

1976

Bioprotein From Banana Wastes.

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BIOPROTEIN FROM BANANA WASTES.

The Louisiana State University and Agricultural
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Home Economics

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BIOPROTEIN FROM BANANA WASTES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by

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May, 1976

ACKNOWLEDGMENTS

The author wishes to express her sincere gratitude and appreciation to Dr. Samuel P. Meyers, Professor, Department of Food Science, for his patient guidance, encouragement, understanding, suggestions and assistance throughout the course of this investigation.

She also wishes to thank Dr. Arthur F. Novak, Head, Department of Food Science, for providing the opportunity to develop this research program.

Gratitude is also expressed to Dr. W. H. James, Professor, Department of Food Science, Dr. J. A. Liuzzo, Professor, Department of Food Science, Dr. W. F. McKnight, Associate Professor, Department of Home Economics, and Dr. R. M. R. Rao, Professor, Department of Food Science, for their assistance in serving as members of the examining committee.

The author also wishes to thank her husband, Chin-Ki Chung, for his understanding and encouragement during the course of this study.

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ABSTRACT

Utilization of banana wastes for production of yeast protein by fermentation to provide a single-cell protein (SCP) for animal feed and potential human food use was studied. Possible large-scale application of the microbial process in banana-producing countries was discussed. Initially proximate composition of banana substrates (designated RPL [ripe banana pulp liquid] and RSL [ripe banana skin liquid]) and optimal growth conditions of Pichia spartinae, the major yeast species examined, were determined. Total sugars as hexoses, protein, ash and phosphate content were 72.8%, 2.6%, 4.3% and 0.184% for RPL, and 44.0%, 3.1%, 28.6% and 0.6% for RSL. Optimal pH for growth of P. spartinae was broad, between 4.0 and 7.0, with the most suitable temperature for maximal production of yeast biomass at 30-35°C before a 36-hr growth period, and 20-25°C after 36 hr. Vitamins and phosphate content of the banana medium appeared to be sufficient for growth of the yeast. The culture medium did not require additional nutrient supplementation except for a metabolizable nitrogen source. Ammonium sulfate was the most suitable nitrogen substrate of the inorganic nitrogen compounds tested, however, yeast extract was superior to ammonium sulfate as a nitrogenous substrate for P. spartinae development.

Maximal yields of yeast biomass under optimal condition from RPL and RSL were 53% and 58%. A non-sterile process of development was possible, since P. spartinae can grow under acid conditions, although

yields were low compared with those obtained from heat-treated media. Protein levels of the dried yeast cell mass were 33% in basal medium, 39% when supplemented with 0.2% yeast extract, and 47% when yeast extract was used as a sole nitrogen source. Amino acid analysis of the microbial protein revealed a high percentage of lysine with an essential amino acid pattern that compared favorably with FAO reference protein.

Electron-capture gas chromatographic studies indicated that ethyl-alcohol, sec-butanol, iso-butanol, n-butanol, iso-amyl alcohol, iso-butyric acid and palmitic acid were present in RPL medium, and sec-butanol, n-butanol, propionic acid and caprylic acid were present in RSL medium. While preliminary evaluation of quantitative aspects of ethanol production suggests that *P. spartinae* is not a noteworthy ethanol producer, further work is needed to evaluate nutritional and physical parameters that may significantly affect total ethanol production.

The study of production of protein-rich fermented bananas for animal feed using waste whole bananas indicated that protein content of waste whole banana could be increased approximately three times over the original banana wastes via the fermentation process. Further optimization of these protein yields appears feasible. Applications are suggested for possible use of protein-enriched fermented bananas as animal feedstuffs in protein-poor countries. Low-technology fermentation approaches are considered in terms of applications in banana-producing areas of the world.

INTRODUCTION

The world population is undergoing a rapid expansion with protein deficiency existing in about 60% of the populated areas of the world, particularly in tropical countries (Bhattacharjee, 1971a; Strasser et al., 1970; Wiken, 1972; Scott et al., 1972; Cooney et al., 1975). Numerous authorities project that it will become more and more difficult to supply the necessary protein by means of conventional agriculture (Strasser et al., 1970) in large part due to increasingly limited cultivated land which continues to be utilized to provide sites for housing, factories, markets, schools and roads. In view of the relevance of a significant increase in world population, undoubtedly greater food supplies, principally those of proteinaceous origin, will be needed in the near future, especially in third world countries. In such economically less-developed areas in which animal proteins may be consumed in meager amounts, especially where religious taboos are prevalent and vegetable proteins often are of poor quality, diseases manifested by protein deficiency are extremely common in children and adults. Therefore, it is essential to examine all possible protein sources, conventional as well as non-conventional, and to seek alternative proteins for both animal feed and human food applications. One of the more important new sources of protein for food and animal feed purposes is single-cell protein, commonly designated "SCP". The term SCP covers a variety of biomass of microbial origin produced by fermentation. Processes

yielding SCP may utilize either bacteria, yeast, fungi, or algae, which can utilize a variety of biodegradable substrates such as methane, alcohols, gas oil, carbohydrates, carbon dioxide, and a diversity of plant and animal waste products. Since several extensive reviews on substrates and processes for producing various SCP products have been published in recent years (Dabbah, 1970; Litchfield, 1968; Lipinsky and Litchfield, 1970; Reed and Peppler, 1973), only a few specific examples of recent developments will be cited. This recent acceleration in research activity on microbial protein is due in large part to the dramatic increase in the price of meat products and animal feed ingredients (Lipinsky and Litchfield, 1974), world-wide agricultural shortages in 1972, and a sharp rise in the cost of fishmeal used as animal feed protein supplement (Gorman, 1974). In the cheese-making industry, the sporogenous yeast, Saccharomyces fragilis, is being used to convert acid and sweet-cheese whey, a high-volume waste product, into yeast protein for an animal feed supplement. The active lactose-utilizing ability of the organism, coupled with supplementation with nitrogen compounds, produces a final protein product at yields of 45-55%. The common food yeast, Candida utilis, has been used in treatment of sauerkraut waste (highly acidic from the lactic acid fermentation process), whereby soluble and suspended solids are rapidly converted into cell biomass. Elsewhere, attention is being given to development of yeast together with protein enrichment of tropical plant products such as cassava, a major component of the diet prevalent in many developing countries. Other high-starch containing tropical plants also are being evaluated as substrates for microbial growth and subsequent production of proteinaceous cell crops. The Symba Yeast Process, designed for conversion of starch into yeast biomass (via

development of torula yeast in symbiosis with an amylase-producing yeast species), has been proposed as a means of economic utilization of waste starch present in effluents from processing of potatoes, corn, rice, etc.

Various advantages of SCP can be briefly summarized: 1) Microorganisms do not depend on agricultural or climatic conditions, but are cultured in large fermentation vessels, therefore, production of SCP is not limited by land surface or sunlight. 2) Microbial systems are a much more efficient producer of protein than are conventional sources (Appendix 1). 3) Proper biodegradation processes reduce industrial effluent disposal problems considerably, since almost all of the starting "waste" material is converted to protein which theoretically can be utilized as food for human or animal consumption (Tannenbaum, 1971). 4) Microorganisms require a comparatively very small growth area (Lipinsky and Litchfield, 1970), and do not necessitate seasonal processing schedules. 5) Microbiological processes permit reduction of environmental pollution since microorganisms utilize waste effluents and a diversity of by-products of industrial processes as growth substrates for bio-transformation (Cook et al., 1963; East et al., 1966; Tannenbaum, 1971). 6) Genetic experimentation for protein improvement of single cell systems can be readily undertaken.

Of the varied microorganisms studied, including bacteria, molds, algae and yeasts, the latter probably have the most desirable characteristics for use as a food substrate. Several factors favor use of yeasts for SCP production. Historically, these microorganisms have been used since ancient times in food processing, therefore, they are more readily acceptable as a food than less exploited organisms, such as bacteria or algae. Other important factors that favor selection of

for SCP production are their high protein levels reported (Appendix 2), the high content of the important B-vitamin group (Appendix 2), and their relatively favorable amino acid balance (Appendix 3). It should be noted that the nucleic acid content in yeast protein is high, causing an increase in uric acid levels of animals consuming large amounts of such material as food. However, recent development of enzymatic processes for reduction of nucleic acid content of yeast cells (Castro et al., 1971) have a direct bearing on minimizing of this problem. All of these considerations among others strengthen the position of yeast as an invaluable source of microbial protein.

Peppler (1967, 1968) described the commercial yeast product industry together with historical background information on yeast production. The total worldwide production of baker's yeast was estimated at 150,000 tons, and of dry yeast, 187,000 tons. Bunker (1966) estimated the worldwide production of food and feed yeast at 250,000 tons per year, 90% of which probably is traditionally used for animal feedstuffs. In the United States, the yeast industry comprises seven manufacturers operating at 16 locations. Fourteen factories (five companies) in 1968 produced about 55,800 metric tons of yeast dry matter as some form of baker's yeast. This represents nearly 65% of the total domestic production (Appendix 4). The remainder of the annual production comprises 18% food yeast, 13% feed yeast and 0.5% miscellaneous extracts and autolysates. Food yeast in the commercial market is represented basically by three species of the genus Saccharomyces and two species of Candida. Four of these yeasts are merchandised in the United States: Saccharomyces carlsbergensis, recovered from beer; S. cerevisiae, molasses-grown strains of baker's yeast; S. fragilis cultured on cheese whey; and

Candida utilis, propagated on spent sulphite liquor.

Yeast can be readily grown on a variety of different metabolizable carbon-containing substrates, including hydrocarbons, sugars, and starches derived from agricultural and domestic wastes. Among the major agricultural wastes, reject and surplus bananas are important commodities in tropical countries, along with molasses, bagasse, coffee pulp, etc. Surpluses of bananas have occurred largely because world production has expanded at a faster rate than actual consumption of the fresh fruit. Reject fruits originate from various sources including 1) harvesting and handling, 2) packing in areas of commercial banana production, and 3) "ripes and turnings" enroute to import countries. Bananas that are bruised and cut during harvesting and handling are left in dumps in the country of origin to rot. Fruits are rejected at the packing plants because of high standards required for the product in the consumer countries. Bananas that are too small or too large, slightly bruised, have spots or off-color, or are not in an optimal stage of maturity for shipment, are rejected for export, leading to considerable culling of the fruit. For example, nearly 200,000 bunches of bananas, at an average weight of 50 pounds each, were lost per week in Panama, increasing to 500,000 bunches of bananas per week in February, March and April (League for International Food Education, 1972). Thus, there is about 250,000 tons/yr of waste banana, of which only 20% is used for commercial banana products, leaving 200,000 tons/yr available as waste. A small portion (i.e., 1-2%) of this material is fed to livestock including pigs and cattle.

The majority of post-harvest losses in banana exports result from "ripes and turnings" on arrival of ships in port. Ripe fruits are

virtually a total loss; "turning" fruits (fruits beginning to show yellow color in the peel) are down-graded in value. Under carefully controlled conditions of culture, harvest and transport, an average of 0.5-3.0% of the boxes in a cargo will contain ripens and turnings; as much as 5-10% is not uncommon and, under less favorable conditions, as much as 20-50% is possible. It should be noted that a loss of 1% of an average cargo is not insignificant, representing a total volume of nearly 40,000 lb of bananas (Palmer, 1971).

Bananas are a major product of commerce in international trade. Total world exports of bananas were approximately 5.9 millions tons annually in 1969 and 1970, with 7 million tons available for export in 1971 (FAO, 1971). Less than one third of the bananas produced are exported from South and Central America and African countries, with approximately one third utilized for food or feed locally, and the remainder wasted (Watkins, 1967).

Since such large volumes of cull bananas are wasted, efforts have been made to produce various manufactured banana products. Such products from surplus bananas have been limited in variety and volume, being mainly dried "figs," canned or quick frozen purees, powder, flour, flakes, chips, canned slices, and jam (Appendix 5). As noted by Kay (1967) the tendency of the banana fruit to lose much of its flavor and to discolor has affected large-scale commercial development of banana products. Furthermore, unless the ripening process is carefully controlled, many banana varieties may develop an astringent off-flavor when processed. Although a portion of the surplus bananas is utilized for the manufactured products, banana skins still remain as a major waste item. Possible utilization of the latter product may have a two-fold

benefit: production of protein via microbial fermentation processes, and reduction of industrial waste disposal problems.

Bananas are high in sugars which comprise about 80% of the dried pulp, but low in protein, about 5% in dry weight. Therefore, the fruit could readily serve as an excellent microbial carbohydrate substrate, along with proper nitrogen sources, for metabolic processes. Various types of closely controlled culture systems are possible in which a specific microorganism, or a properly selected combination of metabolic types, is allowed to develop under defined conditions. The resulting metabolic products and by-products may be recovered and the cell biomass generated used as a source of protein, vitamins and other nutrients.

The purpose of the present study was to investigate the utilization of banana wastes, including rejects and surpluses of pulp and skins, by microbial conversion into food yeast or protein-enriched animal feed for ultimate application in third world countries, particularly in tropical areas where protein deficiency is extremely common and bananas are available in large quantities at very low costs.

The specific objectives of this investigation include:

- 1) Determination of the chemical composition of liquid component of bananas used as substrate for selected yeasts.
- 2) Determination of optimal conditions for growth of certain species of yeasts for maximal production of cell mass.
- 3) Evaluation of the potential nutritional value of the yeast protein by means of amino acid analysis and protein levels.
- 4) Production of protein-enriched animal feed substrate through low-level technology fermentation processes.

5) Analyses of the composition of aromatic compounds produced as a possible source of economically valuable by-products from the fermentation pathway.

REVIEW OF LITERATURE

The literature selected deals with world production and compositional changes during ripening of bananas, both of which are important to proper comprehension of the amount and quality of waste bananas available as a substrate for microbial conversion and to the comprehension of the overall composition of bananas for use as a substrate for selected yeasts at particular stages of fruit ripening. Pertinent reports noting relevant aspects associated with single-cell protein and its significance also are included.

I. BANANA

Introduction

One of the world's most important fruits, the banana is consumed extensively throughout the tropics, where it is grown, and in the temperate zone, where it is popular because of its flavor, its food value and its availability at all times of the year (Encyclopaedia Britannica, 1964).

The banana belongs to the genus Musa, comprising thirty-two or more distinct species and at least one hundred subspecies. The genus is divided into two broad sections: Eumusa, with edible fruits, and Physo-caulis, with inedible fruits. For commercial purposes, Eumusa is divided into bananas and plantains. The standard varieties of bananas handled in commercial trade belong to Musa sapientum (Gros Michel) and

M. cavendishii (Cavendish or Chinese banana). M. paradisica, the plantain or starchy, cooking banana, is used in the locality of its production and seldom enters into international trade.

The bananas of American commerce are largely those of the Gros Michel type and Cavendish mutants. Cavendish varieties (Valery) have recently replaced Gros Michel as the principal export type, mainly because Cavendish cultivars are resistant to the devastating Fusarium wilt disease known as Panama Disease.

Although bananas and plantains are grown largely for export and domestic human consumption, large quantities of fruit are wasted and used for animal feed. At the packing plants in areas of commercial banana production, fruit that are too small or too large, slightly bruised, have spots or off-color, or are not in an optimal stage of maturity for shipping, are rejected for export. These reject or waste bananas, along with smaller quantities of farm-produced bananas and plantains, constitute good sources of metabolizable carbohydrate substrate for microbial processes.

World Production and Trade of Bananas

Trade in bananas is fairly recent, for it was not until the second half of the nineteenth century that the first bananas appeared in the United States and Western Europe. Despite this rather short history in the industrial countries, it is believed that bananas were actually among the first fruits to be cultivated by mankind (Haarer, 1964).

Banana production has been growing extremely rapidly. The average world production was estimated by the Food and Agriculture Organization of the United Nations (FAO) (1971) at about 12.5 million metric

tons in 1948-1952, 21 million in 1961-1965, and 28 million in 1970. More recent data are not presently available. More detailed estimated figures are given in Appendix 6, although these figures, in all likelihood, are conservative. Most probably, the actual production of bananas is much larger. For example, it is extremely difficult to account for the total amount of bananas that farmers grow in small quantities for individual consumption. In addition, since the producing countries are mostly small and less technologically developed, their statistics could be misleading due to the limited financial efforts expended in collecting data on production. Nevertheless, the amount of bananas produced certainly would place bananas in first place among cultivated fruits (Valles, 1968).

Most bananas are consumed locally with only about 20% being transported or exported to distant markets. Bananas represent 40% by weight of all world trade in fruits, either fresh or dried. World exports in 1963 were about 4 million metric tons, with total value estimated at 1.1 billion U.S. dollars. This ranked bananas seventh in tonnage and ninth in value of all world agricultural and fish export crops, exclusive of forest items. Export tonnage increased to 5 million metric tons in 1965 and reached 7 million tons by 1970.

In 1965, 80% of the export bananas came from the Americas, 10% from Africa and the rest from Australasia. Fifty percent of this crop was imported by the United Kingdom and Western Europe, 40% by the United States and Canada. The remainder went to a few countries in South America, Africa and Australasia.

In production of bananas for the fresh fruit trade, considerable quantities of reject fruit are usually available, which are of

satisfactory eating quality but unsuitable for export, being undersized, misshapen, or disfigured by skin blemishes, etc. It is estimated that less than one third of the bananas produced are exported from South America, Central America and African countries, with approximately one third utilized for food or feed locally, and the remainder wasted (Watkins, 1967). Utilization of this reject fruit has been a great problem in most banana-producing countries for many years (Kay, 1967). Recent studies on possible utilization of waste bananas in the Panama animal feed industry have been reported by Cooney and Dunlap (unpublished data). A fermentation process was proposed for converting waste bananas to protein-rich cattle feed. The proposed process would consist essentially of chopping rejected bananas and feeding them to fodder yeast or baker's yeast in an aerobic fermentor. After fermentation, undigested material would be filtered out and the yeast dried and mixed with nutrient salts, vitamins, and a source of fiber, such as chopped banana leaves. The mixture would be pelletized for use. A prime outlet for the protein-rich pellets would be Panamanian cattle for domestic consumption. Currently such animals are raised on low-protein grasses and require four or five years to reach a market weight of 1000 lb.

Post-Harvest Physiology and Biochemistry

Introduction

In commercial practice bananas are never allowed to ripen on the tree, for the peel splits, rendering the fruit easy prey to insects and disease. Therefore, bananas are picked green and ripened under controlled temperature (14.5-21°C), humidity (90-95% relative humidity), and ventilation to prevent excessive accumulation of carbon dioxide.

Accumulation of carbon dioxide retards ripening of the fruit and also causes a reduction in its ascorbic acid content. Commercially, ethylene (about 1000 ppm) is used for producing uniformity in ripening and to accelerate slow ripening fruit. The treatment of bananas with ethylene will accelerate hydrolysis of starch and concurrently increase respiration processes.

Compositional Changes During Ripening

1. Carbohydrate

When harvested, the banana is green and hard with its carbohydrate content, almost entirely starch, constituting approximately 21% of the pulp. During the ripening process, the starch of the green banana is converted into a variety of sugars. The total sugar content of the fresh pulp ranges from 1-2% in the green banana, to approximately 15-20% in the fully ripe fruit. Starch is converted concurrently, i.e., from about 20% in the green fruit to 1-2% in the pulp of the ripe banana. Total carbohydrate decreases 2-5% during ripening, presumably as sugars are utilized in respiration.

The green peel contains about 3% starch, localized mostly in cells adjacent to the pulp. This starch also is hydrolyzed during ripening, with concomitant accumulation of sugars.

Three sugars, glucose, sucrose and fructose, have been identified as the major sugars in banana pulp (Poland et al., 1937). These three compounds all increased during ripening, maintaining nearly constant proportions of 66% sucrose, 14% fructose and 20% glucose (Poland et al., 1938). There is some disagreement in the literature on the proportions of sugars in the ripe banana. Barnell (1940), in his extensive studies

of carbohydrate metabolism, found that reducing sugars (glucose and fructose) exceeded amounts of sucrose at ripeness, whereas several other investigators (e.g., Gane, 1936; Poland et al., 1938) noted that sucrose was the predominant sugar. All workers seem to agree that glucose slightly exceeds fructose. The higher content of glucose is probably due to the formation of the trisaccharide, 6G- β -fructosyl sucrose, by condensation of sucrose with one of the two products of its conversion. Maltose was the only other sugar found, being present in trace amounts in ripe Gros Michel.

2. Pigments

The skin or peel serves, more or less, as a color index of the ripeness of the fruit. When the peel turns yellow, except for a green tip, the pulp begins to soften and about two-thirds of the starch content has changed to sugars. In the next stage, "yellow ripe," when all trace of green has disappeared, the largest part of the starch has become sugar. At the fully ripe stage, when the skin is flecked or spotted with brown, practically all the starch has been converted into fruit sugars, the flavor has developed to its highest delicacy, and the pulp is most digestible.

Loesecke (1950) presented a color chart of the typical color changes and summarized information on the chemistry of the color changes. The green banana peel contains about 50-100 $\mu\text{g/g}$ fr. wt. chlorophyll, 5-7 $\mu\text{g/g}$ fr. wt. xanthophyll and 1.5-3.5 $\mu\text{g/g}$ fr. wt. carotene. During ripening all the chlorophyll disappears, and the total yellow pigment remains approximately constant.

3. Hemicelluloses, Cellulose and Pectic Substances

The hemicelluloses constitute an appreciable fraction of the dry matter of the unripe fruit, and, according to Barnell (1943), represent a significant unstable resource of carbohydrate. Their concentration decreases during ripening from 7-8% of the fresh pulp in the green fruit to about 1% at ripeness. The disappearance of hemicellulose at this time is probably due to hydrolysis.

Barnell (1943) found that the peel of the fruit contained much less hemicellulose than the pulp, and the decrease in absolute amount of hemicellulose during ripening was relatively small. No appreciable differences could be found between fruits stored for long and short periods.

Cellulose in the pulp is more or less constant at around 1-2% and decreases slightly during ripening.

The interconversion of pectic substances, i.e., protopectin to pectin, pectin to pectic acid, is presumed to be involved in the characteristic softening which occurs during ripening. In the pulp of bananas, insoluble protopectin decreases from about 0.5 to about 0.3% fresh weight and soluble pectin shows a corresponding increase during ripening (Loesecke, 1950).

4. Water Relations

Despite transpirational losses, the moisture content of banana pulp normally increases during ripening, from about 69% ($\pm 4\%$) to about 74% ($\pm 3\%$). The water from breakdown of carbohydrates, presumably during respiration, contributes in part to this net increase. Probably a more significant factor is the osmotic withdrawal of moisture from the peel. A marked difference in osmotic pressure between peel and pulp develops

during ripening, largely because sugar content increases more rapidly in the pulp than in the peel (Stratton and Loesecke, 1931).

5. The pH Value

The pH of the fruit varies from 5.0 to 5.6 in green bananas, to from 4.2 to 4.7 in the ripe fruit, although these limits vary (Harris and Poland, 1937). Barnell (1941) showed that in general the pulp was slightly more acid than the peel during ripening. The principal non-volatile acid of the fruit is malic. According to Harris and Poland (1937), this acid occurs as free acid in the ripe fruit. During ripening, the l-malic acid reaches a peak, then gradually decreases as the banana matures. Titratable acidity is a good measure of l-malic acid at any time after the peel is more yellow than green. In ripe bananas, titratable acidity and total malic acid are practically similar; malic acid is almost the only acid present and is entirely in a free state in this fruit (Harris and Poland, 1937). Wyman and Palmer (1963) reported that oxalic acid exceeded malic and citric in the green fruit but decreased at ripening so that malic became the principal component.

6. Lipids

Ether-extractable materials constitute between 0.2 and 0.5% of the fresh weight of bananas at all stages of ripeness (Loesecke, 1950). Palmitic, oleic and linolenic were identified as the major fatty acids in both peel and pulp by Grobois and Mazliak (1964). They reported an increased proportion of fatty acids in the peel and a decreased proportion of unsaturated acids, especially palmitoleic, in the pulp during ripening. The extent of these changes was not reported.

Total lipids showed no significant change during ripening,

averaging about 1% of the dry weight of the pulp and 6.5% of the peel. Data on fatty acid composition of the lipid fraction, determined by gas chromatography of the derived methyl esters, are given in Appendix 7. Palmitic (16:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) were the major acids of the pulp. In the peel, palmitic, linoleic and linolenic acids predominated. The peel acids changed only slightly during ripening.

7. Proteins and Amino Acids

Protein levels in the ripe fruit are relatively low, being between 0.5 and 1.5%. Sacher (1967) stated that a net increase in protein nitrogen apparently does not occur in the banana fruit during ripening. Similarly, a noteworthy change in nitrogen content of the ripening fruit has not been reported.

Investigation of nitrogen content of various parts of banana plants has been described by Steward et al. (1960a). Protein nitrogen makes up 60-65% of the total nitrogen (Steward et al., 1960b; Brady et al., 1970b). Total nitrogen remains constant in both peel and pulp during ripening. Nineteen free amino acids occur in various concentrations in different parts of the plant. The only outstanding feature is the exceptionally high content of histidine in the fruit pulp but not in the skin.

Other nitrogenous compounds contained in bananas are dopamine, serotonin (5-hydroxytryptamine), norepinephrine and tyramine (Waalkes et al., 1958; West, 1958; Marshall, 1959; Udenfriend et al., 1959). In the pulp, serotonin averaged 28 $\mu\text{g/g}$ fr. wt., norepinephrine about 2 $\mu\text{g/g}$ fr. wt., dopamine about 8 $\mu\text{g/g}$ fr. wt. and tyramine about 7 $\mu\text{g/g}$ fr. wt.

The peel concentrations were much higher: 65 $\mu\text{g/g}$ fr. wt. for serotonin, 122 for norepinephrine, 700 for dopamine and 65 for tyramine. The serotonin content of the peel increased sharply during ripening, from 74 to 161 $\mu\text{g/g}$ fr. wt. in the outer peel and 13 to 170 $\mu\text{g/g}$ fr. wt. in the inner peel. The serotonin content of the pulp increased about 50% during ripening, from 24 to 36 $\mu\text{g/g}$ fr. wt. There are, however, some variations of the value among workers. Vettorazzi (1974) examined the levels of 5-hydroxytryptamine in unprocessed bananas and in a number of banana-containing food products and found the amount of 5-hydroxytryptamine in the pulp (unprocessed bananas) decreased during maturation while that in the peel increased. The variation is probably due to the different analytical methods used and to species differences. Dopamine is the principal substrate of banana polyphenol oxidase and thus is responsible for the blackening of damaged or infected fruit (Griffith, 1959).

8. Ash and Vitamins

Apparently there are no marked differences in the total ash content during ripening, or between different varieties (Stratton and Von Loesecke, 1930). The more common constituents of the ash in bananas are silica (SiO_2), sulfur (SO_3), calcium (CaO), magnesium (MgO), iron (Fe_2O_3), phosphorus (P_2O_5), chlorides (Cl), potassium (K_2O) and sodium (Na_2O). The fruit also contain zinc, iodine, copper and aluminum in small amounts.

Few studies of vitamins in bananas have been reported except to tabulate data on vitamin content (McCance and Widdowson, 1960). Thornton (1938) reported that ascorbic acid is quickly destroyed when banana pulp is exposed to air.

9. Volatile Constituents

The banana fruit contains at least 200 individual volatile components (Wick et al., 1969). Although identification of these substances has proceeded quite rapidly, many remain unidentified and little is known about the contribution of individual volatiles to the characteristic banana flavor and aroma.

The main component of the scent of the ripe fruit is amyl acetate; amyl butyrate, acetaldehyde, ethanol and methanol have also been identified. McCarthy et al. (1963) reported that the major volatile components could be classified according to three general sensory impressions, banana-like, fruity and green, woody or musty (Appendix 8).

Ripening is accompanied by hydrolysis of starch to sugars and by a net loss of hemicelluloses. Acidity declines as the fruit ripens as also do the tannins which are responsible apparently for the astringency of the green fruit.

II. SINGLE-CELL PROTEIN (SCP)

The following literature review includes a discussion of the utilization of waste substrate for production of SCP, yield of microbial protein from carbohydrates (based on sugars), and factors affecting SCP processes. Pertinent reports are given dealing with the nutritive value of yeast protein in particular, and problems in the use of the protein important to their actual application in animal feed and as possible sources of human food.

Production of SCP from Waste Carbohydrate

Microbiologists have considered the possibilities of utilizing specific municipal, agricultural and industrial wastes and discharge effluents, particularly those containing significant amounts of carbohydrates, by growing microorganisms (including bacteria, molds, and yeasts) on these materials as metabolizable substrates, with or without addition of supplemental nutrients. In this way, waste disposal would become more feasible from an economic point of view by combining it with a more or less profitable process for production of a utilizable microbial biomass. The latter may be used as human food, as fodder yeast for animals, or as a food or feed supplement, either as such or after appropriate autolysis.

Particular attention has been directed to yeasts which will utilize waste carbohydrate materials, i.e., molasses (Peppler, 1968), sulfite waste liquor (Peppler, 1968), citrus-waste press juice (Nolte et al., 1942), cheese whey (Bernstein and Everson, 1974; Vananuvat and Kinsella, 1975), and sauerkraut waste (Hang et al., 1973).

For instance, beet molasses and cane molasses (blackstrap) supply fermentable sugar, essential minerals (phosphorus, potassium, iron, magnesium, copper, zinc), amino nitrogen (asparagine, aspartic acid, alanine, glutamic acid, glycine), and vitamins (principally biotin, pyridoxine, thiamine, pantothenic acid, and inositol). These nutrients vary in concentration according to the specific type of molasses, its geographical origin, agricultural crop practices, and sugar mill operations. The yeast S. cerevisiae has been used for fermentation purposes along with addition of ammonia and phosphate into the molasses reaction medium. Production of 100 lb of dry yeast, containing about 50% protein

(N x 6.25), would require about 400 lb molasses, 25 lb aqueous ammonia, 15 lb ammonium sulfate, and 7 lb monoammonium phosphate.

In the paper industry using the sulfite process, the yeast, Candida utilis, is being used to convert the waste liquor, which consists of pentoses and hexoses, into yeast protein. The active pentose-utilizing ability of the organism, with addition of potassium chloride and phosphate salts, yields a cell biomass containing 50-55% protein. Nolte et al. (1942) also used C. utilis for the production of feed yeast from the press juice of cannery waste dehydration plants. An average yield of 46% of dry yeast, based on the total content of the press juice, was obtained. In recent years, several workers (Vananuvat and Kinsella, 1975; Bernstein and Everson, 1974) have tried to utilize whey, a high volume waste product of the cheese-making industry, as a substrate for conversion into high quality protein animal feed supplement by using the yeast, Saccharomyces fragilis. The active lactose-utilizing ability of the organism, with addition of nitrogen compounds, yields approximately 45-55% of cell mass. The crude protein content ranged from 25 to 40%. The food yeast, Candida utilis, has been used in treatment of sauerkraut waste (highly acidic from the lactic acid fermentation process), whereby soluble and suspended solids are rapidly converted into cell biomass. Wiken (1972) illustrated the application of yeasts (the Symba Process) for purification of agricultural and industrial wastes, and for utilization of tapioca, etc., with simultaneous production of a microbial biomass of potential economic interests. The Symba process uses an amylase-producing species, Endomycopsis fibuligera, in combination with Candida utilis. The latter organism is unable to attack the starch directly, or waste materials which contain starch. This process has been proposed as

a means of economic utilization of waste starch and other carbohydrates in effluents from processing of potatoes, corn, rice, etc.

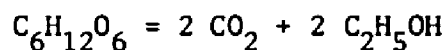
With waste materials such as sulfite liquor or vegetable processing waste and cheese whey, continuing availability of substrate throughout the year is an important consideration. At the present time, sulfite waste liquor is becoming less available, as paper mills in this country convert from the sulfite process to a new process, the kraft process (lipinsky and Litchfield, 1970). Cheese whey is produced in very large volumes, but individual cheese factories are in relatively separate and isolated locations and the volume of whey available at any one site would probably be insufficient to allow establishment of a yeast plant for utilizing this whey. The cost of collecting the whey and concentrating it at a central point in most cases would be prohibitive. This same consideration may be applied to many other agricultural and food processing wastes.

Yield of SCP from Carbohydrates

Carbohydrates in general are among the most abundant sources of carbon compounds available for microbial conversion into proteinaceous biomass. According to Worgan (1973), if the primary aim of high nutritional quality for human consumption, then the following aspects are relevant to the biological efficiency of the process: 1) the yield of protein per unit weight of carbohydrate, 2) the rate of conversion to protein, 3) the nutritional quality of the biomass as a source of protein in the human food chain.

Calculations of the maximum yield of yeast from sugar are based on fermentation of sugar to alcohol. Under anaerobic conditions, sugar

is converted almost quantitatively to alcohol and carbon dioxide, with a negligible increase in yeast cell mass. When oxygen is introduced



into the system the alcohol is almost completely utilized for yeast cell growth. The assumption is made that two carbon atoms are used to form CO_2 and four carbon atoms are used for yeast cell synthesis. Therefore, from 180 g glucose, 48 g of carbon are available for cell synthesis. Since the average yeast cell contains approximately 47% carbon, the yield of cells is 102.1 g, or 56.7% of the weight of glucose used (White, 1954).

It has been proposed that the biological energy required for cell synthesis is similar for all microorganisms. Experimentally, the amount of energy has been determined as 1 mol ATP per 10.5 g cell mass (Senez, 1962). Assuming that during growth under aerobic conditions all of the energy required for synthesis is derived from complete metabolism of the substrate to CO_2 and water, the maximum yield of biomass of any microorganism from a given substrate may be calculated from equation (1).

$$\text{Maximum yield of microbial bio-} \\ \text{mass from 1 mole substrate (Y}_m\text{)} = \frac{10.5 \text{ M} \times \text{C}_s}{\text{M/E} \times \text{C}_s + 10.5 \text{ C}_m} \dots\dots\dots (1).$$

where M = molecular weight of substrate

C_s = carbon content (percent) of substrate

C_m = carbon content (percent) of microbial biomass

E = number of moles of ATP produced by the aerobic metabolism of 1 mole of substrate.

The maximum yield of S. cerevisiae from glucose will be 61.7% by applying equation (1) (calculation shown in Appendix 9). Since the carbon content

of the biomass may vary from one microorganism to another, the maximum yield of microbial cell mass from glucose would also be different.

Yields of various yeasts on different carbohydrate substrate are noted in Appendix 10. These yields vary, based on the sugars in the substrate, from 23.2% for S. cerevisiae grown on an industrial scale, to 61.1% for Rhodotorula gracilis, grown in a laboratory scale fermentor on glucose.

Yields of microbial biomass from most carbohydrates will be the same as those calculated from glucose since hydrolysis of polysaccharides to monosaccharides produces comparatively little biological energy. An estimate of the maximum possible yield of protein from carbohydrates can be made assuming the maximum true protein contents of yeasts and fungi are 50%, and of bacterial cells 70%, and the yield of yeast from carbohydrate is assumed to be 50%. Thus, the maximum yields of true protein from carbohydrate substrates will probably be $50 \times 50/100 = 25\%$ for yeasts and fungi, and for bacteria, $50 \times 70/100 = 35\%$.

Factors Affecting SCP Process

Many factors govern the choice of a process for single cell production, including economic, technological, nutritional, sociological, and food technology aspects. Each of these has been dealt with in some depth elsewhere (Mateles and Tannenbaum, 1968a,b) and are briefly summarized in Appendix 11.

The major problems are substrate cost and availability, processing costs associated with maintaining sterility, cooling and harvesting, and marketing the product at prices competitive with existing plant and animal proteins.

Nutritive Value of SCP

In order for a protein to be most useful for nutritional purposes, not only must all the essential amino acids be present, but they should be present in a certain ratio. If the latter is not correct, the biological value of the protein will be lowered. The ratio generally accepted as more or less ideal is that published by the FAO and reproduced in part in Appendix 3. In yeast protein all of the essential amino acids, except methionine and cystine, are present in adequate quantities (Carter and Phillips, 1944). The lysine content of SCP in general is adequate, and in some cases is extremely good. This is in contrast to many plant proteins and indicates the possibility of supplementing lysine-deficient proteins with SCP.

Protein content is variable, depending not only on species variations but also greatly influenced by cultural conditions. For instance, if microorganisms are grown under nitrogen-limited conditions in the presence of excess carbon substrate, they will tend to deposit energy reserves in the form of glycogen or poly- β -hydroxybutyric acid in the case of bacteria, and fats and other lipids in the case of yeasts and molds. The protein content per unit total mass, therefore, is diminished. This can be readily be avoided by cultivation of the organisms under conditions in which the growth is limited by the energy source. Subject to these variations among species and cultural conditions, yeasts generally will have crude protein contents between 45-55%; bacteria, 50-80%; fungi (molds), 15-45%; and algae, 20-80%. Bacteria thus appear to be relatively rich in protein, whereas molds are distinctly inferior, and algae are variable. These crude protein contents usually are calculated by multiplying total nitrogen by the factor 6.25, assuming that the average

protein contains 16% nitrogen. However, several studies and reviews (Stokes, 1958; Farrer, 1956; Lindan and Work, 1951; Carter and Phillips, 1944) have indicated that 15 to 20% of the total nitrogen can be attributed to purines, pyrimidines, choline, glucosamine, and other non-protein constituents. Therefore, only about 80% of the total nitrogen of the yeast cell is in the form of actual protein. The maximum proportion of true protein in yeast cells is estimated to be 40 to 50%.

Certain yeasts, such as species of Candida, serve as a good source of riboflavin and pantothenic acid. High production of lipids is possible with species such as Trichosporon pullulans, Candida utilis, and Saccharomyces cerevisiae. Rhodotorula gracilis is, however, the most promising microbial lipid producer (Bhattacharjee, 1971b).

Feeding experiments by Bresani (1968) showed that rats could not be maintained on a diet in which yeast was the sole source of protein due to deficiency of the yeast in sulfur-containing amino acids, i.e., methionine, cystine. When food yeast was supplemented with methionine, its repletion value, as measured in protein-depleted adult rats, was equivalent to casein. This suggests that food yeast, under proper application conditions, could be used as a source of protein of high quality (Harris, et al., 1951; Dabbah, 1970). Scrimshaw et al. (1962) fed rats on INCAP Vegetable Mixture 9 diluted with starch to a 10% protein level. They noted that when 3% Candida yeast was added to the INCAP Mixture, growth and feed utilization of the rats were increased. In general, microbial proteins are low in methionine. Supplementation with this amino acid is necessary to achieve protein efficiency ratios (PER) or biological values (BV) equivalent to those of animal protein sources, i.e., casein (PER 2.5, BV 72-75) and whole egg (PER 3.0, BV 97-100). Kosaric

(1973) observed that feeding experiments with rats showed that yeast was readily digested and absorbed and that these nutrients could provide up to 94% caloric value in the diets. Feeding experiments with dogs and men showed utilizations of 80 and 90%, respectively.

Problems in the Use of SCP

The United Nations Protein Advisory Group has set forth detailed guidelines regarding safety standards and procedures for SCP products (Gounelle de Pontanel, 1972). Key problem areas in the use of SCP products in food at present are: 1) potential danger of kidney stone formation or the development of gout, 2) adverse gastrointestinal reactions, 3) possible adverse skin reactions, and 4) possible presence of carcinogenic compounds.

A major limitation in use of yeasts as food sources has been their high nucleic acid content. Uric acid, the end product of the purine portion of nucleic acid catabolism, is only slightly soluble at the pH of body fluids. There is some risk that salts may be deposited in the renal tract and possibly other tissues (e.g., joints) resulting in kidney stones, gallstones, and gout if the diet contains excessive purines (Waslien et al., 1970). Consequently, with many microorganisms, extraction of nucleic acids is a prerequisite in their production for human food purposes.

Yeast SCP may contain as much as 6-11% (dry weight basis) of nucleic acids (a source of purines) that can cause elevations of serum uric acid levels and increased urinary excretion if this product is fed to humans at dietary levels above 20 gper cay (Edozien et al., 1970; Waslien et al., 1970; Scrimshaw, 1972). However, at present, it appears

possible to remove enough of the RNA via processing of cells to eliminate any of the aforementioned hindrances to utilization of the protein (Maul et al., 1970). By subjecting cells to a heat shock, followed by successive periods of incubation at 50 and 60°C, the nucleic acid content has been reduced to 1-2%. The heat processing does not result in loss of protein from cells.

Another problem in the use of SCP is its acceptability. This is caused generally by lack of information, conservatism in nutrition in general, and lack of long-term human nutritional, biological and toxicological studies on effects of such new food products (Kosaric, 1973).

Applications of SCP

Some varieties of SCP already are being sold for animal feed in small amounts, and protein supplements for human beings are expected on the market in the next few years (Gorman, 1974). Yeast grown on ethanol has been offered as a protein extender by Amoco Foods Company in the United States. New Torula Yeast (also called P-10 Torula yeast, Amoco's Torula yeast or Torutein), manufactured by Amoco Foods Company, is produced from ethyl alcohol derived from refined petroleum. The product has been approved by USDA for use in soups, stews, meat patties, and non-specific loaves.

In general, food yeasts are used as a protein supplement and as flavorant and nutrient for human food and animal feed. The most widespread use of food yeast is in its undoubted complementary flavoring effect with meat and savory types of flavor. The nutritional aspects are usually incidental in this type of application. It is thought that the main flavoring compounds in yeast are the amino acids. Some have

distinctive flavors of their own and others act as flavor intensifiers. For instance, glutamic acid, whose sodium salt is now widely used in foodstuffs, is naturally occurring in yeast.

Yeast extracts provide the base meat stock flavor in many new and existing products; soups and gravies benefit greatly by use of yeast extract which is stable under heat processing conditions, and therefore provides a permanent, stable meaty flavor.

In processing of meat goods, especially those incorporating cereal fillers, for example, meat loaf, sausages, hamburgers, etc., yeast extract is used to give body and to supplement the meat flavor. Other uses of yeast extract and dried yeasts are to impart savory flavors to pastry and baked goods, such as biscuits, and cheese products, such as cheese spreads.

For enrichment of breakfast cereals and canned baby foods, food yeast levels of 0.5-2% are commonly used. Supplements of 1-3% food yeast are acceptable in many foods and prepared dishes: biscuits, muffins, crackers, chocolate cake, baked macaroni, meat dishes, peanut butter (up to 20% dried yeast), desserts and puddings (Sure, 1946). Doughnuts of satisfactory palatability can be made with dried yeast replacing 10% of the flour (McCay, 1950). Syrups used on pancakes and waffles, cookies, soups, gravies, confections, salad dressings, snacks, sausages, specialty products and pet foods are excellent vehicles for incorporation of the flavor and nutrient qualities of different food yeasts (Lyal, 1964). INCAPARINA, the cereal mixture used for the people of Central America, is fortified with 3% food yeast (Behar, 1963). Enriched macaroni products and enriched noodle products may be fortified with food yeast to supply all or part of the vitamin and mineral requirements.

Yeast has been used as fodder successfully for certain animals such as horses and cows, as feed for poultry and as a protein supplement in pet food in the United States. When fed to cows, yeast will increase the production of high quality milk (Carter and Phillips, 1944). Pigs also have been noted to utilize yeast satisfactorily. In principle, SCP could be used in all of the major animal feed applications including poultry, swine, and cattle feeding and fish farming.

At present it appears that most of the estimated one million tons of SCP estimated to be available annually by 1978 (Anonymous, 1974) will be used in animal feeds, and only small amounts of this microbial protein will be used for an ingredient of food for human consumption.

MATERIALS AND METHODS

Source and Preparation of Substrates

Banana fruits of M. cavendishii (Valery-Chiquita Brand) species were purchased from a wholesale distributor (Capital Tomato Company, Baton Rouge, La.) or local supermarkets, and were used throughout these studies.

The ripe bananas treated by a standard commercial ripening method (three successive applications of ethylene gas, concentration of approximately one cubic foot to every 1000 cubic feet of air, with adjustment of temperature, 15-19°C, and humidity, 90-95% of relative humidity) were used for this study. The color of the peel, which serves as a rough guide to the stage of ripeness, was yellow or yellow flecked with scattered brown spots.

The chemical composition of bananas is correlated with the particular stage of maturity. The most conspicuous change during maturation is conversion of starch to simple sugars. The sugar content depends upon the ripeness of the fruit, and changes during storage. Therefore, it is necessary to maintain this same stage of ripeness or to obtain a constant composition of substrate throughout the experiments. To accomplish this, volumes of bananas were mixed thoroughly in a blender immediately after purchase and freeze-dried.

Following is the general process for preparation of banana substrates: After peeling, the banana pulp (the edible portion of the banana) and banana peels were chopped and blended separately with 0.8%

NaCl solution, the latter to maintain osmotic integrity of cells. The ratio of pulp to solution was 4:1 and that of skin to the solution 1:1. The blended bananas were processed for 15 min in a refrigerated centrifuge (Sorvall, Model RC2-B) at 10,000 rpm for separation of liquid and solid portions. The resulting designated substrates were freeze-dried, stored in sealed bottles at room temperature, and used when needed as substrates for yeast growth. Sample code is as follows and is used throughout the study:

RPL--ripe banana pulp liquid
RPS--ripe banana pulp solid
RSL--ripe banana skin liquid
RSS--ripe banana skin solid

In the present investigation, only the liquid portion of banana pulp (RPL) and skin (RSL) were used as substrates.

In some experiments, fresh blended bananas (liquid portion of both skin and pulp) and fresh blended whole bananas, including skin and pulp, were used without freeze-drying treatment.

Yeasts

Yeasts were maintained on M-12 agar slants (2.0% diamalt, 0.5% peptone, 0.3% yeast extract and 1.7% agar) at 15°C. Transfers were made monthly and "purity" determinations, on M-12 agar, were conducted throughout the study.

Pichia spartinae was the major yeast species used. This organism was one of the predominant yeasts isolated (Ahearn et al., 1970) from the rhizosphere and tissue of oyster grass, Spartina alterniflora, in the Louisiana marshland. P. spartinae is able to utilize the Spartina plant material as an energy as well as a total nutrient source.

Furthermore, the yeast utilizes a variety of lipids (Goter, 1973), and primary, secondary and tertiary amines (Meyers and Nicholson, 1970). Since bananas contain amines (dopamine, serotonin, norepinephrine and tyramine) and lipids in addition to sugars, these physiological properties of P. spartinae may contribute to the formation of microbial protein from the utilization of amines and lipids as well as sugars in bananas. In this way, the yield of the yeast cell mass may be increased above that expected solely on the basis of sugars present.

Ancillary investigations (C. Chao, unpublished data) of β -glucosidase activity. The medium consisted of a mineral salt (Trichoderma viride) medium containing 0.5% banana peel broth (65 g raw banana skin in 195 ml distilled water). Growth and enzyme elaboration were compared in a comparable mineral medium with 1% sucrose as the sole carbon source. Growth studies were run for a 24-hr period and β -glucosidase activity (expressed as specific activity) was analyzed following previously described procedures (Meyers et al., 1975). The data, shown in Appendix 13, indicate that activity in the banana-supplemented flasks was equivalent to that obtained on the sucrose substrate. With certain Pichia species, especially P. etchellsii, P. wickerhamii and P. toletana, enzyme development on the sugars in the banana broth was nearly double that obtained in the sucrose medium.

Other yeasts used in this study were: Candida guilliermondii (GSU-1), Trichosporon cutaneum (GSU-2), Pichia guilliermondii (NRRL Y-2075), Pichia salictaria (NRRL Y-6780), Pichia angophorae (NRRL Y-7118), Pichia ohmeri (NRRL Y-1932), Pichia pseudopolymorpha (NRRL YB-4229), Pichia wickerhamii (NRRL Y-2436) and Pichia quercuum (NRRL YB-4281). These yeasts were obtained from the Northern Regional Research

Laboratory, Peoria, Illinois.

Media

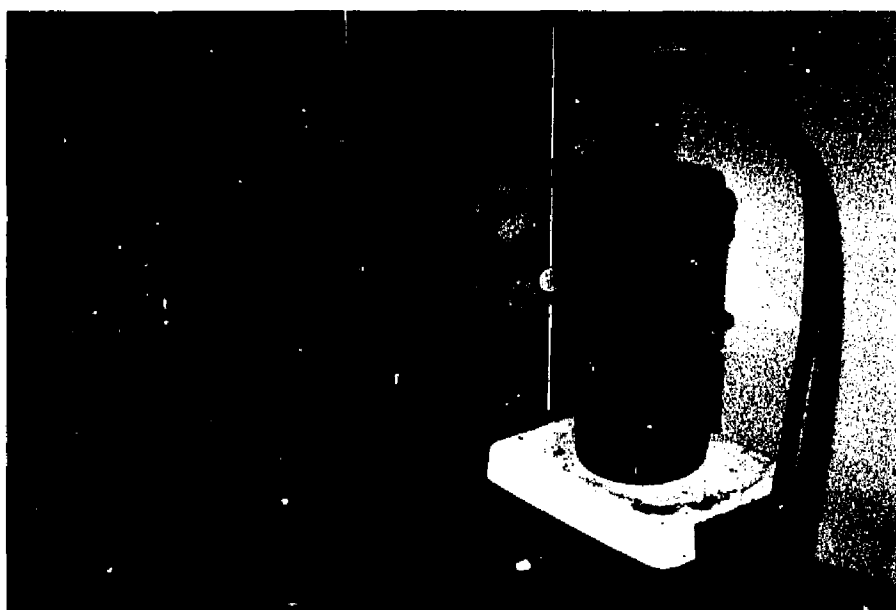
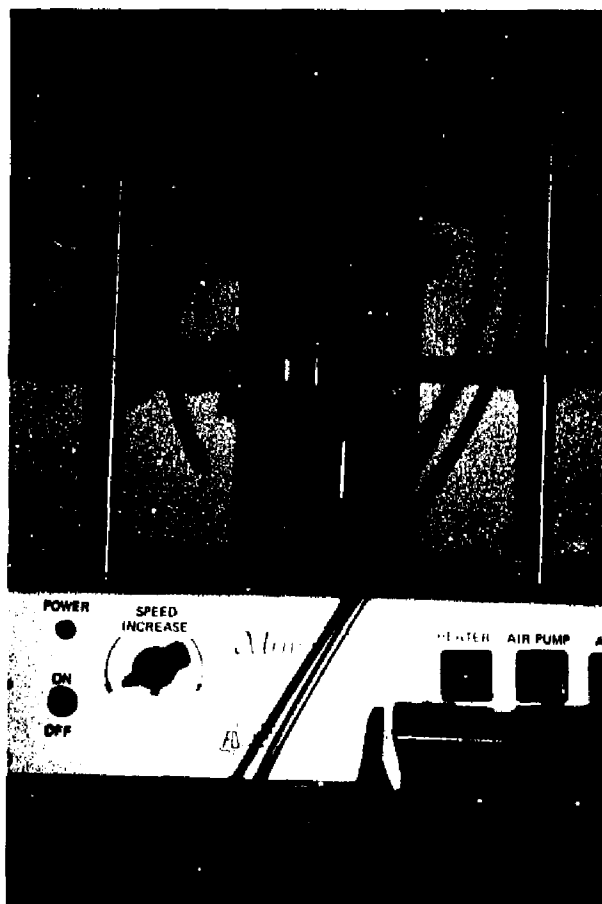
The growth medium contained 1.5% RPL (unless otherwise noted) or 3.0% RSL with 0.2% $(\text{NH}_4)_2\text{SO}_4$ added. In some experiments 0.1% yeast extract (Difco) was added to determine the effect of this supplement on the growth of *P. spartinae*. The substrates and nitrogen sources were sterilized (at 121°C for 15 min) separately to prevent brown color development. Although the browning reaction is caused by a reaction of reducing sugars in bananas with amino acids, peptides and proteins, the addition of $(\text{NH}_4)_2\text{SO}_4$ to the substrate before sterilization also resulted in an intensive brown color formation. Therefore, as noted, the banana substrate and nitrogen sources were sterilized separately. Yeast extract (10%) was filter-sterilized through 0.22 μ cellulose acetate membrane filter (Millipore Filter Corp.) and added to the medium to yield a final concentration of 0.1%. The sterilized substrates and nitrogen sources (and yeast extract in some instances) were combined aseptically, and 500 ml of the combined medium were added to sterile 1000-ml fermentor jars or 100 ml of the medium to sterile 250-ml Erlenmeyer flasks. Polyglycol (0.001%) was added to the medium in the fermentor jar to prevent foaming.

The mini-fermentors used were Mini-Ferm Model M-1000. The 1000-ml beaker and rubber stopper was equipped with sample line glass tubing, exhaustion hole and aeration glass tube with terminal air stone. A small aquarium-type air pump was used for aeration. The apparatus is illustrated in Figure 1.

Figure 1. Micro-fermentor apparatus.

Above: Mini-Ferm Model M-1000

Below: Mini-Ferm Model M-1000 and modified micro-fermentor apparatus. The latter consists of 1000-ml beaker and rubber stopper equipped with sample line glass tubing, exhaustion hole and aeration glass tube with terminal air stone.



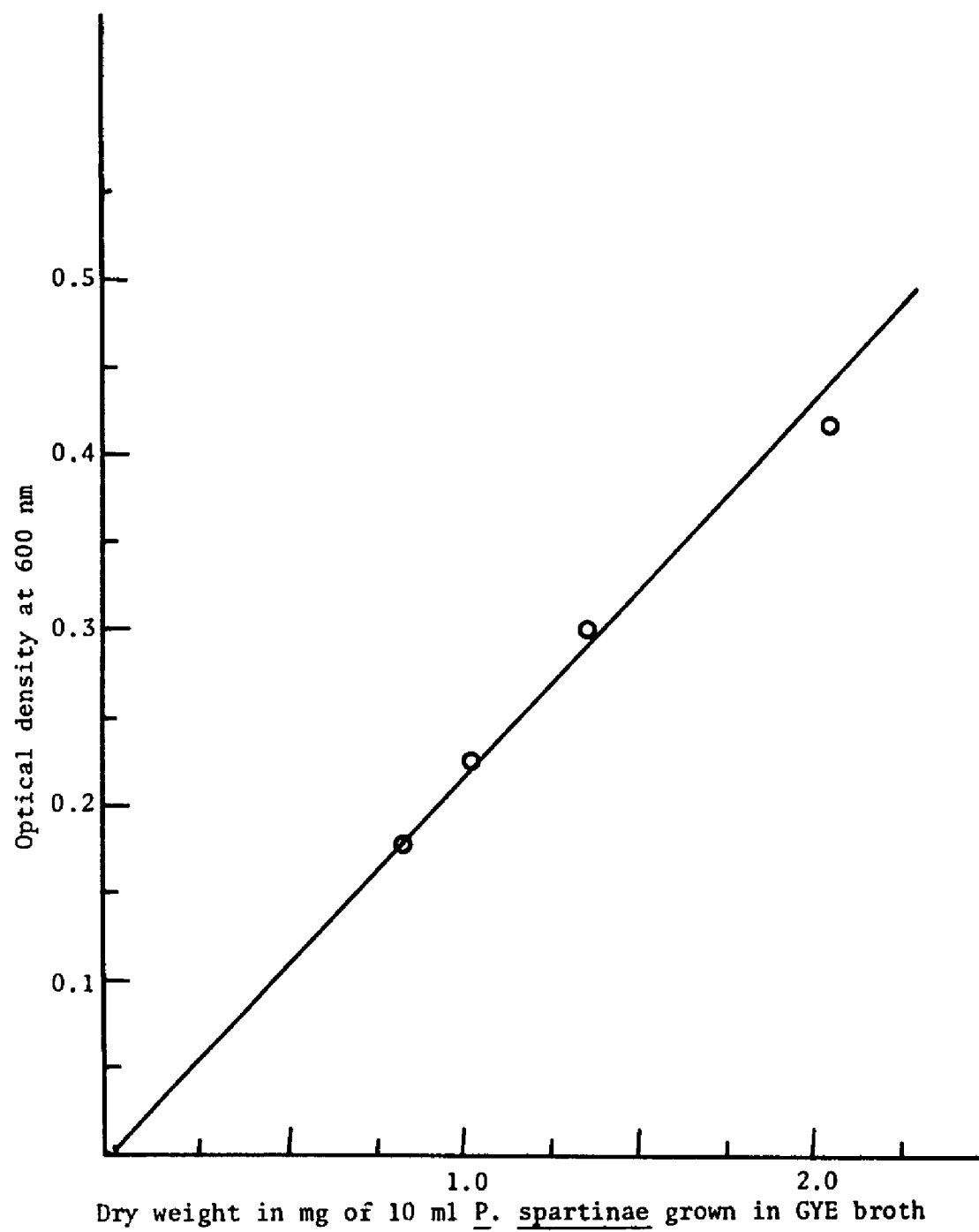
The inoculum for the growth studies was prepared by transferring the appropriate stock yeast culture from M-12 agar slant to 20 ml of GYE broth (1.0% glucose, 0.25% yeast extract) in a 50-ml Erlenmeyer flask with incubation on a Gyrotory Shaker Model G2 (Lab-line Junior Orbit Shaker). The concentrated inoculum (48 hr old) was added to the growth medium, 1% for the flask system and 3.5% for the mini-fermentor. Flasks were then incubated at room temperature on a large gyrotary shaker. Test-tube cultures, containing 10 ml medium, were inoculated with 2% inoculum (about 5 drops) and incubated at room temperature on a roller drum (Model TC-5, New Brunswick Scientific) at approximately 60 rpm to produce agitation and resultant aeration.

Growth was determined spectrophotometrically at 600 nm, measuring optical density (turbidity) of culture broth. The relationship of O.D. and dry weight of P. spartinae is shown in Figure 2.

Harvesting

Yeasts were harvested after 48 hr, except for growth studies, and separated from the culture medium by centrifugation at 10,000 rpm for 15 min. Following washing of the cell crop with distilled water to remove carry-over nutrient from the medium, yeasts were dried under vacuum at 60°C overnight. Dried yeast samples were weighed and yields calculated on the basis of total sugar content in the medium. For example, the 500 ml growth medium with 1.5% RPL contained 5.46 g of total sugar since total sugar content in RPL is 72.8%. If the weight of dried yeast obtained from the 500 ml culture broth (total sugar 5.46 g) was 2.5 g, the yield would be $2.5/5.46 \times 100 = 45.75\%$.

Figure 2. Relationship of optical density and dry weight of P. spartinae.



Proximate Analysis of Substrates

The chemical analyses of substrates including moisture content, ash, fat and crude fiber content were performed according to the official method described by A.O.A.C. (11th ed., 1970).

The protein content of substrates and yeast cells was determined by the macro Kjeldahl procedure, with multiplication of the nitrogen value by 6.25.

Determination of Amino Acids

Amino acids were determined by the method of Block and Weiss (1956). The samples were held in a desiccator over Drierite until constant weight was maintained. The dried samples (2.05 mg of P. spartinae cells) were hydrolyzed with 1.0 ml of 6N-HCl at 110°C for 22 hr. The hydrolysates were evaporated under a vacuum and evaporated samples dissolved in 1.0 ml of pH 2.2 citrate buffer. Analyses were conducted on a Beckman Model 116 C amino acid analyzer; 0.1 ml of the hydrolysates were applied to each column. Because tryptophan is destroyed during acid hydrolysis, this amino acid is not reported.

Phosphate Determination

Phosphate content of substrates were analyzed according to the method of Chen (1968) to determine whether supplementation of phosphate to the growth medium is necessary for growth of P. spartinae. The sample solution, containing about 0.05-1.0 mg P_2O_5 , was diluted to 70 ml with distilled water; 5 ml of 0.75% ammonium molybdate in 2N- H_2SO_4 and 4 ml of reducing solution were added, and the liquid made up to 100 ml volume with distilled water. (Reducing solution: 15 g $NaHSO_3$ and 0.5 g anhydrous Na_2SO_3 were dissolved in 800 ml distilled water. After 0.25 g

of 1,2,4-amino-naphthol-sulfonic acid was added, the solution was diluted to 1 liter. The solution was stored in a polyethylene bottle.) The liquid was allowed to stand for 10 min to permit maximum color development before the solutions were measured at 830 nm in a spectrophotometer, adjusted to give 100% transmittance with blank. A blank and a standard solution containing 0.1-0.7 mg of P_2O_5 were similarly treated to obtain a standard reference graph. The amount of phosphate was determined using the prepared standard reference graph. Standard phosphate solution was prepared by using 0.1916 g pure KH_2PO_4 dissolved in 1 liter distilled water. This solution was stored in a polyethylene bottle and each ml contained 0.1 mg P_2O_5 .

Total Sugar Determination

The total sugar content was determined by phenol-sulfuric acid colorimetric method (Whistler and Wolfrom, 1962). One ml of aqueous solution containing between 10 and 70 μ g of the sugar (hexoses) was pipetted into a colorimetric tube. One ml of 5% phenol solution was added and mixed. Blanks were prepared with 1 ml of water instead of sugar solution. From a fast-flowing pipette, 5 ml of 96% H_2SO_4 was added to each tube so that the stream hit the liquid surface directly to produce good mixing and even heat distribution. Each tube was agitated during the acid addition, and exactly the same mixing procedure was practiced throughout. After 10 min, the tubes were reshaken and placed in a water bath at 25-30°C for 20 min. The yellow-orange color was stable for several hours. Absorbances were measured at 490 nm for hexose. All hexoses, whether simple sugars or in combined form, i.e., disaccharides or hexosans, could be determined by this method. The amount of sugar was calculated using a standard glucose curve.

Gas Chromatography for Determination
of Alcohols and Fatty Acids

Preparation of Sample. The general procedure of Jackson and Kempton (1973) was used for extraction and analysis of microbial metabolites. The alcohols and volatile acids were extracted with ether; non-volatile acids were converted to their volatile methyl esters and extracted with chloroform.

The aqueous culture medium, after removal of yeast cells by centrifugation, was acidified ($\text{pH} < 2$) with 50% H_2SO_4 (v/v). This acidification releases free organic acids from the ether-insoluble salt form. Precipitated proteins were removed by brief centrifugation, i.e., 5 min at 2,000 rpm at room temperature.

To extract volatile fatty acids and alcohols, 1 ml of anhydrous ethyl ether was added to 4 ml of the acidified aqueous extract in a 15-ml conical centrifuge tube. The tube was tightly stoppered with a neoprene rubber bung and contents thoroughly mixed by inverting the tube about 30 times. The ether emulsion formed was broken by brief centrifugation, 1-2 min at 2,000 rpm at room temperature, and the tube placed in a freezer until the aqueous layer was frozen. The ether extract was decanted into a 12 x 75 mm screw-cap culture tube and traces of water removed by the addition of anhydrous MgSO_4 to equal approximately one-half of the volume of ether.

To prepare methyl derivatives of non-volatile acids, 1 ml of BF_3 -methanol (14% w/v) was added to 1 ml of acidified aqueous extract in a 15-ml conical centrifuge tube. The tube was stoppered tightly and placed in a 60°C water bath for 30 min; 0.5 ml of chloroform was added, and the tube inverted 30 times to extract the methyl esters. Brief

centrifugation clarified the chloroform layer, which was then removed with a Pasteur pipette to a 12 x 75 mm screw cap culture tube. Contamination of the chloroform extract with water was avoided by first removing the aqueous layer with a pipette attached to an aspirator pump. The extract was again dried with anhydrous MgSO_4 . Both extracts were stored at -20°C before analysis.

Gas Chromatography Determination. Gas chromatography determinations were done using a Perkin-Elmer 990 Gas Chromatograph with flame ionization detector. Stainless steel columns (1/8 in. O.D., 6 ft long) were packed with 5% Carbowax 20M on Chromosorb W, AW, 80/100 mesh and 5% FFAP (Varian) on Chromosorb W, AW-DMCS, 80/100 mesh. Standard conditions for all analyses are summarized in Table 1. A mixture of pure alcohols and pure volatile fatty acids (Tek-Lab, Baton Rouge, La.), and a mixture of methylated fatty acids containing C_6 - C_{16} were chromatographed to aid in the identification of peaks in the sample. Identification was made by a comparison of retention time of standard alcohol and fatty acid peaks with the sample peaks. Sample peak areas were compared with those of known amounts of ethyl alcohol standards in order to obtain sample concentrations. Peak areas were obtained by multiplying peak heights by widths at one-half peak height.

Table 1. Parameters for gas chromatographic analysis

	Carbowax	FFAP
Carrier gas	Nitrogen	Nitrogen
Carrier flow	25 cc/min	35 cc/min
Detector	Flame ionization	Flame ionization
Injection temperature	175°C	250°C
Manifold temperature	225°C	300°C
Column temperature		
Initial	70°C	60°C
Final	150°C	230°C
Program rate	6 C/min	24 C/min
Initial hold	2 min	2 min
Final hold	4 min	4 min
Chart speed	1 in./min	1 in./min

RESULTS AND DISCUSSION

Yields and Chemical Composition of Substrates

The yields of RPL and RSL from fresh banana pulp and skin, and the proximate composition of freeze-dried banana substrates is shown in Table 2. As noted, a material balance on 100 lb of fresh, ripe banana pulp and skin gave 15 lb RPL (10.9 lb total sugar as hexoses) and 4.5 lb RSL (1.98 lb total sugar as hexoses), respectively. Both substrates supply adequate concentrations of fermentable sugars and essential minerals (probably potassium, magnesium, phosphorus, zinc, iron, copper). Water-soluble vitamins such as B vitamins and ascorbic acid, in all likelihood, are present in both substrates. The banana pulp liquid (RPL) is richer in fermentable sugars than that from the banana skins (RSL); both materials are nearly comparable in amounts of total organic nitrogenous compounds, fat, and fiber. RSL is substantially richer in minerals (as ash, 28.6%). Both RPL and RSL contain relatively large amounts of sugar (72.8% of RPL and 44% of RSL) and minerals as ash (4.3%, RPL; 28.6%, RSL) but are relatively low in protein (3.6%, RPL; 3.1%, RSL). Therefore, a nitrogen source, preferably in an inorganic form, must be added to the growth medium for yeast development. The rather high moisture content of RPL and RSL is probably due to the high hygroscopic properties of the freeze-dried banana samples. Moisture content of both RPL and RSL, therefore, varies more or less throughout these studies which may cause further slight variation in the total sugar content in culture medium and on subsequent calculations of percent yield of yeast cell mass.

Table 2. Yields and proximate composition of freeze-dried banana substrates

Substrate/ingredient	Ripe banana pulp liquid (RPL)	Ripe banana skin liquid (RSL)
Yields from fresh banana pulp and skin	14.97%	4.46%
Protein	3.6	3.1
Fat	0.0	0.1
Fiber	0.3	0.2
Moisture	18.4	22.1
Ash	4.3	28.6
Phosphate	0.18	0.6
Total sugar*	72.8	44.0

*Measured as total hexose content, both single and combined form, in banana substrates. Subsequent yeast yields based on total hexose calculations.

Note: On a final dry weight basis, protein percent is: RPL = 4.4%; RSL = 3.98%. High ash content in RSL is due to large amount of 0.8% NaCl solution used in blending process for preparation of substrate.

The following data give a general picture of the qualitative and quantitative levels of vitamins and minerals in these two substrates. One medium size raw banana, approximately 1 x 6" in size, contains 430 IU vitamin A, 0.04 mg thiamine, 0.05 mg riboflavin, 0.7 mg niacin, and 10 mg ascorbic acid (Peckhan, 1969). According to Von Loesecke (1950), bananas commonly contain such minerals as Ca, Cl, Cu, Fe, Mg, Mn, P, K, Si, N, S. The proximate chemical composition of the fully ripe banana (yellow skin flecked with brown) is shown in Appendix 12. From these data, it can be safely postulated that both RPL and RSL contain concentrations of vitamins sufficient for at least minimal levels of microbial growth. Since the yeast P. spartinae does not grow in a completely vitamin-free medium (Ahearn et al., 1970), the presence of vitamins in the culture medium is necessary for sustained growth of the organism. Whether the absolute concentration of vitamins in the banana substrates is sufficient for optimal yeast development will be discussed subsequently.

Growth Studies of P. spartinae

A growth study was conducted to establish the beginning of the stationary phase of yeast development (i.e., the optimal time of harvest for maximal cell biomass) and to determine the actual rate of growth. Although rapid growth is significant in terms of profuse proliferation of yeast crop within a relatively short period of time, the yield of yeast from the supplied carbohydrate decreases at rapid growth. This is due to oxygen becoming limiting and a change from aerobic metabolism to the less efficient, in terms of cell crop, anaerobic metabolism. According to Worgan (1973) a generation time of 5 hr in the batch process for

the production of baker's yeast is optimal for conversion of carbohydrate to yeast cell mass, or to yeast protein.

Generation time of P. spartinae in growth or basal medium (BM) (1.5% RPL, 0.2% $(\text{NH}_4)_2\text{SO}_4$ in distilled water) was measured as growth rate and calculated by the following equation:

$$\text{Number of generation} = \frac{\log_{10} N_1 - \log_{10} N_0}{0.301}$$

where N_0 = number of cells at initial

N_1 = number of cells at harvest

$$\text{Generation time} = \frac{\text{growth period}}{\text{number of generation}}$$

(Adapted from Stanier et al., 1963)

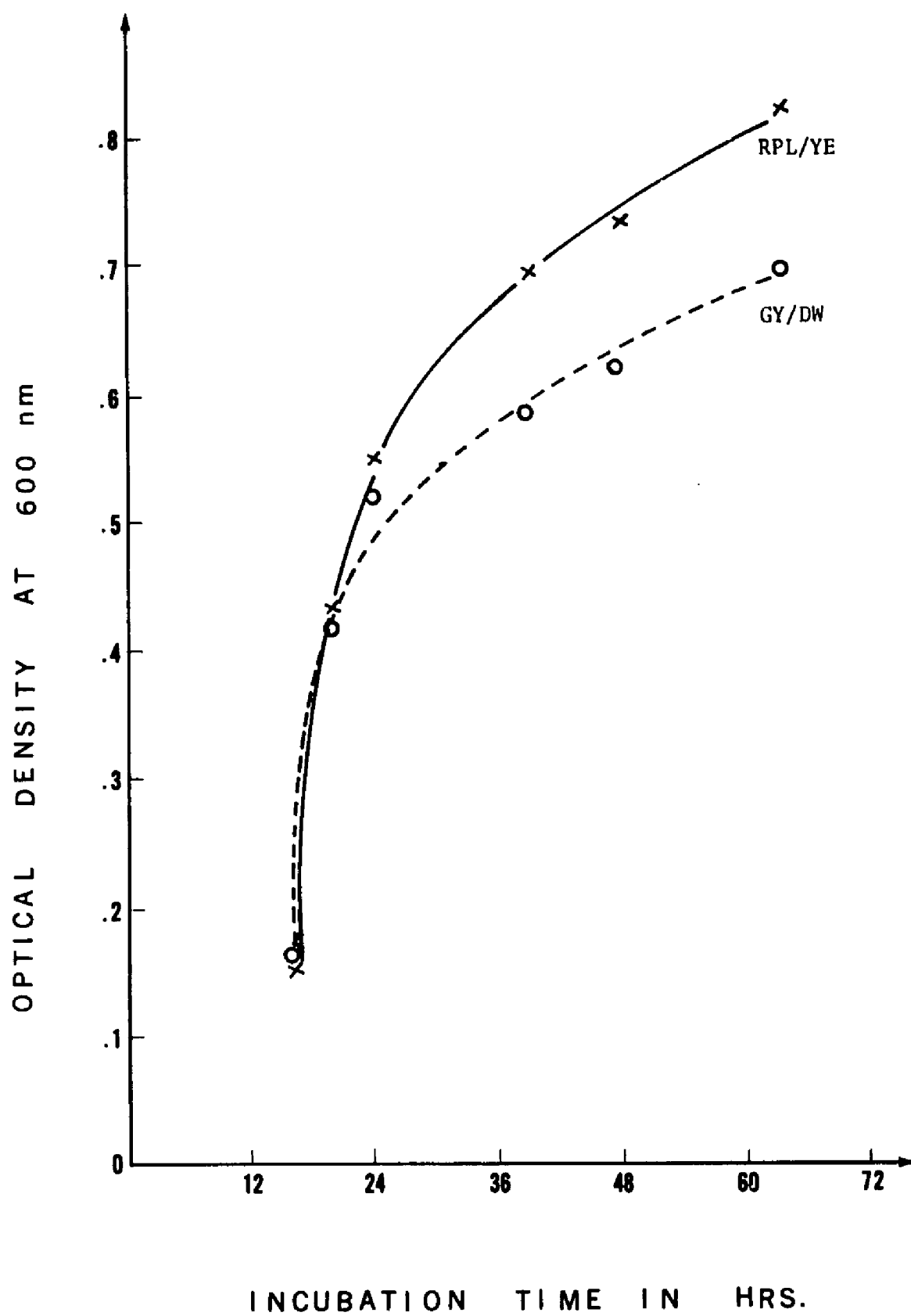
The average generation time of P. spartinae was 2.5 hr (from 2.42 to 2.68 hr) at room temperature. Even though a 5-hr generation time is optimal for maximal production of baker's yeasts, shorter generation times have been reported for other yeasts (Appendix 10). The generation time of various yeasts on carbohydrate substrates varies from 1.5 hr for S. fragilis on cheese whey at 32°C, to 2.5 hr for S. cerevisiae on cane molasses at 30°C.

As Figure 3 shows, the stationary phase of growth of P. spartinae is not well defined. It appears that maximal efficiency is around 36 hr, thus the best harvest period would be during this time. However, in this study, harvesting of cell crops was standardized at 48 hr for convenience. It was also noticed that P. spartinae apparently enters its stationary phase within 30 hr at room temperature, but continues to grow at a slow rate. In order to determine reasons for this continued growth, depleted medium of P. spartinae was tested to establish whether

Figure 3. Growth curve of P. spartinae.

RPL/YE: 1.5% RPL and 0.25% yeast extract in DW

GY/DW: 1.0% glucose and 0.25 yeast extract in DW



it contained sufficient carbon sources for yeast growth, yet was deficient in other nutrients, or contained toxic (inhibiting) metabolites produced by the organism.

The growth or basal medium (BM) in which P. spartinae was grown for 48 hr was used as a depleted substrate after removal of the cell mass by centrifugation. The depleted medium was filter-sterilized (0.45 and 0.22 μ cellulose acetate membrane filters) and transferred aseptically into sterile test tubes. An aliquot of 0.5 ml of 10X filter-sterilized (0.22 μ membrane filter) yeast nitrogen base (YNB) was added to 4.5 ml of the depleted broth and inoculated with P. spartinae. Growth was compared with that obtained in GY/DW.

Results in Table 3 enumerate the development of P. spartinae and other yeasts in the depleted medium. These additional yeasts were examined to establish whether they are able to utilize the metabolic products produced by P. spartinae during its development. As noted, none of the other species grew better than P. spartinae in the depleted medium. Since addition of vitamin mixture and phosphate to the basal medium did not support increased growth of P. spartinae (Table 4), which will be discussed subsequently, it can be assumed that the carbon, vitamins and phosphate sources in the basal medium were sufficient for continued growth of P. spartinae. Since production of some yet unknown metabolic substance may inhibit growth of the yeast, further study is needed to identify such products.

Table 3. Growth of yeasts in depleted medium compared with the growth in GY/DW

Yeast ^a	Dilution ^b	Growth (based on O.D. measurements)	
		Depleted medium	GY/DW
FST 119*	1:10	0.538	0.545
GSU-1	1:10	0.509	-----
GSU-2	1:10	0.444	0.721
NRRL Y-2075	1:10	0.456	0.833
NRRL Y-2436	1:10	-----	0.605
NRRL YB-4229	1:10	0.208 ^c	0.538
NRRL YB-4281	1:10	0.509	0.658
NRRL Y-1932	1:10	0.319	0.678
NRRL Y-6780	1:10	0.523	0.553
NRRL Y-7118	1:10	0.468	0.658

^aSee page 33 for identification of various yeast species.

^bUsed to bring reading into optimal transmittance range.

^c1:5 dilution instead of 1:10.

----- not tested

*P. spartinae

Table 4. Effect of vitamin and KH_2PO_4 supplement on the growth of P. spartinae

Supplement	Growth* (based on O.D.)
Vitamin mixture	
0 %	0.398
0.05%	0.396
0.10%	0.398
0.20%	0.387
0.50%	0.393
KH_2PO_4	
0 %	0.398
0.1%	0.377
0.2%	0.387
0.5%	0.382

*at 48 hr

Growth Conditions

Growth of P. spartinae was examined under a variety of conditions of different temperature, pH, nutrient variation, and aeration. To determine optimum temperature for growth, the yeast was maintained at 15, 20, 25, 30 and 35°C, and growth measured spectrophotometrically. As shown in Figure 4, development occurred at all temperatures tested, with maximum growth at 20 and 25°C. However, the initial growth rate was a little higher at 30-35°C than at 20-25°C. The higher initial growth rate at 30-35°C is significant from an economic standpoint involving cooling costs and development of yeast cell mass in a relatively short period of time. Since fermentation is exothermic, heat must be removed from the system to maintain an optimum growth temperature. Fermentation temperatures are usually less than 45°C, a condition that contributes significantly to operating costs. Since P. spartinae grows at 35°C, maintenance of this temperature is made relatively simple by supplying cooling water to the system. Therefore, since mechanical refrigeration may not be needed to remove the heat produced, additional costs will not be incurred. The high initial growth rate also is significant in terms of profuse proliferation of yeast crop within a relatively short period of time. Within a 24-hr growth period, development of P. spartinae is maximal at 35°C compared with growth at other temperatures.

For determination of optimal pH, the initial pH of the medium was adjusted with sterile HCl and NaOH after sterilization to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. P. spartinae grew readily over a broad pH range, except at pH 9.0. Growth occurred under acid conditions as low as pH 2.0, although less than at pH 3.0-8.0. Optimal pH for growth of P. spartinae is 5.0-8.0 (Figure 5). This broad optimal pH range is

Figure 4. Effect of temperature on the growth of *P. spartinae*.
The yeast was grown in 1.5% RPL, 0.2% $(\text{NH}_4)_2\text{SO}_4$ and distilled water in flasks (100 ml/250-ml Erlenmeyer flask).

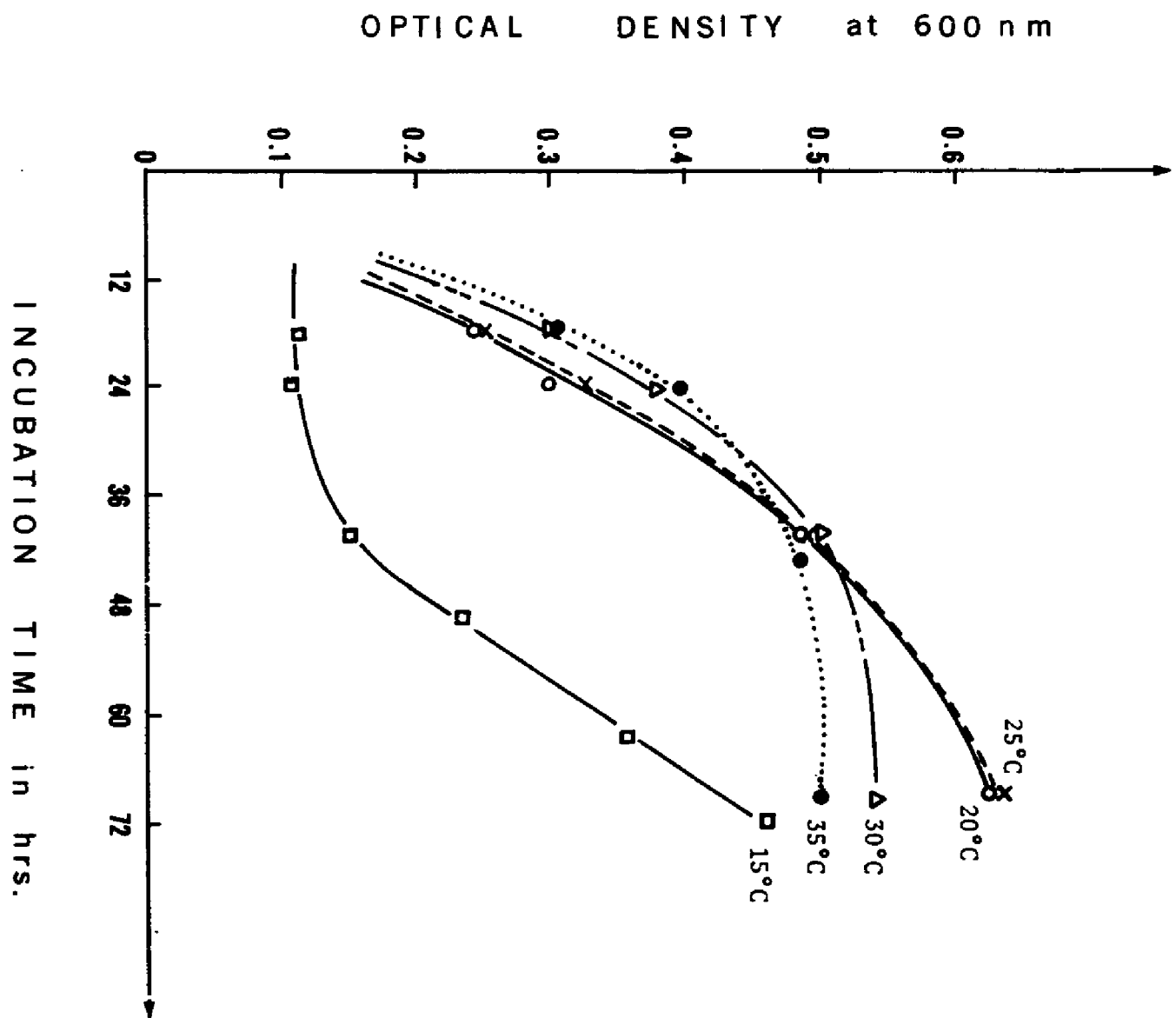
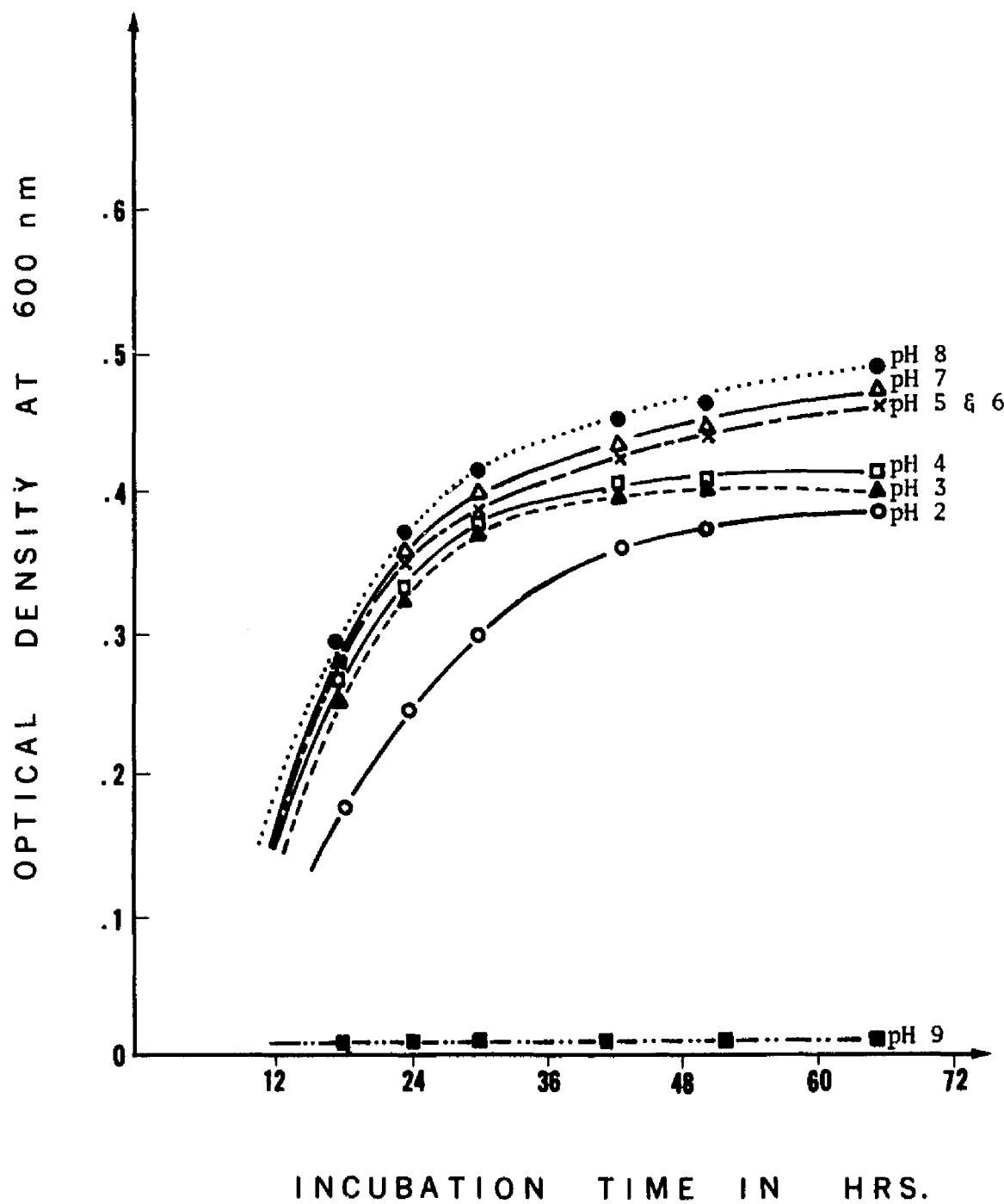


Figure 5. Effect of pH on the growth of Pichia spartinae



probably due to a decrease in pH of the medium caused by production of metabolic acid during growth of the yeast. Therefore, the optimal pH of the medium in all likelihood may be 4.5-5.5. This latter range is preferred in most industrial type operations to minimize the possibility of growth of bacterial contaminants that may be present, while still maintaining good growth rates.

When pH was adjusted to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 throughout the growth periods (pH was adjusted every 12-hr period), P. spartinae also grew readily over a broad pH range, except at pH 2.0. A slow initial growth at pH 8.0 was noticed. Even though pH was adjusted every 12 hr, the pH of the reaction vessel changed so rapidly that maintaining the original corresponding pH was quite difficult. This rapid change of pH for every 12 hr is seen in Table 5. The dramatic change in pH occurred during 24 hr, especially in high pH of the medium (pH 7 and 8). The good growth at pH 6.0-8.0 is probably due to the rapid change in pH (from pH 7 and 8 to 4-6). Therefore, the results (Figure 6) are in agreement with our establishment of the optimal pH of the medium at 4.5-5.5.

Table 6(A) shows pH changes within the growth medium during development of P. spartinae. When the yeast was grown in the basal medium, pH dropped rapidly within the initial 24 hr. However, when the organism was grown in $\text{RSL}/(\text{NH}_4)_2\text{SO}_4$ medium, changes of pH (from 5.3 to 3.2) were not so great as those in the former medium (from 5.2 to 2.75). Slow change of pH in the latter medium was probably due to the high phosphate content which contributes to the buffering action.

Studies were undertaken to determine what types of acids were responsible for the rapid pH depression, and what components of the

Table 5. pH changes of medium with the pH being adjusted every 12 hr during growth of P. spartinae

pH of medium	Growth period in hours					
	12	24	36	48	60	72
2	2.0	2.0	2.0	2.0	2.0	2.0
3	2.62	2.72	3.15	3.0	2.7	2.85
4	3.05	3.75	3.9	3.9	4.0	4.0
5	3.7	3.72	4.9	4.8	5.0	5.0
6	4.2	4.52	6.0	6.0	6.0	6.0
7	5.0	4.1	6.45	6.3	6.25	6.95
8	6.8	5.25	5.65	6.8	7.15	8.0

Note: Readings were made immediately before adjustment of pH.

Figure 6. Effect of pH (adjustment was made throughout growth period)
on the growth of P. spartinae

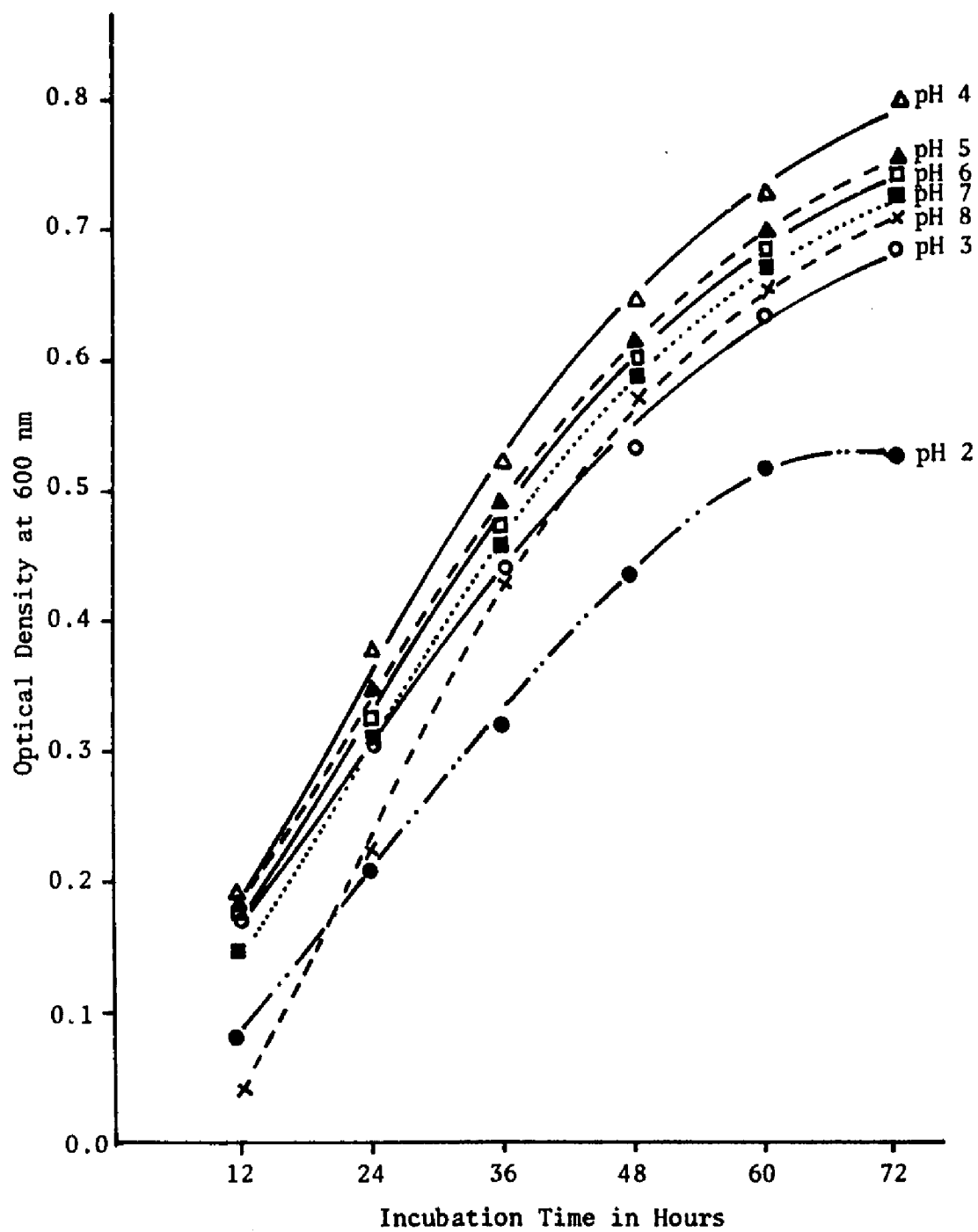


Table 6. pH changes of medium during growth of P. spartinae

Growth medium	Growth period in hours			
	0	12	24	48
(A) RPL- $(\text{NH}_4)_2\text{SO}_4$	5.2	3.3	2.8	2.75
RSL- $(\text{NH}_4)_2\text{SO}_4$	5.3	4.0	3.2	4.35
(B) G-Y	6.4	---	5.22	5.3
F-Y	6.0	---	5.41	5.2
G-F-Y	6.2	---	5.4	5.25
S-Y	6.6	---	5.46	5.7
RPL-Y	4.8	4.8	5.2	6.2

G: glucose
 F: fructose
 S: sucrose
 Y: yeast extract

medium contributed to the production of the acids. Since the growth medium contains RPL and ammonium sulfate, experiments were performed to determine the effect of the major components in RPL (sucrose, glucose, fructose) and ammonium sulfate on pH change. Glucose, fructose, the mixture of glucose and fructose, and sucrose were selected as carbon sources in the growth medium. Table 6(B) shows the pH changes when the yeast was grown in those media. This pH of the media was not changed extensively, suggesting that those sugar compounds were not related to the production of acids.

To determine the effect of ammonium sulfate, the pH of the medium, with ammonium sulfate as a nitrogen source, was compared with that using yeast extract as a nitrogen source. When P. spartinae was grown in yeast extract instead of ammonium sulfate, the pH increased rather than decreased. The pH decreased only when ammonium sulfate was added to the growth medium. It was postulated that the decrease in pH was due to formation of sulfuric acid during yeast growth, formed from the un-utilized sulfate remaining in the medium after the yeast metabolized nitrogen from ammonium sulfate. The presence of sulfuric acid was confirmed by precipitation of barium sulfate when tested with barium hydroxide ($\text{Ba}(\text{OH})_2$). Since production of sulfuric acid caused a pH drop of the culture medium and affected growth of P. spartinae, adjustment of pH is required for maximal yeast crop when ammonium sulfate is used as nitrogen source.

Ahearn et al. (1970) observed that P. spartinae would not grow in a vitamin-free medium. A vitamin mixture (NBC Vitamin Fortification Mixture), therefore, was added to the basal growth medium to ascertain if supplemental vitamins are needed for growth. The filter-sterilized

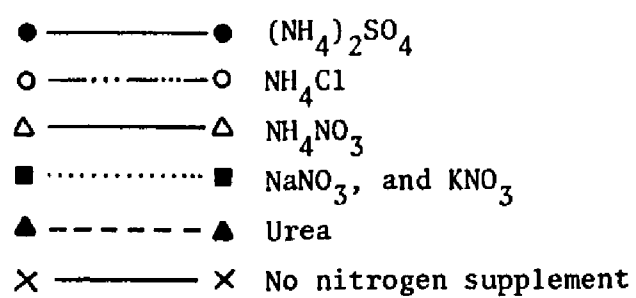
vitamin mixture was added aseptically to the medium at concentrations of 0.05, 0.1, 0.3 and 0.5%, and growth was determined by increase in optical density. Data indicated no significant difference in growth of P. spartinae with the vitamin supplement (Table 4), suggesting that the banana-supplemented medium contains sufficient vitamins for growth of the organism.

Studies were performed to determine whether the phosphate concentration of the banana broth was sufficient for yeast growth since phosphate is important not only for a part of the composition of the cell constituent but is also essential for energy metabolism (ATP, ADP, AMP, etc.). The results of the supplementation of KH_2PO_4 , at 0.1, 0.2 and 0.5%, on the growth of P. spartinae is seen in Table 4. No significant difference is noted on yeast growth, suggesting that the phosphate content of banana broth (0.184%) is sufficient for adequate P. spartinae development.

As noted, bananas are rich in sugars and a good source of vitamins and minerals, but quite low in proteins. Thus, the fruit could serve as an excellent metabolizable substrate for microbial development after supplementation with a suitable nitrogen source. Several such nitrogen compounds, i.e., ammonium sulfate, ammonium chloride, ammonium nitrate, sodium nitrate and urea, were tested. Based on growth response (Figure 7), ammonium sulfate was selected as the most suitable nitrogen compound for P. spartinae.

When yeast extract was added to the basal medium, a significant increase in growth occurred (Figure 8). In addition to providing a source of nitrogen, yeast extract also contains carbon and vitamins. If the increased growth of P. spartinae was due to the carbon and vitamins,

Figure 7. Effect of nitrogen source on the growth of Pichia spartinae



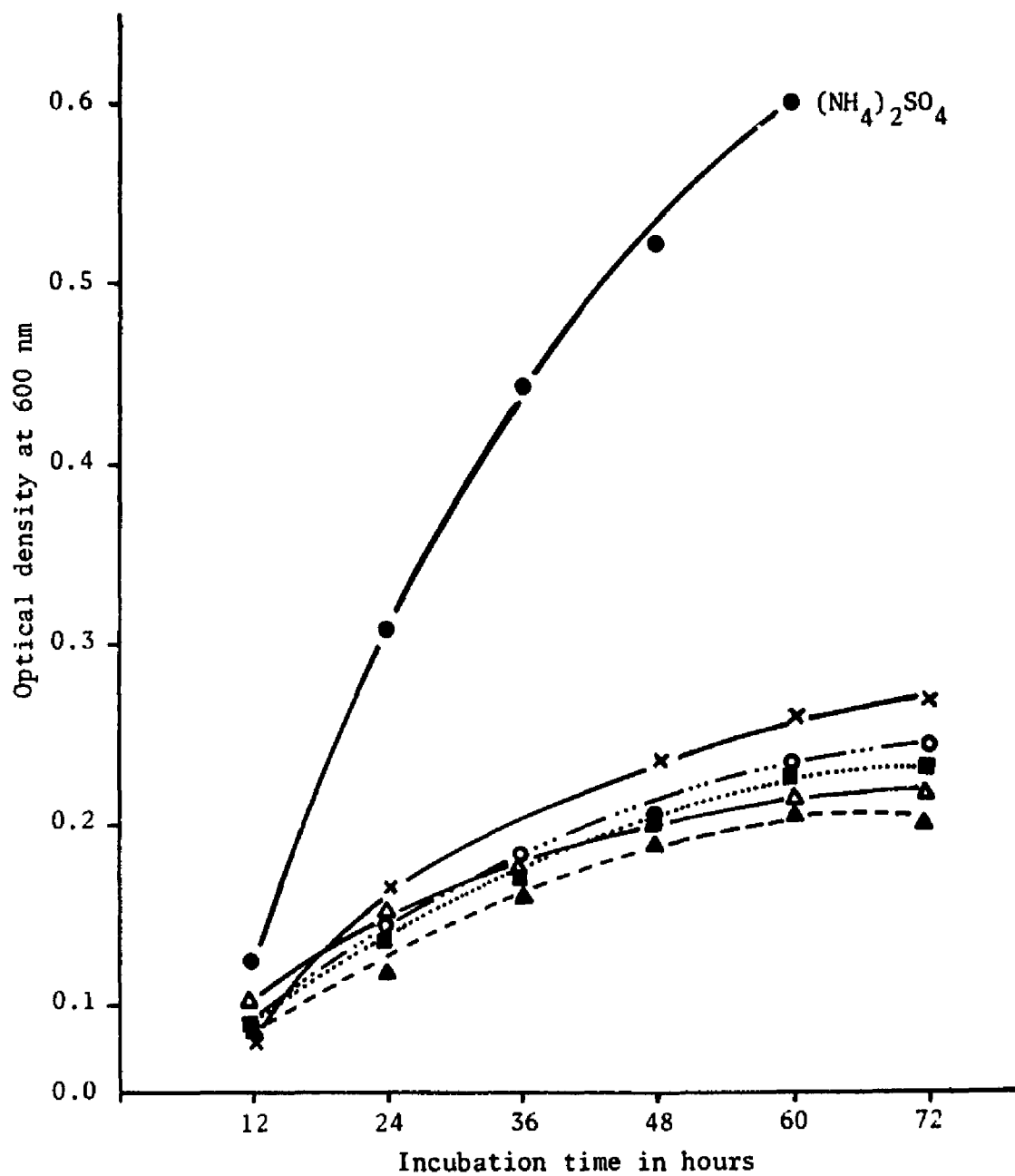
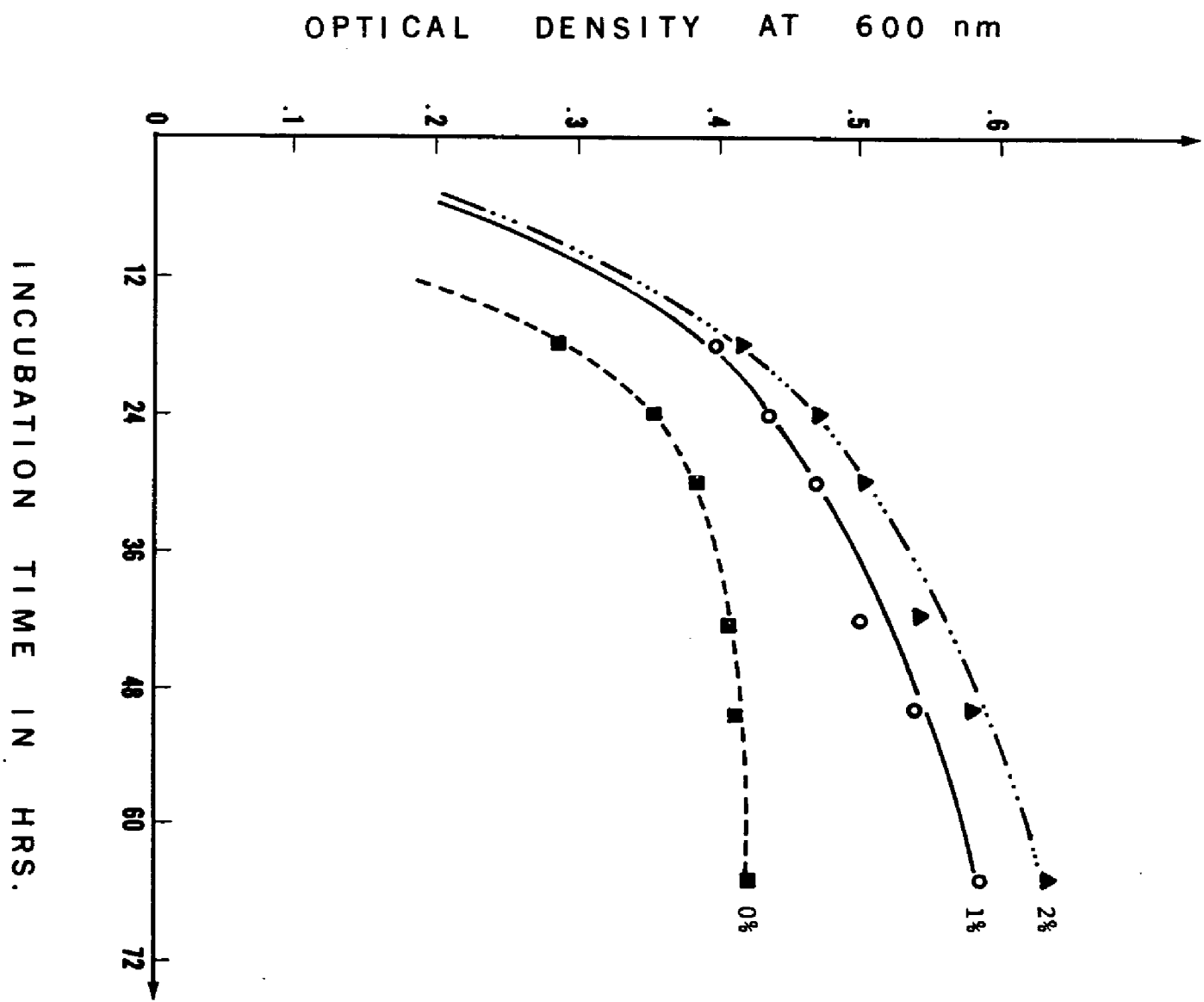


Figure 8. Effect of yeast extract on the growth of P. spartinae



rather than the nitrogen in the yeast extract, initial growth should not be affected by addition of yeast extract since carbon and vitamins are not lacking in the medium at initial stage of growth. The earlier experiment also showed that vitamin supplementation to the growth medium did not affect growth of the organism, therefore, nitrogen in yeast extract is probably responsible for increased growth. It can be concluded that P. spartinae grows better in media containing organic nitrogen rather than an inorganic source.

Table 7 summarizes the effect of supplementation of ammonium sulfate, yeast extract and vitamins on growth of P. spartinae. As noted, supplementation with inorganic nitrogen gave a significant increase in growth; yeast extract was a better nitrogen source than ammonium sulfate for yeast growth. P. spartinae grew better in the medium supplemented with yeast extract alone than in that supplemented with both yeast extract and ammonium sulfate. This is probably due to the resultant low pH when supplemented with ammonium sulfate. Vitamin supplementation did not materially affect growth.

Proper aeration of the growth medium is very important in fermentation. Inadequate aeration in baker's yeasts (S. cerevisiae) propagation (Strohm and Dale, 1961) results in the production of ethyl alcohol rather than cell substance, which is reflected as a loss in cellular yield. Over-aeration, however, serves no useful purpose and results in sizable added expenses for chemical antifungal agents and air compressor operation.

The following experiments showed the effect of aeration on growth of P. spartinae. The organism was grown in 50, 100, 150 and 200 ml of basal medium in 500-ml Erlenmeyer flasks on a shaker. The

Table 7. Effect of nutrient supplement to the medium on growth of Pichia spartinae

Substrate	O.D. at 48 hr	Final pH
RPL	0.288	4.52
RPL-N	0.654	3.00
RPL-Y	0.824	5.69
RPL-V	0.223	4.51
RPL-N-Y	0.698	3.20
RPL-N-V	0.701	3.00
RPL-Y-V	0.796	5.50
RPL-N-Y-V	0.660	3.19

RPL: 1.5% ripe banana pulp liquid (freeze-dried)

N: 0.2% (NH₄)₂SO₄

Y: 0.1% yeast extract

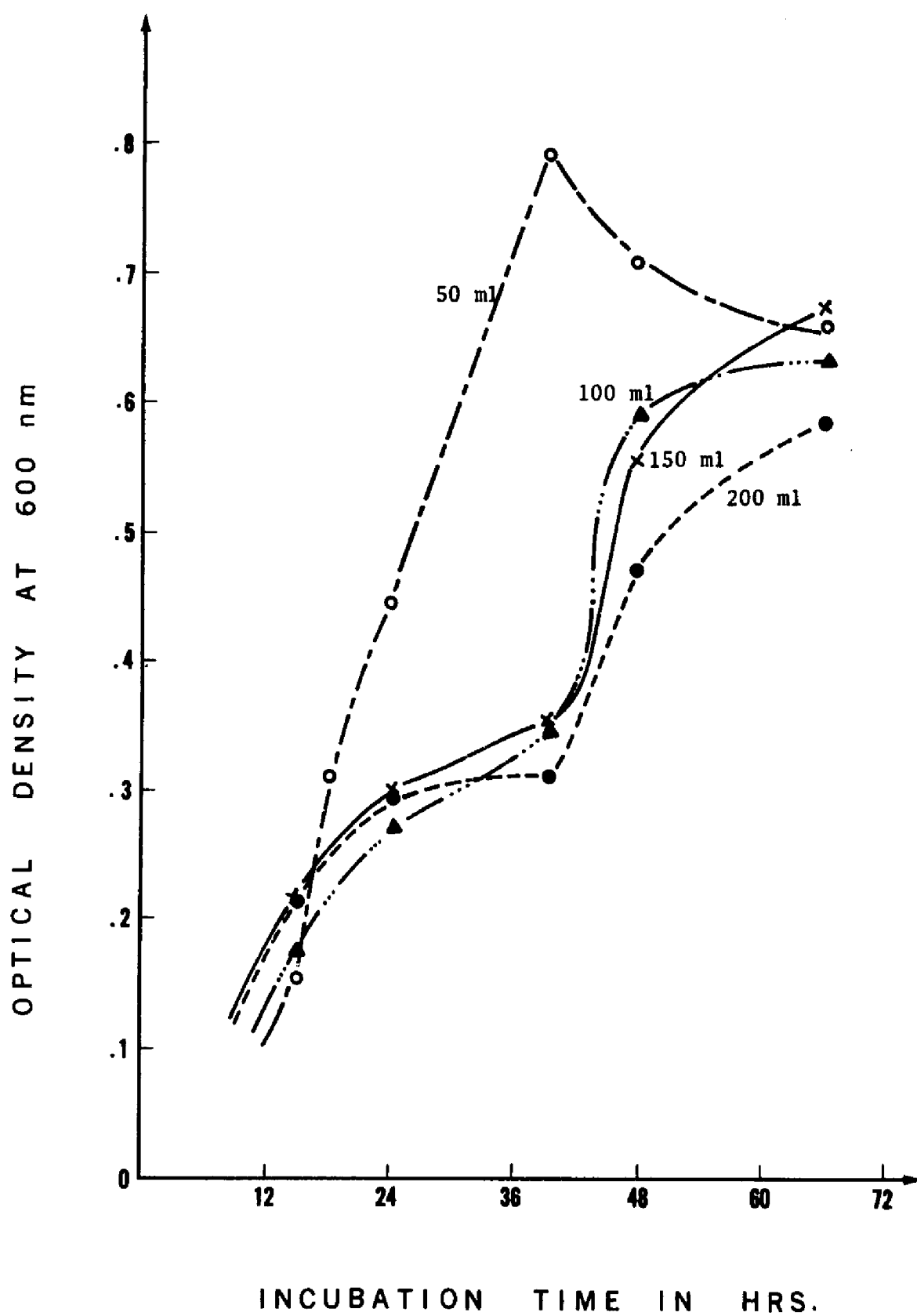
V: 0.1% vitamin mixture (NBC Vitamin Diet Fortification Mixture)

Grown in flasks (100 ml/250-ml Erlenmeyer flask)

degree of aeration to these flasks would be $50 > 100 > 150 > 200$ due to liquid surface area. Aeration to the medium in the 50-ml flasks was sufficient due to the large liquid-surface area present. Therefore, more rapid growth was observed in these vessels as compared with that in other vessels. The sudden decrease in growth after 36 hr is probably due to depletion of nutrients in the medium (Figure 9). Other flasks with 100, 150, 200 ml medium exhibited sluggish growth during 24-40 hr and showed subsequent continual growth (Figure 9). This is probably due to limiting aeration during 24-40 hr and the following availability of air. Sufficient aeration in the growth medium, therefore, is significant in terms of maximal production of yeast protein within relatively short time periods.

Large quantities of oxygen are required since only dissolved oxygen is available to the yeast cell. Since the solubility of oxygen in aqueous solutions is very low and added solutes and defoaming agents interfere in the diffusion of absorbed oxygen to growing cells (Peppler, 1968), only a small fraction of the oxygen sparged into commercial fermentors is utilized by the organism. Strohm and Dale (1961) reported that in the period of maximum molasses (to furnish about 1% of sugar) feeding to fermentor, the oxygen absorption rate is slightly greater than 2 mmole O_2 /liter/min, while the oxygen demand of the yeast is about 0.1 mmole O_2 /g/min dry yeast. Commercial experience indicates that for optimum growth, a five-fold excess of oxygen is required to satisfy this demand. The results in this study indicated that the optimum amount of medium used for flask culture would be 1/10 of the volume of the flask capacity to obtain optimum aeration.

Figure 9. Effect of aeration on the growth of P. spartinae



Yields of the Yeast Cell Mass from Banana

Scale-up studies, to convert banana to yeast protein, were undertaken using a mini-fermentor which can not only increase volume of the reaction system but also can supply increased controlled aeration. As a preliminary test the effect of concentration of substrate and nitrogen source on the growth of P. spartinae was examined. As indicated in Table 8, the combination of 1.5% RPL and 0.2% ammonium sulfate appeared to be optimum for cell production in terms of dry weight of yeast. However, when calculations were made on the basis of total sugar contained in the medium, the combination of 1.0% RPL and 0.5% $(\text{NH}_4)_2\text{SO}_4$ supported maximum yeast yield. The lower yields in media using 1.5% RPL were probably due to insufficient aeration. Aeration should be increased with concentration of substrate; however, in this study, comparable amounts of aeration were applied to both concentrations of substrate, i.e., 1.0 and 1.5%. Since aeration is an important factor for maximal production of yeast cell mass, increased aeration should give better yields. Therefore, the combination of 1.5% RPL and 0.2% $(\text{NH}_4)_2\text{SO}_4$ was chosen as optimum growth conditions for maximal cell yield.

In earlier experiments, it was emphasized that supplementation with ammonium sulfate resulted in a pH lowering in the growth medium. This low pH, i.e., pH 2.7, may partially inhibit growth of P. spartinae but on the other hand, it may give economic benefits in commercial production of yeast protein. By maintaining a low pH (3.0-5.0), contamination is minimized and, therefore, special aseptic equipment or elaborate sterilization techniques may not be necessary. Pasteurization, or even an absence of heat treatment of the medium, may permit the latter to remain free from contamination. If this is possible, and the yield of

Table 8. Effect of the concentration of substrate and nitrogen source on growth of P. spartinae

Concentration of $(\text{NH}_4)_2\text{SO}_4$	Concentration of RPL			
	1.0%		1.5%	
	Dry wt ^a of yeast	Yield ^b	Dry wt of yeast	Yield
0.1%	1.57 g	43.1%	1.83 g	33.5%
0.2%	1.65	45.3	2.06	37.7
0.5%	1.68	46.2	1.82	33.3

^aHarvested at 48 hr from 500 ml medium.

^bBased on total sugar content in substrate; total sugar concentrations of 1.0% RPL--3.64 g, 1.5% RPL--5.46 g.

cell mass is comparable with that in sterile media, costly sterilization processes are not necessary. Therefore, the test was made to determine the effect of heat treatment of the growth medium on yield of P. spartinae.

Soluble portions of banana pulp (RPL) and ammonium sulfate were added to sterile flasks (250-ml Erlenmeyer) containing 100 ml of distilled water. Media were pasteurized at 60, 80 and 100°C for 15 min. Growth of P. spartinae in those media were compared with that in the sterilized and non-heat treated series. The results (Table 9) demonstrated that the yields in the media were lower when compared with that obtained in the sterile medium; however, there were no contamination problems either in pasteurized or the non-heat treated media. These low yields may be due to the less utilizable carbon sources available in the pasteurized and non-heat treated media compared with those in the sterile medium. During heat treatment, certain less metabolizable (in terms of P. spartinae utilization) carbon compounds, such as hemicellulose and pectic substances, may be hydrolyzed to utilizable simple sugars. Data in Table 9 indicate that a positive correlation exists between yield of yeast cells and temperature.

Yields of the yeast crop from bananas under various growth conditions are summarized in Table 10. When pH of the culture medium was adjusted (between 3.7-7.0), final yield was greater than that obtained in the unadjusted medium. Higher yield of cells can also be attributed to each of the following parameters: increased aeration, smaller air bubbles, yeast extract supplement and heat treatment at 121°C/15 min. As noted previously, size of air bubbles was more or less arbitrarily regulated by variation of the porosity of the glass/ceramic air stone.

Table 9. Effect of heat treatment of growth medium on growth of Pichia spartinae

Heat treatment of growth medium	Growth (dry weight in mg ^a)	
	24 hr	48 hr
No heat treatment	--	275 mg
Pasteurized:		
at 60°C for 15 min	184 mg	278 mg
at 80°C for 15 min	212 mg	289 mg
at 100°C for 15 min	236 mg	327 mg
Sterilized at 121°C for 15 min	255 mg	361 mg

^amg in 100 ml growth medium

Table 10. Percent yield of the yeast cell mass from banana under various conditions

Growth condition	Total sugar in medium (g)	Production of cell mass (g/500 ml)	% yield of cell mass based on total sugar in the medium
1. pH			
No adjustment	5.46	2.30	42.12
Adjusted from 3.7 to 7.0	5.46	2.547	46.65
2. Inoculum size			
1%	5.46	1.92	35.16
3%	5.46	2.372	43.44
5%	5.46	2.355	43.13
3. Yeast extract			
With supplement	5.46	2.862	52.42
Without supplement	5.46	2.30	42.12
4. Sterile and non-sterile			
Sterile conditions	18.2	5.432	29.85
Non-sterile conditions	18.2	3.08	16.92
5. Aeration			
A degree (good)	5.46	2.327	42.62
B degree (fair)	5.46	1.914	35.05
6. Size of air			
Small	5.46	2.204	40.37
Large	5.46	1.914	35.05

Note: 5 and 6 as described on pages 70, 72 and 76 (Table 8).

Final yield was not significantly affected by inoculum size (Figure 10), from 1 to 5% of yeast cell suspension. The yield in the heat-treated medium was much higher than in the pasteurized medium and that without heat treatment. This was probably due to the presence of more readily available sugars in the former medium, due to hydrolysis of non-utilizable carbohydrates to simple sugars during sterilization.

Calculations of total yields were based on the amount of sugar in the medium. Maximal yields (Table 11) were 2.9 g/500 ml and 3.2 g/500 ml of RPL and RSL media, respectively. The percent yield of yeast biomass in the RPL medium was 52.9%, while in the RSL medium the yield was 58.0%. The significance of these yields is discussed below. The selected optimal conditions for maximal yield were heat treatment of medium, maximal aeration and yeast extract supplementation. Adjustment of pH was not mandatory. Yields of various yeasts from carbohydrate substrate vary from 23.2% for Saccharomyces cerevisiae on sugar in industrial scale, to 61.1% for Rhodotorula gracilis on glucose under laboratory fermentor conditions (Appendix 10). Compared with these values, the value of 52.9% found in this study is certainly an acceptable level. As noted subsequently, this can be improved considerably under scale-up conditions. In this study the actual requirement of aeration was not measured, but only arbitrary comparison of aeration was made. Therefore, if proper aeration is supplied to the medium by measuring oxygen requirement per unit of cells, the yield can be improved considerably. If the continuous culture condition is used, instead of batch type laboratory fermentor conditions, the pH of the medium will be increased by constant addition of new fresh medium and withdrawal of some biomass to the separator. This continuous process not only supplies nutrients constantly

Figure 10. Effect of inoculum size on the growth of P. spartinae

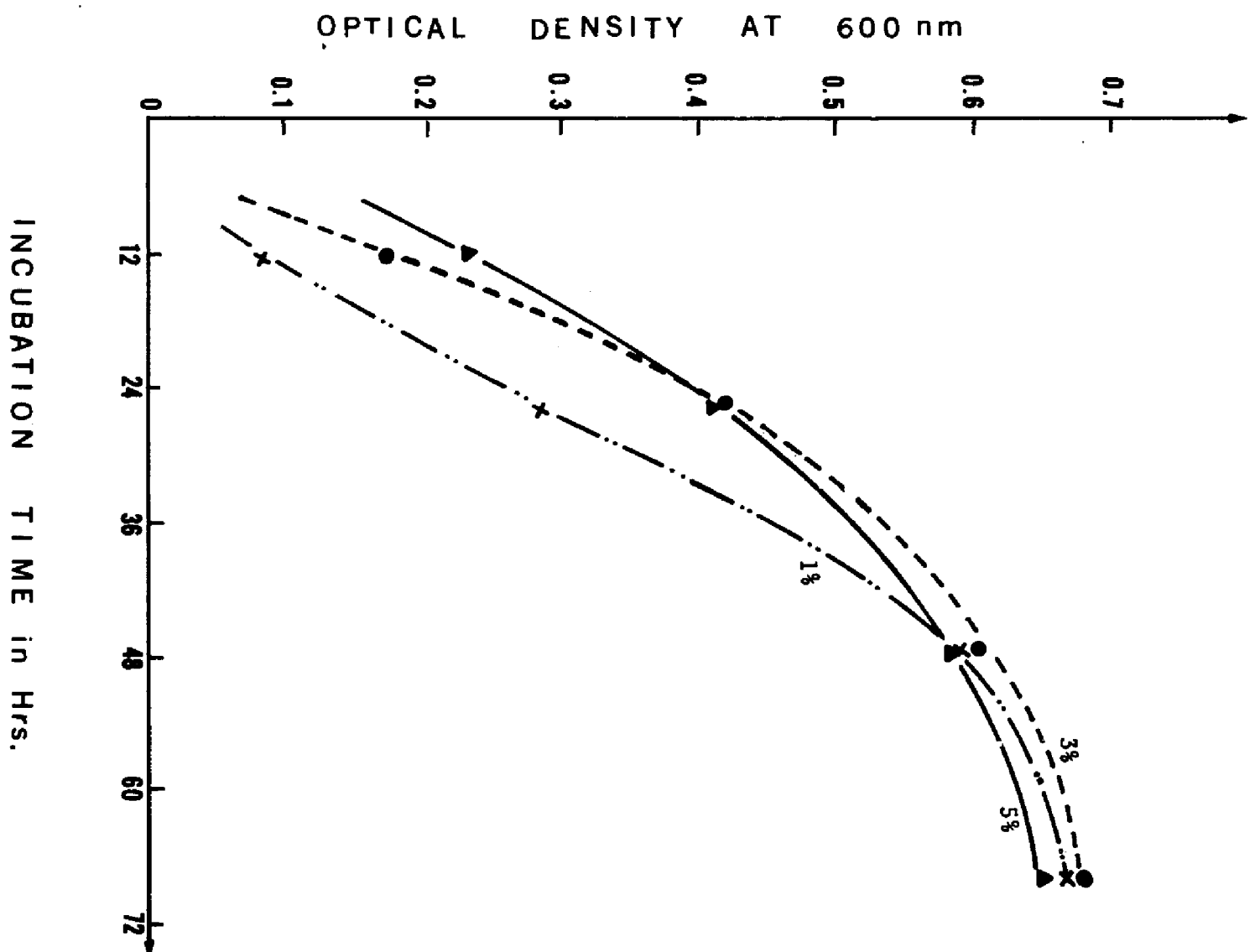


Table 11. Yields of yeast cell mass and yeast protein in the optimal growth condition

Growth condition	Substrate concentration	Total sugar in medium	pH at harvest	Yield of cell mass		Yield of yeast protein	
				(g/500 ml)	(%)*	(g/500 ml)	(%)*
Sterile condition, A degree aeration, small air size, supplement of YE, no pH adjustment	1% RPL	5.46 g	2.5	2.887	52.88	1.126 (2.25 mg/ml)	20.62
	2.5% RSL	5.50 g	7.45	3.188	57.96	1.24 (2.49 mg/ml)	22.60

*Percent yield of cell mass and protein based on the total sugar in the medium.

to the fermentor, but also prevents rapid depression of pH which may partially restrict growth. Therefore, it is reasonable to conclude that the yield can be increased considerably.

Protein Content of *P. spartinae*

Production of yeast protein was correlated with the specific nitrogen compound used in the culture medium. When *P. spartinae* was grown in ammonium sulfate, the cellular protein level was about 33%, but was approximately 47% when yeast extract was used (Table 12). The combination of yeast extract and ammonium sulfate increased the protein level from 33.2 to 39.0%. When *P. spartinae* was grown in the soluble portion of banana skin as a substrate (RSL) the protein of the cell mass was higher in value than in cells from the soluble portion of banana pulp (RPL). The concentration of ammonium sulfate used in the culture medium did not appreciably affect production of yeast protein (Table 13).

The protein content of certain food yeasts has higher values than that found in this study. The protein content ($N \times 6.25$) of *Saccharomyces cerevisiae* from molasses was 50%; debittered brewer's yeast (*Saccharomyces uvarum*), 45%; sulphite-grown *Candida utilis*, 50%; *Candida utilis* from cane molasses, 51%; *Saccharomyces fragilis* from whey, 54%. Since protein content was calculated by multiplying total nitrogen by 6.25, the figure obtained for protein must consider other nitrogenous compounds such as purine, pyrimidine, nucleic acid, amino sugars, etc., in addition to true protein. Therefore, the amino acid profile of the yeast protein is more important than the protein percent itself. This subject of amino acid content takes on considerable importance if yeasts are to be utilized as an inexpensive source of protein to supplement

Table 12. Protein content of Pichia spartinae grown in various media

Medium	Protein content (dry basis)	Mean value
1.5% RPL + 0.2% $(\text{NH}_4)_2\text{SO}_4$	32.63% 33.71%	33.17%
1.5% RPL + 0.2% $(\text{NH}_4)_2\text{SO}_4$ + 0.1% Yeast Extract	39.52% 38.45%	38.99%
1.5% RPL + 0.25% Yeast Extract	46.819%	46.819%
3.0% RSL + 0.2% $(\text{NH}_4)_2\text{SO}_4$	45.885% 42.146%	44.02%

Table 13. Effect of concentration of nitrogen in the growth medium on the production of yeast protein

Concentration of nitrogen	% protein
0.1% $(\text{NH}_4)_2\text{SO}_4$	31.6
0.2% $(\text{NH}_4)_2\text{SO}_4$	32.4
0.3% $(\text{NH}_4)_2\text{SO}_4$	32.3
0.5% $(\text{NH}_4)_2\text{SO}_4$	33.8
1.0% $(\text{NH}_4)_2\text{SO}_4$	31.9

Substrate: 3% blended fresh banana pulp was used instead of 1.5% RPL.

Harvest: 4th day of growth.

poor quality proteins or to serve as components of protein-rich foods. The amino acid content of P. spartinae grown in basal medium supplemented with yeast extract is reported below.

Amino Acid Profile of P. spartinae

The amino acid content of the cell protein of P. spartinae grown in banana wastes was determined and expressed as a percentage of the total crude protein. The values obtained were compared with that of FAO reference protein along with proteins of other single-cell proteins from hydrocarbons and petroleum. As shown in Table 14, the essential amino acid pattern of the cell protein compares favorably with that of FAO reference protein. The lysine content, which is limiting or deficient in a number of foods, particularly in cereal grains, was higher than the reference protein. This suggests the possibility of supplementation of lysine-deficient substrates with microbial protein of yeast origin. The amino acid content of P. spartinae grown in banana wastes was comparable to that of SCP produced from hydrocarbon and petroleum. It is reasonable to assume that methionine would be the first limiting amino acid, and that supplementation by methionine would raise the overall biological value of the yeast protein.

Fermented Banana as an Animal Feed

As mentioned earlier in the literature review, production of bananas on a world-wide basis exceeds the current market need for bananas in hand. Large volumes of cull bananas are wasted. For example, in Panama about 200 million lb of bananas are wasted annually, though some use is made for cattle and pig feeding. The bananas, bruised and cut during harvesting and handling, are left in dumps to rot. The

Table 14. Amino acid content of the cell protein (grams of amino acid per 100 g protein)

Amino acid	<i>P. spartinae</i> cell protein	FAO reference protein ^a	B.P. protein ^b	Torula yeast ^c
Arginine	5.2		5.0	4.4
Histidine	3.3		2.1	2.1
Isoleucine	4.2	4.2	5.3	4.5
Leucine	7.4	4.8	7.8	7.1
Lysine	6.9	4.2	7.8	6.6
Methionine	1.1	2.2	1.6	1.4
Phenylalanine	4.1	2.8	4.8	4.1
Tyrosine	6.5	2.8	4.0	3.3
Threonine	3.6	2.8	5.4	5.5
Valine	5.4	4.2	5.8	5.7
Aspartic acid	11.1			8.8
Serine	4.9			4.7
Glutamic acid	16.0			14.6
Proline	3.1			3.4
Glycine	4.6			4.5
Alanine	7.2			5.5

^aNational Academy of Science-National Research Council.

^bAdapted from Dabbah (1970).

^cA protein derived from a yeast grown on petroleum (Anderson et al., 1974).

utilization of these whole waste bananas is significant from an economic point of view. Therefore, a fermentation process for converting these to protein-rich cattle feed was attempted.

Fresh whole bananas, including peels, were blended in 0.8% NaCl solution (with the ratio of banana to NaCl solution, 1:2 and 1:3) with 0.2% ammonium sulfate added for use as a culture medium for P. spartinae. Rationale for such fermented animal feed substrate can be noted.

Currently, animals are raised on low-protein grasses or rejected bananas and require four or five years to reach market weight of 100 lb (Reese, 1975). Therefore, the protein-rich fermented bananas used for an animal feed can be more effectively used for rapid growth of animals in a relatively short period of time. The percent protein content of fermented bananas compared with non-fermented bananas is given in Table 15. Protein increased about three times in the fermentation process. Since the fermented banana contains increased levels of protein, this may prove to be an excellent animal feed. However, actual feeding tests will have to be conducted to determine its total value as a feed ingredient.

Identification of Possible By-Products Produced in Culture Media by Gas Chromatograph

Very pleasant ester-like aromas were associated with growth of P. spartinae in banana-based media. Therefore, possible production of economically valuable by-products of the yeast in banana medium was explored. Alcohols and fatty acids in culture medium were analyzed by gas chromatograph. A mixture of standard pure alcohols (Figure 11) and a mixture of standard methylated fatty acids, containing C_6 - C_{16} (Figure 12), were chromatographed to aid in identification of sample

Table 15. Protein content of fermented banana for animal feed

Medium	Protein content (dry basis)	Mean value
Control (whole blended banana without fermentation)	9.37% 9.54%	9.46%
Fermented Banana--I ^a	28.49% 29.02%	28.76%
Fermented Banana--II ^b	27.86% 27.57%	27.71%

^aThe blended whole banana including peels and pulps was diluted with 0.8% NaCl solution (2x volume of blended bananas), and 0.2% $(\text{NH}_4)_2\text{SO}_4$ was added.

^bThe blended whole banana was diluted with 0.8% NaCl solution (3x volume of blended banana), and 0.2% $(\text{NH}_4)_2\text{SO}_4$ was supplemented.

Both were harvested at 48 hr.

Figure 11. Gas chromatograph of standard alcohols

- 1 - ethyl alcohol
- 2 - sec-butanol or t-amyl alcohol
- 3 - iso-butanol
- 4 - n-butanol
- 5 - iso-amyl alcohol
- 6 - propionic acid
- 7 - iso-butyric acid

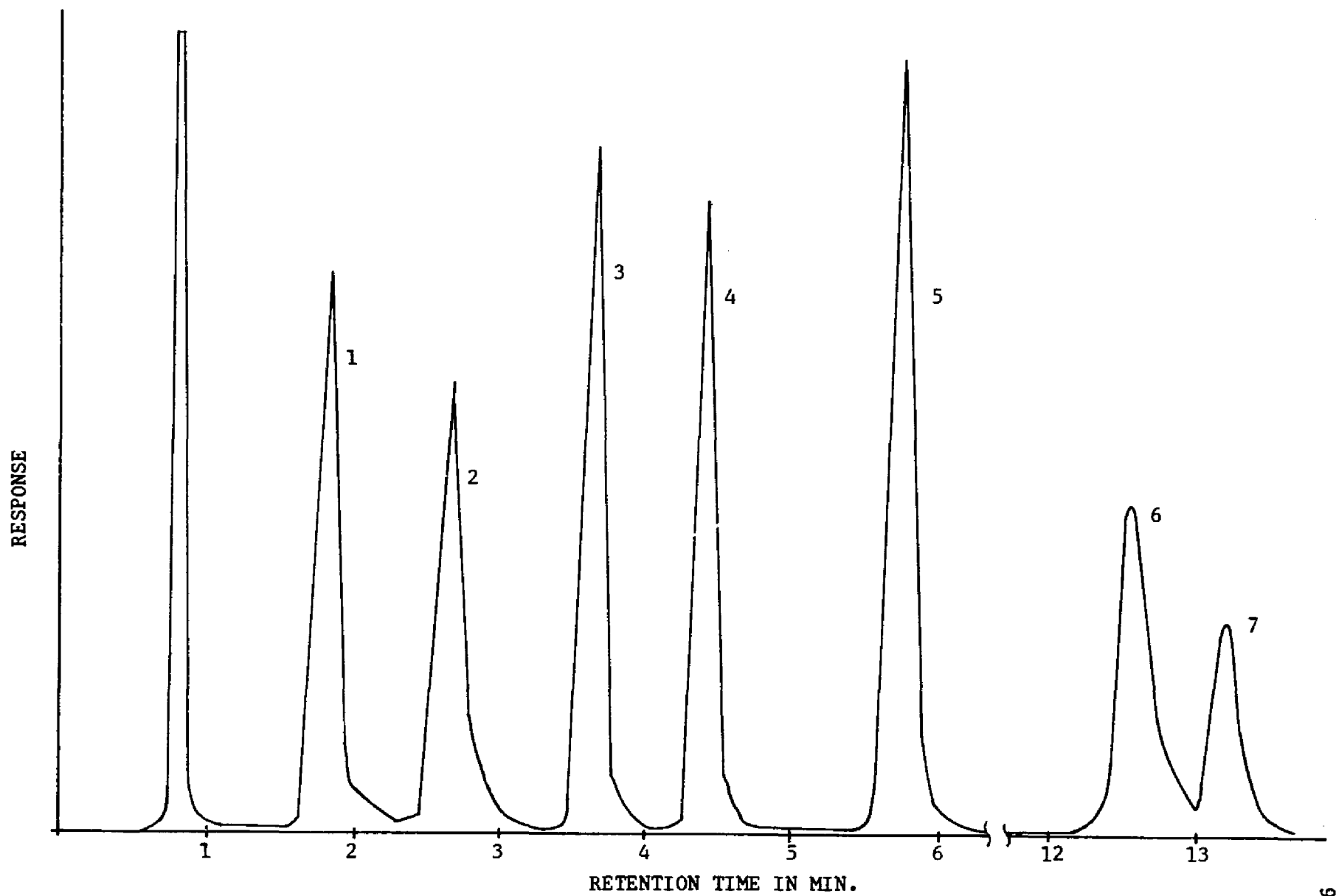
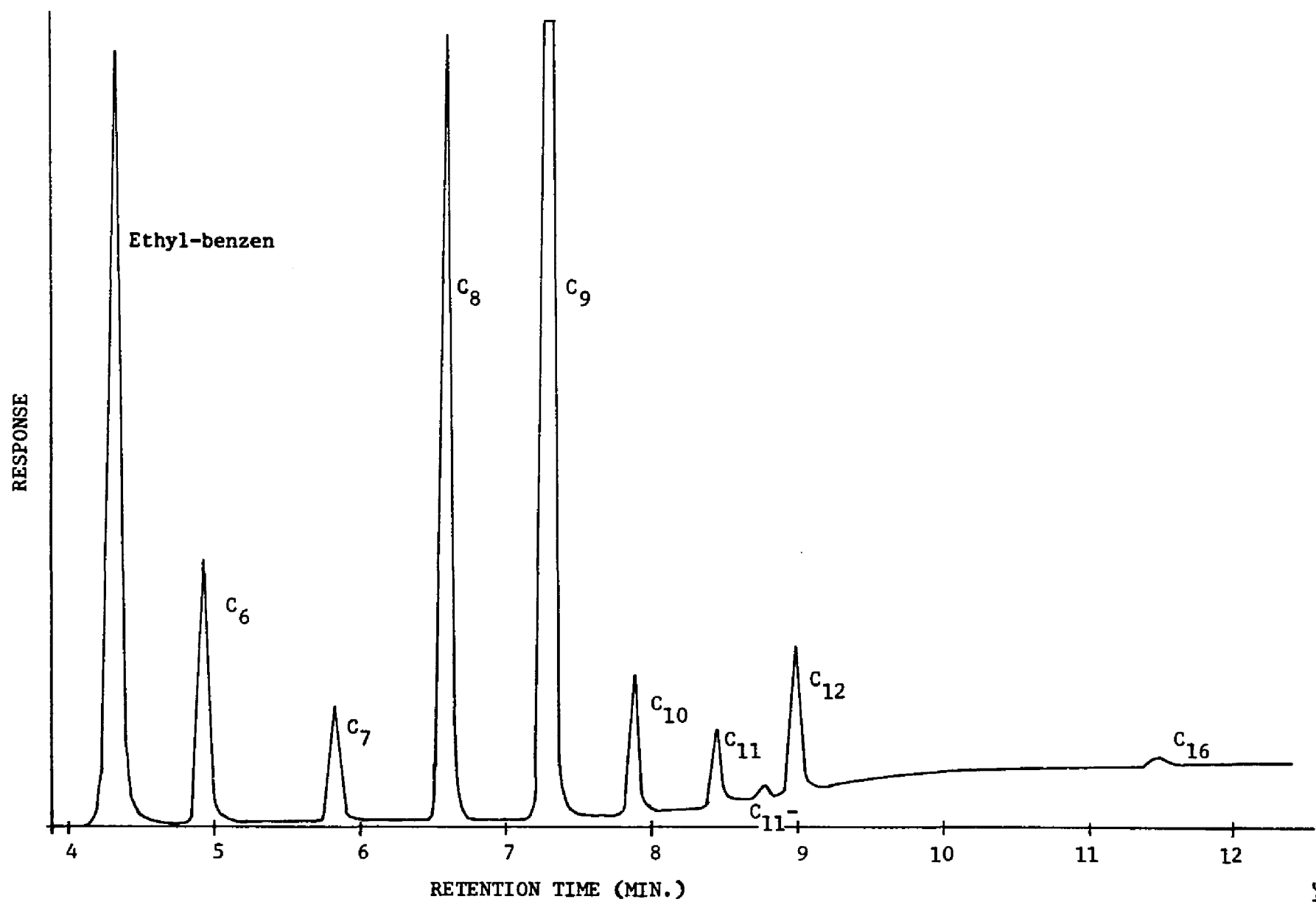


Figure 12. Gas chromatograph of standard methylated fatty acids
(injection volume = 1 μ l x 128 x 10)

- C₆ - methyl caproate
- C₇ - methyl heptanoate
- C₈ - methyl caprylate
- C₉ - methyl nonanoate
- C₁₀ - methyl caprate
- C₁₁ - methyl undecanoate
- C₁₁ - methyl undecylenate
- C₁₂ - methyl laurate
- C₁₆ - methyl palmitate



peaks. Ethyl alcohol, sec-butanol, iso-butanol, n-butanol, iso-amyl alcohol, iso-butyric acid and palmitic acid were identified from the RPL medium (Figures 13 and 14), and sec-butanol, n-butanol, propionic acid and caprylic acid were identified from the RSL medium (Figures 14 and 15).

As noted in Figures 13, 14 and 15, all alcohol and fatty acid peaks were small, except for the ethyl alcohol peak in RPL and sec-butanol peak in RSL media. Since the ethanol peak was the highest, possible production of ethanol by P. spartinae was examined. The organism was grown in 3.0% RPL and 0.2% ammonium sulfate for 48 hr, following which 50% and 100% (v/v) of the original fresh banana broth was added. The fermentor jar was closed tightly to maintain anaerobic conditions. Ethanol production was determined at 24 and 48-hr periods. Results are given in Figure 16 and Table 16. To determine concentration of ethanol produced, sample peak areas were compared with peak areas of a known amount of an ethanol standard. Peak areas were calculated by multiplying heights by width at 1/2 peak heights. As noted (Table 16) the amount of ethanol produced was small, i.e., percent yield of ethanol based on sugars in the medium was 1-2%. While it appears on a preliminary evaluation that P. spartinae is not a particularly outstanding ethanol producer, further work is needed to evaluate nutritional and physical parameters, i.e., pH, temperature, CO₂ pressure, etc., that may significantly affect total ethanol production. Other yeasts must be examined as ethanol producers as well as those yielding high cell crop which can be used for animal feed and possibly as human food.

Figure 13. Qualitative gas chromatograph of alcohol and volatile fatty acid in RPL medium ($2 \mu\text{l} \times 4 \times 10$)

- 1 - ethyl alcohol
- 2 - sec-butanol (or t-amyl alcohol)
- 3 - iso-butanol
- 4 - n-butanol
- 5 - iso-amyl alcohol
- 6 - propionic acid
- 7 - iso-butyric acid

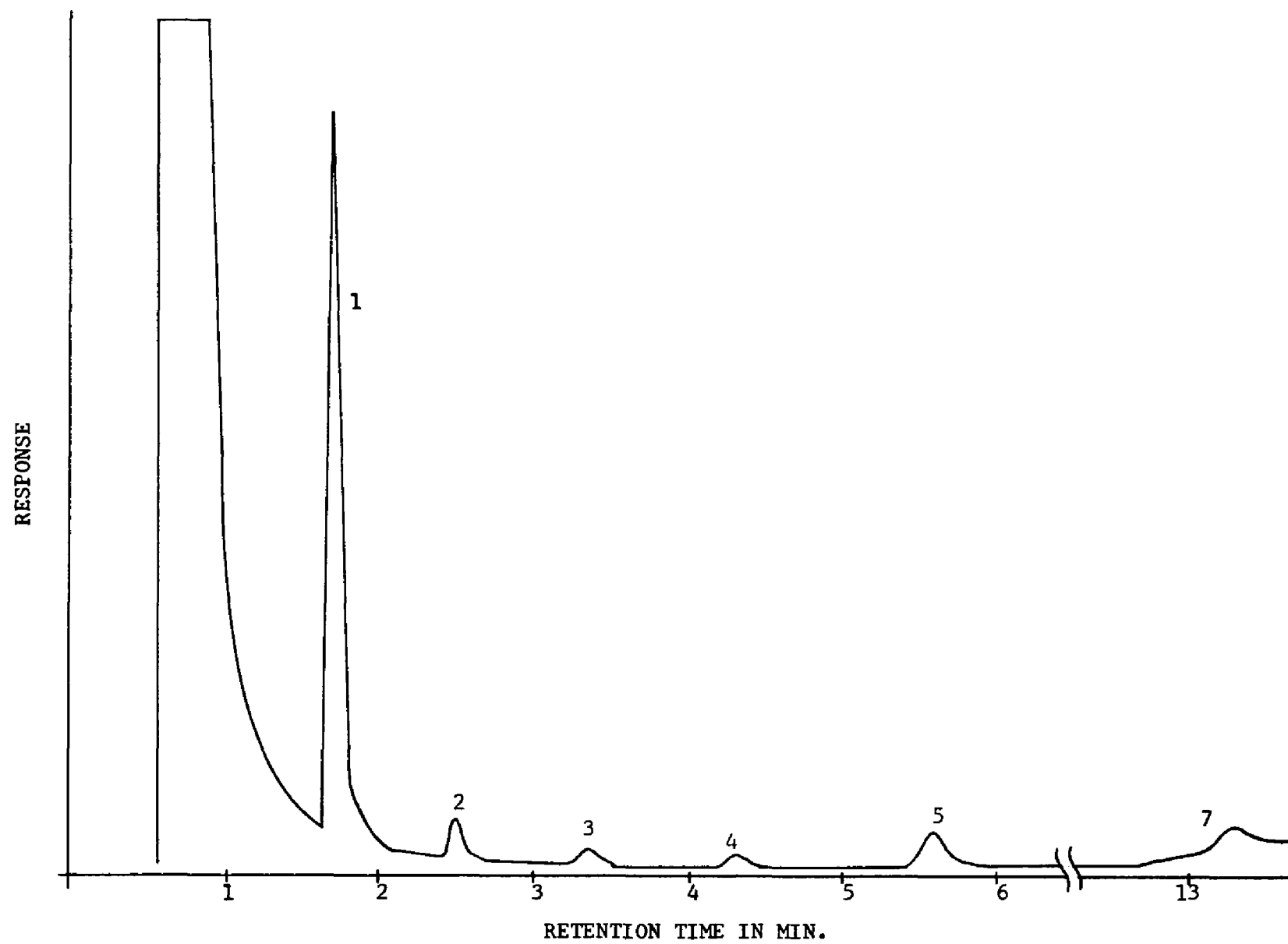


Figure 14. Qualitative gas chromatography of fatty acids in RPL and RSL growth media

(RPL - 1 μ l x 64 x 10)

(RSL - 1 μ l x 128 x 10)

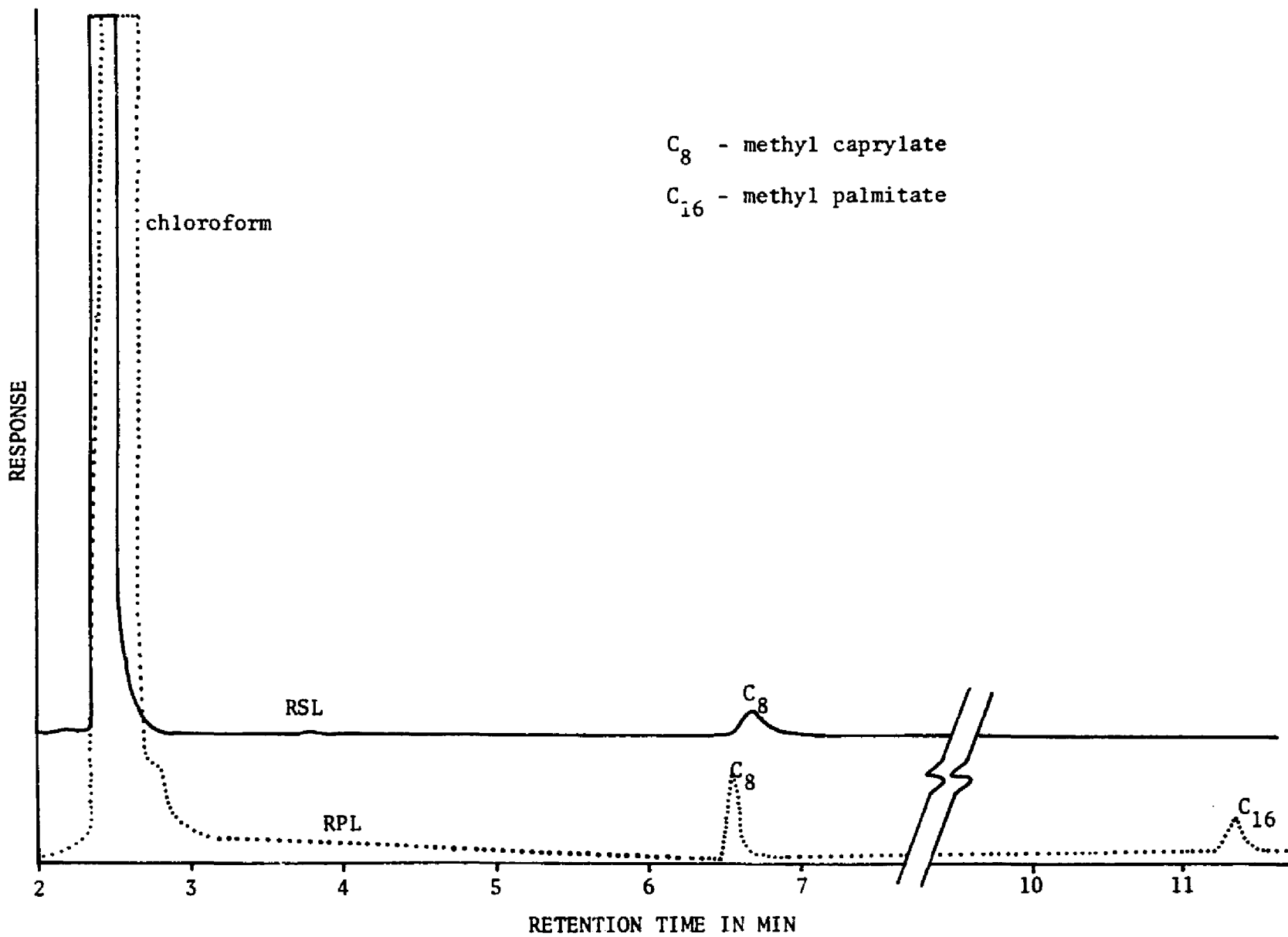


Figure 15. Qualitative gas chromatograph of alcohols and volatile fatty acids in RSL growth medium

(2 μ l x 4 x 10)



Figure 16. Quantitative determination of ethyl alcohol content in growth medium in which Pichia spartinae was grown

- 1 - standard ethyl alcohol, 1 μ l x 512
- 2 - 10X-24, 1 μ l x 64
- 3 - 10X-48, 1 μ l x 64
- 4 - 5X-24, 0.5 μ l x 64
- 5 - 5X-48, 1 μ l x 128

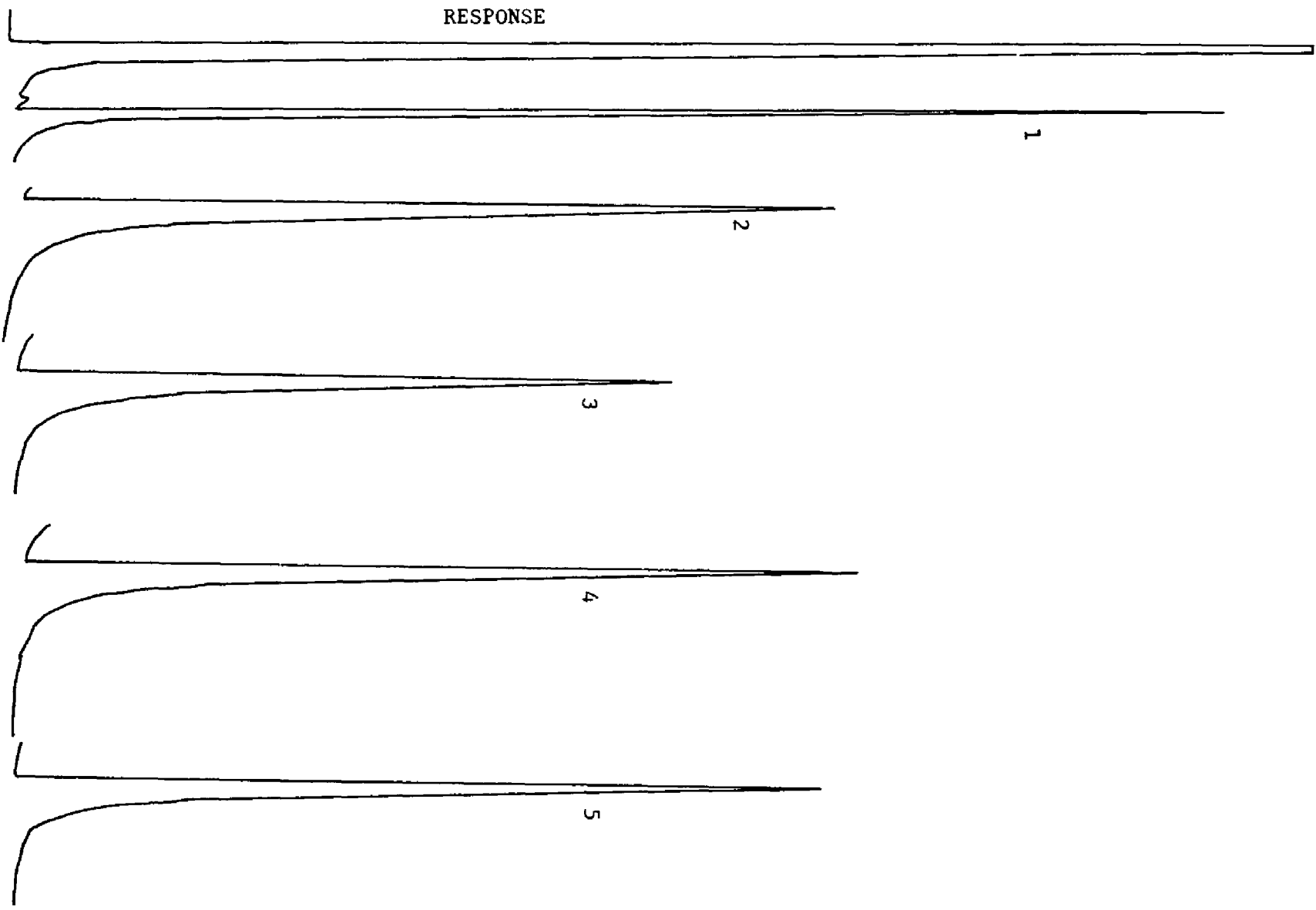


Table 16. Quantitative determination of ethyl alcohol production by gas chromatography

Sample	Volume obtained from 20-ml sample	Injection volume (μ l)	Attenu- ation	Peak height (cm)	Peak width at 1/2 height (cm)	Peak area (cm^2)	Concen- tration (μ l/ μ l)	(ml/l) *	% yield based on sugar
Standard (Et-OH)		1	512 x 10	20.1	0.06	1.206	0.02		
10X-24 ^a	2.0 ml	1	64 x 10	13.5	0.21	2.835	0.00588	0.588	1.546
10X-48 ^b	2.0 ml	1	64 x 10	10.9	0.20	2.18	0.00452	0.452	1.189
5X-24 ^c	1.5 ml	0.5	64 x 10	13.8	0.21	2.898	0.01202	0.901	2.371
5X-48 ^d	1.0 ml	1	128 x 10	13.4	0.19	2.546	0.01056	0.528	1.389

^a10 times fresh medium was added to the inoculum and harvested at 24 hr.

^b10 times fresh medium was added to the inoculum and harvested at 48 hr.

^c5 times fresh medium was added to the inoculum and harvested at 24 hr.

^d5 times fresh medium was added to the inoculum and harvested at 48 hr.

*ml of alcohol/liter of culture medium.

SUMMARY AND CONCLUSION

Bananas are produced in every tropical Central and South American country and are the major export cash crop in many of these regions. The majority of export fruit is grown on company plantations or by large private landholdings and is cultivated with good agricultural practices. The perishable nature of the fruit has necessitated the development of rapid and efficient transportation systems from field to packing house and from packing house to dock.

In spite of the attention bananas receive, almost 25% of the total crop is rejected at the packing house as unsuitable for shipment. Almost all of these rejects are from surface blemishes which have not damaged the pulp at all. Such rejects are usually hauled to one or more central "landfills" and dumped. Waste bananas are also available during cutting and handling. Furthermore, surpluses of bananas have occurred largely because world production has expanded at a faster rate than actual consumption of the fresh fruit. Utilization of this reject fruit has been a great problem in most banana-producing countries, and banana waste serves as a ready substrate for production of SCP by fermentation. The chemical composition of the reject or waste bananas varies with the stage of ripeness. Bananas to be exported are picked green and at this stage, the carbohydrate content constitutes almost entirely starch (approximately 21% of the weight of the pulp which contains about 24% dry matter; 3% of the weight of the skin), and the peel is about equal in weight to the pulp. As the fruit ripens the pulp-to-peel ratio

increases to about 3:2 (60% pulp, 40% peel) and the starch undergoes natural hydrolysis to sugars. The major sugars produced are glucose, sucrose and fructose. A material balance on 100 lb of fresh, ripe banana pulp would typically give 70-75 lb water, and 25-30 lb dry matter (23-27 lb carbohydrate, 1-1.2 lb crude protein, and 1-2 lb ash). In this study, a material balance on 100 lb of fresh, ripe banana pulp and skin gave 15 lb RPL (10.9 lb total sugar as hexoses) and 4.5 lb RSL (1.98 lb total sugar as hexoses), respectively.

In addition to the waste banana fruit, the banana pseudostem (stalk), which is a source of high quality starch (Shantha and Siddappa, 1970a,b), is also a waste material after the fruit is harvested. Possible use of the pseudostem starch (as a waste material), along with whole bananas including skin and pulp, as a substrate for microorganisms to produce a feed-grade SCP by existing local technology is economically important.

The proximate composition of banana substrates (RPL and RSL) for growth of P. spartinae, and optimal growth conditions for production of maximal yeast cell mass, were determined. The total sugar content, expressed as hexoses, in RPL and RSL was 72.8 and 44.0%, respectively. Protein content of RPL and RSL was 3.6 and 3.1%; ash content of RPL and RSL, 4.3 and 28.6%; phosphate content of RPL and RSL, 0.184 and 0.6%, respectively. Both substrates contained sufficient phosphate and vitamins for yeast growth. Therefore, no supplementation of nutrients, except for a nitrogen source, is necessary for growth of P. spartinae. Several nitrogen compounds, i.e., ammonium sulfate, ammonium chloride, ammonium nitrate, sodium nitrate, potassium nitrate and urea, were tested. Based on growth response, ammonium sulfate was selected as the

most suitable nitrogen compound for P. spartinae. However, yeast extract was a better nitrogen source than ammonium sulfate for yeast growth.

Growth studies of P. spartinae have shown that the optimal time of harvest for maximal cell biomass is around 36 hr at room temperature and about 24 hr at 35°C. The optimal temperature for yeast development was 20-25°C, however, the initial growth rate was a little higher at 30-35°C than at 20-25°C. The higher initial growth rate at 30-35°C is significant from an economic standpoint, in terms of cooling costs, and development of yeast cell mass in relatively short periods of time. P. spartinae grows equally well over a broad pH range from 4.0 to 7.0. This broad range is probably due to a decrease in pH of the medium caused by continual production of metabolic acid during growth of P. spartinae. Therefore, the optimal pH of the medium, in all likelihood, may be 4.5-5.5. The latter range is preferred in most industrial type operations in order to minimize the possibility of growth of any bacterial contaminants while still maintaining good growth rates. By operating at a low pH, contamination is minimized, therefore, expensive aseptic equipment or elaborate techniques may not be necessary.

The maximum yields of yeast cell mass under optimal growth conditions were 53% from RPL and 58% from RSL substrates. The pH was not adjusted under these conditions, however, when the pH was adjusted, the yield was improved considerably. P. spartinae was also grown under non-sterile conditions and yields were compared to those obtained in sterile media. Four media, one which had not been heat treated, and three which were pasteurized at 60, 80 and 100°C for 15 minutes were used as representatives of non-sterile conditions. The yields in the sterile medium

were much higher than that in the pasteurized media and in that of the media without heat treatment. A positive correlation between yield of yeast cells and treatment temperature was noticed. The heat treatment apparently altered the medium, in that the sucrose and other non-utilizable carbohydrates were hydrolyzed, liberating amounts of glucose which is more readily utilized by the yeast.

The dried yeast has a crude protein of 33% in basal medium, 39% when supplemented with yeast extract, and 47% when yeast extract was used as a sole source of nitrogen. The protein shows an excellent amino acid profile compared with that of FAO reference protein and high in lysine, although somewhat low in the sulfur-containing amino acids.

The waste whole bananas may be used for development of a protein-rich fermented substrate for animal feed. Currently, animals are raised on low-protein grasses or rejected bananas and require four or five years to reach market weight of 1,000 lb (Reese, 1975). Therefore, the protein-rich fermented bananas can be used for rapid growth of animals in a relatively short period of time. Cooney and Dunlap (unpublished data) have suggested the utilization of waste bananas as a possible source of feed in the Panama animal industry. A fermentation process was proposed for converting waste bananas to protein-rich cattle feed. In the current study, protein of the fermented whole banana increased about three times during fermentation, i.e., from 9.5 to 28.0%.

Electron-capture gas chromatographic studies indicated that ethyl alcohol, sec-butanol, iso-butanol, n-butanol, iso-amyl alcohol, iso-butyric acid and palmitic acid were present in RPL growth medium, and sec-butanol, n-butanol, propionic acid and caprylic acid were present in RSL medium. While the quantitative determination of ethanol

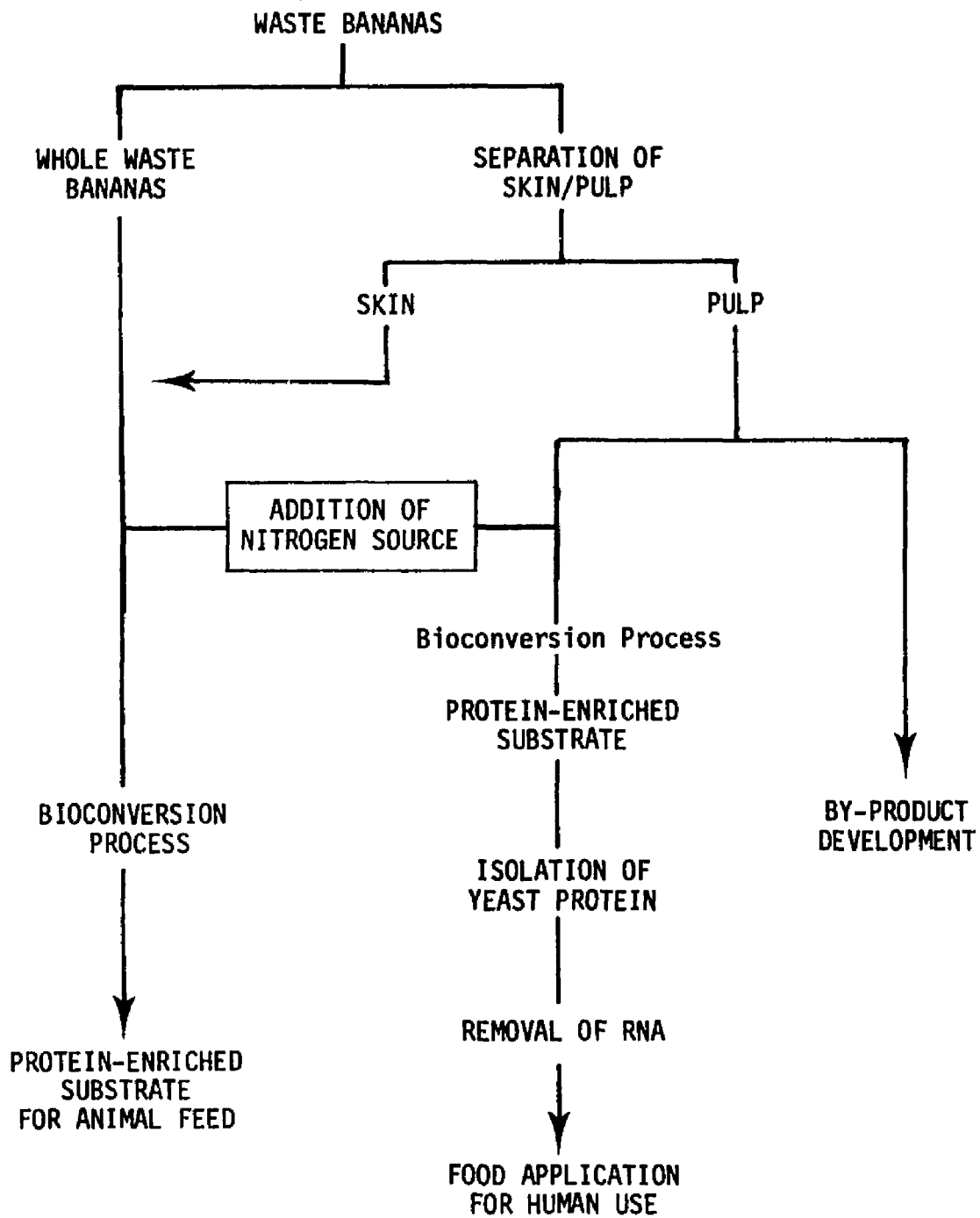
production on preliminary evaluation suggests that P. spartinae is not a noteworthy ethanol producer, further work is needed to evaluate nutritional and physical parameters that may significantly affect total ethanol production. Furthermore, other yeasts must be examined as potential ethanol producers as well as those yielding a high cell crop which can be used as animal feed or possibly human food.

The commercial development of microbial proteins from banana wastes offers distinct advantages over the manufacture of SCP from petrochemicals. First, conversion of hydrocarbons into SCP requires knowledge of an advanced technology of petroleum refining, which is at present lacking in third world countries, where the need for protein is imperative. In spite of the great strides in fermentation of hydrocarbons in the last decade, the process faces a number of problems because of the immiscibility of the substrates in water. Hydrocarbons must be adequately dispersed in the medium so that the organisms come into contact with the substrate during growth. In contrast, banana substrates are readily soluble in the growth medium and the problem of contact of the yeast with the substrate is not encountered. Since the hydrocarbons are in a lower state of oxidation than the usual carbohydrates, oxygen requirements in such systems are high. Use of banana substrate does not entail separation or solvent removal and requires less agitation and cooling than hydrocarbon fermentation. Hydrocarbons release more heat per unit of biomass than other substrates and need greater cooling capacity. Furthermore, there is a possible carcinogenic risk involved in hydrocarbon-grown SCP, this being an important factor in terminating the rapid Japanese development of SCP (Anonymous, 1973).

The cost of production of yeast protein is primarily dependent on the cost of the substrate and the capital investment. In calculating the actual cost of such substrates, one must include all associated costs such as collection, transportation, purification or separation, and sterilization. Since tremendous amounts of waste bananas are available in banana-producing countries, the cost of the raw material will be low or negligible. Transportation costs can be minimized if a pilot plant could be established near banana plantations and packing houses where most waste bananas are available. Since banana substrates do not need purification and separation, preparation costs would be minimal compared with use of hydrocarbons or other wastes. When cellulose is used as a substrate, pre-treatment, i.e., acids, alkalis and size reduction, is necessary to improve enzymatic hydrolysis rates. The direct fermentation processes of native, insoluble cellulose for generation of protein containing biomass are slow, and cellulose utilization is relatively low, making these processes economically questionable as well.

In summary, the fermentation process discussed here to convert banana wastes to yeast protein is relatively simple, especially in that it does not require sophisticated equipment and highly advanced techniques. These considerations are of considerable significance in cases where incorporation of low-technology approaches is essential. Since the growth medium does not need nutrient supplementation except for ammonium sulfate or another suitable nitrogen source, and also a non-aseptic methodology for yeast growth is possible, the process may be technically feasible for large-scale application in banana producing countries. A possible schematic diagram illustrating approaches to utilization of banana wastes by fermentation is shown in Figure 17.

Figure 17. Schematic diagram for utilization of banana wastes by fermentation



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APPENDICES

APPENDIX 1

MAXIMUM MASS DOUBLING TIMES OF CERTAIN ORGANISMS

<u>Organism</u>	<u>Time to double the mass in hours</u>
Bacteria and Yeasts	0.3 - 2
Molds and Algae	2 - 6
Green Vegetables	170 - 350
Chicken	350 - 700
Hogs	700 - 1000
Cattle	700 - 1400

APPENDIX 2

COMPARISON OF PROXIMATE ANALYSIS AND VITAMIN CONTENT
OF DRY WHOLE MILK, FLUID WHOLE MILK, TORULA YEAST,
AND BRITISH PETROLEUM CONCENTRATE (B-P)^a

	P e r c e n t a g e			
	Dry whole milk	Fluid whole milk	Torula yeast	B-P protein*
Protein	26.5	3.5	50.0	43.6
Carbohydrates	38.0	4.8	30.0	21.9
Fat	27.0	3.8	2.0	18.5
Ash	6.5	0.9	7.0	4.4
Moisture	2.0	87.0	4.9	7.0
Vitamins (mcg/gm)				
Thiamine	3.4	0.44	150.0	3-16
Niacin	7.3	0.94	500.0	180-200
Biotin	0.3	0.031	1.0	-
Choline	862.0	121.0	2500.0	-
Cobalamine	3.9	0.64	0.003	0.11
Riboflavin	15.5	1.75	50.0	75.0
Pantothenic acid	7.4	3.46	100.0	150-192
Pyridoxine	3.9	0.64	35.0	23.0
Folic acid	0.018	0.0028	30.0	-

^aAdapted from Dabbah, 1970.

*Derived from a hydrocarbon-grown yeast.

APPENDIX 3

COMPARISON OF AMINO ACID COMPOSITION
OF PROTEINS FROM DIFFERENT SOURCES^a

Amino Acid	FAO reference protein	Meat	Bread	Milk	Torula yeast	B-P protein*
Arginine	-	7.5	4.0	4.28	7.22	5.0
Histidine	-	2.1	2.0	2.57	2.62	2.1
Isoleucine	4.2	3.3	3.5	4.28	7.50	5.3
Leucine	4.8	12.5	12.0	16.28	7.14	7.8
Methionine	2.2	4.2	4.0	4.0	1.68	1.6
Phenylalanine	2.8	4.6	5.5	5.71	4.82	4.8
Threonine	2.8	4.6	2.5	4.57	5.16	5.4
Tryptophan	1.4	1.3	1.0	1.71	1.32	1.3
Valine	4.2	3.3	3.0	5.43	5.96	5.8
Cystine	2.0	-	-	-	-	0.1

^aAdapted from Dabbah, 1970.

*Derived from a yeast grown on hydrocarbons.

APPENDIX 4

YEAST PRODUCTION IN THE UNITED STATES^a
(1968 estimate)

Product	Annual production (metric tons dry matter)	Sugar Sources
Baker's yeast		
Compressed	53,500	Molasses
Active dry	2,300	Molasses
Food yeast		
<u>Saccharomyces</u> sp.	14,100	Grain, molasses, whey
<u>Candida utilis</u>	1,000	Spent sulphite liquor
Feed yeast		
<u>Saccharomyces</u> sp.	9,500	Grain, molasses, whey
<u>Candida utilis</u>	1,800	Spent sulphite liquor
Autolysates, extracts	3,500	Grain, molasses
TOTAL	85,700	

^aAdapted from Peppler, 1968.

APPENDIX 5
EXPORTS OF BANANA PRODUCTS^a

Banana products	Major producers	Total amounts of exports in 1964
Banana Figs	Ecuador	1341 tons
	Madagascar	147 tons
	Angola	65 tons
Banana Powder	Brazil	89 tons
Banana Flour	Ecuador	760 tons (proposed plant)
Banana Flakes	Israel	----
	United States	----
Banana Puree	Dominican Republic	3872 tons
Canned Banana Slices	Jamaica	limited quantities
	Australia	
	Republic of South Africa	
	India	
Banana Chips	several countries	----
Banana Jam	Brazil (for local sale)	small quantities
Freeze-dried Banana Slice	United States	----

^aAdapted from Kay, 1967.

---- No data available

APPENDIX 6

WORLD PRODUCTION OF BANANAS (1000 metric tons)

	1948-1952	1961-1965	1966	1967	1968	1969	1970
Europe	242	372	422	409	425	471	362
North America	4	4	4	5	4	4	4
Latin America	7,154	13,125	14,409	15,555	15,873	16,976	17,862
Near East	68	154	196	176	176	202	193
Far East	3,863	5,733	8,053	7,552	7,393	7,471	7,530
Africa	947	1,434	1,425	1,553	1,507	1,745	1,930
Oceania	112	165	150	161	144	156	153
World Total	12,500	21,139	24,829	25,581	25,692	27,198	28,214

Source: FAO Production Yearbook, 1971.

APPENDIX 7
FATTY ACIDS OF BANANAS^a
(mg/10 g dr.wt.)

Acid	Pulp		Peel	
	Unripe	Ripe	Unripe	Ripe
14:0	0	0	1.35	1.43
15:0	0.33	Trace	0	0
16:0	10.89	11.92	56.30	62.80
16:1	2.21	0.84	0	0
16:2	1.16	Trace	0	0
18:0	0.63	1.68	7.32	6.46
18:1	4.44	4.08	8.70	9.50
18:2	12.85	4.88	38.00	26.70
18:3	6.08	6.84	19.80	18.40
Total sat.	11.85	13.60	64.97	70.69
Total unsat.	26.47	16.64	66.50	54.60

^aFrom Goldstein and Wick (1970).

APPENDIX 8

SENSORY IMPRESSIONS OF MAJOR BANANA VOLATILES^a

Banana-like	Fruity	Green, woody or musty
Isoamyl acetate	Butyl acetate	Methyl acetate
Amyl acetate	Butyl butyrate	Pentanone
Amyl propionate	Hexyl acetate	Butyl alcohol
Amyl butyrate	Amyl butyrate	Amyl alcohol
		Hexyl alcohol

^aFrom McCarthy et al. (1963).

APPENDIX 9

CALCULATION OF THE MAXIMUM YIELD OF S. CEREVISIAE
FROM GLUCOSE

Maximum yield of microbial biomass from 1 mole substrate (Y_m)

$$= \frac{10.5 M \times C_s}{M/E \times C_s + 10.5 C_m} \dots\dots\dots (1)$$

Applying the above equation to the production of S. cerevisiae cells from glucose,

Molecular weight of glucose (M)	= 180 g
Carbon content of glucose (Cs)	= 40%
Carbon content of yeast cells (Cm)	= 47% (White, 1954)
Number of moles ATP per mole of glucose (E)	= 38

$$\therefore \text{Yield of yeast cells } (Y_m) = \frac{10.5 \times 180 \times 40}{180/38 \times 40 + 10.5 \times 47} = 111 \text{ g}$$

The maximum yield of yeast from glucose will therefore be

$$111/180 \times 100 = 61.7\%$$

APPENDIX 10

GROWTH CHARACTERISTICS OF SELECTED YEASTS*

Organism	Substrate	Temperature (°C)	pH	Scale aeration & agitation	Generation time (hr)	Cell density (g/l) (dry wt)	Cell yield g (dry wt) per 100 g substrate
<u>Candida</u> <u>intermedia</u> HD-5	glucose	30	5.5	1-1 vessel	2.0	-	35.4
<u>Candida</u> <u>utilis</u>	sulphite waste liquor	32	5.0	0.50 cfm	2.0	-	39.2 ^a
<u>Rhodotorula</u> <u>gracilis</u>	glucose (corn sugar) (20 g/l)	32	4.5	18-1 vessel 3 g 2/l/hr	2.0	8.49	61.1
<u>Saccharomyces</u> <u>fragilis</u>	cheese whey	32	5.5-5.7	1600 gal tank 15 mM O ₂ /gal/min	1.5	14.6	55.0 ^b
<u>Saccharomyces</u> <u>cerevisiae</u>	cane molasses (1.0 g reducing sugar/l)	30	4.5-5.0	50,000 gal tank 2 nM O ₂ /l/min	2.5	-	23.2 ^c

*Adapted from CRC Critical Reviews in Food Technology, Vol. 1(4), 1970.

^abased on total sugar

^bbased on lactose

^cbased on reducing sugar

APPENDIX 11

FACTORS INFLUENCING THE CHOICE OF MICROORGANISM
FOR SCP PRODUCTION

I. Economic and Technological Factors

- Yield of protein per unit of substrate
- Cost of substrate
- Cost of nutrients other than carbon substrate
 - Oxygen
 - Minerals
 - Vitamins and other growth factors
- Productivity (mass of protein per unit volume per day)
- Cost of sterilization
- Recovery cost, type of recovery operation

II. Nutritional Factors

- Amino acid pattern
- Protein digestibility
- Effects of extraneous materials
 - Cell wall
 - Nucleic acid
 - Other
- Protein content

III. Food Technological Factors

- Flavor
- Texture
- Solubility
- Color
- Possible processing to improve nutritional, organoleptic, or technological qualities

APPENDIX 12

PROXIMATE CHEMICAL COMPOSITION OF THE FULLY RIPE BANANA
(YELLOW SKIN FLECKED WITH BROWN)

	<u>Percent</u>	<u>Dry wt bases</u>
Moisture	75.6	
Total sugars	19.9	81.56%
Dextrose	4.5	18.44%
Levulose	3.5	14.34%
Sucrose	11.9	48.77%
Starch	1.2	4.92%
Crude fiber	0.6	2.46%
Protein (N x 6.25)	1.3	5.33%
Fat	0.6	2.46%
Ash	0.8	3.28%
Ca	0.009	
Cl	0.125	
Cu	0.0002	
Fe	0.0006	
Mg	0.028	
Mn	0.0008	
P	0.0310	0.127%
K	0.401	
Si	0.0238	
Na	0.0348	
S	0.010	

APPENDIX 13

β-GLUCOSIDASE ACTIVITY OF PICHIA SPECIES
ON BANANA PEEL SUGARS AND SUCROSE

Species of <u>Pichia</u>	Specific activity ^a (enzyme unit/mg protein)	
	Banana broth	Sucrose
<u>P. spartinae</u>	0.495	0.495
<u>P. guilliermondii</u>	0.495	0.304
<u>P. angophorae</u>	0.521	0.314
<u>P. ohmeri</u>	0.261	0.248
<u>P. etchellsii</u>	0.810	0.568
<u>P. pseudopolymorpha</u>	0.539	0.264
<u>P. wickerhamii</u>	0.478	0.284
<u>P. toletana</u>	0.670	0.396

^aOne unit of enzyme = amount necessary to hydrolyze 1 mole of p-nitrophenyl-D-glucoside (PNPG) per minute (see Meyers et al., 1975).

VITA

Soomi Lee Chung was born on June 30, 1943, in Seoul, Korea. She was graduated from Ewha girls' high school in February 1961 and entered Sung Kyun Kwan University in March of the same year. In February 1965 she received her Bachelor of Science degree in Chemistry.

She was married in September 1966 and came to the United States in 1968. She began graduate work in the Department of Food Science at Louisiana State University in September 1970 and received her Master of Science degree in December 1972. She continued her studies at Louisiana State University where, majoring in food science and minoring in nutrition, she is presently a candidate for the degree of Doctor of Philosophy.


EXAMINATION AND THESIS REPORT


Candidate: Soomi Lee Chung

Major Field: Food Science

Title of Thesis: Bioprotein from Banana Wastes

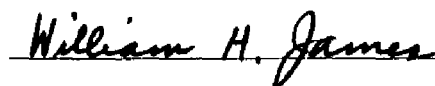
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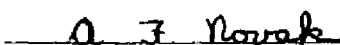

Major Professor and Chairman

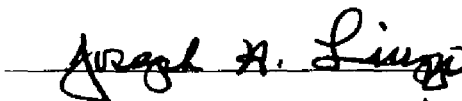

Dean of the Graduate School

EXAMINING COMMITTEE:











Date of Examination:

April 19, 1976