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COMPREHENSIVE BIOTECHNOLOGY

The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine

EDITOR-IN-CHIEF
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University of Waterloo, Ontario, Canada

Volume 3

The Practice of Biotechnology: Current Commodity Products

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OXFORD · NEW YORK · TORONTO · SYDNEY · FRANKFURT

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Production of Baker's Yeast

S. L. CHEN and M. CHIGER
Universal Foods Corporation, Milwaukee, WI, USA

20.1 INTRODUCTION

The first written record of the actual existence of bread dates to around 2600 BC in Babylonia. An extant clay tablet from the period of Urukagina, King of Lagash, revealed that a priest performing the burial rite would take for himself seven urns of beer, 420 pieces of bread, 60 quarts of grain, *etc.* By the time of Hammurabi, who ruled during the 12th century BC, baking had developed into a specialized craft. The discovery of leavened bread was generally attributed to ancient Egyptians. At the time of the early Pharaohs, the beginning of the third millennium BC, the peasant and the slave laborer received a daily ration of four loaves of bread and two jugs of beer as his compensation. Mixing of a fermenting beer with the wheat flour used in the palace household was probably done by a royal baker, leading to the development of a sour-dough process generally accepted by the Egyptian bakers (Jacob, 1944). The knowledge of making leavened bread spread from Egypt to other areas of the Mediterranean world in the 13th century BC. In ancient Jerusalem, for example, public bakeries were established which produced small breads similar to the present day bread rolls (Pyler, 1958). The practice of using beer yeast in sour-dough fermentation continued into the 19th century, when the commercial bakers obtained their yeast supplies from local breweries (Frey, 1930). Due to its bitter taste and variable fermentation activity, brewer's yeast was gradually replaced by distiller's yeast which in turn was replaced by baker's yeast.

The earliest production of pressed baker's yeast probably occurred around 1781 in Holland. With the so-called Dutch process, the yield of pressed yeast was equivalent to only 4–6% of the weight of the raw material used. In 1846, a Vienna process was developed by Mautner. In this process, yeast was recovered from the entire batch by continuously collecting the foam produced during fermentation. The yield of compressed yeast was increased to about 14%, plus a concurrent yield of 30% spirits. Aeration of the grain mash was introduced by Marquardt in 1879, resulting in the yield of compressed yeast gradually increasing to 50–60% and the amount of spirits falling to 20%. In 1919, a process was invented by Sak in Denmark and Hayduck in Germany in which sugar solution was fed to an aerated suspension of yeast instead of adding yeast to a diluted sugar solution. This process was known as 'Zulaufverfahren'. An incremental-feeding or fed-batch process was thus born. At about the same time, the traditional grain mash was replaced by molasses, because of the food shortage during World War I. These refinements gradually raised the yield of compressed yeast to the theoretical maximum of 50% by weight of the raw material used, with no concomitant spirit formation. Such accomplishments eventually led to the development of a baker's yeast industry independent of alcoholic beverage production (Pyke, 1957; Paturau, 1982). Several excellent books and reviews have been published on this subject matter in recent years (Burrows, 1970; Harrison, 1971; Peppler, 1979; Reed, 1982; Reed and Peppler, 1973; Rosen, 1977; White, 1954).

20.2 PHYSIOLOGY OF YEAST GROWTH

20.2.1 Species and Cultures of Baker's Yeast

Historically, beer yeast or brewer's yeast (*Saccharomyces uvarum*, sym. *S. carlsbergensis*) was used for baking purposes, but it was subsequently replaced by distiller's yeast and baker's yeast (*S. cerevisiae*). Attempts have been made periodically to evaluate the baking properties of other yeast strains and cultures. For example, Mitchell (1957) evaluated the baking properties of some 75 cultures including *Candida arborae*, *C. pseudotropicalis*, *C. tropicalis*, *Hansenula subpelliculosa*, *Saccharomyces chevalieri*, *S. chodati*, *S. diastaticus*, *S. ellipsoideus thermophilus*, *S. fragilis*, *S. italicus*, *S. intermedius*, *S. logos*, *S. marxianus*, *S. osmophilus*, *S. oviformis*, *Schizosaccharomyces pombe*, *Torula colliculosa*, *T. dattila*, *Zygosaccharomyces lactis*, *Z. drosophilae*, *etc.* Except possibly for the last one, none of these species and cultures has been found to be superior to the *S. cerevisiae*.

20.2.1.1 Preservation of yeast cultures

Maintenance of desirable yeast cultures is a major concern of the yeast industry. Several methods of preserving yeast cultures are in use at various organizations (Martin, 1964; Dalby, 1982; Hayner, *et al.* 1955; Beech and Davenport, 1971; Collins and Lyne, 1976).

(i) Periodic transfer

The storage of conventional agar slant or stab cultures at refrigerated temperature (*e.g.* 4–5 °C) has long been used as a means of reducing the metabolic activity of cultures and thereby increasing the duration for periodic transfer to 3–6 months. This method is commonly used for yeasts, algae, filamentous fungi, but seldom for bacteria. Several all purpose growth media, such as malt extract agar, yeast extract agar, beer wort agar, MYPD agar (malt yeast extract–peptone–dextrose), *etc.*, are being used for carrying stock cultures. Other media, such as potato-dextrose agar, yeast extract–peptone–dextrose agar, *etc.*, are commonly used for isolation and detection of yeasts and determination of yeast counts. Bacterial growth in these media is controlled by pH regulation or by addition of an antibiotic, such as tetracycline. Synthetic media, such as yeast morphology agar, yeast nitrogen base, yeast carbon base, vitamin-free yeast base, *etc.*, have been formulated and used for identification, characterization and classification of yeast (Wickerham, 1951). Most of these growth media are available commercially.

(ii) Storage under oil

The practice of covering actively growing slant or stab cultures with sterile mineral oil and subsequently storing them under refrigeration has frequently been used to increase the longevity of cultures and thereby lengthen the transfer intervals. Overlaying with oil prevents dehydration and slows down metabolic activity by reducing oxygen availability.

(iii) Storage in water

This method is extremely strain specific. Some bacteria have been reported to have survived suspension in distilled water for ten years at room temperature. Strains of *S. cerevisiae* stored in dilute buffer at 4 °C showed 2–19% survival after one year.

(iv) Storage in soil

Storage in sterile soil has been used quite commonly for extending the longevity of microorganisms, such as spore-forming bacteria, conidiating fungi, and some algae; however, it has been seldom used for yeasts. In its simplest form, this method consists of adding a suspension of cells or conidia to sterile soil, drying the mixture at room temperature, and then storing it in the refrigerator.

(v) Dehydration

Dehydration, without preliminary freezing, is a method which has taken many forms, from the simple drying of conventional slant cultures of sporulating molds, to some rather complex techniques of vacuum drying. Desiccation with silica gel or on cellulose material has also been used by some workers.

(vi) Freezing

With this method, a small volume of cell suspension is cooled slowly (*i.e* about 1 °C min^{-1}) to −20 °C and then as rapidly as possible until storage temperature (*i.e.* below −55 °C) is reached. Addition of a protective agent, such as glycerol (10–20%) or dimethyl sulfoxide (10%) to the cell suspension prior to freezing is recommended. Thawing should be accomplished as rapidly as possible. Use of liquid nitrogen for ultralow temperature (−130 °C) storage of cells has been successfully used for a variety of cells including fungi, bacteriophages, protozoa, algae, mammalian cells and bacteria (Norris and Ribbons, 1970).

(vii) Lyophilization or Freeze-drying

This is probably the most satisfactory method for long term preservation of yeast cultures. There are many advantages of this method: it requires no subculturing of cells; it causes no change in biochemical properties of the culture; the freeze-dried cells are genetically stable; there is no contamination problem once the ampoule is sealed; the finished ampoule can be shipped by mail; and the initial cost is low. This process consists of introducing a few drops of propagating yeast suspension (in a protective medium containing one part of fresh skim milk and one part of distilled water) into an ampoule, placing a piece of sterile cotton plug into the upper portion of the ampoule, freezing the yeast suspension to −35 °C and connecting the ampoule to a vacuum of 200 μm Hg for moisture removal by sublimation. After drying, the ampoule is sealed under vacuum and stored under refrigeration. During the revival of the yeast culture, the ampoule is opened under aseptic conditions, a small volume of sterile water is added to dissolve the pellet. After 30 minutes, the suspension is streaked in a growth agar medium. As an alternative, the pel-

let in the ampoule may be emptied into a sterilized flask, containing about 50 ml of a suitable culture medium such as malt extract.

20.2.2 Nutrition and Biosynthesis

The nutritional requirement for the growth of baker's yeast may be estimated from its elemental composition. Based upon the average values published by several authors (Frey *et al.*, 1936; White, 1954; Olbright, 1956; Harrison, 1967 and 1971; Wang *et al.*, 1977), baker's yeast contains (on a dry solids basis) about 46% carbon, 32% oxygen, 8.5% nitrogen, 6% hydrogen and 7.5% ash. On the assumption that 200 g of sucrose is required for the production of 100 g of yeast solids under efficient growth conditions, the following material balance equation may be established:

$$\begin{aligned} &200 \text{ g sucrose} + 10.32 \text{ g } NH_3 + 100.44 \text{ g oxygen} + 7.5 \text{ g 'ash'} \rightarrow \\ &\quad 100 \text{ g yeast solids} + 140.14 \text{ g } CO_2 + 78.12 \text{ g } H_2O \end{aligned} \quad (1)$$

This equation shows that in addition to sucrose, 10.32 g of ammonia, 7.5 g of 'ash' and 100.44 g of oxygen (equivalent to 1 g of oxygen per g of yeast solids produced) are required. It also shows an overall respiratory quotient (RQ) of 1.02 during the course of efficient yeast propagation. The chemical constituents of the growth medium must supply all elemental and energy requirements. Assimilation of these nutrients is discussed in the following sections.

20.2.2.1 Carbon assimilation

The carbon source for baker's yeast propagation usually consists of assimilable sugars, such as glucose, fructose, mannose, galactose, sucrose, maltose and hydrolyzed lactose. Ethanol has also been used, at least partially, as a substrate for yeast production. In order to be assimilated, these compounds must be transported into the yeast cells. Monosaccharides are transported by carrier-mediated or facilitated diffusion. Three carrier systems have been identified: a specific carrier preferring pyranoses with an equatorial hydroxyl at C-4 (such as glucose), a non-specific one taking up all pyranose-type sugars, and an inducible one preferring pyranoses with an axial hydroxyl at C-4 (such as galactose). For disaccharides, sucrose is hydrolyzed in the periplasmic space by invertase before being transported into baker's yeast cells, while unhydrolyzed lactose is not taken up at all. Maltose enters baker's yeast cells either by facilitated diffusion or by active transport. Four transport systems are known. These systems may be induced by their respective substrates, such as maltose, trehalose, α-methyl-D-glucoside and glucose. Their half-lives are very short, of the order of 1 h (Alonso and Kotyk, 1978). Unlike sugars, ethanol enters yeast cells by simple diffusion (Barnett, 1976; Kotyk and Horak, 1981.)

Aerobic assimilation of glucose substrate is either by the glycolytic-TCA cycle pathway or by the pentose phospate pathway. By using C^{14}-labelled glucose substrates, Chen (1959a) demonstrated that aerobically growing *S. cerevisiae* cells catabolize 94% of the substrate through the glycolytic-TCA cycle pathway and only 6% through the pentose phosphate pathway. These results are in sharp contrast to those obtained with other oxidative yeasts, *e.g.* 35% through the pentose phosphate pathway in *Candida utilis* (Blumenthal *et al.*, 1954), 50% in *C. albicans* (Chattaway *et al.*, 1973) and 80–100% in *Rhodotorula gracilis* (Hofer *et al.*, 1971). The initial step in the assimilation of intracellular fructose and mannose is phosphorylation by the constitutive hexokinase. Fructose 6-phosphate is an intermediate for both pathways, while mannose 6-phosphate is either isomerized to fructose 6-phosphate or epimerized to glucose 6-phosphate (Slein, 1950; Noltmann and Bruns, 1958). The ability of baker's yeast to utilize galactose depends on the carbon source on which the yeast is grown. Transferring the yeast grown on D-galactose to a glucose medium leads to a loss of the D-galactose fermenting ability. Conversion of D-galactose to D-glucose 6-phosphate requires the participation of several enzymes. Galactokinase, hexosyl phosphate uridyl transferase, and UDP-D-glucose 4-empimerase are present in galactose-grown yeast, but not in glucose-grown baker's yeast (de Robichon-Szulmajster, 1958a, 1958b; Wilkinson, 1949).

Utilization of sucrose presents no problem, since it is hydrolyzed prior to being transported into the yeast cells as glucose and fructose. Unlike non-fermentable monosaccharides, lactose is not taken up by *S. cerevisiae* cells. However, hydrolyzed lactose has been used as a substrate for baker's yeast production (Anon, 1982; Stineman *et al.*, 1980). Assimilation of intracellular maltose involves the hydrolytic action of α-D-glucosidase, which is highly specific for the sugar moiety. It has no activity when the configuration of the sugar moiety is modified by (i) inversion at C-2 (α-

D-mannopyranosidyl), or C-4 (α-D-galactopyranosyl), (ii) substitution on the 6-hydroxyl group (raffinose), or (iii) other substitutions or replacement on C-2 to C-6. This enzyme also hydrolyzes maltotriose, which is known to be transported intact into baker's yeast (Yamamoto and Inone, 1961).

Oligo-(1,6)-D-glucosidase is also present in *S. cerevisiae* cells. It hydrolyzes isomaltose, methyl α-D-glucopyranoside but not maltose. Induction of these two α-D-glucosyl hydrolases has been investigated. Maltose induces both hydrolases, but α-D-methyl glucopyranoside induces only oligo-(1,6)-D-glucosidase, not α-D-glucosidase. Glucose often suppresses the synthesis of both enzymes. Another trisaccharide, raffinose, is known to be present in molasses. Baker's yeast can utilize only the fructose moiety of raffinose following its hydrolysis by invertase in the periplasmic space (Barnett, 1976).

Ethanol also has been used, at least partly, as a substrate for the production of *S. cerevisiae* yeast. The pathway for assimilating ethanol substrate into glycolytic intermediates and carbohydrates is *via* oxaloacetate (Haarasilta and Oura, 1975). The cell yield is reported to be 40–70 g of yeast solids per 100 g of ethanol (Mor and Fiechter, 1968; Suomalainen and Oura, 1978). In a mixed ethanol–glucose substrate (*i.e.* 15% ethanol–85% glucose), the yield obtained from ethanol is nearly 85 g yeast solids per 100 g of ethanol.

Ethanol carbon is as efficient as hexose carbon, *i.e.* 3 mol of ethanol are equivalent to 1 mol of hexose up to a mixture of 40% ethanol–60% molasses. If the proportion of ethanol is increased further, lower cell yields are obtained. Ridgeway *et al.* (1975) also reported on the use of ethanol for yeast production, but their results were obtained mainly with *C. utilis* yeast.

Yeasts are known to be capable of fixing CO_2. However, under efficient aerobic growth conditions reabsorption of metabolic CO_2 is insignificant, *e.g.* 1–3% (Chen, 1959a; Oura and Haarasilta, 1977).

20.2.2.2 Nitrogen assimilation

Assimilable nitrogen for commercial baker's yeast production is supplied in the form of aqueous ammonia, ammonium salts (such as phosphate, sulfate, chloride, bicarbonate, carbonate, *etc.*), and urea. Occasionally, amino acid mixtures, such as protein hydrolysates or autolysates, may be added. Nitrate and nitrite are not assimilated by *S. cerevisiae*. Ammonium ions are transported by the K^+-carrier system, while undissociate ammonium hydroxide and urea enter yeast cells by simple diffusion (Suomalainen and Oura, 1971). Ammonia is assimilated into glutamate and glutamine before being incorporated into other amino acids and nucleotides (Sims and Folkes, 1964; Witt *et al.*, 1964). Urea is hydrolyzed by urea amidolyase to ammonia, which is then assimilated (Roon and Levenberg 1968, 1972), and CO_2, which can be quantitatively recovered (Chen, 1959a).

The nitrogen of compounds such as allantoin and allantoic acid which can be converted to urea is available for yeast growth. However, the ureido nitrogen in purine bases is not available (DiCarlo *et al.*, 1953).

Amino acid uptake is mediated by ten active transport systems (Kotyk and Horak, 1981). The amino and amido groups of several L-amino acids (such as alanine, arginine, aspartic acid, asparagine, glutamic acid, leucine and valine) can be readily assimilated by baker's yeast, while those of other amino acids, such as cysteine, glycine, histidine, lysine and threonine, are not utilized (Schultz and Pomper, 1948). Mixtures of amino acids can be assimilated more rapidly than individual amino acids (Thorne, 1949). In the presence of assimilable sugars, the carbon skeletons of certain amino acids (such as aspartic acid, asparagine, proline and possibly glutamic acid) can also serve as carbon sources for the growth of *S. cerevisiae* yeast (Schultz *et al.*, 1949; White, 1954).

20.2.2.3 Inorganic elements

The total ash content of baker's yeast varies from 4.7 to 10.5%, which is made up of 1.9–5.5% of P_2O_5, 1.4–4.3% of K_2O, 0.1–0.7% MgO, less than 0.2% of CaO and SiO_2, and less than 0.1% of Al_2O_3, Fe_2O_3, SO_3, Cu and Cl (White, 1954). Trace elements, such as Ba, Cr, Au, Co, Mo, Ni, La, Pb, Mn, Rb, Pt, Ag, Tl, Sn and Zn, have been detected (Richard and Troutman, 1940). While some of these elements, such as P, K, Mg, Na, S, Fe, Cu and Zn must be supplied for

proper yeast growth, other elements, such as B, Mn, Ca, Tl, Co, I and Sn have no effect on yeast growth (Olson and Johnson, 1949).

(i) Phosphorus

In commercial yeast production, phosphorus is supplied in the form of phosphoric acid, ammonium phosphate, potassium phosphate, sodium phosphate, *etc.* Orthophosphate uptake in *S. cerevisiae* is mediated by at least two active transport systems. The first one has a relatively low affinity for its substrate and takes up two protons together with $H_2PO_4^-$ (a potassium ion leaves the cell to compensate for electric charge movement); this system is not affected by Na^+ (Cockburn *et al.*, 1975). The second carrier has a higher affinity for phosphate and is driven by a putative Na^+ gradient. Two Na^+ions are bound per phosphate ion. Na^+ can be replaced by Li^+, but not by K^+ or Rb^+. This system is stimulated by Mg^{2+} and Ca^{2+} (Roomans *et al.*, 1977). Phosphorus is essential to the growth and survival of yeast cells, since it is involved in all phases of cellular metabolism. It is taken up by yeast cells only in the presence of an energy source such as glucose (Kotyk and Horak, 1981). Upon its absorption, ^{32}P-orthophosphate labels ten organic phosphate esters within 0.1 s (Miettinen, 1964). Orthophosphate is also stored as metaphosphate in volutin granules (Wiame, 1946, 1947).

Yoshida and Yamataka (1953) have established that metaphosphate could well be the store of phosphate energy in yeast cells. Trimeta- and tripoly-phosphate have also been isolated from baker's yeast (Kornberg, 1956). However, Suomalainen and Pfaffli (1961) believed that polyphosphate did not constitute any appreciable energy reserve in baker's yeast cells.

(ii) Potassium and sodium

Potassium ions enter yeast cells through an active transport system. Absorption of K^+ occurs in the presence of a metabolizable substrate and is accompanied by an efflux of protons from the cells. Baker's yeast can take up K^+ from the medium against a concentration gradient of 1000:1. As the substrate becomes depleted, part of the intracellular potassium will be excreted in exchange for the H^+ in the medium. Uptake of sodium ions may involve the same carrier system (Foulkes, 1956) or two separate carrier systems (Conway *et al.*, 1954; Reilly, 1967). Unlike animal cells, active ion transport in yeast is not dependent on the energy derived from ATP, but is effected by means of the 'redox pump' (Conway, 1955). Potassium is necessary for yeast growth and for fermentation. When K^+ is absent from the medium, phosphate cannot be absorbed (Schmidt *et al.*, 1949). Attempts have been made to replace K^+ with sodium and ammonium ions. The 'sodium yeast' and the 'ammonia yeast' had lower growth rates and higher resting oxygen consumption than the reference 'potassium yeast' (Conway and Moore, 1954; Conway and Breen, 1945).

(iii) Magnesium and calcium

Magnesium and calcium are taken up by an active transport system at the expense of ATP. Conway and his coworkers (Conway and Beary, 1956, 1958; Conway and Duggan, 1958) reported that Mg^{2+} was transferred into yeast cells by the same carrier as that for K^+ ions and that the uptake involved H^+ secretion. The activity of this carrier is dependent on the presence of oxygen. Magnesium is essential to yeast growth and cellular activities, since it serves as enzyme activator for many enzyme systems, but calcium is believed to be non-essential for yeast growth (Morris, 1958).

(iv) Sulfur

Sulfur is usually supplied in the form of sulfate for yeast production. Its uptake is mediated by an active transport system and is enhanced by the presence of glucose in the medium. Breton and Surdin-Kerjan (1977) found that two permeases are involved: permease I has a high affinity and permease II has a low affinity for the substrate. Sulfate may be replaced by other inorganic compounds, such as sulfite and thiosulfate (Schultz and McManus, 1950), and organic compounds, such as methionine and glutathione (Maw, 1960). However, *S. cerevisiae* cannot utilize the sulfur in certain amino acids such as cysteine, or vitamins such as biotin and thiamine (Suomalainen and Oura, 1971). Assimilation of the sulfate ion in *S. cerevisiae* yeast involves its reduction to sulfite ion and H_2S through the formation of adenine monophosphate-SO_2 and phosphoadenosine phosphosulfate. Hydrogen sulfide is then incorporated into cysteine and homocysteine through sulfhydrylation of *O*-acetylserine and *O*-acetylhomoserine respectively. Methionine is formed by methylation of homocysteine (de Robichon-Szumajster and Surdin-Kerjan, 1971).

(v) Trace elements

Yeast cells are relatively impermeable to bivalent cations, but elements such as Ba^{2+}, Zn^{2+}, Mn^{2+}, Sr^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+} and Cu^{2+}, can equilibrate rapidly with the cell surface by combining with its phosphate and carboxyl groups. The binding of exogenous bivalent cations is rapid and reversible (Rothstein and Hayes, 1956; Van Steveninck and Booji, 1964). Like Mg^{2+} and Ca^{2+}, transport of these bivalent cations into yeast cells is mediated by an active transport system having the following affinities: $Mg^{2+}>Co^{2+}>Zn^{2+}>Mn^{2+}>Ni^{2+}>Ca^{2+}>Sr^{2+}$ (Fuhrman and Rothstein, 1968). Except for phosphate and sulfate, no data on the transport of other anions is available as yet (Kotyk and Horak, 1981).

Among these trace elements, *S. cerevisiae* requires 200 μg of Zn^{2+}, 75 μg of Fe^{2+}, and 12–15 μg of Cu^{2+} per liter of medium for optimum growth (Olsen and Johnson, 1949). Trace elements function, in general, as catalysts or activators in enzyme or vitamin systems. Zinc is known to be a constituent of alcohol dehydrogenase, carbonic anydrase, carboxypeptidase, glutamic –dehydrogenase, lactic dehydrogenase, *etc.* Addition of Zn^{2+} to the growth medium results in a higher rate of fermentation (Frey *et al.*, 1967; Stone, 1965; Densky *et al.*, 1966) and protein autolysis (Maddox and Hough, 1970). Several metalloenzymes and proteins, such as cytochromes, cytochrome c reductase, catalase, peroxidase, *etc.*, contain iron, while others, such as ascorbic acid oxidase, tyrosinase, uricase, *etc.*, contain copper. On the other hand, many heavy metals are found to be inhibitory to yeast growth in trace quantities (White, 1954).

20.2.2.4 Vitamins

Due to the minute quantities of various vitamins in yeast cells, these compounds are ignored in material balance calculations. However, their contents in yeast cells have been well documented (Eddy, 1958). As many as six compounds in the vitamin B complex (*i.e.* biotin, pantothenic acid, inositol, thiamine, pyridoxine and niacin) have been found to be essential for the growth of certain yeast species and strains (Burkholder *et al.*, 1944). An attempt has been made to classify baker's yeast strains on the basis of their vitamin requirements (Schultz and Atkin, 1937). Among these vitamins, biotin (Bios 2B) is required by most species and strains. While its uptake into yeast cells is mediated by a carrier transport system, its efflux is probably by simple diffusion. Thiamine is transported actively by a system that includes a membrane-bound receptor protein, while pyridoxine and pyridoxal are transported by a constitutive system that is stimulated by K^+ and has a pH optimum of 3.5 (Kotyk and Horak, 1981). The uptake of both niacin and its amide is dependent upon the metabolic activity of yeast cells (Oura and Suomalainen, 1978).

Biotin requirements for yeast growth vary with growth conditions (Oura, 1978; Oura and Suomalainen, 1978). Increased aerobicity lowers the need for biotin. During anaerobic growth with glucose–urea substrate, three biotin-containing enzyme systems, *i.e* pyruvate carboxylase, acetyl-CoA carboxylase and urea amidolyase, are functioning; the biotin requirement is highest. When urea is replaced by an ammonium salt, only two enzymes, *i.e.* pyruvate carboxylase and acetyl-CoA carboxylase, are essential and the biotin requirement is lower. During aerobic growth on glucose, oxaloacetate formation through pyruvate carboxylation is partly supplemented by the glyoxylate cycle; the biotin requirement should be further decreased. The lowest biotin requirement occurs during aerobic growth on ethanol, when only acetyl-CoA carboxylase is needed. Biotin may be replaced by biocytin, biotine-D-sulfoxide, D-desthiobiotin and D-biotin methyl ester. According to Oura (1978), 100 μg of biotin/100 g sugar is required for aerobic yeast growth.

Pantothenic acid (Bios 2A) is required by many yeast species for growth. It is a component of coenzyme A, which participates in the transfer of the acyl group in carbohydrate and fatty acid metabolism. White (1954) found that the maximum yield of baker's yeast was achieved at 44 p.p.m. of pantothenic acid in the yeast cells. Only relatively few yeast species require inositol for growth. For these yeasts, deficiency of inositol (Bios 1) leads to morphological changes in the cell wall (Challinor and Power, 1964; Power and Challinor, 1969) and weakened glucose metabolism (Ridgeway and Douglas, 1958; Lewin, 1967). Ghosh and Bhattacharrya (1967) believed that phosphofrutose kinase activity is affected by inositol deficiency. Several yeast species, particularly the lactose-fermenting yeasts, require thiamine for growth (Rogosa, 1944). Baker's yeast can synthesize thiamine from thiazole and pyrimidine components (Schultz *et al.*, 1941). The thiamine added to the medium is taken up by the cells and esterified to thiamine pyrophosphate or cocarboxylase. It has been reported that thiamine stimulates the fermentation rate of certain baker's yeasts (Schultz *et al.*, 1937; Suomalainen and Axelson, 1956). Pyridoxine has been reported to promote growth of *S. cerevisiae* (Schultz *et al.*, 1939). Its requirement may be partly

replaced by pyridoxal or pyridoxamine (Melnick *et al.*, 1945). Pyridoxal phosphate acts as a coenzyme of aminotransferases in amino acid metabolism. Only a few yeast species require niacin for growth. The ability of baker's yeast to synthesize niacin is limited under anaerobic conditions so it must be considered a necessary growth factor. Under aerobic conditions a considerable amount of niacin is secreted from the yeast cells. There may be a decreased need of NAD for yeast growth under aerobic conditions (Suomalainen *et al.*, 1965a, 1965b).

20.2.2.5 Oxygen requirement

On the basis of material balance calculations, 1 g of oxygen is required for the production of 1 g of yeast solids under efficient growth conditions (equation 1). This figure is in agreement with actual experimental values (Maxon and Johnson, 1953). Thus, oxygen, like other nutrients, must be supplied for efficient yeast growth. In commercial production of baker's yeast, oxygen is supplied by sparging an air stream through the fermenter broth. Under oxygen-limiting conditions, the amount of yeast which can be produced per unit fermenter volume and unit time is dependent upon the amount of oxygen which can be transferred from the gas phase to the liquid phase per unit volume and unit time. For example, for a volumetric productivity of 5 g of yeast solids l^{-1} h^{-1} the amount of oxygen required is 5 g or 156 mmol of O_2 l^{-1} h^{-1}.

20.2.3 Kinetics of Yeast Growth

In a batch process, where adequate nutrients are present, the exponential growth of yeast may be described by the following equation:

$$dX/dt = \mu X \text{ or } dN/dt = \mu_n N \tag{2}$$

where X = concentration of cell mass (g l^{-1}); N = number of cells l^{-1}; t = time; μ = specific growth rate for cell mass (h^{-1}); and μ_n = specific growth rate for cell number (h^{-1}).

The doubling time for cell mass is equal to 0.693/specific growth rate. The maximum specific growth rate for yeast has been reported to be 0.6 h^{-1} (Aiyar and Luedeking, 1966), equivalent to a doubling time or generation time of 1.16 h.

Since a fed-batch process, rather than a batch process, is normally used for commercial production of baker's yeast, application of these equations must be made with discretion. In the fed-batch process, yeast growth takes place under substrate-limiting conditions in order to achieve a maximum yield of biomass. The specific growth rate is not constant, but decreases progressively during fermentation. Thus, any calculations of the specific growth rate and/or doubling time merely reflect the instantaneous values for that moment. As such, they have little value in projecting and describing the course of a fed-batch fermentation. The ever-changing growth rate in the fed-batch fermentation may be estimated from the feeding schedule of the substrate. By assuming an appropriate yield factor for the substrate used, one may estimate the cell mass produced at various time intervals and thence the specific growth rates during the course of fed-batch fermentations.

The progressive decrease in the specific growth rate in the fed-batch process is probably due to the continuous increase of cell mass concentration and decrease of substrate concentration in the fermenter broth as dictated by the oxygen transfer capability of the fermenter system. As the cell mass increases and the yeast productivity approaches the limit of the oxygen transfer capability, the growth efficiency of biomass will begin to decrease, since aerobic growth will tend to shift gradually toward anaerobic growth. In order to maintain a desirable growth efficiency, the substrate concentration must be reduced to a proper level commensurate with the oxygen supply. The relationship between growth rate and substrate concentration has been found to be similar to the saturation kinetics exhibited by monomolecular absorption (Monod, 1949). For *S. cerevisiae*, K_s has been reported to be 25 mg glucose l^{-1}. Thus μ becomes a strong function of substrate concentration below $S = 10K_s$ or 250 mg glucose l^{-1} (Wang *et al.*, 1979).

In addition to substrate concentration other operating conditions, such as temperature, oxygen supply, pH, *etc.*, affect the growth rate μ. White (1954) has determined μ at different temperatures as follows: 20 °C, 0.149 h^{-1}; 24.5 °C, 0.207 h^{-1}; 30 °C, 0.311 h^{-1}; 32.5 °C, 0.324 h^{-1}, and 40 °C, 0.094 h^{-1}. Similar results have been reported by Keszler (1967). The critical dissolved oxygen concentration for yeast growth has been found to be 0.004 mM (Winzler, 1941).

20.2.4 Cellular Yields

While the information on the oxygen yield of yeast growth may provide useful data on the productivity of a fermenter system, the performance of a fermentation process as a whole must be evaluated on the basis of the efficiency of yeast growth, *i.e.* cell mass produced per unit weight of energy substrate, or substrate yield. Two methods have been proposed for calculating the theoretical substrate yields.

The first method is based on the 'available electrons' in the substrate. Mayberry *et al.* (1967) found the mean yield of cell mass per equivalent of available electrons to be 3.14 ± 0.11 g, regardless of the substrate or organism. Since four equivalents of electrons were required to reduce 1 mol of oxygen, complete oxidation of glucose required 24 electron equivalents. On the basis of their data, these authors predicted a substrate yield of 75.4 g cell mass of *Candida utilis* yeast per mole or 180 g of glucose, *i.e.* 41.9%. This predicted value is considerably lower than those reported in the literature. The low predicted value may be due to the fact that in their calculations the fraction of substrate being assimilated into biomass was not separated from the fraction of substrate actually oxidized.

The second method uses the amount of ATP produced from the substrate. Bauchop and Eldsden (1960) found that, under anaerobic conditions, the cell yield of *S. cerevisiae*, *Streptococcus fecalis* and *Pseudomonas lindneri* was proportional to the amount of ATP synthesized. The mean value of cell mass per mole of ATP (*i.e.* yield_{ATP}) was found to be 10.5 g (range: 8.3–12.6). An attempt has been made to estimate yield_{ATP} under aerobic growth conditions by a material balance method. Assuming a P/O ratio of 3.0, the yield_{ATP} was calculated to be 10.96 for *Candida utilis* and 6.21 for *S. cerevisiae* (Chen, 1964). After correcting the P/O ratio to 2.0 for *S. cerevisiae*, its yield_{ATP} was recalculated to be 9.82 (Reed and Peppler, 1973, p. 69). These data are in general agreement with 7.0 for *C. utilis* at P/O = 3, 7.5 for *S. carlsbergensis* at P/O = 2 (Hernandez and Johnson, 1967), 8.7 for *S. cerevisiae* at P/O = 1.93 (Oura, 1972a) and 9.5–12 at P/O = 1.89 (Oura, 1973).

Maximum efficiency of yeast growth, whether it is expressed as cell mass per unit weight of substrate, or per mole of ATP synthesized, or per equivalent of available electron, can be achieved only under optimal conditions. Even in the presence of adequate nutrient supply, the efficiency of yeast growth is affected by many operating conditions, such as oxygen supply, substrate concentration, temperature, pH, as well as growth rate. The effect of oxygen supply on substrate yield is probably best understood. In the absence of oxygen, only 2 mol of ATP are synthesized per mole of glucose assimilated. Using Bauchop and Eldsden's (1960) data of 10.5 g cell mass per mole ATP for *S. cerevisiae*, a cell yield would be equal to: $(2 \times 10.5 \times 100)/180 = 11.65$ g cell mass per 100 g of glucose substrate. White (1954) determined a substrate yield of 10.5 under anaerobic conditions, while Reed (1982) reported a substrate yield of 7.5 g cell mass per 100 g of fermentable sugar. As oxygen supply increases, the growth efficiency increases according to Monod's saturation kinetics to about 50–54 g cell mass per 100 g of energy substrate (Reed, 1982).

Increased substrate concentration also leads to higher specific growth rate, but unlike oxygen supply, it lowers cell yield. This is due to the inhibition of yeast respiration and enhancement of alcoholic fermentation at higher substrate concentration even in the presence of oxygen. This phenomenon is known as the Crabtree effect (DeDeken, 1966; Rickard and Hogan, 1978). In order to achieve high efficiency, the fed-batch process, where calculated amount of substrate is fed continuously during the course of fermentation, is generally used in yeast production.

Cell yield is also a function of specific growth rate μ. Dellweg *et al.* (1977) reported that as μ exceeds 0.18 in a continuous fermenter, the substrate yield decreases sharply. This is due to increased substrate concentration at higher dilution rate, leading to higher alcoholic fermentation, as shown by greater RQ. von Meyenburg (1969) also reported lower substrate yield as μ exceeds 0.23. Thus, μ should not exceed 0.2 if a high yield of yeast is to be achieved.

The optimal temperature for maximum cell yields is considerably lower than that for maximal specific growth rate; μ is found to be highest at 32.5 °C, while the maximum growth efficiency occurs at 20 °C (White, 1954). Eroshin *et al.* (1976) reported the optimal temperature for growth efficiency at 28.5 °C. Lower cell yield at high temperatures is probably due to the greater maintenance energy requirement. Eroshin *et al.* (1976) also reported the optimal pH for growth efficiency to be 4.1 in their fermentation system.

In addition to various fermentation conditions, cell yield is also affected by the presence of metabolic by-products and other substances in the fermentation system. Chen and Gutmanis (1976) reported significantly lower yield as the CO_2 concentration exceeds 1.6×10^{-2} g l^{-1} in the fermenter broth. Molasses substrate used for yeast production usually contains several inhibitory

substances, such as SO_2, aconitic acid, nitrite, heavy metals, *etc.* Lower substrate yields are obtained as the SO_2 concentration exceeds 100 p.p.m. in the fermenter broth. Similar results are obtained at high concentrations (*i.e.* 1–2%) of aconitic acid. Notkina *et al.* (1975) reported a loss in yield at 0.004–0.001% of nitrite.

20.3 COMMERCIAL PRACTICE IN FERMENTATION

20.3.1 General Description of Production Processes

Commercial baker's yeast is usually produced in a multiple-stage process. The early stages, one or more, are batch fermentations in that all ingredients are in the fermenters before yeast inoculum is added. The later stages are fed-batch fermentations, where the ingredients are added to the fermenters in a predetermined way before and during fermentation. The yeast cells produced in the later stages are separated by centrifugation; portions are used to inoculate the subsequent stage. Suomalainen (1963) reported an eight-stage process leading to a final production of 100 000 kg of compressed yeast in two weeks. Burrows (1970) reported a five-stage process with a final production of 125 000 kg of compressed yeast in 65 h, not counting the turn-around time between fermentations.

A variation of the multi-stage process is the DeLoffre process (DeLoffre, 1964), which normally involves a single batch fermentation stage followed by a three-stage fermentation, designated as A, B, C. Stage A is a highly alcoholic fermentation resembling a batch fermentation. Stage B is transitory to the highly aerated stage C, which corresponds to the final fermentation of the fed-batch process described above. Due to the constant adjustment of feeding rates based upon the yeast solids and ethanol contents in stages B and C, the results of the DeLoffre process can be quite variable. This process is not widely practiced.

Continuous fermentation processes have been used successfully in the production of *Candida* yeasts from spent sulfite liquor, hydrocarbons and ethanol substrates (Chen and Peppler, 1979), but its application to the production of baker's yeast from molasses substrate is limited (Olsen, 1960; Sher, 1960). The disadvantages of the continuous fermentation are as follows (Burrows, 1970): (1) inferior baking quality due to undesirable physiological changes, such as elongation of yeast cells, in continuous fermentation; (2) contamination problems, particularly with the fast-growing *Candida krusei* yeast; and (3) unfavorable economics because of costly equipment and lower productivity in the multi-vessel system.

20.3.2 Oxygen Supply and Fermenter Systems

It is generally recognized that the rate of transfer of atmospheric oxygen into the fermenter liquid is the limiting factor in yeast propagation. The major concern in fermenter design is the maximization of oxygen transfer capacity and economy (Finn, 1969).

The fermenters with mechanical agitation usually have higher oxygen transfer capacity, as measured by oxygen transfer rate or K_La. An oxygen transfer rate close to 1 mol O_2 l^{-1} h^{-1} has been reported in a submerged turbine fermenter (Nyiri, 1974), even though the normal operating aeration capacity is lower. The K_La of this type of fermenter can be estimated from the power consumption per unit volume, air velocity, number and speed of impellers. A yeast concentration of 8–10% solids (Reed, 1982) or even 15% solids (Skiba, 1966) can be attained in this type of fermenter. Thus, the yeast productivity is expected to be very high in the submerged turbine fermenter, which has been used for commercial production of baker's yeast in many countries including the USA. The Vogelbusch fermenter with a rotating aeration wing also has been used in commercial-scale fermentations, particularly in European countries (Suomalainen, 1963; Hospodka *et al.*, 1962). The Waldhof fermenter with a rotating aeration wheel has been used commercially in the production of *Candida* yeast from spent sulfite liquor (Inskeep *et al.*, 1951), but seldom in the commercial production of baker's yeast from molasses substrate. The self-priming fermenter (Frings aerator), which sucks air through a hollow vertical shaft and aspirates through a rotating turbine, has been used for yeast production, but mostly on a pilot scale (Ebner *et al.*, 1967; Ebner and Enenkel, 1974). The Vogelbusch deep jet aeration system uses rotary pumps outside the fermenter to recirculate continuously the fermenter broth to the top of the fermenter where air is sucked into a hollow overflow shaft (Schreier, 1974). It has been used successfully for

aerobic treatment of industrial and community waste water, but its application to baker's yeast production is not known with certainty.

Among the diffused-air fermenters, the gas-sparged fermenter with perforated horizontal pipes is used around the world for commercial production of baker's yeast. In this type of fermenter, the air is blown by a compresser–blower through a large number of small holes in the horizontal pipes near the bottom of the fermenter. Rosen (1977) reported a fermenter system of 150 m^3 which was aerated through a center pipe with 24 side tubes provided with 30 000 holes of 1.5 mm diameter. The aeration capacity, in terms of $K_L a$, of a gas-sparged fermenter may be predicted from the dimension of the fermenter, airflow rate, air velocity at the orifice and the calculated air bubble size (Bhavaraju, *et al.*, 1978). Although the aeration capacity of the gas-sparged fermenter is lower than the submerged-turbine fermenter, its aeration economy as measured by lbs oxygen transferred per energy unit may be higher, particularly at low oxygen transfer rates (Hatch, 1975). The aeration tower system is similar in principle to the gas-sparged fermenter except for the dimension of the fermenter. An aeration capacity equivalent to a $K_L a$ value of 640 h^{-1} has been reported by Yoshida and Akita (1965) for this system. The air-lift fermenter has been used for semi-commercial production of single-cell protein from *n*-paraffins (Cooper *et al.*, 1975), but it is not known to be used for commercial production of baker's yeast.

The oxygen transfer rate required depends upon the fermentation process in use. If the maximum feed rate during the course of fermentation is 10 g of sugar l^{-1} h^{-1}, a yeast productivity of 5 g solids l^{-1} h^{-1} is expected. Thus, a minimum oxygen transfer rate of 5 g O_2 or 156 mol O_2 l^{-1} h^{-1} must be provided. A higher yeast productivity can not be achieved in a fermenter system which can not provide higher aeration capacities.

In evaluating competing aeration systems, the following factors must also be considered: (1) the power cost per unit volume of oxygen transferred; (2) the heat energy required per unit volume of oxygen transferred, since this will have an effect on the capital and operating costs of the system selected; (3) the ratio of the working volumes of various systems under consideration; (4) the effect of the air–liquid emulsion generated on the heat-transfer efficiency in the heat removal from the fermenter; and (5) the cost of installation.

There is considerable variation in the size of baker's yeast fermenters. The smaller ones have volumes in the neighborhood of 50 m^3 while the largest may be over 350 m^3. They have diameters in excess of 7.5 m and heights of up to 10 m. The fermenter size influences the type of aeration system selected. In fermenters with diameters up to about 4 m, mechanical or sparger aeration systems work well. In fermenters with diameters over 4 m, gas sparged systems are generally used because the distribution of air by a properly designed sparger is nonradial and therefore there is greater assurance of uniform air distribution and mixing throughout the fermenter. Fermenter size is an important factor in the selection of the fermenter cooling system and the method of heat removal should be compatible with the aeration system selected. Coils and/or plate heat exchangers are most commonly used for heat removal. The cooling area required for a given fermenter is a function of the yeast solids to be produced, the mechanical energy inputs, the temperature of the nutrient solutions and the temperature of the cooling water available. The quanitity of heat liberated during the production of baker's yeast is in the order of 20×10^3 kJ per kilogram of yeast solids (Fengl, 1969). The heat input from the fermenter mechanical systems, mainly aeration, and nutrient feed systems, mainly molasses, can amount to between 10 and 20% of the total heat load. Since natural cooling water temperature and availability are a function of location, the heat exchanger surface area required for a given fermenter will vary with location. The aeration system and heat removal surface are an integral part of any fermenter design. All other fermenter connected systems are auxiliary in that they can be by-passed in operation.

The aeration capacity and economy of various types of fermenters are tabulated in Table 1. For the high-price and small volume pharmaceuticals where productivity is a major consideration, fermenters with high aeration capacities, *e.g.* submerged turbine or similar mechanically-agitated fermenters, should be used. For the low-price and high volume products, such as baker's yeast, fermenters with high aeration economy, *e.g.* diffused-air fermenters, may have some advantages.

20.3.3 Preparation of Raw Materials

20.3.3.1 Energy substrates

Molasses has replaced grain mash as the primary energy substrate for yeast production since World War I. Beet and cane molasses are used singly or in combination. Decisions regarding the

Table 1 Aeration Capacity and Economy of Fermenter Systems[a]

Fermenter system	*Normal operating O_2-transfer capacity* (mmol O_2 l^{-1} h^{-1})	*Aeration economy* (lb O_2 HP^{-1} h^{-1})	*Air utilization* (%)
Mechanically agitated systems			
Submerged turbine	300–350	2.4	14–45
Waldhof aeration wheel	—	2.4–3.2	—
Phrix	—	1.7–2.5	14–22
Self-priming (Frings)	—	2.4	14
Vogelbusch aerating wing	140	2.3–2.7	14–19
Vogelbusch deep jet aerator	315–385	3.3	28
Diffused-air systems			
Gas-sparged fermenter	100–150	2.7	10–15
Air-lift fermenter	120	4.3–7.0	7–8

[a] Compiled from Cooney *et al.* (1977); Hatch (1973, 1975); Hospodka *et al.* (1962); Schreier (1974); Reed and Peppler (1973); Reed (1982).

type of molasses to be used are based on molasses availability and economics. When both types of molasses are available at comparable cost, higher proportions of beet molasses are used because it is easier to process and normally yields a lighter colored yeast.

Raw beet and cane molasses enter the factory at 80 to 90 °Brix and contain 80 to 85% solids of which up to 60% may be sugar. Dilution is necessary to facilitate pumping, clarification and sterilization. Dilution is accomplished in one of several ways. The simplest is to weigh a given quantity of molasses and add it to or add to it a given quantity of water. More complex systems use special pumps or blending systems, utilizing analog or digital instruments, to blend molasses and water to the desired Brix.

Factories using both cane and beet molasses may blend the two during the initial dilution, or at any stage of molasses treatment up to feeding to the fermentations, or the molasses may not be blended but fed to the fermentations in separate streams. The reasons why different manufacturers blend at different stages are based on experimentation which has indicated that benefits are to be derived from processing in a particular way, or on one method being the most consistent with plant processing conditions.

Cane and blends of cane and beet are usually diluted to between 30 and 40 °Brix for processing. Pure beet molasses may be processed at a lower dilution. The greater dilution of cane molasses helps clarification by providing a greater gradient between the specific gravities of the suspended solids and the liquid phase. The manufacture of sugar from cane starts with the crushing of the sugar cane to produce a sugar rich juice. The crushing and washing of the fibrous husk liberates inorganic and non-sugar organic compounds some of which are colloidal in nature. Beet sugar production starts with the slicing of the thin skinned beets and the resulting molasses contains less suspended solids.

The sludge and colloidal material in cane molasses cause problems in various stages of the manufacturing process and cane molasses is almost always clarified. Beet molasses on the other hand may or may not be clarified. Whether or not to clarify beet molasses is based on preference and perceived need on the part of each manufacturer. In general molasses is clarified to improve final yeast colors. In addition, yeast made from clarified molasses is easier to dewater and dry. Molasses clarification probably aids fermenter oxygen transfer and reduces the formation of fermentation foam.

Early clarification procedures involve precipitation of suspended matter by forming flocculents using alumina or phosphates and/or filtration with diatomaceous earth. Cane molasses is difficult to filter even after precipitation, and when necessary basket centrifuges were used to polish the decanted supernatant. Chemical clarification and filtration were later replaced by clarification in nozzle clarifiers equipped with sludge recycle. More recently, intermittent discharge centrifuges have come into use for molasses clarification. The movement to centrifugal clarification reduced losses, materials and labor, and improved overall process control.

Clarification can be done before or after sterilization depending on manufacturing philosophy. Clarifying after sterilization removes the precipitate formed in heating to sterilization temperatures but to some extent increases the possibility of recontaminating the sterile molasses in the clarifying equipment, while clarifying before sterilization reduces fouling of the sterilization equipment and reduces the risk of recontamination. Molasses sterilization is accomplished by direct steam injection on a batch or continuous basis. Batch sterilizations involve the heating of molasses in a tank, usually, but not always, at atmospheric pressure, to specified time and tem-

perature conditions. Continuous sterilization is done in closed loops at higher temperatures with very short residence times, *e.g.* 1 s at 140–145 °C (Rosen, 1977). Continuous systems sometimes incorporate preheating in plate type heat exchangers, which are used in various configurations to save energy, and flash tanks that receive the heated molasses from the high pressure sterilization loop.

Recently, hydrolyzed cheese whey has been suggested as a substrate for baker's yeast production (Stineman *et al.*, 1980; Anon, 1982). The whey proteins and suspended solids are partially removed by centrifugation and ultrafiltration; the lactose in the permeate is hydrolyzed by lactase, either free or immobilized, and concentrated to a solids content of 65–70% or a fermentable sugar content of 50%. This substrate also has to be diluted, clarified and sterilized prior to use for baker's yeast production.

20.3.3.2 Nutrient supplementation

A comparison of the composition of molasses with that of baker's yeast shows that the molasses substrate is deficient in certain essential nutrients such as N, P, K, Mg, Zn and S (Harrison, 1971). These elements must be supplied for optimal yeast growth.

The nitrogen levels in baker's yeast are usually dictated by the leavening activity and the storage stability desired in the final yeast. Nitrogen normally makes up from 6.5 to 10% of the yeast solids. Beet molasses contains approximately 1.5% nitrogen one third of which is available to the baker's yeast. Cane molasses has a low nitrogen content and for practical purposes it contains no biologically available nitrogen for yeast growth. Baker's yeast preferentially takes up inorganic nitrogen. Aqueous ammonia, anhydrous ammonia, ammonium salts and urea are the usual sources of nitrogen in commercial fermentations. They are used either singly or in combination. The nitrogen source is selected on the basis of cost, availability and the effect of these compounds on fermentation pH control. In an efficient fermentation system, most of the ammonia fed is taken up by the yeast cells.

The P_2O_5 content in baker's yeast ranges from 1.5 to 3.5% of the yeast solids. While the yeast cells can take up larger quantities of phosphate, the commercial practice is to limit the P_2O_5 content to 25–30% of the nitrogen content. Small quantities of K, Mg and Zn are usually supplied in the form of chloride or sulfate, while S is supplied either as sulfuric acid or as sulfate, depending upon the fermentation process in use. There is no need to supply other trace elements, since molasses usually contains adequate quantities of these elements.

As pointed out in a previous section, baker's yeast requires some members of the vitamin B-complex, particularly biotin, for optimal growth. Since beet molasses is deficient in biotin (*i.e.* 0.01–0.13 $\mu g\ g^{-1}$), it is a common practice to blend beet molasses with blackstrap cane molasses which has a higher biotin content (*i.e.* 0.6–3.2 $\mu g\ g^{-1}$). Pure biotin may be used when cane molasses is not available. Oura (1978) showed that 100 μg biotin/100 g sugar is required for aerobic yeast growth. Addition of other vitamins, such as thiamine and pantothenic acid, is often practiced as well.

20.3.4 Multistage Fermentation

Peppler (1979) reported a six-stage fermentation process for the commercial production of baker's yeast. The first stage (F1) was a flask culture stage, the second (F2), third (F3) and fourth (F4) stages were batch fermentations, while the fifth (F5) and sixth (F6) stages were fed-batch fermentations. The procedure for the multi-stage process is described in the following sections.

20.3.4.1 Flask culture stage (F1)

Yeast cultures used for daily production of baker's yeast are maintained by periodic transfer on molasses or malt agar slants. Production cultures may be periodically renewed from stock cultures maintained under more stringent control procedures in a central quality control laboratory. These cultures are grown for 2–4 days through one or more flasks containing molasses or malt media with approximately 5% sugar for introduction to the factory; Erlenmeyer, Carlsberg or Pasteur flasks are commonly used. In some processes, the quantity of flask culture may be as

much as 75 l and in others it may be as little as 1 l. Most processes use less than 5 l to inoculate the initial plant fermentation, the first pure culture stage.

20.3.4.2 Pure culture stages (F2, F3 and F4)

The plant production process begins with a pure culture fermentation (F2). The number of pure culture fermentations depends on the process in use and the quantity of contamination-free inoculum required. The pure culture fermentations are run in small fermenters which are easy to sterilize and the process is basically a continuation of the flask cultures except that the pure culture fermenters have provisions for sterile aeration and aseptic transfer to the next stage. The fermentations are batch in that all the nutrients are in the fermenters prior to inoculation with the flask culture or with the yeast from a previous pure culture stage. Rigorous sterilization of the fermentation media prior to inoculation is conducted by heating under pressure or by boiling at atmospheric pressure for extended periods. The critical factor in pure culture operations is sterility. Since *S. cerevisiae* has a long generation time compared to other microorganisms, a contaminating microorganism can easily outgrow the baker's yeast strain of interest.

Sterility and the exponential nature of yeast growth make it unnecessary to inoculate a pure culture fermentation with a large quantity of flask culture. If the quantity of yeast solids available from 1 l of flask culture is 5 g and the average doubling time is 2.5 h, this pure culture inoculum will produce, in the presence of adequate substrate and nutrients, 5.12 kg yeast solids in 25 h or 10 generations. Doubling the inoculant will reduce the fermentation time by 2.5 h, and a 100-fold increase in the inoculum will reduce the fermentation time only by a little over one half. The usual fermentation time for the first pure culture stage is 24 h and those for the subsequent stages are 9–11 h, according to Burrows (1970). Whether to use one or more pure culture fermentations depends on the quantity of yeast needed to inoculate the subsequent fed-batch fermentations. Generally, a single pure culture stage will be used for 5.0 kg of yeast solids or less, and a multivessel system will be used for greater quantities. In this case, the entire contents of a pure culture vessel is aseptically transferred to inoculate a subsequent fermentation (*i.e.* F3 or F4). The size relationship of one pure culture vessel to another varies with the manufacturer's scheme over a considerable range and depends to some extent on time considerations. The poor substrate yield and the practice of inoculating one fermentation with the entire contents of the previous fermentation limit the size of pure culture vessels to less than 30 m^3.

The need for process control in the pure culture operation is limited. Microbiological testing of the media before, during and after each fermentation is essential. The substrate concentration of the initial fermenter is often standardized to between 5 to 7.5% sugar. This is most easily done by manual measurement with a Brix hydrometer. The Brix measurement can also be used to monitor the progress of the fermentation, which is deemed complete when the Brix reading declines to a steady point. The pH may be monitored over the course of fermentation but any pH adjustment is made prior to sterilization of the media. Once the pure culture fermentation is started, the only controllable parameters are temperature and aeration. Control of aeration is not critical because of the excess sugar substrate. Temperature control is meaningful but control can be over a broad range.

20.3.4.3 Fed-batch fermentation (F5 and F6)

The main fermentations are of the fed-batch type and they are highly aerobic. The size of the fermenters and the auxiliary equipment required for incremental substrate supply, aeration and cooling make sterilization and the maintenance of sterility difficult. In practice, contamination limits the number of fed-batch fermentations after the pure culture stage. Some producers use the pure culture to inoculate a single fed-batch fermentation, while others may produce a seed yeast fermentation (F5) followed by the final fermentation (F6). Sometimes, as many as three to four successive seed yeast fermentations may be conducted prior to the final fermentation. One process calls for the drying of the seed yeast for long term storage. The dried seed yeast is then rehydrated as needed to inoculate three to four additional fermentations and the need for routine pure culture fermentations is eliminated.

The primary objective in the seed yeast fermentations, other than growing the biomass, is to limit contamination. This usually means greater attention to sterilization, operating at lower pH, shorter fermentation time and high growth rate at the expense of substrate yield. The final stage

of commercial fermentation is conducted to maximize leavening activity, storage stability, substrate yield and the appearance of the final yeast product. Most of these objectives are achieved by manipulating various fermentation parameters including the pattern of substrate and nutrient addition.

The baker's yeast production process developed empirically, as an art, and while it is now possible to design *S. cerevisiae* fermentations with respect to the efficient production of a given amount of biomass, the development of fermentations to yield given physiological characteristics in the final yeast is still empirical. In practice, baker's yeast is not propagated with strictly exponential substrate additions nor is it propagated at the highest specific growth rate compatible with maximal substrate yield. Rather, the baker's yeast propagation is conducted to produce specific properties in the final yeast product. The substrate feed pattern and consequently the specific growth rate are a function of the conditions necessary to obtain the end results. Since the substrate feeding pattern in the fed-batch fermentations is developed empirically, it is expected to be different for different manufacturers. Some feeding patterns have been reported by Reed and Peppler (1973).

In order to achieve an acceptable substrate yield, the sugar concentration in the fermenter should be kept below certain levels. Wang *et al.* (1977) reported glucose repression of respiration at a glucose concentration of 0.13 g l^{-1} when the specific growth rate corresponded to $\mu = 0.25$ h^{-1} in a fed-batch fermentation. Dellweg *et al.* (1977) placed the critical concentration at 1.1 mmol l^{-1} (*i.e.* 0.2 g l^{-1}) in continuous fermentations with growth rates in the range of $\mu =$ 0.14–0.18 h^{-1}. Exceeding the critical glucose concentration results in the production of ethanol and intermediate organic acids which tend to lower fermentation pH. These conditions have bacteriostatic effects and they are used by some manufacturers to limit contaminant growth in their seed yeast fermentations and in the early phase of their final commercial fermentations. The loss of substrate yield accompanying ethanol formation in the final fermentations can be minimized by allowing the yeast to metabolize the ethanol present late in the fermentation.

Aside from substrate concentration and feeding pattern, other fermentation parameters, such as pH and temperature, are also established empirically, not necessarily based upon their effects on specific growth rate and growth efficiency. Usually the pH lies between 4.0 to 6.5 and the temperature ranges between 28 to 32 °C. A properly designed final fed-batch fermentation should also permit the yeast cells to mature. This can be accomplished by stopping the feeding of nutrients at the end of fermentation but allowing a slight aeration to continue for an hour to 'ripen' the yeast, according to Oura *et al.* (1974). During this period, the unused substrate is assimilated; the cells with buds grow from two daughter cells and mature. These investigators also pointed out that the yeast samples taken at the middle of ripening period had the best leavening power and that the unripened yeast cells with buds were less stable in that they autolyzed easily.

The fermentation time for the final stage may vary from 10 to 20 h, during which the yeast may multiply six- to seven-fold (Burrows, 1970; Soumalainen, 1963). The yeast solids content at the end of fermentation may vary from 3 to 8% depending upon the aeration capacity of the fermenter system. In order to achieve a high productivity, the substrate concentration and the feeding pattern should be compatible with the aeration capacity of the fermenter.

20.3.5 Instrumental Control of Fermentation Process

In order to produce baker's yeast economically, a proper environment must be provided in the fermenter, so the efficiency and productivity of yeast growth can be maximized. Instrumental control of the physical environment in the fermenter, such as temperature, power input, gas flow, liquid flow, pressure, *etc.*, is relatively straightforward; adequate sensors are generally available. With the exception of pH and oxygen measurements, instrumental control of the chemical environment is in a less advanced state.

20.3.5.1 Physical parameters

(i) Power input

Two main systems have been used for measurement of shaft power: torsion dynamometer and strain gauge. The latter is preferred because of its greater accuracy. In this system, balancing strain gauges are mounted on the impeller shaft in the fermenter broth. Lead wires from the

gauges are passed out of the vessel *via* an axial hole in the shaft. The electrical signal is picked up from the rotating shaft by an electrical slip ring.

(ii) Impeller speed

This is routinely measured by a magnetic proximity sensor which counts revolutions of a gear or a magnetic spot on the shaft. The output of the sensor is electronically manipulated to give the r.p.m. or tip speed on a digital or meter readout.

(iii) Liquid flow

Liquid flow may be effected by a centrifugal pump, a positive displacement pump, or a diaphragm metering pump, such as Interpace's Pulsafeeder. The pump is generally coupled with an electric actuator and a controller. The liquid feeding pattern is dictated by a curve follower. The actual flow rate may be monitored by a flow meter or by mounting the liquid reservoir on a load cell system, which weighs and records continuously the content in the reservoir.

(iv) Pressure

Relatively simple diaphragm gauges can be used to measure the pressure in a fermenter. The resulting pneumatic signal can be either relayed directly or transduced through a simple device to an electronic signal.

20.3.5.2 Chemical parameters

(i) pH

Sterilizable combination electrodes suitable for continuous operation are commercially available. *e.g.* Ingold's pressurized pH electrode (Buhler and Ingold, 1976). It may be coupled to a pH controller, such as New Brunswick's model 121 or 122, for monitoring and control of yeast fermentations.

(ii) Dissolved oxygen

Two types of membrane probe are used: polarographic (or amperometric) and galvanic. The former requires an applied polarizing voltage of -0.7 to -0.9 V, while the latter is self-polarizing. The electrical signal from the oxygen controller is used to monitor, to record and to maintain the pre-set DO level by regulating the air-flow rate and/or impeller speed (Vincent, 1974).

(iii) Oxygen in effluent air

The oxygen content in the exit gas may be monitored by a paramagnetic analyzer or by an oxygen probe. For the paramagnetic analyzer, the moisture in the exit gas must be removed, but this operation is not necessary for the oxygen probe. Satisfactory results have been obtained in the monitoring of yeast fermentations with a polarographic probe (Chen and Gutmanis, 1976). It is also possible to monitor effluent oxygen with a mass spectrometer, such as the Perkin-Elmer multiple gas analyzer MGA-1200.

(iv) Residual glucose

There is no reliable method at the present time. Attempts have been made to monitor the residual glucose content in the fermenter broth by using an L and N's glucose analyzer coupled with a continuous sampling probe attachment (Chen and Gutmanis, 1976). Since this method is based upon the production of H_2O_2 by the immobilized glucose oxidase, any contamination by the catalase-containing yeast cells will seriously affect the accuracy of this method.

(v) Other chemical constituents

Although several specific ion electrodes and enzyme electrodes have been developed in recent years, none of them are suitable for continuous monitoring operation at the present time.

20.3.5.3 Fermentation products

(i) CO_2 in effluent gas

The CO_2 in the exit gas can be successfully monitored by an IR analyzer. Development of a membrane CO_2 probe suitable for monitoring dissolved CO_2 in the fermenter broth and the CO_2

in the exit gas has been reported by Shoda and Ishikawa (1981). The mass spectrometer may also be used to monitor the CO_2 in the exit gas.

(ii) Ethanol in exit gas

The ethanol content in the exit gas can be monitored by a long path IR analyzer, such as Miran II Analyzer, at 3.2 and 3.4 μm. Quantitative measurements were obtained by calibrating the instrument against ethanol solutions of known concentrations. The electrical signal from the IR analyzer can be used to control the substrate feeding rate in a fed-batch fermentation. For precise on-line measurement of ethanol in the exit gas, a mass spectrometer may be used. Monitoring of seven volatile compounds in the gas and liquid phases of fermenters by this method has been reported by Weaver *et al.* (1980). For approximate monitoring purposes a hydrocarbon analyzer may be adequate.

(iii) Biomass of baker's yeast

There is no satisfactory method for direct on-line monitoring of microbial biomass in the fermenter. Continuous measurement of turbidity with a turbidimeter or spectrophotometer has been attempted, but the results are not satisfactory. Indirect monitoring of microbial mass is based upon material balance (Cooney *et al.*, 1977), oxygen balance or the determination of certain cell constituents, such as ATP, NAD, *etc.* A constant chemical composition in the biomass is assumed in all indirect monitoring methods; the validity of this assumption remains to be established and confirmed.

20.3.5.4 Computer-coupled fermentation

In this application, a computer may perform the following functions: data acquisition and processing as well as process monitoring, control and optimization (Flynn, 1974). It becomes possible to obtain instantaneously many indirect measurements relating to the physiological state of the baker's yeast and to the fermentation conditions, such as the respiratory quotient (RQ), growth rate, cell density, K_La, C/N ratio of the nutrient solution, *etc.* It appears that the crucial problem is to develop a control strategy for the computer-coupled fermentation, *i.e.* which parameters are to be selected for process control and optimization.

The respiratory quotient (RQ) is often used for the control of baker's yeast fermentation. As shown in equation (1), the RQ is calculated to be 1.02 under efficient growing conditions. Aiba *et al.* (1976) used a constant RQ of 1.0 to 1.2 to regulate the substrate feeding rate in a fed-batch culture of *S. cerevisiae*. A substrate yield of 0.55 g cell/g glucose and a specific growth rate of 0.24 h^{-1} were obtained.

If one accepts the fact that the substrate yield is determined mainly by the metabolic pathways of the microorganism and that the volumetric productivity is limited mainly by the aeration capacity of the fermenter, it becomes obvious that the computer aided fermentation will not be expected to make a significant impact on the efficiency and the productivity of baker's yeast production. The advantages of a computer-coupled system will lie in the greater uniformity of the product, better reproducibility of the fermentation process and the substantial reduction of manufacturing cost through more efficient utilization of raw material, fermenter and manpower.

20.3.6 Handling of Baker's Yeast

20.3.6.1 Separation, washing and cream storage

At the end of each fed-batch fermentation stage the yeast cells are centrifugally separated from the fermented media and subjected to one or more washing separations. Washing separations are run to reduce the non-yeast solids in the fermenter liquor which remain with the cells after the initial separation. This is necessary because the non-yeast solids hinder filtering and drying, darken the color of the final yeast, and affect the rehydration characteristics of active dry yeast. The number of washing separations depends on the fermentation stage and on the efficiency of the washing system. In practice the seed yeast fermentations usually receive fewer washings than the final commercial fermentations. Washing system efficiency is determined by the concentration of the yeast solids, the quantity of dilution water used and the solids content of the dilution water. The last factor operates when the washing water runs counter current to the yeast stream. This is usually done to reduce water usage.

If the final fermenter liquor contains 4% yeast solids and 4% non-yeast solids, and the yeast solids are 40% of the cell, then the 4% non-yeast solids will be contained in 90% of the fermenter volume. If the separator yeast discharge contains 20% yeast solids, the concentrate will consist of 50% yeast cells and 50% of the original fermenter liquor containing 4% non-yeast solids. Diluting the concentrate back to 110% of the original volume will result in the liquid phase solids being reduced to 0.4%. Reconcentration will yield a cream yeast containing 0.4% non-yeast solids in the liquid phase and each time the washing process is repeated a ten-fold reduction in the non-yeast solids is obtained. Washing obviously requires large volumes of water. Where water is scarce or effluent considerations limit its use, the supernatant discharge from the final washing separation can be used as the diluent in the previous stage. In this case the efficiency of the washing operation is reduced by the fraction of original solids running in the wash water when the system reaches equilibrium.

The separation process yields a light colored yeast cream containing up to 22% yeast solids. At this concentration the cells occupy 55% of the liquid volume and the cream appears to be highly viscous. Cream yeast is stored in agitated tanks at 2 to 4 °C for use in seeding additional fermentations or for additional dewatering if it is the final trade yeast. While cream yeast can be held for several weeks without appreciable deterioration it is seldom held for more than a few days. The pH of seed yeast creams is usually adjusted to between 2.5 and 3.5, and sodium bisulfite or ammonium persulfate may be used as a bacteriostat.

20.3.6.2 Filtration

While baker's yeast is occasionally sold in the cream form, the greatest portion is sold either as compressed yeast containing 27 to 30% yeast solids (Europe and America respectively) or as dry yeast containing from 92 to 96% solids (worldwide). There are many disadvantages to distributing cream yeast, primary among these are its greater water content, and attendant higher hauling costs, and the investment required to install a liquid yeast distribution system. A further 10% reduction in the water content of the cream yeast produces a yeast cake containing approximately 25% more yeast solids. The compressed yeast can be molded into blocks or bulked in bags for distribution.

Dewatering takes the cream yeast from 18–22% solids up to 28–33% solids. Filter press or rotary vacuum filters are used for this operation. The filtered yeast may or may not be mixed with emulsifiers prior to being extruded into yeast cakes or packaged in large multi-walled paper bags. Fresh baker's yeast may also be marketed in the form of free-flowing particles. This type of product is produced by adding certain hydrophobic SiO_2 and hydrophilic SiO_2, modified starch, micronized cellulose, *etc.* to the filtered yeast (Luca *et al.*, 1979; Pomper and Akerman, 1980). Yeast cakes may range in weights from approximately 10.5 g to 2.25 kg. Multi-walled bags vary in weight up to about 22.5 kg. The compressed yeast is then refrigerated at 2 to 4 °C until used.

20.4 COMMERCIAL PRACTICE OF ACTIVE DRY YEAST (ADY) PRODUCTION

Successful commercial production of active dry yeast started during World War II, even though many attempts to prepare this type of product had been patented in the 1920s. Frey (1957) reviewed the historically significant studies leading to the development of ADY; Thorn and Reed (1959) described the production and baking techniques for ADY.

20.4.1 General Description of Drying Processes

The production of ADY begins with the selection of baker's yeast strains which will yield the desired characteristics on drying. The selected strains are propagated using fermentation protocols known to condition the yeast cells for dehydration. The yeast so produced is filtered to as high a solids content as possible. The yeast cake is then extruded into strands or particles of various sizes, which are dried in an air stream under controlled conditions.

In order to produce an ADY product with acceptable leavening activity and storage stability, the following factors should be taken into consideration during dehydration: drying temperature, drying rate and the final moisture content in ADY. As a living microorganism, the vegetative

yeast cell is rapidly killed at temperatures exceeding 50 °C (Reed and Peppler, 1973). Obviously, yeast should not be dried at such elevated temperatures. The equilibrium moisture content of ADY may be predicted from the predetermined desorption isotherms. A series of these curves has been published by Josic (1982).

20.4.2 Drying Methods and Systems

Although many methods have been explored, only a few are in use for the commercial production of ADY. Selection of a drying system is based upon the desired physical appearance and properties of the ADY as well as the cost of installation and operation. Some of these commercial drying systems are discussed below:

20.4.2.1 Roto-Louvre dryer

This dryer was originally developed in Sweden for drying of wood chips, paper pulp, *etc.* It was later used for dehydration of foods, chemicals, coal and cement (Erisman, 1938). The dryer consists of a hollow cylinder with radial louvre fin plates attached to the inside wall, dividing the cylinder into a number of small compartments. To these fin plates are attached the tangential louvres. During drying operation, the extruded yeast strands are fed into the cylinder, which rotates at 1 to 4 r.p.m. Heated air, at about 50–60 °C, is blown into the cylinder through the louvres and the tumbling yeast particles. The temperature of the yeast particles does not exceed 45 °C however. For batch operation, the total drying time may vary from 10–20 h (Reed and Peppler, 1973). The roto-louvre dryers range in size from 2.5 to 11.5 ft in diameter, and 8 to 35 ft in length. The largest unit is capable of evaporating 12 000 lb water h^{-1} (Marshall and Friedman, 1950). Operation of a roto-louvre dryer 4.85 m in length and 2.2 m in diameter for ADY production has been reported by Sysojewa and Gorochova (1965). It is possible to use a roto-louvre dryer for continuous operation.

20.4.2.2 Through-circulation dryer

This type of dryer may be used for continuous or batch operation. For continuous operation, the extruded yeast strands are spread as a layer, 1–6 in deep, on an endless perforated belt. It moves through several drying chambers, where heated air is blown alternately upward and downward through the yeast bed to avoid over- and under-drying. The size of each chamber may vary from 30 to 60 ft long and from 6 to 10 ft wide. Each chamber may be considered as an individual unit, complete with fan and heating coils, arranged in series to form a housing or tunnel through which the conveying belt travels. The air velocity, humidity and temperature in each chamber are individually controlled to achieve the desired drying rate and the final moisture content in the final ADY product. Belokon (1962) reported a drying time of 2 to 4 h for this type of dryer with inlet air temperatures of 42, 37, 32 and 28 °C, respectively, for four drying chambers.

In batch operation, the extruded yeast strands are placed on removable screen-bottom trays, suitably supported in the dryer. It is similar to a standard tray dryer except that the heated air passes through the yeast bed instead of across it. The pressure drop through the bed usually does not exceed 1 in of water (Marshall and Friedman, 1950). The direction of air flow may be reversed at regular time intervals. Again, the air velocity, humidity and temperature are controlled during the entire drying operation to achieve the desired properties in the ADY product. Chulina (1969) employed an inlet temperature of 50 °C at the beginning and 35 °C at the end of the drying period.

20.4.2.3 Air-lift (fluidized-bed) dryer

For batch operation, the extruded yeast strands are fed into a drying chamber with a metal screen or perforated plate at the bottom. Heated air is blown from the bottom through the yeast

particles at velocities capable of suspending the yeast particles in a fluid bed. Use of this type of dryer for ADY production has been described by Simon (1976). Emulsifiers and swelling agents are often added to the yeast suspension prior to drying (Clement and Rossi, 1982). The drying time may vary from 10 min to 4 h. For rapid drying between 10 to 30 min, Langejan (1972, 1974) used an air temperature of 100–150 °C at the beginning of the drying period, while keeping the yeast temperature at 24–40 °C. The use of a multichamber air-lift dryer for continuous operation has been patented by Pressindustria (1971). The operating conditions are as follows: air velocity, 4000 m h^{-1}; airflow volume, 4000 m^3 h^{-1}; air temperature, 46, 36, 32 and 30 °C in four chambers; retention time, 3 h; yeast productivity, 160–350 kg h^{-1}. The final moisture content of the ADY was about 7%.

20.4.2.4 Spray dryer

With this process, the yeast suspension, containing 10–20% yeast solids, is atomized into a drying chamber where a stream of heated air is introduced. The moisture from the yeast suspension evaporates into the air stream; the dried yeast powder is separated from the air and collected while the moist cool air is exhausted. Use of this process for ADY production has been patented by Aizawa *et al.* (1968). The yeast suspension was dried at an inlet temperature of 100–120 °C and an outlet temperature of 65–67 °C. The moisture content of the powdery ADY was 6–7%. These investigators found that the addition of certain additives, such as alkali metal salts of inorganic and organic acids, polyhydric alcohols, non-fermentable sugars, urea, *etc.*, to the yeast suspension prior to spray drying resulted in higher fermentation activities of the final product. Presumably, these compounds imparted certain changes in the osmotic properties of the spray-dried ADY. Some other additives, including gelatin, carboxymethylcellulose, *etc.*, have been used by Wakamura *et al.* (1973). These authors used a drying temperature of 60–120 °C in their process; the moisture content of the powdery ADY was 5–6%.

20.4.2.5 Other drying systems

Johnston (1959) patented a process for ADY production by suspending yeast cells in a finely divided state in an edible oil. Water was removed from the yeast suspension by blowing a heated air stream (100 °F; 38 °C) from the bottom of the oil-bath chamber. At the end of drying, oil was removed and extracted from the yeast product. Fermentation activity was found to be quite high in the powdery ADY, which had a moisture content of 7–8%.

Grylls *et al.* (1978) patented a process and an apparatus for the production of powdery ADY by subjecting the yeast particles to disintegration forces while they are being dried in a fluidized-bed dryer. Vacuum drying of the yeast cream spread on an endless steel belt was reported by Hartmeier (1977).

Combination of two drying methods has also been used. Van 'Triet and De Bruijn (1981) patented a process of drying yeast initially in a fluidized-bed dryer at a temperature of 90–130 °C with an air velocity of 0.8–2.0 m s^{-1} and a loading of 100–1000 kg cm^{-2}. As the moisture content in the yeast reduced to 10–25%, the yeast particles were dried in a vacuum dryer at 25–45 °C and at a pressure of 1–10 mm Hg. The final moisture content may be as low as 2%.

20.4.3 Properties of Active Dry Yeast

The properties of active dry yeast are functions of its propagating conditions as well as its drying conditions. It was recognized at the early stage of ADY development that high protein yeast was not suitable for drying; activity loss was substantial and storage stability was poor (Frey, 1957). Until recently, ADY was prepared from yeast propagated under nitrogen limitation, resulting in a product with a low protein content of 40–45% and a relatively high level of carbohydrates. Since the fermentation activity of the yeast is related to its protein content, a low-protein yeast is less active, on a dry matter basis, than the compressed yeast grown to a 50–55% protein level.

The importance of high carbohydrate level in ADY, particularly trehalose, was also recognized at about the same time. Pollock and Holmstrom (1951) reported 16–18% of trehalose in ADY

with good gassing activity. Simultaneous syntheses of trehalose and glycogen were observed in batch culture (Suomalainen and Pfaffli, 1961) and in non-proliferating culture (Grba *et al.*, 1975). A high incubation temperature of 45 °C favored trehalose formation, while a lower temperature of 30 °C was optimal for glycogen synthesis. In fed-batch fermentation, glycogen and trehalose were not formed in parallel, however. The former was formed during the initial exponential phase of growth, while the latter was synthesized at the late exponential growth phase (Grba *et al.*, 1979) or during the depletion of glucose substrate (Panek, 1975). Trehalose accumulated in baker's yeast during transfer from anaerobic to aerobic growth; its amount was highest at the final commercial fermentation stage (Suomalainen and Oura, 1956).

The physical appearance of ADY depends on the drying process. It is important with regard to the application and storage stability of the product. Pellet ADY is produced by roto-louvre dryers. This type of ADY has high storage stability due to the coating developed by the tumbling yeast particles during the drying process. It must be fully rehydrated prior to use. The granular ADY is produced by the through-circulation dryers. Since the yeast particles are not as dense as the pellet ADY, storage stability is somewhat inferior. The friability of the granular ADY may be increased by incorporating certain surfactants (Chen *et al.*, 1966) or by entrapping air (Carduck *et al.*, 1982) in the yeast strands prior to drying, or by controlling the rate of drying. Such friable yeast granules may be pulverized and blended directly with flour for baking (Cooper and Chen, 1966). Extremely fine yeast particles 0.2–3.0 mm long are produced in fluidized-bed dryers. Due to its uniform drying conditions, this drying process can be used to dry high protein yeast (Langejan, 1976, 1980). As a result, high activity dry yeast can be produced by this process (Clement and Hennette, 1982). The disadvantage of this type of product is its poor storage stability, due to its large specific surface area. Also, it should not be rehydrated in water directly due to its high leaching characteristics. The properties of powdery ADY produced in the spray dryers have not been fully investigated, because this product is not widely distributed.

20.5 QUALITY OF BAKER'S YEAST

While a good deal of information is available on the growth and production of baker's yeast biomass, the interrelationship between its propagating conditions and its baking quality is far from being understood. The baker's yeast biomass produced at the highest efficiency and productivity does not necessarily have the most desirable properties for baking. The primary functions of baker's yeast are two-fold: imparting an appetizing flavor and aroma to the baked products and leavening the baked products. Fulfillment of these requirements by baker's yeast will be discussed in this section.

20.5.1 Flavor and Aroma Development in Baked Products

There is little doubt that the predominant flavor and aroma of baked products originate from panary fermentation by baker's yeast. A loaf of bread with physical characteristics similar to yeast leavened bread can be produced by using proper chemical leavening agents such as glucono-Δ-lactone and $NaHCO_3$. Yet the resulting loaf is totally devoid of the flavor and aroma expected of yeast-leavened bread, in spite of the fact that identical ingredients and baking conditions were used. Similarly, the short fermentation continuous-mixing processes used for bread production in the United States during the 1960s are gradually being phased out, due to insufficient flavor and aroma in their products, even though higher levels of yeast are generally used in such processes. Many of the flavor compounds are produced during fermentation (Suomalainen and Lehtonen, 1978). Volatile and nonvolatile compounds, such as acids, alcohols, aldehydes, esters, ether derivatives, furan derivatives, hydrocarbons, ketones, lactone derivatives, pyrazines, pyrrole derivatives and sulfur compounds have been found in bread (Coffman, 1967; Maga, 1974). However, except for isolated cases, little information is available to relate the formation of these compounds to the origin and propagating conditions of baker's yeast. Sugihara *et al.* (1971) reported that *Saccharomyces exiguus* yeast was responsible for the leavening action in San Francisco sour-dough bread, but the bread's characteristic flavor was of bacterial origin (Kline and Sugihara, 1971). Chen and Peppler (1956a) reported the development of an off-odor in cinnamon breads due to the conversion of cinnamaldehyde to styrene by a mutant strain of baker's yeast.

20.5.2 Leavening of Baked Products

There are two facets of yeast leavening: CO_2 production during panary fermentation and CO_2 retention in dough systems.

20.5.2.1 Carbon dioxide production during panary fermentation

Yeast-leavened baked products are made from dough systems containing flour, water, yeast, salt and optional ingredients, such as sugar, shortening, emulsifier, milk solids, flavoring substances, yeast food, *etc.* The rate of CO_2 production (or fermentation activity) is dependent upon the intrinsic properties of yeast, concentration and composition of dough ingredients as well as environmental factors such as temperature, pH, *etc.* The effects of these factors will be examined separately.

(i) Intrinsic properties of yeast

Baker's yeast biomass is normally produced under aerobic conditions to achieve optimal growth efficiency and productivity, yet the panary fermentation in dough system is essentially anaerobic. It has been found that the fermentation activity of baker's yeast decreases when the yeast is transferred from anaerobic to aerobic growth conditions. This phenomenon is related to the decrease in the activities of several glycolytic enzymes in the aerobic yeasts, such as pyruvate decarboxylase (Suomalainen, 1963; Polakis and Bartley, 1965), hexokinase and alcohol dehydrogenase (Oura, 1972b; 1976). Besides aerobicity during yeast propagation, the glycolytic enzyme activities also are affected by the concentration and composition of growth substrates. In the presence of a high sugar concentration in the growth medium, yeast propagation shifts toward anaerobic growth, due to the Crabtree effect. As the glucose concentration increases from 0.6 to 20% in the growth medium, for example, the activities of several glycolytic enzymes, such as aldolase, triosephosphate isomerase, pyruvate kinase and pyruvate decarboxylase, increase 25 to 100 fold (Hommes, 1966). Oura (1972a, 1972b) reported that the highest fermentation activity and pyruvate decarboxylase activity were obtained from baker's yeast grown with glucose substrate, followed by those grown with ethanol and pyruvate substrates. Replacement of glucose substrate by acetate, ethanol, pyruvate or glycerol led to reduced enzyme levels for practically all glycolytic enzymes (Maitra and Lobo, 1971). Polakis and Bartley (1965) reported that the pyruvate decarboxylase activity of the glucose-grown cells was about five times that of the galactose-grown cells. Incubation of molasses-grown baker's yeast in a maltose solution resulted in higher contents of α-glucoside permease and α-glucosidase (Hautera and Lovgren, 1975b). To some extent, the level of glycolytic enzymes is reflected in the nitrogen content of yeast cells; high nitrogen content usually leads to higher fermentation activity. On the other hand, the baker's yeast grown at high temperature, high CO_2 tension (Chen and Gutmanis, 1976) or high aconitic acid content in the molasses substrate usually has lower fermentation activities.

All these data show that the fermentation activity of baker's yeast is related to its propagating conditions. For active dry yeast (ADY), other crucial factors, such as dehydration and rehydration conditions, must also be considered. During dehydration, the moisture content of the yeast cells is reduced to below 10%. It must be rehydrated to restore its full activity for baking. Due to the changes in the semi-permeability of the plasma membranes in the dehydrated cells (Harrison and Trevelyan, 1963), certain low molecular weight cell constituents leach out from the cells upon rehydration (Herrera *et al.*, 1956; Ramnietse *et al.*, 1978). The amount of cell constituents leached during rehydration is regulated by three factors: moisture content of ADY, rehydration temperature and rate of rehydration. The interrelationship of these factors is shown in Figure 1, based upon the data reported by Chen *et al.* (1966). This graph shows that (1) a greater amount of cell constituents leach out from a low-moisture (*i.e.* 5.3% H_2O) ADY than from a high moisture (*i.e.* 8.1%) ADY; (2) greater amount of cell constituents leach out at low rehydration temperature (*i.e.* 10 °C) than at higher rehydration temperature (*i.e.* 43 °C); and (3) addition of an emulsifier, such as sorbitan monostearate, can reduce the amount of leached substances from a low-moisture ADY by slowing down its rehydration rate (Mitchell and Enright, 1959). Blending of ADY with flour prior to rehydration can also slow down water absorption by yeast particles and thus minimize the leaching of their cell constituents (Cooper and Chen, 1966; Bruinsma and Finney, 1981). Even the properly rehydrated ADY has a longer lag phase than compressed yeast; it requires a longer recovering time to achieve its maximum fermentation activity, sugar consumption, oxygen consumption, specific growth rate as well as budding. Beker *et al.* (1974) believed that this was due to the structual damage incurred during dehydration; longer lag phase

was required for the restoration of the polyphosphate and nucleic acids in rehydrated ADY. Generally speaking, the fermentation activity of the rehydrated ADY is inversely proportional to the amount of cell constituents leached during rehydration (Thorn and Reed, 1959; Ponte *et al.*, 1960; Kraus et al, 1981).

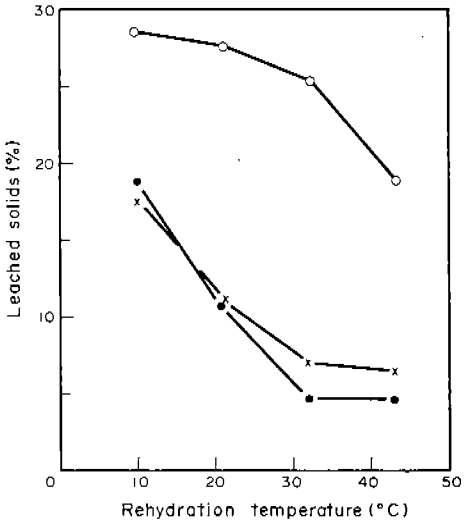

Figure 1 Effect of rehydration temperature, moisture content and surfactant treatment on the leaching characteristics of ADY: ●-●-●, 8.0% moisture content; o-o-o, 5.3% moisture content; x-x-x, 5.1% moisture content + 2% sorbitan monostearate

(ii) Dough ingredients and osmotic pressure

It is well recognized in the baking industry that yeast fermentation activity is substantially lower in dough systems containing high levels of sugar and/or salt. This phenomenon is interpreted as inhibition of yeast fermentation by the high osmotic pressure due to dough ingredients. The quantitative effect of osmotic pressure on yeast fermentation activity is shown in Figure 2. This graph shows that yeast fermentation activity decreases as the osmotic pressure in the dough system increases. Furthermore, D-xylose, a nonfermentable sugar, and NaCl have similar inhibitory effects at the same osmotic pressure, while glucose and sucrose have similar effects. These two lines run parallel to each other but with different ordinate intercepts, due to the additional CO_2 produced from glucose or sucrose supplementation. Thus, the inhibitory effects of fermentable sugars, nonfermentable sugar and salt all can be explained on a physicochemical basis.

The osmosensitivity of baker's yeast in dough systems is dependent on the yeast strain and its propagating conditions. Certain strains have greater tolerance to high osmotic pressure; these strains usually perform well in sweet dough systems containing 20–30% sugar. They tend to have lower invertase contents (Sato *et al.*, 1961; Reed and Peppler, 1973). The baker's yeast grown in a fed-batch process is less osmosensitive than that grown in a batch process; a slow-growing yeast is less osmosensitive than a fast-growing yeast (White, 1954). Pomper and Akerman (1970) propagated baker's yeast in the presence of an effective amount of non-nutritive salts, such as NaCl, Na_2SO_4, NaBr, Na_2CO_3, Na acetate and $SrCl_2$, to impart to the yeast superior leavening activity in sweet dough. Presumably, the yeast so produced adapts to the high osmotic pressure in dough systems. High salt concentration during propagation usually results in lower growth rate and substrate yield, due to a higher maintenance energy requirement (Watson, 1970).

In contrast to the inhibitory effect of high sugar concentration on yeast fermentation activity, the effect of sugar composition is difficult to demonstrate in panary fermentation. The formulas of various baked products may consist of no sugar supplementation in lean-dough systems to 30% sucrose or glucose in sweet-dough systems. The predominant fermentable sugar in the lean-dough system is maltose, even though small quantities of sucrose, glucose, fructose, *etc.* are present in the flour (Reed and Peppler, 1973). Many authors have reported low fermentation activity of commercial baker's yeast in synthetic maltose media (Atkin *et al.*, 1946; Seeley and Ziegler, 1962; Schultz, 1965). Correlation between maltose fermentation activity and α-glucoside per-

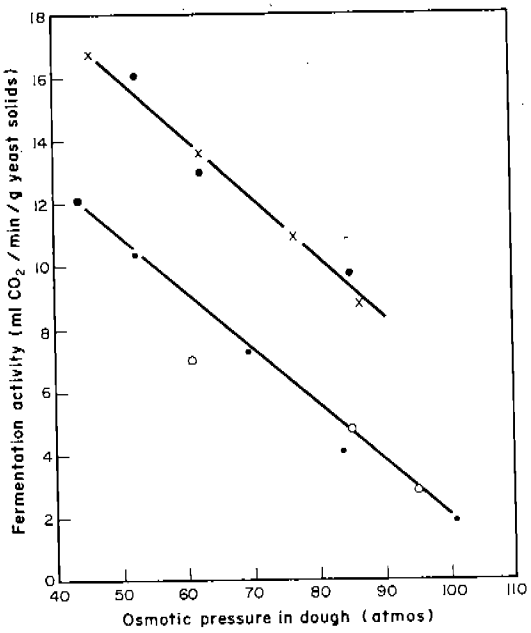

Figure 2 Yeast fermentation activity in relation to the osmotic pressure in the dough systems containing various amounts of ingredients: ●-●-●, sucrose, x-x-x, glucose; ·-·-·, D-xylose; o-o-o, NaCl

mease or α-glucosidase content has been demonstrated by Hautera and Lovgren (1975b), yet none of these criteria has any correlation to the leavening activity in lean-dough fermentation (Lovgren and Hautera, 1977; Suomalainen, 1975). These authors believed that the CO_2 produced in the early phase of lean-dough fermentation did not derive from maltose, but from other fermentable sugars in the flour. On the other hand, a high fermentation rate has been observed in the dough system containing no sugar supplementation (Figure 2), suggesting that the slow fermentation of maltose in the flour may have been counterbalanced by the favorable low-osmotic pressure in the lean-dough system.

With regard to other dough ingredients, a slight stimulating effect by nitrogen-containing yeast food on yeast fermentation was reported by Peppler (1960), while the inhibitory effect of mold inhibitors, such as calcium propionate and sodium diacetate, has been reported by Seeley and Ziegler (1962). The inhibition by the ethanol produced during panary fermentation has also been reported. Blish and Hughes (1932) reported alcohol contents of 2.79, 3.50 and 5.11% (w/w on flour weight) at the end of 24-hour fermentation in straight-doughs containing 0, 2.5 and 10% sugar (on flour weight), respectively. During the regular fermentation time of 3 h, the respective alcohol contents were 1.40, 1.38 and 1.25%. White (1954) reported about 13% inhibition of gassing rate toward the end of straight-dough fermentations, when 4 g of EtOH was added to 280 g flour (*i.e.* 1.42% w/w of ethanol on flour) and 156 ml of water. Inhibition of fermentation rate by ethanol in various liquid systems has been reported by many investigators (Brown *et al.*, 1981; Franz, 1961; Gray and Sova, 1956; Rosá, 1963).

(iii) Environmental factors

The effect of dough temperature on the fermentation rate is well documented. Conventional sponge is generally set at 25–27 °C. After a four-hour fermentation and remixing, the dough temperature rises to about 30 °C; it is then proofed at 35–37 °C. Liquid preferment is usually set at 30 °C. After one to one and a half hour fermentation and mixing, the temperature of the dough rises to about 35–40 °C; it is proofed at about 45 °C. White (1954) reported an increased fermentation rate with temperature in a straight-dough system between 20 and 34 °C; the Q_{10} for gas production is calculated to be between 1.8 to 2.2. Harbrecht and Kautzmann's results (1967) on sponge fermentation between 27.5 and 35 °C showed a higher Q_{10} of 2.9 during the first 30 min. After a two-hour fermentation, the Q_{10} dropped to 2.4. Garver *et al.* (1966) reported increased CO_2 production rate at increased temperatures in a preferment system; it reached a maximum rate of 26 mmol $CO_2\, l^{-1}\, g^{-1}$ yeast solids at 38 °C, beyond which the rate began to decline.

With the exceptions of sour-dough bread and soda crackers, the conventional dough systems normally have a pH of 5.0–5.4 and they are well-buffered. Deviation from this range is seldom observed in actual baking practice. However, this is not true in preferments. Unless they are sufficiently buffered by the addition of inorganic buffering salts and/or milk solids, the pH of the preferment may drop to 3.0, due mainly to the dissolved CO_2 produced in fermentation. Garver *et al.* (1966) reported a significantly lower fermentation rate in a preferment system at pH 3.5; maximum fermentation rate was observed at pH 4.0–5.2. In a straight-dough system, the maximum fermentation rate was observed at pH 5.3, above which declining rates were noted (Seeley and Ziegler, 1962). Similar results were reported by Franz (1961) with molasses solutions.

20.5.2.2 Carbon dioxide retention in dough systems

In order to retain the CO_2 produced during panary fermentation in dough systems, the gluten in the flour must be properly hydrated and developed into a coherent and extensible film with desirable viscoelastic properties, otherwise much of the CO_2 will be lost from the fermented dough, resulting in low bread volume and inferior leavening of the baked products. To some extent, both baker's yeast and its fermentation action play important roles in the proper development of the flour constituents for gas retention.

It is well established that disulfide and hydrogen bonds are of primary importance for gluten structure, while electrostatic bonds and van der Waals forces are of minor significance (Bloksma, 1978). Yeast cells normally contain from 4–14 mg glutathione per gram of yeast solids, depending upon yeast strain and propagating conditions (Schultz and Swift, 1955). While little glutathione leaches out from the compressed baker's yeast during panary fermentation, a substantial portion of this tripeptide does leach out during rehydration of active dry yeast, varying according to the rehydration temperature and the moisture content of active dry yeast (Chen *et al.*, 1966). Glutathione has been found to participate in the disulfide–sulfhydryl interchange reaction in flour (Kuninori and Sullivan, 1968), leading to the cleavage of gluten molecules, lowering of relaxation constants in dough and reduction of development time during mixing (Ponte *et al.*, 1960). While such effects may be desirable in certain applications requiring prolonged mixing, such as pizza dough, bun dough and sweet dough, more precise control in dough mixing is required to avoid overdevelopment. The effect of yeast glutathione can be duplicated by the addition of crystalline glutathione to dough systems. Similarly, addition of reduced thiotic acid, a normal flour constitutent, also results in weakened mixing behavior (Dahle and Hinz, 1966). The presence of thiotic acid reductase in yeast cells has been demonstrated (Black *et al.*, 1960).

Besides yeast cells *per se*, the substances produced during yeast fermentation also play an important role in modifying the physical properties of dough systems (Nagao *et al.*, 1981b). Incorporation of a prefermented dough piece into an unleavened dough led to marked improvements in gas retention and rheological properties, as measured by a Do-Corder instrument. Such an improvement was not likely to be due to the lowering of dough pH from 5.7 to 5.0 by the dissolved CO_2, since the addition of acetic acid to the same pH level had little beneficial effect. The function of CO_2 lies in the transformation of the developed gluten film into a vesicular structure by the expanding gas cells. In addition to CO_2, several organic acids, keto acids, glycerol, *etc.* are also produced in appreciable quantities in yeast fermentation (Cole *et al.*, 1962, 1966; Suomalainen and Keranen, 1967). Incorporation of several organic acids in the unleavened dough results in better rheological properties (Nagao *et al.*, 1981a). Decrease in sulfhydryl content has been observed during panary fermentation. This may also account for some improved physical properties of the fermented dough, as the addition of a fermented dough piece to a fresh sponge or dough produces a rheological change similar to the incorporation of potassium bromate, an oxidizing dough improver (Nagao *et al.*, 1981b). Little information is available on the effect of ethanol, a major fermentation product, on dough development. Blish and Hughes (1932) concluded on the basis of loaf volume that alcohol, up to 5.11% (w/w on flour) in the dough, produced no serious changes that might be regarded as gluten degradation. No rheological measurement was made, however.

20.5.3 Storage Stability of Baker's Yeast

Aside from flavor development and dough leavening, storage stability is also an important criterion for the quality of baker's yeast. A compressed baker's yeast usually contains 68–72%

water. As such, it is a perishable product requiring refrigeration during storage. The effect of storage temperature on yeast activity has been studied by many investigators. Hautera and Lovgren (1975a) reported little decrease in yeast activity after it was stored at 5 °C. for 28 days. At 23 °C, the fermentation activity remained reasonably constant for 16–18 days, while at 35 °C, yeast activity decreased linearly with storage time and approached zero activity after 7–9 days. Ginterova *et al.* (1966) found that the sucrose-fermenting activity of compressed yeast remained reasonably constant for 36 days at 7 °C, 5 days at 20 °C, and less than 3 days at 30 °C. However, the decline of maltose-fermenting activity occurred sooner in all cases.

Compressed yeast may also be stored in the frozen state in commerce. Thiessen (1942) found 2–6% yeast activity loss over 16 to 20 weeks in frozen storage, while Peppler (1960) reported little activity loss over 19 weeks at −30 °C. Although the freezing and thawing rates affected yeast viability (Mazur, 1970), Hsu *et al.* (1979) showed that the final freezing temperature was more important. At a freezing temperature of −10 to −20 °C, the prooftime of the frozen-dough was 71–72 min, while at −40 °C and −78 °C, the proof time increased to 132 and 360 min, respectively.

The effect of storage atmosphere on the fermentation activity of compressed yeast is not clear-cut. Edelman *et al.* (1978) reported lower leavening ability of commercial baker's yeast after it was stored under anaerobic conditions for 4 days at 30 °C. Similarly, the compressed yeast stored in 50 g pieces had higher leavening ability than that stored in 1 kg blocks (Edelman *et al.*, 1980). On the other hand, Takakuwa (1962) found that the liquefaction of compressed yeast occurred much later when it was stored in nitrogen than in oxygen or an air atmosphere. It was often observed that granulated compressed yeast was more difficult to store than the consolidated blocks (Burrows, 1970; Reed and Peppler, 1973), presumably due to higher endogenous carbohydrate breakdown under aerobic than under anaerobic conditions (Eaton, 1960).

In addition to endogenous consumption of reserve carbohydrates, such as glycogen and trehalose, the decrease of fermentation activity is also accompanied by an increase of dead cells (Suomalainen, 1975), proteolytic activity (Tohoyama and Takakuwa, 1971) and NADase activity (Takakuwa *et al.*, 1975) in the compressed yeast during storage. Its nucleic acid content shows a slight increase during the early phase of storage, followed by a rapid decrease in the late phase when autolysis is observed. Concurrently, the acid-soluble nucleotides increase slowly in the early phase and more rapidly in the late phase, when softening and autolysis of compressed yeast begin (Parkkinen *et al.*, 1973, 1974). These data suggest that when the reserve carbohydrates have been used up, the yeast cells start to attack other vital cellular constitutents, leading to rapid hydrolysis of proteins and nucleic acids, resulting in eventual autolysis.

Besides temperature and aerobicity, the storage stability of compressed yeast is also related to its propagating conditions: aerobically-grown yeast is more stable than anaerobically-grown yeast; low-protein yeast is more stable than high-protein yeast and a mature cell is more stable than a less mature yeast (Suomalainen, 1975). An attempt has been made to improve the storage stability of compressed yeast by post-harvest treatment with metabolic inhibitors (Takakuwa and Tohoyama, 1975); some degree of success has been reported with biuret treatment (Takakuwa, 1962).

The storage stability of active dry yeast (ADY) is significantly better than that of compressed yeast, since it contains less than 10% moisture. Its shelf life may be as short as 1 to 2 months to as long as 1 year, depending upon the storage temperature and atmosphere as well as its moisture content. Generally speaking, the storage stability is inversely proportional to storage temperature and moisture content. Dehydration of ADY to a lower moisture content (*i.e.* 4–6%) greatly improves its thermostability (Mitchell and Enright, 1957), but it does not eliminate the deteriorative effect of oxygen. Under an oxygen or air atmosphere, a pellet ADY with a rather impervious surface is more stable than a granular or finely particulated ADY. For improved shelf life, ADY is often packaged *in vacuo* or under an inert atmosphere such as nitrogen or CO_2. Incorporation of certain phenolic antioxidants, such as BHA, BHT or propyl gallate, also leads to improved storage stability in an air atmosphere (Chen and Cooper, 1962; Chen *et al.*, 1966). The biochemical changes for the deterioration of ADY are not fully understood. Chen and Peppler (1956b) showed an excellent correlation between pyruvate decarboxylation activity, anaerobic fermentation and baking activity of the ADY stored at 48 °C. The deterioration of pyruvate decarboxylation activity was found to be due to destruction of cocarboxylase, and not to denaturation of the apo-enzyme.

20.6 CONCLUSIONS

Due to the world-wide attention to single-cell protein and fuel alcohol, a good deal of effort has been devoted in recent years to the improvement of fermentation equipment and optimization of the fermentation process. Significant progress has been made toward the production of microbial biomass from various substrates (Mateles and Tannenbaum, 1968; Tannenbaum and Wang, 1975). However, in the production of baker's yeast, biomass formation is only a part of the picture. The quality of baker's yeast is equally, if not more, important. This is evidenced by the lack of acceptance of the continuous fermentation process in the baker's yeast industry. The current knowledge on the quality of baker's yeast is empirical and incomplete. For example, it is not known whether the panary fermentation activity of baker's yeast is determined mainly by its enzyme constitution, its sugar permeation rate, its osmotic sensitivity, or its drying tolerance. This basic information must be available before baker's yeast fermentation can be scientifically optimized.

ACKNOWLEDGEMENT

The authors are grateful to Messrs Gerald Reed, Henry Peppler, Richard Raymond, Mrs Aileen Mundstock and Mrs Betty Blue for their suggestions, comments and assistance during the preparation of this manuscript.

20.7 REFERENCES

Aiba, S., A. Humphrey and N. Mills (1973). *Biochemical Engineering*, 2nd edn. Academic, New York.

Aiba, S., S. Nagai and Y. Nishizawa (1976). Fed batch culture of *Saccharomyces cerevisiae*: A perspective of computer control to enhance the productivity in baker's yeast cultivation. *Biotechnol. Bioeng.*, **18**, 1001–1016.

Aiyar, A. S. and R. Luedeking (1966). A kinetic study of the alcoholic fermentation of glucose by *Saccharomyces cerevisiae*. *Chem. Eng. Prog., Symp. Ser.*, **62** (69), 55–59.

Aizawa, M., T. Matsuda, S. Kawabata, I. Omura, I. Amano and T. Nakamura (1968) Process for preparing an active dry powdery yeast. *US Pat.* 3 407 072.

Alonso, A. and A. Kotyk (1978). Apparent half-lives of sugar transport proteins in *S. cerevisiae*. *Folia Microbiol. (Prague)*, **23**, 118–125.

Anon (1982). Corning–Kroger combine technology to exploit lactose-hydrolyzed whey. *Food Dev.*, January, 34–35.

Armiger, W. B. and A. Humphrey (1979). Computer applications in fermentation technology. In *Microbial Technology*, ed. H. J. Peppler and D. Perlman, 2nd edn., vol. 2, chap. 15, pp. 375–401. Academic, New York.

Arnold, B. H. and R. Steel (1958). Oxygen supply and demand in aerobic fermentations. In *Biochemical Engineering*, ed. R. Steel, pp. 149–182. Heywood, London.

Atkin, L., A. S. Schultz and C. N. Frey (1946). Yeast fermentation. In *Enzymes and Their Role in Wheat Technology*, ed. J. A. Anderson. Interscience, New York.

Barnett, J. A. (1976). The utilization of sugars by yeasts. *Adv. Carbohydr. Chem. Biochem.*, **32**, 125–234.

Bauchop, T. and S. R. Eldsden (1960). The growth of micro-organisms in relation to their energy supply. *J. Gen. Microbiol.*, **23**, 457–469.

Beech, F. W. and R. P. Davenport (1971). Isolation, purification and maintenance of yeasts. In *Methods in Microbiology*, ed. B. Booth, vol. 4, chap. 5, pp. 153–182. Academic, New York.

Beker, M. E., B. E. Damberga, A. A. Upit, Y. E. Blumberg, I. Y. Krause, E. Y. Ventinya, I. K. Brivkalne and M. V. Popova (1974). Some peculiarities of the lag phase of the development of *Saccharomyces cerevisiae* after dehydration. *Mikrobiologia*, **43**, 1028–1033.

Belokon, V. N. (1962). Yeast drying on a belt drier. *Spirt. Promst.*, **1**, 40–42.

Bhavaraju, S. M., T. W. F. Russell and H. W. Blanch (1978). The design of gas sparged devices for viscous liquid systems. *AIChE J.*, **24**, 454–466.

Black, S., E. M. Harte, B. Hudson and L. Wartofsky (1960). A specific enzymic reduction of L(-)-methionine sulfoxide and related nonspecific reduction of disulfides. *J. Biol. Chem.*, **235**, 2910–2916.

Blish, M. J. and R. C. Hughes (1932). Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance. *Cereal Chem.*, **9**, 331–356.

Bloksma, A. H. (1978). Rheology and chemistry of dough. In *Wheat Chemistry and Technology*, ed. Y. Pomeranz, 3rd edn. American Association for Cereal Chemistry, Inc., St. Paul, MN.

Blumenthal, H. J., K. F. Lewis and S. Weinhouse (1954). An estimation of pathways of glucose catabolism in yeast. *J. Am. Chem. Soc.*, **76**, 6063–6097.

Breton, A. and Y. Surdin-Kerjan (1977). Sulfate uptake in *Saccharomyces cerevisiae*: Biochemical and genetic study. *J. Bacteriol.*, **132**, 224–232.

Brown, S. W., S. G. Oliver, D. E. F. Harrison and R. C. Righelato (1981). Ethanol inhibition of yeast growth and fermentation: Difference in the magnitude and complexity of the effect. *Eur. J. Appl. Microbiol. Biotechnol.*, **11**, 151–155.

Bruinsma, B. L. and K. F. Kinney (1981). Functional (bread-making) properties of a new yeast. *Cereal Chem.*, **58**, 477–480.

Buhler, H. and W. Ingold (1976). Measuring pH and oxygen in fermentors. *Process Biochem.*, **11**, 19–24.

Burkholder, P. R., I. McVeigh and D. Moyer (1944). Studies on some growth factors of yeasts. *J. Bacteriol.*, **48**, 385–392.

Burrows, S. (1970). Baker's yeast. In *The Yeasts*, ed. A. H. Rose and J. S. Harrison, vol. 3. chap. 7, pp. 349–420. Academic, New York.
Carduck, F. J., D. Kloetzer and G. Veldman (1982). Preparation of porous active yeast granules. *US Pat.* 4 335 144.
Challinor, S. W. and D. M. Power (1964). Effect of inositol deficiency on yeast in particular reference to chemical composition of the cell and of the cell wall. *Nature* (*London*), **203**, 250–251.
Chattaway, F. W., R. Bishop, M. R. Holmes, F. C. Odds, and A. J. E. Barlow (1973). Enzyme activities associated with carbohydrate synthesis and breakdown in the yeast and mycelial forms of *Candida albicans*. *J. Gen. Microbiol.*, **75**, 97–109.
Chen, S. L. (1959a). Carbohydrate assimilation in actively growing yeast, *S. cerevisiae*. I. Metabolic pathways for C^{14}-glucose utilization by yeast during aerobic fermentation. *Biochim. Biophys. Acta*, **32**, 470–479.
Chen, S. L. (1959b). Carbohydrate assimilation in actively growing yeast, *S. cerevisiae*. II. Synthesis of polysaccharides from C^{14}-glucose. *Biochim. Biophys. Acta*, **32**, 480–483.
Chen, S. L. (1964). Energy requirement for microbial growth. *Nature* (*London*), **202**, 1135–1136.
Chen, S. L. and E. J. Cooper (1962). Production of active dry yeast. *US Pat.* 3 041 249.
Chen, S. L. and F. Gutmanis (1976). Carbon dioxide inhibition of yeast growth in biomass production. *Biotechnol. Bioeng.*, **18**, 1455–1462.
Chen, S. L. and H. J. Peppler (1956a). Conversion of cinnamaldehyde to styrene by a yeast mutant. *J. Biol. Chem.*, **221**, 101–106.
Chen, S. L. and H. J. Peppler (1956b). Destruction of cocarboxylase in active dry yeast. *Arch. Biochem. Biophys.*, **62**, 299–304.
Chen, S. L. and H. J. Peppler (1979). Single-cell proteins in food applications. *Dev. Ind. Microbiol.*, **19**, 79–94.
Chen, S. L., E. J. Cooper and F. Gutmanis (1966) Active dry yeast: Protection against oxidative deterioration during storage. *Food Technol.*, **20**, 79–83.
Chulina, E. P. (1969). The effect of drying temperature on the quality of yeast. *Khlebopek. Konditer., Promst.*, **13**(3), 27–28.
Clement, P. A. and A. Hennette (1982). Strains of yeast for breadmaking and novel strains of yeast thus prepared. *US Pat.* 4 318 930.
Clement, P. and J.-P. Rossi (1982). Active dried baker's yeast. *US Pat.* 4 328 250.
Cockburn, M., P. Earnshaw and A. A. Eddy (1975). The stoichiometry of the absorption of protons with phosphate and L-glutamate by yeast of the genus *Saccharomyces*. *Biochem. J.*, **146**, 705–712.
Coffman, J. R. (1967). Bread flavor. In *Chemistry and Physiology of Flavors*, ed. H. W. Schultz, E. A. Day and L. M. Libby. AVI Publishing Co., Westport, CT.
Cole, E. W., W. S. Hale and J. W. Pence (1962). The effect of processing variations on the alcohol, carbonyl, and organic acid contents of pre-ferments for bread baking. *Cereal Chem.*, **39**, 114–122.
Cole, E. W., V. Helmke and J. W. Pence (1966). Alpha-keto acids in bread preferments. *Cereal Chem.*, **43**, 357–361.
Collins, C. H. and P. M. Lyne (1976). *Microbiological Methods*, 4th edn. Butterworths, London.
Conway, E. J. (1955). Evidence for a redox pump in the active transport of cations. *Int. Rev. Cytol.*, **4**, 377–396.
Conway, E. J. and M. E. Beary (1956). Active transport of magnesium. *Nature* (*London*), **178**, 1044.
Conway, E. J. and M. E. Beary (1958). Active transport of magnesium across yeast cell membrane. *Biochem. J.*, **69**, 275–280.
Conway, E. J. and J. Breen (1945). An 'ammonia' yeast and some of its properties. *Biochem. J.*, **39**, 368–371.
Conway, E. J. and P. F. Duggan (1958). A cation carrier in the yeast cell wall. *Biochem. J.*, **69**, 265–274.
Conway, E. J. and D. T. Moore (1954). A sodium yeast and some of its properties. *Biochem. J.*, **57**, 523–528.
Conway, E. J., H. Ryan and E. Carton (1954). Active transport of sodium ions from yeast cells. *Biochem. J.*, **58**, 158–167.
Cooney, C. L., H. Y. Wang and D. I. C. Wang (1977). Computer-aided material balancing for production of fermentation parameters. *Biotechnol. Bioeng.*, **19**, 55–67.
Cooper, E. J. and S. L. Chen (1966). Yeast leavened pre-mix for bakery products. *US Pat.* 3 231 389.
Cooper, E. J. and G. Reed (1968). Yeast fermentation. *Baker's Digest*, **42** (6), 22–24, 26, 28–29, 63.
Cooper, R. G., R. S. Silver and J. P. Boyle (1975). Semi-commercial studies of a petroprotein process based on *n*-paraffins. In *Single Cell Protein II*, ed. S. R. Tannenbaum and D. I. C. Wang, chap. 23, pp. 454–466. MIT Press, Cambridge, MA.
Dahle, L. K. and R. S. Hinz (1966). The weakening action of thioctic acid in unyeasted and yeasted doughs. *Cereal Chem.*, **43**, 682–688.
Dalby, D. K. (1982). Pure culture methods. In *Prescott and Dunn's Industrial Microbiology*, ed. G. Reed, 4th edn., chap. 3, pp. 44–62. AVI Publishing Co., Westport, CT.
DeDeken, R. H. (1966). The Crabtree effect: A regulatory system in yeast. *J. Gen. Microbiol.*, **44**, 149–156.
Dellweg, H., W. K. Bronn and W. Hartmeier (1977). Respiration rates of growing and fermenting yeast. *Kem.-Kemi*, **4**, 611–615.
DeLoffre, M. C. H. (1964). Improvements in multiple stage production of yeast. *US Pat.* 3 120 073.
Densky, H., P. J. Gray and A. Budat (1966). Further study on the determination of zinc and its effect on various yeasts. *Proc. Am. Soc. Brew. Chem.*, 93–100.
de Robichon-Szulmajster, H. (1958a). Uridine-diphosphogalactose-4-epimeriase, an adaptive enzyme in *Saccharomyces fragilis*. *Biochim. Biophys. Acta*, **29**, 270–272.
de Robichon-Szulmajster, H. (1958b). Induction of enzymes of the galactose pathway in mutants of *Saccharomyces cerevisiae*. *Science*, **127**, 28–29.
de Robichon-Szulmajster, H. and Y. Surdin-Kerjan (1971). Nucleic acid and protein synthesis in yeasts: regulation of synthesis and activity. In *The Yeasts*, ed. A. H. Rose and J. S. Harrison, vol. 2, chap. 9, pp. 336–418. Academic, New York.
DiCarlo, F. S., A. S. Schultz and A. M. Kent (1953). The mechanism of allantoin catabolism by yeast. *Arch. Biochem. Biophys.*, **44**, 468–474.
Eaton, N. R. (1960). Endogenous respiration of yeast. I. The endogenous substrate. *Arch. Biochem. Biophys.*, **88**, 17–25.
Ebner, H. and A. Enekel (1974). Device for aerating liquids. *US Pat.* 3 813 086.

Ebner, H., K. Pohl and A. Enekel (1967). Self-priming aerator and mechanical defoamer for microbiological processes. *Biotechnol. Bioeng.*, **9**, 357–364.
Eddy, A. A. (1958). Aspects of chemical composition of yeast. In *The Chemistry and Biology of Yeasts*, ed. A. H. Cook, chap. 5, pp. 157–249. Academic, New York.
Edelmann, K., P. Stelwagen and E. Oura (1978). Quality of baker's yeast stored under aerobic and anaerobic conditions. *6th International Specialist Symposium of Yeasts, Montpellier, France*, Abstr. SVII, pp. 15–16.
Edelmann, K., P. Stelwagen and E. Oura (1980). The influence of temperature and availability of oxygen on the carbohydrates of stored baker's yeast. In *Advances in Biotechnology*, ed. G. G. Stewart and I. Russell, vol. 4, Current Devel-opments in Yeast Research. Proceedings of the 5th International Yeast Symposium, London, Ontario, Canada. pp. 51–56. Pergamon, Toronto.
Erisman, J. L. (1938). Roto-Louvre dryer. *Ind. Eng. Chem.*, **30**, 996–997.
Eroshin, V. K., I. S. Utkin, S. V. Ladynichev, V. V. Samoylov, V. D. Kuvshinnikov and G. K. Skoyabin (1976). Influence of pH and temperature on substrate yield coefficient of yeast growth in chemostat. *Biotechnol. Bioeng.*, **18**, 289–295.
Fengl, Z. (1969). Production of microbial protein from carbon sources. In *Global Impacts of Applied Microbiology II*, ed. E. L. Gaden, Jr., pp. 63–70. Interscience, New York.
Finn, R. K. (1969). Energy cost of oxygen transfer. *Process Biochem.*, **4**, 17, 22.
Flynn, D. S. (1974). Computer control of fermentation processes. In *Advances in Microbial Engineering*, ed. A. Prokop and M. Novak, part 2, pp. 597–605. Wiley, New York.
Forsander, O. and H. Suomalainen (1957). Thiamine in baker's yeast. *Acta Chem. Scand.*, **11**, 1094.
Foulkes, E. C. (1956). Cation transport in yeast. *J. Gen. Physiol.*, **39**, 687–704.
Franz, B. (1961). Kinetics of the alcoholic fermentation during the propagation of baker's yeast. *Die Nahrung*, **5**, 458–481.
Frey, C. N. (1930). History and development of the modern yeast industry. *Ind. Eng. Chem.*, **22**, 1154–1162.
Frey, C. N. (1957). History of the development of active dry yeast. In *Yeast — Its Characteristics, Growth and Function in Baked Products*, ed. C. S. McWilliams and M. S. Peterson, pp. 7–33. Quartermaster Food and Container Institute for the Armed Force, Chicago.
Frey, C. N., G. W. Kirby and A. Schultz (1936). Yeast — physiology, manufacture and uses. *Ind. Eng. Chem.*, **28**, 879–884.
Frey, S. W., W. G. DeWitt and B. R. Bellamy (1967). The effect of several trace metals on fermentation. *Proc. Am. Soc. Brew. Chem.*, 199–205.
Fuhrmann, G. F. (1973). Dependence of divalent cation transport on ATP in yeast. *Experientia*, **29**, 742.
Fuhrmann, G. F. and A. Rothstein (1968). The transport of Zn^{2+}, Co^{2+} and Ni^{2+} into yeast cells. *Biochim. Biophys. Acta*, **163**, 325–330.
Garver, J. C., I. Navarine and A. M. Swanson (1966). Factors influencing the activation of baker's yeast. *Cereal Sci. Today*, **11**, 410–418.
Ghosh, A. and S. N. Bhattacharyya (1967). Changes in respiratory and glycolytic behaviour of *S. carlsbergensis* due to inositol deficiency. *Biochim. Biophys. Acta*, **136**, 19–26.
Ginterova, A., L. Mitterhauszerova and O. Janotkova (1966). Effect of temperature on the agglutination of stored baker's yeast. *Branntweinwirstschaft*, **106** (3), 57–59.
Gray, W. D. and C. Sova (1956). Relation of molecular size and structure to alcohol inhibition of glucose utilization by yeast. *J. Bacteriol.*, **72**, 349–356.
Grba, S., E. Oura and H. Suomalainen (1975). On the formation of glycogen and trehalose in baker's yeast. *Eur. J. Appl. Microbiol.*, **2** (1), 29–38.
Grba, S., E. Oura and H. Suomalainen (1979). Formation of trehalose and glycogen in growing baker's yeast. *Finn. Chem. Lett.*, 1979, 61–64.
Grylls, F. S. M., S. D. Rennie and M. Kelly (1978). Process and apparatus for producing active dried yeast. *US Pat.* 4 081 558.
Haarasilta, S. and E. Oura (1975). On the activity and regulation of anaplerotic and gluconeogenetic enzymes during the growth process of baker's yeast. *Eur. J. Biochem.*, **52**, 1–7.
Harbrecht, A. and R. Kautzmann (1967). Vergleichende Untersuchungren zur Triebkraftbestimmung von Backhefen. *Branntweinwirtschaft*, **107**, 21–23.
Harrison, J. S. (1967). Aspects of commercial yeast production. *Process Biochem.*, **2** (3), 41–45.
Harrison, J. S. (1971). Yeast production. *Prog. Ind. Microbiol.*, **10**, 129–177.
Harrison, J. S. and W. E. Trevelyan (1963). Phospholipid breakdown in baker's yeast during drying. *Nature (London)*, **200**, 1189–1190.
Hartmeier, W. (1977). Active dry yeast and method of its production. *Ger. Pat.* 2 515 029.
Hatch, R. T. (1973). Experimental and theoretical studies of oxygen transfer in the airlift fermentor. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA.
Hatch, R. T. (1975). Fermentor design. In *Single-Cell Protein II*, ed. S. R. Tannenbaum and D. I. C. Wang. MIT Press, Cambridge, MA.
Hautera, P. and T. Lovgren (1975a). The fermentation activity of baker's yeast. *Baker's Digest*, **49**, 36–37, 49.
Hautera, P. and T. Lovgren (1975b). α-Glucosidase, α-glucoside permease, maltose fermentation and leavening ability of baker's yeast. *J. Inst. Brew.*, **81**, 309–311.
Hayduck, F. (1919). V. D. Spiritusfabrikanten in Deutschl., *Ger. Pat.* 300 662.
Hayner, W. C., L. J. Wickerham and C. W. Hesseltine (1955). Maintenance of cultures of industrially important microorganisms. *Appl. Microbiol.*, **3**, 361–368.
Hernandez, E. and M. J. Johnson (1967). Energy supply and cell yield in aerobically grown microorganisms. *J. Bacteriol.*, **94**, 996–1101.
Herrera, T., W. H. Peterson, E. J. Cooper and H. J. Peppler (1956). Loss of cell constituents on reconstitution of active dry yeast. *Arch. Biochem. Biophys.*, **63**, 131–143.
Hofer, M., K. Brand, K. Deckner and J. U. Becker (1971). Importance of the pentose phosphate pathway for D-glucose catabolism in the obligatory aerobic yeast *Rhodotorula gracilis*. *Biochem. J.*, **123**, 853–855.

Hommes, F. A. (1966). Effect of glucose on the level of glycolytic enzymes in yeast. *Arch. Biochem. Biophys.*, **114**, 221–233.
Hospodka, J., Z. Caslavsky, K. Beran and F. Stross (1962). The polarographic determination of oxygen uptake and transfer rate in aerobic steady-state yeast cultivation on laboratory plant scale. In *Continuous Cultivation of Microorganisms*, ed. I. Malke, K. Beran and J. Hospodka, pp. 353–368. Academic, New York.
Hsu, K. H., R. C. Hoseney and P. A. Seib (1979). Frozen dough. II. Effects of freezing and storing conditions on the stability of yeasted doughs. *Cereal Chem.*, **56**, 424–426.
Inskeep, G. C., A. J. Wiley, J. M. Holderby and L. P. Hughes (1951). Food yeast from sulfite liquor. *Ind. Eng. Chem.*, **43**, 1702–1711.
Irwin, R. (1954). Commercial yeast manufacture. In *Industrial Fermentation*, ed. L. A. Underkofler and R. J. Hickey, vol. 1, pp. 273–246. Chemical Publishing Co., New York.
Jacob, H. E. (1944). *Six Thousand Years of Bread*. Doubleday, Doran and Co., Garden City, NY.
Johnston, W. R. (1959). Active dry yeast product and processes for producing the same. *US Pat.* 2 919 194.
Jones, R. P. and P. F. Greenfield (1982). Effect of carbon dioxide on yeast growth and fermentation. *Enzym. Microbiol. Technol.*, **4**, 210–223.
Josic, D. (1982). Optimization of process conditions for the production of active dry yeast. *Lebensm.-Wiss. u.-Technol.*, **15**, 5–14.
Keszler, H. J. (1967). Some factors determining growth of yeast. *Zbet. Bakt. II*, **121**, 129–178.
Kline, L. and T. F. Sugihara (1971). Microorganisms of the San Francisco sour dough bread process. II. Isolation and characterization of undescribed bacterial species responsible for souring activity. *Appl. Microbiol.*, **21**, 459–465.
Kornberg, S. R. (1956). Tripolyphosphate and trimetaphosphate in yeast extract. *J. Biol. Chem.*, **218**, 23–31.
Kotyk, A. and J. Horak (1981). Transport processes in the plasma membrane. In *Yeast Cell Envelopes: Biochemistry, Biophysics and Ultrastructure*, ed. W. N. Arnold, vol. 1, chap. 4, pp. 49–64. CRC Press, Boca Raton, FL.
Kraus, J. K., R. Scopp and S. L. Chen (1981). Effect of rehydration on dry wine yeast activity. *Am. J. Enol. Vitic.*, **32**(2), 132–134.
Kuninori, T. and B. Sullivan (1968). Disulfide–sulfhydryl interchange studies of wheat flour. II. Reaction of glutathione. *Cereal Chem.*, **45**, 486–495.
Langejan, A. (1972). A novel type of active dry yeast baker's yeast. In *Fermentation Technology Today*, ed. G. Terui, pp. 669–671. Society of Fermentation Technology, Osaka, Japan.
Langejan, A. (1974). Preparation of active dried baker's yeast. *US Pat.* 3 843 800.
Langejan, A. (1976). High protein active dried baker's yeast. *US Pat.* 3 993 782.
Langejan, A. (1980). Active dried baker's yeast. *US Pat.* 4 217 420.
Lewin, L. M. (1967). Effect of *meso*-inositol deficiency on some important biological and chemical characteristics of yeasts. *J. Gen. Physiol.*, **41**, 215–244.
Lovgren, T. and P. Hautera (1977). Maltose fermentation and leavening ability of baker's yeast. *Eur. J. Appl. Microbiol.*, **4**, 37–43.
Luca, S. L., T. Thommel and W. K. Brown (1979). Freely-flowing powdered fresh baker's yeast preparation and method of producing it. *US Pat.* 4 160 040.
Maddox, I. S. and J. S. Hough (1970). Effect of zinc and cobalt on yeast growth and fermentation. *J. Inst. Brew.*, **76**, 262–264.
Maga, J. A. (1974). Bread flavor. *CRC Crit. Rev. Food Technol.*, **5**, 55.
Maitra, P. K. and Z. Lobo (1971). A kinetic study of glycolytic enzyme synthesis in yeast. *J. Biol. Chem.*, **246**, 475–488.
Marshall, W. R., Jr. and S. J. Friedman (1950). Drying. In *Chemical Engineers' Handbook*, ed. J. H. Perry, 3rd edn., pp. 799–884. McGraw-Hill, New York.
Martin, S. M. (1964). Conservation of microorganisms. *Annu. Rev. Microbiol.*, **18**, 1–16.
Mateles, R. I. and S. R. Tannenbaum (1968). *Single Cell Protein*. MIT Press, Cambridge, MA.
Maw, G. A. (1960). Utilization of sulphur compounds by a brewer's yeast. *J. Inst. Brew.*, **66**, 162–167.
Maxon, W. D. and M. J. Johnson (1953). Aeration studies on propagation of baker's yeast. *Ind. Eng. Chem.*, **45**, 2554–2560.
Mayberry, W. R., G. J. Prochazka and W. J. Payne (1967). Growth yields of bacteria on selected organic compounds. *Appl. Microbiol.*, **15**, 1332–1338.
Mazur, P. (1970). Cryobiology: The freezing of biological systems. *Science*, **168**, 939–949.
Melnick, D., M. Hockberg, H. M. Himes and B. L. Oser (1945). The multiple nature of vitamin B_6. Critique of methods for the determination of the complex and its components. *J. Biol. Chem.*, **160**, 1–14.
Meyenburg, von, H. K. (1969). Energetics of the budding cell of *S. cerevisiae* during glucose-limited aerobic growth. *Arch. Mikrobiol.*, **66**, 289–303.
Miettinen, J. K. (1964). Three fast sampling techniques for biokinetics experiments with radioisotopes. In *Rapid Mixing and Sampling Techniques in Biochemistry*, ed. B. Chances, *et al.*, pp. 303–310. Academic, New York.
Mitchell, J. H., Jr. (1957). Evaluation of the bread baking properties of various genera and species of yeasts. In *Yeasts — Its Characteristics, Growth, and Function in Baked Products*, ed. C. S. McWilliams and M. S. Peterson, pp. 67–69. Quartermaster Food and Container Institute for the Armed Force, Chicago.
Mitchell, J. H., Jr. and J. J. Enright (1957). Effect of low moisture levels on thermostability of active dry yeast. *Food Technol.*, **11**, 359–362.
Mitchell, J. H., Jr. and J. J. Enright (1959). Thermostable active dry yeast composition. *US Pat.* 2 894 842.
Monod, J. (1949). The growth of bacterial culture. *Annu. Rev. Microbiol.*, **3**, 371–394.
Mor, J. R. and A. Fiechter (1968). Continuous cultivation of *S. cerevisiae*. I. Growth on ethanol under steady-state conditions. *Biotechnol. Bioeng.*, **10**, 159–176.
Morris, E. O. (1958). Yeast growth. In *The Chemistry and Biology of Yeasts*, ed. A. H. Cook, chap. 6, 251–321. Academic, New York.
Morris, J. R. and D. W. Ribbons (1970). *Methods in Microbiology*, vol. 3A. Academic, New York.
Nagao, S., S. Endo, K. Takeya and K. Tanaka (1981a). The Do-Corder as a tool to evaluate the breadmaking properties of a dough. *Cereal Chem.*, **58**, 384–387.

Nagao, S., S. Endo and K. Tanaka (1981b). Effect of fermentation on the Do-Corder and bread-making properties of a dough. *Cereal Chem.*, **58**, 388–391.
Noltmann, E. and F. H. Bruns (1958). Phosphamannose isomerase. *Biochem. Z.*, **330**, 514–520.
Notkina, L. G., L. M. Balyberdina and L. D. Lavrenchuk (1975). The effect of nitrite in baker's yeast manufacture. *Khlebopek. Konditer. Promst.*, **19**(2), 28–31.
Nyiri, L. K. (1974). *Oxygen Transfer Rates in 270L Pilot-plant Fermentor*. Report of Fermentation Design, Inc. Bethlehem, PA.
Nyiri, L. K., G. M. Toth, C. S. Krishnaswami and D. V. Parmenter (1976). On-line analysis and control of fermentation processes. In *Workshop Computer Applications in Fermentation Technology*, ed. R. P. Jefferis, III, pp. 37–46. Verlag Chemie, Weinheim.
Olbright, H. (1956). *Die melasse*. Institut für Garungsgewerbe, Berlin.
Olbright, H. (1972). Baker's yeast production. In *International Molasses Report*, ed. F. O. Lights, vol. 9, no. 11, 12, 13, 15, 16.
Olsen, A. J. C. (1960). Manufacture of baker's yeast by continuous fermentation. I. Plant and process. *Soc. Chem. Ind. Monograph*, **12**, 81–93.
Olson, B. H. and M. J. Johnson (1949). Factors producing high yeast yields in synthetic media. *J. Bacteriol.*, **57**, 235–246.
Oura, E. (1972a). The production of energy during aerobic growth of baker's yeast on glucose and ethanol. *Proceedings of the 2nd Congress on Yugoslav Microbiology, Opatija*, pp. 237–246.
Oura, E. (1972b). The effect of aeration on the growth energetics and biochemical composition of baker's yeast. *Res. Lab. State Alcohol Monopoly, Helsinki*.
Oura, E. (1973). Energetics of yeast growth under different intensities of aeration. *Biotechnol. Bioeng. Symp. No. 4*, 117–127.
Oura, E. (1976). The effect of aeration intensity on the biochemical composition of baker's yeast: activities of enzymes of the glycolytic and pentose phosphate pathways. *Biotechnol. Bioeng.*, **18**, 415–420.
Oura, E. (1978). The effect of culture conditions on the requirement of biotin by baker's yeast. *6th International Specialist Symposium on Yeast, Montpellier, France*. Abstr. pp. SI. 1–2.
Oura, E. and S. Haarasilta (1977). Carbon dioxide fixation in yeast grown under gluconeogenic conditions. *Abstracts of the 11th FEBS Meeting, Copenhagen*.
Oura, E. and H. Suomalainen (1978). Biotin and the metabolism of baker's yeast. *J. Inst. Brew.*, **84**, 283–287.
Oura, E., H. Suomalainen and E. Parkkinen (1974). Changes in commercial baker's yeast during its ripening period. *Proceedings of the 4th International Symposium on Yeasts, Part I*, pp. 125–126.
Panek, A. D. (1975). Trehalose synthesis during starvation of baker's yeast. *Eur. J. Appl. Microbiol.*, **2**, 39–46.
Parkkinen, E., E. Oura and H. Suomalainen (1973). Changes in RNA fractions during storage of baker's yeast. *Proceedings of the 3rd International Specialist Symposium on Yeasts, Finland, Part I*, abstr., pp. 62–63. Print OY, Helsinki.
Parkkinen, E., E. Oura and H. Suomalainen (1974). Effect of storage on the nucleic acid composition of baker's yeast. *J. Inst. Brew.*, **80**, 271–277.
Payen, R. (1949). Variations des teneurs en glycogene et trehalose pendant le sechage de la levure. *Can. J. Res.*, **27B**, 749–756.
Paturau, J. M. (1982). *By-Products of the Cane Sugar Industry*. Elsevier, New York.
Peppler, H. J. (1960). Yeast. In *Bakery Technology and Engineering*, ed. S. A. Matz. AVI Publishing Co., Westport, CT.
Peppler, H. J. (1979). Production of yeasts and yeast products. In *Microbial Technology*, ed. D. Perlman and H. J. Peppler, 2nd edn., vol. 1, pp. 157–185. Academic, New York.
Polakis, E. S. and W. Bartley (1965). Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. *Biochem. J.*, **97**, 284–297.
Pollock, G. E. and C. D. Holmstrom (1951). The trehalose content and the quality of active dry yeast. *Cereal Chem.*, **28**, 498–505.
Pomper, S. and E. Akerman (1970). Propagation of yeast. *US Pat.* 3 617 306.
Pomper, S. and E. Akerman (1980). Preparation of free-flowing particulate yeast. *US Pat.* 4 232 045.
Ponte, J. G., R. L. Glass and W. F. Geddes (1960). Studies on the behaviour of active dry yeast in breadmaking. *Cereal Chem.*, **37**, 263–279.
Power, D. M. and S. W. Challinor (1969). The effects of inositol deficiency on the chemical composition of the yeast cell wall. *J. Gen. Microbiol.*, **55**, 169–176.
Pressindustria, SpA (1971). Process for drying bakery yeast. *Br. Pat.* 1 230 587.
Pyke, M. (1957). Bakers' yeast. In *Yeasts*, ed. W. Roman. Dr. W. Junk Publishers, The Hague.
Pyler, E. J. (1958). *Our Daily Bread*. Siebel Publishing Co., Chicago, IL.
Ramnietse, V. E., I. V. Skard and M. V. Popova (1978). Effect of glutamic acid on permeability of *Saccharomyces cerevisiae* cells during their rehydration after dehydration. *Mikrobiologia*, **47**, 430–435.
Reed, G. (1982). Production of bakers' yeast. In *Prescott and Dunn's Industrial Microbiology*, ed. G. Reed, 4th edn., chap. 14, pp. 593–633. AVI Publishing Co., Westport, CT.
Reed, G. and H. J. Peppler (1973). *Yeast Technology*. AVI Publishing Co., Westport, CT.
Reilly, C. (1967). Sodium and potassium transport in normal and mutant yeast: inhibition by *O*-phenanthroline. *Nature (London)*, **214**, 1330–1331.
Richard, O. W. and M. C. Troutman (1940). Spectroscopic analysis of the mineral content of yeast grown on synthetic and natural media. *J. Bacteriol.*, **39**, 739–746.
Richards, J. E. (1961). Studies in aeration and agitation. *Prog. Ind. Microbiol.*, **3**, 143–172.
Rickard, P. A. D. and C. B. J. Hogan (1978). Effect of glucose on the activity and synthesis of fermentative and respiratory pathways of *Saccharomyces* species. *Biotechnol. Bioeng.*, **20**, 1105–1110.
Ridgeway, G. J. and H. C. Douglas (1958). Unbalanced growth of yeast due to inositol deficiency. *J. Bacteriol.*, **76**, 163–166.
Ridgeway, J. A., T. A. Lappin, N. M. Benjamin, J. B. Corns and C. Akin (1975). Single-cell protein materials from ethanol. *US Pat.* 3 865 691.
Rogosa, M. (1944). Vitamin requirements of lactose-fermenting and certain other yeasts. *J. Bacteriol.*, **47**, 159–170.

Roomans, G. M., F. Blaso and G. Borst-Pauwels (1977). Cotransport of phosphate and sodium by yeast. *Biochim. Biophys. Acta*, **467**, 65–71.
Roomans, G. M., A. P. R. Theuvenet, T. Van DenBerg and G. Borst-Pauwels (1979). Kinetics of Ca^{2+} and Sr^{2+} uptake by yeast; effects of pH, cations, and phosphate. *Biochim. Biophys. Acta*, **551**, 187–196.
Roon, R. J. and B. Levenberg (1968). An adenosine triphosphate dependent, avidin sensitive enzymatic cleavage of urea in green algae. *J. Biol. Chem.*, **243**, 5213–5215.
Roon, R. J. and B. Levenberg (1972). Urea amidolyase. *J. Biol. Chem.*, **247**, 4107–4113.
Rosa, M. (1963). The fermentation activity of yeast during discontinuous and continuous alcoholic fermentations. *Die Nahrung*, **7**, 508–517.
Rosen, K. (1977). Production of baker's yeast. *Process Biochem.*, **12** (3), 10–12.
Rothstein, A. and A. D. Hayes (1956). The relationship of the cell surface to metabolism. XIII. The cation-binding properties of the yeast cell surface. *Arch. Biochem. Biophys.*, **63**, 87–99.
Sak, S. (1919). *Danish Pat.* 28 507.
Sato, T., N. Tsumura, Y. Tanaka, T. Okada and Y. Koyanagi (1961). Comparison of practical characters of baker's yeast of the world. *Nippon Jozo Kyokai*, **57** (1) 74–80; (2) 67–76.
Schmidt, G., L. Hecht and S. J. Thannhauser (1949). The effect of potassium ions on the absorption of orthophosphate and the formation of metaphosphate by baker's yeast. *J. Biol. Chem.*, **178**, 733–742.
Schreier, K. (1974). Bio-reactors: Stage of development and industrial application, especially with regard to systems for transfer of gas. *4th International Symposium on Yeasts*.
Schultz, A. (1965). Investigations of leavening activity of baker's yeasts. *Brot Gebaeck*, **19** (4), 61–65.
Schultz, A. S. and L. Atkin (1947). The utilization of Bios response in yeast classification and nomenclature. *Arch. Biochem.*, **14**, 369–380.
Schultz, A. S. and D. K. McManus (1950). Amino acids and inorganic sulfur as sulfur source for the growth of yeasts. *Arch. Biochem.*, **25**, 401–409.
Schultz, A. S. and S. Pomper (1948). Amino acids as nitrogen source for the growth of yeasts. *Arch. Biochem.*, **19**, 184–192.
Schultz, A. S. and F. R. Swift (1955). Manufacture of yeast. *US Pat.* 2 717 837.
Schultz, A. S., L. Atkin and C. N. Frey (1937). A fermentation test for vitamin B_1. *J. Am. Chem. Soc.*, **59**, 948.
Schultz, A. S., L. Atkin and C. N. Frey (1939). Vitamin B_6, a growth promoting factor for yeast. *J. Am. Chem. Soc.*, **61**, 1931.
Schultz, A. S., L. Atkin and C. N. Frey (1941). Synthesis of thiamin and product. *US Pat.* 2 262 735.
Schultz, A. S., D. K. McManus and S. Pomper (1949). Amino acids as carbon source for the growth of yeasts. *Arch. Biochem.*, **22**, 412–419.
Seeley, R. D. and H. F. Ziegler (1962). Yeast: Some aspects of its fermentative behaviours. *Baker's Digest*, **36** (4), 48–52.
Sher, H. N. (1960). Manufacture of baker's yeast by continuous fermentation. II. Instrumentation. *Soc. Chem. Ind. Monograph*, **12**, 94–115.
Shoda, M. and Y. Ishikawa (1981). Carbon dioxide sensor for fermentation systems. *Biotechnol. Bioeng.*, **23**, 461–466.
Sims, A. P. and B. F. Folkes (1964). A kinetic study of the assimilation of ^{15}N-ammonia and the synthesis of amino acids in an exponentially growing culture of *Candida utilis*. *Proc. R. Soc. London, Ser. B*, **159**, 479–502.
Simon, E. J. (1976). Drying of microorganisms in fluid bed driers under mild conditions. *Chemie-Tkechnik*, **7**, 277–280.
Skiba, M. (1966). *Abstracts of the 2nd International Congress on Food Science and Technology Warsaw*, p. 53. Cited by Burrows, S. (1970). *Baker's Yeast*, p. 363. Academic, New York.
Slein, M. W. (1950). Phosphomannose isomerase. *J. Biol. Chem.*, **186**, 753–761.
Stineman, T. L., J. D. Edwards and J. C. Grosskopf (1980). Production of baker's yeast from acid whey. *US Pat.* 4 192 918.
Stone, I. N. (1965). Treatment of wort with zinc. *US Pat.* 3 164 472.
Sugihara, T. F., L. Kline and M. W. Miller (1971). Microorganisms of the San Francisco sour dough bread process. I. Yeasts responsible for the leavening action. *Appl. Microbiol.*, **21**, 456–458.
Suomalainen, H. (1963). Changes in the cell constitutions of baker's yeast in changing growth conditions. *Pure Appl. Chem.*, **7**, 634–654.
Suomalainen, H. (1975). Some enzymological factors influencing the leavening capacity and keeping quality of baker's yeast. *Eur. J. Appl. Microbiol.*, **1**, 1–12.
Suomalainen, H. and E. Axelson (1956). Effect of thiamin on the rate of fermentation of zymohexose and of maltose by baker's yeast. *Biochim. Biophys. Acta*, **20**, 315–318.
Suomalainen, H. and A. J. A. Keranen (1967). Keto acids formed by baker's yeast. *J. Inst. Brew.*, **73**, 477–484.
Suomalainen, H. and M. Lehtonen (1978). Yeast as a producer of aroma compounds. *Proceedings of the 1st European Congress on Biotechnology, Interlaken, Monograph 82*, pp. 207–220.
Suomalainen, H. and E. Oura (1956). The fermentability of maltose by baker's yeast containing trehalose. *Biochim. Biophys. Acta*, **20**, 538–542.
Suomalainen, H. and E. Oura (1971). Yeast nutrition and solute uptake. In *The Yeasts*, ed. A. H. Rose and J. S. Harrison, vol. 2, chap. 2, pp. 3–74. Academic, New York.
Suomalainen, H. and E. Oura (1978). Ethanol as substrate for baker's yeast. *XII International Congress of Microbiology, München*, p. 60.
Suomalainen, H. and S. Pfaffli (1961). Changes in carbohydrate reserves of baker's yeast during growth and on standing. *J. Inst. Brew.*, **67**, 249–254.
Suomalainen, H., A. Boorklund, K. Vihervaara and E. Oura (1965a). Nicotinic acid and nicotinamide adenine dinucleotide contents of baker's yeast in changing culture conditions. *J. Inst. Brew.*, **71**, 221–226.
Suomalainen, H., T. Nurminen, K. Vihervaara and E. Oura (1965b). Effect of aeration on the syntheses of nicotinic acid and nicotinamide adenine dimucleotide by baker's yeast. *J. Inst. Brew.*, **71**, 227–231.
Sysojewa, J. L. and N. W. Gorochova (1965). Production of active dry yeast. *Khlebopek, Konditer. Promst.*, **9** (6), 37–41.
Takakuwa, M. (1962). Effect of biuret on preservability of pressed yeast and its mechanism. *Mem. Ehime Univ., Sect. VI*, **8**, 443–447.

Takakuwa, M. and H. Tohoyama (1975). Effect of metabolic inhibitors on the preservability of pressed baker's yeast and on the increase of intracellular proteinase activity during its storage. *J. Ferment. Technol.*, **53**, 895–897.
Takakuwa, M., K. Takeuchi and N. Yamasaki (1975). Increase of NADase activity during storage of pressed baker's yeast. *Agric. Biol. Chem.*, **39**, 2235–2236.
Tannen, L. P. and L. K. Nyiri (1979). Instrumentation of fermentation systems. In *Microbial Technology*, ed. H. J. Peppler and D. Perlman, 2nd edn., vol. 2, chap. 14, pp. 331–374. Academic. New York.
Tannenbaum, S. R. and D. I. C. Wang (1975). *Single Cell Protein II*. MIT Cambridge, MA.
Thiessen, E. J. (1942). The effect of temperature and viability and baking properties of dry and moist yeast stored for varied periods. *Cereal Chem.*, **19**, 773–784.
Thorn, J. A. and G. Reed (1959). Production and baking techniques for active dry yeast. *Cereal Sci. Today*, **4**, 198–201.
Thorne, R. S. W. (1949). Nitrogen metabolism of yeast. A consideration of the mode of assimilation of amino acids. *J. Inst. Brew.*, **55**, 201–222.
Tohoyama, H. and M. Takakuwa (1971). Increase in intracellular proteinase activity during storage of pressed baker's yeast. *J. Ferment Technol.*, **50**, 397–404.
Van Steveninck, J. and L. Booji (1964). The role of polyphosphates in the transport mechanism of glucose in yeast cells. *J. Gen. Physiol.*, **48**, 43–60.
Van 'Triet, J. and J. De Bruijn (1981). Process for the preparation of active dried microorganisms. *Int. Pat.* WO-81 01 415.
Vincent, A. (1974). Control of dissolved oxygen. *Process Biochem.*, **9**, 30–32.
Wakamura, A., Y. Yokoyama and K. Ueno (1973). A method to prepare powdery dry yeast for bread mix. *Jpn. Pat.* 48 35 475.
Walker, J. A. H. and A. B. Holdsworth (1958). Equipment design. In *Biochemical Engineering*, ed. R. Steel, pp. 223–274. Heywood, London.
Wang, H. Y., C. L. Cooney and D. I. C. Wang (1977). Computer aided baker's yeast fermentation. *Biotechnol. Bioeng.*, **19**, 69–86.
Wang, H. Y., C. L. Cooney and D. I. C. Wang (1979). Computer control of baker's yeast production. *Biotechnol. Bioeng.*, **21**, 975–996.
Watson, T. G. (1970). Effect of sodium chloride on steady state growth and metabolism of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.*, **64**, 91–99.
Weaver, J. C., E. Pungor and C. L. Cooney (1980). Mass spectrometer monitoring of volatile compound in the gas and liquid phases of fermentors. *Am. Chem. Soc. Annu. Meeting*, Las Vegas.
White, J. (1954). *Yeast Technology*. Chapman and Hall, London.
Whitney, P. A. and T. G. Cooper (1970). Urea carboxylase and allophanate hydrolase: Two components of a multienzyme complex in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.*, **40**, 814–819.
Wiame, J. M. (1946). Basophile et metabolisme du phosphore chez la levure. *Bull. Soc. Chim. Biol.*, **28**, 552–556.
Wiame, J. M. (1947). Etude d'une substance polyphosphorée, basophile et meta-chromatique chez les levures. *Biochim. Biophys. Acta*, **1**, 234–255.
Wickerham, L. J. (1951). *Taxonomy of Yeasts*. US Dept. of Agriculture Tech. Bull. 1029.
Wilkinson, J. F. (1949). The pathway of the adaptive fermentation of galactose by yeast. *Biochem. J.*, **44**, 460–467.
Winter, R. (1956). Technical development problems in the production of yeast from sulfite liquor. *Chem. Technol.*, **8**, 144–157.
Winzler, R. J. (1941). The respiration of baker's yeast at low oxygen tension. *J. Cell. Comp. Physiol.*, **17**, 263–276.
Wise, W. S. (1951). The measurement of the aeration of culture media. *J. Gen. Microbiol.*, **5**, 167–177.
Witt, I., P. G. Weiler and H. Holzer (1964). Steigerung der CO_2 fixierung in glucose oxydierender hefe durch NH_4^+-salze. *Biochem. Z.*, **339**, 331–337.
Yamamoto, Y. and T. Inone (1961). Studies of the poor-attenuative yeast. I. Permeation of maltotriose through the cell wall of poor-attenuative yeast. *Rep. Res. Lab. Kiri Brew. Co.*, 49.
Yoshida, F. and K. Akita (1965). Performance of gas bubble columns: Volumetric liquid-phase mass transfer coefficient and gas hold-up. *AIChE J.*, **11**, 9–13.
Yoshida, A. and A. Yamataka (1953). On the metaphosphate of yeast. I. *J. Biochem.* **40**, 85–94.