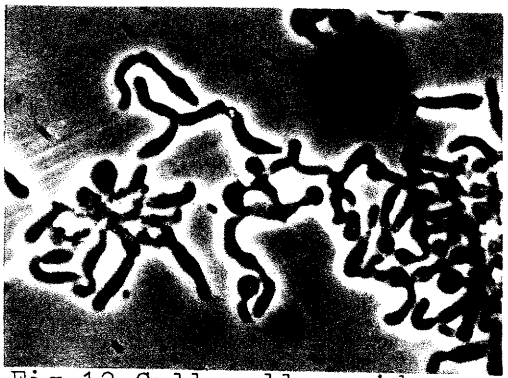


Fig.12.Cell wall residues of S. cerevisiae JD7(x625) grown in YPD.

Fig. 5. Three dimensional structure of mycelium-like JD7 aggregates(x2000).

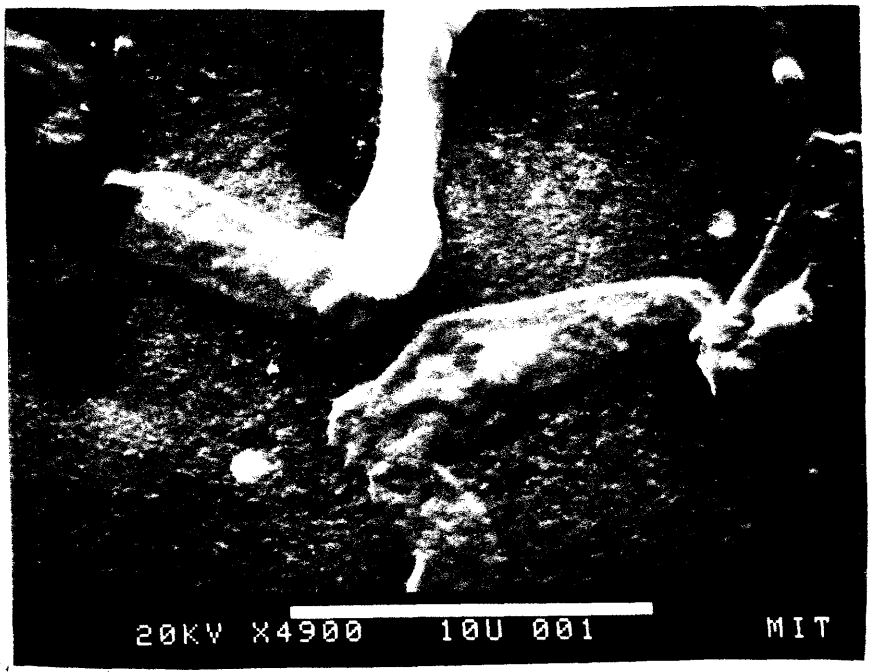


Fig. 6. Collapsed mycelium-like cell wall. Picture taken by scanning electron microscope(x4900).

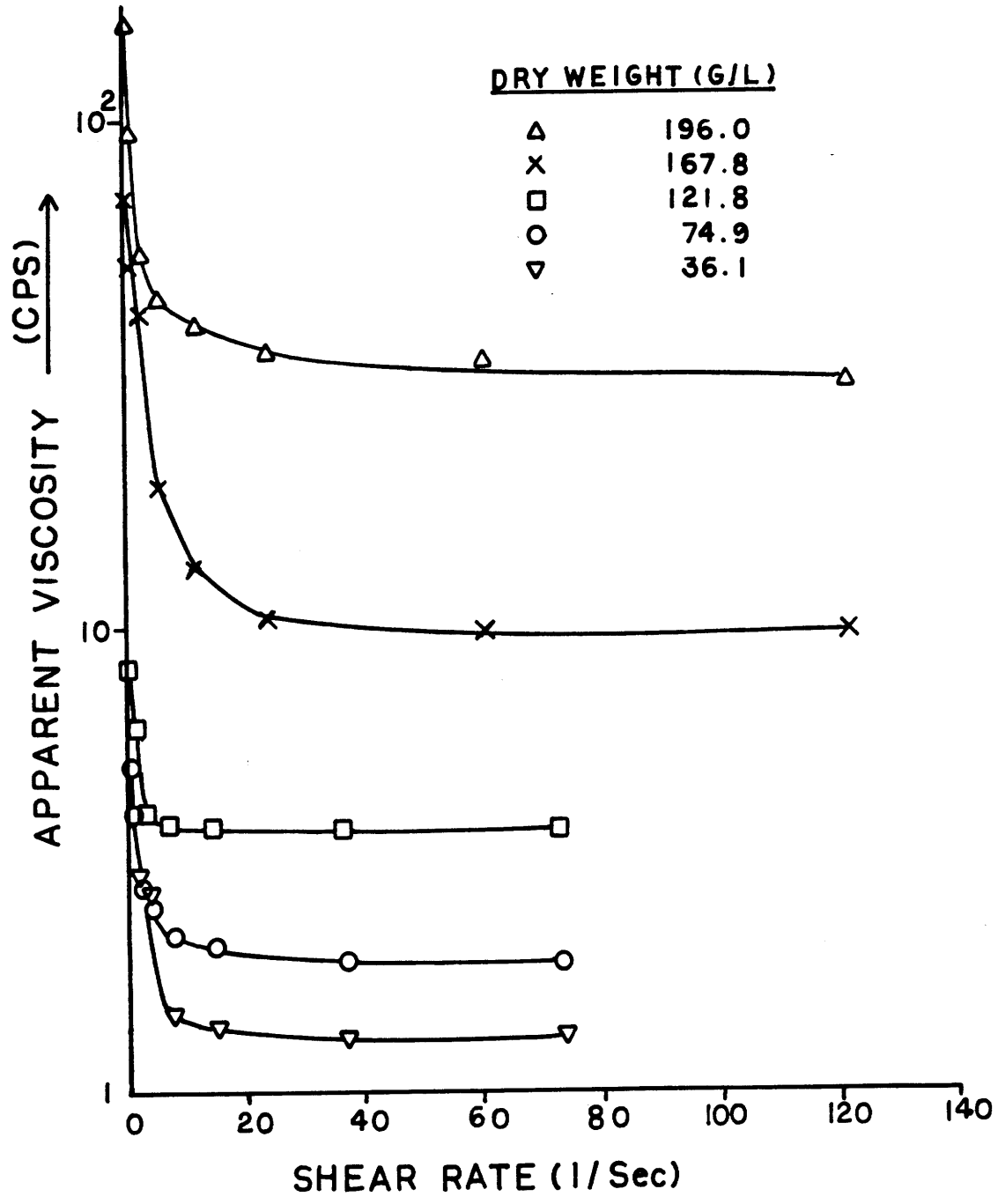


Figure 13: Effect of Shear Rate on Apparent Viscosity of Saccharomyces cerevisiae A364A Cell Suspensions

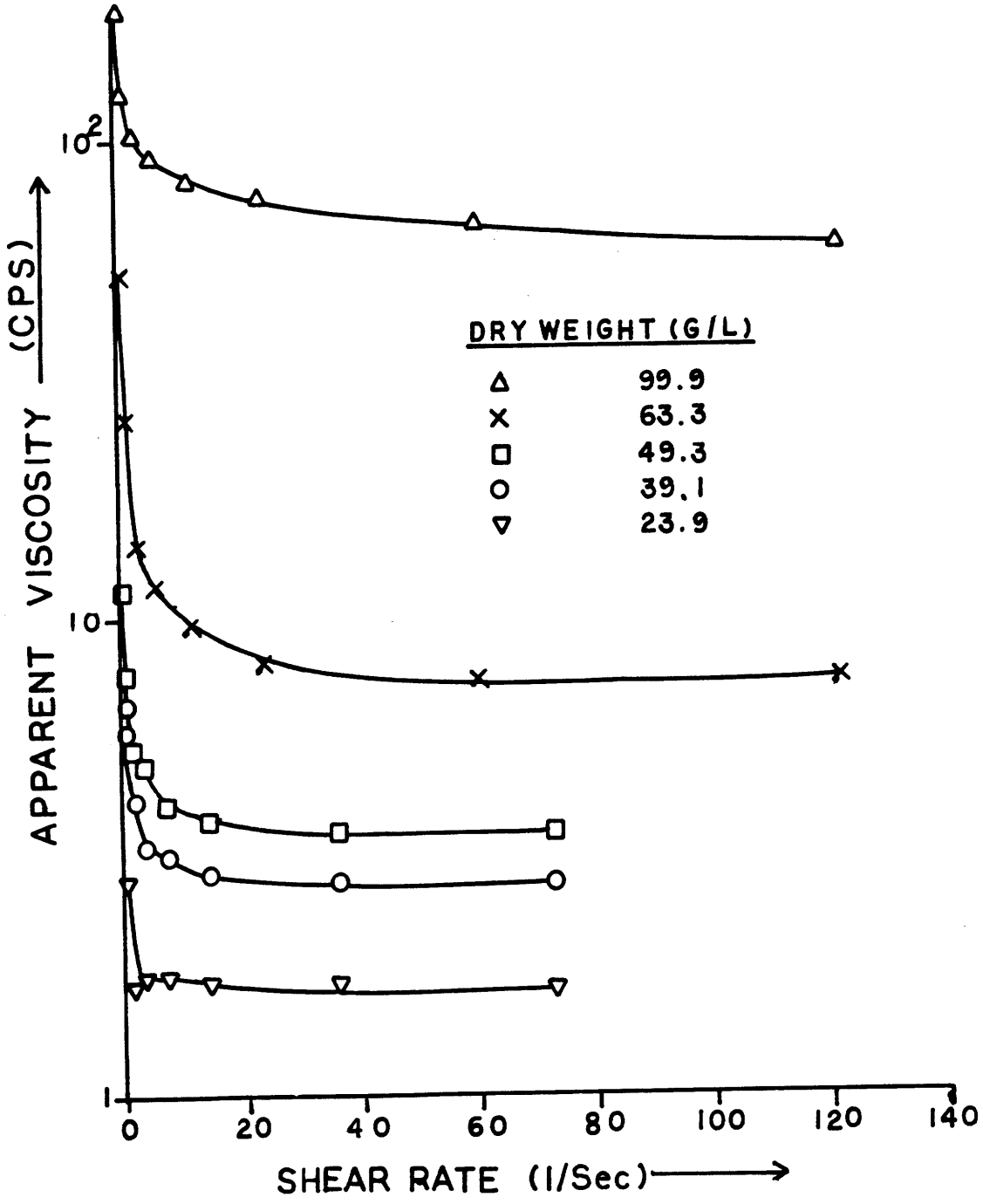


Figure 14: Effect of Shear Rate on Apparent Viscosity of Yeast-like Saccharomyces cerevisiae JD7 cell Suspensions

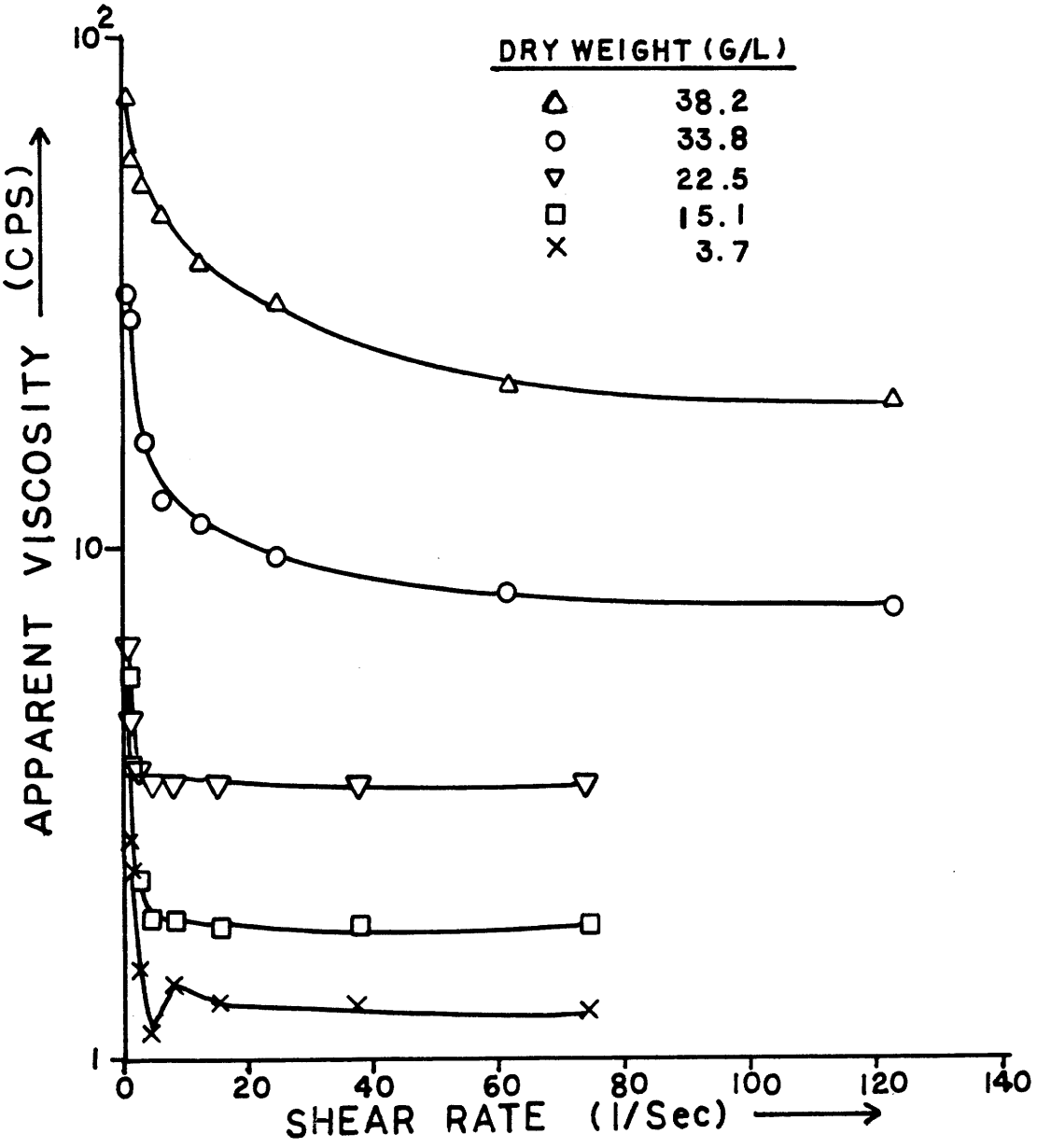


Figure 15: Effect of Shear Rate on Apparent Viscosity of Mycelium-like Saccharomyces cerevisiae JD7 Cell Suspensions

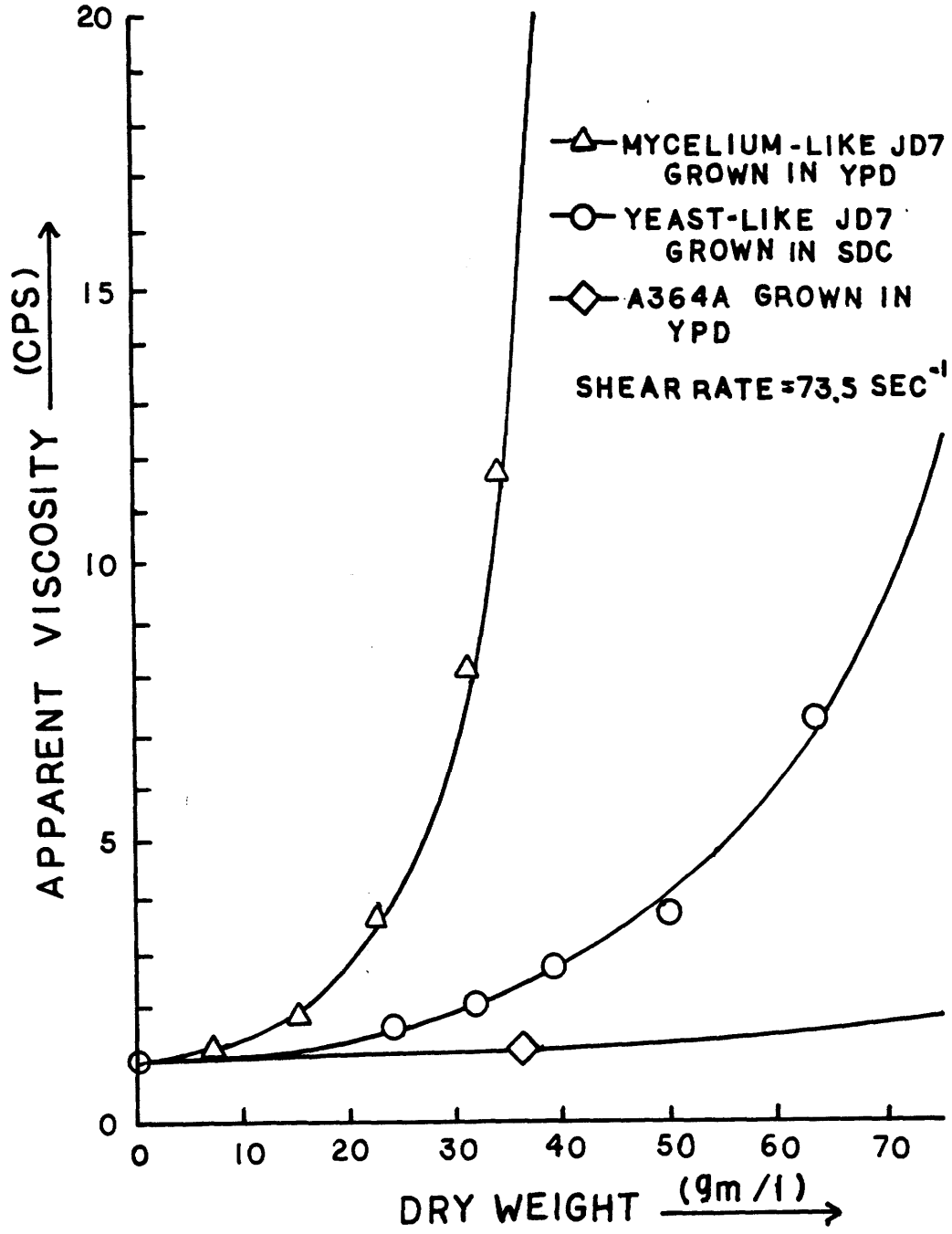


Figure 16: Viscosity Profiles of Three Different Morphologies

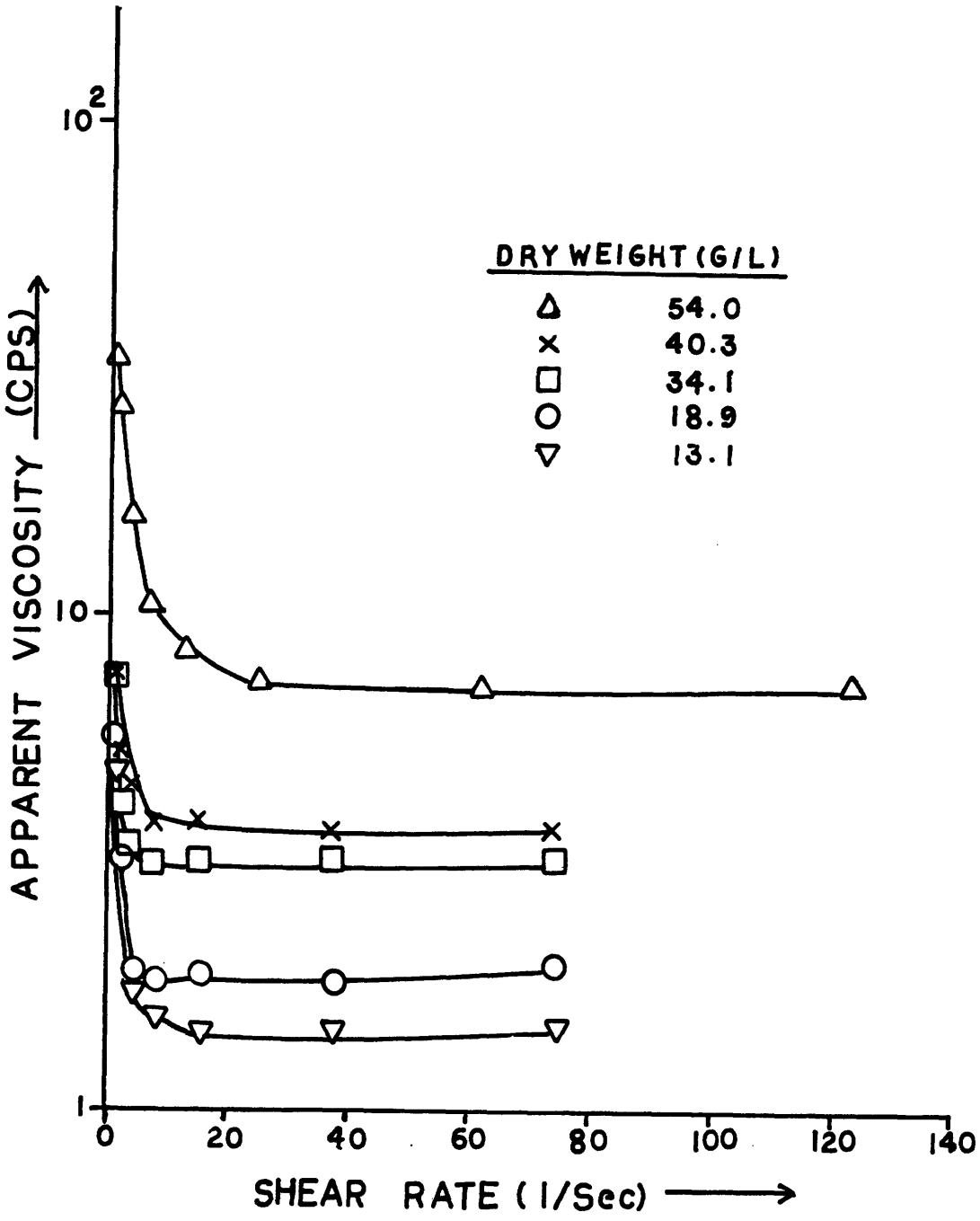


Figure 17: Apparent Viscosity-Shear Rate Plot of Cell Wall Suspensions of Saccharomyces cerevisiae A364A

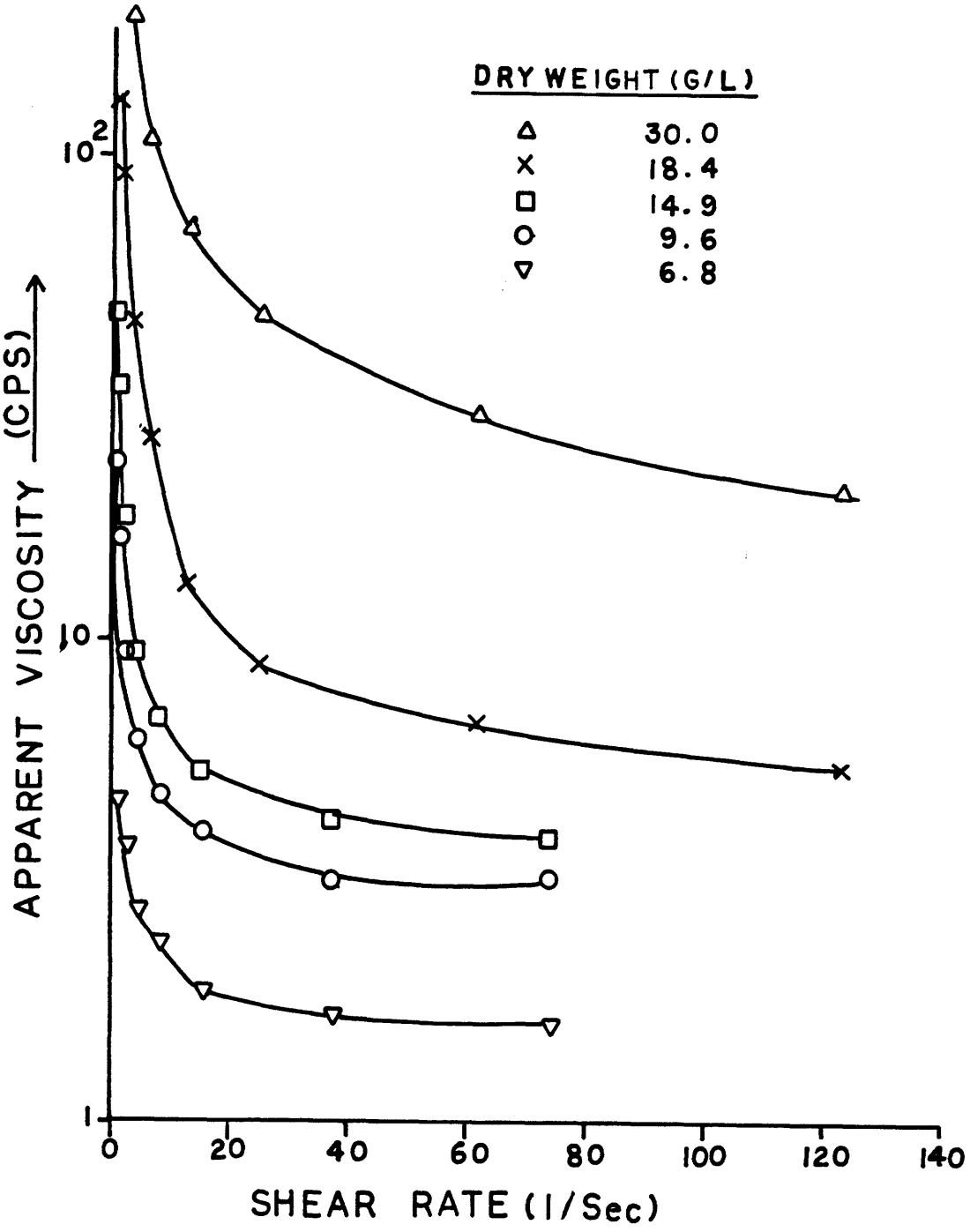


Figure 18: Apparent Viscostiy-Shear Rate Plot of Cell Wall Suspensions of Yeast-like Saccharomyces cerevisiae JD7

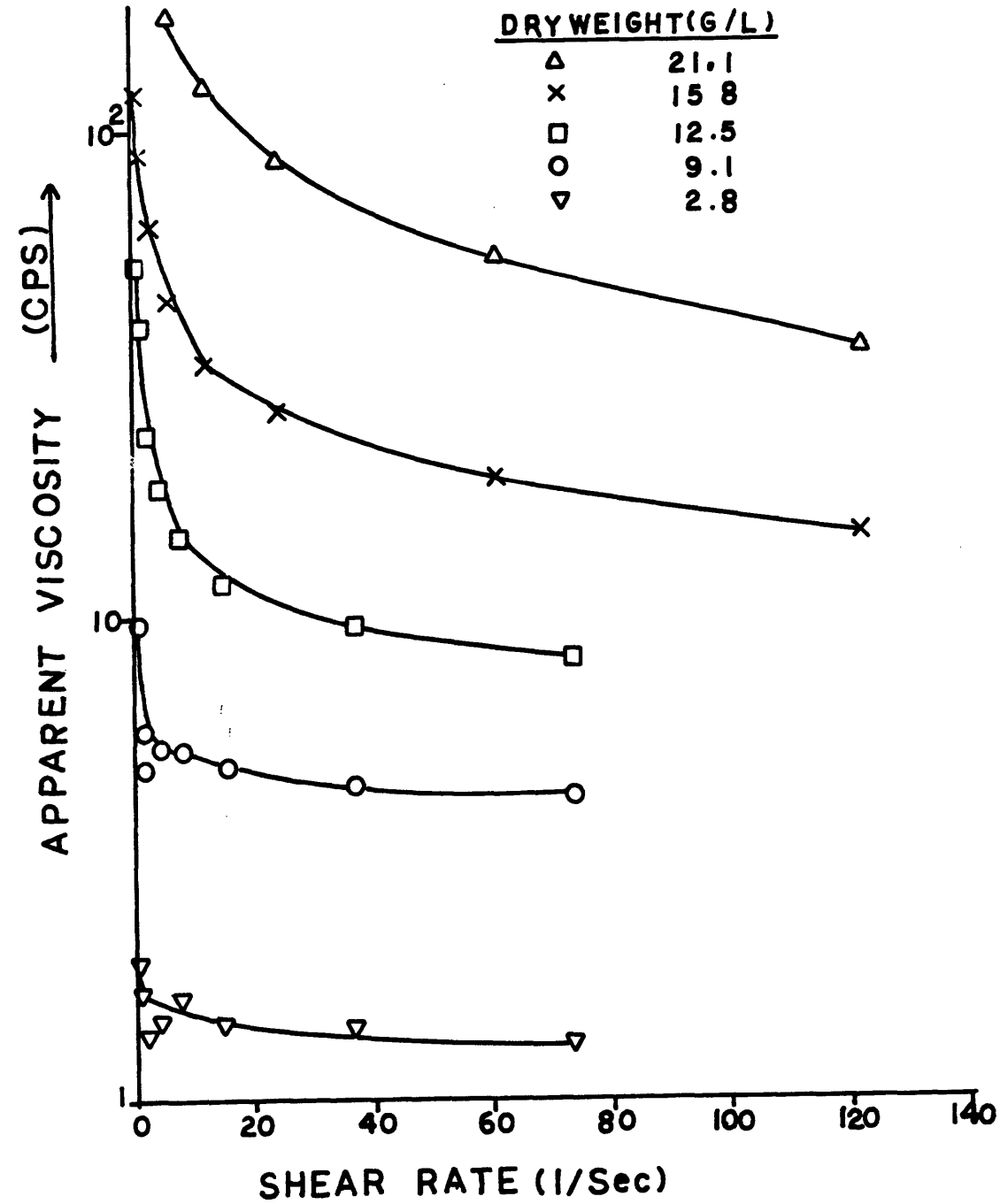


Figure 19: Apparent Viscosity-Shear Rate Plot of Cell Wall Suspensions of Mycelium-like Saccharomyces cerevisiae JD7

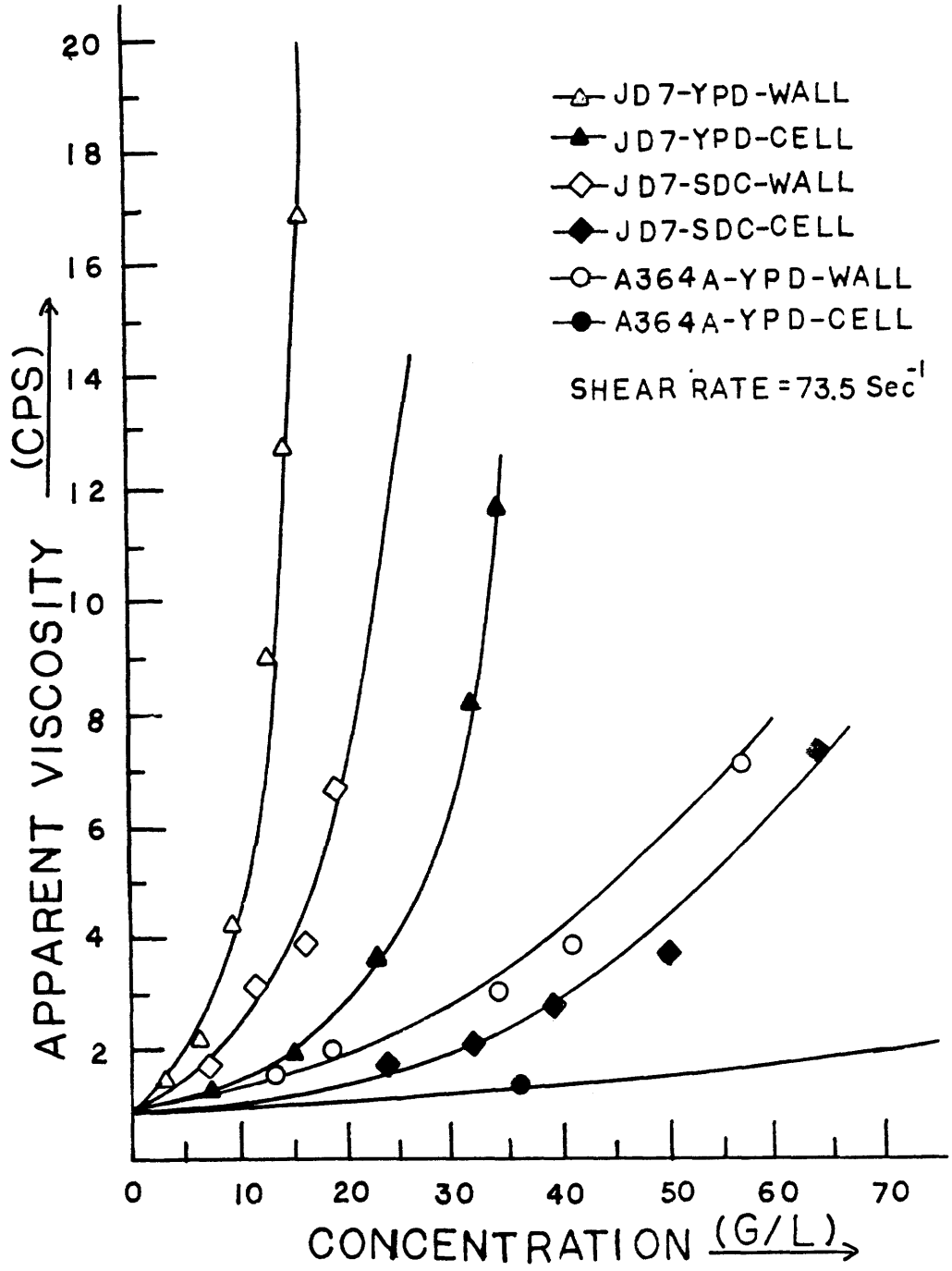


Figure 20: Summary of Viscosity Profiles of Yeast Cells and Cell Walls Suspensions

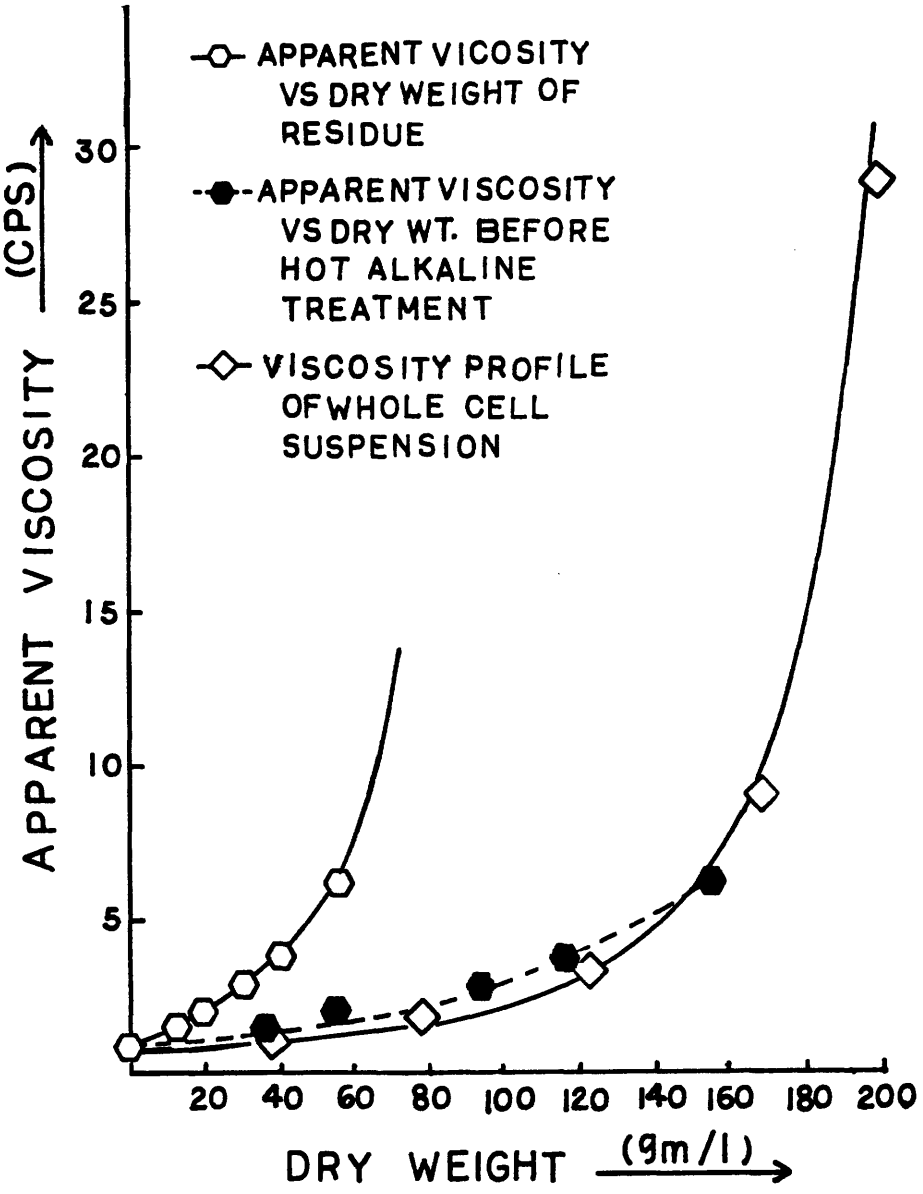


Figure 21: Viscosity Profile of Cell Wall Suspensions of Saccharomyces cerevisaie JD7 Grown in SDC

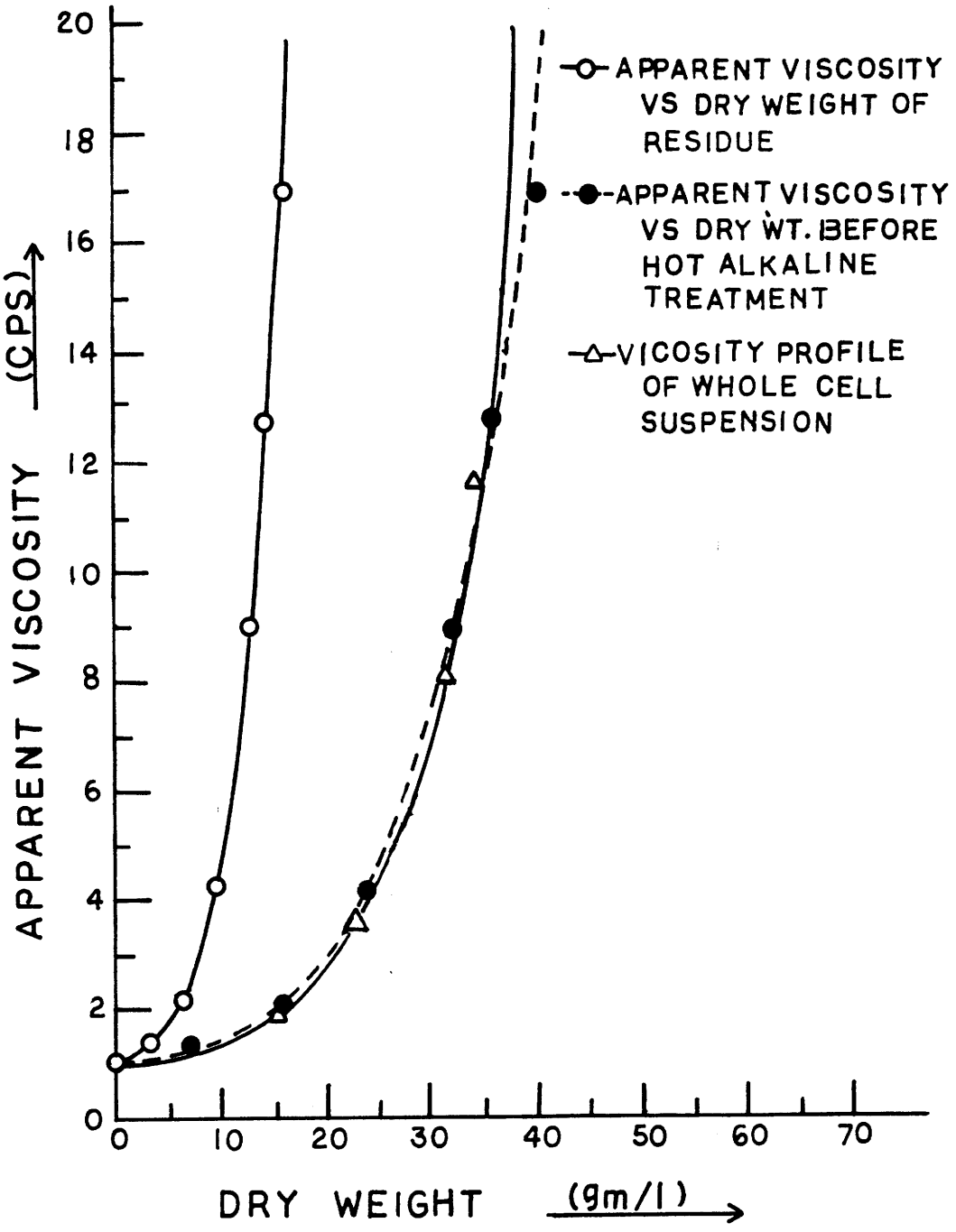


Figure 22: Viscosity Profile of Cell Wall Suspensions of Mycelium-like Saccharomyces cerevisiae JD7

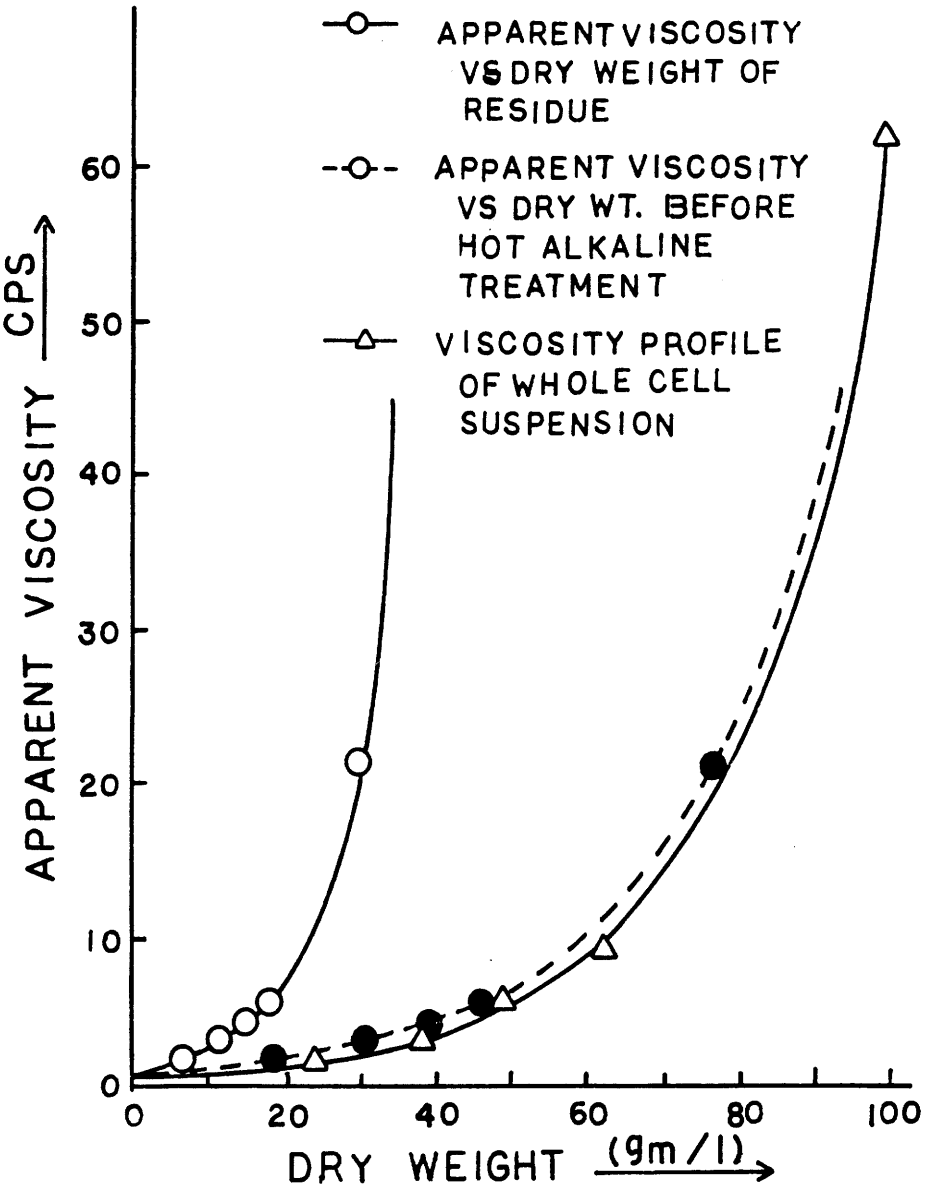


Figure 23: Viscosity Profile of Cell Wall Suspensions of Saccharomyces cerevisiae A364A

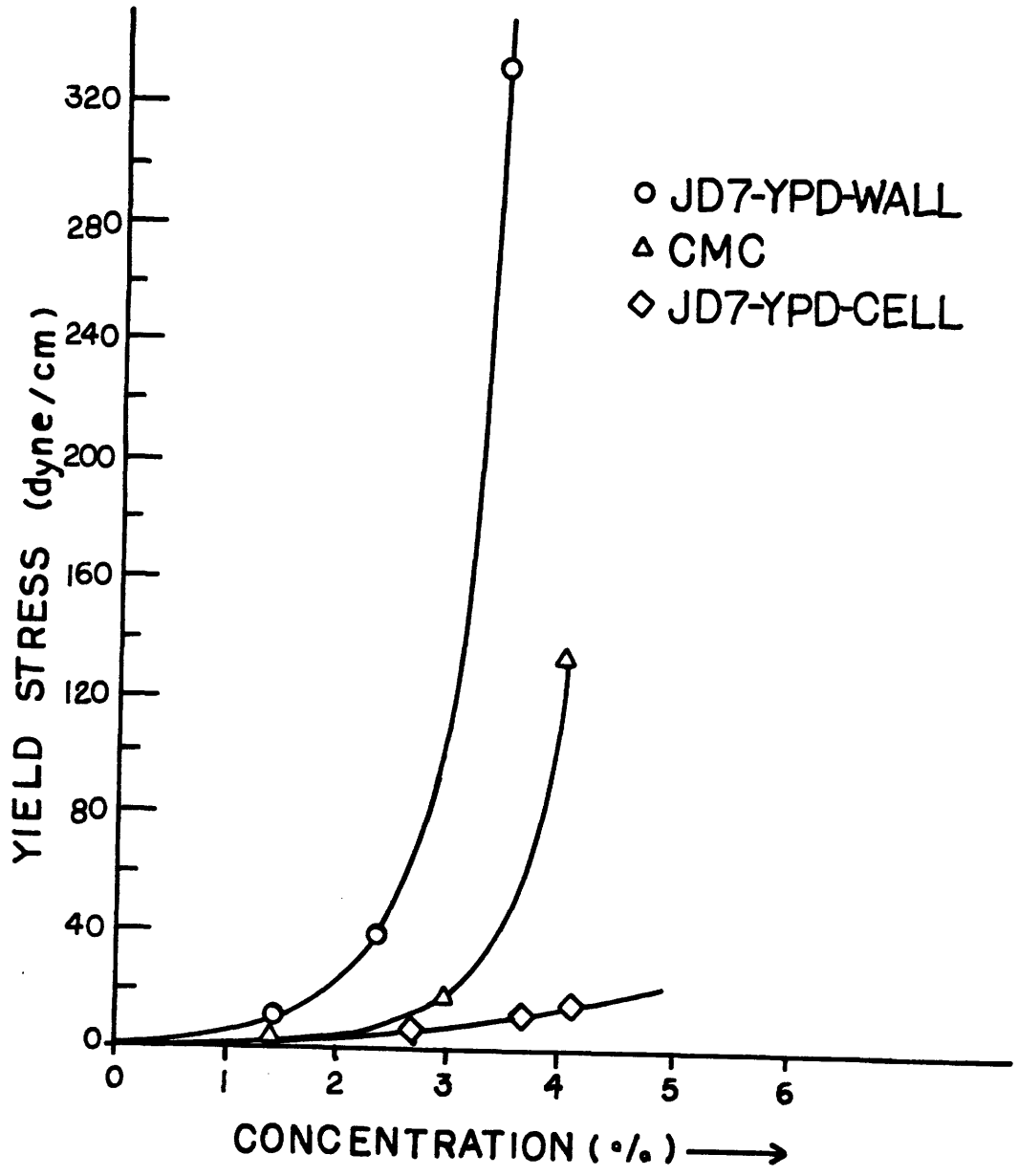


Figure 24: Yield Stress-Concentration Relationships of Mycelium-like Cell Wall Suspensions and CMC

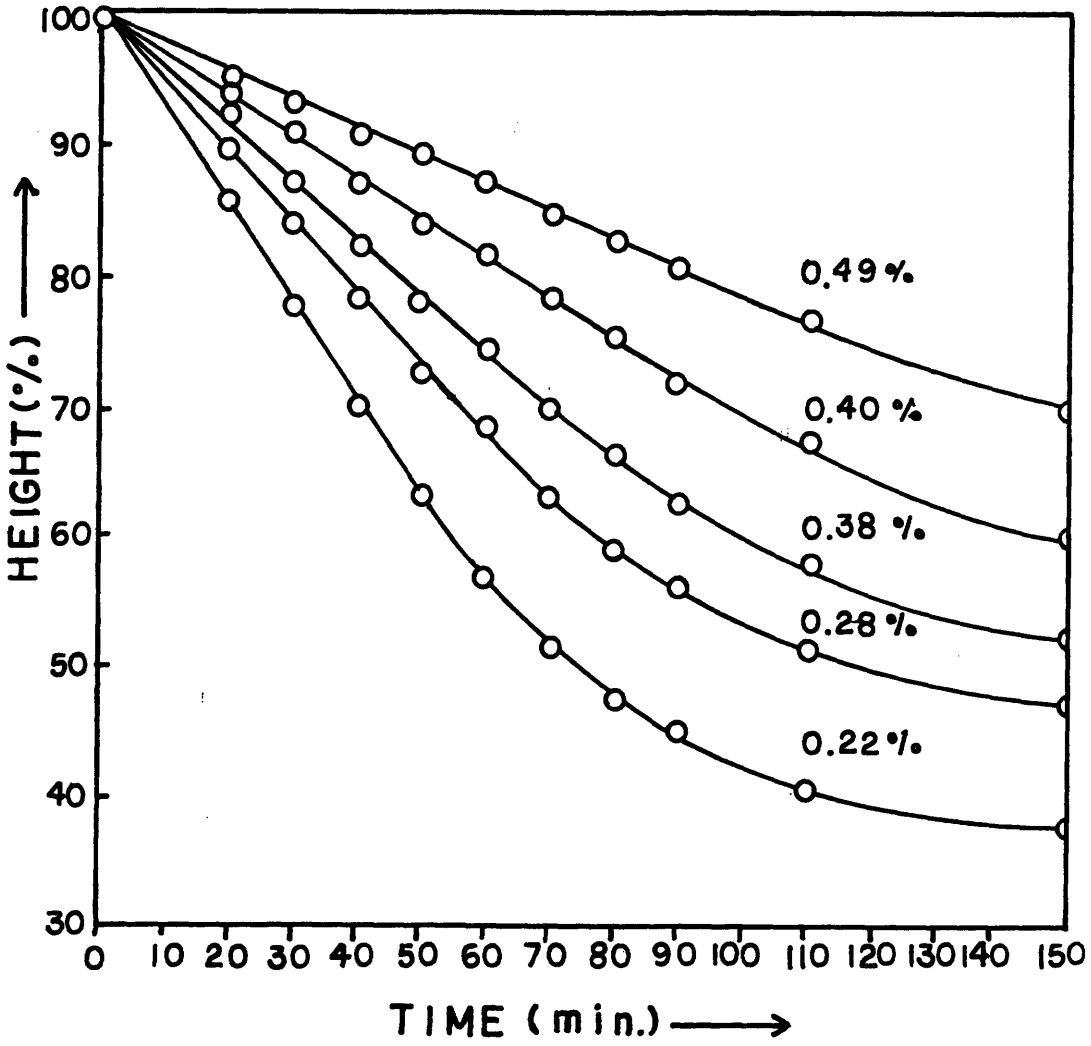


Figure 25: Sedimentation Rate of Yeast-like Saccharomyces cerevisiae JD7 Cell Wall Residues

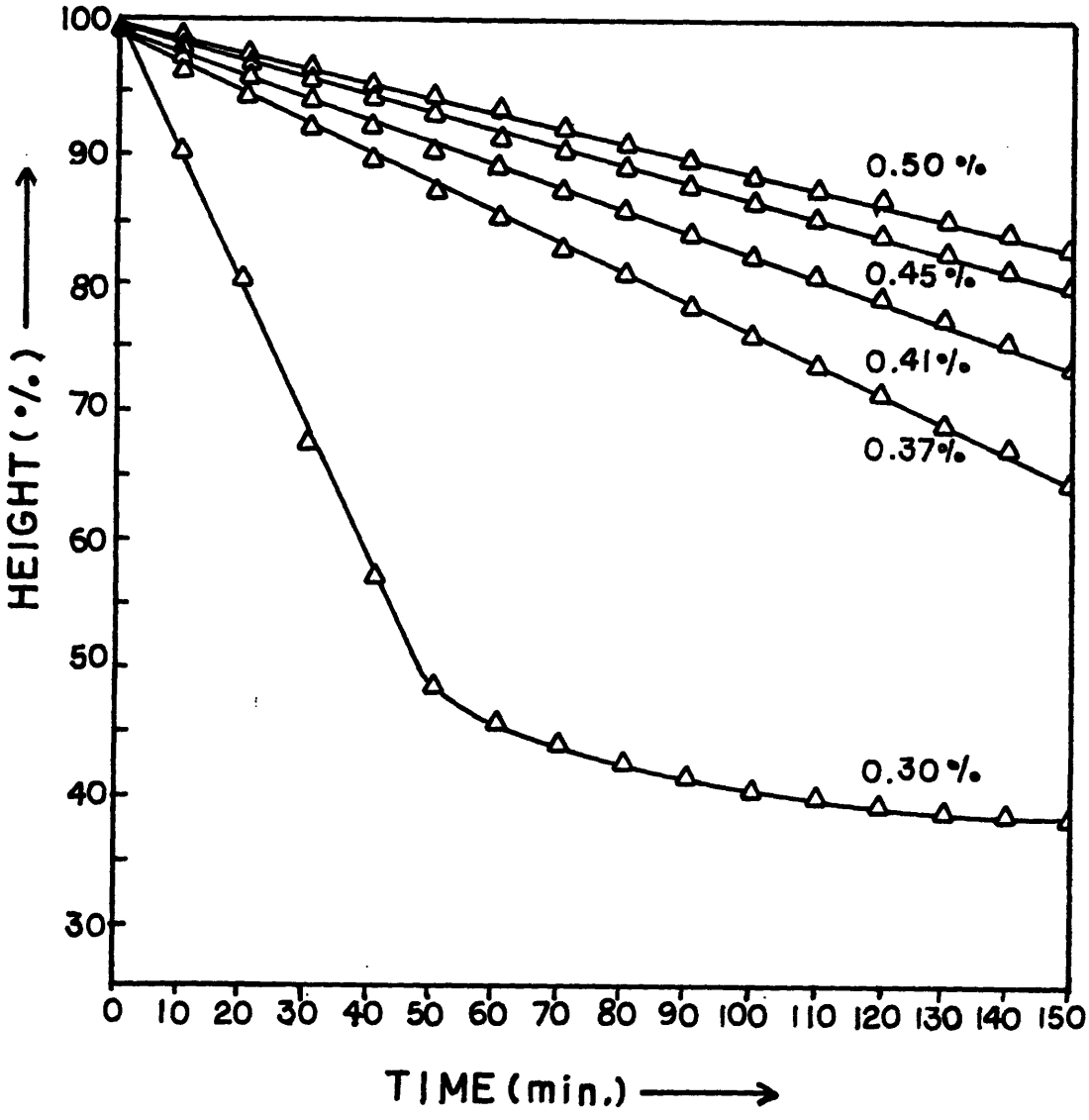


Figure 26: Sedimentation Rate of Mycelium-like *Saccharomyces cerevisiae* JD7 Cell Wall Residues

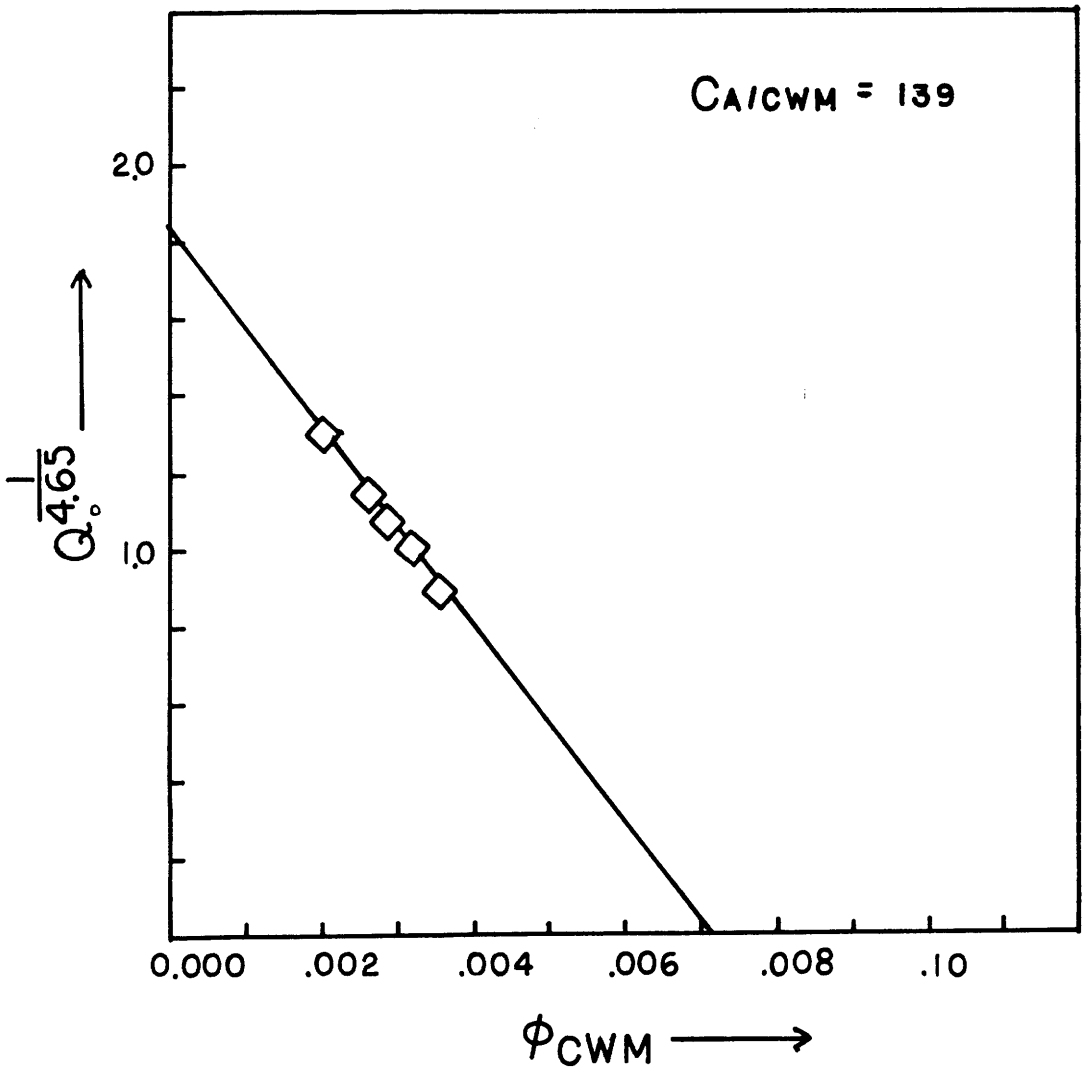


Figure 27: $Q_{\circ}^{\frac{1}{4.65}}$ Versus Volume Fraction for Cell Wall Residues of Mycelium-like Saccharomyces cerevisiae JD7

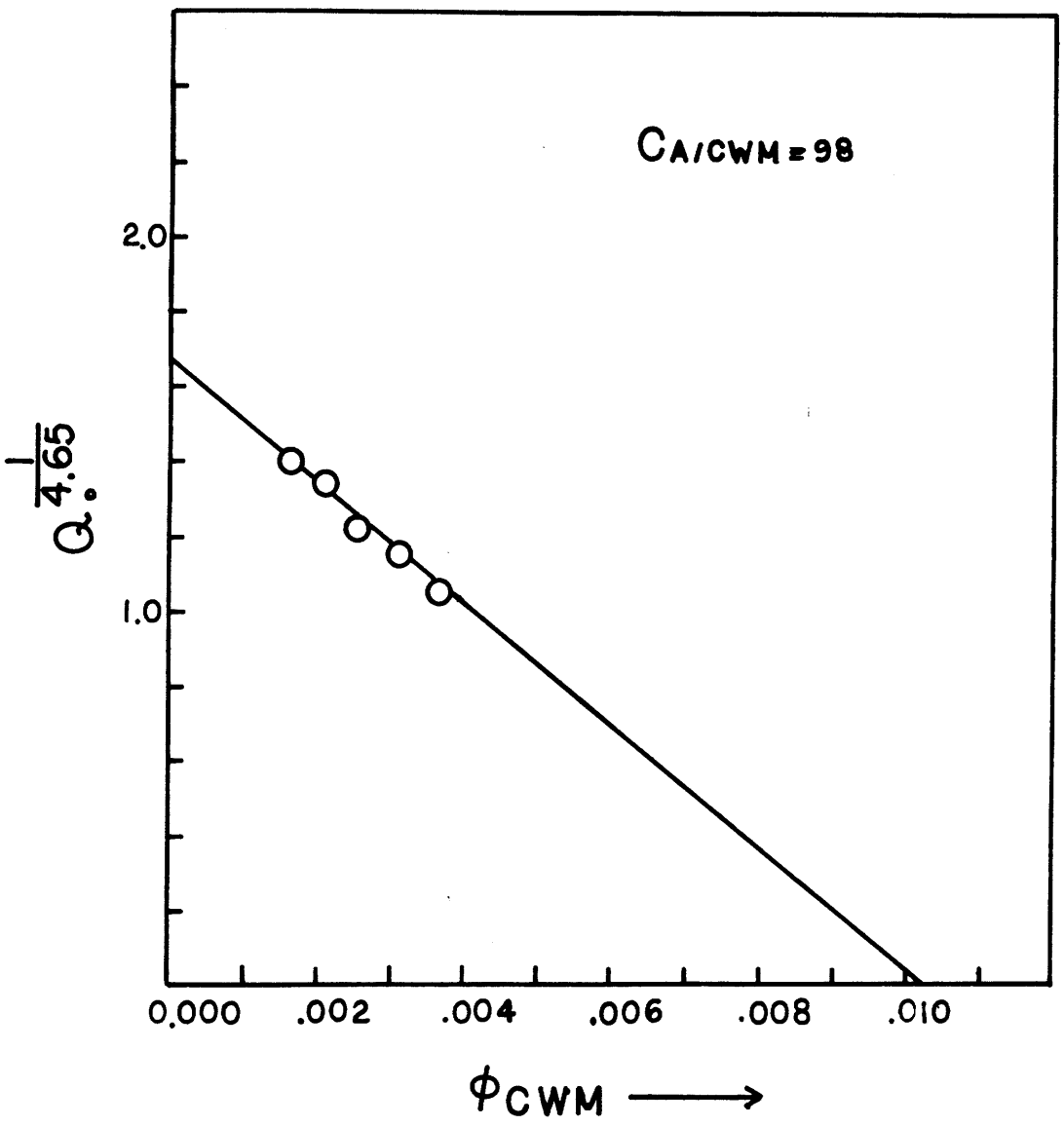


Figure 28: $Q_o^{\frac{1}{4.65}}$ Versus Volume Fraction for Cell Wall Residues of Yeast-like Saccharomyces cerevisiae JD7

REFERENCES

Aiba, S., S. Kitai and N. Ishida(1962). Density of yeast cell and viscosity of its suspension. J. Gen. Appl. Microbiol. 8(2):103-108.

Bartnicki-Garcia, S. (1968). Cell wall chem., morphogenesis and taxonomy of fungi. Ann. Rev. Microbiol. 22:87-108.

Bartnicki-Garcia, S. (1970). Cell wall composition and other biochemical markers in fungal phylogeny, pp.81-103. In J.B. Hasborne (ed.) Phytochemiacal Phylogeny, Academic Press Inc., New York and London.

Bongenaar, J.J.T.M, N.W.F. Kossen, F.W. Meijboom & B. Metz (1973). A method for characterizing the rheological properties of viscous fermentation broths. Biotechnol. Bioeng. 15:201.

Brinkman, H.C. (1952). The viscosity of concentrated suspension and solutions. J. Chem. Phys. 20:571.

Blanch, H.W. and S.M. Bhavaraju (1976). Bioengineering report. Non-Newtonian fermentation broths: rheology and mass transfer. Biotech. Bioeng. 18:745-780.

Blumauerova, M., M. Mraeek, J. Vondraekova, M. Podojil, Z. Hostalek, and Z. Vanek (1969). Regulation of biosynthesis of secondary metabolites, IX:the biosynthetic activity of blocked mutants of Streptomyces aureofaciens. Folia Microbiol. 15:215-225.

Ballou C.E. and W.C. Raschke (1974). Polymorphism of the somatic antigen of yeast. Science 184:127-134.

Ballou, C.E. (1976) Structural and biosynthesis of the mannan compnent of the yeast cell envelope. Adv. Microbiol. Physiol. 14:93-158.

Cabib, E., and B. Bowers (1971). Chitin and yeast budding. Localization of chitin in yeast bud scars. J. Biol. Chem. 246:152-159.

Cabib, E. (1975). Molecular aspects of yeast morphenesis. Ann. Rev. Microbiol 29:191-214.

Cabib, E., B. Bowers (1975). Timing and function of chitin synthesis in yeast. J. Bacteriol. 124:1586-1593.

Callaja, G.B. and B. Johnson (1977). A comparism of quantitative methods for measuring flocculation. Can. J. Microbiol. 23:68-74.

Carilli, A., Chain, E.B., G. Gualandi, G. Morisi, (1961) Sci. Rep. 1st. Super. Sanita 1, 177.

Casson, N. (1959). A flow equation for pigment-oil suspensions of the printing ink type. In: C.C. Mile(ed.), Rheology of Disperse Systems. London:Perger.

Charles, M. (1978). Technical aspects of the rheological properties of microbial cultures. In:Ghose, T.K., A. Fiechter, N. Blakebrough (ed.) Adv. Biochem. Eng. 8:1-62. Springer, Berlin Heidelberg New York.

Charm S.E. (1963). Effect of yield stress on the Power low constants of fluid food materials determined in low shear rate viscometers. Ind. Eng. Chem. Proc. Des. Dev. 2:62-65.

DeAngelo, J. (1978). Isolation and characterization of morphological mutant of Saccharomyces cerevisiae. S.M. thesis. Massachusetts Institute of Technology.

Deindoerfer, F.H. and J.H. West (1960). Rheological properties of fermentation broths. Adv. Appl. Microbiol. 2:265-273.

Deindoerfer, F.H. and J.H. West (1960) Rheological examination of some fermentation broths. Biotech. Bioeng. 2:165-175.

Einstein, A. (1906). Eine neue bestimmung der molekuldimmensionen. Ann. Phys. Paris IV. 19:289-306.

Eirich, V.F., M. Bunzl and H. Margaretha (1936). Untersuchungen uber die viskositat vow suspension und losungen. 4. Uber die viskositat vow Kugel suspension Kolloid-Z. 74:276-285.

Farkas, V. (1979). Biosynthesis of cell wall of fungi. Microbiol. Rev. 43(2):117-144.

Gander, J.E. (1974). Fungal cell wall glycoproteins and peptido-polysaccharides. Ann. Rev. Microbiol. 28:103-119.

Gunja-Smith, Z., N.B. Patil, and E.E. Smith (1974). Evidence for periplasmic location of glycogen in Saccharomyces. Biochem. Biophys. Res. Commun. 56:588-592.

Gunja-Smith, Z., N.B. Patil, and E.E. Smith (1977). Two pools of glycogen in Saccharomyces. J. Bacteriol. 130:818-825.

Herbert, D., P.J. Phipps, and R.E. Strange (1971). Chemical Analysis of Microbial Cells. In: J.R. Norris and D.W. Ribbons (ed.), Method in Microbilogy, Vol. 5B, London and New York:Academic Press.

Holmes, A.B., S.L. Gilbert and C.K. Rha (1977). Rheological properties of cranberry cell wall material. J. Food Sci. 42:29.

Holmes, A.B. and C.K. Rha (1978). Structural of chemical composition of cranberry cell wall material. J. of Food Sci. 43:112-115.

Koga, S., C. Kuribayashi, and K. Nunonura (1959). Studies on the rheological volume of cell suspension. J. Gen. Appl. Microbiol. 5:33-42.

Lampen, J.O. (1968). External enzymes of yeasts:their nature and formation. Antonie van Leeuvenhoek J. Microbiol. Serol. 34:1-18.

Lee, D.I. (1969). The viscosity of concentrated suspensions. Trans. Soc. Rheol. 13(2):273-288.

Manners, D.J. and A.J. Masson (1969). The structure of two glucans from yeast cell walls. FEB Lett. 4:122-124.

Manners, D.J., A.J. Masson and J.C. Patterson (1973). The structure of a -(1,3)-D-Glucan from yeast cell walls. Biochem. J. 135:19-30.

Metz, B., Kossen, N.W.F. (1977). The growth of molds in the form of pellets. Biotechnol. Bioeng. 19:781.

Michaels, A.S. and J.C. Bolger (1962). Settling rates and sediment volumes of flocculated kaolin suspensions. Ind. Eng. Chem. Fund. 1:24-33.

Miyasaka, Y. (1979). The application of temperature-sensitive mutants for single cell protein production. S.M. thesis, Massachusetts Institute of Technology.

Miyasaka, Y., A.J. Sinskey, J. DeAngelo, C.K. Rha (1980). Characterization of a morphological mutant of Saccharomyces cerevisiae for single-cell protein production. J. Food Science, 45:558.

Mooney, M. (1951). The viscosity of a concentrated suspension of spherical particles. J. Collid Sci. 6:162.

Raschke, W.C., K.A. Keron, C. Antalis, C.E. Ballou(1973). Genetic control of yeast mannan structure. J. Biol. Chem. 248:4660.

Reols, J.A., J. Van den Berg and R.M. Voncken (1974). The rheology of mycelial broths. Biotech. Bioeng. 16:181-208.

ReuB, B., M. Josic, M. popovic and W.K. Bronn (1979). Viscosity of yeast suspension. European J. Appl. Microbiol. Biotechnol., 8:167-175.

Rha, C.K. (1978). Rheology of fluid foods. J. Food Tech., July:77-82.

Rha, C.K. (1975) Ultilization of SCP for human food. In: S.R. Tannenbaum and D.I.C. Wang (editors). Single Cell Protein II. Cambridge: MIT Press.

Rho, M.A. and Y.D. Hang (1975). Flow behavior of concentrated suspensions of yeast grown on sauerkraut waste. J. Milk Food Technol. 38(6):315-318.

Saito, N. (1950). Concentration dependence of the viscosity of high polymer solution. I. J. Phys. Soc. Japan, 5:4-8.

Schaffeld G. (1980). Effect of some environmental parameters on protein release from lysis mutant. S.M. thesis. Massachusetts Institute of Technology.

Sherman, P. (1960). Proc. 3rd Intern. Congr. Surface Activity II, 596-609.

Shimmons, B.W., W.Y. Sorcek and J.E. Zajic (1976). Cell concentration control by viscosity. Biotech. Bioeng., 18:1793-1805.

Sherman, P. (1970). Industrial Rheology, Ch. 2, London and New York:Academic Press.

Spizek, J., I. Malek, M. Vondraeek and Z. Vanek (1965). Metabolites of Streptomyces moursei :IV. Formation of secondary metabolite by producing mutants. Folia. Microbiol., 10:259-266.

Solomons, G.L. and G.O. Weston (1961). The reduction of oxygen transfer rates in the presence of mould mycelium. Biotechnol. Bioeng. 3:1.

Sucher, R.W., E.A. Robbins, D.R. Sidotl, E.h. Schuldt,Jr., R.D. Seeley (1975). Yeast glucan and process of making same, US Patent 3,867,554.

Stanffer, J.T., and M.P. Backus (1954). Spontaneous and induced variation in selected stocks of the Pencillium chrysogenum, Ann. N.Y. Acad. Sci., 60:35-49.

Trevelyan, W.E. and Harrison, J.S. (1956). Studies on yeast metabolism. Biochemical J., 63:23-33.

Vand, V. (1948). Viscosity of solutions and suspensions. I. J. Phys. Coll. Chem. 52:277-321.

Wickerham, L.J., C.P. Kurtzman and A.T. Herman (1970). Sexual reproduction in Candida lipolytica. Science 167:1141.