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CHARACTERIZATION AND BIOCONVERSION
OF GREAT NORTHERN BEAN
BLANCHING EFFLUENT

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CHARACTERIZATION AND BIOCONVERSION OF GREAT NORTHERN BEAN BLANCHING EFFLUENT

By

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ABSTRACT

CHARACTERIZATION AND BIOCONVERSION
OF GREAT NORTHERN BEAN
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Characterization of blanching/cooling effluent, including mass balance, chemical analyses and waste strength assessment, was performed. Results were used in choosing Hansenula anomala for single cell protein bioconversion.

The wastewater contained high amounts of total solids and BOD. Included in the solid portion were starch (49.5%), protein (23.6%), and ash (18.7%). Nitrogen and phosphorus levels were above those necessary for good microbial growth. A high BOD to COD ratio (0.95) indicated a waste easily and completely degradable.

A series of batch fermentations utilizing whole, filtered, nitrogen-enriched, and pH-controlled blancher effluent as substrates for growth of H. anomala was carried out. Cell protein and BOD reduction were measured. Controlling pH resulted in the greatest BOD reduction (93% at 36 hours) and the highest cell yield (14.51g dry cells/l). Nitrogen enrichment gave the highest cell protein content, 55.8%. Great Northern bean blanching effluent was a suitable growth medium for H. anomala.

To Carla

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INTRODUCTION

Two current and long-term problems facing mankind are pollution of the environment and production of adequate food and feed. Both of these concerns are aggravated by increasing world population. The use of non-toxic food processing and other agricultural wastes to support the growth of protein-rich microorganisms for human and animal consumption is a process which addresses both of these problems.

Improvement and maintenance of surface and ground waters are important activities of government and industry. Research efforts generally aim to identify major pollution sources, develop alternative processes to reduce or eliminate effluent streams, and develop effective and economical treatments and utilizations for wastes. The food industry is technologically advanced in conversion of raw agricultural goods into acceptable food products, but less attention has been given to water usage and waste generation. Considerable effort will need to be expended if a trend toward a cleaner environment is to develop.

Recent water pollution control legislation has shifted emphasis from stream-by-stream pollution accounting to controlling pollutants at each source. Legal procedures

for prosecution of violators have been streamlined and heavier penalties have been provided. Limitations on effluent strength are based on levels of cleanup possible employing the best conventional pollution control technology. In many cases, modification or replacement of existing waste handling equipment and/or procedures is necessary. Conversion of waste into a marketable by-product would not only reduce the amount of waste discharged, but would also generate supplemental income. Recovery of nutrients previously lost in the wastewater and of newly synthesized protein would help alleviate food and feed shortage problems.

The present study concerned wastewater generated by the blanching operation of a small bean processing plant. Traditionally, this waste was handled by the public wastewater treatment facility located in the same village as the processing plant. The cost to the plant operator of this waste treatment rose significantly when the town recently restructured its sewage rate assessment policy. The new rate structure equitably divides treatment costs among the users on the basis of their proportionate share of the waste. Operating capacity of the current lagoon waste stabilization system, built in 1969, has been surpassed and the town may ask the plant owners to finance renovations and improvements to the system or to pretreat their wastes before discharging.

The current study was undertaken to determine whether the effluent from the bean blanching operation was suitable for bioconversion. Fifty-three percent of the total organic load of this plant's waste was found to originate in the blanching

operation (PEDCo. Environmental, Inc., 1979). This is typical of fruit and vegetable processing wastes (Bomben, 1977; National Canners Association, 1971). Objectives of this research were: (1) calculation of solid mass balance for the bean blanching operation, (2) chemical characterization of the blancher effluent, (3) use of wastewater composition information to determine the biological treatability of the waste and to select a suitable organism for growth, and (4) analysis of the benefits derived from growth of the organism in the waste. The last objective was assessed by determining the reduction in the biochemical oxygen demand (BOD) of the waste and the production of microbial protein occurring during growth of the organism in the waste.

Bioconversion of waste would be practical only if economically attractive, therefore, supplementation and manipulation of the waste, adding to overall treatment costs, were to be kept to a minimum. The goal of this project was to evaluate raw, unsupplemented Great Northern bean blanching effluent as a potential growth medium for a selected microbe.

REVIEW OF LITERATURE

Food Processing and Water Pollution

Food processing wastewaters are a major source of non-toxic organic pollution, frequently containing high energy organic matter such as sugars and fats. These wastes represent not only a burden on receiving waters, but also a substantial loss of nutrients for human consumption. Eckenfelder (1976) reported that in the U.S., over half of these wastes are handled by municipal waste treatment facilities. However, due to recent legislation, the food industry is faced with selecting or developing alternative waste treatment methods.

The Federal Water Pollution Control Act Amendments of 1972 required sweeping federal and state campaigns to prevent, reduce and eliminate water pollution (Ninety-second U.S. Congress, 1972). Also known as Public Law 92-500, this act represented a fundamentally new approach to the nation's water pollution problem. While previous laws dealt with pollution as it became a problem in individual waters, P.L. 92-500 established uniform national standards which controlled pollutants at the source (Willis, 1973). The act also provided for more effective prosecution and stiffer penalties for violators.

The two main objectives of the law were: (1) to achieve wherever possible, by 1983, water quality good enough for swimming and other recreational uses, and clean enough for the production and propagation of fish, shellfish and wildlife, and (2) the elimination of discharges of pollutants into all waters by 1985 (Ninety-second U.S. Congress, 1972). Within provisions for waste management plans were requirements that all users of a treatment system pay an equitable share of operation and maintenance costs (Willis, 1973). Industrial users were mandated to pay an additional user charge reflecting their share of the Federal funding for original capital assets of new projects.

Deadlines for application of new technology to water pollution control from industrial sources were established. Industries discharging into the nation's waters were required to apply the "best practicable control technology currently available" (BPT) by July 1, 1977 and the "best available technology economically achievable" (BAT) by July 1, 1983 (Ninety-second Congress, 1972). The Environmental Protection Agency was to issue guidelines defining these technologies, taking into consideration the cost of pollution control, the age of the facility, the process used, and the economic impact (Willis, 1973).

Studies on the effect of P.L. 92-500 on various industries were performed. Olson et al. (1974) reported estimates of expected fruit and vegetable processing plant closings due to pollution control costs to vary between 296 and 468, depending on the extent of cleanup required. The original

act provided for midcourse corrections after three years of research, hearings and debates. These changes were made into law as the Clean Water Act of 1977, P.L. 95-217 (Schafer, 1978). The emphasis of the 1972 law remained, with P.L. 95-217 categorizing wastes as toxic, conventional or non-conventional, each having its own technology-based controls. Typical food wastes (biochemical oxygen demand, suspended solids, pH, etc.) were classified as conventional wastes. A new level of technology was defined -- "best conventional pollutant control technology" (BCT) including factors used to define BPT and BAT, plus a new cost test (Schafer, 1978). This test called for a reasonable relationship between costs and benefits. Industries discharging conventional pollutants were to achieve effluent reduction by application of BCT by July 1, 1984.

The food industry utilizes more public treatment and ground disposal for handling of its waste than do other U.S. manufacturers (National Canners Association, 1971). Fruit and vegetable processing, especially blanching and cooling operations, results in a large amount of wastewater (Bomben, 1977). Most cannery wastes contain more than ten times the biochemical oxygen demand (BOD) found in domestic sewage (Weckel, 1967). Quantities of wastewater and pollution loads per unit of production vary greatly depending on the commodity processed, the product style, the percentage of plant capacity used and the method of conveying the product and solid wastes (Olson et al., 1974).

Analysis of individual unit operations in canning indicated that blanching was a major source of effluents (Lund, 1974).

Ralls and Mercer (1973) reported that an average of 40% of cannery wastes were due to blanching. Depending on the commodity, blanching serves to expel gases from the tissue, inactivate enzymes, reduce microbial load, cleanse the product, elevate the product temperature prior to retorting, facilitate further operations such as peeling or dicing, set natural colors, and in some cases, wilt or soften the product to facilitate packaging (Lopez, 1981).

Blanching of dry legumes results in hydration and softening prior to further processing. Hot water and/or steam blanching are most common, with both methods producing liquid wastes high in volume, BOD and water soluble nutrients (Lund, 1974). Several studies of fruit and vegetable processing wastes have reported relatively high effluent strengths for dry bean wastewaters. Olson et al. (1974) reported that of twenty-six fruit and vegetable processes investigated, dry bean processing resulted in the largest amount of wastewater and the highest BOD per ton of raw product. The values reported were 30% and 50% higher, respectively, than for the commodity ranked second in each category. In a similar study, Detroy and Hesseltine (1978) observed thirteen fruit and vegetable processing operations and found legume waste loads among the highest for BOD and total solids per ton. Legume cooking water was found to contain measurable amounts of all nine minerals tested, and relatively high amounts of magnesium, phosphorus and potassium (Meiners, et al, 1976b). Hang and Woodams (1979) reported

high BOD in baked bean processing wastewater and noted that municipal activated sludge processes produced foul odors when this waste was treated. A similar odor problem was noted during investigation of lagoon treatment of the wastewater from the present study (PEDCo. Environmental, Inc., 1979).

Food processing wastewater can be characterized as being readily biodegradable, non-toxic, and of relatively high organic concentration (Eckenfelder, 1976). The real and present danger of toxic pollutants has recently overshadowed the concern shown in the late 1960's for general improvement of water quality. If man is to be considered the organism ultimately affected, than all types of water pollution are of biological significance. Organic wastes effect changes in subtler and slower ways than do toxins. Biochemical oxygen demand is a useful measurement for determining the relative oxygen requirements of wastewaters and for evaluating waste treatment procedures (APHA, 1971). However, an understanding of how organic wastes deteriorate normal habitats of streams and other receiving waters can give a greater appreciation of BOD's broader meaning. Clean waters contain a well-balanced population, usually comprised of a large number of species with relatively few individuals of each. Generally, the number of individuals is inversely proportional to the organism size, with millions of bacteria, hundreds of insects, several fish, etc., existing in a balanced food pyramid. Pure waters usually contain minerals from soil

and rocks, particles from erosion, and organic matter from decaying plants and animals. The waters are cool and contain high levels of dissolved oxygen. Soluble organic material is in low supply. The wasting of soluble food materials into streams can greatly stimulate the growth of aquatic organisms. Huge numbers of bacteria and fungi soon overwhelm previous population levels, utilizing much more than their share of available dissolved oxygen to oxidize the sudden abundance of readily available food. Higher species in the food chain are not able to reproduce quickly enough to control microbial populations and are soon stressed by oxygen deficiency. This effect rises through the food chain and a complete overthrow of the kinds and numbers of organisms present results. New conditions promote development of large populations of a few species of organisms. Low-oxygen requiring species invade and if conditions become anaerobic, the only living species present will be anaerobic bacteria. As waste moves downstream and is oxidized, a point is eventually reached where natural aeration will provide oxygen sufficient to exceed demand. Here the stream begins recovery. Although in the U.S., probably little raw food plant effluent is dumped into streams, this is not the case everywhere. It is hoped that this discussion will help the reader to understand BOD as more than a waste treatment parameter. It represents the oxygen required to meet the metabolic needs of aerobic microbes living in water rich in organic matter.

Chemical oxygen demand (COD) is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant (APHA, 1971). COD values are almost always larger than BOD values for a particular waste as they include some compounds, e.g., cellulose, which are not part of the immediate biochemical load on the oxygen of the receiving water. COD determinations can be performed much more rapidly (hours) than can BOD determinations (5 days), making them useful for waste treatment monitoring.

Protein Production from Agricultural Wastes

The terms "biomass", "microbial biomass" and "single cell protein" (SCP) are used interchangeably throughout this thesis unless otherwise noted. Cooney et al. (1980) defined single cell protein as a generic term for crude or refined sources of protein whose origin is unicellular or multicellular microorganisms, including bacteria, yeasts, molds, and algae. Reed (1981) preferred to refer to these whole cells as biomass, and reserve SCP for proteins and protein concentrates extracted from the cells. The author feels that the first definition is receiving greater current use and will use it herein.

Yeasts and other microbes have been consumed for tens of thousands of years, traditionally unnoticed as part of a fermentation process. The first full scale effort at producing microbes for human consumption came about 1910 in Germany,

with drying of spent brewer's yeast (Rose, 1961). Traditional substrates for SCP production have been carbohydrates, either as sugars or starchy materials readily degraded to sugars (Reed, 1981). Recognition of severe worldwide protein shortages in the 1960's, as well as the need to combat high BOD pollution from industrial wastes, spawned interest in a wide range of substrates and organisms. Methane, petrochemicals and paraffin hydrocarbons were investigated (Litchfield, 1977). Cellulose, lignin and related materials were seen as potentially vast untapped reserves of fermentation substrates (Dunlap, 1975). Most solid agricultural wastes contain cellulose as the principal carbon source, but the low growth rate of cultures capable of converting cellulose and similar carbohydrate polymers to fermentable sugars has "made biomass production from untreated cellulosics difficult to envision" (Dunlap, 1975). Humphrey et al. (1977) have been able to produce up to 45g cell solids per 100g of cellulose by using a thermophilic organism grown on a pure cellulose (Avicel) substrate, however this hardly seems to be a practical waste treatment.

Research on starchy or mixed agricultural wastes has been more fruitful. During the last decade many substrates and organisms have been investigated. Some lab-scale investigations (substrate, plus organism in parentheses) are presented here: cheese whey (Aeromonas hydrophilia), tapioca starch (Candida utilis), carob bean waste (Aspergillus niger), corn and pea wastes (Trichoderma viride, Geotrichum sp.),

brewery wastes (Calvatia gigantia), baked bean processing waste (Aspergillus foetidus), sauerkraut brine (Candida utilis) and potato processing waste (various bacteria) (Butany and Ingledew, 1973; Thanh and Wu, 1975; Imrie and Vlitos, 1975; Church et al., 1973; Shannon, 1973; Hang and Woodams, 1979; Kyung, 1978; Sistrunk et al., 1979). Many of the studies of this period were aimed at reduction of BOD in effluents rather than production of biomass. Generally these studies proved successful at reducing BOD in the range of 50-90% and producing biomass of 35-83% protein (Anderson et al., 1975).

Few organisms and substrates have been scaled-up to commercial production (Moo-Young, 1976). Yeasts have been the organism of choice for most operations, being grown predominately on paraffins and sulphite liquor. Commercial-scale food waste bioconversion is currently being done with whey and potato starch wastes.

Use of Single Cell Protein as Food and Feed

Incentive for research into utilization of microorganisms as food and fodder comes from concern over increasing world population and its effect on food supplies. General advantages offered by microbes over plants and animals as protein producers are: (1) short generation time allowing rapid mass increase, (2) easy modification by genetic manipulation, (3) protein content higher than most common foodstuffs, (4) production based on raw materials locally available in large quantities,

and (5) continuous cultivation independent of climatic changes and requiring little land area and water (Kihlberg, 1972). Microbial biomass for use in the feed industry is blended with other dry feed ingredients or introduced through wet culture of grain mashes by yeasts (Reed, 1981). Special preparations called "yeast cultures" are prepared by seeding cereal grain mashes with yeast cells, incubating and then drying the fermented mash so as to preserve live yeast cells, enzymes and other heat sensitive nutrients. Reed (1981) reported that a majority of the spent brewer's yeast produced in the U.S. was used in feeds. Yeasts are also commonly known as a rich source of B vitamins.

One of the major limitations to use of microbial biomass as food for humans is the high content of nucleic acids (Cooney et al., 1980; Litchfield, 1977; Viikari and Linko, 1977). However, these compounds present no problem to livestock. Humans lack the enzyme urate oxidase (uricase) and ingestion of purine compounds may lead to elevated blood levels of uric acid. This metabolite is only slightly soluble at physiological pH and crystals may form in the joints (gout) or urinary tract (Calloway, 1974; Lehninger, 1975). In most mammals, uric acid is oxidized to soluble allantoin and is excreted in the urine.

As a food for human consumption, SCP can be viewed as a protein supplement or as a source of protein isolates and concentrates with functional attributes useful in food systems (Litchfield, 1977). Seeley (1975) reported that anticipated

hesitance in acceptance of microbial proteins by humans meant that commercial success of SCP depended upon post-fermentation processing treatments to develop functional food ingredients. Many studies have been conducted in this field. In the form of dried whole cells, SCP may be used for improving the quality of cereal proteins (by helping balance available essential amino acids), enrichment of vitamin and mineral content of foods, improvement of physical properties of food (e.g., water-binding capacity), and as a dietary protein source (Chen and Peppler, 1978). Yeast protein isolates, prepared by disruption of the cell wall, extraction and concentration of proteins, can be used to add or enhance desirable properties in foods. Flavor and texture improvement have been areas of investigation. Reed and Peppler (1973) reported on the preparation of the potent flavor enhancers 5'-inosine monophosphate and 5'-guanidine monophosphate from nucleic acid extracted from yeast biomass. In a variety of products, yeast extract has been used to partially replace the flavor enhancer monosodium glutamate, resulting in savings of 50-60% (Anon., 1981). Seeley (1975), Chen and Peppler (1978) and Reed (1981) reported textural improvements in a variety of foods incorporating SCP concentrates. Success in these trials was attributed to the isolates' functional properties, including: fiber-forming property, whippability, foaming property, dispersibility, elasticity, viscosity and interaction with other protein systems. Another subcellular component, referred to as bakers yeast cell wall glycan, has been reported to rapidly and markedly lower the

serum cholesterol of hypercholesterolemic rats (Robbins and Seeley, 1977). Isolated comminuted yeast cell walls comprised this glycan fraction.

Problems limiting the use of SCP by humans are the presence of toxic factors, high nucleic acid content, and poor digestibility (Calloway, 1974). A series of guidelines concerning toxicity evaluation of SCP was published by the Protein Advisory Group of the United Nations (Chen and Peppler, 1978). Particular emphasis was placed on testing for toxic residues introduced with the substrate (e.g., pesticides), testing of culture conditions and treatments (heat damaged protein, contamination), and testing for absence of pathogens, viable cells, mycotoxins, substrate and solvent residues. Chen and Peppler compiled data from various sources showing SCP preparations to have a range of digestibility values of 32-95, as compared to 97 for egg and 79 for oatmeal. Addition of 0.03% methionine to three yeast strains raised the digestibility an average 43.8%.

High nucleic acid content must be reduced if SCP products are to become major protein sources for humans. At current safe levels of nucleic acid intake, only about twenty grams of yeast SCP (10% nucleic acid) can be consumed daily (Waslein et al., 1970). This accounts for less than one-third of the RDA for a 70kg adult male.

Much has been published about the technology of biomass protein production, including a profusion of techniques for reducing nucleic acid content. Viikari and Linko (1977) divided these processes into five cagetories: (1) limitation

of nucleic acid synthesis during fermentation, (2) production of protein isolates or concentrates with reduced nucleic acid content, (3) hydrolysis and extraction of nucleic acids from whole cells by the action of various chemicals, or by (4) exogenous or (5) endogenous enzymes. A partial list of techniques includes: succinylation of protein after cell disruption (protein removal is facilitated by binding succinyl groups, nucleic acid remains in supernatant), cell suspension in NaOH or aqueous ammonia, heat shock treatments, and cell suspension in methanol/HCl mixtures (Shetty and Kinsella, 1979; Viikari and Linko, 1977; Tannenbaum et al., 1973; Tamura et al., 1972).

The field of biomass fermentation research is full of variables and room for optimization. Each substrate offers a unique nutrient profile and the challenge of finding a suitable organism for biological treatment. Gaining consumer acceptance, especially in sophisticated markets, may prove to be the greatest barrier to increased SCP utilization.

MATERIALS AND METHODS

Source of Wastewater

Wastewater samples for all analyses and fermentations were obtained from the Randall Foods, Inc. plant in Tekonsha, Michigan. Effluent from three consecutive commercial blanchings of Great Northern beans (*Phaseolus vulgaris* L.) was collected on February 16, 1982. Samples were stored at -20°C until needed. Dried and blanched bean samples were also obtained for use in calculation of mass balance.

Nature of Study

Objectives of this study included:

a. calculation of solid mass balance for the bean blanching operation,

b. chemical characterization of the blancher effluent,

c. use of wastewater composition information to assess biological treatability of the waste and to select a suitable organism for growth, and

d. analysis of benefits derived from the fermentations, e.g., reduction of BOD and production of high-protein biomass.

Wastewater was collected from the effluent stream of three consecutive blanchings (from here referred to as runs A, B, C.) Water from each run was analyzed, in triplicate,

for total solids, volatile solids, ash, simple and complex carbohydrates, protein, pH, and chemical and biochemical oxygen demands (COD, BOD). Duplicate analyses were performed to determine levels of eleven minerals.

Given the nutrient profile of the wastewater, a suitable organism for bioconversion was sought. Due to the very favorable growth potential offered by the water, the literature indicated that several organisms might do well. The yeast Hansenula anomala was chosen primarily because of its ability to utilize starch without pretreatment of the waste.

Review of the literature indicated optimal physical parameters for the fermentations (temperature, aeration, agitation). These selected parameters were held constant throughout the experiments. A series of batch fermentations, each of three replications, was conducted with the common goals of reduction in BOD and production of single-cell protein. Wastewater pretreatments investigated were filtration, nitrogen supplementation and pH control.

Statements in this thesis which indicate differences between results of analytical procedures do not imply statistical significance. Rather, data from experiments was observed and the trends or differences stated represent nonstatistical assessments by the author. The presentation of data in tables and graphs is discussed more fully in the Results and Discussion section.

Analytical Procedures

Material Balance

In order to illustrate the great usage of water and loss of solids which occurs during the processing of dry beans, it is useful to calculate a material, or mass, balance. This can show the uptake of water by the beans as well as generation of wastewater containing leached bean solids. To collect the necessary data, the industrial processing procedure was observed to familiarize the researcher with the various operations during which contact between beans and water allowed exchange. Both dry and blanched beans were collected along with wastewater for analysis of moisture content. AOAC method 14.003 (AOAC, 1970) was used to determine total solids and moisture of beans. Approximately 2g of well mixed sample were accurately weighed into a dried, cooled, and previously weighed dish. Following drying at 100°C to a constant weight in a partial vacuum oven, the residue was allowed to cool in a desiccator, and was weighed. Triplicate determinations were made on the dry and blanched beans.

Results of these moisture determinations, data from wastewater analyses, recorded input of dry beans and fresh water and output of blanched beans and wastewater were combined to develop the mass balance.

Wastewater Analysis

Gathering of wastewater samples was done at a single location (the blancher effluent stream) following three

consecutive blanch runs. All of the following analyses were performed in triplicate on each of the runs.

Total Solids, Volatile Solids, Ash. Methods 224A and 224B of the Standard Methods for the Examination of Water and Wastewater (APHA, 1971) were used to characterize the solids present in the waste. One-hundred ml portions were dried to a constant weight at 103°C. After cooling in a desiccator, the residue of the sample was weighed and recorded as mg/l total solids. The residue was then ignited at 550°C in an electric muffle furnace to a constant weight. Material volatilized during this operation was referred to as volatile solids and that remaining was ash.

Carbohydrates. Two procedures were utilized for the measurement of simple and complex carbohydrates. The sugars glucose and sucrose, the sugar alcohol m-inositol, and the oligosaccharides raffinose and stachyose were analyzed using high pressure liquid chromatography (HPLC), according to the procedure of Agbo (1982). A portion of sample containing 1g of solids was mixed with 10ml of 80% ethanol in an 80°C shaking water bath for 10 minutes. This mixture was then centrifuged for 3 minutes at 2000 rpm and the supernatant was collected in a separate tube. This extraction was performed twice more, with 5ml of 80% ethanol used the last (third) time. The precipitate was saved for starch determination. To the extract were then added 2ml of 10% lead acetate. This mixture was shaken and centrifuged

at 2000 rpm for 3 minutes to precipitate proteins. The supernatant was removed and to it 2ml of 10% oxalic acid were added to remove the lead acetate. Again, the mixture was shaken and centrifuged and the final supernatant was brought to 55ml with deionized distilled water. Passage of this solution through a SEP-PAK C_{18} cartridge (Waters Associates, Inc., Milford, MA) was done prior to injection into the HPLC. The chromatography system was comprised of a Solvent Delivery System 6000A, a Universal Chromatograph Injector U6K, a Differential Refractometer R401, and a Data Module Model 730 (Waters Associates, Inc., Milford, MA). The solvent used was a 75:25 v/v acetonitrile:water mixture at a flow rate of 2.5ml/min. Resolution of sugars from this aqueous extraction was accomplished using a Carbohydrate Analysis (μBONDAPAK) column (Waters Associates, Inc., Milford, MA). An external standard method programmed into the Data Module was used to identify and quantitate the sugars. The injected sample size for each determination was 30μl.

The following procedure for starch analysis, taken from Agbo (1982), utilized the precipitate remaining after the third ethanol extraction. These steps served to hydrolyze the starch pellet into glucose which was then measured by the YSI Model 27 Industrial Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). The precipitate was allowed to air dry before being transferred to a 350ml centrifuge tube. Fifty ml of 0.5N NaOH solution were added. After the pellet was completely dissolved, 50ml of 0.5N acetic acid

were added to neutralize the slurry. This was then centrifuged at 1500 rpm for three minutes and the supernatant was collected. To 0.4ml of the supernatant were added 1.8ml of α-amylase solution (2mg/ml). This incubated for one hour at which time 1.8ml amyloglucosidase solution (5mg/ml) were added for an additional hour. Following calibration of the YSI Model 27, injections of 25 μl were made to assay for glucose.

The percentage starch was calcluated according to the following formula:

$$\text{percent starch} = \frac{\text{amount shown on data module (mg/ml)} \times \text{original volume (ml)} \times 100 \times 0.9}{\text{sample weight (mg)}}$$

In this formula, the factor 0.9 accounts for the water gained during starch hydrolysis.

Protein. Measurement of organic nitrogen as an indicator of crude protein was done using AACC Method 46-13; the Micro-Kjeldahl Method (AACC, 1962). Into a Kjeldahl digestion flask were placed 1.1ml wastewater (containing 34.1mg total solids), 1.30g K_2SO_4, 40mg HgO and 2.0 ml H_2SO_4. This mixture was digested on an electric heater at a boil sufficiently vigorous to distill the acid into the neck of the flask. After cooling and bringing into solution with a minimum of distilled deionized water, the digest mixture, with 8ml of sodium hydroxide - sodium thiosulfate solution added, was

steam-distilled until about 20ml of distillate were collected. A 125ml Erlenmeyer flask containing 5ml of 4% boric acid solution and 4 drops of methyl red-methylene blue indicator solution was used to receive the distillate. Titration of NH_3 to a faint pink endpoint was done using 0.0224 N HCl. Blank determinations, following the same procedure less the wastewater, were also made to determine the amount of N in the reagents.

Minerals. The following eleven minerals were analyzed by direct-coupled plasma emission spectroscopy: boron, zinc, iron, manganese, copper, molybdenum, aluminum, phosphorus, magnesium, calcium and potassium. Sample size for these analyses was 32ml (0.992g total solids) of wastewater. Sample preparation was based on recommendations of Jones and Warner (1969) and Jones and Steyn (1973). The wastewater sample was pipetted into a previously dried and weighed crucible, dry-ashed in an electric muffle furnace at 500°C for 10 hours, and allowed to cool. The residue was dissolved in 5ml of 6N nitric acid and allowed to stand 1 hour. The ash solution, brought to a volume of 10ml with distilled deionized water containing 1000ppm LiCl, was then filtered into labeled vials for analysis of Al, B, Cu, Fe, Mn, Mo and Zn. The solution was then diluted 50-fold with 1000ppm LiCl solution prior to analysis for Ca, K, Mg and P.

The spectrophotometer used was a Spectraspan IIIA Emission Spectrophotometer (Spectrometrics, Inc., Andover, MA) and included an automatic sampler, direct coupled plasma

emission spectrophotometer, microprocessor, and multielement capacity integrator. Interfaced was a TI Silent 700 Printer (Texas Instruments, Dallas, TX). A computer program converted spectrograph counts to percent concentration for Ca, K, Mg, and P, and parts per million for Al, B, Cu, Fe, Mn, Mo and Zn. These analyses were performed at the Soil Chemistry Laboratory, Department of Crop and Soil Science, Michigan State University.

pH. Measurement of pH was made using an Orion Research Model 901 Microprocessor Ionalyzer equipped with a pH electrode attachment (Orion Research, Cambridge, MA).

Biochemical Oxygen Demand (BOD). All BOD determinations were made using the standard 5-day incubation period in accordance with the method described in the Standard Methods for the Examination of Water and Wastewater (APHA, 1971). Several trial runs were undertaken to allow tailoring of the procedure to the wastewater. Samples were diluted with deionized distilled water so as to contain 500 to 1500mg/l BOD (Michigan Bureau of Environmental Protection, 1978). One ml of this water was then pipetted into a standard BOD bottle. The bottle was then filled with dilution water prepared according to Section 219.4a of the Standard Methods for the Examination of Water and Wastewater (APHA, 1971). Half of the bottles were incubated for 5 days in the dark at 20°C while the others were immediately analyzed for dissolved oxygen. After incubation, dissolved oxygen measurements were made on the sample bottle contents. BOD values were calculated from the amount of oxygen consumed during the incubation period. As

a check on the quality of the dilution water, trials were run by the same procedure less the waste sample. The precision of the method was checked by determining the BOD of a glucose-glutamic acid solution and comparing it with expected results (APHA, 1971).

Dissolved oxygen measurement was made utilizing a Beckman Model 0206 Oxygen Analyzer (Beckman Instruments, Inc., Irvine, CA). The sensor was placed directly into the sample allowing oxygen in the sample to diffuse through a membrane separating the sample from an internal polarographic cell, resulting in current flow proportional to the partial pressure of oxygen in the sample. Calibration of the instrument was made using the air-saturated water method as described in the operator's manual (Beckman Instructions 015-555375-A).

Chemical Oxygen Demand (COD). COD determinations were done in accordance with Section 220 of the Standard Methods for the Examination of Water and Wastewater (APHA, 1971). The wastewater was diluted to 300 to 800mg COD/l, of which 20ml were added to a 350ml ground glass 24/40 neck Erlenmeyer flask. To this were added 0.4g $HgSO_4$, 10ml of 0.250N potassium dichromate and several glass beads. After attachment to the condenser, 30ml of concentrated H_2SO_4 (containing 9.73g Ag_2SO_4/1 H_2SO_4) were carefully added through the condenser while swirling the refluxing flask. This mixture was allowed to reflux for 2 hours after which it was diluted and cooled. The excess dichromate was titrated with standardized 0.1000N ferrous ammonium sulfate solution and ferroin indicator to a

red-brown endpoint. The quantity of oxidizable organic matter present in the waste is proportional to the potassium dichromate consumed. Evaluation of technique and reagent quality was performed by determining the COD of a standard solution of potassium acid phthalate and comparing it to expected values.

Fermentations

All fermentations were carried out in a 7 liter capacity bench-top Microferm Fermentor (New Brunswick Scientific Co., Inc., Edison, NJ) which included automatic temperature, aeration and agitation control. Fermentations were done as batch operations and employed the following parameters: steady temperature of 30°C, aeration rate of 1 liter air/liter wastewater/minute, and agitation rate of 800 rpm. Agitation was achieved by movement of an impeller shaft with flat paddles and vertical baffles, magnetically coupled to a precision drive mechanism. Impeller speed was regulated by an electronic feedback control to compensate for voltage fluctuation and viscosity changes in the culture medium. Air was metered through a pressure regulator, needle valve, flow meter and glass wool packed filter. Incoming air was fed through a sparger line with a single opening and outgoing air was passed through a water-cooled exhaust gas condenser where vapors and aerosols were condensed and returned to the culture. Regulation of temperature occurred via activation of an in-line immersion heater or of a valve in the cold water inlet line. Cool or warm water passed through a network of hollow baffles to help prevent temperature surges and improve control accuracy.

The fermentation vessel was made of Pyrex glass fabricated for repeated autoclavings (New Brunswick Scientific Co., Inc., 1979).

Each batch fermentation utilized three liters of wastewater. Analyses of BOD, COD and pH were performed on each batch prior to sterilization by autoclaving at 121°C for 15 minutes. Autoclaving was performed with the wastewater already in the fermentation vessel so that upon cooling only inoculation would be required before beginning the fermentation. The inoculum for each batch consisted of 300ml of a 30-hour culture of H. anomala in acidified malt extract broth (Dehydrated Bacto-Malt Extract, Difco B186). This resulted in an inoculation of approximately 5 x 10^6cells/ml.

A portion of the fermentation mixture was aseptically removed every 3 hours for 36 hours. Determination of COD and pH were conducted on these scheduled samples according to the methods outlined in the wastewater analysis section. These analyses permitted a close monitoring of the progress of the fermentation insofar as changes in the culture medium were concerned. To follow the growth of the yeast cells, enumeration by plating on acidified potato dextrose agar was performed at the same intervals (Dehydrated Bacto Potato Dextrose Agar, Difco B13). Flannigan (1974) suggested addition of sterile 10% tartaric acid solution subsequent to autoclaving, until a pH of 3.5 was reached. This acidification would inhibit bacterial growth while not affecting yeast growth. Duplicate pour plates, representing an appropriate range of dilutions of

the fermentation broth, were prepared and incubated at 25°C for 5 days (Koburger, 1973). Enumeration of colonies was performed manually. This data was reported as viable count, cells/ml.

In order to assess the effectiveness of the fermentations in achieving the goal of reducing the organic load of the wastewater, BOD and COD determinations were made on the water after completion of the fermentations and removal of the yeast cells. Along with pH, these were analyzed according to the methods outlined in the previous sections.

Harvesting of yeast cells from the fermentation broth involved centrifugation at 1300 x g for 10 minutes, and twice washing with distilled deionized water and recentrifuging (Thanh and Wu, 1975). Harvested cells were washed into a dried and tared aluminum dish using 10ml deionized distilled water. Determination of yeast dry weight (total solids) was performed according to AOAC Method 10.173 (AOAC, 1970). Cells were dried to a constant weight, cooled in a desiccator and weighed.

There exists no generally accepted unit of measure for expressing the relationship between cell growth and substrate utilization. Depending upon the researcher's discipline and primary aim of his project, one of many reasonable systems could be chosen, including, for example, g cell solids/g substrate (Abbott and Clamen, 1973), g yeast/g COD removed (Thanh and Wu, 1975), and g yeast/l substrate (Shannon, 1973). In this study, yield was expressed as g dried cells/l substrate.

Protein analysis on the dried yeast was performed in

accordance with AACC Method 46-13, as previously discussed. Sample size was 30mg of dried cells.

Design of Fermentation Experiment. This study was originally planned to include fermentation of whole wastewater, as well as a series of fermentations set up to evaluate the benefit of possible wastewater pretreatments. It was planned that all fermentations and analyses involved would be performed in triplicate. Results of chemical analysis and fermentation of whole wastewater would be assessed in planning further investigations. Thus, pretreatments of filtration, nitrogen supplementation and pH control (using phosphate buffer) were performed. The procedures employed are presented here.

Filtration. To determine the percent of BOD which would be removed from the wastewater by a primary settling or screening step, filtration through Whatman filter paper No. 1 was done prior to fermentation. This treatment also allowed harvesting of a cell pellet free of residual solid material from the waste. Accuracy of the cell protein and dry weight analyses for this treatment was thus enhanced.

Nitrogen Supplementation. Although analysis and fermentation of whole wastewater showed no nitrogen deficiency, several investigators claimed benefits from nitrogen enrichment. Obviously, much more cell growth occurs in media containing no nutritional deficiencies, however Thanh and Wu (1975) reported that addition of N, while not appreciably increasing the yield, resulted in cells containing 39.4%

more protein. Helmers et al. (1952) claimed that on the basis of BOD removal, little is to be gained by adding supplementary nitrogen in excess of some critical amount. Hansenula anomala required less supplemental N to achieve maximum yield than did three common food yeasts grown on molasses (El Sawy et al., 1977). In the present study, 0.1% (w/v) ammonium sulfate (21.2%N) was added to the water, increasing the nitrogen by 18.14% (212 mg/l).

pH Control. Nitrogen and phosphorus are the main nutrients required for efficient stabilization of organic waste (Thanh and Wu, 1975). Generally, if a fermentation substrate requires buffering, a phosphate buffer should be employed as it provides needed phosphorus as well as buffering action (Symons et al., 1960). Based on pH changes noted in whole wastewater fermentations, buffering at pH 6.5 was chosen. Following acidification of the wastewater to pH 6.5 by addition of HCl, 28.359g $NaH_2PO_4 \cdot H_2O$ and 13.419g Na_2HPO_4 were added to each three liter batch to achieve 0.1M phosphate buffer concentration (Gueffroy, 1978). Addition of these compounds (22.5% P and 21.8% P, respectively) also resulted in a dramatic increase in the phosphorus content of the water; a nutrient already far in excess of demand (Sawyer, 1956). Fermentations for this treatment were carried out for 48 hours to assess the value of the buffer.

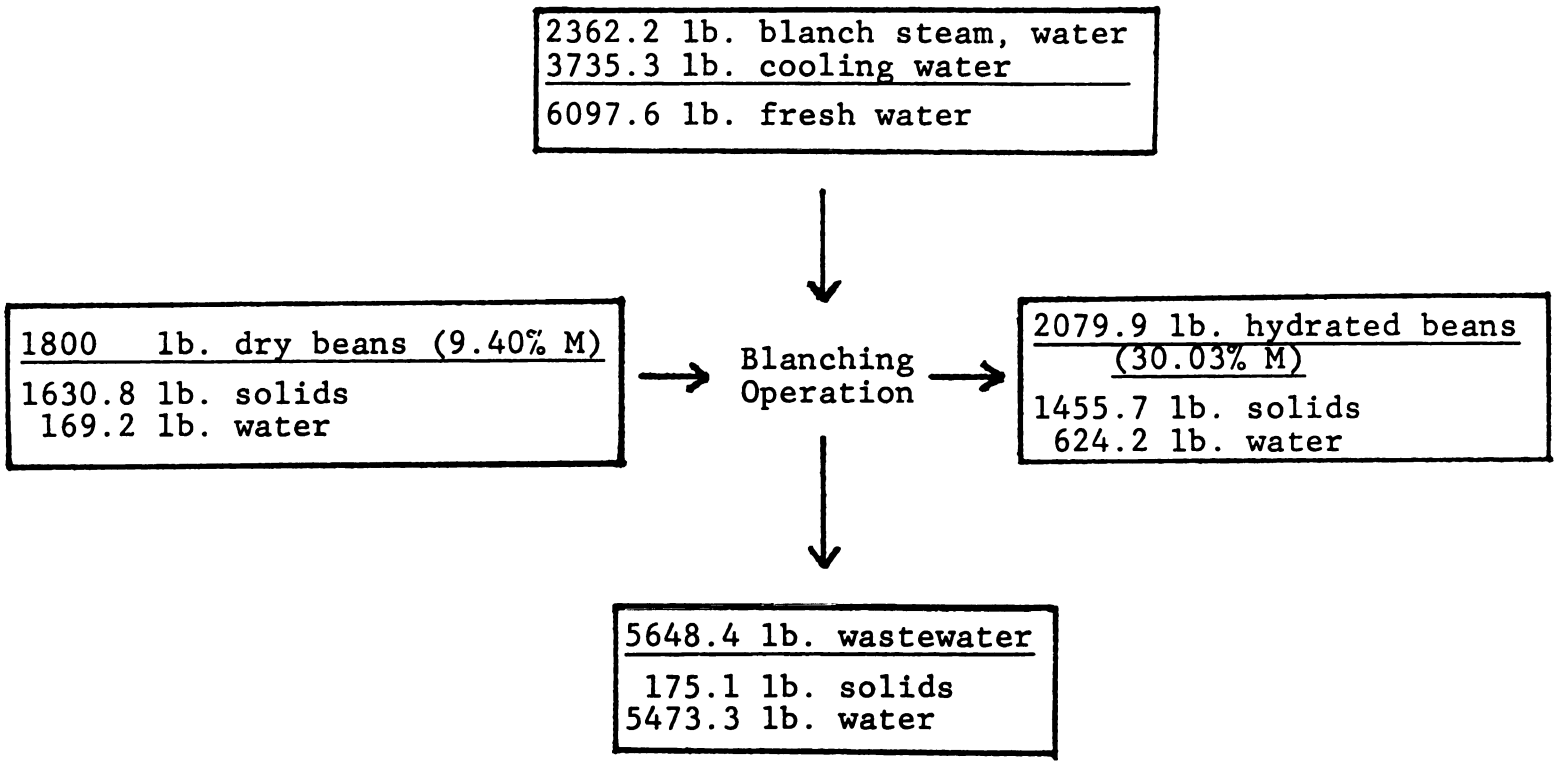
RESULTS AND DISCUSSION

Material Balance

A large portion of the total wastewater generated by the vegetable processing industry results from blanching and cooling of vegetables for canning and freezing (National Canners Assoc., 1971). An average of 40% of the plant effluent BOD was due to blanching, reported Ralls and Mercer (1973). Figure 1 shows the moisture and solids balance for the blanching operation involved in the present study. Material balances are useful in comparisons of blancher performance with theoretical values and for the mapping of nutrient loss and waste generation (Bomben et al., 1975; Bomben, 1977).

A typical blanch run is represented in Figure 1. Dry beans, at 9.40% moisture, were gravity-fed into the blancher from an outdoor storage silo. Water was added to the beans and they were steam cooked for 23 minutes, followed by cooling in an upflow rinse. Displaced water (blancher effluent) was trapped by overflow drains and piped to a temporary holding tank. After blanchig, the beans (30.03% moisture) were conveyed to a packing line where they were packed in jars containing a salt and sugar solution. Some of this packing brine spilled onto the floor and added to the total plant effluent BOD.

Figure 1. Typical moisture-solids balance for the twenty-three minute steam/hot water blanching and cooling operation for Great Northern beans, at the Tekonsha plant.



After pressure-cooking, the beans were labeled, packed and shipped.

Water added during the twenty-three minute steam blanch hydrated and softened the beans prior to canning and retorting. Little waste was generated during this hydration period but high temperatures and rapid swelling of the beans caused some splitting. Following blanching, cold water piped into the bottom of the blanch tank flowed up through the beans and out of the blancher as a hot translucent yellow liquid. This was the waste stream sampled for this study.

Bomben et al. (1975) used the following calculation as a crude measure of the nutrient loss from a vegetable during blanching and cooling:

$$\%\ \text{solids loss in blancher and cooler} = 100 \times \frac{\text{blancher/cooler effluent, lb./ lb. vegetable}}{} \times \frac{\%\ \text{total solids in effluent}}{\%\ \text{total solids in soaked vegetable}}$$

Using data from Figure 1, a 13.90% loss was calculated for this operation. This value is somewhat higher than that reported for peas by Bomben et al. (1975), probably due to the greater degree of splitting in Great Northern beans.

Wastewater Analysis

Results of chemical analyses of the effluents as well as composite data are given in Table 1. Measurements on three

Table 1. Composition of effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant, reported as mg/l (except pH).

	Blanch Run, Means (n=3)			Composite Characteristics (n=9)		
	A	B	C	Mean ± 1s.d.	Range	CV, %
	mg/l					
BOD	27607	27396	27237	27413 ± 489	1500	1.8
COD	29183	28987	28758	28976 ± 233	804	0.8
Total Solids	31104	31090	30806	31000 ± 899	2286	2.9
Nitrogen	1174	1166	1167	1169 ± 25	75	2.2
Carbohydrates:						
Starch	15536	15431	15044	15337 ± 261	860	1.7
Glucose	571	720	822	704 ± 145	453	20.6
Sucrose	111	106	102	106 ± 13	44	11.9
m-Inositol	475	470	466	470 ± 11	34	2.3
Raffinose	49	45	44	46 ± 3.8	14	8.3
Stachyose	580	527	516	541 ± 48	160	8.8
Ash	5890	5800	5707	5799 ± 165	554	2.8
pH	6.94	6.99	7.01	6.98 ± 0.03	0.11	0.4

separate runs were not made so as to differentiate between the runs, but rather to develop a composite characterization, i.e., the typical waste from this operation. The purpose of these measurements was to define an average nutrient pool which would be provided by the blanching operation for growth of an organism. In Table 1, composite values are presented as a mean $\pm$ one standard deviation, the range of the data collected (maximum value minus minimum value), and the coefficient of variability (CV, %). The CV is a relative measure of variation independent of the unit of measurement, whereas standard deviation is reported in the same units as the observations. Coefficient of variability can be useful in evaluating results of separate measurements of the same character (Steel and Torrie, 1980). Unless otherwise noted, reference is made to composite values.

Because of various factors influencing composition of food plant wastes (raw product quality, processing methods, waste collection techniques), it is difficult to make meaningful statements comparing results of different studies. Indeed, some studies and reviews report concentration ranges so broad that it is difficult to interpret the nature of these wastes at all. Values reported in Table 1 for BOD, N, total solids, and ash fall within ranges published for baked bean processing wastewater by Hang and Woodams (1979). Protein content (N x 6.25) is similar to that found in legume cooking water by Meiners et al. (1976a).

Table 2 shows the levels of 11 minerals in the wastewater.

Table 2. Mineral content of effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant, reported as mg/l.

	Blanch Run, Means (n=3)			Composite Characteristics (n=9)		
	A	B	C	Mean ± 1s.d.	Range	CV, %
	mg/l					
Potassium	3815	3703	3584	3700 ± 112	335	3.0
Phosphorus	1223	1154	1088	1155 ± 58	116	5.0
Magnesium	500	474	443	472 ± 24	70	5.1
Calcium	257	245	218	240 ± 17	54	7.3
Zinc	43.1	42.1	41.7	42.3 ± 3.4	11.8	8.1
Iron	25.9	25.5	25.1	25.5 ± 1.6	5.1	6.3
Aluminum	11.2	11.0	10.8	11.0 ± 1.0	3.1	8.8
Copper	5.3	5.2	5.1	5.2 ± 0.3	1.0	6.1
Manganese	4.7	4.6	4.5	4.6 ± 0.4	1.2	7.6
Molybdenum	0.2	0.2	0.2	0.2 ± 0.01	0.05	4.4
Boron	0.1	0.1	0.1	0.1 ± 0.02	0.05	16.7

Although ash values compared favorably with those of Hang and Woodams (1979), phosphorus levels in the current study were much greater. However, even higher levels of phosphorus and potassium were reported by Meiners et al. (1976b). Some of the dry beans entering the blancher were coated with dried mud, which probably contributed to variabilities noted in the mineral analysis. Coefficients of variability are well within the ranges reported for multiple determinations made on five standard solutions and eight plant tissues (Jones and Warner, 1969). Over half (62%) of the ash was potassium and the four "macrominerals", potassium, phosphorus, magnesium and calcium, together accounted for 95.8% of the total.

Choice of Organism

Results of wastewater characterization showed it to have a very favorable potential as a fermentation substrate. Several investigators have identified nutritional requirements for biological treatment of wastewaters (Helmers et al., 1952; Symons et al., 1960; Sawyer, 1956). A comparison of these criteria and levels provided in the current study are presented in Table 3.

Values listed as requirements in Table 3 represent the minimum ratios of N and P to BOD or COD necessary for unimpeded microbial growth. The ratio of BOD:COD gives an indication of the extent of degradation which can be expected using unacclimated organisms. A ratio of 0.6 or higher indicates a waste which can be easily and completely treated (Symons

et al., 1960). Some reported BOD:COD ratios are: 0.5-0.8 for various brewery wastes (Shannon, 1973), 0.67 for sauerkraut brine (Kyung, 1978), and over 0.95 for tapioca starch wastewater (Thanh and Wu, 1975). Nutritional requirements of various microorganisms differ but all require carbon, nitrogen and phosphorus. As shown in Table 3, the substrate in this experiment exceeded these general requirements for growth.

Table 3. Comparison of literature values for nutritional requirements for biological treatability of wastes with values provided by effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant.

	Required Values from Literature	Provided Values in Effluent
BOD:N:P[1]	100 : 3.1 : 0.67	100 : 4.3 : 4.2
BOD:N[2]	25- 33 : 1	23.4 : 1
BOD:P	143-200 : 1	23.7 : 1
BOD:COD[3]	0.6	0.95
COD:N	20 : 1	24.8 : 1
COD:P	100 : 1	25.1 : 1

[1](Sawyer, 1956)
[2](Helmers et al., 1952)
[3](Symons et al., 1960)

Bacteria, yeasts and filamentous fungi are all suitable for biomass conversion; each having advantages and disadvantages. Bacteria exhibit faster growth rates, higher protein

levels and higher sulfur amino acid levels. However, they are less traditional as a food source, they contain high levels of nucleic acids, and most importantly, their smaller cell size makes cell recovery a major technical and economic problem (Cooney et al., 1980). The higher fungi, only relatively recently investigated for single cell protein potential, possess potent enzymatic capabilities, are easily harvested and can grow on very inexpensive carbohydrate wastes. A major drawback of these organisms is their relatively slow growth, making contamination a concern (Cooney et al., 1980). Yeasts are the most popular bioconversion microorganisms for several reasons: (1) they are familiar to humans as food (leavening, vitamin supplements, flavoring additives), (2) they have a good protein content (47-56%), (3) they are easier to recover than bacteria, and (4) they contain fewer toxic compounds than do higher fungi and bacteria. Yeasts do contain higher nucleic acid levels than the filamentous fungi (Reed, 1981).

Hansenula anomala was chosen for this study based on a comparison of this organism's biochemical abilities with the nutrient profile of the wastewater substrate. H. anomala is able to aerobically assimilate glucose, sucrose, raffinose, and starch (Lodder, 1970). Although most of the carbohydrate present may not be immediately assimilable, the action of yeast amylases almost invariably breaks down starch more rapidly than the yeast can metabolize the resulting sugars (Harrison, 1971). H. anomala is capable of vigorous growth

on vitamin-free media, synthesizing all necessary vitamins (Phaff et al., 1978). Although of little importance in the present study, an interesting metabolic by-product of H. anomala grown in liquid cultures is the ester, ethyl acetate (Gray, 1949). This compound is a frequent component of the odors of fruits, particularly pineapples, strawberries and wines, and esters in general are responsible for various odors of fermented culture media (Phaff et al., 1978; Williams et al., 1974). Phaff et al. (1978) reported that H. anomala produced large amounts of acetic acid from glucose. This acid is enzymatically esterified with ethanol to form ethyl acetate, a transitory by-product which upon longer incubation and aeration is taken up by the cells and subsequently disappears from the media.

In a series of studies on protein production by yeasts, H. anomala fared well compared to 89 other strains tested (Mahmoud et al., 1977). High total nitrogen (10.32%) and yield (19.43g/l fresh weight, 3.3g/l dry weight) were reported, and H. anomala was one of 28 strains selected for further study. A subsequent investigation into protein, non-protein nitrogen and amino acid content of yeast strains was conducted (Abdel-Hafez et al., 1977). Of the 28 strains tested, H. anomala ranked fourth in protein percentage (52.69%) and fifth in protein per liter of medium (1.75g/l). Although all strains tested contained all of the essential amino acids, the sulfur amino acids were found to be relatively low. Other investigators have reported this trend (Reed, 1981; Harrison,

1971). H. anomala contained a higher amount of methionine (9.03mg/g dry yeast) than all but two of the yeasts tested. Total sulfur amino acids were also high in this organism (17.43mg/g dry yeast). In the last of this series of studies, El-Sawy et al. (1977) showed H. anomala to require less supplemental nitrogen to achieve maximum yield, maximum total N, maximum non-protein N and maximum protein content than did three other strains included in the study. In all, H. anomala proved to be metabolically well-suited for protein production when compared to a wide variety of other yeast strains.

Growth Studies

Three fermentations were carried out for each of three pretreatments plus whole wastewater. Table 4 presents data collected to show the overall changes resulting from a 36-hour fermentation by H. anomala. Also given are the results of 48-hour fermentations of water receiving pH control. Tabular values are the means of three replicate batch fermentation runs. Graphs showing changes in COD, cell number and pH vs. time are presented in Figures 2 through 5. The points plotted in these figures are mean values for three replicate runs. Statements regarding differences between results do not imply statistical significance, but rather nonstatistical observations by the author.

Table 4. Characteristics of effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant, before and after 36 or 48 hour fermentation with H. anomala.

Treatment	Before Fermentation			After Fermentation			%-decrease in BOD
	BOD (mg/l)	COD (mg/l)	pH	BOD (mg/l)	COD (mg/l)	pH	
Whole Water	27055	28596	7.08	4245	5816	7.36	84.3
Filtered Water	21060	21495	7.01	1874	2183	7.48	91.1
N-supp. Water	27267	29006	6.99	4008	5758	7.28	85.3
Buffered Water	27135	29032	6.50	1898	2709	6.56	93.0
Buffer/48 hr.	-----	-----	----	698	2104	7.07	97.0

n=3

Whole Wastewater

Waste conversion into useful biomass is often viewed as a system for reducing disposal costs while at the same time producing a saleable by-product (microbial protein). It is often undertaken more to recoup losses than for full-scale yeast production. Therefore, addition of chemicals to enhance the nutrient profile of a waste may defeat the original premise of the venture, namely, saving money. In an investigation of five biomass production facilities, Moo-Young (1977) found that the cost of supplements (phosphorus, nitrogen, other minerals, miscellaneous) ranged from 13.2 - 38.1% of the total production costs. A good medium for industrial fermentations consists of an inexpensive carbon and energy source, an inexpensive nitrogen source and sufficient biological adjuncts to meet specific nutritional requirements of the organism in question (Weinshank and Garver, 1967). Any other materials employed are used to shape the pH profile of the run or to otherwise direct the course of the fermentation.

The goal of the present study was not to determine the optimal nutrient supplementation levels for growth of H. anomala in this waste, but rather to investigate whether plain, unsupplemented waste could serve as a suitable substrate. Chemical analysis of whole wastewater indicated this potential. Experiments to test the effect of slight supplementation and modification of the waste were carried out to see if a real gain could be obtained through slight manipulation of the waste. Small scale biomass conversion would be

attractive to individual plant owners only if investment of labor and materials would be outweighed by reduced disposal costs and/or income from the sale of the by-product.

A graph depicting changes during fermentation of whole wastewater is found in Figure 2. There was a lag phase during which cell number increased only slightly. This period was marked by active metabolism and synthesis of new protoplasm; however, growth occurred in cell size, not cell number. A new environment required cells to synthesize new enzymes in amounts sufficient for optimal metabolism. The cells present were active; the lag was in cell reproduction. Following the lag phase was the exponential, or logarithmic, growth phase. As cells reproduced steadily at a constant rate, the log of the number of cells plotted against time was a straight line. Growth rate was at a maximum during this phase. After the log growth period, marked by transition from a straight line through a curve to another straight line, a period of stationary growth began. The population remained steady as a result of cessation of reproduction or by balancing of reproduction and death rates. This trend can be attributed to exhaustion of some nutrients or production of toxic waste products. A downward sloping line following the stationary phase signaled the death phase, during which cells died faster than new cells were produced. This resulted from a continuation of the factors leading to the termination of log growth.

Removal of COD from the medium paralleled the growth of the cells. After a lag phase, COD disappeared steadily

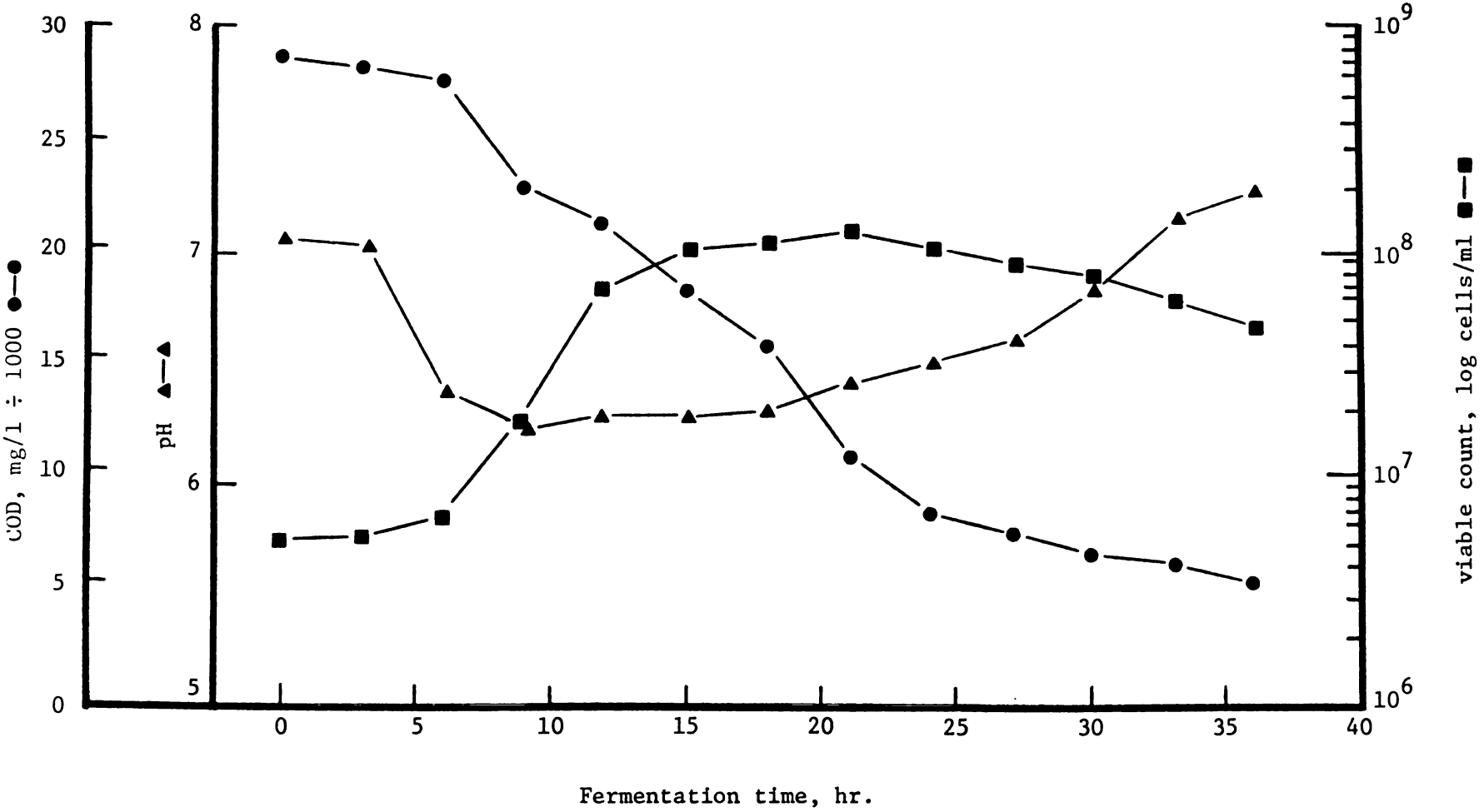


Figure 2. Changes in cell count, COD and pH during 36 hour H. anomala fermentation of effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant.

n=3

until the beginning of the cell death phase, at which time its rate of removal slowed. A rapid drop in pH was noted early in the run. In 6 hours, pH dropped from 7.08 to about 6.3. This probably resulted from the release of acids as glucose utilization by-products. Stabilization of pH at 6.3 occurred and lasted until 18 hours at which time pH began to steadily increase, finally reaching 7.36 at the end of the 36-hour fermentation. This increase in pH preceded the cell death phase and may represent the accumulation of toxic (basic) compounds resulting in reduced growth rate. The toxicity of these compounds was expressed as an increase in pH above that favoring good yeast growth.

A reduction of 84.3% of the BOD (79.7% of COD) of whole wastewater was accomplished. The resulting effluent had a BOD of 4245g/ml and a pH near neutrality.

Filtered Wastewater

Filtration of blancher effluent removed 23.2% of the BOD (25.8% of COD) prior to fermentation. The higher degree of COD removed may be due to filtration of bean skin particles which were not biodegradable. The remaining liquid proved to be a good fermentation substrate. Lag time appeared shorter and the maximum growth less with this water than with whole wastewater (see Figure 3). The pH at 36 hours was somewhat higher for filtered (7.48) than for whole water (7.36), perhaps reflecting removal of substances with buffering capacity. Another reason may be that the relatively low

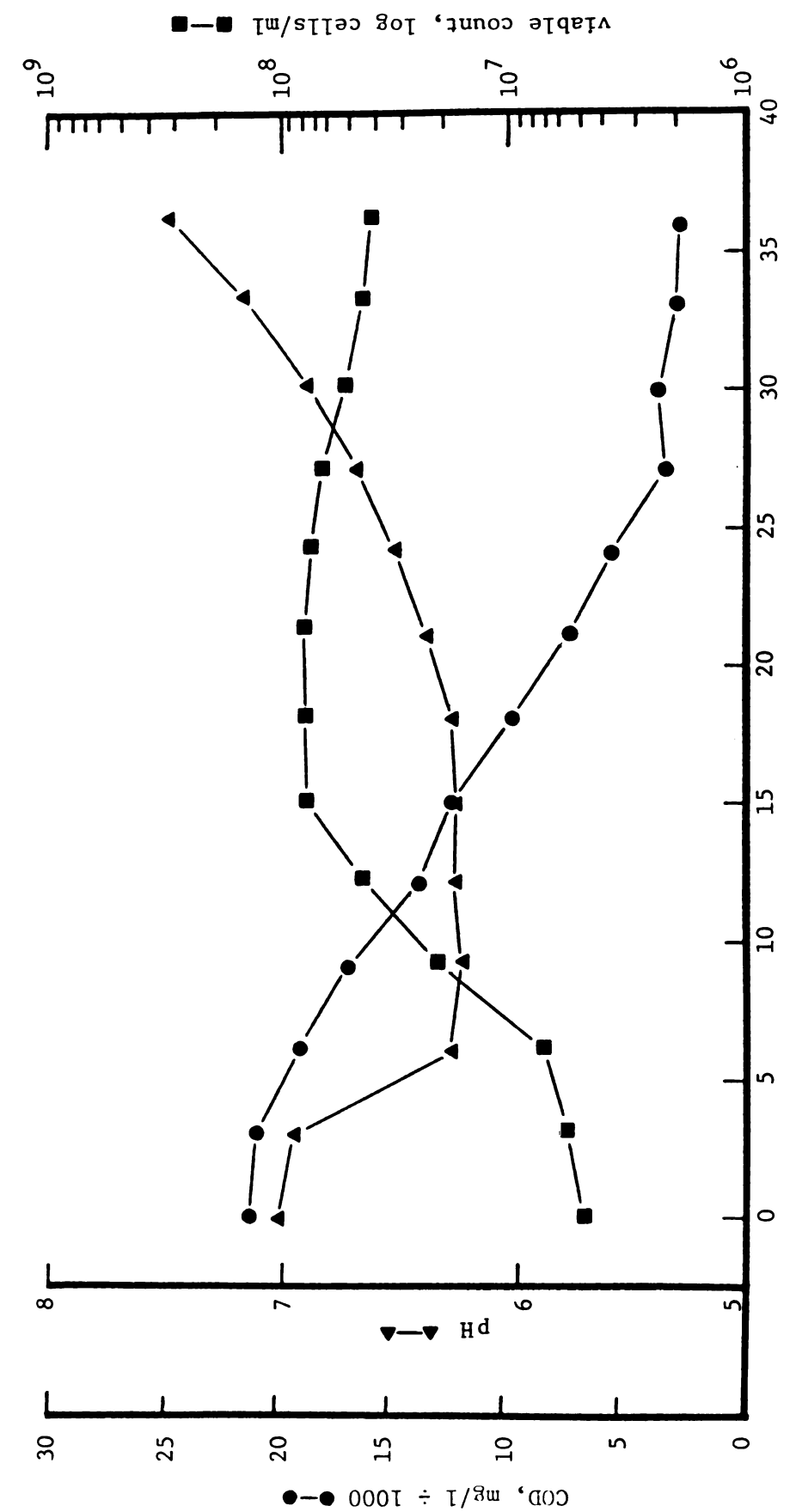


Figure 3. Changes in cell count, COD and pH during 36 hour H. anomala fermentation of filtered effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant.

n=3

energy supply of the medium at this late stage of the run was causing cells to deaminate amino acids for energy thereby releasing free NH_3 (Weinshank and Garver, 1967).

BOD and COD reduction, 91.1% and 89.8% respectively, were greater than those for whole wastewater. The combination of filtration and fermentation resulted in 93.2% reduction of BOD (92.5% of COD) found in whole wastewater.

Nitrogen-supplemented Wastewater

As seen in Figure 4, the growth pattern on N-supplemented water was similar to that on whole water, with maximum cell number slightly higher for the supplemented waste. One appreciable difference is that in the N-supplemented runs, pH did not begin to rise until after 21 hours, as compared to 18 and 15 hours, respectively, for whole and filtered water. When N is supplied as an ammonium salt ($(NH_4)_2SO_4$), the utilization of ammonia liberates free acid (H_2SO_4) into the medium. This is also reflected in the lower final pH (7.28) of these fermentations. Reductions of BOD and COD in this waste were 85.3% and 90.7%, respectively.

pH-controlled Wastewater

Results of whole wastewater fermentations showed the possibility of extending the stationary growth phase if the rise in pH preceding the cell death phase could be controlled. It also appeared as if log growth required a lower pH than that of the whole wastewater. To test the effect of starting

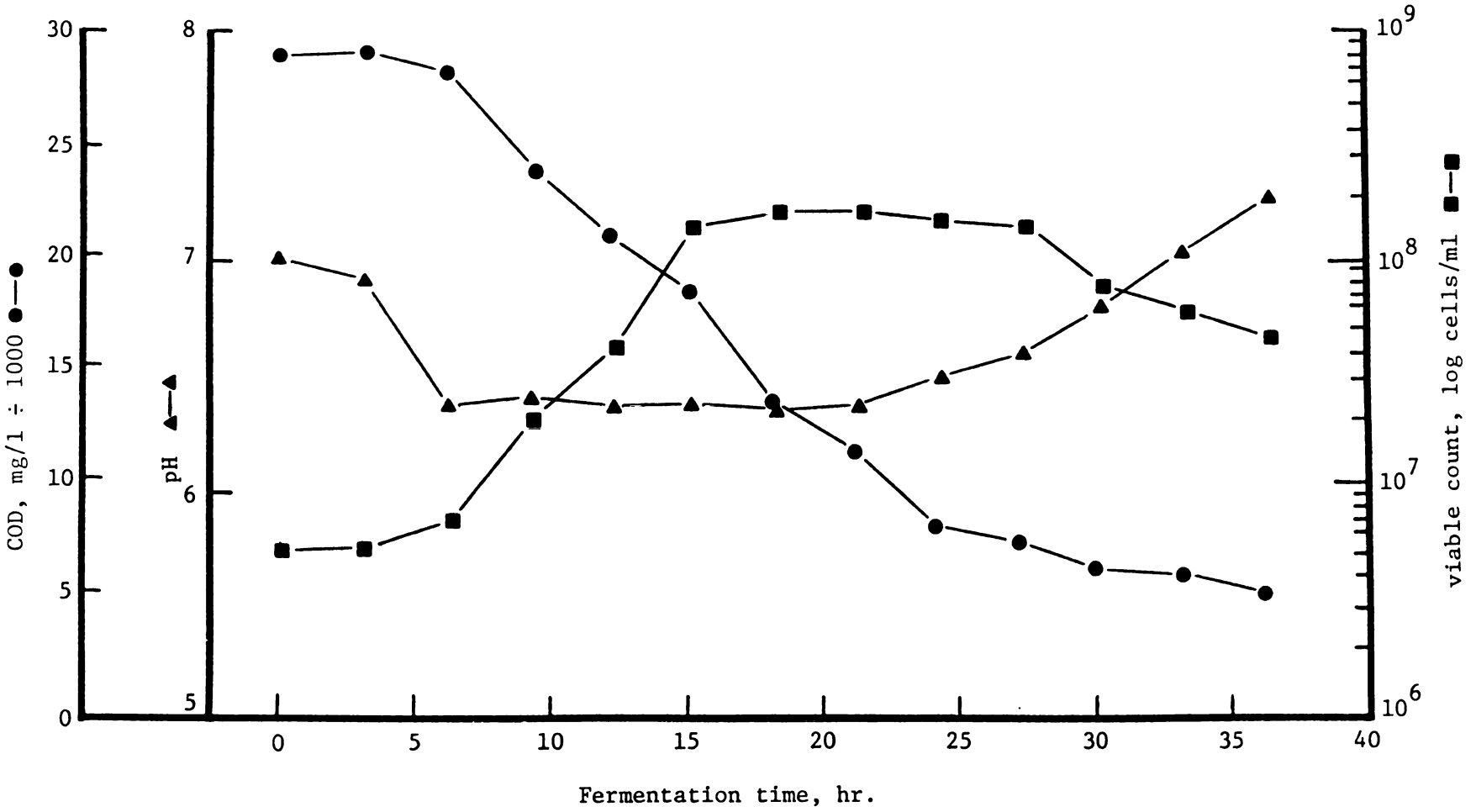


Figure 4. Changes in cell count, COD and pH during 36 hour H. anomala fermentation of N-supplemented effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant.

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n=3

with a lower pH and extending the period spent at the lower pH, the fermentation media was acidified to pH 6.5 and buffered at this pH with 0.1M phosphate buffer. The growth pattern for this pretreated waste is found in Figure 5. Lag phase in this water was nearly eliminated and stationary growth lasted well past those of un-buffered wastes. Removal of COD, however, decreased greatly approximately 6 hours prior to the start of the death phase. These data suggested that cell growth and reproduction may have nearly ceased but the stable pH allowed cells to remain alive, thereby giving a steady cell count. Further, new cells may have been utilizing compounds released by lysed cells and were therefore not assimilating as much COD.

The extended period of high metabolic activity resulted in a further reduction of BOD. However, even at 36 hours, 93.0% of the BOD (90.7% of COD) had been removed; a value greater than that for any previous treatments. This is likely due to the very stable low pH (approximately 6.47) maintained throughout this period. Allowing the fermentation to continue past 36 hours to gain an additional 4% reduction in BOD would depend on a cost-benefit analysis of the increased utility and space (fermentor) utilization. After 48 hours, BOD and COD had been reduced 97.0% and 92.8%, respectively. The final effluent had a pH of 7.07.

Cell Yield and Protein Content

Data for cell yield and protein content are presented in Table 5. Water receiving pH control and a 48-hour

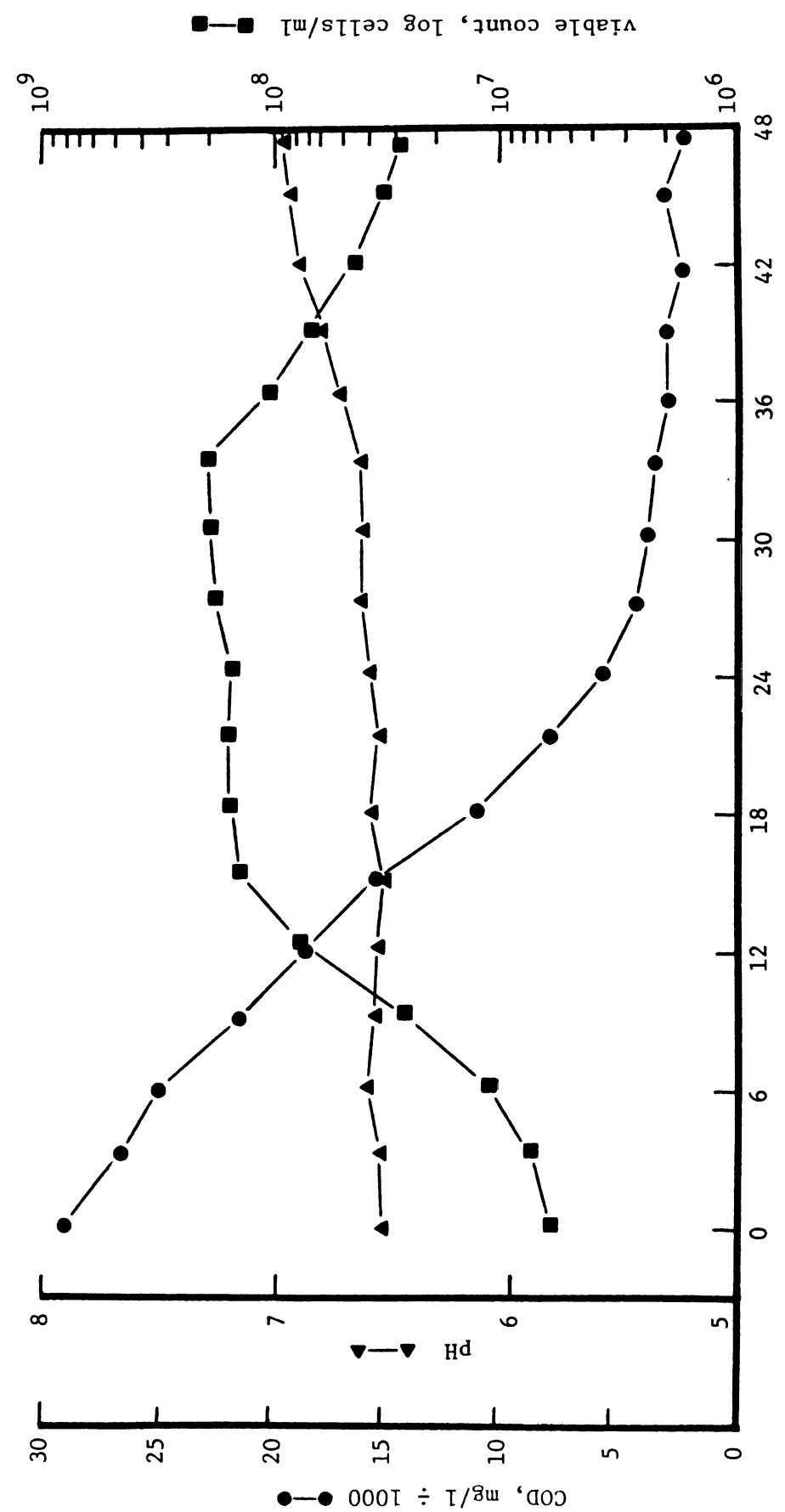

Figure 5. Changes in cell count, COD and pH during 48 hour H. anomala fermentation of pH-controlled effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant.

n=3

Table 5. Cell yields and protein contents of H. anomala biomass harvested following 36 or 48 hour fermentation of whole and treated effluent obtained from the Great Northern bean blanching and cooling operation at the Tekonsha plant.

Treatment	Yield (g dry cells/ l)	% N (Kjeldahl)	% Protein (% N x 6.25)
Whole Water	12.64	8.54	53.4
Filtered Water	10.53	8.69	54.3
N-supp. Water	12.91	8.93	55.8
Buffered Water	14.51	8.43	52.7
Buffer/48 hours	14.84	8.38	52.4

n=3

fermentation produced the greatest yield while filtered water gave the least. This may be due in part to less suspended solids being removed with the cell pellet in the filtered water. Yeast solids from the filtered water contained more N than did whole and buffered treatments, again indicating a more pure harvest. Cell yields are similar to those reported for yeasts in general by Abbott and Clamen (1973). Many different cell yields are reported in the literature and they vary considerably depending on substrate and organism, ranging from 1.07g cells/g substrate for Pseudomonas sp. grown on n-paraffins (Wodzinski and Johnson, 1968) to 0.37g cells/g substrate for Hansenula polymorpha grown on methanol (Levine and Cooney, 1973). Calculated in this way, yields from this

study ranged from 0.41g cells/g substrate from whole wastewater to 0.48g cells/g substrate from buffered wastewater.

Percent protein, calculated as Kjeldahl N x 6.25, was highest in cells grown on N-supplemented wastes (55.8%) and lowest in cells grown on buffered wastes (52.4%). All protein values are similar to those generally reported for yeasts (Cooney et al., 1980; Kihlberg, 1972). As reported by Thanh and Wu (1975), addition of N above adequate levels for growth resulted in cells containing a greater percent protein.

In this study, protein was expressed as Kjeldahl N x 6.25 in keeping with the convention used by almost all workers in this field. However, a considerable fraction of the nitrogen in microbial biomass is non-protein nitrogen. This fact is generally recognized by experts in the field but often overlooked in practice (Reed, 1981). Yeast cells contain 6-12% nucleic acids (Kihlberg, 1972), as well as lesser amounts of choline, glucosamine and other N-containing non-protein constituents. H. anomala is reported to contain 1.78% total non-protein N (Abdel-Hafez et al., 1977). Application of the 6.25 factor may lead to overestimates of protein content by 10-20%.

SUMMARY AND CONCLUSION

Results of material balance calculation indicated a 13.90% crude nutrient loss during blanching and cooling of Great Northern beans at the processing plant investigated in this study. This value is higher than that reported for peas (Bomben et al., 1975), probably because of a greater extent of bean splitting during blanching.

Chemical analyses of effluent from Great Northern bean blanching and cooling operations showed this waste to be high in total solids. Of these solids, almost half (49.5%) was found to be starch. Other carbohydrates analyzed and found present were glucose, sucrose, m-inositol, raffinose and stachyose. Crude protein content (Kjeldahl N x 6.25) of the solid portion of the waste was 23.6%, and ash, 18.7%. Of this ash, potassium, phosphorus, magnesium and calcium were present in the greatest quantities, together accounting for 95.8% of the total. Varying levels of seven other minerals were found, however, the presence of dried mud on beans entering the blancher probably affected these values.

Results of wastewater characterization indicated the waste to be suitable as a fermentation substrate without addition of supplemental nutrients. Ratios of nitrogen and

phosphorus to BOD or COD were above that required for efficient biological stabilization. A high ratio of BOD to COD (0.95) indicated that the waste could be easily and completely treated via microbial growth. A review of the literature was undertaken to identify a microorganism capable of assimilating the bulk of the solid portion of the waste. Hansenula anomala was chosen because of its ability to utilize starch and to grow in vitamin-free media. Previous studies had shown this organism to be metabolically well-suited for protein production when compared to a wide variety of yeasts grown on molasses.

A series of batch fermentations was undertaken to determine the effectiveness of H. anomala in reducing the BOD of the wastewater and in producing a high-protein microbial biomass. Whole and filtered effluent, as well as wastewater receiving nitrogen supplementation or pH control, served as substrates for these trials. All four types of waste proved to be good fermentation substrates, with BOD reduction ranging from 84.3% in whole wastewater to 97.0% in pH-controlled wastewater fermented for 48 hours. Growth of the organism in these wastes followed the typical pattern, passing through lag, exponential growth, stationary growth, and death phases. A decrease in pH preceded exponential growth and a rise in pH preceded the death phase. This trend indicated that control of pH within a certain range might extend the period of active growth thereby enabling further reduction of BOD. Low initial pH plus buffering against a rise in pH resulted

in the greatest reduction in BOD after 36 hours of fermentation. Allowing this buffered waste to undergo an additional 12 hours of fermentation resulted in further reduction of BOD, however, increased cost of this extended fermentation might render it commercially unattractive.

The effects of the treatments previously mentioned on cell yield and protein content were investigated. Buffering resulted in the highest yield (14.5lg dry cells/l) while nitrogen supplementation gave the greatest protein content, 55.8%. Determination of the practicality of nutrient supplementation and/or pH control would require a cost-benefit analysis. These treatments did produce better results, but perhaps not so much better as to recoup their expense.

In summary, Great Northern bean blanching effluent proved to be a good substrate for bioconversion by H. anomala. This waste contained adequate nitrogen and phosphorus for unimpeded microbial growth. Supplementation of nitrogen and control of pH enhanced the protein content of harvested cells and the reduction of BOD, respectively.

RECOMMENDATIONS FOR FURTHER STUDY

There is an ongoing need for research into economical methods for dealing with industrial pollution. The importance of a pragmatic approach cannot be overemphasized. New procedures and equipment will have to provide some savings or benefit in order that plant operators adopt or purchase them. Unfortunately, altruism is bad business.

Investigations into alternative blanching methods, e.g., hot gas or microwave, are appropriate because they address the cause of the waste. These methods would probably be less effective for legumes because a hydration period would still be required.

Studies on organisms and substrates for biomass conversion will probably continue to provide a ready list of possibilities for commercial scale-up. However, until some moderately high-volume food applications are developed, it is doubtful much commercial interest will be shown. Here again is the need for research with profit as an objective.

Research into bioconversion of wastes prevalent in regions suffering from protein malnutrition is warranted. Low cost nucleic acid removal would be necessary if SCP were to satisfy a major portion of the protein requirement.

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