PRODUCTION OF MICROBIAL PROTEIN FROM BREWERY WASTES

THESIS FOR THE DEGREE OF M.S.
MICHIGAN STATE UNIVERSITY

BY
LYLE JOHN SHANNON

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ABSTRACT

PRODUCTION OF MICROBIAL PROTEIN

FROM BREWERY WASTES

By

Lyle John Shannon

Three brewery wastes, grain press liquor, trub press liquor and fermentation tank sludge liquor, were employed in a series of experiments for the production of single cell protein. Eight microorganisms were evaluated on the basis of their ability to grow, produce protein and reduce biochemical oxygen demand (BOD) in these wastes.

Preliminary analysis indicated that the press liquors contained relatively large concentrations of reducing sugars and only small amounts of nitrogen, while the converse was found to be true of sludge liquor. BOD values ranged from 32,000 mg/l in grain press liquor to 133,000 mg/l in trub press liquor.

Growth studies demonstrated that all the unsupplemented wastes were capable of supporting some degree of microbial growth, although considerable differences were observed. Trub press liquor was by far the best substrate. Of the microorganisms used, the yeast Candida steatolytica and the mushroom Calvatia gigantea were judged to be most suitable for further study.

As the yields and the cellular protein content of organisms grown on unsupplemented wastes were generally lower than those previously reported for similar organisms, experiments were conducted with C. steatolytica and C. gigantea using wastes supplemented with nitrogen

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and phosphorus. Both sludge liquor and ammonium sulfate were investigated as nitrogen sources, while potassium phosphate was used as a source of phosphate and a buffer.

Significant improvements in yield, protein concentration and BOD reduction were obtained on supplemented samples. Yields of up to 39.7 g/l of C. gigantea and 12.7 g/l of C. steatolytica were obtained. Cellular protein concentrations in the area of 44% were achieved for both organisms while BOD reductions up to 75% were observed for C. gigantea as compared with a maximum 54% reduction in BOD with C. steatolytica.

In general, best results were obtained at .05 to .10% added nitrogen. With respect to nitrogen source, BOD reduction was generally greater with ammonium sulfate, while protein production was greater in C. gigantea with sludge liquor and in C. steatolytica with ammonium sulfate. The type of nitrogen source had essentially no effect on total yield.

PRODUCTION OF MICROBIAL PROTEIN FROM BREWERY WASTES

By

Lyle John Shannon

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INTRODUCTION

With the problems of expanding population and static land resources facing much of the world, the task of maintaining sufficient food supplies has become increasingly difficult. The widespread use of new, high yield varieties of wheat and rice has averted massive famine in many areas, but this can only provide a temporary measure if population growth continues at the present rate. This is particularly evident in developing countries where, in many cases, malnutrition and starvation exist.

In the so-called 'developed' countries of the world, where economic and industrial development have risen to great heights, the problems of proper diet are generally not as pressing. Highly developed systems are used for processing and storage of food products, thereby permitting more uniform distribution and less wastage of surplus crops. These systems, however, exert an increased demand on the environment as various types of processing wastes are released. Treatment of these wastes is usually costly and often presents special problems.

Both of these areas of concern are affected by many complex, interrelated issues which defy any simple solutions. Indeed, it has become quite apparent that there is no ready solution for these problems. In this regard much attention has been given in recent years to the uses of microbial protein (single-cell protein or SCP) as a human food, and many exaggerated claims have been made. Yet in

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spite of the difficulties associated with use of SCP, the lack of acceptance in many parts of the world, the high nucleic acid levels, the low methionine content and the sometimes poor digestibility, there are many promising aspects. Unlike the growing of crops, the production of SCP does not necessarily require agricultural land, and unlike the harvesting of marine resources, no limits must be set in the interest of conservation of species. Another attractive feature is the variety of readily available substrates for microbial growth. Numerous investigators have shown the feasibility, at least on a laboratory scale, of using carbohydrate rich waste products to produce SCP. Thus, ideally many of the polluting components of the wastes are transformed into food products, and the cost of waste treatment is considerably reduced.

It was in an effort to examine the possibility of utilizing brewery wastes as a substrate for SCP, that this study was undertaken. In many areas, particularly smaller cities, treatment of brewery waste may present serious problems, for the total biochemical oxygen demand (BOD) produced by a large brewery may equal that produced by the remainder of the town. In such cases the biological functions of a treatment plant may be easily upset, and the brewery may be forced to find an alternate means of disposing of its waste.

At present, however, the use of municipal treatment facilities is the most economical means of waste disposal for the majority of breweries. In general, brewery effluent is readily biodegradable and in large cities it may be successfully processed with domestic sewage. In fact, the alkaline character of brewery wastes may be helpful in neutralizing acid wastes from other industries.

The economics of this situation may change in the future, however. As stricter anti-pollution laws are enacted, cities may place a surcharge on the industrial wastes sent to municipal plants. It is important, then, that breweries consider methods for reducing both the volume and the BOD of their wastes. If techniques can be developed which will allow production of a saleable protein product from these wastes, the BOD of the effluent sent to municipal treatment plants might be significantly reduced and a portion of treatment costs might be recovered by sale of the protein. It is the purpose of this investigation to determine the feasibility of such a system.

LITERATURE REVIEW

The Use of Yeasts as Food and Feed

According to Wiley (1954), interest in the use of yeasts as human food dates to 1875, when Delbruck and his co-workers in Germany began to investigate the nutritional qualities of certain yeast strains. Later Völtz and Baudrexel (1911) reported that yeast protein had a digestibility coefficient of 90% when incorporated into the diets of human subjects. During World War I workers at the Institut für Garüngsewerbe obtained good yields of food yeast on a substrate of molasses and ammonium salts. Commercial production was initiated, but plants were soon forced to close due to molasses shortages (Rose, 1961).

Further research led to the development of wood sugar hydrolysates and spent sulfite liquor as substrates for yeast production. Candida utilis (then known as Torula utilis and later as Torulopsis utilis) was favored over the Saccharomyces species used by brewers and bakers, as C. utilis could utilize the pentose sugars and simple nitrogen sources present in these materials (Bunker, 1963). Commercial production of C. utilis on wood sugar began in Germany prior to World War II and continued throughout the war. At the height of the war, at least eight plants, with a reported annual capacity exceeding 25,000 metric tons of dry yeast, were in operation (Saeman et al., 1946). France, Switzerland and Sweden also opened plants during the war, using spent sulfite liquor to grow food yeast. Aries (1946b)

reported that food yeast produced during the war was used mainly as a meat substitute or meat extender in extracts, soups, sauces, sausages and stuffings and as a flavoring for vegetable dishes.

Following the war, a number of countries began developing programs for food yeast production. A British plant, built in Jamaica in 1944, produced a reported five tons of C. utilis per day using a molasses substrate (Thaysen, 1956). Other plants opened in Finland, South Africa, Canada and Formosa (Wiley, 1954). In the United States, interest in yeast production was centered around waste product utilization. Using ideas and techniques developed in Germany, the first U.S. plant, which was built in Rhinelander, Wisconsin in 1948, grew strains of C. utilis on spent sulfite liquor (Harris et al., 1948). A second plant began production in 1955, but has since ceased operation (Peppler, 1968).

Post-war investigators have examined a number of waste products as substrates for yeast growth. Dunn (1952) points out that any waste or surplus material with a significant carbohydrate content is a potential substrate for yeast production. Although C. utilis has been employed in most studies, numerous other species have been grown successfully on a variety of waste products. Saeman et al. (1946) reported that, in addition to C.utilis, Monilia candida (Candida tropicalis) and Torula (Metschnikowia) pulcherrima were employed in early German sulfite liquor and wood hydrolysate operations. Kurth (1946) reports growing Mycotorula (Candida) lipolytica and Hansenula suaveolens (saturnus) on wood sugar. Molasses has proven to be a good substrate for several strains of C. utilis (Thaysen and Morris, 1943; Thaysen, 1956; Agarwal and Peterson, 1949), Saccharomyces

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cerevisiae (Peppler, 1968) and Rhodotorula gracilis (glutinis var. glutinis) (Thatcher, 1954). Good yields of Saccharomyces fragilis (Porges et al., 1951; Wasserman et al., 1958), C. utilis and Torula cremoris (Candida pseudotropicalis) (Graham et al., 1953) have been obtained from whey. Wiley et al. (1950) grew C. utilis on citrus peel juices and pear processing waste. Pear, apple and cherry juice and maraschino cherry brine were used by Adams and Hungate (1950) to grow an unidentified yeast. Reiser (1954) used potato processing wastes for the propagation of C. utilis.

Considerable study has been given recently to the use of hydrocarbons as substrates for yeast (Raymond, 1961; Champagnet et al., 1963; Miller et al., 1964), and in several countries pilot plants are now producing hydrocarbon-grown yeast, mainly Candida lipolytica (Evans, 1968; Ko and Yu, 1968; Iyengav, 1968; Dostalek et al., 1968). At present, the only commercial primary food and feed yeast operations involve production of S. cerevisiae from molasses, C. utilis from sulfite liquor and S. fragilis from whey (Peppler, 1968).

Nutritive Value of Yeasts

Protein Content

The composition of a yeast cell varies with the strain, the conditions of propagation and the substrate on which it is grown. Under most conditions the major cell component is protein, which may range from 25-60% (calculated as % nitrogen x 6.25) of the dry weight (Agarwal et al., 1947). The average protein content of a yeast cell is usually between 45 and 55% of the dry weight. Aries (1946a) reported that the protein contents of baker's, brewer's and food

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yeasts produced on wood sugar varied between 45 and 56%. Bunker (1963) found C. utilis grown on molasses had a protein content of 53%, while S. cerevisiae grown on the same substrate contained 56% protein. Peppler (1968) lists values of 50% protein for S. cerevisiae grown on molasses and 54% protein for S. fragilis from whey. Wiley et al. (1950) found 51.2% protein in C. utilis grown on molasses and 50.4% protein in the same strain grown on sulfite liquor.

Amino Acid Content

Preliminary amino acid analyses of yeast proteins indicated a relatively high sulfur amino acid content. Aries (1946_a) noted that Fink and Just (1938) found as high as 1.7% cysteine in C. utilis strains. Skinner and Muller (1940), however, reported a deficiency of both cysteine and methionine in yeast proteins. These findings were supported by numerous investigators (Block and Bolling, 1945; Kurth and Cheldelin, 1946; Linden and Work, 1951). Spanyer and Thomas (1956) and Mojonnier et al. (1955) noted that, although deficient in sulfur amino acids, yeast proteins were good sources of lysine and other essential amino acids. They recommended dried yeast as a supplement for grain products, which tend to be low in lysine. Bressani (1968) emphasized that yeast protein contained a high ratio of essential to total amino acids.

Feeding Studies

It is well known that amino acid analyses may not provide a true picture of protein quality. Biological availability of amino acids must be determined by feeding trials. Preliminary work indicated that yeast protein could not be used to completely replace animal protein. Growth and metabolic disturbances were noted in rats

fed diets containing more than 40% yeast. Such problems were relieved, however, by the addition of 2% cysteine to the diet (Nelson et al., 1923; Still and Koch, 1928). It was found that yeasts provided an excellent protein and vitamin source for animals when they were either supplemented with methionine or mixed with other foods high in methionine (Kon and Markuze, 1931; Klose and Fevold, 1945). Robbins et al. (1973) reported protein efficiency ratio (PER) values ranging from 2.1-2.4 for proteins isolated from baker's yeast and 1.5-1.6 for protein from C. utilis. The addition of .1-.2% dl-methionine increased the PER of baker's yeast proteins to 2.9-3.2. This compares with a PER of 2.5 for casein. Good reviews (although dated) of the use of dried yeast as animal feed are given by Braude (1942) and Aries (1946b).

Aries (1946b) also reviews a number of feeding studies on children and adults in England, and concluded that yeast provides a good protein supplement and vitamin source and that humans may ingest at least 1/4 oz (7 g) of dried yeast daily without metabolic disturbance. Dirr (1942) reported good assimilation of protein when human subjects were fed 130 g of dried torula yeast per day. However, he found elevated levels of uric acid in his subjects. Subsequent investigations have shown that the high uric acid levels are caused by the high purine content in yeast cells (Carter and Phillips, 1944). Floch (1956) and others (Lal, 1956; Goyco, 1959) have noted increases in growth and development in children fed diets supplemented with yeast.

There remains considerable controversy on the desirable level of yeast intake. Aries (1946b) and Von Loesecke (1946) found that intake levels exceeding 15 g daily may cause digestive upset.

Bunker (1968) maintained that larger amounts may be safely consumed. According to Bressani (1968) some subjects have been given up to 85 g of yeast three times daily with no ill effects. At present dried yeast is included in human diets in relatively small amounts, usually as a supplement in breads and cereal foods.

Higher Fungi as Food and Feed

Sporophores of the common field mushroom, Agaricus campestris were first cultivated in horse manure beds in France during the seventeenth century (Lambert, 1955). This practice did not reach the United States until the latter part of the nineteenth century, when greenhouse operators began to grow mushrooms under greenhouse benches and some farmers made use of empty barns and cellars to cultivate mushrooms (Litchfield, 1967a). Subsequent experiments (Duggar, 1905; Styer, 1928 and 1930) demonstrated that the mycelia of A. campestris could be cultivated on liquid media.

The concept of growing mushrooms for their mycelia rather than for their sporophores was furthered by the development of submerged culture techniques in the antibiotic industry. Humfeld (1948) was the first to report the successful production of mushroom mycelia (A. campestris) by submerged culture. Further investigations (Humfeld and Sugihara, 1949 and 1952) demonstrated that only certain strains were suited for growth in agitated media; these strains had fairly simple nutrient requirements and could utilize a wide variety of carbon and nitrogen sources. Reusser et al. (1958a) found that mushrooms can utilize lignins, cellulose, hemicellulose, pectins and other polymers in addition to simple sugars. Other investigators reported

on the ability of a large number of mushroom species to grow in submerged culture (Block et al., 1953; Szuecs, 1956; Reusser et al., 1958b). Block (1960) and Robinson and Davidson (1959) pointed out the potential value of muchroom mycelia as food or feed.

The relatively simple nutrient requirements of the mushrooms led to the experimental use of many waste products for their cultivation. Sugar beet molasses has been used as a substrate for Agaricus campestris, Collybia velutipes, Cantharellus cibarius, Morchella hybrida, Boletus indecisus, Xylaria polymorpha, Tricholoma nudum (Reusser et al., 1958a) and Morchella esculenta (Szuecs, 1956). Block et al. (1953) used corn steep liquor and citrus press water to produce mycelia of Agaricus blazei. Cheese whey, corn canning waste and pumpkin canning waste have been used to grow mycelia of Morchella crassipes, Morchella esculenta and Morchella hortensis (Litchfield and Overbeck, 1965). Agaricus campestris, Boletus indecisus, Cartharellus cibarius, Morchella hybrida, Xylaria polymorpha and Tricholoma nudum have been grown on sulfite liquor (Reusser et al., 1958a).

Falange et al. (1964) used soybean whey to culture mycelia of Boletus indecisus, Cantharellus cibarius, Collybia velutipes, Morchella hybrida, Tricholoma nudum and Xylaria polymorpha. Vinasse, a waste product from the Brazilian brandy industry, has been used as a growth medium for mycelia of Agaricus campestris, Tricholoma nudum and Boletus indecisus (Falange, 1962). Humfeld and Sugihara (1949) have grown Agaricus campestris on asparagus and pear processing wastes.

One of the problems associated with production of mushroom mycelia concerns the development of "mushroom flavor." Flavor is generally not a problem in yeast production; most yeasts have a

typically bland flavor and are used mainly as protein and vitamin supplements in foods. However, one of the major anticipated uses of mushroom mycelia is as a flavoring agent in soups, gravies, sauces and vegetables (Litchfield, 1967a). For such uses it is desirable that the product have a flavor similar to that of the sporocarp of the mushroom.

At present, very few species have been found which possess a flavor typical of their fruiting bodies. Humfeld (1948) and Sugihara and Humfeld (1954) claim to have developed mushroom flavor in Agaricus campestris. This is challenged by Eddy (1958), but later work by Moustafa (1960) supports the findings of Humfeld and Sugihara. Other species which are said to produce a typical mushroom flavor include Agaricus blazei (Block, 1960), Pleurotus ostreatus (Eddy, 1958), Lepiota rachodes (Sugihara and Humfeld, 1954) and various Morchella species (Szuecs, 1956 and 1958; Robinson and Davidson, 1959; Litchfield, 1963a).

Several techniques have been suggested for improving flavor of mushroom mycelia. Humfeld and Sugihara (1949) and Block et al. (1953) advised allowing a slight autolysis of the mycelium by permitting the fermentation to continue one to two days after the sugar is completely utilized. Block et al. (1953) also recommended heating the mycelium and Szuecs (1956) patented a technique for treating the mycelia with NaCl. Litchfield (1967a) stressed, however, that the most important factors in flavor development are proper selection of mushroom strain, substrate and aeration rate. He stated further that complex nitrogen sources yield better flavor than simple inorganic sources.

Nutritive Value of Higher Fungi

Protein Content

In general, mushroom mycelia are lower in protein than yeast cells. There also appears to be a difference in protein concentration between the sporocarp and the mycelium, although this may vary between species. Block *et al.* (1953) found the mycelia of *Agaricus blazei* contained less protein than the sporocarp, while Terramoto *et al.* (1966) found the reverse to be true for *Lentinus edodes*.

Protein contents of mushroom mycelia range from 27.5 to 60% depending on the strain and the nitrogen content of the medium (Litchfield, 1967a). Humfeld and Sugihara (1949) studied two strains of *Agaricus campestris*, one containing 35.5% protein and the other containing 45.3% protein. Four species of *Morchella* were observed to contain between 30 and 35% protein (Litchfield, 1963a; Reusser *et al.*, 1958a). Falange (1962) reported a protein content of 27.9% for *Tricholoma nudum* grown on vinasse, while Reusser *et al.* (1958b) obtained 49.8% protein with the same strain grown on a molasses medium.

Amino Acids

There have been few amino acid analyses of mushroom mycelia. Block *et al.* (1953) reported that *A. blazei* mycelia contained all the essential amino acids, but did not give quantitative values. Analyses of the mycelial proteins of *T. nudum* (Reusser *et al.*, 1958b) and several *Morchella* species (Litchfield, 1963b) have indicated that the amino acid profiles of these proteins are quite similar to those of other microbial proteins, i.e., they contain adequate levels of all the essential amino acids with the exception of methionine.

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Feeding Studies

Fitzpatrick et al. (1946) fed rats the sporocarps of Agaricus campestris and observed the rats did not grow as well as controls maintained on soybean meal and casein. In experiments with human subjects, Lintzel (1941 and 1943) reported that sporocarps of Morchella esculenta, Agaricus campestris, Boletus edulis and Cantharellis sp., when fed at 100% of dietary protein level, were equivalent to muscle protein in nutritional value. However, there have been no subsequent reports of human feeding experiments conducted with either fruiting bodies or mycelia, to confirm these results.

Effectiveness of Fungi in Reducing BOD of Waste Substrates

A number of factors are involved in determining the effectiveness of an organism in reducing BOD in a given substrate. A major consideration is the concentration of nitrogen, phosphorus and trace elements in the medium (Helmers et al., 1952; Reiser, 1954). Wiley (1954) noted that the concentration of nonfermentable carbon compounds in the medium and the characteristics of the metabolic wastes released by the organism are also important in determining the degree of BOD reduction. Thus, even though 90-96% of the reducing sugars in sulfite liquor is utilized by C. utilis, BOD is reduced an average of only 50-60% due to the presence of unfermented carbonaceous material and fermentation by-products. Reiser (1954) obtained similar results when C. utilis was grown on potato starch wastes. Inskeep et al. (1951) reported that C. utilis could remove up to 70% of the BOD from some batches of sulfite liquor. However, the average reduction in BOD was only 59.7%.

There is no published data on the effectiveness of mushroom cultures in reducing BOD, although Church et al. (1973) reported using a mixed culture of Fungi Imperfecti to degrade corn and pea canning wastes. Using a mixture of Trichoderma viride, Gliocladium deliquescens, Geotrichium sp. and Fusaruim sp. they achieved a 95% reduction in BOD. Investigators working with mushroom cultures have, in most cases, measured only reducing sugar utilization, which may be a poor indicator of BOD. Falange (1962) noted 98% utilization of reducing sugars with Agaricus campestris and with Boletus indecisus grown on vinasse. Similar results were obtained with Tricholoma nudum and with Boletus indecisus grown on soybean whey (Falange et al., 1964). Both Morchella crassipes and M. esculenta were found to utilize only 50% of the reducing sugars in corn canning waste while M. hortensis was able to utilize 74% (Litchfield, 1963c). Reusser et al. (1958a) found that reducing sugar utilization in sulfite liquor varied from 26.7% with Cantharellus cibarius to 88.5% with Boletus indecisus. Considerably more work is needed in this area.

In summary, the development and use of yeast and mushrooms as food and feed and the use of waste products as substrates for these organisms have been reviewed. It has been shown that fungi are generally rich sources of protein and vitamins, although the proteins tend to be deficient in the sulfur amino acids. Significant reductions in BOD have been obtained by growing fungi on waste products, although in many substrates the concentration of nonfermentable carbon compounds precludes removal of more than 60% of the BOD.

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This study is being undertaken to evaluate the ability of selected yeasts and mushrooms to reduce the BOD of brewery wastes and to produce protein.

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MATERIALS AND METHODS

Microorganisms

Eight microorganisms were used in this investigation. A culture of *Saccharomyces cerevisiae* (var. *ellipsoideus*) No. Y-25 was obtained from the Department of Food Science and Human Nutrition, Michigan State University. Another culture from the same source was marked *Saccharomyces carlsbergensis* ATCC 9080, however, this organism was recently reclassified as *Saccharomyces uvarum* (Walt, 1970). Accordingly, this organism is henceforth referred to as *S. uvarum*. Cultures of *Candida utilis* C-25 and *Candida steatolytica* 70-40, a new species described by Yarrow (1969), were obtained from the Department of Food Science and Technology, University of California at Davis.

Strains of the morel mushroom, *Morchella esculenta* and the 'oyster mushroom', *Pleurotus ostreatus*, were obtained from Dr. Wm. G. Fields, Department of Botany and Plant Pathology, Michigan State University. A culture of the commercial mushroom, *Agaricus bisporus*, was provided by Dr. E. Beneke, Department of Botany and Plant Pathology, Michigan State University. A strain of the giant puff ball, *Calvatia gigantea*, was isolated from a specimen collected by the author near Baldwin, Michigan.

Prior to use in this investigation all yeast cultures were streaked on yeast extract-malt extract-peptone-glucose-agar (YMPGA) plates and incubated at 25 C. Isolated colonies on these plates were

transferred to YMPGA slants for maintenance. The mushroom cultures were inoculated into YMPG broth and incubated at 25 C on a gyrotary shaker (New Brunswick Scientific, New Brunswick, New Jersey) at 100 rpm. When discrete mycelial balls had formed after approximately 8 days, one of the mycelial balls was transferred to a YMPGA slant. The mushroom cultures were also maintained on YMPGA.

Waste Preparation

Quantities of three brewing wastes were obtained from the Stroh's Brewery, Detroit, Michigan. Prior to use, the wastes were all frozen in Cry-O-Vac bags (W. R. Grace Co., Simpsonville, South Carolina) and held at -20 C. The bags, containing approximately 4-l portions, were thawed as needed, by holding them at 10 C for 24 hr. Preparation of the wastes for use as microbial substrates is described below. In order to obtain accurate assessments of the dry weight of cells grown on the wastes, as much solid material as possible was removed.

Spent grains, recovered from the mashing process, and trub, a precipitate from the hot wort tank, were transferred to closely woven nylon press bags placed in the sausage stuffer (F. Dick Co.) and subjected to the maximum pressure which could be manually exerted. The press liquids were filtered through several layers of cheesecloth to remove contaminating particles and adjusted to pH 5.3 with 1N HCl. It should be noted that the composition of these press liquors probably differs from those produced in commercial feed drying operations where hydraulic presses are used.

Sludge from the fermentation tank was treated by batch centrifugation at 10,000 x g, followed by vacuum filtration through

Hy-Flo Supercel (Johns-Manville Corp., Manville, N.J.). This served to remove most of the spent yeast and cell debris from the sludge. The clarified fluid was then adjusted to pH 5.3 using 1N NaOH.

All the waste liquors were subjected to a heat treatment to destroy any vegetative cells which might be present. One hundred-ml aliquots of the wastes were transferred to 250-ml erlenmeyer flasks and the flasks were placed in a steam bath at 100 C for 10 min. After cooling, these flasks were inoculated with 1 ml of a prepared inoculum as described below.

In some experiments nitrogen was added to the grain press liquor and trub press liquor. This was accomplished by adding quantities of either ammonium sulfate or sludge liquor to three aliquots of each waste, such that the final concentration of added nitrogen was .05, .10 and .15%, respectively. Those wastes containing added nitrogen were buffered with 3 g/l of potassium phosphate, as it has been indicated that the use of $(NH_4)_2SO_4$ as a nitrogen source in unbuffered media results in a low pH and poor substrate utilization (Reusser et al., 1958b).

Growth Studies

All the studies were carried out using 250-ml flasks containing 100 ml of waste liquor. The inoculated flasks were held at 25 C on a shaker operating at 200 rpm on a gyrotary shaker (New Brunswick Scientific).

In studies with yeasts, substrates were inoculated with 1 ml of a 72-hr culture of the organism in YMPG broth. The higher fungi could not be inoculated in this manner, however, due to their peculiar spherical growth under conditions of agitated culture. After

incubation for 8 days, mushroom cultures in YMPG broth were filtered through a fine wire screen. The mycelial spheres were then aseptically transferred to glass-stoppered 250-ml erlenmeyer flasks containing sterile glass beads and 50 ml of sterile deionized water. These flasks were vigorously shaken for 1 hr to break up the mycelia. One-ml aliquots of the resultant suspensions were used for inoculation.

Yeast cells were harvested by centrifugation for 10 min at 10,000 x g. The supernatant fluid was decanted from the cell pellet and saved for analysis. The cells were suspended in 50 ml of deionized water and centrifuged at 10,000 x g for 10 min. Mushroom cultures which formed discrete mycelial balls were harvested by filtration through a fine wire screen. The filtrate was saved for further analysis and the mycelia were washed with 50 ml of deionized, distilled water. Occasionally mushroom cultures grew in a dispersed form. In this case, the mycelia were harvested by centrifugation at 10,000 x g for 10 min.

The harvested cells were washed from a centrifuge bottle or wire screen into tared aluminum weighing dishes using ca. 15 ml of deionized, distilled water. These dishes were dried in a convection oven at 60 C for 48 hr after which they were transferred to a dessicator for 24 hr prior to weighing.

Analytical Techniques

All wastes were analyzed before and after microbial growth, to determine the effects of reducing sugar and nitrogen content on growth, and evaluate the effectiveness of these organisms in reducing COD and BOD. In addition, the dried cells were assayed for crude protein content. Unless otherwise noted, all analyses were run in

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Reducing sugars were determined by the method of Somogyi (1945) and Nelson (1944). Samples were diluted with deionized, distilled water to a final reducing sugar concentration of 5 to 25 µg/l. One ml of the diluted sample was mixed with 1 ml of Somogyi's copper reagent and held 10 min in a boiling water bath. After cooling, 1 ml of Nelson's arsenomolybdate reagent was added and mixed well. The reducing sugar content (as glucose) was determined by measuring the absorbance of the resultant color complex on a Beckman DB-G spectrophotometer at 660 nm and compared with the absorbances of glucose standards which were run concurrently.

Nitrogen was determined by a modification of the micro-Kjeldahl method of AOAC (1970). For the wastes, 4 ml of a digestion mixture (containing 5 g $CuSO_4 \cdot 5H_2O$, 5 g SeO_2 and 500 ml conc. H_2SO_4), was added to 2 ml of undiluted sample in a micro-Kjeldahl flask and the mixture was heated over an open flame in a micro-Kjeldahl digestion apparatus. When the sample cleared, the sides of the flask were rinsed with deionized, distilled water, and 1 ml of 30% H_2O_2 added. Heating was continued for another hour. After cooling, the sample was diluted with 10 ml of deionized, distilled water and attached to a distillation apparatus. Approximately 25 ml of 40% NaOH was added and the nitrogen was steam-distilled into a dilute boric acid trap solution. The nitrogen (as NH_3) was determined by titration with .020N HCl to a light gray endpoint with Kjeldahl indicator (AOAC, 1970). Tryptophan standards containing 13.76% nitrogen were run periodically as controls.

Total crude protein in the dried cells was also determined by

the micro-Kjeldahl method. The procedure is the same as for total nitrogen with the exception of sample size, which was approximately 0.1 g of dried cells. Protein was estimated by multiplying the % nitrogen by 6.25, based on the assumption that fungal protein contains 16% nitrogen and that all nitrogen is protein nitrogen.

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were determined according to the methods described in Standard Methods for Examination of Water and Wastewater (APHA, 1971). In the COD technique a flask containing 20 ml of sample (diluted to 200-800 mg/l COD), 0.4 g $HgSO_4$ and 10 ml of .250N potassium dichromate was refluxed for 2 hr with a concentrated H_2SO_4-Ag_2SO_4 solution (9.73 g Ag_2SO_4 per 1 H_2SO_4). Following the reflux, the remaining unoxidized dichromate was titrated with freshly standardized 0.100N $Fe(NH_4)_2SO_4$ solution. From this, the COD, based on the amount of dichromate oxidized, was calculated. An aqueous glucose solution containing 468.6 mg/l or 500 mg/l COD was used as a standard.

For BOD determinations, samples were diluted with deionized, distilled water so as to contain 300 to 1800 mg/l BOD. One ml of these diluted samples was then pipetted into standard BOD bottles. Using a siphoning technique, so as to avoid entrapment of air, the bottles were filled with seeded dilution water, prepared according to Section 219.4a of Standard Methods for the Examination of Water and Wastewater (APHA, 1971), to a final concentration of 1 to 6 mg/l BOD. Primary effluent obtained from the East Lansing Sewage Treatment Plant was used at a concentration of 2 ml/l as a seeding agent. Half of the sealed BOD bottles were incubated in the dark for 5 days at 20 C, while the remaining bottles were analyzed after 15 min to

determine the initial concentration of dissolved oxygen in the bottles. Following incubation, the dissolved oxygen content was determined in the sample bottles. BOD values were calculated from the amount of oxygen utilized by the sample less corrections for the amount of oxygen used by the seed alone.

Dissolved oxygen was determined by the modified azide method, Section 218b, Standard Methods for the Examination of Water and Wastewater (APHA, 1971). This is an iodometric method based on the oxidation of manganese ions in alkaline solutions containing iodide ions, and the subsequent release of iodine upon acidification of the solution and reduction of the oxidized manganese ions. The iodine liberated is directly proportional to the dissolved oxygen content of the sample. Standard .025N sodium thiosulfate is used to titrate the iodine using 1% soluble starch as an indicator. As a check on technique and seed quality, a standard containing 150 mg/l of both glucose and glutamic acid was run with each set of samples.

Experimental Design

The growth characteristics of eight microorganisms, four yeasts and four mushrooms, were investigated on grain press liquor, trub press liquor and fermentation vat sludge liquor from the brewing industry. The evaluation of these organisms was based on three major criteria: net yield in terms of dry weight, protein content of the mycelia or cells, and relative amount of BOD reduction. Based on the preliminary investigations on unsupplemented brewery wastes, one yeast and one mushroom culture were selected for additional experiments concerning the effects of supplementation with nitrogen and phosphorus. In these studies the two organisms were grown on grain press liquor

and trub press liquor buffered with phosphates and containing .05, .10 and .15% added nitrogen either as ammonium sulfate or fermentation sludge liquor.

RESULTS AND DISCUSSION

Waste Analysis

Carbohydrate and Nitrogen Content

Several investigators (Helmers et al., 1951; Thiel and duToit, 1965; Ault, 1969) have noted that brewery effluent, while containing large quantities of fermentable organic material, tends to be low in nitrogen. Preliminary analysis of the wastes used in this study confirmed these findings for the press liquors. Sludge liquor, however, was found to contain relatively large amounts of nitrogen. Table 1 shows the characteristics of the wastes prepared for use in the growth studies. Other samples of these wastes varied in composition, particularly in reducing sugar content, by as much as 20% from the values shown in the table.

The table indicates that ratios of reducing sugars to nitrogen were on the order of 70:1 in the press liquors, while only about 3.5:1 in sludge liquor. Total carbon in the wastes was not measured, so C:N ratios cannot be calculated. It can be inferred, however, considering the high COD values of the wastes, that C:N ratios would be equal to, or greater than, the reducing sugar:N values.

Fungi will grow over a latitude of C:N ratios, however, highest yields are usually obtained in a narrow range determined by the species, the growth medium and the cultural conditions.

Table 1. Characteristics of brewery wastes.

	Grain Press Liquor	Trub Press Liquor	Fermentation Sludge Liquor
Reducing Sugar (mg glucose/1)	30,000	55,000	9,900
Nitrogen (mg/1)	434	764	2,870
Red. Sugar:N	69.1:1	72.0:1	3.45:1
COD (mg/1)	56,100	169,000	133,000
BOD (mg/1)	31,800	132,000	67,500
BOD:COD	.57	.78	.51
pH	5.96	5.30	4.43

A. campestris, for example, has been grown over a C:N range of 20:1 to 73.3:1, although highest yields were obtained in the area of 20:1 to 25:1 (Reusser et al., 1958a). On the other hand, Litchfield (1963a) obtained optimum yields of M. hortensis at C:N ratios between 5:1 and 10:1. Moustafa (1960), who calculated reducing sugars to nitrogen ratios, found the best yields of A. campestris at ratios between 20:1 and 30:1.

Oxygen Demand

Depending on the brewery and the sample on which the determinations are made, wide ranges of BOD and COD values have been reported for brewery wastes. Thiel and duToit (1965) reported a COD range of 4,000 to 15,100 mg/l for a combined brewery effluent. Ault (1969) found an average BOD of 693 mg/l for a combined brewery effluent, while Kosaric (1972) cited a figure of 1,300 mg/l BOD for an "average" brewery effluent. Grain press liquor has a reported BOD of up to 39,000 mg/l (Ault, 1969). No information is available on trub press liquor. Fermentation sludge liquor appears to be of relatively uniform composition with a BOD of approximately 69,000 mg/l (Dietrich, 1961).

As shown in Table 1, the BOD values of grain press liquor and sludge liquor used in these experiments fall within the range reported by previous investigators. Of particular note is the extremely high BOD of trub press liquor. BOD:COD ratios have been calculated to demonstrate the difference in composition of the wastes. Trub has a BOD:COD ratio of nearly 0.8, indicating a high percentage of biologically degradable organic matter. Grain press liquor and sludge liquor have BOD:COD ratios of about 0.6 and 0.5, respectively,

implying a greater content of non-biologically utilizable compounds.

pH

The wastes had varying degrees of acidity, in apparent contrast to the alkaline pH values often reported for total brewery effluent. Several investigators have noted, however, that most of the wastes from brewing operations are acidic (Thiel and duToit, 1965; Ault, 1969). It has been shown that the alkalinity in the final combined effluent comes largely from the washing and bottling operations.

Prior to the growth experiments grain press liquor and sludge liquor were adjusted to pH 5.3 with 1N HCl and 1N HaOH, respectively. Trub press liquor required no pH adjustment.

Growth Studies

Yield

All of the wastes were capable of supporting microbial growth, although there was a considerable difference in the total yield obtained with each of the substrates. A significant variation in total yield among different species grown on the same substrate was also noted. There is evidence to suggest that some organisms are utilizing other carbon sources in the substrates in addition to reducing sugars. Where significant quantities of carbon sources other than reducing sugars are assimilated, the yield factor in terms of g cells/g reducing sugar is of questionable value. This calculation is included in Tables 2-4, however, as an aid in comparing these data with those of previous investigators who favored this notation.

All the yeast cultures were harvested after a 72-hr

incubation period, since preliminary growth studies with S. cerevisiae and C. steatolytica indicated that both species had similar growth patterns and produced maximum yields at ca. 72 hr. The mushrooms, however, showed considerable variation in growth rate, as has been noted by a number of investigators. Maximum growth of A. campestris in agitated culture has been obtained over incubation periods ranging from 8 days (Reusser et al., 1958a) to 12 days (Falange, 1962). Optimum incubation times for Morchella species have been reported at 5 days for M. esculenta (Litchfield, 1967c), 7 days for M. crassipes (Litchfield, 1967c) and 8 days for M. hybrida (Reusser et al., 1958a). Work by Eddy (1958) indicated that up to 24 days is required for maximum yields of Coprinus comatus. In the present experiments, incubation of the mushroom cultures was limited to 8 days. It was felt that even though greater yields of some organisms might have been obtained by longer incubation, the excessive time interval would preclude any sort of commercial operation.

With respect to time requirements for commercial production, it should be noted that such operations normally use large fermentors, which can be operated on a continuous flow basis. Under these conditions, maximum mycelial yields are obtained after considerably shorter periods than those required for agitated flasks. Indeed, incubation times as short as 30 hr have been reported (Block, 1960).

Table 2 shows that the net dry weights of cells and mycelia ranged between 3.28 and 6.28 g/l for organisms grown in grain press liquor. Similarly, reducing sugar utilization varied from 69.2 to 89.4% With the exception of C. gigantea, the mushrooms generally produced smaller cell masses than the yeasts, although the overall

Table 2. Yields of yeasts and mushrooms grown in grain press liquor.

Organism	Net Dry Wt.* (g/l)	% of Reducing Sugar Used	Yield Factor (g cells/g sugar)
S. cerevisiae	5.02	88.5	.189
S. uvarum	4.94	89.4	.177
C. utilis	5.09	73.5	.231
C. steatolytica	6.28	74.2	.282
P. ostreatus	3.81	69.2	.184
M. esculenta	3.28	78.3	.139
A. bisporus	3.45	83.2	.138
C. gigantea	6.25	83.8	.248

*Average of 3 determinations.

difference in utilization of reducing sugar was less marked. Of the yeasts, C. steatolytica produced the greatest cell mass: 6.28 g/l of dry cells. Cell yield from all the other yeasts was about 5 g/l. It is interesting to note that the Candida species, while producing equivalent or greater cell masses than the Saccharomyces species, utilized about 16% less reducing sugar. This may be due to the utilization of non-reducing carbon sources in the substrate.

C. gigantea, the fastest growing of the mushroom cultures, produced a dry mycelial weight of 6.25 g/l, significantly higher than the other species. Little difference was observed among the other mushrooms grown on grain press liquor. They all produced 3 to 4 g/l of dried mycelium.

Trub press liquor (Table 3), being a rich medium, proved to be the best substrate for all the organisms. Dry cell and mycelial weights ranged from 8.94 to 27.72 g/l, with the mushroom cultures producing better yields than the yeasts. C. gigantea afforded the greatest net dry weight of all the organisms, yielding 27.72 g/l. All mushroom cultures, however, produced 4 to 5 times the mycelial mass produced in grain press liquor. The cell mass did not increase as dramatically with the yeast cultures, although significant gains were made. C. steatolytica produced 10.56 g/l, the largest dry cell mass among the yeasts.

The percentage of reducing sugar utilization, varying from 52.7 to 73.5%, was generally lower in trub press liquor than in grain press liquor. It should be noted, however, that 50% utilization of the 5.5 g/l of reducing sugar in trub represents an amount of sugar equal to 92% utilization of the 3.0 g/l of sugar in grain press

Table 3. Yields of yeasts and mushrooms grown in trub press liquor.

Organism	Net Dry Wt.* (g/l)	% of Reducing Sugar Used	Yield Factor (g cells/g sugar)
S. cerevisiae	8.94	66.4	.245
S. uvarum	8.95	73.5	.221
C. utilis	9.86	58.1	.308
C. steatolytica	10.56	63.6	.302
P. ostreatus	20.05	52.7	.691
M. esculenta	16.88	53.6	.570
A. bisporus	11.32	54.5	.377
C. gigantea	27.72	63.6	.749

*Average of 3 determinations.

liquor. Therefore, a greater amount of reducing sugar was actually assimilated in trub. This accounts for the apparent contradiction of increased cell yields and decreased percentages of sugar utilization observed in trub press liquor as compared to grain press liquor.

The mushrooms assimilated less sugar than the yeasts, even though they produced significantly greater yields. This appears to be further evidence of the use of alternate carbon sources; a function, perhaps, of a larger complement of degradative enzymes in mushrooms.

Cell and mycelial yields ranging from 2.45 to 6.46 g/l were obtained from sludge liquor. These were not as small as might have been predicted from the low sugar content of the medium. In fact, as shown in Table 4, S. cerevisiae, C. steatolytica, P. ostreatus and A. bisporus essentially grew as well in sludge as they had in grain press liquor. Sludge liquor proved to be the least suitable waste for the growth of the other organisms, however. Of particular note is the relatively poor growth of C. gigantea from which the highest yields had been obtained on the other substrates.

Reducing sugar utilization varied from 72.2 to 87.1%, approximately the same range observed in grain press liquor. As previously noted, this does not indicate that equal amounts of sugar were used in grain press liquor and in sludge. Rather, it is an indication that considerably less sugar was metabolized in the latter substrate. Since growth in sludge was only slightly less prolific than that in grain press liquor, it appears evident that carbon sources other than reducing sugars were also assimilated. If this is in fact the case, the yield calculations, in terms of g cells/g

Table 4. Yields of yeasts and mushrooms grown in fermentation sludge liquor.

Organism	Net Dry Wt.* (g/l)	% of Reducing Sugar Used	Yield Factor (g cells/g sugar)
S. cerevisiae	5.21	83.3	.631
S. uvarum	4.02	84.5	.480
C. utilis	3.65	78.7	.468
C. steatolytica	6.46	81.0	.805
P. ostreatus	3.42	74.7	.462
M. esculenta	2.45	72.2	.343
A. bisporus	3.38	87.1	.392
C. gigantea	3.04	78.8	.390

*Average of 3 determinations.

reducing sugar, should not be regarded as significant.

Even on unsupplemented wastes, yields of several organisms compare favorably with those obtained by previous investigators on a variety of enriched substrates. Reusser et al. (1958a) found that A. campestris produced only 3.4 g/l of dried mycelia on sulfite liquor, yet yielded 17.1 g/l on a beet molasses medium. The same strain generated 7.2 g/l of dry mycelia on a malt syrup, cane molasses medium (Moustafa, 1960). This compares with a high of 11.32 g/l of A. bisporus mycelia obtained from unsupplemented trub in the present experiments.

M. esculenta yielded 7.92 g/l of dried mycelia when grown on pumpkin canning waste (Litchfield and Overbeck, 1965). In the current study, significantly greater yields, up to 16.88 g/l were obtained. Sugihara and Humfeld (1954) used a synthetic medium to produce up to 25 g/l of P. ostreatus. The highest yield of this organism obtained in the present study was 20.05 g/l.

In much of the available data on yeast production yields are expressed in terms of g cells/g reducing sugar. Harris et al. (1948) reported obtaining .296 to .392 g cells/g reducing sugar of C. utilis from sulfite liquor. Yields of another strain of C. utilis, grown on wood sugar stillage, ranged from .53 to .63 g cells/g reducing sugar (Kurth and Cheldelin, 1946). Inskeep et al. (1951) reported yields of 10 g/l for C. utilis grown on sulfite liquor. Using a culture identified as Torula, Gray et al. (1964) obtained 8.2 g/l of dried cells from a synthetic medium. Agarwal et al. (1947) obtained yields of S. cerevisiae, grown on beet molasses, ranging from .427 to .543 g cells/g reducing sugar. In the present experiments the optimum

yields of yeasts were 8.94 g/l of S. cerevisiae and 9.86 g/l of C. utilis in trub press liquor and .631 g S. cerevisiae/g reducing sugar and .468 g C. utilis/g reducing sugar in sludge liquor. However, as previously discussed, these latter figures may be misleading.

Yields obtained by previous investigators should be interpreted and compared with caution, as a number of different drying techniques have been used. The drying method and temperature determine the final moisture content of the cells and thus affect the net dry weight. In the data shown above, some cells were dried on drum dryers, while others were dried in vacuum, forced air or convection ovens at temperatures ranging from 50 to 110 C. All samples in this study were dried in convection ovens at 60 C.

These preliminary growth studies on unsupplemented wastes have shown that all the waste substrates will support microbial growth. Trub press liquor provides the best growth medium, probably due to its higher carbohydrate content. Grain press liquor, which contains 30 g/l of reducing sugars, produces only slightly better yields than sludge liquor, which contains 10 g/l of reducing sugar. The relatively good growth observed in sludge would appear to be a function of the high nitrogen levels present in that substrate. Conversely, the low nitrogen concentration in grain and trub press liquors may be an important factor in limiting growth in those media.

Protein Production

In addition to yield, another important criterion in this study concerned the cellular protein content of the organisms. Table 5 lists the protein concentration in the dry cells grown on

grain and trub press liquors (calculated as Kjeldahl N x 6.25). Total protein production of each organism is also shown, so that comparisons may be made between species which grow poorly on the wastes, but yield large amounts of protein, and those which grow well while producing relatively small amounts of protein.

From the data shown in Table 5 several generalizations can be made. As noted by previous investigators (Reusser et al., 1958a; Litchfield et al., 1963a; Litchfield, 1967) mushroom mycelia generally have lower protein concentrations than yeast cells although this varies markedly with the organism. P. ostreatus, for example, yielded only 6.88% protein when grown in grain press liquor, whereas C. utilis produced 27.11% in the same substrate. It was also found that the protein contents of all organisms were increased 1 to 6% when trub press liquor was the substrate. Thus, S. cerevisiae increased its cellular protein content from 26.77% in grain press liquor to 32.91% in trub press liquor.

C. steatolytica had the lowest protein concentration of the yeasts in both substrates, although it produced essentially the same quantity of total protein. More samples would be required to detect a significant difference in protein content among the other yeasts, all of which produced between 26 and 27% protein on grain press liquor and 28 to 33% protein on trub press liquor.

Considerably more variation in protein content was observed among the mushrooms. P. ostreatus, as previously noted, was extremely low in protein, containing a maximum of only 7.14% on trub press liquor. Mycelia of C. gigantea also contained comparatively small amounts of protein; however, its total protein production equaled or

Table 5. Crude protein of organisms grown on unsupplemented brewery wastes.

Organism	Grain Press Liquor		Trub Press Liquor	
	Crude Protein (% dry wt.)	Total Protein Production*	Crude Protein (% dry wt.)	Total Protein Production*
S. cerevisiae	26.77	1.34	32.91	2.94
S. uvarum	26.40	1.30	27.74	2.48
C. utilis	27.11	1.38	28.67	2.83
C. steatolytica	21.30	1.34	23.49	2.48
P. ostreatus	6.88	.26	7.14	1.43
M. esculenta	22.50	.74	27.12	4.58
A. bisporus	20.31	.70	22.28	2.59
C. gigantea	13.69	.86	17.50	4.85

*Calculated as net dry wt./per liter of cells or mycelia x % protein/100.

exceeded that of all other organisms. M. esculenta and A. bisporus both produced good yields of protein, with the former organism actually exceeding the yeast C. steatolytica in protein content.

Overall there appeated to be little difference in total protein production between the yeasts. Among the mushrooms, M. esculenta and C. gigantea were approximately equal in total protein production, both yielding significantly greater quantities than P. ostreatus and A. bisporus.

All organisms grown on unsupplemented wastes had consistently lower protein contents than those reported in the literature. C. utilis and S. cerevisiae, for example, have both been reported to contain 45 to 55% protein when grown under optimum conditions (Aries, 1946a; Bunker, 1963). However, on unsupplemented trub they had protein contents of only 28.67 and 32.91%, respectively. Similarly, the mycelia of A. bisporus grown on trub were found to consist of only 22.28% protein in contrast to reported protein contents ranging from 35 to 45% (Humfeld and Sugihara, 1949). Of the eight test organisms, only M. esculenta, with a protein content of 27.12%, attained a concentration of protein near its suggested maximum of 31.1% (Litchfield et al., 1963b).

Reusser et al. (1958b) showed that the mycelial protein concentration of Tricholoma nudum could be increased from 18% to a maximum of 52% by increasing the ammonium tartrate concentration of the medium from .025 to 1%. Similar results have been obtained by other investigators using a variety of organisms and nitrogen sources on various substrates (Humfeld and Sugihara, 1952; Falange, 1962; Litchfield et al., 1963a; Litchfield et al., 1963b). Among the

more commonly used inorganic nitrogen supplements are ammonium phosphate, ammonium sulfate and ammonium tartrate. Organic supplements have included urea, corn steep liquor and soybean whey. A range of nitrogen concentrations from .012% (Block et al., 1953) to .56% (Falange et al., 1964) have been used, although the usual values are from .04 to .15% nitrogen, whether inorganic or organic materials are used. These figures are based on C:N ratios in the area of 5:1 to 30:1. If C:N ratios are significantly higher, as they appear to be in brewery wastes, additional nitrogen may be required.

Reduction of Oxygen Demand

The reduction in BOD and COD achieved by growing the organisms in unsupplemented wastes are shown in Table 6. Originally BOD measurements were to be made only on samples showing significant reductions in oxidizable material as determined by the COD method. It was found, however, that COD was not a reliable indicator of the degree of BOD reduction. In trub press liquor, for example, the reduction in COD was about 45% of the reduction in BOD. In grain press liquor BOD and COD were reduced by approximately the same percentage, while BOD reduction was 25% greater than COD reduction in sludge liquor. For this reason it was felt that a more accurate picture of the biological oxidations occurring should be obtained by determining BOD values for all the samples.

Most of the organisms grown on unsupplemented grain press liquor removed 28 to 34% of the BOD. P. ostreatus and M. esculenta were slightly less effective, reducing BOD by only 21.9 and 22.7%, respectively. The most remarkable results, however, were obtained with C. gigantea, which reduced BOD by 56.2%. On unsupplemented trub press

Table 6. Effectiveness of yeasts and mushrooms in reducing COD and BOD of unsupplemented brewery wastes.

	Grain Press Liquor		Trub Press Liquor		Fermentation Sludge Liquor	
	% COD Reduction	% BOD Reduction	% COD Reduction	% BOD Reduction	% COD Reduction	% BOD Reduction
S. cerevisiae	30.5	34.4	14.7	42.4	35.3	25.9
S. uvarum	30.3	34.0	17.1	43.2	30.1	21.5
C. utilis	28.6	28.0	18.3	45.5	26.3	20.0
C. steatolytica	30.4	28.2	20.7	45.5	33.1	24.4
P. ostreatus	19.6	21.9	18.7	43.9	24.1	17.0
M. esculenta	19.7	22.7	18.0	42.5	20.3	15.5
A. bisporus	33.9	34.4	24.3	49.2	33.1	24.4
C. gigantea	57.1	56.2	27.8	56.1	34.6	25.9

liquor, BOD was reduced, on the average, about 45%. There was essentially no difference among the organisms with the exception of C. gigantea which removed 56.1% of the BOD. Lower BOD reductions, in the area of 16 to 26%, were obtained in sludge liquor. P. ostreatus and M. esculenta were at the low end of this range, while S. cerevisiae and C. gigantea removed the largest amounts of BOD.

The reductions in BOD obtained in the unsupplemented wastes, particularly in grain press liquor and in sludge liquor were substantially less than the maximum values obtained by other investigators. The greatest degree of BOD removal was obtained in trub press liquor, where an average reduction of 45% was noted. This, however, was still short of the 60 to 70% reductions reported by Inskeep et al. (1951) and Reiser (1954). Improved BOD removal in brewery wastes would appear to be related to improved growth of the organisms in the wastes, and thus to nitrogen supplementation.

Supplementation of Wastes

From the results obtained in the preliminary studies, it appeared evident that supplementation of the wastes was required for improved growth, higher protein concentrations and increased BOD removal. As previously discussed, it was felt that nitrogen was the major supplement required. Two nitrogen sources were finally chosen for use, based on their low cost and easy availability; considerations of primary importance in any commercial operation. The most obvious nitrogen source was sludge liquor, which contained over 2 g/l of nitrogen, far in excess of any growth requirements. In addition to nitrogen, however, sludge liquor should be a good source of vitamins

and minerals from autolysed yeast cells. Further, since only mediocre growth was obtained when sludge liquor was used alone as a substrate, this would provide a convenient method for utilizing it. As a comparison of the effectiveness of sludge liquor as a nitrogen source, a second set of samples was supplemented with ammonium sulfate.

Wastes were supplemented at levels of .05, .10 and .15% added nitrogen. This corresponds to 2.36, 4.72 and 7.08 g/l of ammonium sulfate, or to 174, 349 and 523 ml/l of sludge liquor, respectively. The addition of ammonium sulfate has essentially no effect on the reducing sugar content or BOD of the wastes. Sludge liquor is added in such large quantities, however, that reducing sugar concentration in both press liquors is significantly reduced. BOD is decreased slightly in trub press liquor and increased slightly in grain press liquor. These effects are summarized in Table 7.

In experiments with a synthetic medium, Humfeld and Sugihara (1949) reported on the nitrogen, phosphorus, potassium, sulfur, magnesium, iron and zinc requirements of A. campestris. They found the largest mineral requirements were for phosphorus and potassium. Litchfield (1968) noted that while vitamin and mineral supplements are required for growth in synthetic media containing purified carbohydrates, most crude substrates are rich in minerals such as iron, manganese, zinc, copper, cobalt, magnesium and calcium and contain amounts of phosphorus and sulfur adequate for good growth. Indeed, most investigators who have worked with waste substrates have found that only nitrogen, and in some cases, phosphorus additions were required. Falange (1962) reported increases in growth of A. campestris on vinasse with the addition of ammonium sulfate. Supplements of

Table 7. Changes in composition of grain and trub press liquors with sludge supplementation.

Sample	Red. Sugar (mg/l)	BOD (mg/l)
Grain Press Liquor (GPL)	30,000	56,100
GPL + .05% Sludge N (17.4% Sludge)	26,000	58,000
GPL + .10% Sludge N (34.9% Sludge)	23,000	59,000
GPL + .15% Sludge N (52.3% Sludge)	20,000	61,900
Trub Press Liquor (TPL)	55,000	132,000
TPL + .05% Sludge N (17.4% Sludge)	47,000	121,000
TPL + .10% Sludge N (34.9% Sludge)	39,000	109,000
TPL + .15% Sludge N (52.3% Sludge)	32,000	98,000

magnesium sulfate and potassium phosphate had little or no effect, however. In similar experiments using soybean whey, Falange et al. (1964) noted no effect on the growth of T. nudum with the addition of iron, sulfur, zinc, phosphorus or potassium and only slight growth increases with magnesium additions. Peppler (1968) on the other hand, observed that both phosphorus and potassium supplements are used in the commercial production of C. utilis from sulfite liquor.

Selection of Organisms for Further Studies

From the eight original organisms, C. steatolytica and C. gigantea were chosen for further experimentation. Selection was based on the net yield, the degree of BOD reduction, the protein content of the cells, and the total amount of protein produced.

With respect to the yeast cultures, selection was based primarily on net yield, as the preliminary data did not indicate any consistent differences in either BOD removal or total protein production. Thus, although S. cerevisiae removed the greatest quantity of BOD from grain press liquor, there was essentially no difference in BOD reduction among the yeasts grown on the other wastes. C. steatolytica had the lowest cellular protein concentration. However, because of higher yields, its total protein production was equivalent to that of the other yeasts in grain press liquor, and only slightly less than S. cerevisiae and C. utilis in trub press liquor. The only distinct differences were observed in net yield where the best growth on all wastes was obtained with C. steatolytica. On this basis the decision was made to use C. steatolytica for further studies.

More pronounced differences were noted in evaluating the mushroom cultures. C. gigantea clearly produced the best yields and achieved the greatest reductions in BOD. Although its mycelial protein concentration was lower than M. esculenta and A. bisporus, its total protein production was slightly higher than any of the other cultures. It seemed then, that C. gigantea was definitely the best suited of the mushrooms for cultivation on brewery wastes.

Growth Studies

Yield

As shown in Table 8, significantly greater yields of C. gigantea were obtained on supplemented wastes. The improvement in growth due to supplementation was particularly notable in grain press liquor and was less pronounced in trub. Optimum net yields were

Table 8. Yields of Calvatia gigantea on supplemented brewery wastes.*

Waste	Red.Sugar:N	Net Dry Weight (g/l)	% Red.Sugar Used	Yield Factor (g cells/g sugar)
Grain Press Liquor (GPL)	69.1:1	6.25	83.8	.248
GPL + .05% N as $(NH_4)_2SO_4$	32.1:1	15.19	90.3	.660
GPL + .10% N as $(NH_4)_2SO_4$	20.9:1	13.03	86.7	.501
GPL + .15% N as $(NH_4)_2SO_4$	15.5:1	10.17	73.3	.462
GPL + .05% N as Sludge Liquor	28.4:1	10.67	83.0	.498
GPL + .10% N as Sludge Liquor	16.0:1	9.96	87.0	.498
GPL + .15% N as Sludge Liquor	10.1:1	9.23	82.1	.576
Trub Press Liquor (TPL)	72.0:1	27.72	68.4	.749
TPL + .05% N as $(NH_4)_2SO_4$	43.5:1	39.74	70.9	.883
TPL + .10% N as $(NH_4)_2SO_4$	31.2:1	36.61	76.4	.872
TPL + .15% N as $(NH_4)_2SO_4$	24.3:1	15.65	74.5	.382
TPL + .05% N as Sludge Liquor	37.3:1	31.44	81.8	.816
TPL + .10% N as Sludge Liquor	22.6:1	28.21	83.9	.881
TPL + .15% N as Sludge Liquor	13.9:1	25.52	85.5	.945

*All samples buffered with 3 g/l of potassium phosphate and adjusted to pH 6.0.

obtained on samples supplemented with ammonium sulfate. As expected, slightly lower yields were recorded in the samples supplemented with sludge. It was determined that maximum growth in both substrates, with both nitrogen sources was obtained at a level of .05% added nitrogen. At nitrogen concentrations exceeding this, decreases in yield were observed. With ammonium sulfate supplementation, this may indicate that the substrate did not have sufficient buffer capacity to maintain suitable pH ranges at higher nitrogen levels. As noted by Reusser et al.(1958b), the use of ammonium sulfate tends to increase acid production in the medium. At higher concentrations of sludge nitrogen lower yields are at least partly related to substrate dilution.

Actually, as shown in Table 9, the yields in all samples containing sludge were in excess of the amounts which would be expected from the sum of the yields on the separate components of the sample. Thus, in GPL supplemented with 52.3% sludge (.15% N), the expected growth, as calculated from growth in unsupplemented GPL and unsupplemented sludge, would be 4.57 g/l (6.25 g/l in GPL x .478 + 3.04 g/l in sludge x .523). The actual yield, however, was 9.23 g/l, a 102% increase over the calculated yield. This improvement in growth is attributed to the added nitrogen.

The reducing sugar:N ratios which afforded maximum yield were found to be independent of the nitrogen source. In grain press liquor, the best growth was observed at reducing sugar:N ratios in the area of 30:1, while the figure for trub press liquor was closer to 40:1.

Reducing sugar utilization was slightly improved over that in unsupplemented wastes. The difference was probably not significant, however. The data show no relationship between nitrogen content and

Table 9. The effectiveness of sludge liquor in increasing yields of C. gigantea.

	Calculated* Yield (g/l)	Actual Yield (g/l)	% Change
GPL + .05% Sludge N	5.69	10.67	+ 88
GPL + .10% Sludge N	5.14	9.46	+ 94
GPL + .15% Sludge N	4.57	9.23	+102
TPL + .05% Sludge N	23.42	31.44	+ 34
TPL + .10% Sludge N	19.13	28.21	+ 42
TPL + .15% Sludge N	14.83	25.52	+ 72

*Calculated on the basis of % GPL x yield in unsupplemented GPL + % sludge x yield in unsupplemented sludge.

utilization of reducing sugar, which provides a strong indication that non-reducing sugars and other carbon sources are being assimilated.

Improvements in the growth of C. steatolytica (Table 10) were not as dramatic as those observed with C. gigantea. A maximum of 8.08 g/l of dry cells was obtained in grain press liquor, while total yields up to 12.70 g/l were recorded in trub press liquor. This represented increases of only 29% and 20% respectively over growth in the control samples.

In contrast to the results shown in Table 8, net yields of C. steatolytica in grain press liquor were found to be independent of nitrogen source, i.e., growth obtained using sludge nitrogen was equivalent to or greater than that obtained with ammonia nitrogen. It would be tempting to attribute this to the presence of growth factors

Table 10. Yields of Candida steatolytica on unsupplemented brewery wastes.*

Waste	Red.Sugar:N	Net Dry Weight (g/l)	% Red.Sugar Used	Yield Factor (g cells/g sugar)
Grain Press Liquor (GPL)	69.1:1	6.28	74.2	.282
GPL + .05% N as $(NH_4)_2SO_4$	32.1:1	6.51	76.7	.283
GPL + .10% N as $(NH_4)_2SO_4$	20.9:1	7.89	78.3	.336
GPL + .15% N as $(NH_4)_2SO_4$	15.5:1	5.45	76.0	.239
GPL + .05% N as Sludge Liquor	28.4:1	8.08	79.2	.384
GPL + .10% N as Sludge Liquor	16.0:1	7.84	80.4	.424
GPL + .15% N as Sludge Liquor	10.1:1	6.93	82.1	.433
Trub Press Liquor (TPL)	70.0:1	10.56	63.6	.302
TPL + .05% N as $(NH_4)_2SO_4$	43.5:1	10.71	68.1	.286
TPL + .10% N as $(NH_4)_2SO_4$	31.2:1	12.70	70.0	.330
TPL + .15% N as $(NH_4)_2SO_4$	24.3:1	11.03	65.4	.306
TPL + .05% N as Sludge Liquor	37.3:1	10.25	72.3	.301
TPL + .10% N as Sludge Liquor	22.6:1	9.06	71.8	.324
TPL + .15% N as Sludge Liquor	13.9:1	8.04	71.0	.365

*All samples buffered with 3 g/l of potassium phosphate and adjusted to pH 6.0.

or other required nutrients in sludge. If this is the case, however, these growth factors are evidently present at optimum levels in trub, as shown by the fact that the addition of sludge liquor actually results in apparent decreases in net yield (assumed to be due to substrate dilution as noted with C. gigantea).

This is demonstrated further in Table 11, which shows that yields in GPL supplemented with sludge were 9-28% higher than would be expected on an additive basis, while yields in trub were essentially equivalent to calculated yields (i.e., sludge addition had little effect on growth of C. steatolytica in TPL). From these data it appears that ammonium sulfate is the preferred N source for C. steatolytica.

The optimum nitrogen concentration appears to be slightly higher with ammonium sulfate than with sludge liquor. Thus, maximum yield in both wastes was obtained at .10% ammonia nitrogen and between .05 and .10% sludge nitrogen. A greater number of samples, at smaller increments of nitrogen supplementation, should be analyzed to determine whether a difference does indeed exist.

Maximum yield was obtained on grain press liquor at reducing sugar: N ratios in the area of 20:1 to 25:1. On trub press liquor, optimum production occurred on reducing sugar:N ratios of 30:1 to 35:1. In general these ratios were higher for sludge nitrogen than for ammonium sulfate. As noted previously with C. gigantea, reducing sugar utilization was improved slightly with supplementation, although there was no obvious relationship between nitrogen level and degree of sugar utilization.

Table 11. The effectiveness of sludge liquor in increasing yields of C. steatolytica.

	Calculated* Yield (g/l)	Actual Yield (g/l)	% Change
GPL + .05% Sludge N	6.31	8.08	+28
GPL + .10% Sludge N	6.34	7.84	+24
GPL + .15% Sludge N	6.37	6.93	+ 8.8
TPL + .05% Sludge N	9.84	10.25	+ 4.2
TPL + .10% Sludge N	9.12	9.06	- .66
TPL + .15% Sludge N	8.42	8.04	- 4.5

*As before.

Since neither C. gigantea nor C. steatolytica has been extensively studied, there is no data available on their growth characteristics in other substrates. However, yields obtained by previous investigators working with similar organisms, indicate that the yields observed in this study are quite high. The maximum yields of mushroom mycelia reported previously were 26.6 g/l of Agaricus blazei (Block et al., 1953) and 29.6 g/l of Morchella hybrida (Reusser et al., 1958a). The yield of 39.74 g/l of C. gigantea is considerably higher than these values. Yields of yeast cultures are usually on the order of 8-10 g/l (Inskeep et al., 1951; Gray et al., 1964). Again the yield of 12.7 g/l of C. steatolytica obtained in these experiments compares well with these figures. As noted previously, the yields reported in this study may be slightly high due to the use of a convection oven in the drying process.

Protein Production

As shown in Table 12, significant increases in protein content were achieved with nitrogen supplementation. Maxima of 44.25% and 44.52% protein were obtained in C. steatolytica and C. gigantea, respectively. As noted in the preliminary studies, protein concentrations obtained in trub press liquor were higher than those obtained in grain press liquor.

In grain press liquor, C. steatolytica produced maximum protein at .10% added nitrogen from either nitrogen source. Optimum protein was obtained in trub press liquor at .10% added ammonium nitrogen and at .05% added sludge nitrogen. With both wastes, the yields of protein at optimum nitrogen levels were considerably greater with ammonia nitrogen nitrogen than with sludge nitrogen.

Conversely, maximum protein production in C. gigantea was attained with sludge rather than ammonium sulfate supplementation. The optimum levels of nitrogen addition in both wastes ranged from .10% to .15%, with the highest yields obtained at .15% added sludge nitrogen. The differences in mycelial protein concentration between .10% and .15% added nitrogen were generally quite small.

The maximum protein concentration obtained in C. steatolytica was 44.25%, which is at the lower range of protein concentrations most investigators have observed in yeast cells. A similar protein content of 44.52% was attained by C. gigantea. This value compares quite favorably with the usually reported mycelial protein concentrations. Falange (1962) has reported 44.6% protein in a strain of A. campestris and 48.3% protein in M. hybrida. Cantharellus cibarius has the highest protein content reported for mushroom mycelia: 53.8% (Reusser et al.,

Table 12. Effect of nitrogen supplements on protein content of C. gigantea and C. steatolytica grown on grain press liquor and trub press liquor.*

Waste	C. steatolytica		C. gigantea	
	Crude Protein (% dry wt.)	Total Protein Production** (g/l)	Crude Protein (% dry wt.)	Total Protein Production** (g/l)
Grain Press Liquor (GPL)	21.30	1.34	13.69	.85
GPL + .05% N as $(NH_4)_2SO_4$	28.75	1.87	29.40	4.40
GPL + .10% N as $(NH_4)_2SO_4$	40.13	3.17	35.62	4.64
GPL + .15% N as $(NH_4)_2SO_4$	34.87	1.90	37.37	3.80
GPL + .05% N as Sludge Liquor	27.75	2.24	33.54	3.59
GPL + .10% N as Sludge Liquor	34.87	2.73	37.94	3.78
GPL + .15% N as Sludge Liquor	34.65	2.40	44.40	4.10
Trub Press Liquor (TPL)	23.49	2.48	17.50	4.85
TPL + .05% N as $(NH_4)_2SO_4$	27.75	2.97	28.56	11.35
TPL + .10% N as $(NH_4)_2SO_4$	44.25	5.62	38.27	14.01
TPL + .15% N as $(NH_4)_2SO_4$	34.81	3.84	29.70	4.65
TPL + .05% N as Sludge Liquor	37.14	3.81	40.27	12.66
TPL + .10% N as Sludge Liquor	33.60	3.04	44.33	12.50
TPL + .15% N as Sludge Liquor	29.56	2.37	44.52	11.36

*All samples buffered with 3 g/l of potassium phosphate and adjusted to pH 6.0.
**Calculated as net dry wt./per liter of cells or mycelia x % protein/100.

1958a). In interpreting these values it is important to again note the effects of drying technique. Presumably, samples dried in a convection oven have a higher moisture content than samples dried by other methods. If this is the case, the protein concentrations reported here may be slightly low.

BOD Reduction

Table 13 shows the effects of nitrogen supplementation on BOD removal. In general, C. gigantea was found to be much more efficient than C. steatolytica in this respect. The addition of ammonium sulfate to grain press liquor had a negligible effect on the removal of BOD by C. steatolytica. Sludge liquor, however, permitted BOD reductions up to 42%, a 12% increase over the control. In trub press liquor, there was very little difference between the nitrogen sources in effecting reductions in BOD. Both supplements permitted 52% to 54% BOD removal, a 7-10% increase over the control. It was generally observed with C. steatolytica that increasing nitrogen additions beyond .05% had little additional effect on BOD removal.

Ammonium sulfate permitted the largest reductions in BOD attained with C. gigantea. Maxima of 75% BOD reduction in grain press liquor and 65% BOD reduction in trub press liquor were obtained at .05% added ammonia nitrogen. With sludge supplements, BOD reductions of only 65% and 63% were recorded on grain press liquor and trub press liquor, respectively. These values were obtained at .10% added nitrogen.

As noted in the growth studies, the effects of sludge addition on BOD removal were significantly greater than could be accounted for on a simple additive basis. Table 14 shows that BOD was reduced up to

Table 13. Effect of nitrogen supplements on BOD reduction in grain press liquor and trub press liquor.*

Waste	% BOD Reduction	
	C. steatolytica	C. gigantea
Grain Press Liquor (GPL)	28.2	56.2
GPL + .05% N as $(NH_4)_2SO_4$	28.1	75.0
GPL + .10% N as $(NH_4)_2SO_4$	31.2	67.2
GPL + .15% N as $(NH_4)_2SO_4$	29.7	66.4
GPL + .05% N as Sludge Liquor	40.0	55.3
GPL + .10% N as Sludge Liquor	41.8	65.1
GPL + .15% N as Sludge Liquor	39.2	59.1
Trub Press Liquor (TPL)	45.5	56.1
TPL + .05% N as $(NH_4)_2SO_4$	50.7	65.2
TPL + .10% N as $(NH_4)_2SO_4$	54.5	62.1
TPL + .15% N as $(NH_4)_2SO_4$	50.7	55.3
TPL + .05% N as Sludge Liquor	52.1	54.5
TPL + .10% N as Sludge Liquor	50.0	63.3
TPL + .15% N as Sludge Liquor	48.5	59.8

*All samples buffered with 3 g/l of potassium phosphate and adjusted to pH 6.0.

Table 14. The effectiveness of sludge liquor in increasing BOD reduction by C. steatolytica and C. gigantea.

	C. steatolytica			C. gigantea		
	Calculated* BOD Red. (%)	Actual BOD Red. (%)	Change in % BOD Red.	Calculated* BOD Red. (%)	Actual BOD Red. (%)	Change in % BOD Red.
GPL + .05% Sludge N	27.5	40.0	+ 12.5	50.9	55.3	+ 4.4
GPL + .10% Sludge N	26.8	46.8	+ 15.0	45.6	65.1	+ 9.5
GPL + .15% Sludge N	26.3	39.2	+ 12.9	40.4	59.1	+ 18.7
TPL + .05% Sludge N	41.8	52.1	+ 10.3	50.8	54.5	+ 3.7
TPL + .10% Sludge N	38.1	50.0	+ 11.9	45.5	63.3	+ 17.8
TPL + .15% Sludge N	34.5	48.5	+ 14.0	40.3	59.8	+ 19.5

*Calculated as: (% GPL x % BOD reduction in unsupplemented GPL + % Sludge x BOD reduction in unsupplemented Sludge).

19.5% more than would be caluclated on the basis of BOD reduction in unsupplemented wastes.

Maximum BOD reductions obtained with C. steatolytica were in the area of 55%, which is still somewhat less than the 60% BOD reductions reported by Inskeep et al. (1951) and Reiser (1954). C. gigantea was considerably more successful in this respect, removing up to 75% of the BOD in grain press liquor and 65% of the BOD in trub press liquor.

Feasibility of Growing Mushrooms on Brewery Wastes

As shown in the preliminary investigations, all the wastes are capable of supporting microbial growth. Grain and trub press liquors, due to their sugar concentrations, produced the highest yields. Growth was generally rather poor on sludge liquor, however, and it was found that the best means of utilizing this waste was in combination with the press liquors, where its high nitrogen content actually stimulated growth.

It has been demonstrated that suitable microorganisms can significantly reduce the BOD of supplemented brewery wastes. If nitrogen requirements of the organisms are met, it would be reasonable to expect to obtain BOD reductions of 60 to 70%. Of all the organisms studied, C. gigantea has proven to be the most satisfactory in this respect, removing up to 75% of the BOD in some samples. C. steatolytica, the most promising of the yeast cultures, was considerably less effective, achieving a maximum BOD reduction of 54%. Similarly, cell yields and total protein production obtained with C. gigantea were far greater than those obtained with C. steatolytica. On this basis, it

would seem that further study of C. gigantea is warranted.

A number of factors are involved in assessing the feasibility of using microorganisms to reduce the BOD of brewery wastes. In determining whether a 60 or 70% BOD reduction would economically justify the costs of constructing, operating and maintaining an installation to produce microbial protein, the costs for municipal processing of untreated wastes and the potential reduction in these costs which could be achieved must be considered. The market price for microbial protein must be considered, along with the fact that there is not currently a large demand for this product, so that substantial marketing and advertising costs would probably be incurred. At present the only commercially produced mushroom mycelia is that of a Morchella species. The dried powder sold for $3.60 per lb in 1967 (Litchfield, 1967a). Food yeasts, on the other hand, are considerably cheaper, ranging from $0.15 per lb for dried Torula yeast (C. utilis) to $0.42-0.48 per lb for dried S. cerevisiae (Peppler, 1968). The slow growth of mushroom mycelia (which could probably be reduced to 3 or 4 days by the use of a fermentor) is a further economic disadvantage which must be weighed against the faster growth (but often poorer yields) of the yeasts.

In spite of all the potential problems, however, the results of these experiments indicate that further investigations employing C. gigantea and perhaps several other organisms are merited. Such work should concentrate on increasing yield and decreasing incubation time. This should include more detailed supplementation experiments and would require the use of a fermentor in growth studies so that

conditions could be standardized. Other important studies include a nutritional evaluation of the proteins, both through amino acid analyses and feeding studies.

SUMMARY AND CONCLUSIONS

This study was undertaken to investigate the utilization of brewery wastes as a substrate for the growth of microorganisms. Eight microorganisms - four yeasts and four mushroom species - were evaluated for their ability to grow, produce protein and reduce the BOD of the wastes. Three wastes, grain press liquor, trub press liquor and fermentation tank sludge were employed in these experiments.

Analysis of the wastes indicated that the press liquors contained relatively large concentrations of reducing sugars and only small amounts of nitrogen, while the converse was found to be true of sludge. BOD values ranged from 32,000 mg/l in grain press liquor to 133,000 mg/l in trub press liquor.

Preliminary growth studies indicated that all the wastes were capable of supporting some degree of microbial growth, although trub press liquor was by far the best substrate. In general, C. steatolytica, of the yeasts, and C. gigantea, of the mushrooms, were found to be best suited for growth in these media. They both were equivalent to or exceeded all the other yeasts or mushrooms in net yield, total protein production and reduction of BOD. On this basis, further experiments, using these two organisms, were performed to determine the effectiveness of nitrogen supplementation on growth in the wastes.

Two nitrogen sources were used. Since sludge liquor was high in nitrogen, and low in sugar, it was felt that a convenient method of

utilizing it would be to combine it with the press liquors. As a comparison of the effects of sludge nitrogen on growth, a second set of samples was supplemented with ammonium sulfate. Potassium phosphate was also added to all samples, serving both as a buffer and a source of phosphorus and potassium.

It was found that nitrogen addition caused a significant increase in growth. Yields of up to 39.7 g/l of C. gigantea and 12.7 g/l of C. steatolytica were obtained. In addition, protein content of the cells of both organisms was doubled and maximum BOD reductions of 54% and 75% were achieved by C. steatolytica and C. gigantea, respectively.

There was no great difference in yield with either nitrogen source. C. gigantea produced more protein using sludge, but removed greater quantities of BOD with ammonium sulfate as a nitrogen source. C. steatolytica generally produced slightly more protein and achieved greater BOD reductions with ammonium sulfate. The optimum level of nitrogen supplementation was found to vary depending on the criterion being evaluated. Growth and BOD reduction were usually greatest at .05% N, while protein production was highest at .10 or even .15% N. In general, however, the best results were obtained between .05 and .10% added N. This reflected optimum reducing sugar:N ratios of 20:1 to 30:1 in grain press liquor and 30:1 to 40:1 in trub press liquor.

It has been shown that significant yields of microbial protein and corresponding reductions in BOD can be obtained in brewery wastes. These results definitely justify further work in the area, and indeed considerably more data is needed before any feasibility judgment can be made from an economic standpoint.

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