YEAST TECHNOLOGY

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Bakers' Yeast Production

HISTORY

Yeast grows and multiplies in doughs if the initial concentration of yeast is low, and if sufficient time is allowed during the dough fermentation period. This means that it is possible to propagate bakers' yeast indefinitely by inoculating a fresh dough with a piece from the preceding, fermented dough. This traditional method of preserving and propagating yeast for bread making is still practiced in some households. In the U.S. this method is not practiced commercially except for the production of San Francisco sour-dough bread (Sugihara *et al,*, 1971). This industrial method gives some insight into the propagation of bakers' yeast as it must have been practiced before the beginning of the 19th century.

Before 1800 brewers' top-fermenting yeast was used widely after separation from the beer foam and pressing. The production of bakers' yeast from grain mashes (usually in conjunction with the production of distilled spirits) dates from the beginning of the last century. Malted cereal grains and unmalted grain were the principal raw materials. The yield of yeast was very low and the yield of ethanol was high. Advances in the art of making baker's yeast (or grain yeast, as it was called) were made in Holland, Germany, and Austria during the last century. This work was stimulated by the change in Central Europe from top-fermenting brewers' yeasts (*S. cerevisiae*) to bottom-fermenting yeasts (*S. carlsbergensis*), the latter being unsuited for bread production. Bakers were unable to obtain satisfactory brewers' yeast for making bread and began to depend on the separate production of grain yeast.

During the last hundred years the major changes in the production of bakers' yeast have been (a) the use of aeration, (b) incremental feeding, and (c) the replacement of grain with molasses as a source of carbon and energy. The stimulating effect of aeration was well known towards the end of the 19th century, and continuous aeration was used in Britain in 1886. In the following years yeast yield could be substantially increased by the use of more dilute worts and greatly increased aeration. Incremental feeding (the *Zulauf* process) was introduced between 1915 and 1920 by Danish and German scientists. It is generally practiced today, and it is the only method which permits production of yeast biomass without simultaneous production of sizable quantities of ethanol. Finally, the traditional mash bill at the turn of the century consisted of corn, malt and malt sprouts. During the 1920's and 1930's it was slowly replaced with less expensive molasses sugar.

Experiments in the drying of compressed yeast to yield active dry yeast date back to the first half of the 19th century when compressed yeast was dried on adsorbent paper, but the development of commercially satisfactory processes had

to wait for nearly 100 years. Beginning in the 1920's, the foundations were laid for the production of active dry yeast as we know it today, and following World War II production of active dried yeast increased substantially. At the present time active dry yeast dominates the consumer market; it is used widely in commercial bakeries, particularly in areas where transportation of refrigerated, compressed yeast poses a problem.

Comprehensive reviews of the history of bakers' yeast have been written by Kiby (1912) and Butschek and Kautzmann (1962). The development of active dry yeast has been reviewed by Frey (1957).

OUTLINE OF THE MANUFACTURING PROCESS

The principal raw material in the production of bakers' yeast is beet or cane molasses, which supply fermentable sugars as the major source of carbon and energy, together with minerals, sulfur, vitamins, trace elements, and some organic nitrogen. Additional nitrogen (ammonia or ammonium salts) and phosphate must be supplied. Occasionally addition of vitamins (biotin) and of mineral salts or trace metals is required.

Large-scale commercial fermentations are carried out in fermenters equipped with cooling coils and with means for vigorous aeration, under conditions which minimize anaerobic or aerobic fermentation to ethanol. This requires addition of molasses by incremental feeding and adequate aeration of the fermenter (0.5 to 1 volume of air per fermenter volume per minute). It also requires restriction of the growth rate coefficient to not more than 0.25. About 4 to 4.5% of yeast solids can be produced in the fermenter liquid.

Further processing includes concentration of the yeast by centrifuging, washing, and pressing or filtration. The press or filter cake can be extruded in the form of semisolid blocks (at 30% solids). The blocks or yeast cakes are wrapped in wax paper, cooled, and shipped refrigerated to bakeries.

For the production of active dry yeast the press or filter cake can be extruded in the form of thin ribbons. These are dried on tray, belt, rotolouver, or air-lift dryers, with air at 40–60°C. Active dry yeast contains about 92% solids.

RAW MATERIALS

Molasses

Bakers' yeast can be grown on glucose, fructose, maltose or sucrose as the principal carbon and energy source. In many parts of the world grain mashes, after hydrolysis to maltose, are still used for the production of bakers' yeast, but in the developed countries such mashes have been replaced with molasses. This by-product of the sugar industry is at present the least expensive source of sugar and, in contrast to grain, it does not require hydrolysis of the starch. A detailed study of sugar-containing or sugar-yielding raw materials was made by Becker and Weber (1962).

Corn grits are only slightly higher in cost than molasses if the two materials are compared on an equivalent glucose basis, but the cost of hydrolysis with bacterial and fungal amylases is appreciable. In the past, attempts have been made to use "hydrol," the mother liquor of acid-hydrolyzed cornstarch, after glucose crystallization. It contains more than 5% NaCl and by itself it is not a satisfactory substrate for fermentation.

Klaushofer (1971) reports that it may be used if it is diluted with equal parts of cane or beet molasses. However, the mother liquors from the enzymatic hydrolysis of cornstarch are entirely suitable for yeast production. At present they are slightly more expensive than molasses. Other possible substrates are raisins, dates, or sugar-containing waste of the confectionery industry. In general, these sugar-containing materials command a sufficiently high price on the feed market so that they become unattractive for the production of yeast. Sulfite waste liquor may be used for the production of yeast (Piš, 1970). However, since *S. cerevisiae* does not metabolize the pentoses of the waste liquor, the concentration of fermentable sugars is too low for economical operation. Concentration of the sulfite waste liquor or the use of exceptionally large fermenters would be required.

Wood hydrolyzate or hydrolyzates of vegetable waste may also be used for the production of bakers' yeast. However, these raw materials are more suitable for the production of feed yeast. These raw materials are discussed in Chapter 11.

The gross chemical composition of beet and cane molasses is shown in Table 5.1. Since many of the values cited in earlier publications are more than 20 years old, recent data on typical molasses have been included (columns 1 and 4). The values shown in these two columns are those of U.S. beet molasses and of U.S., Central American, South American, West Indian, Hawaiian and Philippine cane molasses. In general, beet molasses are higher in nitrogen, ash, potassium and SO_2, and lower in nonfermentable reducing sugars, slightly lower in fermentable sugars, and lower in phosphate content. The pH of beet molasses is appreciably higher. Other references to molasses composition can be found in Olbrich (1956) and Becker and Weber (1962).

In addition to sugars, yeast may assimilate other organic compounds and use them as carbon sources. The most important of these is ethanol. For instance, ethanol produced anaerobically or aerobically may be later assimilated through the respiratory mechanism, and ethanol carbon can thus be incorporated into the cell content of the yeast. Yields of dry cell mass with ethanol are as high as with sucrose if the two compounds are compared on the basis of equivalent carbon content. But bakers' yeast produced with ethanol as the principal carbon source has poor fermenting activity in doughs.

Acetic acid and acetaldehyde may also serve as carbon sources. Lactic acid is a better source of carbon, but yields are still lower than with monosaccharides. The fermenting activity of yeasts grown on media containing both sucrose and lactic

TABLE 5.1

COMPOSITION OF MOLASSES

	Cane[1]	Cane[2]	Cane[3]	Beet[4]	Beet[5]	Beet[6]
Brix	84.0–90.0	78.0–85.0	93	80.0–84.0	78.0–85.0	83.5
% Reducing sugar (as invert)	58.0–62.0	50.0–58.0	57	52.0–57.0	48.0–58.0	52.0
% non-ferm.	3.0–4.0	–	3.5	1.5–1.8	–	–
% N_2	0.3–0.7	0.08–0.5	–	1.3–1.9	0.2–2.8	1.4
% NH_2	0.07–0.14	–	–	0.23–0.24	–	–
% P_2O_5	0.08–0.11	0.01–0.07	0.05	0.01–0.04	0.02–0.07	0.06
% SO_2	0.01–0.02	–	–	0.03–0.08	–	–
% ash	5.5–8.5	3.5–7.5	13.9–14.5	8.2–10.0	4.0–8.0	11.5
% CaO	0.2–0.55	0.15–0.8	0.55–0.68	0.4–1.1	0.15–0.7	0.35
% K_2O	2.4–3.0	0.8–2.2	–	3.2–5.0	2.2–4.5	4.7
% MgO	0.5–1.0	0.25–0.8	0.05–0.08	0.03–0.09	0.01–0.1	0.27
pH	4.9–6.0	–	–	6.7–8.3	–	–

1. U.S. cane molasses from Louisiana, cane molasses from Central America, South America, Hawaii and the Philippines.
2. Refinery cane molasses (White, 1954).
3. Cane molasses (Honig, 1953).
4. U.S. beet molasses.
5. Beet molasses (White, 1954).
6. Beet molasses (McGinnis, 1951).

acid was better than that of yeast grown on sucrose alone. Pyruvic acid has an effect similar to lactic acid (Kautzmann, 1969A).

Other organic acids, such as succinic, tartaric and α-keto-glutaric, may serve as carbon sources. Many amino acids are readily assimilated. These will be discussed later, since they serve principally as sources of yeast nitrogen. Glycerol is partially assimilated in the presence of mono- and disaccharides.

Minerals

Yeast contains from 6 to 9% of ash based on dry weight. About half the ash consists of phosphates, and values of 2.2 to 3.6% (as P_2O_5) are common. The required amount of phosphate in a bakers' yeast depends somewhat on the nitrogen content, and yeasts high in nitrogen should contain more phosphate. As a rule of thumb, 1 part of P_2O_5 is required for each 3 parts of nitrogen. Both beet and cane molasses are grossly deficient in phosphate; and in commercial fermentations this anion is supplied in the form of ammonium phosphate or alkaline phosphate salts.

About 2.4 to 2.8% K_2O is found in bakers' yeast (dry basis). With few exceptions cane molasses contain enough potassium to supply this element, and beet molasses contain a considerable excess. About 0.45% of MgO and 0.15% CaO can be found in yeast, but the amounts of these alkaline-earth metals are quite variable. Molasses always supplies enough calcium but not always sufficient magnesium. The latter element can be supplied in the form of magnesium sulfate. In addition, yeast ash contains sodium (0.4 to 0.5% Na_2O) and sulfate (0.2 to 0.25% SO_3). These elements are readily supplied by molasses.

All the above calculations are based on the assumption that 100 kg of molasses with a fermentable sugar content of 50–55 kg yield about 25–27.5 kg of yeast solids. In practice yeast yields rarely exceed this figure.

Yeast requires the presence of some trace elements. Olson and Johnson (1949) worked with shake flasks and with media containing low sugar concentrations. Their yields indicate that they obtained essentially aerobic growth. A strain of U.S. bakers' yeast required 70 μg Fe, 200 μg Zn, and 12 to 15 μg of Cu in 1 liter of medium. Thallium, boron, cobalt, iodine and tin did not affect yeast growth.

Others have found stimulating effects of Co, Fe, I, Mn, Mo and Zn (Pečulis *et al.*, 1969; Augustatienė, 1966). Maddox and Hough (1970) working with *S. carlsbergensis* found that 50 mg of Zn per liter stimulated yeast growth, particularly in a medium containing protein. Cobalt in amounts of 5 to 50 mg/l stimulated protein utilization. The mechanism of Zn uptake has been studied by Ponta and Broda (1970). Some of the cited publications would have been of greater practical value if trace metal content had been related to the actual amount of yeast grown.

In almost all instances, sufficient amounts of these trace elements are supplied with molasses media. Some of the microelements are toxic to yeast. White (1954) complied a list of these elements and determined their effect on yeast growth. Cd, Cu, Ag, Os, Hg and Pd are the substances with the highest toxicity (in the order given). For instance, the presence of 0.175 mg of Cu per liter of medium reduced yeast growth by 50%. This work was done with synthetic media; when molasses is used, higher percentages of Cu can be tolerated.

Vitamins

Yeast requires several vitamins for growth. Those generally lacking in growth media containing molasses have been studied more extensively, particularly biotin. Bakers' yeast contains approximately 0.75 to 2.5 ppm of biotin. However, the requirement for optimum growth is not higher than 1 ppm (based on dry weight). The earlier literature indicates that cane molasses contain from 0.5 to 0.8 ppm of biotin and beet molasses from 0.01 to 0.13 ppm. A growth medium based on beet molasses is obviously grossly deficient in biotin. For the commercial production of bakers' yeast, biotin must be added to a beet molasses medium, or the wort must contain at least 20% cane molasses. The biotin content of cane molasses is sufficiently high to make it a preferred source of this vitamin. Recent cane molasses samples from North, Central and South America had biotin concentrations of 0.5 to 0.8 ppm, while beet molasses from the 1970 harvest contained about 0.015 to 0.02 ppm of biotin.

Most of the work with biotin has been carried out with ammonium salts or ammonia as nitrogen sources. If urea is used, higher levels of biotin are required to obtain adequate yield (Atkin, 1948). Supplementation with 1.25 μg biotin/g yeast solids produced appears to be sufficient to obtain maximum yields.

The requirement of bakers' yeast for biotin is absolute, that is, the yeast does

not adapt to the absence of the vitamin. L(+)-aspartic acid can partially replace biotin. Together with oleic acid it can replace biotin completely (Suomalainen and Keränen, 1963). Therefore, one should take the contents of the medium in these acids into account when calculating the amount of biotin fortification required.

Using a synthetic growth medium White (1954) found that 40 μg pantothenate (per g yeast solids) were required to obtain optimum yield. The value determined by Olson and Johnson (1949) is close to that reported by White. The earlier literature indicates that cane molasses contains about 20 to 120 ppm pantothenate and beet molasses between 40 and 100 ppm. Actually the range encountered in practice is somewhat wider, but pantothenate deficiencies in molasses media are rare.

The same is true of inositol, of which cane molasses contains about 6,000 ppm and beet molasses slightly more. The requirement reported by the above authors is between 2,000 and 5,000 μg inositol per g yeast solids grown. Yeasts will adapt to the absence or deficiency of pantothenate and inositol.

Yeasts may partially adapt to the lack of thiamine. In practice thiamine is frequently added to fermentations to supplement the thiamine content of molasses. Without addition of thiamine, bakers' yeast contains variable amounts of thiamine with a median value in the neighborhood of 15 ppm (based on solids). This is quite satisfactory for optimum growth of yeast. But thiamine is a potent stimulant for the fermentation of doughs and for this reason it is frequently added to the fermenter feed. In recent years the thiamine content of U.S. bakers' yeast has reached levels between 50 and 150 μg per g yeast solids. This is important in the fermentation of liquid brews which contain fermentable sugar but which may not contain flour.

Other vitamins are either not required, or are supplied by the common molasses media in sufficient quantity to permit optimum growth. It should be added that literature references to vitamin requirements of yeast are not particularly useful unless both the vitamin concentration in the medium and the amount of yeast grown are given.

Nitrogen

Both beet and cane molasses contain nitrogenous materials, a fraction of which is assimilated by yeast. Various values are given in the literature for the percentage of assimilable nitrogen in molasses, but in practice this percentage has to be determined for each new molasses sample. Even for beet molasses, which contain higher concentrations of α-amino nitrogen than cane molasses, the amount of available nitrogen covers only a very small fraction of that required for yeast growth. Nitrogen is generally added in the form of ammonia or ammonium salts, such as ammonium sulfate or ammonium phosphate, or sometimes in the form of urea. Nitrogen from such sources is readily assimilated, and with the exception of urea (see above) they are interchangeable. But it must be kept in mind that the

ammonium salts, particularly ammonium sulfate, have an acidifying effect because assimilation of the $[NH_4]^+$ ion leaves the $[HSO_4]^-$ anion in solution.

Nitrogen and the required mineral salts are fed incrementally during commercial fermentations. However, the rate of addition is not as critical as the carbohydrate feed. All the nitrogen may be added several hours before the completion of the fermentation.

Amino acids present in molasses are assimilated by yeast but the use of added amino acids is not economical. A short discussion of the effect of individual amino acids follows, since protein hydrolyzates have sometimes been used as sources of nitrogen. For instance, the use of wheat starch (after hydrolysis) as sugar source and of hydrolyzed wheat gluten as nitrogen source have been practiced. The assimilation of amino acids by yeasts under anaerobic conditions is discussed in Chapters 3 and 7.

Assimilation of amino acids may change the amino acid composition of yeasts. Aspartic acid in the medium increases the aspartic acid content of the yeast protein from 6 to 12% if it replaces 25 to 50% of the ammonia nitrogen in the feed. Similarly, alanine, glycine, phenyl alanine, and lysine in yeast protein are increased by partial replacement of ammonia nitrogen in the feed with these individual amino acids, respectively. Glutamic acid and α-aminobutyric acid do not change the amino acid composition of the yeast even at high replacement levels (Kautzmann, 1969B). The effect of individual amino acids on yield of yeast and its bake activity is shown in Table 5.2.

Fermentation Activators

In many instances activating effects have been claimed for particular substances. Usually, such substances are waste products of the food or feed industry and merely supply a known nutrient which stimulates yeast growth. For instance,

TABLE 5.2

EFFECT OF AMINO ACIDS IN GROWTH MEDIA FOR BAKERS' YEAST

Amino Acid	Replacement Value[1]	Bake activity (in relation to standard)
Glutamic acid	100%	as good as standard or better
Alpha amino butyric acid	100%	10–16% less
Aspartic acid	up to 75%	considerably less
Alanine	up to 75%	satisfactory
Glycine	up to 75%	satisfactory
Lysine	up to 50%	better than standard
Phenyl alanine	up to 25%	worse
Leucine	5–10%	better
Valine	not utilized	
Methionine	not utilized	

Kautzmann, 1969B.
[1]Percent ammonia nitrogen replaced in the medium.

Palagina (1968) attributed the stimulating effect of waste from the flour-milling industry to the presence of biotin.

A more direct effect is exerted by plant growth factors, such as the auxin compounds. Jakubowska and Wlodarczyk (1969) added 1 ppm of β-indolylacetic acid to the growth medium and enhanced the rate of sugar uptake and the rate of propagation. Yanagishima (1966) obtained cell elongation with indole-3-acetic acid and α-naphthalene acetic acid in a respiration deficient mutant of *S. ellipsoideus* (*S. cerevisiae?*).

Fermentation Inhibitors

Sulfite inhibits yeast growth. Concentrations up to 800 ppm in molasses can generally be tolerated. Bergander (1969) mentions a limit of 1,500 ppm of SO_2 in molasses. This figure is naturally variable because there is adaptation of yeast to the presence of SO_2.

In recent years more attention has been paid to the presence of herbicides, pesticides and fungicides in molasses, but such studies have not found their way into the scientific literature. Bergander (1.c.) discusses other factors which may limit yeast growth in commercial fermentations with molasses as carbon source.

PRINCIPLES OF AEROBIC GROWTH OF BAKERS' YEAST

Introduction

Under anaerobic conditions yeast grows rapidly while producing at the same time ethanol and carbon dioxide. A 10- to 20-fold increase in the weight of yeast during a wine fermentation and a 4- to 8-fold increase during a beer fermentation are common. Yeast even grows in bakers' doughs. But the yield of yeast, based on the amount of available fermentable sugar, is low, often not more than 10%. In aerobic systems a yield of up to 50% of the weight of fermentable sugar can, however, be obtained under rather special conditions. All these conditions must be fulfilled to achieve such yields. Apart from an adequate supply of nutrients these conditions are as follows:

Oxygen must be supplied to the yeast in such a manner that the liquid surrounding the cells always shows a partial oxygen pressure.

Fermentable sugar must be added in such a way that it is growth-limiting, that is, in concentrations generally below 0.0004%. This can be achieved by continuous or continual addition of molasses to a strongly aerated fermenter, a system known as incremental feeding (or *Zulauf* process). In practice one cannot demonstrate the presence of any sugar in the fermenter liquor with available analytical techniques; this means that sugar is assimilated by the yeast as fast as it is added.

The rate of growth of the yeast must not exceed a given value. Otherwise "aerobic" fermentation occurs, ethanol is formed and the yield of yeast is reduced.

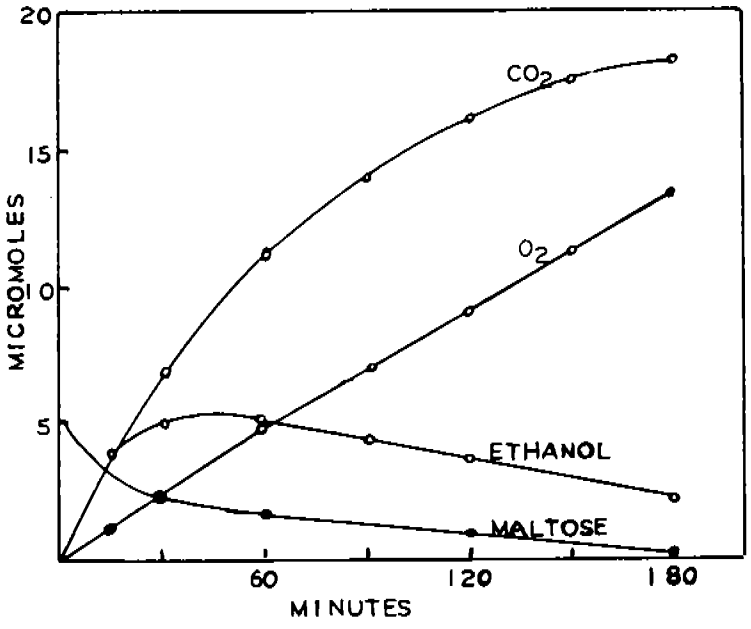
Concentration of Fermentable Sugars

Glucose is fermented faster under anaerobic than under aerobic conditions. This is the well-known Pasteur effect. The presence of glucose inhibits respiration, even in the presence of excess oxygen, i.e., under aerobic conditions.

At a level of 5% glucose in the medium, formation of enzymes of the respiratory chain is suppressed. That means that yeast produces considerable quantities of ethanol even under aerobic conditions if the medium contains a sufficient amount of glucose or other fermentable sugar. However, with limited supplies of fermentable sugars as carbon source *and* under aerobic conditions the process is essentially respiratory. This is reflected in the increased activity of enzymes of the electron transport system and of the citric acid cycle (Suomalainen, 1969).

Fig. 5.1 shows the aerobic growth of yeast in a medium (2.2 ml) containing 5 μM maltose. During the first 30 minutes 5 μM ethanol are produced and the respiratory quotient (Q_{CO_2}/Q_{O_2}) is higher than 2. After 60 minutes when the amount of maltose has dropped to 2 μM (320 mg/l) there is no further ethanol production and the respiratory quotient approaches a value of 1 (Görts, 1967). Similar results have been obtained by von Franz (1964), who found absence of ethanol and a respiratory quotient of 1 at sucrose concentrations below 300 mg/l.

For the production of bakers' yeast it is essential to suppress fermentation to ethanol and to obtain all the required energy for yeast growth by respiration. The



Görts (1967)

FIG. 5.1. O_2 UPTAKE, AEROBIC CO_2 PRODUCTION AND AMOUNT OF MALTOSE AND ETHANOL IN THE REACTION MIXTURE DURING WARBURG EXPERIMENTS WITH *S. CEREVISIAE*

carbon and energy source, glucose or fructose, must, therefore, be provided in extremely small concentrations. This can be achieved by incremental feeding in either batch or continuous fermentations, provided the supply of sugars is the limiting factor in yeast growth. If a diluted molasses solution is fed continuously into an aerated, agitated solution this purpose can be readily achieved. Under such conditions the concentration of fermentable sugars in the medium is so low that there is no formation of ethanol.

Limitation of Yeast Growth Rate

In order to obtain maximum yields of bakers' yeast the supply of fermentable sugars must not only be limiting to yeast growth, but it must limit yeast growth to a specific growth rate not higher than 0.2 to 0.25.* This means practically that the amount of yeast solids in the fermenter must not increase by more than 25% per hour. At higher specific growth rates the yield of yeast is drastically reduced as shown in Fig. 5.2 (von Meyenburg, 1969). The reason for the reduction in yield is also apparent from the figure. Above a specific growth rate of 0.25, the respiratory quotient rises rapidly above 1, indicating the occurrence of aerobic fermentation. The figure demonstrates what yeast producers have known for a long time from practical experience, namely, that one can grow yeast at appreciable higher growth rates, but only at a considerable loss in yield.

Oxygen Requirements and Aeration

The amount of oxygen required to grow a given amount of yeast can be calculated from the chemical composition of yeast. For instance, if one assumes that yeast is grown on sucrose as the carbon source and that 50 g of yeast solids can be produced from 100 g of sucrose, one arrives at a figure of approximately 1 g O_2 per g yeast solids. It is further assumed that the portion of the substrate not used for growth is completely oxidized and supplies energy for growth. For this case Harrison (1967) arrives at a figure of 1.025 g O_2 required for the growth

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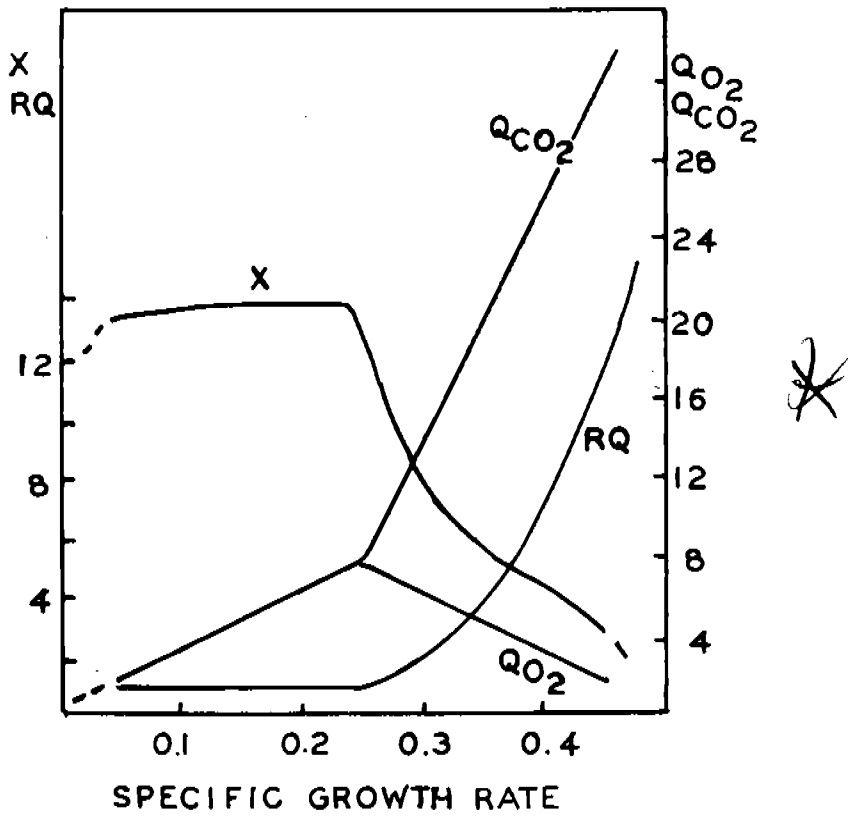
*The specific growth rate (u) for exponential growth may be defined by

$$\frac{dP}{dt} = u \times P$$

in which P is the mass of yeast and t is time. The equation can also be written as

$$\frac{dP}{P} = u \times dt$$

Upon integration one obtains $\ln (P_t/P_O) = u \times t$. When $t = 1$ one obtains $u = \ln (P_t/P_O)$ or $2.31 \times \log_{10} (P_t/P_O)$. This particular constant is called the specific growth rate for exponential growth. White (1954) has called this rate the "modulus of increase" and has defined the hourly growth rate as P_t/P_O. This has led to some confusion, and in studying reports of various authors one must ascertain whether they are referring to the specific growth rate (as defined above), to the hourly growth rate as defined by White, or to the percentage increase per hour.



Meyenburg (1969)

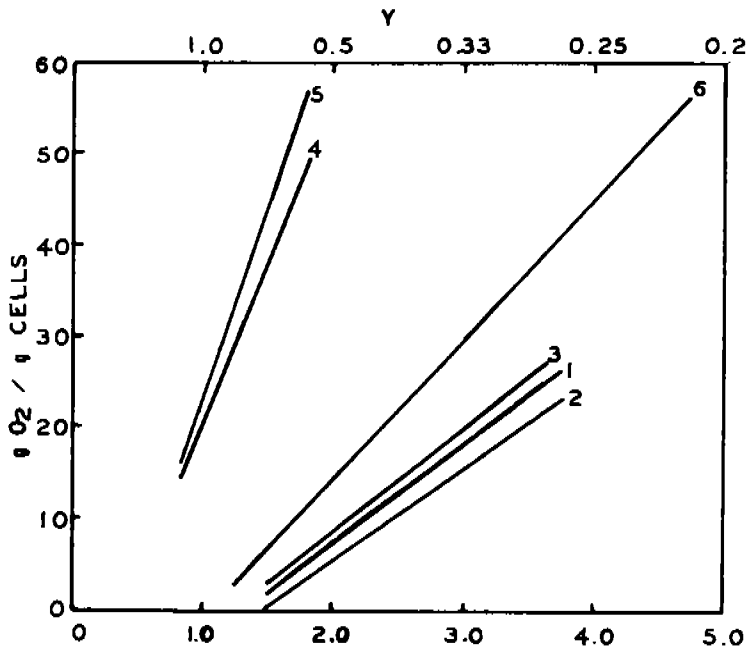
FIG. 5.2. AEROBIC GROWTH OF *S. CEREVISIAE* IN CONTINUOUS CULTURE

X = dry weight of yeast in mg/ml
Q_{CO_2} = CO_2 production in mM/g dry weight/hr
Q_{O_2} = O_2 uptake in mM/g dry weight/hr
RQ = respiratory quotient Q_{CO_2}/Q_{O_2}

of 1 g yeast solids which contain 44.6% carbon, 6.1% hydrogen, 31.2% oxygen, and 8.5% nitrogen. The general formula of Mateles (1971) gives a figure of 0.91 g O_2 per g yeast solids produced. The difference is due to the assumption of a chemical composition of yeast higher in carbohydrate and lower in nitrogen of the yeast.

These calculations depend greatly on assumed yields; for lower yields higher amounts of O_2 are required, as shown in Fig. 5.3 (Mateles, 1971). The above calculations are based on the growth of yeast with sucrose as the sole carbon source, and the formation of by-products such as ethanol and acetaldehyde has been neglected. Therefore, it seems sufficient to use a figure of 1 g O_2/g yeast solids grown as an adequate approximation.

In commercial fermentations propagation may be carried on to yeast solids levels of 4 to 4.5%. With an expected hourly growth rate of 1.1 (P_t/P_o) an additional 0.4 to 0.45% yeast solids would be grown during the last hour of



Mateles (1971)

FIG. 5.3. PLOT OF OXYGEN REQUIRED VS YIELD FOR MICROORGANISMS GROWN ON VARIOUS SUBSTRATES

1. Yeast on glucose, 2. bacteria on glucose, 3. yeast on sucrose, 4. yeast on hexadecane, 5. bacteria on methane, 6. bacteria on methanol.

Y = yield of cell solids as a fraction of substrate.

fermentation. This would require 400 to 450 g O_2 per 100 l of fermenter volume per hour.

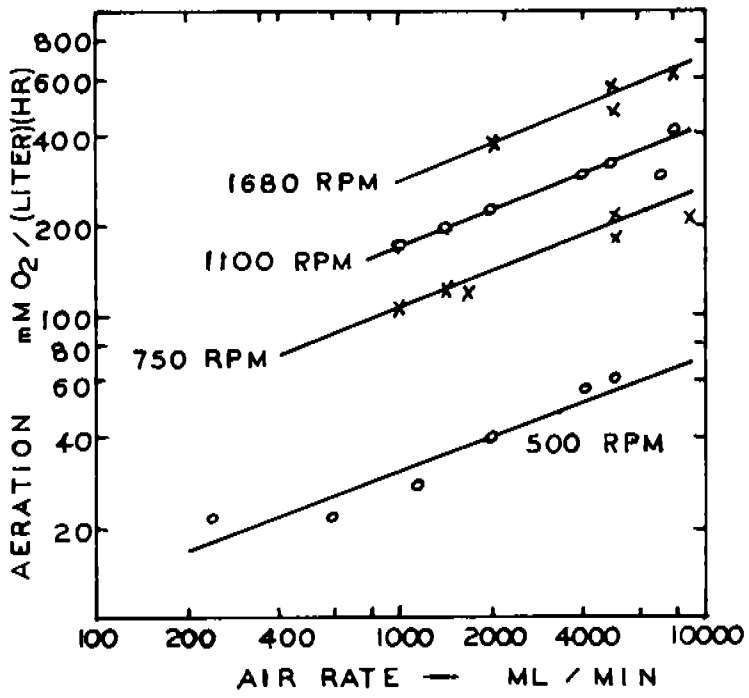
Even a large commercial fermenter of 100 m^3 capacity can be aerated so that 1 volume of air per fermenter volume is passed through the liquid per minute. Since 100 l of air contain about 30 g O_2, 1,800 g O_2 would become available per hour per 100 l. This is not quite sufficient to grow 400 g yeast solids, since not more than about 20% of the available O_2 is transferred to the liquid.* This is based on the use of an aeration system consisting of horizontal perforated pipes, and located at the bottom of the fermenter so that air blown into the fermenter has to pass through the entire height of the liquid. Hence, it appears that for most

*In the utilization of O_2 in commercial fermentations it is assumed that the mass transfer of O_2 from the gas to the liquid phase is the limiting step. This transfer depends on the product of the mass transfer coefficient and the total surface area of the air bubbles. Better dispersion of the incoming air, and hence smaller bubbles and a larger bubble surface area, can be obtained by use of mechanical agitation at the point at which the air enters the fermenter. With such systems 40 to 50% of the available O_2 can be transferred to the liquid phase and utilized by the yeast. Such systems are described later on page 75 (Aiba *et al.*, 1965).

commercial systems the amount of yeast that can be grown is limited by aeration requirements.

The amount of O_2 supplied to a fermenter can be increased by use of gaseous O_2 or by mixtures of air and O_2. The cost of O_2 has prevented this use up to the present time. It is possible to supply O_2 in the form of hydrogen peroxide which may be added directly to the fermenter liquid. Yeast contains enough catalase to release O_2 from H_2O_2, and bakers' yeast can actually be grown in this manner. Again, cost considerations keep this method from actual use. Finally, one can increase the O_2 transfer rate to the liquid by raising the pressure in the fermenter, but it is too costly to build fermenters able to withstand the required pressure, and it is also costly to pump large volumes of air into a vessel kept at high pressure. It has sometimes been claimed that excessive levels of aeration lower the yield of bakers' yeast. But Maxon and Johnson (1953) suggest that loss of fermentable carbon sources (principally acetaldehyde) in the exit gas can account for the reported losses.

The capability of an aeration system to transfer O_2 from air to the liquid phase of a fermenter can be determined by the so-called sulfite oxidation method (Cooper *et al.*, 1944). This is based on the rate at which a sulfite solution of known concentration is oxidized to sulfate by the air stream in the presence of a copper catalyst. The method has been widely used to assess the efficiency of aeration systems. For instance, Fig. 5.4 shows the effect of air rate and agitator



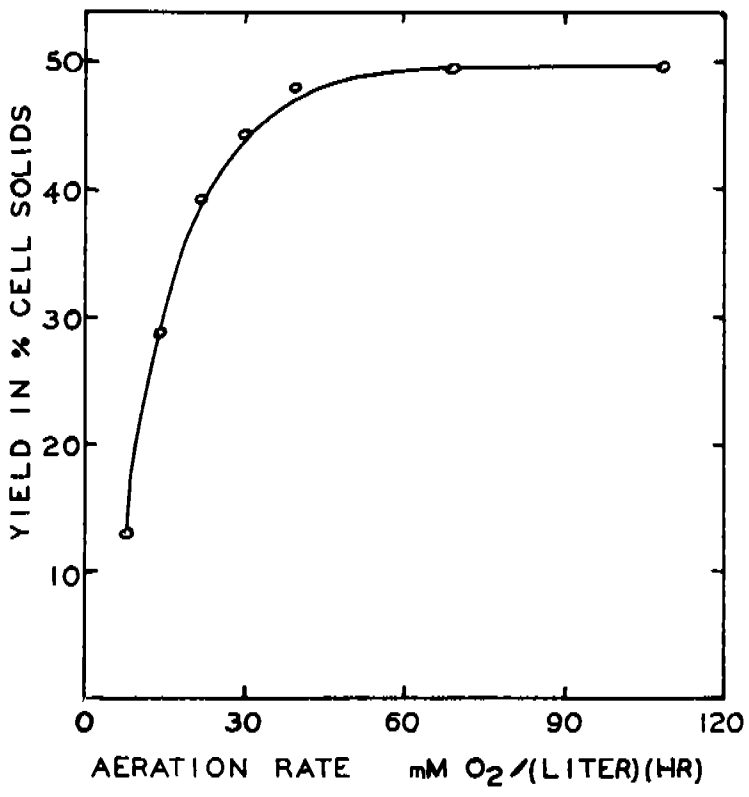
Maxon and Johnson (1953)

FIG. 5.4. EFFECT OF AIR RATE AND AGITATOR SPEED ON EFFECTIVE AERATION IN A FERMENTER

speed in a 3.5-l fermenter (1.5 l working volume). Sulfite oxidation values are expressed as mM of O_2 absorbed per liter per hour. Multiplication of this figure by 32 gives the O_2-transmitting capacity of the system in mg O_2 per l per hr. Improvements in the method of determining sulfite oxidation values have been suggested by Vincent and Allcock (1967).

However, it has been known for some time that determination of sulfite oxidation values of a fermenter is not an infallible guide to the actual air requirements of a fermentation. Fig. 5.5 shows the relationship of sulfite oxidation values to yeast yields in a shake flask system in which the medium was fed incrementally (Chen, 1959). But much higher sulfite oxidation values seem to be required for commercial fermenters. The discrepancy may well be explained by the fact that both pH and the presence of small proportions of impurities (particularly fatty acids) affect the sulfite oxidation value (Bell and Gallo, 1971).

The O_2 mass transfer coefficient (K_L) as well as the surface area of the air bubbles are affected by the addition of surface-active agents. With very small concentrations of surface-active agents in the fermenter, K_L, and with it the product K_L times a (surface area), decrease sharply. At higher levels of surface-active agents the product $K_L \times a$ increases somewhat because the smaller bubble size (and larger bubble surface) becomes the overriding factor. The absorption of



Chen (1959)

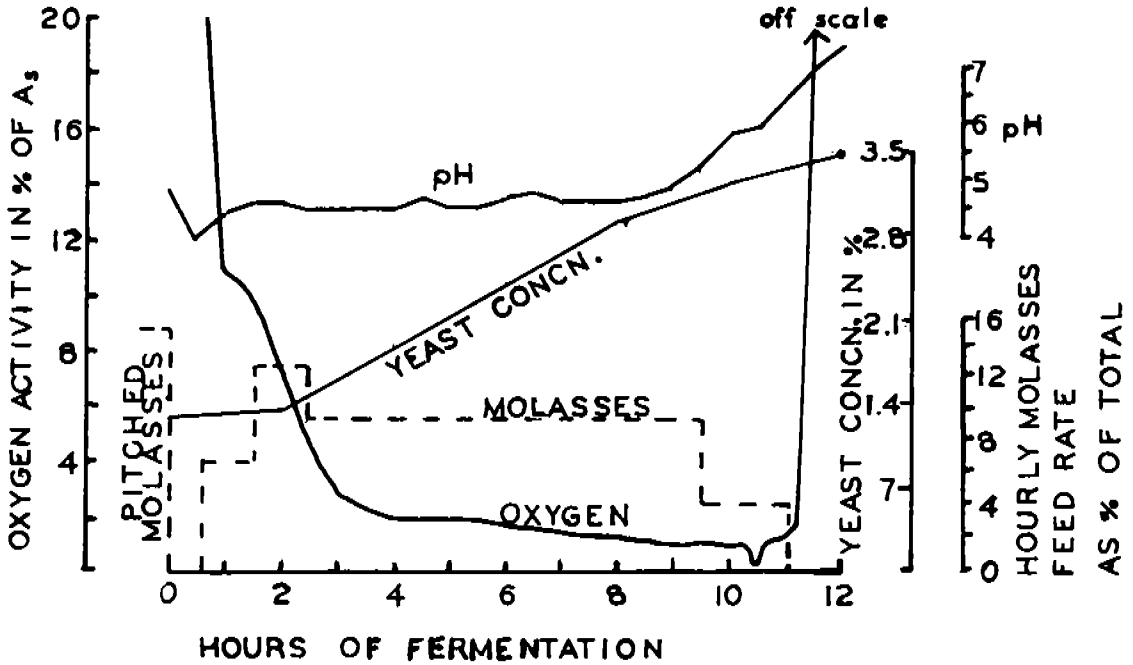
FIG. 5.5. EFFECT OF AERATION RATE ON GROWTH EFFICIENCY OF YEAST IN SHAKER FERMENTATIONS

O_2 from the air stream is affected by the presence of proteins and ethanol, and it is temperature-dependent. Therefore, the actual aeration requirements of a fermentation cannot be accurately predicted from measurement of the sulfite oxidation value of a given system.

Actual levels of dissolved O_2 in a fermenter can be determined by O_2 electrodes (see Aiba *et al.*, 1965). Several such electrodes are commercially available. They are frequently calibrated by saturating the fermenter liquor (without yeast) with air and by equating the instrument response with "air saturation." Instrument readings during the actual fermentation can then be expressed as "% of air saturation." A figure of 7 ppm O_2 in an air-saturated fermenter is a useful approximation of the actual amount of O_2 present. Fig. 5.6 shows O_2 values obtained during a 12-hour fermentation of bakers' yeast in a sparged (non-agitated) fermenter. The O_2-transfer rate of the system had previously been determined to be 150 m*M* O_2 per l per hr. The actual levels of O_2 during the fermentation are quite low (about 1.5 ppm). If the air supply is turned off O_2 disappears immediately from the liquid. The same effect is obtained by overfeeding the fermenter with sugar (not shown on the figure).

Effect of pH

Over a pH range of 3.6 to 6.0 bakers' yeast gives the best yields at pH values between 4.5 and 5. In experimental and commercial fermentations pH can be



Strohm et al., (1961)

FIG. 5.6. SUPERIMPOSED PLOTS SHOW RELATION OF RAW MATERIAL FEED, YEAST CONCENTRATION, pH, AND DISSOLVED O_2 ACTIVITY IN PILOT PLANT SPECIAL *S. CEREVISIAE* PROPAGATION

partly controlled by the ratio of ammonia to ammonium sulfate in the feed. The total amount of nitrogen supplied by these compounds must, of course, be kept constant and calculated to correspond to the desired level of nitrogen in the yeast. Higher concentrations of ammonium sulfate lower the pH as the ammonium ion is utilized by the yeast. Additions of ammonia temporarily increase the pH. When all the required nitrogen for a fermentation has been supplied the pH may be regulated by adding sodium carbonate.

Generally, it is desirable to grow yeast at low pH values in order to minimize contamination. On the other hand, yeast readily absorbs pigments from molasses at low pH values. In commercial operation the pH is changed slowly during the fermentation as shown in Fig. 5.6. This permits growth at a reasonably low pH throughout most of the fermentation; and raising of the pH towards the end of the fermentation yields a reasonably light-colored yeast. Kautzmann (1969C) suggests a starting pH of 4.2 to 4.5 and a pH of 4.8 to 5.2 at the end of the fermentation.

Temperature

It is surprising how little work on the effect of temperature on yeast growth has been published during the past 15 years. White (1954) is still the most widely quoted authority. Using this graph to calculate the generation time during the exponential growth phase, the following times can be determined: at 20°C, 5 hr; at 24.5°C, 3.5 hr; at 27°C, 3 hr; at 30°C, 2.2 hr; at 36°C, 2.1 hr; at 40°C, 4 hr; and at 43°C, approximately 8 hr.

Keszler (1967) determined the specific growth coefficient for a top-fermenting strain of brewers' yeast (*S. cerevisiae*) as a function of temperature. He found the following values for the specific growth coefficient: 0.15 at 20°C; 0.21 at 24°C; 0.30 at 28°C; 0.31 at 30°C and 32°C; 0.29 at 36°C; and 0.19 at 38°C. The yeast did not multiply at 40°C. These values lead to the same conclusion as those of White (l.c.), namely, that the optimum growth rate is between 30 and 36°C. But yeast yields are somewhat higher at lower temperatures and in practice yeast fermentations are carried out at 30°C.

Yield, Energy, and the Development of Heat

It has been mentioned several times that a yield of 50% of yeast solids (based on fermentable carbohydrate) can be obtained in commercial fermentations. Higher yields have sometimes been claimed for experimental work (see compilation by Butschek and Kautzmann, 1962). Chen (1959) obtained a yield of 50% based on the carbon source, and Oura (1969) obtained a yield of 52% based on the weight of sucrose. It appears that a yield of 50% in commercial fermentations is quite adequate. In evaluating yield data care has to be taken to relate such data to a common solids level. European workers often report their results as compressed yeast with a 27% or 28% solids basis. In the U.S. compressed yeast is reported on a 30% solids basis.

In 1960 Bauchop and Elsden suggested that the growth yield of a microorga-

nism, such as *S. cerevisiae*, is proportional to the amount of adenosine triphosphate (ATP) produced by its catabolic processes. They defined Y_{ATP} as the number of grams of cells (dry weight) produced per mole of ATP synthesized. Under anaerobic and substrate-limiting conditions they found the Y_{ATP} to be constant, about 10.5 g dry cells per mole of ATP.

Attempts have been made to extend this concept to the aerobic growth of microorganisms. For example, Chen (1964), on the basis of material balance data, calculated Y_{ATP} to be 10.96 for *Candida utilis* and 6.21 for *S. cerevisiae*. It should be pointed out that Chen's calculations were based on the assumption that 38 moles ATP were produced per mole glucose oxidized (i.e., a P/O ratio of about 3.17). While this assumption may be valid for *Candida* yeast (Ohnishi *et al.*, 1966A), it is too high for *Saccharomyces* yeast. Ohnishi *et al.* (1966B) reported that the P/O ratio for *Saccharomyces* mitochondria was one unit lower than that of mammalian and *Candida* mitochondria. Thus, by using this lower P/O ratio of 2.0 for *Saccharomyces* yeast, Chen's calculation of Y_{ATP} for *S. cerevisiae* should be 9.82 (unpublished data). With this correction both Y_{ATP} values, i.e., 10.96 for *Candida* and 9.82 for *Saccharomyces*, are in good agreement with those reported by Bauchop and Elsden (1960).

In similar attempts Hernandez and Johnson (1967) found the maximum Y_{ATP} to be 7.0 for *C. utilis* (assumed P/O ratio of 3.0) and 7.5 for *S. carlsbergensis* (assumed P/O ratio 2.0). At a P/O ratio of 1.1, von Meyenburg (1969) reported the Y_{ATP} for *S. cerevisiae* to be 12.0.

A knowledge of the O_2 requirements (see above) permits calculation of the heat evolved during the aerobic growth of yeast. Cooney *et al.* (1969) used *Candida intermedia* and found a factor of 3.44 kcal per g O_2 consumed. If we assume further that 1 g O_2 is required for the aerobic production of 1 g yeast solids, we obtain 3.44 kcal per g yeast solids. Harrison (1967) calculated heat production and found it to be approximately 3.87 kcal per g yeast solids, or 15.6 million BTU per ton yeast solids. In an independent study Levit and Galashov (1970) calculated the amount of heat evolved per g yeast solids as 3.8 kcal. The calculation is based on sucrose (in molasses) as the principal carbon source and on the assumption that 10% of the carbon is contributed by molasses compounds other than fermentable sugar.

In small experimental vessels of 5 to 20 l capacity, temperature may be controlled by immersing the vessels in a water-bath. For commercial fermenters cooling coils inside the fermenter are used. The cooling requirement must be calculated for the period during which the largest absolute increase in yeast dry matter occurs. This is usually not the last hour of fermentation. Although total cell concentration is highest at this time, the rate of growth is slower than earlier during the fermentation.

Osmotic Pressure

White (1954) investigated the osmosensitivity of yeast in detail. Osmosensitivity refers to the adverse effect of osmotic pressure on the ability of the yeast to

ferment sugars anaerobically (i.e., in doughs). The osmotic pressure may be due to high concentrations of sugars, as in sweet doughs, or to high salt concentrations. As expected, the effect of 6% glucose is approximately the same as that of 1% salt (percentage figures based on flour). For nominal amounts of salt (2.25%) or sugar (8 to 9%) the effect is not very great. White (1.c.) finds that yeast grown with incremental feeding has low osmosensitivity compared to yeast grown in a set (non-incremental) fermentation.

He also finds that growth rate affects osmosensitivity, yeast grown at high specific growth coefficients having high osmosensitivity. It is not clear whether growth rate is really an important factor since all the yeast grown in White's experiments was grown in set fermentations and with widely different pitching rates.

An increase in the osmotic pressure of the fermenter liquor by addition of sodium chloride decreases the maximum specific growth coefficient (Watson, 1970).

In anaerobic fermentations the osmotic pressure of the system rises because of the formation of ethanol. In aerobic commercial fermentations it rises because of the increase in non-assimilated molasses compounds as the molasses are fed incrementally throughout the fermentation period (see also Koppensteiner, 1970). The effect of yeast invertase on the osmotic pressure of doughs and its relation to yeast performance in sweet doughs is discussed in Chapter 6.

Yeast Concentration in the Fermenter

In commercial practice yeast concentrations of 3.5 to 4.5% (yeast solids) are reached at the end of the fermentation. That means that from 10 to 13% of the liquid volume consists of yeast cells. Drews *et al.* (1962) obtained a final concentration of 4.6% yeast solids in several fermentations in which various patterns of incremental feeding were used.

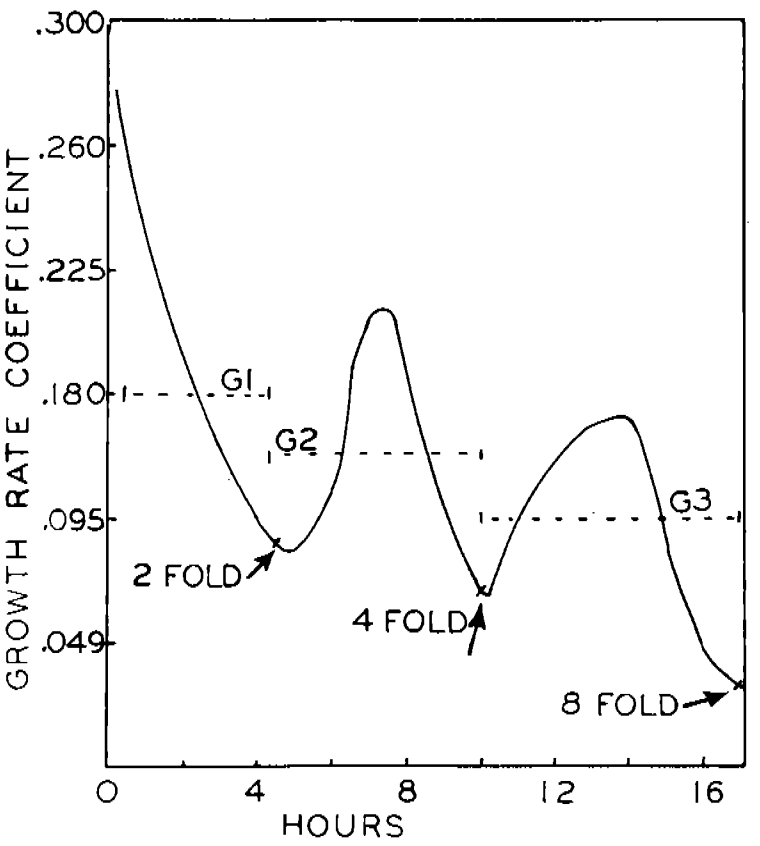
It is well known that exponential feeding of yeast cannot be continued beyond the first part of the fermentation because the growth rate decreases rapidly. For instance, in a commercial fermentation one may find a generation time of 4 hr at the beginning of the fermentation, of 6 hr during the middle, and of 8 hr during the final portion of an 18-hr fermentation (von Fries, 1962).

Various reasons have been given for this increase in generation time and for the inability to reach yeast solids concentrations exceeding about 4.5% as follows: Inability to supply enough O_2 (air rate); increase in viscosity and decrease in the rate of diffusion of O_2 and of other nutrients in solution; exhaustion of growth factors which are abundant in the pitching yeast; higher osmotic pressure; accumulation of inhibitors which may be present in molasses or may be excreted by the yeast; and finally, the growth inhibition which occurs from direct contact of cells (in tissues). None of these factors has been sufficiently explored to permit formulation of a convincing theory. Hennessy (1964) found self-inhibition by yeast, but Keszler (1967) could not find any inhibitors in

media which had been repeatedly exhausted by yeast growth and whose content of nutrients had been replenished before each new growth phase. However, total concentration of yeast solids in these experiments did not exceed 0.5% at the end of the growth period; that is, they were far below solids concentrations obtained in commercial practice.

Periodicity and Budding

The final trade fermentations which produce yeast for commercial sale are sometimes pitched with yeast which has been fairly well matured; that is, it contains a rather low number of budding cells. This leads to synchronization of yeast growth at least in the earlier stages of the fermentation. Therefore, all the cells of the pitching yeast begin to bud at about the same time after the start of the fermentation, and the mother cells separate from the daughter cells at the same time. This periodicity can be shown if one plots specific growth rates as a function of fermentation time, using small time increments rather than average



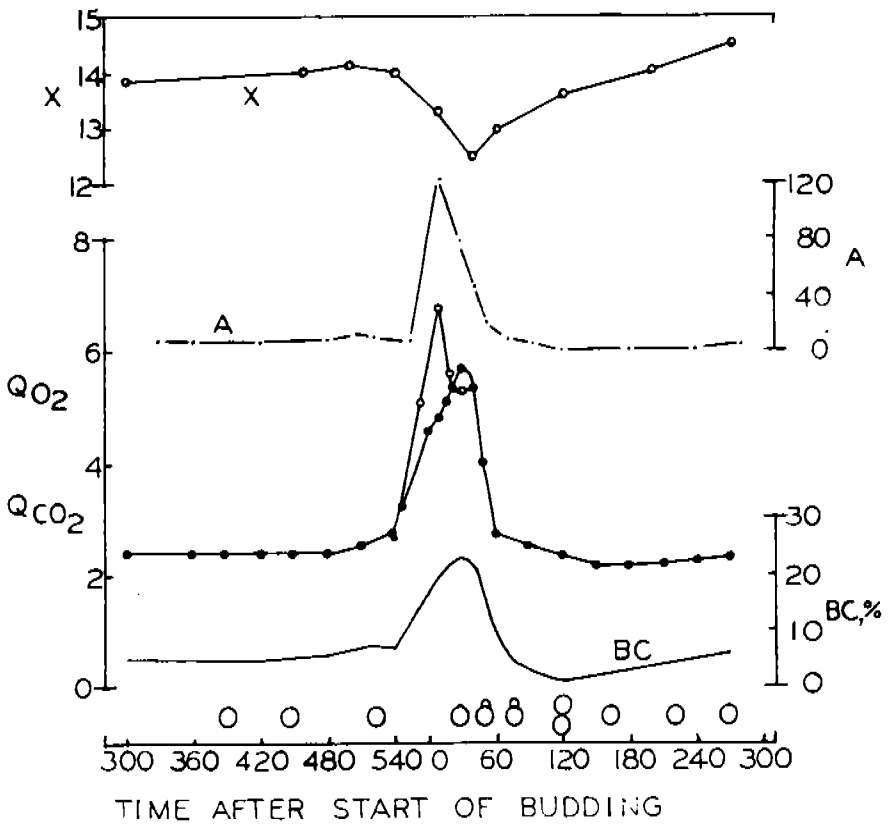
Fries (1962)

FIG. 5.7. RELATIONSHIP BETWEEN GROWTH RATE COEFFICIENT AND YEAST MULTIPLICATION DURING A 17 HOUR FERMENTATION AS FUNCTION OF TIME

G1 = time for 2-fold multiplication
G2 = time for second 2-fold multiplication
G3 = time for third 2-fold multiplication

growth rates. Fig. 5.7 shows such a curve for a commercial yeast fermentation. (von Fries, 1962). The specific growth rate coefficient decreases to a minimum value during budding and increases again as the separate mother and daughter cells resume growth. The point at which the yeast population doubled, quadrupled and became eightfold is clearly visible on the curve.*

Fig. 5.8 shows in more detail the relationship between respiratory quotient and weight of cell solids as a function of the budding cycle. At the onset of budding, O_2 consumption rises drastically, but CO_2 output rises more steeply and the



Meyenburg (1969)

FIG. 5.8. SYNCHRONOUS GROWTH OF *S. CEREVISIAE*

X = cell dry weight in mg/ml
A = ethanol concentration in mcg/ml
Q_{CO_2} = CO_2 production in mM/g dry weight/hr
Q_{O_2} = O_2 uptake in mM/g dry weight/hr
BC = % of initial budding cells

(mean generation time 9.5 hr (D = 0.073).

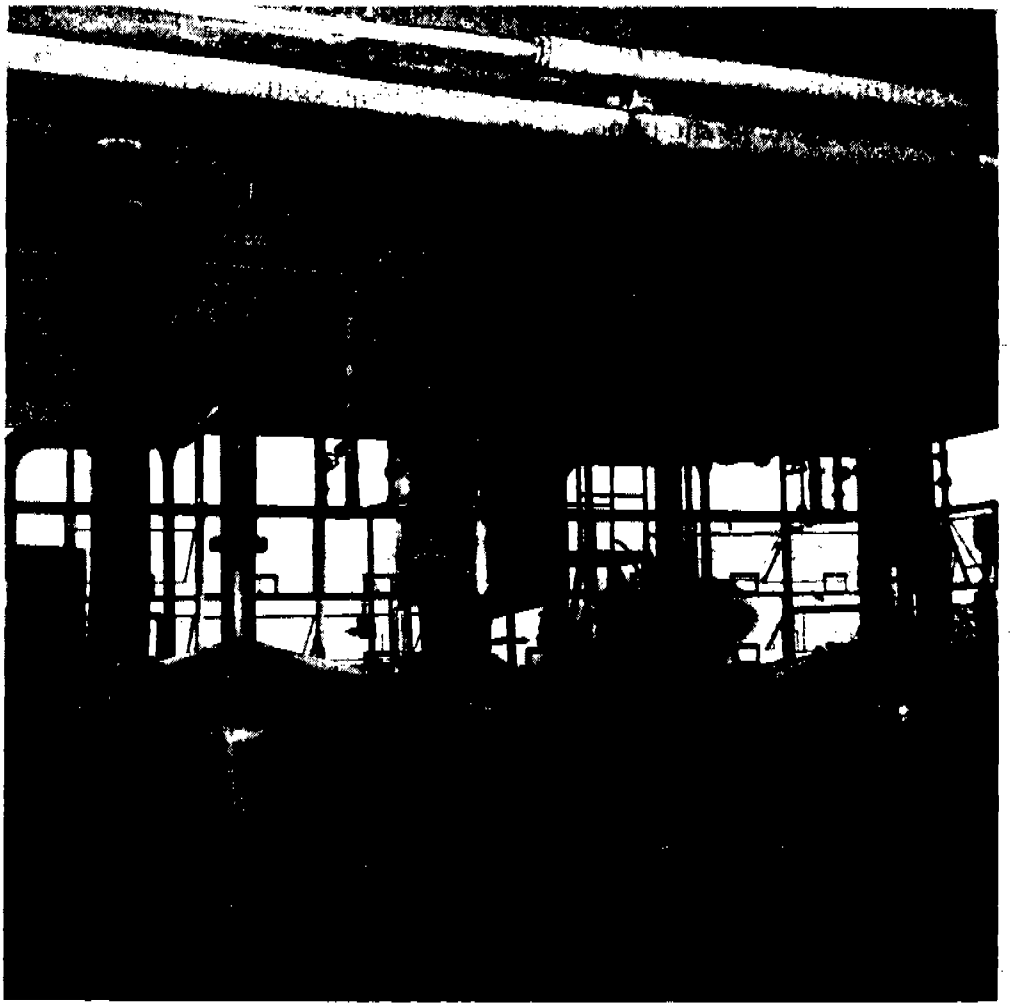
*This does not mean that the weight of yeast becomes eightfold. Both cell volume and cell weight are somewhat smaller after eightfold multiplication of the population, and the increase in the weight of yeast solids may only be sevenfold.

respiratory quotient is higher. At the same time some ethanol is produced, nitrogen uptake is increased, and the weight of cell solids decreases. Toward the end of the budding cycle, which lasts about 2 hr, these variables return to the values they had before the onset of budding. It should be noted that the values shown in Fig. 5.8 were obtained under conditions of continuous culture and with synchronous growth of the yeast. They cannot be simply correlated with the growth rate figures in Fig. 5.7. However, it is tempting to speculate that the periodicity shown in both figures is closely related to the budding cycle.

PRACTICE OF THE AEROBIC GROWTH OF BAKERS' YEAST

Fermentation Tanks

In discussing the growth of bakers' yeast in commercial fermenters it is advantageous to start with the "trade" fermentation and to treat the preparation



Courtesy Basic Foods Corp., Manila

PLATE 5.1. FERMENTERS AND FEED TANK

The upper portions of two fermenters are shown on the lower left and right. A feed tank holding nutrient solution is shown in center.

of the pitching yeast subsequently. "Trade" fermentation is the designation of the final stage which produces yeast for sale. It is generally carried out in the largest available fermenters, which may range in size from 75 to 225 m^3. Only about 75% of this volume can be utilized because of the lowering of the actual density of the content by air bubbles and because of foaming.

The fermentation tanks may be made of wood, copper or stainless steel. Today stainless steel is generally used. In contrast to fermentations which require complete sterility (such as antibiotic or enzyme fermentations) bakers' yeast may be propagated under conditions which permit some contaminants to enter with the medium, with the air, or with the pitching yeast. Therefore, the fermentation vessels need not be placed under pressure for sterilization of the tank and construction of such vessels is less expensive. Such fermenters are often called "open" fermenters in contrast to the "closed" fermenters of the antibiotics industry. However, it is desirable to keep contamination to a minimum by methods of construction which permit thorough cleaning and sanitizing of the fermenter walls, of all pipes and valves, as well as of the instruments (thermometers, oxygen probes, pH electrodes) which may be in contact with the fermenter contents. Plate 5.1 shows the upper portions of the fermenters.

Cooling

Bakers' yeast fermentations are carried out at 30°C. Heat generation during the growth of the yeast is about 3.5 kcal per g of yeast solids. Cooling is usually carried out with cold water flowing through stainless steel pipes of 2 to 2.5-in. diameter wound in spiral fashion along the inside of the fermenter wall. It is advisable to place the pipes at a sufficient distance from the fermenter wall to permit thorough cleaning. Cooling requirements vary with the concentration of yeast in the fermenter and with its specific growth rate. The rate of evaporative cooling at high aeration rates must also be taken into account.

Aeration Systems

A large number of aeration systems are available, but for commercial use only three of these have found wide application. Each of these systems has certain advantages and disadvantages. These cannot be discussed in detail but a general outline of such systems follows.

Horizontal, Perforated Pipes.—In this system air is blown through a large number of horizontal pipes which are strung in parallel near the bottom of the fermenter. The diameter of the pipes is between 1 and 2 in., and the openings drilled along the top or the bottom of the pipes are about 1/32 in. Agitation of the fermenter liquid is provided solely by the stirring action of air bubbles as they pass through the liquid.

Air is supplied to such perforated pipes by a vertical air supply tube of large diameter in the center of the fermenter (Fig. 5.9A).

The total amount of air required to grow 1 lb yeast varies, of course, with the

size and construction of the fermentation vessel, as well as with the size and number of air orifices in the perforated tubes. Anywhere from 25 to 50 m^3 of air are required per lb yeast solids grown, but modern installations generally do not require more than 25 to 30 m^3 (de Becze and Liebmann, 1944; Butschek and Kautzmann, 1962). One can readily achieve a throughput of 1 volume of air per fermenter volume per minute with such perforated tubes. This method of aeration is the most practical because of low initial cost, simplicity, and ease of cleaning; but it requires larger amounts of air than the other two systems discussed below, and its operation is somewhat more costly than systems with mechanical agitation.

The efficiency of air utilization can be measured by determining the percentage of O_2 in the air supply and in the effluent air, and it can also be calculated by determining the total amount of air required to grow 1 lb yeast solids. In the aeration system described above air utilization is somewhere between 10 and 15%.

Compressed Air and Mechanical Agitation.—The efficiency of aeration with a given volume of air can be greatly increased by mechanical agitation. The simplest systems employ a circular, horizontal air pipe with sufficiently large air outlets. Air is supplied to the circular pipe under pressure. Directly above the air outlets a horizontal turbine disc provides mechanical agitation and distributes the air in fine bubbles through the fermenter. Such systems require baffles in the fermenter to prevent the liquid from rotating in the direction of the motion of the disc.

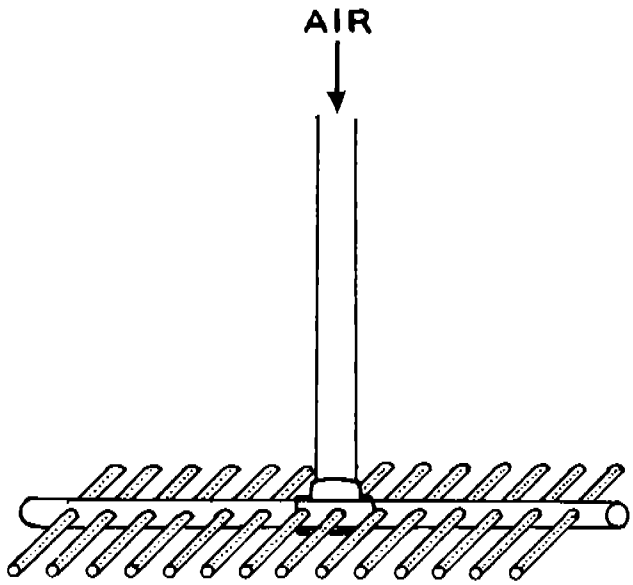
Štros *et al.* (1968) used this system in Czechoslovak yeast factories; 10 to 15 m^3 air were required per lb yeast solids produced, which is considerably less than the amount of air required without mechanical agitation. The efficiency of air utilization was between 25 and 30%, which means that this percentage of O_2 was removed from the air by passage through the fermenter.

Satisfactory results can also be obtained with the use of rotating (horizontal) wings in which compressed air passes through the hollow wing and is sheared off as it leaves the small orifices in the trailing edge of the wing (Fig. 5.9B).

The efficiency of air utilization in such a system is also of the order of 25 to 30%.

Self-Priming Aerator.—This device operates with a turbine which sucks air through a hollow, vertical shaft into the fermenter liquid. Air is aspirated through the vertical shaft of the turbine (at 1450 or 1700 rpm) and the design works without a compressor. Hence, the pressure of air at the outlets is not very high and the depth of submergence of the turbine is limited. The openings in the turbine through which the air enters the fermenter liquid are arranged radially, as shown in Fig. 5.9c. In commercial use these aerators gave satisfactory performance at air rates of 0.25 to 0.3 volume air per fermenter volume per minute, which is about 25 to 30% of the air volume required with perforated pipes (Ebner *et al.* 1967).

Finn (1969) has addressed himself to the energy costs of O_2 transfer. This question has been treated in considerable detail by bioengineers concerned with



deBecze and Liebmann (1944)

FIG. 5.9A. NETWORK OF PERFORATED AIR TUBES

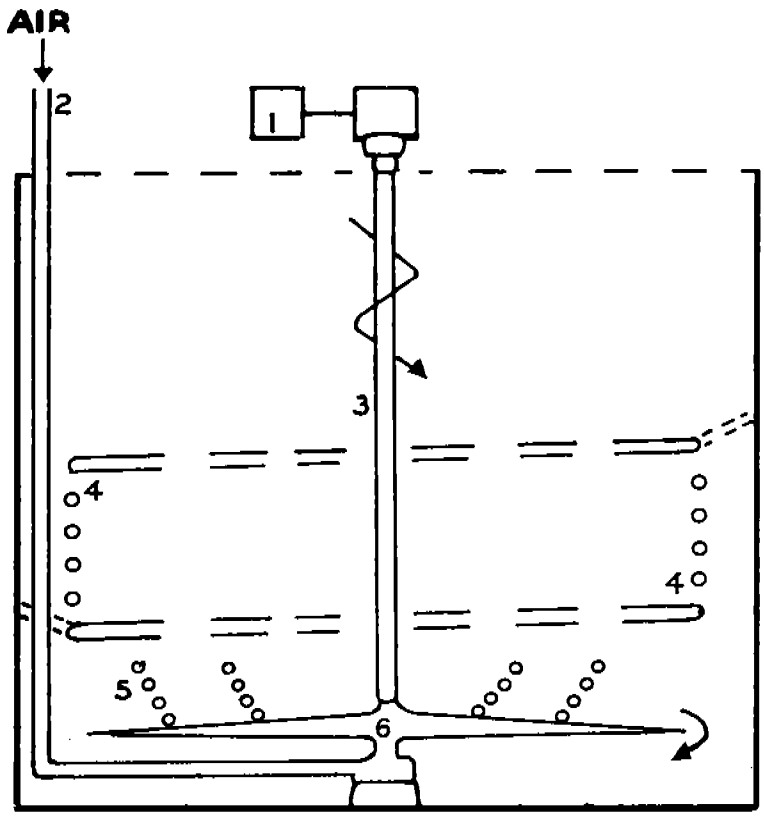
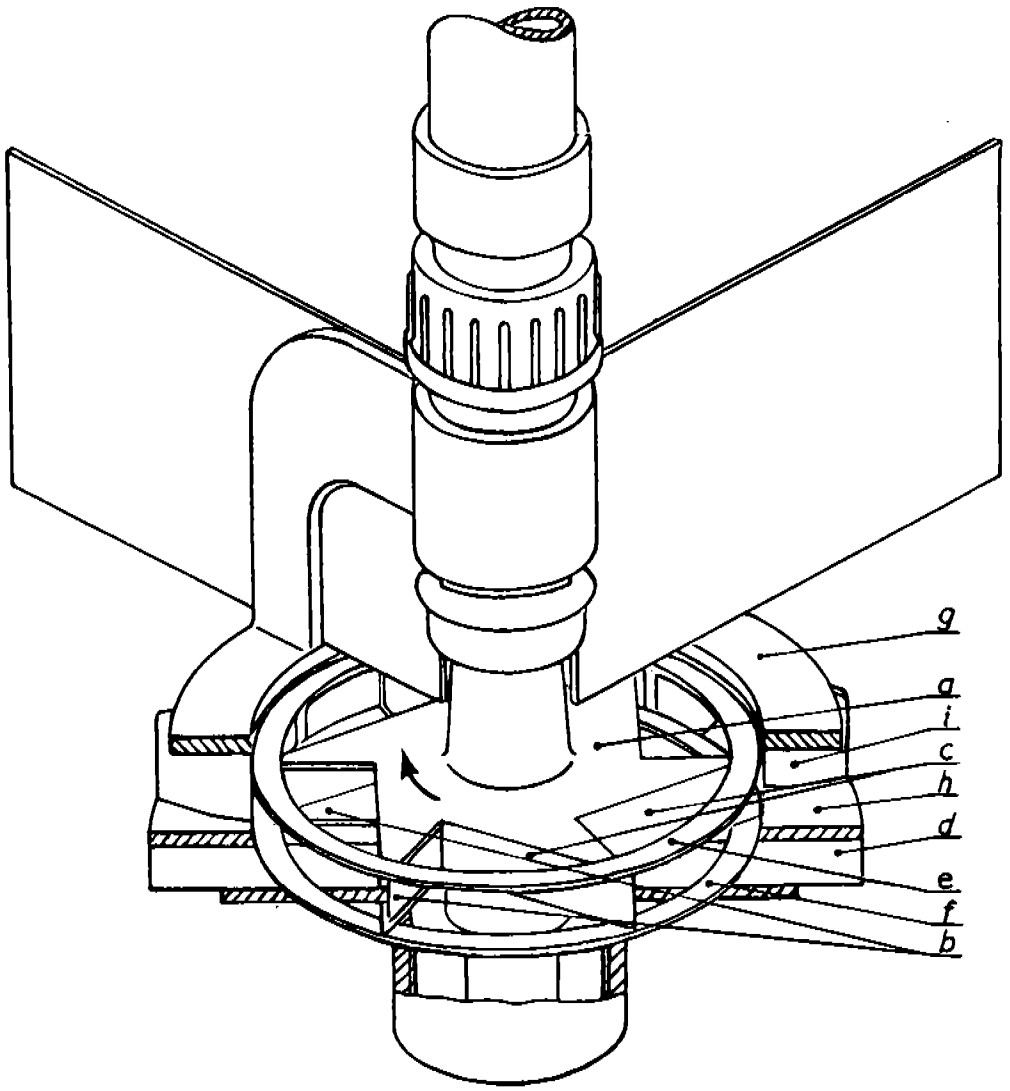


FIG. 5.9B. FERMENTER WITH VOGELBUSCH AERATION WING

1. Motor, 2. Air inlet, 3. Drive shaft for aerator wing, 4. Cooling coils, 5. Air bubbles escaping through aerator wing, 6. Rotating aerator wing.



Enenkel et al. (1967)

FIG. 5.9C. SELF PRIMING AERATOR

a. Turbine hollow body, b. Radial openings (open against the direction of rotation), c. Vertical shields for openings, d. Stator, e. and f. Upper and lower rings on turbine, g. and h. Upper and lower rings on stator, i. Vertical sheets connecting g and h.

(Speed of rotation of turbine 1450 or 1750 rpm).

the aeration of biodegradable liquid wastes. But little is known about these costs in the production of bakers' yeast except for the possible inverse relation of economy and fermenter capacity.

Feed Rates

Commercial fermenters are also equipped with a device for adding molasses solutions. This device may be a simple pipe or a system of pipes which permits distribution of the molasses wort over the entire surface area of the fermenter liquid. The feed rate may be controlled by rotometers or by pumps which deliver

a given volume of molasses at regulated time intervals. Nutrient solutions are usually kept in small, separate tanks and are fed through rotometers into the fermenter.

The duration of a trade fermentation may be varied within rather wide limits. Generally, it is of the order of 10 to 13 hr for a fourfold multiplication of the yeast and 17 to 20 hr for an eightfold multiplication. It must be kept in mind than an eightfold multiplication of cells does not result in an eightfold growth of cell solids since the cells are slightly smaller at the end of a trade fermentation (Sato *et al.*, 1964).

Propagation may be continued until the level of yeast has reached 3.5 to 4.5%. Throughout the fermentation the specific growth rate decreases and, therefore, a truly exponential feed rate cannot be used. Fig. 5.10 shows several feed curves

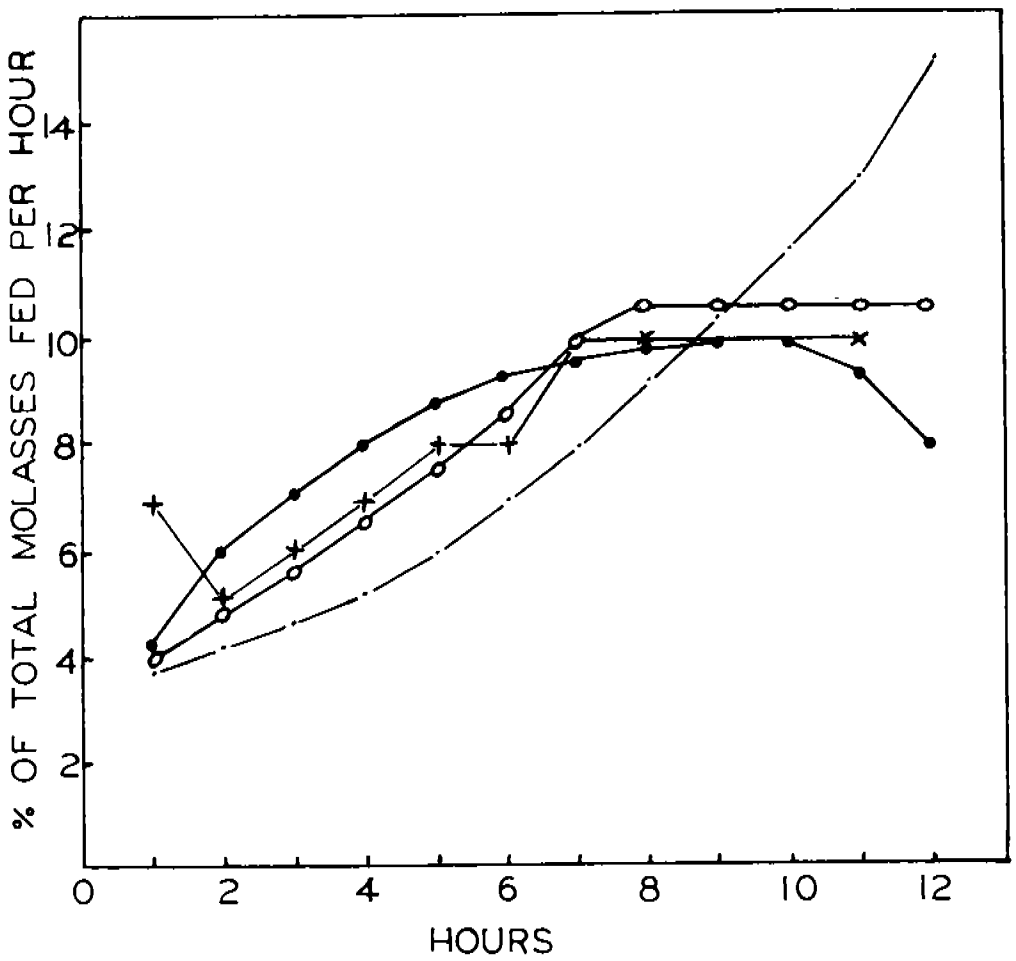


FIG. 5.10. MOLASSES FEED CURVES (EXPERIMENTAL AND COMMERCIAL)

Exponential, exptl (1) ●——●
Exponential and constant, exptl (1) X——X
Commercial (1) O——O
Commercial (2) |——|
(1) Drews *et al.*, 1962.
(2) Butschek and Kautzmann, 1962.

published within the last 10 yr. Curve No. 1 and 2 as well as the feed curve shown in Fig. 5.6 are representative of commercial practice. After all the required molasses has been fed into the fermenter, the liquid is still-aerated for periods of ½ to 1½ hr (at reduced air rates). This permits further "maturing" of the yeast in the sense that it produces a yeast that is more stable in refrigerated storage. It does not reduce the number of budding cells, which means that the process of separation of the daughter cells from the mother cells must be essentially complete at the time the molasses feed is stopped. The rate of molasses feed is critical in yeast propagation and underfeeding or overfeeding lowers the yield of yeast.

The rate of feed of other nutrients is not quite as critical. The nitrogenous salts and phosphates may be fed into the fermenter in a shorter time than the molasses. However, if ammonia is used as a source of nitrogen, additions must be made in such a manner that sudden changes of pH are avoided. Generally, enough nitrogen is added to produce bakers' compressed yeast with 8 to 9% N_2 and active dry yeast with 6.5 to 7% N_2. The level of phosphate in the yeast (expressed as P_2O_5) should be about one-third that of the nitrogen. It should also be pointed out that uniform yeast yields are important. If the yield of yeast based on the weight of molasses is low, the levels of nitrogen and P_2O_5 in the final yeast are likely to be too high, and vice versa.

It has already been mentioned that commercial fermentations are carried out at 30°C and that the pH may vary between 4.5 and 6.5. Fig. 5.6 shows typical changes in pH during a commercial fermentation.

Sequence of Fermentations

The trade fermentation is preceded by a series of smaller fermentations in which the pitching yeast is grown. The process starts in the laboratory, where Pasteur flasks containing a rich medium (malt or malt sprouts) are inoculated from slants of the pure culture (Lincoln, 1960). The content of the Pasteur flasks, after a 2-day incubation, is then inoculated into small pure culture fermenters. Generally there is a series of two pure culture fermenters with capacities of, e.g., 20 and 100 gal. The yeast is grown in these fermenters in a medium rich in the growth factors mentioned above, including some source of organic nitrogen such as corn-steep liquor. The medium is sterilized directly in the pure culture fermenters and there is, of course, no incremental feeding. There is very little aeration and the air is sterilized (see stage R1 and R2 in Table 5.3).

The pure culture stages are followed by another stage of yeast growth without incremental feeding (F1). The entire fermenter contents of the F1 stage is pumped into a larger tank which is equipped for incremental feeding and which has good aeration (F2). This stage is often called the stock fermentation because after completion of the fermentation the yeast is separated from the bulk of the fermenter liquid (beer) by centrifuging, producing a stock of yeast for the next stage.

TABLE 5.3

SUCCEEDING STAGES IN THE PROPAGATION OF BAKERS' YEAST

Fermentation Stage	R1 P.C.*	R2 P.C.*	R3 open	F1 open	F2 open	F3 open	F4 open	F5 open
Yeast per fermenter in kg**	0.2–0.8	0.8–3.5	3.5–25	25–120	120–420	420–2500	833–5000 (3 times)	1666–11000 (3 times)
Total yeast grown in kg (Suomalainen, 1963)	0.8	3.5	25	120	420	2500	15,000	100,000
Yeast per fermenter in kg**		0–8	8–50	50–250	50–250 (5 times)	250–1700	566–3000 (3 times)	1500–4500 (twice)
Total yeast grown in kg		8	50	250	1250	8500	45,000	270,000
Length of fermentation (Butschek & Kautzmann, 1962)		20 hrs.	15 hrs.	16 hrs.	14 hrs.	12 hrs.	12 hrs.	11 hrs.

*P.C. = pure culture.
**At 27% solids.

The next stage (F3) is usually carried out in fermenters as large as those used for the trade fermentation. Aeration is vigorous and molasses and other nutrients are fed incrementally. The fermenter liquor of a completed F3 stage fermentation is usually divided into several parts for pitching of the final trade fermentation. Alternately the yeast may again be separated by centrifuging. The final trade fermentation has the highest degree of aeration and molasses and other nutrients are fed incrementally (F4).

The sequence of fermentations described above is shown schematically in Table 5.3. In the earlier stages of yeast propagation the medium is richer in nutrients and there is less aeration. Consequently, the fermenter liquor contains more alcohol and yields of yeast are lower. This is not necessarily a drawback, since the overall economy of the operation depends largely on the yield of the final trade fermentation.

Few authors have bothered to explain this peculiar sequence of propagation stages except to suggest that the earlier stages require a better supply of nutrients in order to yield a healthy pitching yeast. This is not true, or it need not be true, since one can readily grow satisfactory bakers' yeast in continuous fermentation by supplying all the required nutrients to the continuous fermenter. A more likely reason is the growth of contaminants in the stages following the pure culture stages. This growth limits the number of stages that can be carried out in practice and requires a fresh start with pure culture yeasts after the beginning of the cycle. The fact that earlier stages of propagation do not have the features of full aeration and incremental feeding merely means that it is not economical to equip smaller fermenters with the required devices.

There are some purely operational reasons which make this sequence of propagations and the separation of the stock yeast from stage F2 practical. It is usually not possible to operate many large-scale trade fermentations with the same starting time, since the requirement for maximum aeration would occur in all the fermenters at the same time, and would require the installation of larger air compressors than would otherwise be required. Also, the number and capacity of available centrifuges may not be sufficient to separate yeast from more than one or two fermenters at the same time. Therefore, separation of the stock yeast from the F2 stage and storage in the form of yeast cream (about 18% yeast solids) permits staggering of the large fermentations in the F3 and F4 stages.

Defoaming

The use of substantial air levels during fermentation leads to the formation of foam, which must be controlled chemically or mechanically. In the production of bakers' yeast chemical control is common.

Chemical defoaming agents are surface-active materials that break the foam by replacing the proteins of the liquid-gas interface, which are responsible for foam formation. Defoaming agents by themselves must be unable to produce a stable foam (Solomons, 1967). Synthetic compounds such as silicones, as well as fatty

acid derivatives, are often effective defoaming compounds; other synthetic compounds which have been used are octadecanol and polypropylene glycols. Glyceride oils and fatty acids are used widely because they are rather inexpensive. Often these are proprietary compounds whose exact composition is not fully known. Andreev *et al.* (1968) describe the use of antifoam agents consisting of mixtures of fish oil or oleic acid with sulfuric acid. Alkoxylated fatty acid bases and alkylene glycols have also been used.

The effect of surface-active agents on oxygen transfer is complex. These substances decrease O_2-transfer coefficients, but they also tend to increase the total interfacial area between gas and liquid (Finn, 1969).

Utilization of Ethanol

Oxygen transfer rates are often inadequate, and under such conditions some ethanol is formed. An alcohol level of up to 0.05% is a sign of satisfactory performance, but ethanol levels above 0.1%, as reported for instance by Syhorova and Štros (1966), result in some loss of yield. Ethanol, once it has been formed, may be later aerobically assimilated by the yeast, particularly after a period of adaptation (Drews and Hessler, 1967).

In the DeLoffre process (DeLoffre, 1964) the fermentation is deliberately started with a large amount of molasses wort and little aeration, so that the fermenter liquid contains about 2.5% ethanol (by vol) at the end of the early phase. During later phases of the fermentation the amount of ethanol decreases, as it is assimilated by the yeast, and at the end of the trade fermentation the ethanol concentration is negligible. The process is used commercially but not very widely. It is claimed that it results in higher yields of yeast than fully aerobic fermentations, but it is difficult to see why this should be so. The process may have some advantage because of the slower growth of contaminants during the first (alcoholic) stages of the fermentation.

Automatic Process Control

The fermentation process may be controlled by regulating the flow of molasses feed with various mechanical devices. Considerable refinement can be introduced by sensors which determine the concentration of ethanol in the effluent air, and which may be used to regulate the rate of aeration, the molasses feed rate, or the rate of mechanical agitation. In practice it is preferable to regulate molasses feed, since air flow rates and mechanical agitation are often at their peak rates. The principles of such control devices have been described by Rungaldier and Braun (1961). Alcohol is determined in the effluent air, which is assumed to be saturated with water vapor. For a fermenter liquid containing 0.2% ethanol (by vol) and at a temperature of 28°C the effluent air will contain 32.1 g water and 0.638 g ethanol in 1 m^3 (at STP). This means that the alcohol concentration of the gas is much higher (in relation to water) than that of the fermenter liquid, which facilitates the ethanol assay. The rate of molasses addition may then be

controlled by restricting molasses feed to the point where the concentration of ethanol in the fermenter does not exceed 0.2%. Because of the need for an alcohol assay the response of the system is somewhat slow.

The above system works quite well for fermenter control at the 0.2% ethanol level. Control at lower ethanol concentrations is difficult. Control of the air flow rate by a similar device has been suggested by Rosenquist and Egnell (1966). This system is less desirable because of the above mentioned limitation of air flow rates in a given fermenter.

Automatic pH control during a fermentation is relatively easy to achieve, and most industrial fermenters are equipped with such control devices. An extensive description of instrumentation for automatic control of the process has been given by Sher (1961).

Continuous Aerobic Propagation of Bakers' Yeast

The continuous production of *Candida utilis* yeast in a homogeneous, one-stage fermentation (open or partly closed) will be described in Chapter 11. For the production of food or feed yeast, cell mass and its chemical composition are the only criteria of economy and quality, but in the production of bakers' yeast quality depends also on the fermenting activity and keeping quality. This requires maturing of the yeast to assure stability in storage; hence, a single-vessel, homogeneous, continuous fermentation is not adequate. Bakers' yeast can, however, be produced continuously in a series of at least two vessels. (For a non-homogeneous system see Prokop *et al.*, 1969).

A great deal of experimental and pilot plant work on such continuous fermentations has been published, but the ultimate success of commercial operation remains in doubt unless one can work in pure culture systems. The principal drawback to continuous operation is the increase of contaminants in fermenters which cannot be fully sterilized.

Plevako (1962) described a two-stage continuous fermentation which operated at a steady state with yeast concentrations of 1.35% solids, a temperature of 28 to 30°C, a pH of 4.5 to 5.5, and a specific growth rate coefficient of 0.15. Fiechter (1968) obtained better yields of bakers' yeast in continuous culture with a specific growth rate constant of 0.2 and a productivity of 0.25 g yeast solids per l per hr. In almost all instances experimental work has been done at yeast solids concentrations considerably below those attained in the batch process.

A two-stage continuous fermentation has also been used by Beran and Zemanová (1969). With dilution rates of 0.3 per hour they obtained a constant yeast concentration of 1.5% solids. They also reported on the cell elongation, which has received attention in the continuous production of beer. The morphological changes became apparent as soon as they started to operate their fermenters continuously. The phenomenon could not be explained.

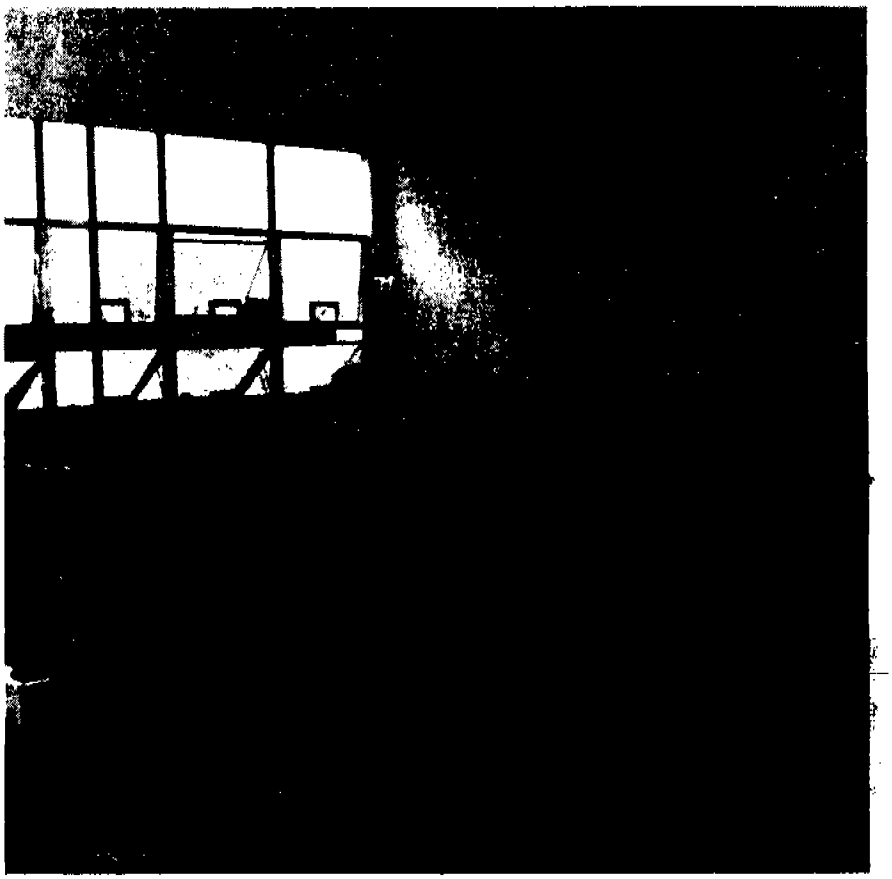
A commercial plant producing bakers' yeast by continuous fermentation has

operated for several years successfully in Britain. Its operation has been described by Olson (1961) and Sher (1961, 1962, 1968). The system operates as a five-stage, homogeneous, open, continuous fermentation. The concentration of yeast solids in the steady state was 1.9% and the specific growth rate coefficient was 0.14. The process begins with a batch fermentation with incremental feed in the first fermenter until a volume of 9000 gal and a yeast concentration of 1.9% solids has been obtained. From this time on, the first fermenter is operated on a continuous basis. The fermenter liquor of the first fermenter is then fed continuously into the second fermenter. The continuous addition of molasses wort and of other nutrients to the second fermenter begins at the same time. In a similar fashion all the fermenters are filled to a capacity of 9000 gal. The total time until all the fermenters have been filled is called the "start-up" phase. It lasts 27 hours. This is followed by the "on-stream" or "steady-state" phase, in which all the fermenters were operated continuously. The steady-state phase lasted from 33 to 70 hr, depending on the demand for yeast during any one week. This was followed by the "closing down" phase, which took about 17 hr, until all the fermenters had been emptied. The total operating time of a continuous run was thus from 5 to 7 days. The factor ultimately limiting the length of the steady-state phase was contamination with microorganisms commonly encountered in the production of bakers' yeast. Burrows (1970) has given an excellent description of the process.

Harvesting of Yeast Cells

Cells of bakers' yeast have a water content of 0.62 g/g of cells and a true density of 1.133 g/cm^3 (Sambuichi *et al.*, 1971). Yeast is routinely recovered from the fermenter liquor by centrifuging, followed by pressing or filtration. The centrifuges used in this process are continuous dewatering centrifuges which develop approximately 4000 to 5000 G. During the first pass through the centrifuges a yeast solids concentration of 8 to 10% can be obtained from a fermenter liquor having 3.5 to 4.5% solids. After washing the yeast with water and a second pass through the centrifuges, a yeast concentration of 18 to 21% solids can be reached. The whitish liquid resulting from this process is called yeast cream. It may be stored at or slightly above 0°C for several days without deterioration. At higher solids concentrations the yeast cream becomes viscous. It is pumpable at solids concentrations up to 23%. Lozenko (1967) reported that storage stability depends on the nitrogen level of the yeast, and he found optimum stability at 8.9% nitrogen. In practice yeast at any nitrogen level may be stored satisfactorily.

Yeast solids are further concentrated by pressing or filtration. In the traditional pressing operation plate and frame filters or recessed frame filters are used. The filter cloth consists either of cotton duck or of a combination of cotton duck and synthetic fibers so tightly woven that the use of any filter aid is unnecessary. Filter presses having frames of 24 to 48 in. are commonly used, and the applied pressure varies between 125 and 150 psi. Yeast with a solids level



Courtesy Basic Foods Corp., Manila

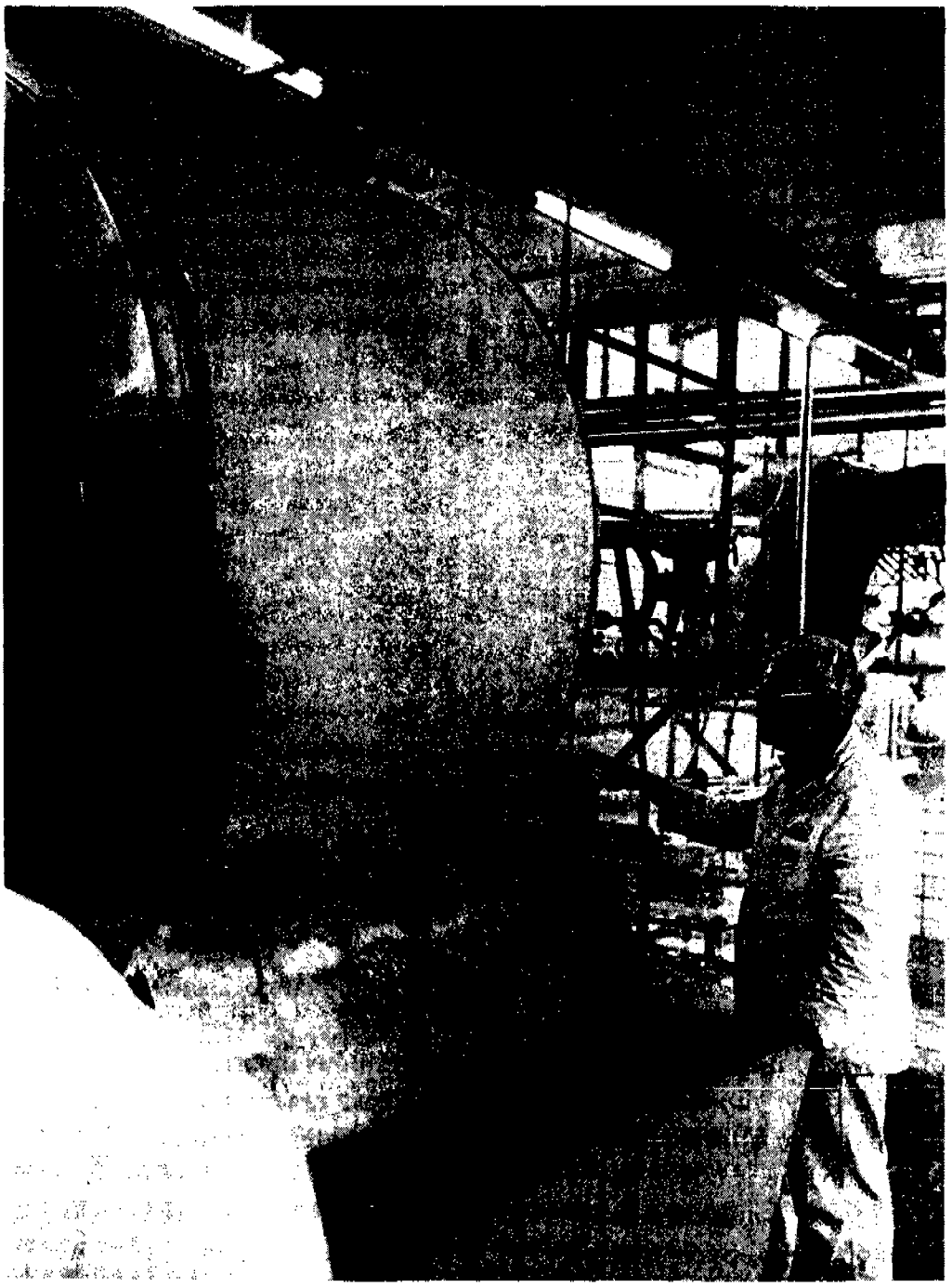
PLATE 5.2. BAKERS' YEAST PRESS

between 27 and 32% may be obtained by pressing. Plate 5.2 shows a picture of a yeast press.

Rotary vacuum filters may be used for continuous operation. These filters are generally of the dip-trough type, but the yeast cream may also be sprayed directly onto the filter surface. The filter wheel rotates at a rate of 15 to 22 rpm. The filter surface is coated with a 3/4 in. layer of potato or other large-granule starch.* Doctor knives remove the filter cake, which usually contains very small amounts of starch. Such filters permit the production of a press cake with 27 to 28% solids. Plate 5.3 shows a view of the rotary vacuum filter.

Higher levels of yeast solids can be obtained if the yeast cream is salted just before filtration with 0.3 to 0.6% NaCl (based on the weight of cream). After formation of a filter cake and while the filter wheel continues to rotate, the excess salt is removed by spraying water onto the filter cake. A filter cake with up to at least 33% solids can be obtained by this process (Kuestler and Rokitansky, 1960).

*Starch is not invariably used, but must be applied if cane molasses is included in the mash bill.



Courtesy Deutsche Hefewerke GMBH, Hamburg

PLATE 5.3. ROTARY VACUUM FOR BAKERS' YEAST

Mixing, Extruding, and Packaging Compressed Yeast

The yeast press or filter cake is blended in ribbon mixers with small amounts of water, emulsifiers, and cutting oils. The emulsifiers are added to give the yeast a white or creamy appearance and to prevent water-spotting of the yeast cakes. Such emulsifiers may be mono- or diglycerides, lecithin, or sorbitan esters (MacDonald and Geisler, 1965). A small amount of an oil, usually soy bean oil,

Courtesy Deutsche Hefewerke GMBH, Hamburg

PLATE 5.4. YEAST CUTTING OPERATION

is added to assist in the extrusion and cutting of the yeast. The mixed press cake is then extruded through nozzles with a tapered (Teflon-coated) throat in the form of continuous ribbons with a height and width of several inches. These ribbons are cut to yield the well-known yeast cakes. In the U.S. the individual cakes weigh 1 lb. In other countries they are available in several weights based on the metric system. Plate 5.4 shows the cutting operation.

The yeast cakes are wrapped with wax paper and the ends of the wrappers are heat-sealed. The wrapped cakes are placed in boxes and cooled in refrigerators until a temperature below 4°C has been reached. They are then ready for shipment in refrigerated trucks.

During the past five years increasing amounts of yeast are also marketed in bulk form as so-called crumbled yeast. For this purpose the press or filter cake is extruded through a screen or perforated plate with large holes so that it is broken up into irregular-shaped pieces. These are packaged in polyethylene-lined, multiwall bags which may contain 25 to 50 lb of crumbled yeast. The bags are carefully sealed since it is important to minimize gas exchange to prevent respiration and heating of the product. The bags are cooled to a temperature of less than 4°C (Schuldt and Seeley, 1966; Takakuwa, 1966).

In the U.S. the solids level of compressed yeast is generally 30%, while it may vary between 27 and 30% in other countries.

Contamination

Bakers' yeast normally contains other microorganisms, since it is grown in open fermenters which do not guarantee sterility. Great efforts are made to keep this contamination at a minimum. For instance, molasses worts are generally sterilized by heating in bulk or in continuous heat exchangers (with steam injection). The wort must be stored hot, which entails some loss of fermentable sugar; it is subject to renewed infection if it is stored cold. The air used for supplying oxygen to the fermenters is filtered through depth filters, membrane filters, or it is heated. However, it is rarely sterile at the time it reaches the fermenter pipes which serve to distribute the air.

Fermentation tanks are generally scrubbed with detergents or strongly alkaline solutions and steamed. In a similar manner pipes, valves, pumps and other accessory equipment must be thoroughly cleansed. The filter cloths of the yeast presses are washed, dried, and stored dry before reuse. It is particularly important to consider the possibility of infection by accessory equipment, such as leaky cooling coils, air pipes, pH electrodes or other instruments, small containers with which samples are removed from the fermenter, or condensate which may drip back into the fermenter from ducts for the effluent air.

Finally, general sanitation of the plant is important. Infection of the yeast may arise from mold growing on moist walls or in corners on moist floors, on equipment which has been cleaned in the wet state and has not been thoroughly dried, and from similar sources. Dead yeast represents an excellent growth medium and deposits of wet or dry yeast on equipment become a ready source of infection, if wetted.

In spite of the precautions mentioned above, compressed yeast contains considerable numbers of contaminating organisms. The word "contaminating" microbes does not mean that these organisms are actually or potentially harmful.

Until fairly recently it has been believed that the presence of some lactic acid-producing organisms in bakers' yeast was desired to give the bread improved flavor. Nevertheless, the presence of any microorganisms other than yeast is undesirable. Contaminants are generally organisms which multiply as rapidly as or more rapidly than yeast at relatively low pH values. At a pH between 5 and 6, that is, towards the end of the fermentation, these contaminants often multiply very much faster than yeast. Total bacterial counts of commercial samples of bakers' yeast may show values between 10^4 and 10^9 per g yeast. Carlin (1958) has reported values of 2 to 3 X 10^9 per g yeast for 3 U.S. brands of compressed yeast. The organisms always or almost always belong to groups of heterofermentative lactic acid-producing bacteria (genus *Leuconostoc*) or to homofermentative organisms of the genus *Lactobacillus.* Some coliform organisms, usually *Aerobacter aerogenes,* and occasionally *E. coli* are found. Active dry yeast contains basically the same kinds of bacteria, but the total count decreases on drying. There is a further decrease in total counts when ADY is stored. The fate of the above-mentioned microorganisms in doughs has been described by Carlin (1959). Rope spores (*B. subtilis*) occur in yeast, but flour is the more likely source of dough infection. According to specifications of the U.S. Army, the number of rope spores is limited to not more than 200 per g of active dry yeast.

Wild yeasts and molds also occur in commercial bakers' yeast, and actual mold growth can be seen on cakes of compressed yeast after 4 weeks of refrigerated storage. Wild yeasts, such as *Candida utilis* or *Rhodotorula* species, can be detected with media which contain lysine as the only source of nitrogen. "Culture" yeasts do not grow on such media and the method is used in the brewing industry for the detection of wild yeasts in beer. Von Lacroix (1963) has applied this method to the investigation of bakers' yeast. Čejková (1967) described the well-known use of actidione to suppress growth of yeast in media used for the identification of coliform bacteria.

Occasionally there are gross infections of bakers' yeast with *Oidium lactis* or *Monilia.* Goncharova *et al.* (1965) described a massive infection of bakers' yeast with other yeasts in the Leningrad area. They found that the following species grew faster than *S. cerevisiae* in mixed culture: *S. paradoxus, C. utilis, T. minor, C. krusei,* and *C. mycoderma.* In relatively small concentrations (5 to 10% of the cell population) the wild yeasts increased yield but decreased gassing activity and yeast stability. *S. paradoxus* has almost the same gassing activity as *S. cerevisiae* and its presence hardly affected total gassing activity. In Britain the following species have been reported as contaminants of bakers' compressed yeast: *C. krusei, C. mycoderma, C. tropicalis, Trichosporon cutaneum, Torulopsis candida* and *Rhodotorula mucilaginosa* (Fowell, 1965).

Fowell (1967) has reviewed the media and microbiological techniques required for the detection and estimation of various bacteria, yeasts, and molds in bakers' compressed yeast.

Stability of Compressed Yeast

Bakers' yeast in the U.S. requires refrigerated storage and transportation. At temperatures between 3 and 8°C there is a loss of about 5% of the initial activity within a week, and smaller losses thereafter. After 3 to 4 weeks the yeast becomes unusable because of mold development. Bakers' yeast with low nitrogen levels may be stored at higher temperatures or for longer time periods. There is a trend in Europe towards the use of yeasts with a higher nitrogen content and faster fermentation activity. Schulz (1970) recommends that yeasts with "normal" leavening activity be stored at less than 18°C and yeasts with rapid rising action at less than 4°C.

During storage of bakers' yeast at elevated temperature (35°C) the concentration of glycogen drops rapidly, and this is followed by a drop in trehalose. These two compounds are largely responsible for the endogenous metabolism of the yeast. Autolysis sets in when the level of trehalose has dropped to about 15% of its original value (Mitterhauszerová and Ginterová, 1969, 1971). Burrows (1970) also noted the immediate loss of carbohydrate on storage at 25°C. Fermentation activity did not decrease during storage for one day, but decreased sharply thereafter. The excretion of α-amino nitrogen remained almost linear for the first 4 days of storage.

The stability of compressed yeast in frozen storage is discussed in Chapter 6.

Active Dry Yeast

Introduction.—The production of active dry yeast (ADY) is of great practical and theoretical interest. It is the only microorganism which is dried on a large industrial scale with little or no loss of viability. The bake activity of ADY is lower than that of compressed yeast at equivalent solids values. It has only partly replaced compressed yeast in wholesale bakeries which can be readily served by distribution of refrigerated goods. It has largely replaced compressed yeast in home baking, and in all areas—foreign or domestic—where bakeries cannot be cheaply or conveniently served with compressed yeast.

ADY is generally produced with a strain typified by strain No. 7752 in the American Type Culture Collection. This strain gives better yields than that used in the production of compressed yeast, and it is sometimes called the "dry yeast strain." The strain is hardy but does not generally reach nitrogen levels above 8.5%. For the manufacture of ADY it is grown to a nitrogen content of 6.5 to 7.5%; therefore, its bake activity on an equivalent solids basis is lower than that of compressed yeast. There is some loss in solids due to respiration during the early phase of drying. Loss of bake activity on drying is minimal (0 to 5%) and the difference in bake activity between ADY and compressed yeast is largely due to the lower nitrogen content of ADY, as already mentioned.

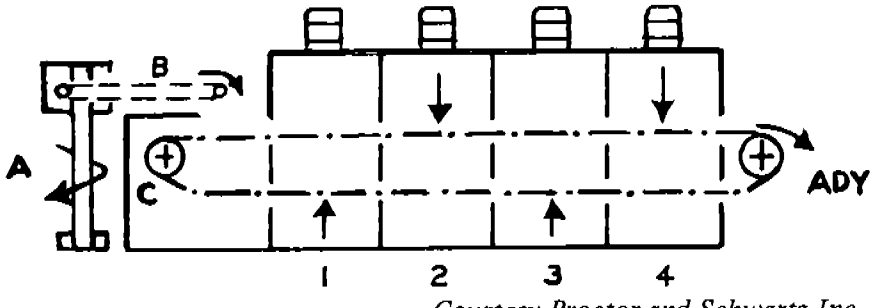
Experimentally, the strain normally used for the production of compressed yeast has been dried, for instance, by Johnston (1959) and the use of a new strain for the production of ADY has been reported (Lodder *et al.*, 1969). This latter strain can be dried satisfactorily at rather high levels of yeast nitrogen.

Drying Methods.—***Early Drying Methods.***—There have been many attempts to dry yeast by mixing it with edible materials which absorb water. For instance, Klein (1922) dried bakers' compressed yeast to a moisture level of 50 to 60% and then added starch to it; Jensen (1923) dried to a moisture level of 10 to 20% with the addition of calcium salts. In 1952 Buré and Matti suggested the addition of flour to bakers' compressed yeast before drying; and more recently it has been proposed to mix 1 kg bakers' compressed yeast with 5 kg low moisture starch or flour. This mixture was used without further drying, and good storage stability has been claimed for this yeast concentrate (Rupprecht and Popp, 1970).

Commercial Drying Processes.—The drying of yeast press cake can be carried out in many ways. For all these processes the yeast is usually pressed or filtered as in the production of compressed yeast. The press cake is extruded through a perforated metal plate. The diameter of the strands of yeast leaving the extruder is usually between 1.5 and 4.5 mm. The strands are often cut by a blade into 1 to 3-cm length before entering the drying equipment. The moisture content of the press cake is of little importance except insofar as it affects the ability of the strands to keep their proper shape and to support a bed of yeast strands. Actual drying may be carried out in equipment in which the yeast layer is quiescent or in which the yeast is tumbled during drying. In either case drying may be continuous or on a batch basis.

Continuous Tunnel (Chamber) Drying.—In this method the strands of yeast leaving the extruder are deposited on the wire screen of an endless belt. This belt carries the yeast bed, which may be several inches high, through several drying chambers. The direction of air flow through the yeast bed is changed from chamber to chamber to prevent overdrying or underdrying of the top and bottom layers of the yeast bed. A drying time of 2 to 4 hours and inlet air temperatures of 42°, 37°, 32° and 28°C respectively, for 4 drying chambers has been reported by Belokoñ (1962).

Fig. 5.11 shows the arrangement of such a dryer, which is generally used in the U.S. The drying time of the continuous process varies between 3 and 6 hours;



Courtesy Proctor and Schwartz Inc.

FIG. 5.11. TUNNEL DRIER (CONTINUOUS)

A—Oscillating feeder, B—Feeder belt, C—Endless wire mesh belt.
1, 2, 3, 4—Drying chambers.
Arrows indicate direction of air flow.

the temperature of the make-up air does not exceed 45°C, and the temperature of the yeast bed does not exceed 40°C. A schedule in which the air inlet temperature is 50°C at the beginning and 35°C at the end of the drying period has been reported by Chulina (1969). Humidity of the air is also often regulated either by drying the inlet air or by humidification. Drying rate depends not only on air temperature and relative humidity but also on the velocity of the air. This indicates that drying rate is controlled by diffusion of moisture vapor from the surface of the yeast particles (Otero de la Gandara and Marzo Rodrigo, 1964).

The ADY obtained by this process has the characteristic shape of "noodles" with a diameter of approximately 2 to 3 mm and a length varying from 3 to 6 mm. The noodles are uneven in shape and highly fissured (see Fig. 6.18).

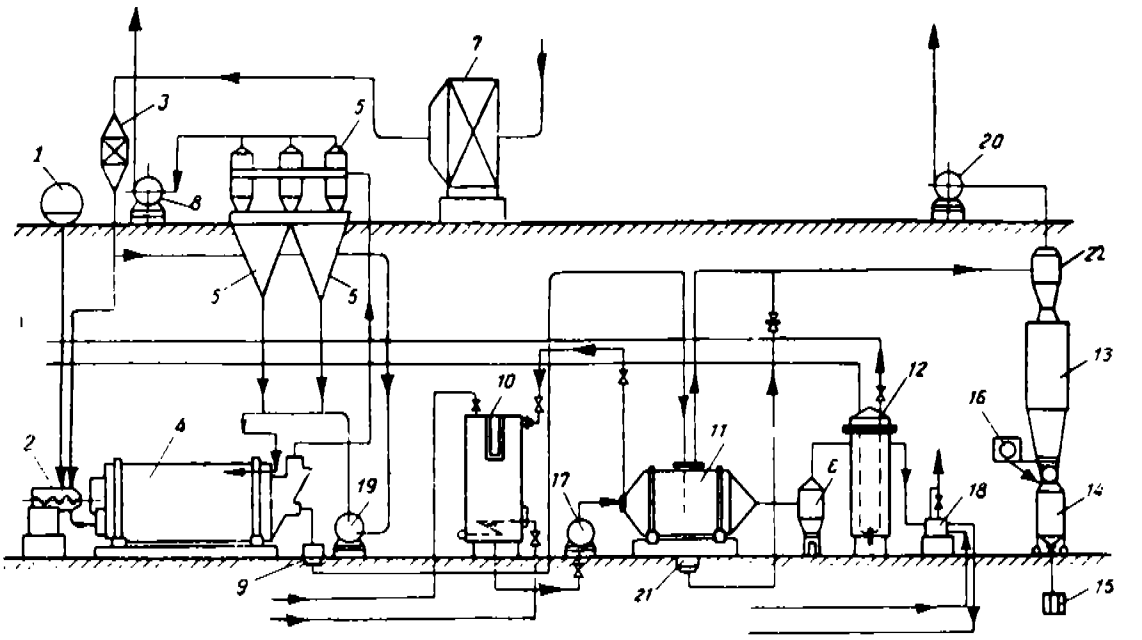
Batch-wise Tunnel (Chamber) Drying.—In this method yeast strands are layered on metal screens inserted into racks. The racks are mounted on a cart which can be pushed into a single drying chamber. Air is blown through the yeast bed and the temperature of the air, its humidity, and the direction of air flow may be changed from time to time. With this method one can obtain results identical to those obtained with the continuous dryer described above. Drying time and temperatures used are also similar to those used in the continuous process. This batch process can be used successfully where the total volume of production does not warrant the capital outlay required for a continuous installation.

Rotolouver Drying.—This is a batch process. The extruded yeast strands are fed into a large, hollow, metal cylinder equipped with baffles on the inside. The cylinder rotates slowly so that the yeast tumbles constantly during the drying period. Warm air (up to 60°C) is blown through louvers into the cylinder and through the tumbling yeast particles.

Total drying in such a system may be from 10 to 20 hr. The temperature of the yeast does not exceed 45°C. At the end of the drying period the yeast, which consists of small pellets of about 2 mm diameter, is conveyed out of the dryer. Fig. 5.12 shows a schematic diagram of such a dryer whose method of operation was as follows: length of cylinder 4.85 m; inner diameter 2.2 m; rotation of cylinder 4 rpm; length of time 13 to 15 hr; temperature of inlet air 50°C (Sysojewa *et al.*, 1965).

The quality of the ADY produced by this process is equal to that obtained by tunnel drying so far as baking activity is concerned. The yeast pellets are more stable on storage, probably because of the smaller surface area per weight of yeast and because of the smooth surface. Rehydration of the pellets takes a few minutes longer than that of noodle or ground noodle yeast. Pellet yeast does not lend itself readily to grinding.

Air-Lift (Fluid-bed) Dryers.—Extruded strands of yeast are fed into a single chamber for batch drying and deposited on a metal screen or perforated plate. Air is blown from the bottom through the yeast layer at velocities which suspend the yeast particles in a fluid bed. Drying time in air-lift dryers may be from 0.5 to 4 hr and the conditions of air temperature and humidity are similar to those



Sysojewa and Gorochowa (1965)

FIG. 5.12. ROTO LOUVER AND VACUUM DRIER FOR ACTIVE DRY YEAST

1. filter, 2. extruder, 3. filter, 4. rotary drier, 5. cyclone, 6. filter, 7. compressor-blower, 8. ventilator, 9. conveyor, 10. boiler, 11. vacuum drier, 12. heat exchanger, 13. collecting bin for *ADY*, 14. hopper, 15. scale, 16. grinder, 17. ventilator, 18. steam jet evacuator, 19. ventilator, 20. ventilator, 21. conveyor, 22. cyclone.

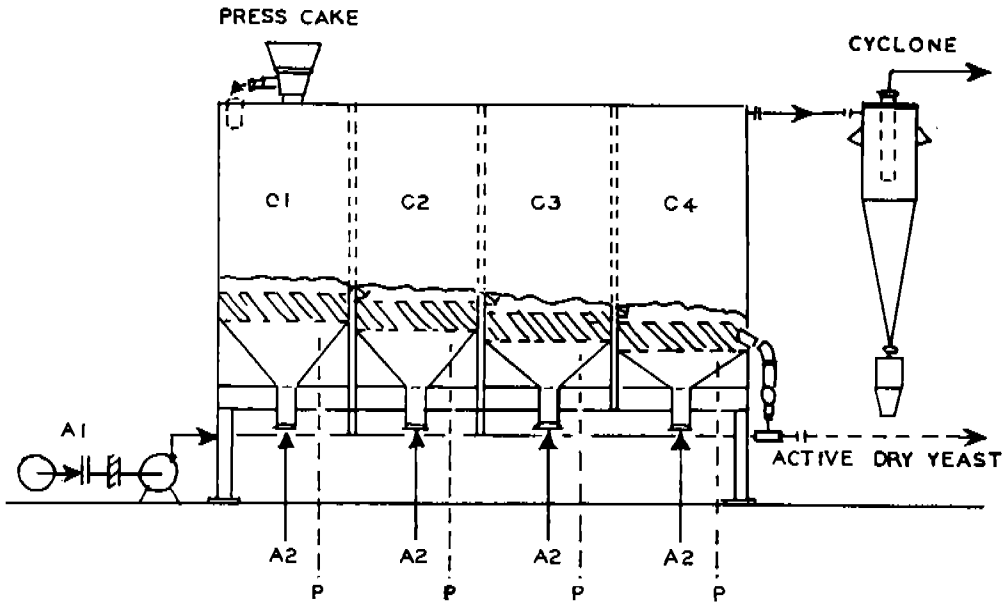
used in tunnel dryers. Appreciably shorter drying times can be achieved by the use of yeast strands of smaller diameter. In a small fluid bed dryer Otero de la Gandara and Marzo Rodrigo (1964) dried compressed yeast to 11.6% moisture in 40 min (air temp. 32°C; relative humidity 26%).

Continuous air-lift dryers are also available for commercial operation. Fig. 5.13 shows the schematic arrangement of such a dryer.

Other Dryers.—Spray-drying of a dispersion of compressed yeast (with the use of additives) has been suggested by Aizawa *et al.*, (1968). In general spray-drying processes have not been successful on a commercial scale. Vacuum-drying may be used following an initial rotolouver drying, as described by Sysojewa *et al.* (1965). Freeze-drying of yeast press cake results in a considerable loss in viability and yeast activity. It may be used in the preparation of yeast cultures (Bréchot and Croson, 1961; Atkin *et al.*, 1962).

A unique process which produces ADY of exceptional quality has been described by Johnston (1959). In this process yeast cream is dispersed evenly in a medium of warm, edible oil and a stream of warm air is passed through the suspension. The ADY recovered in this process contains considerable amounts of oil and must be extracted with oil solvents to yield a suitable end product. The process is probably expensive in operation.

Activity, Stability and Moisture Levels of ADY.—One may remove water from



Courtesy Pressindustria, Milan

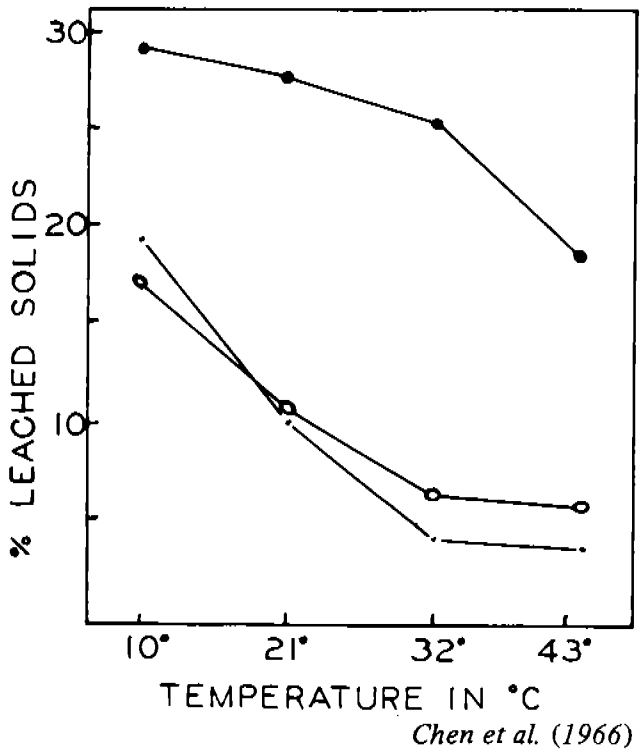
FIG. 5.13. CONTINUOUS FLUID BED DRIER

C1, C2, C3, C4—Drying chambers, A1—Air for pneumatic transport of *ADY*, A2—Air for yeast drying, P—Steam.

compressed yeast to a moisture level of 15 to 30% without impairment of its activity or viability. The production of such yeast concentrates has been suggested by Sumner *et al.* (1963) and Semikhatova *et al.* (1969). Such concentrates can be dispersed in cold water without impairment of their activity, in contrast to ADY of lower moisture content which requires rehydration in warm water. However, these yeast concentrates require refrigerated storage to avoid excessive respiration. This type of concentrate is not produced in Western countries but may be produced in the USSR.

As the moisture level of ADY is reduced below the 12 to 15% range there is a qualitative change in properties. Storage stability at ambient temperatures increases sharply, but the yeast does not exhibit good baking activity if it is rehydrated in cold water. If ADY with a moisture level of 7.5 to 8.3%, which is the common level for ADY, is rehydrated in cold water, about 25% of the soluble solids of the yeast leach into the rehydration water. Fig. 5.14 shows a curve relating temperature of the rehydration water to the concentration of leached solids.

ADY with moisture levels lower than 7.5% leaches more readily even if rehydrated with water of 40°C. Such leaching can be reduced by the use of emulsifiers to coat the yeast particles. It can be eliminated completely by water-vapor rehydration of the yeast. Indeed if one attempts to determine the viability of ADY of low moisture content, it is expedient to rehydrate it with water vapor to prevent damage during this step. But water-vapor rehydration is not a practical method for commercial use of ADY. At low moisture levels stability of



Chen et al. (1966)

FIG. 5.14. LEACHED SOLIDS OF *ADY* AS A FUNCTION OF REHYDRATION TEMPERATURE

·*ADY*, 8.1% moisture
•*ADY*, 5.3% moisture
○*ADY*, 4.0% moisture, with 2% sorbitan monostearate

ADY increases considerably, but so does damage on rehydration. The normal moisture level of 7.5 to 8.3% represents, therefore, a compromise between the demand for stability and bake activity.

Low-moisture ADY of excellent stability can be produced by addition of an emulsifier and an antioxidant. Suitable emulsifiers are sorbitan esters (Mitchell and Enright, 1957, 1959) and sucrose diesters (Pomper and Ackerman, 1968). The most valuable antioxidant is butylated hydroxyanisole. It may be incorporated into the yeast press cake (Pomper and Ackerman, 1969) or it may be emulsified with sorbitan esters and added to the yeast cream before pressing (Chen and Cooper, 1962; Chen *et al.*, 1966). A common level of these additives is 1% of sorbitan ester and 0.1% of butylated hydroxyanisole. Such yeasts are available in the trade as so-called protected ADY. They have better stability in air than regular ADY. Addition of antioxidant to ADY of a moisture level higher than 7.5% has no protective effect.

For use in baking ADY may be rehydrated in water of 40 to 45°C. After a 5-min rehydration period the yeast can be dispersed in the water without difficulty. At least 4 parts of water for each part of ADY must be used to obtain proper rehydration, but larger proportions of water may be used if desired. Stirring

during rehydration may lead to lumping of yeast particles and prevents proper rehydration unless agitation is very vigorous.

Fine-ground ADY may also be added directly to the flour and other dry ingredients, and rehydration proceeds when water is added to form a dough. The requirement for rather warm water restricts use of this process to relatively small doughs which do not heat up additionally during high-speed mixing.

ADY in mixture with flour increases in moisture content during storage. This limits the shelf life of such baking pre-mixes to about 4 weeks. However, with low-moisture flour (less than 10% moisture) and with the use of protected ADY a one-year shelf life of such complete baking pre-mixes can be achieved (Cooper *et al.*, 1966).

ADY of normal composition has good storage stability if it is kept at relatively low temperatures or if it is packed in vacuum or under nitrogen gas. For such gas packs the presence of 0.5% O_2 can be tolerated. Under such conditions ADY loses about 1% of its activity per month at ambient temperatures, or about 10% per year. On storage in air ADY loses about 7% of its activity during the first month and slightly less thereafter. For shipment to bakeries ADY is usually packed in drums without gas protection since it is generally used within a 1- to 3-month period. For consumer sales in 7-g envelopes, for institutional use in 2-lb cans, and for bakery use requiring a longer shelf life, the yeast is protected by an inert atmosphere or vacuum (Felsher *et al.*, 1955; Thorn and Reed, 1959).

The envelopes of consumer yeast consist of an inner layer of polyethylene or Pliofilm and an outer layer of aluminum foil. Such envelopes are flushed with nitrogen gas after filling, and heat-sealed. They provide a useful shelf life of 1 to 1½ yrs at ambient temperature. The reliability of the heat seal and the efficiency of flushing with nitrogen can be readily tested by micro-gas analysis (Dale, 1956).

Chemical Composition of ADY.—The regular ADY of commerce has a nitrogen level of 6.5 to 7.5%. At higher nitrogen levels one can achieve somewhat better bake activity, and at lower levels one can achieve better stability. Again, this figure represents a compromise between the demands for bake activity and stability. The phosphate content expressed as P_2O_5 is about one-third of the nitrogen concentration. Regular ADY has a moisture content of 7.5 to 8.5%; but the protected ADY mentioned above has moisture levels below 7.5%.

Biology of ADY.—Little is known about the factors causing loss of bake activity or viability during the drying of yeast or about the causes of leaching of yeast cell solubles during rehydration. Also, the particular mechanism by which atmospheric O_2 hastens yeast deterioration is not known. Chen and Peppler (1956) studied the destruction of cocarboxylase in ADY, and Harrison and Trevelyan (1963) studied phospholipid breakdown during drying.

The leaching phenomenon has been studied by Herrera *et al.* (1956) and by Kuninori and Sullivan (1968). While the effect of variables such as temperature of the rehydration water or the effect of yeast moisture can be ascertained, the

underlying mechanism remains unknown. Equally unexplained is the absorption of CO_2 by active dry yeast (Amsz *et al.*, 1956). Koga *et al.* (1966) have studied the nuclear magnetic resonance spectra of water in partially dried ADY in order to elucidate the kind of binding of water. They defined a "solution" region at moisture levels above 10%; a "gel" region between 7 and 10%; a "mobile absorption" region between 5 and 7%; and a region of "localized" water at moisture levels below 5%. An attempt to relate these regions to the physiological properties of ADY has been less than successful.

General References on the Production of Bakers' Yeast

Two books have been devoted entirely to the description of the production of bakers' yeast, Walter (1953) and White (1954). The latter has stood the test of time very well and remains the main reference work in the field. Extensive reviews are available, the latest one by Burrows (1970). Others are by Reid (1969), Rosen (1968), Harrison (1963), Butschek and Kautzmann (1962), Peppler (1960), Pyke (1958), and Nickerson and Schultz (1957).

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