To the Graduate Council:

I am submitting herewith a thesis written by Ellen Thomson Vigdorth entitled "Microbial Reduction of the Chemical Oxygen Demand of Cottage Cheese Whey." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

Hugh O. Jaynes, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

L. Evans Ford
Vice Chancellor
Graduate Studies and Research

# MICROBIAL REDUCTION OF THE CHEMICAL OXYGEN DEMAND OF COTTAGE CHEESE WHEY

A Thesis

Presented for the

Master of Science

Degree

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Ellen Thomson Vigdorth

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## ABSTRACT

The objective of the study was to analyze the microbial reduction of the Chemical Oxygen Demand (COD) of cottage cheese whey by Kluyveromyces fragilis, Geotrichum candidum, Streptococcus faecalis subspecies liquefaciens and a Candida species.

The four starter cultures were added singly and in combinations of two, three and four making 15 different culture treatments. A 10 percent inoculum of the culture treatments was added to 400 milliliters of whey. The culture treatments were grown in the whey for 96 hours at 30°C. ± 1°C. on a Burrell shaker. Samples taken at 6, 12, 24, 48, and 96 hours were analyzed for pH, total solids and then centrifuged. The resulting supernatant was analyzed for total solids, titratable acidity, lactose, protein, cell weight and COD. The effects of culture treatment and time were studied. Using the analysis of variance data, regression equations were derived to follow the fate of the several parameters over time.

Effect of culture treatments was significant on all parameters except protein which overall averaged 50 percent reduction. Several treatments showed an outstanding ability to utilize the organic contents of whey and thereby reduce the COD of the whey. Up to 96 percent of the lactose was utilized. Typically titratable acidity was neutralized and pH approached 7. When these factors were combined with 50 percent utilization of protein, the COD was reduced by up to 82 percent. Six culture treatments reduced the COD to below 15,000 ppm. in 96 hours. One treatment reduced the COD to 16,000 ppm. in 48 hours.

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# CHAPTER I

## INTRODUCTION

During 1975 the United States produced over one billion pounds of cottage cheese (4). In Tennessee ten million pounds of cottage cheese were manufactured (3). In addition to the cheese produced, five to ten pounds of a yellowish-green fluid known as whey were produced per pound of cheese. The pH range of this fluid by-product is between 4 and 5. Approximately 70 percent of the nutrients in skim milk are lost into the acid whey. These nutrients include: proteins, lactose, lactic acid, vitamins and minerals.

The unused organic nutrients of whey place a costly burden on sewage systems. The Chemical Oxygen Demand (COD) of whey has been noted as ranging from 60,000 to above 70,000 ppm. depending upon the specific cheese-making process (19). Every 1,000 gallons per day of raw whey accepted by a city sewage system is equal to the waste of 1,800 people. For the oxidation of 1,000 gallons of raw whey discharged into a stream 4,500,000 gallons of unpolluted water are required (22). Environmental Protection Agency has set effluent guidelines listing maximum 5-day Biological Oxygen Demand (BOD) values during thirty consecutive days, maximum BOD values for any single day, maximum value for total suspended solids and a limiting pH range for wastewater discharge in navigable waters (25).

Efforts have been made to characterize the pollution load of cottage cheese whey and the individual wash waters. Each commercial

method used to drain the whey and wash the curd had an effect on the overall COD of the wastewater (26).

Methods of utilization of cottage cheese whey have included foam spray-drying. The resulting whey solids have found application in food products (24, 41). Processes were developed which used ultrafiltration to recover a protein concentrate followed by reverse osmosis to recover a lactose concentrate (18, 22, 40). The lactose and protein may be further concentrated and purified prior to use in food products. Whey has been used in the production of beverages, bakery products, processed meat products, dairy products and confections (16, 20, 21).

All of these industrial processes pose difficulties with regard to the initial equipment and production and the limited markets for the products. The small dairy needs a process that is economically practical and simple due to their limited resources. Due to this need, it was the purpose of this study to investigate the reduction of the COD of cottage cheese whey through microbial assimilation into microbial protoplasm. This microbial process may be a step toward a satisfactory effluent and salable microbial product.

# CHAPTER II

# LITERATURE REVIEW

## I. MICROBIAL UTILIZATION OF WHEY YIELDING BY-PRODUCTS

Microbial utilization of whey for production of by-products has been studied for many years. Whey is inexpensive and supply is usually locally available. Microorganisms convert the components of whey into new cells and by-products. Industrial production of alcohol, lactase and protein by microorganisms have been the main areas of product development.

### A. ALCOHOL PRODUCTION

Prior to World War II, Rogosa et al. (30) studied the feasibility of using whey as a raw material for alcohol production. These authors reported that Torula cremoris was found to be the most efficient alcohol producer among a wide collection of lactose fermenting yeasts. With an inoculum equal to 2 percent of the weight of lactose in acid whey, all of the lactose was fermented by Torula cremoris within 55 hours. Optimum temperature for fermentation was 30°C. No adjustment in the pH of the whey was necessary. In fact, the low pH of the acid whey was found to prevent growth of unwanted contaminants. Alcohol yields averaged 90 percent of theoretical and were good quality. Whey protein and yeast cells could be separated from the still to be sold for feed with a high vitamin content.

## B. LACTASE PRODUCTION

Utilization of whey by microbial cells for the production of lactase enzyme has been investigated. Microbial enzyme sources have found application in the food industry and in metabolic and genetic research.

Wierzbicki and Kosikowski (43) reported a high level of lactase activity with Lactobacillus helviticus in deproteinized whey. The rapid growth and lactic acid production of Lactobacillus helviticus have been used commercially in the production of fermented milk. Continuous fermentation at 45°C. for 48 hours allowed approximately 80 percent of the lactose to by hydrolyzed. Molds demonstrated a lesser ability to hydrolyze lactose. At an acid pH at 25°C. for 5 hours, a maximum of 34 percent of the lactose was hydrolyzed by Neurospora crassa. Kluyveromyces fragilis, also known as Saccharomyces fragilis, utilized 60 percent of the lactose at 30°C. under continuous culturing conditions. High yeast cell yields also occurred.

Aeromonas hydrophila was selected by Butany and Ingledew (9) for lactose hydrolysis due to the high activity of its β-galactosidase. Upon quantitation, cells of Aeromonas hydrophila contained seven to eight times higher levels of lactase than Kluyveromyces fragilis. In experimental procedures, Aeromonas hydrophila utilized the lactose more efficiently when the lactose concentration in diluted whey equalled 1.5 percent. The ph was buffered by phosphate and sulfate supplements to a value between 6.7 and 6.8. After 11 hours, all lactose was utilized and a large cell yield occurred.

In other efforts to hydrolyze the lactose in acid whey, Wierzbicki and Kosikowski (42) made a lactase preparation by concentration of Aspergillus niger's β -galactosidase. At 55°C. 90 percent of the lactose was converted to the glucose and galactose monosaccharides creating a hexose whey. The hexose whey was a more acceptable product than concentrated whey due to its sweetness and lack of tendency toward crystallization. Upon concentration to 70 percent total solids, a yellow syrup was made. Acid whey was also treated with the lactase enzyme and deproteinized or deproteinized and demineralized prior to concentration to yield two other sweet syrups. The syrups were acceptable in taste and application in food products.

## C. FOOD OR FEED PRODUCTION

Many microorganisms are capable of converting the lactic acid, lactose, and soluble proteins in whey into energy for growth and components for cell production. Assimilation of the organic nutrients in whey by microorganisms in the production of new cell mass may be used to produce a high protein food or feed. Not only may the new product be rich in protein, but it has the added advantage of being low in lactose content.

Although the protein differs according to the species, it averages between 47 percent and 87 percent (13). The limiting amino acids, methionine, tryptophan and cystine, are present in bacterial proteins in greater amounts than in yeasts or molds. Yeast cells average 50 percent protein content while molds vary from 30 to 60 percent in protein. Both yeasts and molds may contain high amounts of B vitamins.

Porges et al. (29) made a comparison of cell yields of yeasts grown in whey at 30°C. Under continuous propagation, Kluyveromyces fragilis gave the highest yield in cell mass and utilized a large amount of lactose. The yeast cells were rich in B vitamins. Separation of yeasts cells from the whey created an enriched protein product.

Amundson (1) also used Kluyveromyces fragilis for rapid conversion of whey to yeast cells. As a source of vitamins and yeast nutrients, corn steep liquor was added to the whey. Fermentation occurred at 30°C. with aeration and adjustment of the pH to 3.5 for maximum growth. Yeast cells and precipitated whey proteins were harvested by a self-cleaning clarifier. Once dried the yeast product contained approximately fifty percent protein and little lactose. Amundson suggested the feasibility of acceptance of the yeast product as a feed supplement in pet foods.

Trichosporon cutaneum, a non-fermenting yeast, was used by Atkin et al. (7) to convert whey to yeast cells. Trichosporon cutaneum was grown at 25°C. in continuous and batch systems with the addition of ammonium sulfate and corn steep liquor to maximize growth. One part of whey was diluted with four parts water for maximum lactose assimilation. Under identical conditions, Trichosporon cutaneum exhibited two times the dry cell weight produced as Kluyveromyces fragilis. The lower cell yields by Kluyveromyces fragilis were attributed to loss of growth materials created by fermentation of the lactose. The volatile compounds were lost prior to possible assimilation by the cells. Compared to Kluyveromyces fragilis, Trichosporon cutaneum showed a higher cell yield and greater efficiency in conversion of whey to yeast cells.

Bechtle and Claydon (8) used mixed yeast and lactose fermenting

bacterial cultures to increase cell mass yields from whey. Starter cultures included Torulopsis, Candida and Kluyveromyces and the bacterial cultures Lactobacillus, Streptococcus and Leuconostoc. Starter cultures were added in equal volumes to nutrient-supplemented cottage cheese whey of single strength, three times single strength and four times single strength concentrations. Mutual growth stimulation occurred at pH 5.3 for mixed yeast and bacterial inocula. Adequate air flow was most critical for fermentation. Fermentations were performed at 32°C. with an aeration rate of 110 milliliters per liter of whey per 1 percent lactose per minute. Accumulation of volatile metabolic wastes seemed to be toxic to the cells and slowed fermentation and cell synthesis rates. The mixed inoculum of lactose fermenting bacteria and yeasts in concentrated whey resulted in improved rates of fermentation and production of cell mass. The mixed cultures utilized nearly all the lactose and greater than half of the protein.

While yeasts and mixed cultures of yeasts and lactose fermenting bacteria have been successfully used to ferment whey, single cultures of lactose fermenting bacteria have not been studied until recently. Michigan State University scientists added Lactobacillus cultures to whey to rapidly convert lactose to lactic acid. In a continuous fermentation process, the lactic acid was neutralized by the addition of ammonia, forming ammonium lactate. Once the fermented whey was condensed by evaporation, the total nitrogen content was increased many times, creating a protein-rich product acceptable for cattle feed. The product called "Bactolac" includes "crude protein" in the form of ammonium lactate, lactoalbumin and bacterial cells (5).

## II. MICROBIAL REDUCTION OF CHEMICAL OXYGEN DEMAND OF WHEY

A great deal of knowledge was obtained from research using microorganisms in whey to produce alcohol, lactase and a high protein food or feed. Technology was based on production of a large cell mass. Application of this knowledge has recently been turned toward reduction of the pollution load of whey. By utilizing the components of whey for energy and cell growth, microorganisms may reduce the organic content of whey and its corresponding Chemical Oxygen Demand (COD).

### A. REDUCTION OF CHEMICAL OXYGEN DEMAND OF WHEY BY YEASTS

Early studies were made on reduction of the pollution load of a dairy's milk waste with four yeast species. Kluyveromyces fragilis, Torulopsis cremoris, Candida lypolytica and Torulopsis utilis were grown in 0.1 percent skim milk solution at 30°C. with aeration. Porges et al. (28) found Kluyveromyces fragilis to be the most active species in reduction of COD. With reduction of the COD of lactose from 640 to 0 ppm., the COD of the total waste was reduced from 1,060 to 630 ppm. When the cells were removed from the whey, the supernatant had a COD of 200 ppm. The availability of nitrogen was the limiting factor in the speed of lactose fermentation. Addition of ammonium sulfate resulted in a reduction of the COD at 24 hours equal to the 72 hour COD of unsupplemented whey.

Wasserman et al. (38) began determining the optimum conditions

for the growth of Kluyveromyces fragilis in whey. When the whey was sterilized and the precipitated proteins removed, no advantage in yeast cell yield over the yield in raw whey occurred. Kluyveromyces fragilis was capable of using the lactose and lactic acid for growth. Thirty-five percent of the whey's organic nitrogen was utilized. Amino acids were deaminated and decarboxylated with the resulting ammonia assimilated. Ammonium sulfate, potassium phosphate and yeast extract stimulated growth.

Wasserman et al. (39) reported that the rate of removal of whey nutrients was directly related to inoculum size, but the final results were equivalent regardless of cell number if given a sufficient amount of time. Wasserman (33) reported on the oxygen requirements for Kluyveromyces fragilis grown in whey at 32°C. The peak oxygen demand required for assimilation of nutrients equalled 100 - 120 milliliters of oxygen per liter of whey per minute. The volume of oxygen required was greater than the amount necessary for dissimilation of lactose. The additional oxygen was believed to be necessary for lactic acid and protein oxidation and removal of toxic wastes produced by the cells.

In order to meet the dissolved oxygen requirement for maximum growth of Kluyveromyces fragilis in whey, the oxygen absorption rates of various propagator systems were studied by Wasserman and Hampson (36, 37). Design of the propagators determined aeration and agitation efficiency which was directly related to the efficiency of lactose assimilation. Conflicting results were obtained due to the foaming of the whey. When air bubbles trapped in the foam were dispersed by agitation, an increase

in the rate of available dissolved oxygen occurred which was greater than the expected rate.

Determination of the source of nitrogen assimilated by Kluyveromyces fragilis in cottage cheese whey was completed by Wasserman (34). Twenty-five percent of the total whey nitrogen was used by the yeasts in deproteinated whey. Although the yeast cells were capable of utilizing 50 percent of the ammonia nitrogen, they could only use 25 percent of the organic nitrogen. The organic nitrogen was composed of non-coagulable soluble nitrogen and heat and acid precipitable nitrogen. The portion assimilated by the yeasts was the soluble nitrogen consisting of proteose-peptones, amino acids and other substances. Kluyveromyces fragilis appeared to be incapable of using lactoalbumin and lactoglobulin.

Cells of Kluyveromyces fragilis grown in cheese whey were analyzed for amino acid and vitamin content by Wasserman (35). The amino acid and vitamin content of Kluyveromyces fragilis compared favorably with strains of brewers' yeasts and other yeasts grown for food or feed purposes.

Since the work of Wasserman and associates outlined the growth of Kluyveromyces fragilis in cheese whey, Knight et al. (19) used this information to maximize COD reduction. Heat deproteinized whey was reported at pH values between 4.0 and 6.0. Optimum temperature for growth was between 35° and 40°C. Air flow rate equalled one volume per minute. Under these conditions, a 70 percent reduction in the COD to 20,000 ppm. was accomplished. Lactose was completely utilized. As the inoculum increased, the rate of COD reduction increased. Added nitrogen in the form of peptones did not increase the rate of COD

reduction. Unused nitrogen actually had a detrimental effect in that it increased the final COD of the whey.

Mickle et al. (23) made further studies on increasing the efficiency of COD reduction by Kluyveromyces fragilis and on producing fat by Rhodotorula gracilis in cottage cheese whey. Under the same conditions as in the previously reported study, a maximum of 80 percent COD reduction was realized in 10 - 11 hours using a 30 percent inoculum (v/v) of Kluyveromyces fragilis. Utilization of Rhodotorula gracilis for fat production was found to be impractical in reduction of the COD of whey.

Based on the past success of COD reduction by Kluyveromyces fragilis, this species was chosen to be included in the thesis plan.

## B. REDUCTION OF CHEMICAL OXYGEN DEMAND OF WHEY BY MOLDS

Mold growth in whey has mainly been studied as a means of fat production. Cook and Woodbine (12) examined the growth of Penicillum frequentans in whey. As lactose was utilized, the Biological Oxygen Demand (BOD) of the whey was reduced. Supplementation of the whey with ammonium nitrate and potassium sulfate increased felt weight and fat production. After forty days, the BOD of unsupplemented cheese whey was reduced from 6,000 to 60 ppm., while the BOD of supplemented whey was reduced from 65,000 to 114 ppm. Five day BOD (Biological Oxygen Demand) is generally accepted as being equivalent to 60 percent of the COD (Chemical Oxygen Demand) of the whey.

Forty mold species were examined for lactose utilization and fat

production by Wix and Woodbine (44). One of the cultures included in the study was Geotrichum candidum. Although Geotrichum candidum was considered to be unsatisfactory for fat production, the species demonstrated an ability to utilize the lactose and lactic acid in whey.

The use of Geotrichum candidum for BOD reduction of waste has been determined in acid brines such as sauerkraut, pickle and olive waste. The initial BOD of 24,000 ppm. was reduced by 88 percent to 3,000 ppm. in approximately four days. Mycelial weight of 13 grams per liter of acid brine agrees with the results of Cook and Woodbine (12). The pH was raised from 3.4 to a slightly alkaline pH of 7.4. A temperature of 25°C. was maintained with an initial inoculum of 1 percent (v/v) (17).

In Trinci's (31) studies of the use of cell turbidity to measure mold growth, he states that, "The specific growth rate of Geotrichum candidum at 30°C. is believed to be the fastest recorded for a eucaryotic organism." Geotrichum candidum has been mentioned frequently as a naturally occurring contaminant on dairy products (11, 32). The utilization of lactose and lactic acid and rapid growth by Geotrichum candidum suggest that a role in COD reduction of cottage cheese whey may be possible (10, 17).

## C. MICROBIAL REDUCTION OF CHEMICAL OXYGEN DEMAND OF WHEY

The more rapid growth and metabolic rates of bacteria seem to make them a more favorable choice than yeasts or molds for reduction of the COD of whey. Edmondson and Brazis (15) reported a study of bacterial

utilization of dairy waste. Three species of Pseudomonas, supposedly non-pathogenic, were chosen for the studies. By acting on the proteins, the Pseudomonas species were good decomposers of dairy waste. They did not actively ferment lactose as a carbohydrate source. Moderate reduction in BOD was accomplished under an alkaline pH when aerobic conditions were maintained. The system required the addition of buffered nutrient broth for continuous pH standardization.

According to Bergey's Manual of Determinative Bacteriology (14), Streptococcus faecalis subspecies liquefaciens grows well at approximately 30°C. and demonstrates the ability to ferment lactose and utilize milk proteins. Therefore, Streptococcus liquefaciens was chosen for the study as a possible means of reducing the COD whey.

# CHAPTER III

## MATERIALS AND METHODS

### I. SOURCE OF WHEY AND STOCK CULTURES

One lot of cottage cheese whey was obtained from Mayfield Dairy Farms, Athens, Tennessee, at the time of each fermentation trial to insure freshness and uniformity of medium.

A strain of Geotrichum candidum isolated from raw milk was obtained from Dr. Woodrow Overcast, Department of Food Technology and Science at the University of Tennessee, Knoxville.

Kluyveromyces fragilis NRRL No. Y1156 was obtained from Northern Regional Research Laboratory of the U. S. Department of Agricultural Research Service.

A yeast culture, tentatively identified as a species of Candida, was isolated during routine laboratory analysis of milk samples on Violet Red Bile Agar where apparent proteolytic capabilities of the culture were observed.

### II. PREPARATION OF INOCULUM

Stock cultures were maintained on 4 percent lactose agar slants and stored at 4°C. Cultures were transferred to 100 milliliters of 4 percent lactose broth and incubated at 32°C. ± 1°C. for 24 hours. A 10 percent inoculum (v/v) of the actively growing cultures was transferred from the lactose broth to 400 milliliters cottage cheese whey for fermentation at 30°C. ± 2°C. for 24 hours on a Burrell Shaker. These

cultures in whey represented the starter cultures for each fermentation trial. The four cultures were added individually and in combinations of two, three and four making possible 15 different culture treatments.

A 10 percent by volume inoculum was added for each culture treatment to 400 milliliters of cottage cheese whey. When a single culture was used, 40 milliliters of the starter culture were centrifuged at 1,000 x g. for 15 minutes. The supernatant was removed and the cells were resuspended in 40 milliliters of fresh whey. When a combination of two starter cultures was used, 40 milliliters of each starter culture were centrifuged and the cells resuspended in 20 milliliters of fresh whey. When three starter cultures were used in a combination, 40 milliliters of each culture were centrifuged, supernatant was removed and cells were resuspended in 13.3 milliliters of fresh whey. When all four cultures were combined to make a culture treatment inoculum, 40 milliliters of each of the starter cultures were centrifuged, supernatant was removed and cells resuspended in 10 milliliters of fresh whey. In this manner, each culture treatment inoculum was made to total 40 milliliters or 10 percent of the whey volume.

For each culture treatment, a 10 percent inoculum was added to 400 milliliters of cottage cheese whey. Culture treatments were randomly chosen and incubated at 30°C. $\pm$ 2°C. on a Burrell Shaker for a total of 96 hours.

## III. SAMPLING

Thirty milliliters of sample were removed from each culture treatment at 6, 12, 24, 48 and 96 hours for analysis. A sample of

uninoculated whey substrate was analyzed at the initiation of each trial for comparison.

## IV. ANALYSIS OF THE WHEY

### pH

pH was measured on each sample at the time of removal with a Corning pH meter.

### Total solids

Total solids were determined by the vacuum drying method (6). Approximately 2 grams of each culture treatment of whey were dried in duplicate on a steam bath to remove moisture. To obtain a constant weight, the samples were then dried at 60°C. for 24 hours at a vacuum of 381 - 508 torr.

### Total solids - centrifuged samples

Total solids were determined on the culture treatment supernatants after centrifugation at 1,000 x g. for 15 minutes in 50 milliliter tubes in an International Number 2 Centrifuge. Determinations on the supernatants were made in duplicate as previously described.

### Cell weight and precipitated solids

Cell weight and precipitated solids were determined indirectly as the difference in the weight of total solids of the culture treatment and the weight of total solids of the culture treatment supernatant:

Cell weight and precipitated solids = Total solids of culture treatment - Total solids of supernatant

Lactose

Lactose was determined on culture treatment supernatants according to the picric acid colorimetric method of Perry and Doan (27). Five-tenths of a milliliter of supernatant was mixed with 49.5 milliliters of saturated picric acid. The sample was filtered through No. 1 Whatman filter paper to remove precipitated protein. One milliliter of the filtrate was added to 2 milliliters of $NaCO_3$, and the color developed in a boiling water bath for 20 minutes. A blank consisting of 1 milliliter of saturated pricric acid and 2 milliliters of $NaCO_3$ was used. Samples were diluted to 20 milliliters with distilled water. Absorbance of a portion of the sample was read on a Bausch and Lomb Spectronic 20 at 520 nm. A standard curve had previously been constructed using standard solutions of lactose in 1 percent incremental concentrations from 1 to 6 percent (w/v).

$$\text{Absorbance} = -0.01089 + 0.08133 \ (\% \text{ lactose})$$

Chemical Oxygen Demand

Chemical Oxygen Demand (COD) was determined on culture treatment supernatants according to the method outlined in Agricultural Handbook 176 (2). A 1 to 50 dilution of the sample was made with distilled water. Five milliliters of the diluted sample were added to 50 milliliters of the dichromate oxidizing solution. The solution was heated on a hot plate to 165°C. within 6 minutes. Samples were cooled, 200 milliliters of distilled water added, cooled and 10 milliliters of potassium iodide solution added. Samples were titrated immediately with a standard 0.05 N. sodium thiosulfate solution. COD was calculated as follows:

$$\text{COD (ppm. } O_2) = (\text{ml. titrated}_{\text{blank}} - \text{ml. titrated}_{\text{sample}}) \times \frac{(0.05 \text{ meq./ml.}) (8 \text{ mg. } O_2/\text{meq.}) (1000 \text{ ml./l.}) (50)}{5 \text{ ml.}}$$

or $\text{COD (mg./l.)} = (\text{Titr.}_{\text{blank}} - \text{Titr.}_{\text{sample}}) (80) (50)$

Protein

Protein was determined on 2 milliliters of culture treatment supernatants by the Kjeldahl method (6). A factor of 6.38 x N. was used.

Titratable acidity

Titratable acidity was determined on 5 milliliters of culture treatment supernatants (6). The sample was diluted with two times its volume of distilled water. A few drops of phenolthalein were added, and the sample titrated with a standard sodium hydroxide solution.

Statistical analysis

The 15 culture treatments were handled in blocks of 12 with replication. The experiment was randomized incomplete block design with 3 replications of 12 treatments and 4 replications of 3 treatments. Data were subjected to least squares analysis of variance to measure treatment effects, block effects, and time effects.

# CHAPTER IV

## RESULTS AND DISCUSSION

In a dairy plant, maintenance of sterile conditions for microbial cultures in whey waste would not be practical. Inoculations used in the present study had cell counts of greater than $1.0 \times 10^7$. With such large, actively growing cultures, no appreciable contamination was expected while maintaining a "normal" degree of cleanliness. An incubation temperature of $30^{\circ}C. \pm 1^{\circ}C.$ was used because this temperature was desirable for optimum cell growth and was approximately the same temperature as the whey upon removal from the curd. The 400 milliliters of whey for each culture treatment were placed in a liter size Erhlenmeyer flask and stoppered with cotton plugs. Flasks were placed on a Burrell shaker to provide agitation and aeration. In this manner, laboratory conditions were designed to simulate conditions which could be practically met in a dairy plant.

When each lot of cottage cheese whey was received, the pertinent components were determined prior to addition of inoculation. Composition of the whey is shown in Table I including the standard deviation for each component. An average of all batches was used to graphically illustrate 0 hours when parameters are plotted against time. The greatest variation in the composition of the whey occurred in the total solids and precipitated solids. Such variation may be explained by the amount of fines left in the whey after removal of the curd. All of the other parameters show very little variation.

At 6, 12, 24, 48 and 96 hours, the composition of the culture

TABLE I

COMPOSITION OF BATCHES OF WHEY

| Batches | pH | Total Solids* | Precipitated Solids* | Titratable Acidity | Lactose | Protein | COD |
|---|---|---|---|---|---|---|---|
| | | ---------- | ---------- | % | ---------- | ---------- | ppm |
| 1 | 4.79 | 5.0204 | 0.1041 | 0.55 | 5.000 | 0.938 | 58,480 |
| 2 | 4.67 | 4.5964 | 0.0730 | 0.58 | 4.850 | 0.916 | 55,080 |
| 3 | 4.75 | 6.4646 | 0.1707 | 0.52 | 5.000 | 0.988 | 59,840 |
| 4 | 4.71 | 5.1788 | 0.1004 | 0.54 | 4.941 | 0.931 | 59,400 |
| Means | 4.73 | 5.1120 | 0.1120 | 0.55 | 4.948 | 0.943 | 58,200 |
| Std. Dev. | ±0.05 | ±0.8048 | ±0.0415 | ±0.02 | ±0.071 | ±0.031 | ±2,156 |

*Means of duplicates

treatments was determined. Lactose, total solids of the supernatant (hereinafter referred to as total solids), cell weight and precipitated solids (hereinafter referred to as cell weight), protein and titratable acidity were determined and recorded on a percentage basis. Chemical Oxygen Demand (COD) was calculated in ppm. $O_2$ and the pH of the culture treatments measured. The data were subjected to analysis of variance (ANOVA) to measure treatment, block and time effects on each of the seven measured parameters. "F" values from the ANOVA are listed in Table II. Block effects were found to be significant overall for four parameters: protein, total solids, lactose and COD. When individual culture treatments were studied, no significant difference among replicates was found with the parameters of culture treatments composed of a single microbial species. A number of culture treatments composed of two and three microbial species did show significant differences among replicates. Such variation in replicates seems to point toward competition for growth and energy sources in the whey. Antagonistic and synergistic effects among microbial cultures are to be expected with mixed cultures.

Time was found consistently to have the greatest significant effects on the seven parameters for all fifteen culture treatments. In order to represent graphically the changes in the parameters over time, several mathematical models were necessary. Significant linear, quadratic and higher effects of time for the parameters pH, cell weight, titratable acidity and protein were observed. Linear and polynomial regression equations were derived to fit the data. Data from determinations of lactose, COD and total solids best fit equations derived from

TABLE II

F VALUES FROM STATISTICAL ANALYSIS OF CULTURE TREATMENT VARIABLES

| Source | pH | Total Solids | Cell Weight | Titratable Acidity | Lactose | COD | Protein |
|---|---|---|---|---|---|---|---|
| Treatment (T) | 7.33** | 61.10** | 1.55 | 7.68** | 55.64** | 21.78** | 1.33 |
| Replication (R) | 1.33 | 14.03** | 2.20 | 1.89 | 6.88** | 8.14** | 11.97** |
| Time (t) | 154.28** | 503.72** | 44.68** | 127.11** | 178.48** | 175.43** | 127.13** |
| T x t | 1.65* | 6.67** | 0.87 | 2.22** | 5.67** | 3.33** | 0.65 |
| T x R | 2.51** | 2.04** | 0.74 | 2.34** | 3.49** | 1.54 | 0.66 |
| Regression | 8.33** | 31.28** | 2.60** | 7.63** | 18.09** | 11.81** | 5.79** |
| $r^2$ | 0.8710 | 0.9621 | 0.6780 | 0.8608 | 0.9362 | 0.9054 | 0.8243 |

**Significant at the 1% level
*Significant at the 5% level

exponential transformation of the data. The rate of lactose utilization seems to depend on its concentration in the whey which may be expressed as a first order reaction as follows:

$$\text{Lactose concentration} = Ae^{kt}$$

In first-order reactions when the concentration of the variable, in this case lactose, is plotted over time, the concentration decreases rapidly at first with time. The rate of disappearance of the variable slows as concentration decreases until concentration finally approaches zero asymptotically. Although theoretically complete utilization of a whey component may be possible, experimentally complete utilization does not usually occur. Since lactose is the limiting carbohydrate source for energy in whey, it may restrict complete utilization of total solids and therefore, limit reduction of the COD of the whey. The restriction in utilization or reduction of a component may be represented by adjustment of the exponential equation with the addition of an asymptote greater than zero. Data from determination of COD and total solids fit best to exponential equations with an adjusted asymptote represented as follows:

$$\text{COD or total solids concentration} = A + Be^{kt}$$

After the exponential equations for lactose content were graphed and the means from the data plotted, it was discovered that a slightly better fit would be possible with the addition of an asymptote greater than zero to the equations. Since the exponential equations adequately expressed general trends in utilization of lactose reflected by detailed statistical analysis, no further adjustment was made.

An example of the exponential equations used to graph changes over time in lactose and COD for two culture treatments which demonstrate

extremes in utilization and reduction ability are shown in Figure 1. A complete listing of the equations used for graphing the seven parameters against time for each culture treatment is shown in Table IV, Appendix.

The microorganisms composing the fifteen culture treatments are listed in Table III. Changes in the seven parameters over time are graphed for each culture treatment. Means statistically determined from replicates of each culture treatment are plotted over time. When percent reduction, utilization or change observed for a parameter is discussed, values stated were determined from the means and are approximate values.

Culture treatment 7 was comprised of a yeast, tentatively identified as a Candida species, C. C demonstrated an ability to utilize an average of fifty percent of the protein in the whey (Figure 2). The pH was slightly alkaline by 48 hours with an odor of ammonia present. The titratable acidity was reduced to zero by 48 hours, demonstrating rapid lactic acid assimilation. Growth rate slowed after 48 hours with cell weight increasing only slightly. A small reduction of 7 percent in total solids and 12 percent in lactose is reflected in a minimal reduction in the COD from approximately 58,000 ppm. to 48,000 ppm. by 96 hours. As may be seen in culture treatment 7 an initial increase in COD from the value prior to addition of the inoculum may be explained by the addition of cells to the whey and an early accumulation of waste products in the whey. Total solids data did not fit the exponential model well; a linear equation fit better and was used. Lactose and COD also did not fit exponential equations very well.

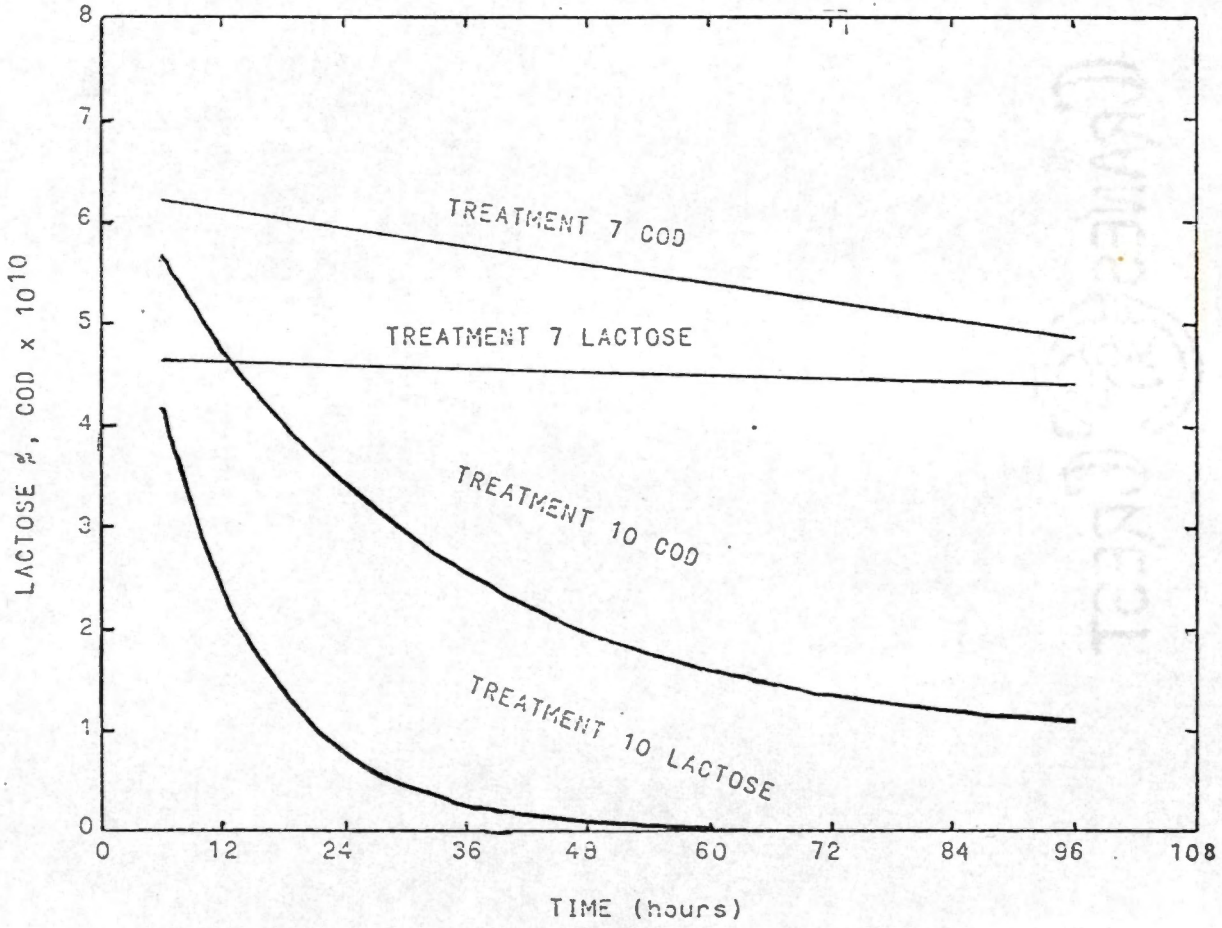



Figure 1. Variation Over Time of Lactose and COD with Culture Treatment 7 and Culture Treatment 10.

TABLE III

MICROORGANISMS COMPRISING CULTURE TREATMENTS

| Treatment | Microorganisms | Symbols |
|---|---|---|
| 1 | Streptococcus liquefaciens | S |
| | Geotrichum candidum | G |
| | Candida species | C |
| 2 | Geotrichum candidum | G |
| | Kluyveromyces fragilis | K |
| 3 | Geotrichum candidum | G |
| | Candida species | C |
| 4 | Streptococcus liquefaciens | S |
| | Kluyveromyces fragilis | K |
| 5 | Streptococcus liquefaciens | S |
| | Geotrichum candidum | G |
| | Kluyveromyces fragilis | K |
| 6 | Kluyveromyces fragilis | K |
| | Candida species | C |
| 7 | Candida species | C |
| 8 | Streptococcus liquefaciens | S |
| | Candida species | C |
| | Kluyveromyces fragilis | K |
| 9 | Geotrichum candidum | G |
| | Candida species | C |
| | Kluyveromyces fragilis | |
| 10 | Streptococcus liquefaciens | S |
| | Geotrichum candidum | G |
| | Candida species | C |
| | Kluyveromyces fragilis | K |
| 11 | Streptococcus liquefaciens | S |
| | Geotrichum candidum | G |

TABLE III (continued)

| Treatment | Microorganisms | Symbols |
|---|---|---|
| 12 | *Geotrichum candidum* | G |
| 13 | *Kluyveromyces fragilis* | K |
| 14 | *Streptococcus liquefaciens*<br>*Candida* species | S<br>C |
| 15 | *Streptococcus liquefaciens* | S |

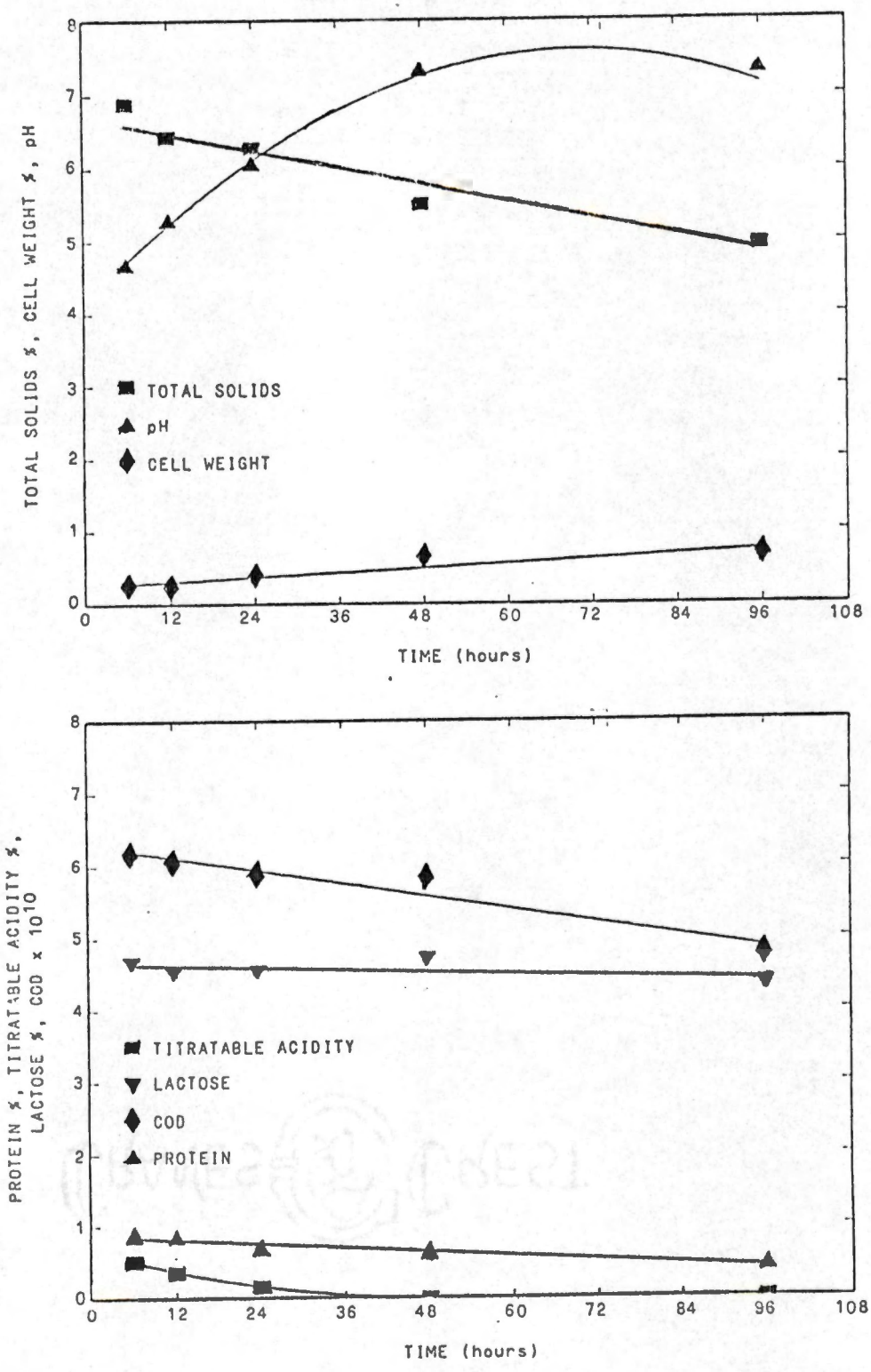



Figure 2. Variation Over Time of Seven Parameters with Culture Treatment 7.

The "k" value, -0.00054965, for lactose was extremely small and the "k" value, -0.014865, for COD was also small compared to the value of other culture treatments which fit the model well. The small percent change over time in these parameters may explain why the equations did not follow the means closely.

Culture treatment 12 contained an inoculum of Geotrichum candidum, G. G was capable of using 50 percent of the available protein (Figure 3). At the end of the fermentation, the pH and titratable acidity were neutralized. G was able to use lactic acid as an energy source. At 24 hours the greatest value in COD occurred suggesting the presence of toxic wastes and metabolites accumulating in the whey. Cell weight actually decreased to its lowest mean value at 24 hours. The greatest reduction in COD and lactose occurred between 24 and 48 hours reflecting the rapid growth rate of cells during this time period. Cell weight increased four-fold to its maximum value during the 24 to 48 hour time period demonstrating a stimulated growth period. By 24 hours only 20 percent of the lactose was utilized, but by 48 hours a maximum of 95 percent lactose assimilation occurred. With a 75 percent reduction in total solids and a similar reduction in other whey components, the COD of the whey was finally reduced by 78 percent to approximately 13,000 ppm. Lack of fit of the exponential equations to means may be explained by the fact that the equations do not take into account a lag phase in cell growth. After 24 hours lactose, COD and total solids did appear to be reduced at an exponential rate. The ability of G to use lactic acid as an energy

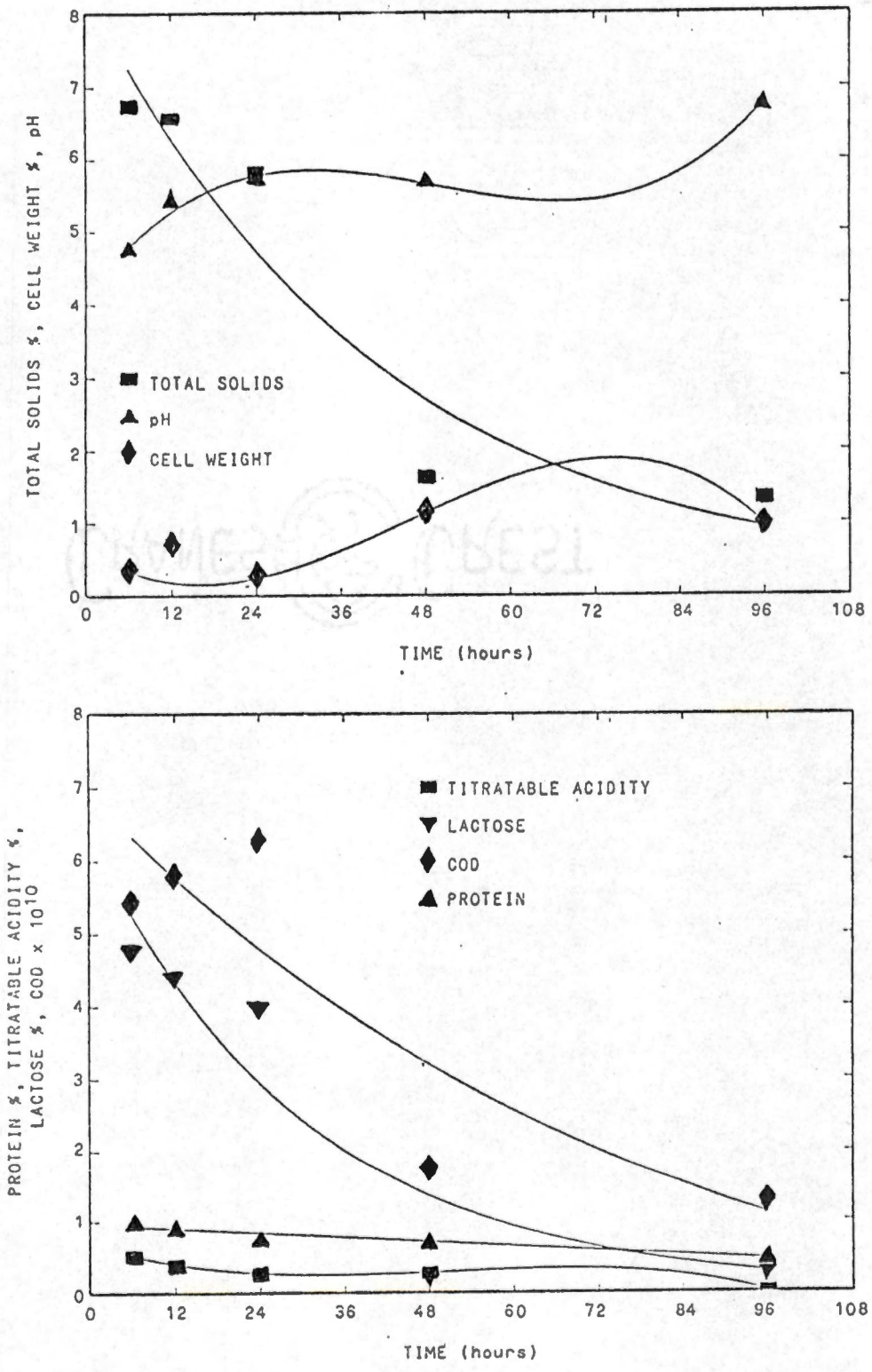



Figure 3. Variation Over Time of Seven Parameters with Culture Treatment 12.

source possibly in preference to lactose may also aid in explanation of the delay in exponential component utilization.

Kluyveromyces fragilis, K, in culture treatment 13 is known as a rapid lactose-fermenting yeast. Lactose utilization proceeded at an exponential rate to 93 percent reduction by 24 hours (Figure 4). A rapid reduction in total solids, lactose and COD by 24 hours is reflected in the high "k" values of -0.08608576, -0.10037908 and -0.07668377 respectively. Lactic acid was utilized by 48 hours and by 96 hours the pH was raised to 6.8. Cell weight increased greatly during the 24 to 48 hour time period. The final COD was 17,350 ppm. which was a decrease of 1,400 ppm. from the COD at 48 hours. In all parameters there was very little change from 48 to 96 hours which suggests that there may be no advantage in continuing the fermentation past two days.

Streptococcus faecalis, S, in culture treatment 15 grew very slowly in whey until after 24 hours (Figure 5). Since the titratable acidity averaged 0.58 percent during this time period, it may be that the acid condition inhibited growth somewhat. There was also very little increase in cell weight. By 96 hours 43 percent of the protein and total solids were utilized. Since reduction in total solids was gradual and small compared to many other treatments, data best fit a linear equation. A 64 percent reduction in lactose and a 40 percent reduction in COD stress the limited ability of S to utilize the components of whey.

When the microorganisms were combined in pairs, the culture treatment responded with varying results. Competition for energy and

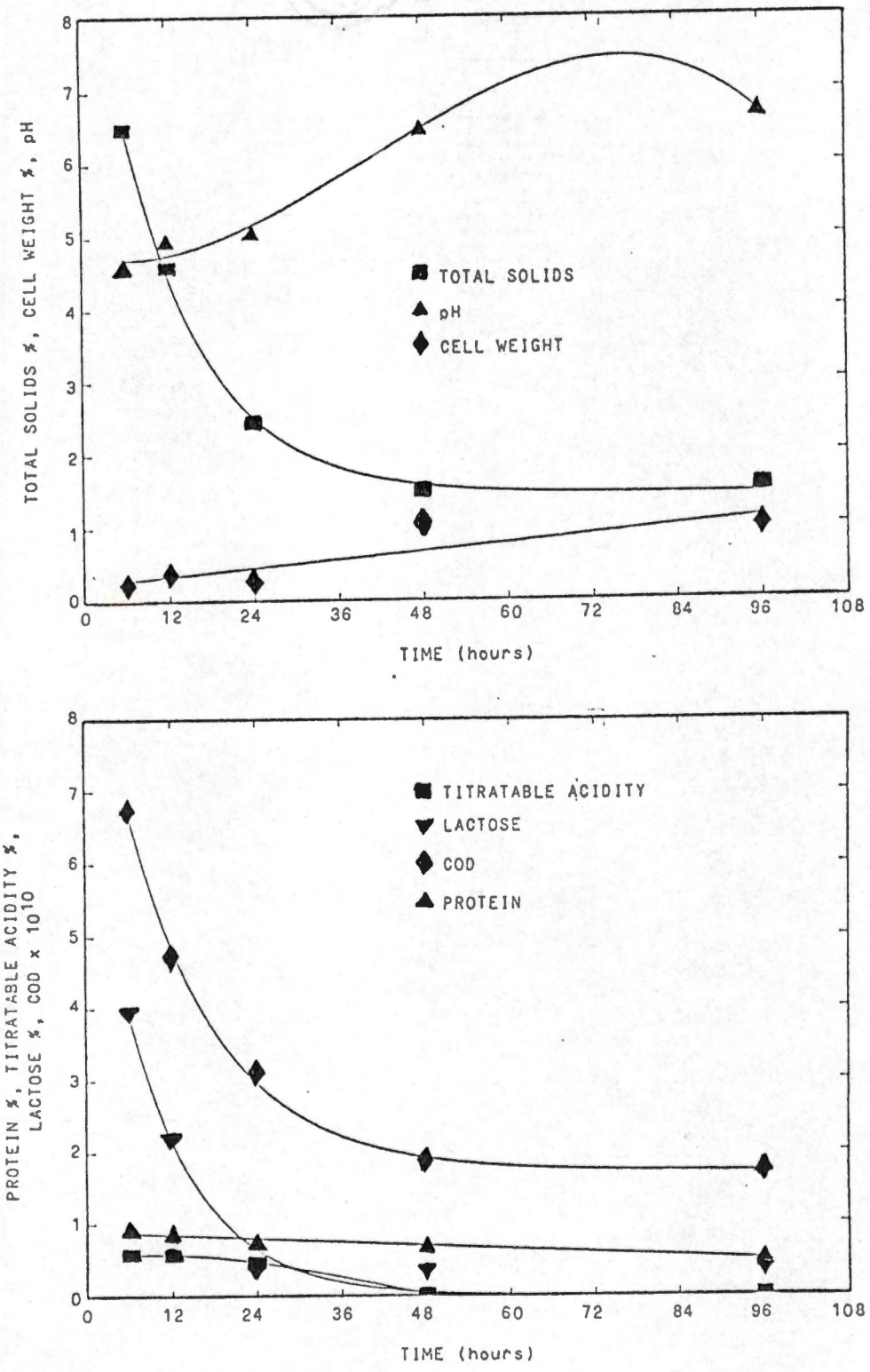
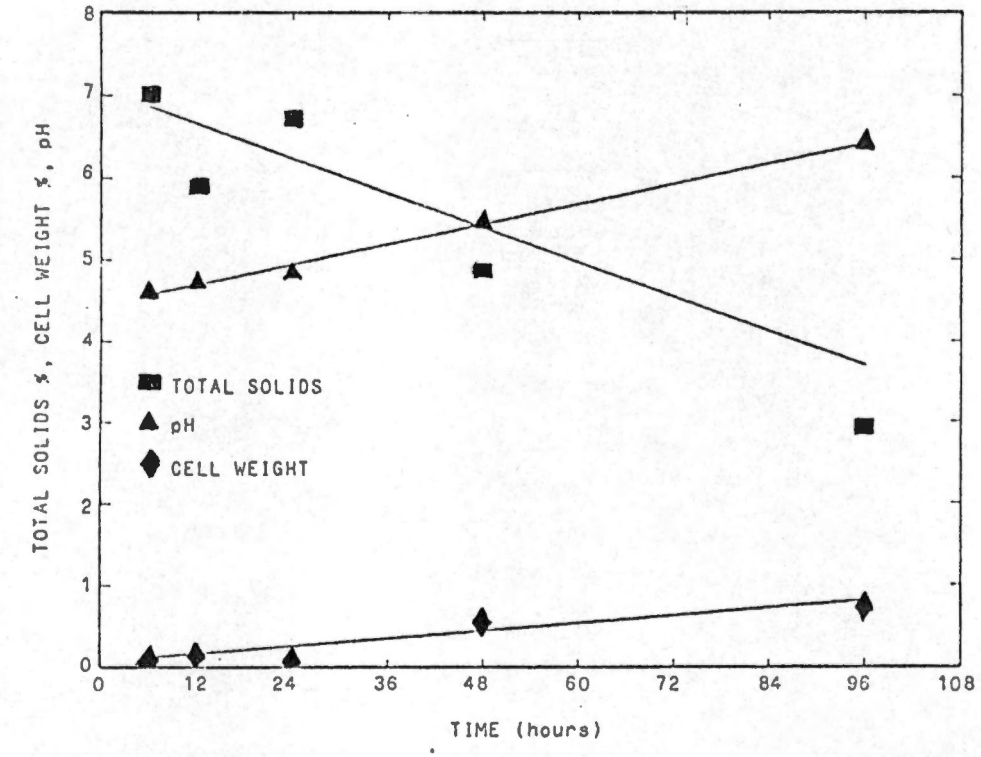



Figure 4. Variation Over Time of Seven Parameters with Culture Treatment 13.



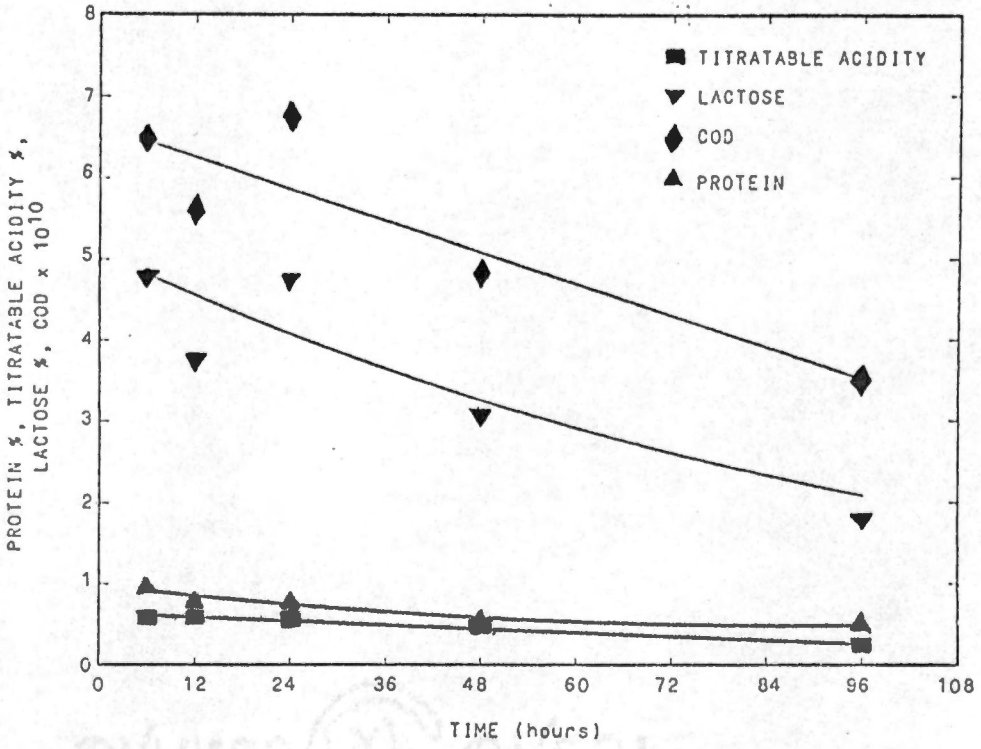



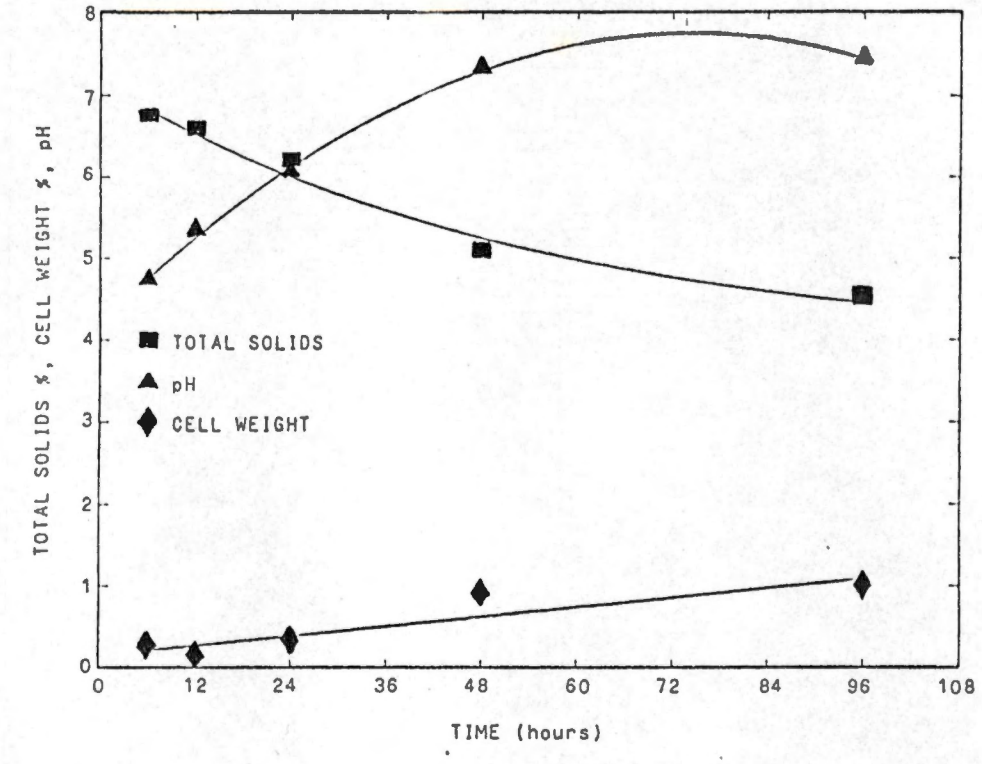

Figure 5. Variation Over Time of Seven Parameters with Culture Treatment 15.

growth sources resulted in some cases of antagonism. Stimulation of growth was also indicated in some mixed cultures which created synergistic effects on component utilization.

Culture treatment 14 consisted of a mixed culture of S and C. The C species seemed to dominate S since there was little lactose utilization (Figure 6). Typical of the observed activity of C alone in whey, there was a rapid reduction of titratable acidity and the pH became alkaline by 48 hours. Some stimulation between the two species seemed to occur since a slightly greater utilization of protein and total solids occurred with the mixed culture compared to utilization by the single cultures. Although minimal, the reduction of the final COD was greater with the mixed culture than with C alone, but less than when S was the sole culture in whey. The difference in COD may be attributed to lower lactose utilization caused by C's prevention of reduction of lactose content by S. Final COD averaged 40,000 ppm.

When G and C were combined in culture treatment 3 some competition seemed to occur. Since both G and C are utilizers of lactic acid, the titratable acidity was quickly neutralized by 48 hours (Figure 7). The pH was neutral by 96 hours but did not become alkaline as displayed by C in culture treatment 7. Lactose was utilized to a greater extent than by C alone but not to the extent observed with G. The final COD of 40,000 ppm. suggests limited removal of organic contents from the whey.

When C was combined with K in culture treatment 6 a synergistic effect in utilization of whey components may have occurred. Total solids, lactose and COD were reduced rapidly by 24 hours (Figure 8). Such activity is reflected in the high "k" values of their exponential



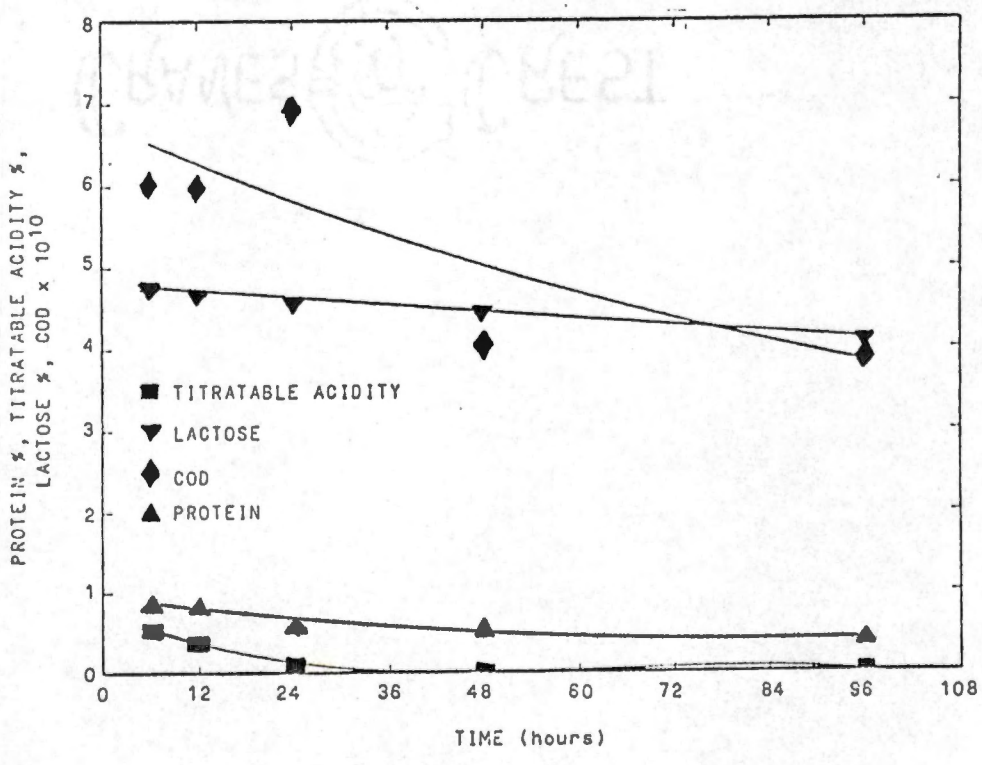




Figure 6. Variation Over Time of Seven Parameters with Culture Treatment 14.

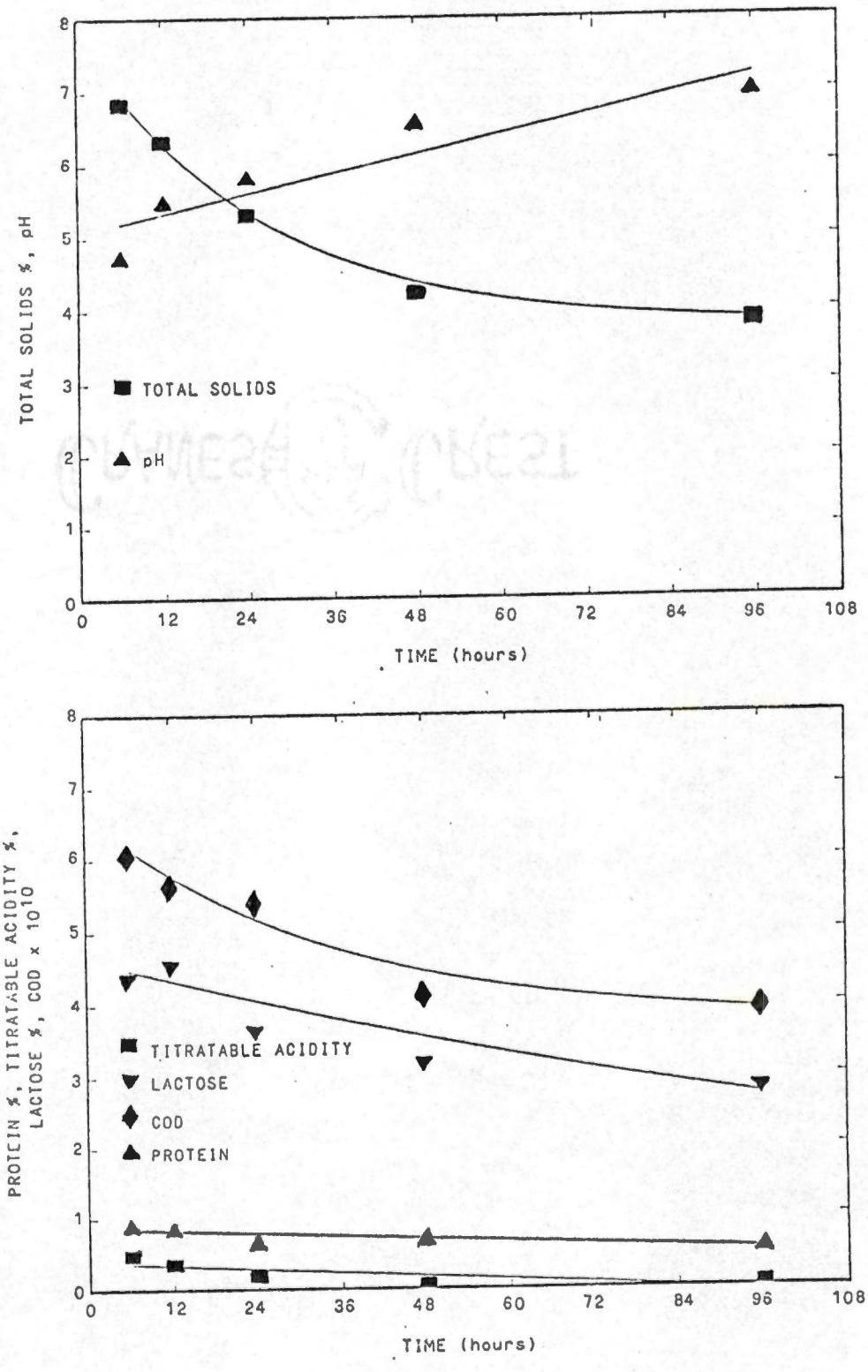



Figure 7. Variation Over Time of Seven Parameters with Culture Treatment 3.

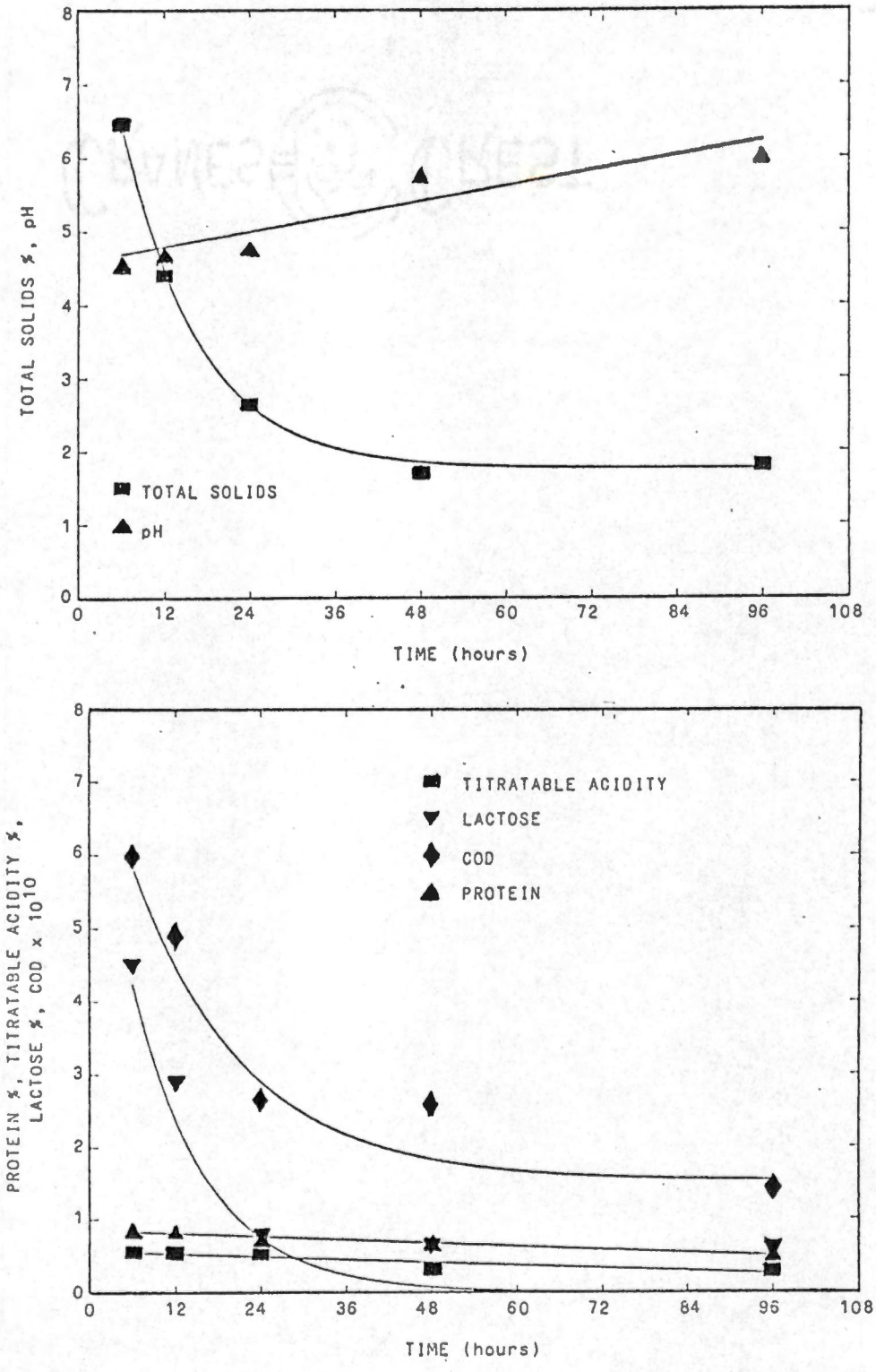



Figure 8. Variation Over Time of Seven Parameters with Culture Treatment 6.

equations in the Appendix. A gradual lactose, lactic acid and protein utilization continued until the end of the fermentation. Such action on the organic content of the whey resulted in a 75 percent reduction of the COD to 14,500 ppm. Of all combinations of two microorganisms, C and K demonstrated the largest reduction in COD.

An enhancement in reduction of the COD of whey also occurred when S and K were combined in treatment 4. Lactose, protein, total solids and lactic acid utilization increased over the amount observed when S and K were present individually in whey (Figure 9). The effect of mutual stimulation was evidenced in all parameters studied. With rapid reduction in lactose, total solids and COD and high "k" values (Appendix) the means fit the exponential equations very well. The pH became alkaline by 96 hours and COD was reduced by 71 percent. Although COD reduction was not increased greatly over the culture treatment with K alone, it may be that due to greater utilization of whey components a larger accumulation of toxic wastes and metabolites were remaining in the whey.

In culture treatment 11, a combination of S and G, both enhancement and antagonism may have occurred. S and G are both capable of lactose utilization and 88 percent of the lactose disappeared (Figure 10). Titratable acidity apparently followed the same pattern of little reduction as found in the culture treatment with S alone. Only 38 percent of the proteins was utilized in the mixed culture, but S and G were individually capable of using 50 percent of the protein protein. The pH only reached 6 by 96 hours. "K" values for lactose, total solids and COD were smaller than for treatments with equations

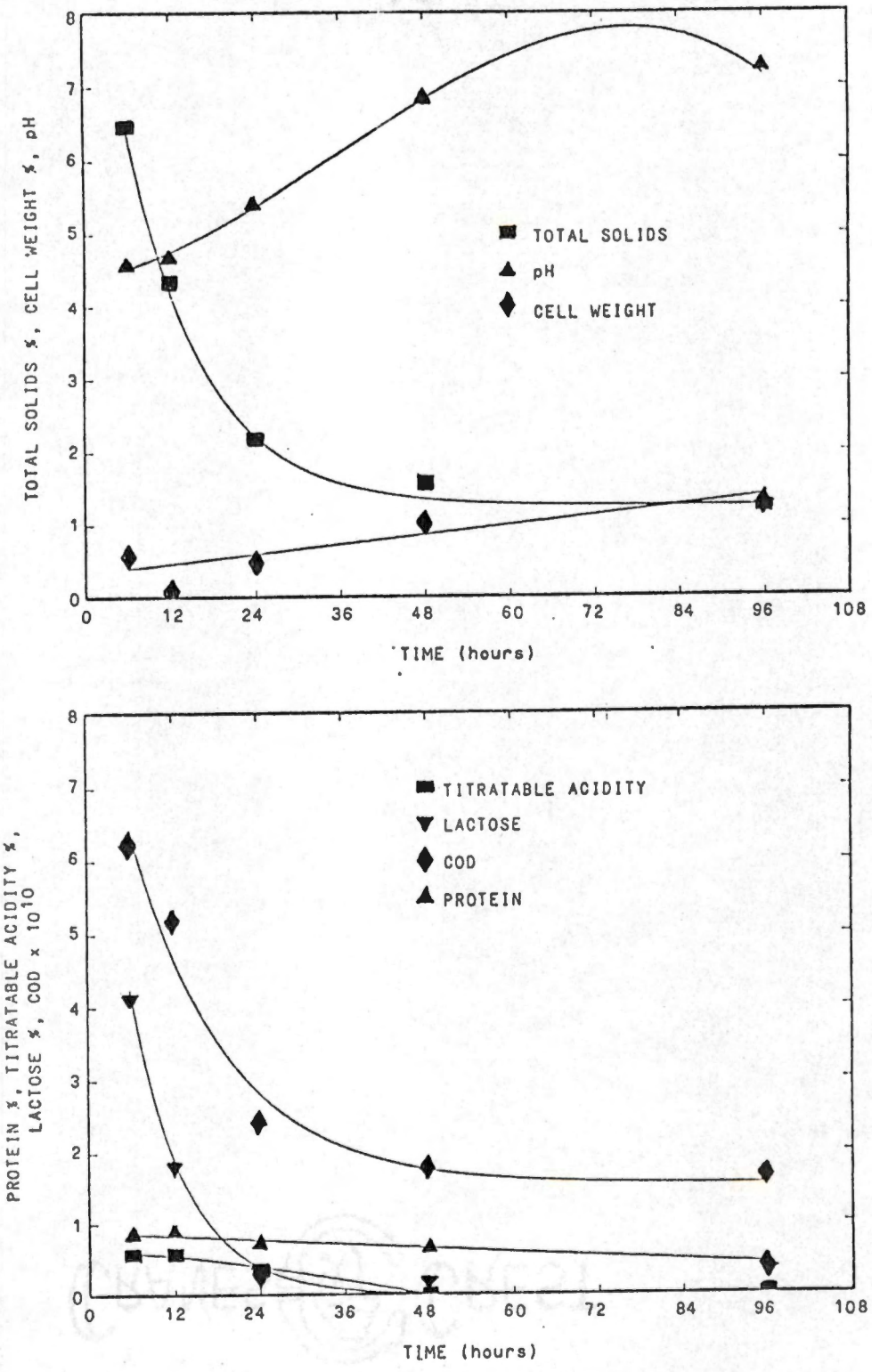



Figure 9. Variation Over Time of Seven Parameters with Culture Treatment 4.

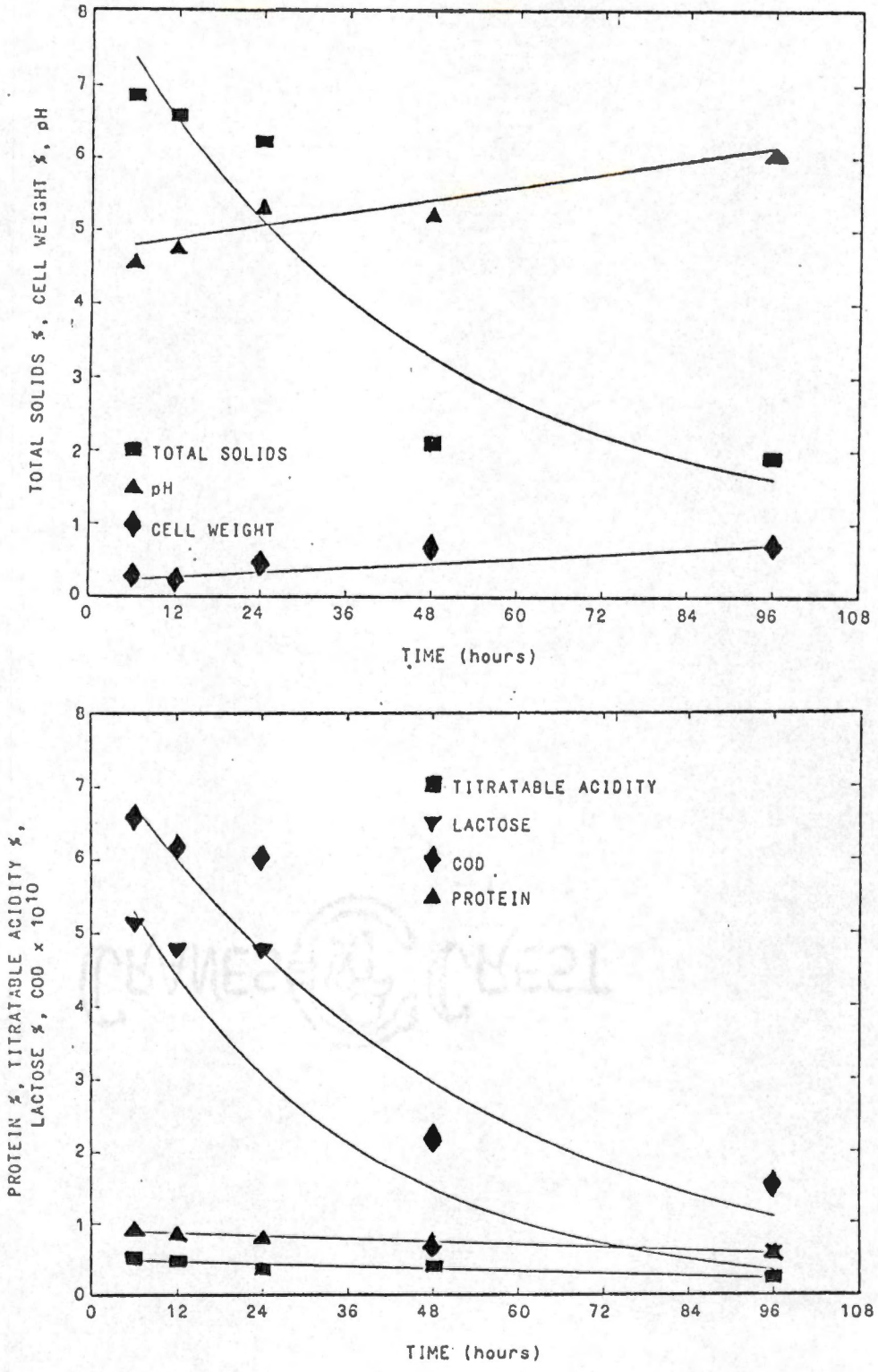


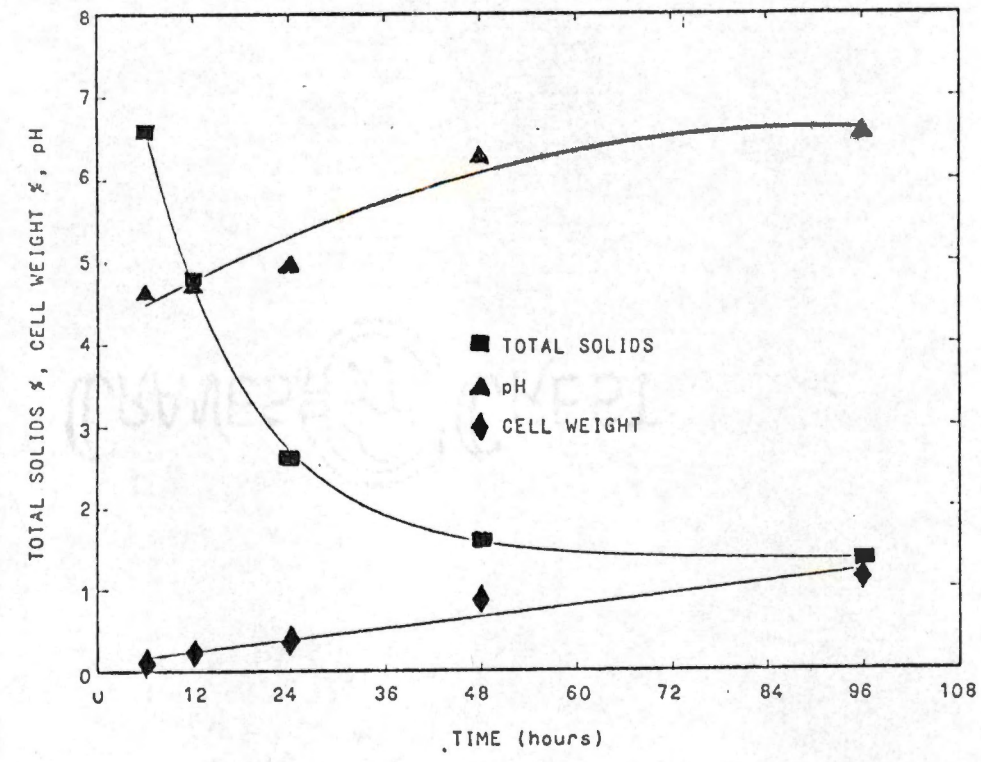

Figure 10. Variation Over Time of Seven Parameters with Culture Treatment 11.

closely fitting the means (Table IV, Appendix). Reduction of the organic content was slow initially, similar to treatment 15 with S, which may account for lack of close fit. Final COD was 15,300 ppm., a reduction of 74 percent.

Culture treatment 2 using cultures G and K (Figure 11) had a rapid reduction of organic contents and COD by 48 hours. High "k" values for lactose, total solids and COD substantiate this observation (Table IV, Appendix). Only slight reduction in COD was observed after 48 hours which may be due to continued protein utilization. Continuation of the fermentation past 48 hours may not be worth the extra time involved. The COD was reduced by 70 percent by the combination of G and K to 16,000 ppm.

When the microorganisms were added to the whey in combinations of three cultures, the specific role each organism played in the fermentation is more difficult to postulate. The patterns observed are difficult to interpret and leave much to be explained.

Treatment 1 was composed of G, S and C. By 96 hours titratable acidity actually increased over the original value in whey and cell weight and pH decreased from their values at 48 hours (Figure 12). It would appear that C had the most antagonistic effect, since the combination of G and S was effective in reducing the COD by 74 percent. When C was combined with either G or S, as in culture treatment 3 and 14 respectively, only a 33 percent reduction in COD occurred. The culture combination of G, S and C only resulted in a 42 percent reduction of COD. Treatment 1 was the least effective in COD reduction of culture treatments composed of three organisms.



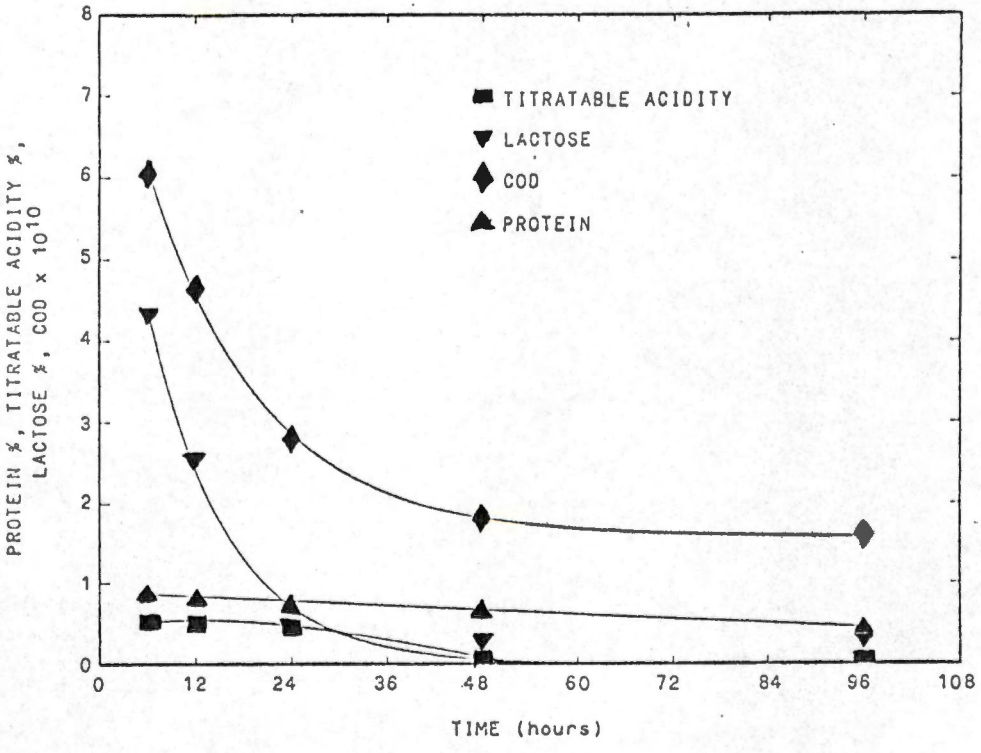




Figure 11. Variation Over Time of Seven Parameters with Culture Treatment 2.

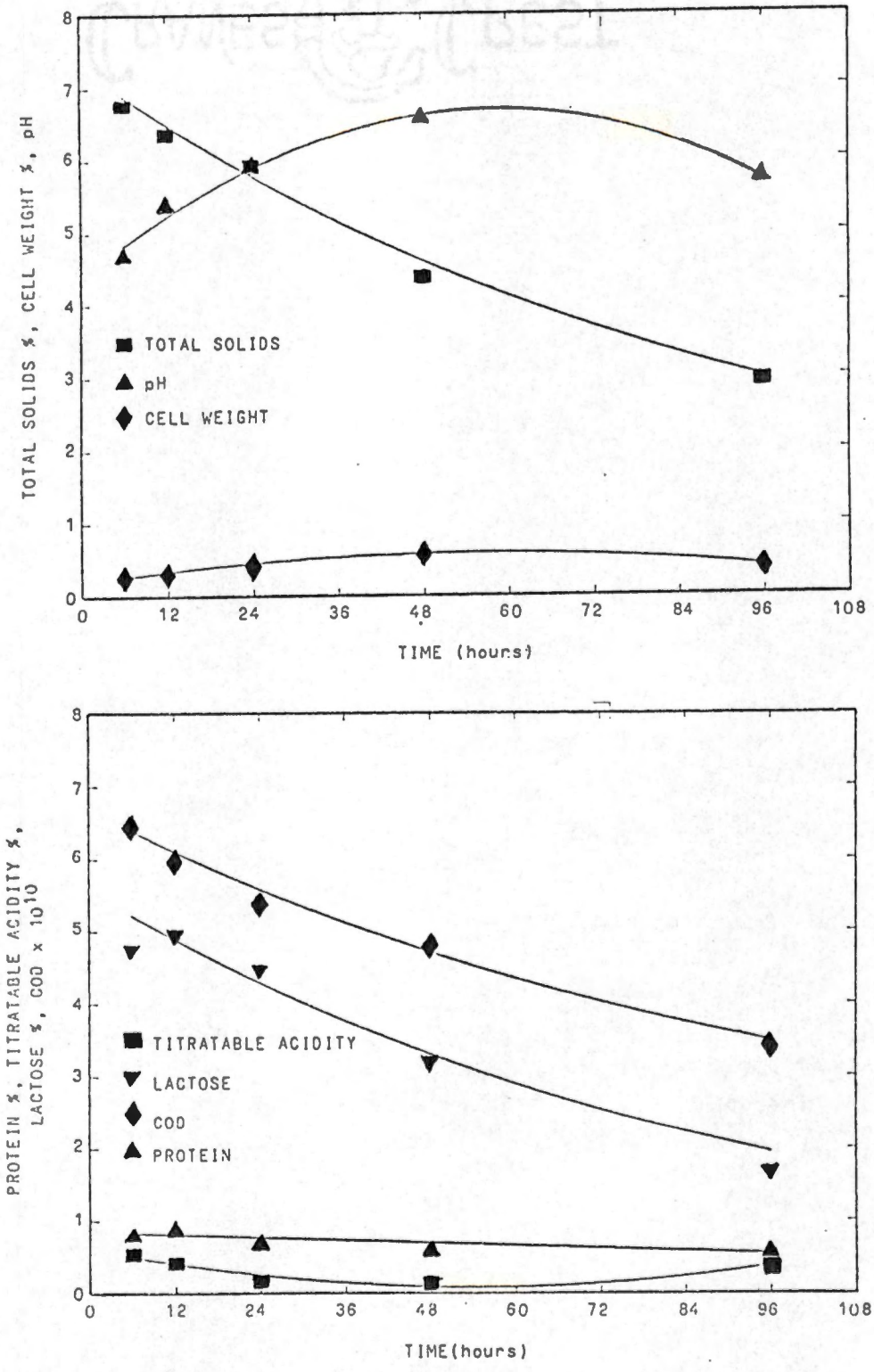



Figure 12. Variation Over Time of Seven Parameters with Culture Treatment 1.

Treatment 5 was composed of S, K and G. Approximately 73 percent of the COD was reduced when S, K and G were combined in pairs in culture treatments. Treatment 5 (Figure 13) showed no advantage in the use of all three microorganisms in a combination over the simpler use of any two in combination. The high "k" value of lactose supports the findings of 96 percent utilization by 24 hours (Table IV, Appendix). Total solids and COD follow the same pattern of rapid utilization also. Fermentation after 24 hours was advantageous in that continued utilization of protein, lactic acid and total solids occurred. At the end of the fermentation 74 percent of the COD of the whey was removed.

Treatment 9, composed of G, C and K, demonstrated a greater effect in utilization of the organic content of whey than when the microorganisms were combined in pairs. By 24 hours lactose was reduced to the lowest observed mean (Figure 14). Titratable acidity was neutralized and pH was 6.8 by 96 hours. At the end of the fermentation, protein was reduced to the lowest observed mean and cell weight had the largest observed mean of one and 0.25 percent. The COD was finally reduced to 13,500 ppm. which represents a 77 percent reduction. Lactose, total solids and COD follow the exponential equations extremely well with high "k" values (Table IV, Appendix). Treatment 9 resulted in the lowest organic content of all culture treatments by 96 hours.

Culture treatment 8 showed a rapid lactose and total solids utilization by 24 hours (Figure 15). Such activity is substantiated by the high "k" value in their exponential equations (Table IV, Appendix). By 24 hours COD was reduced by 56 percent due to the rapid utilization

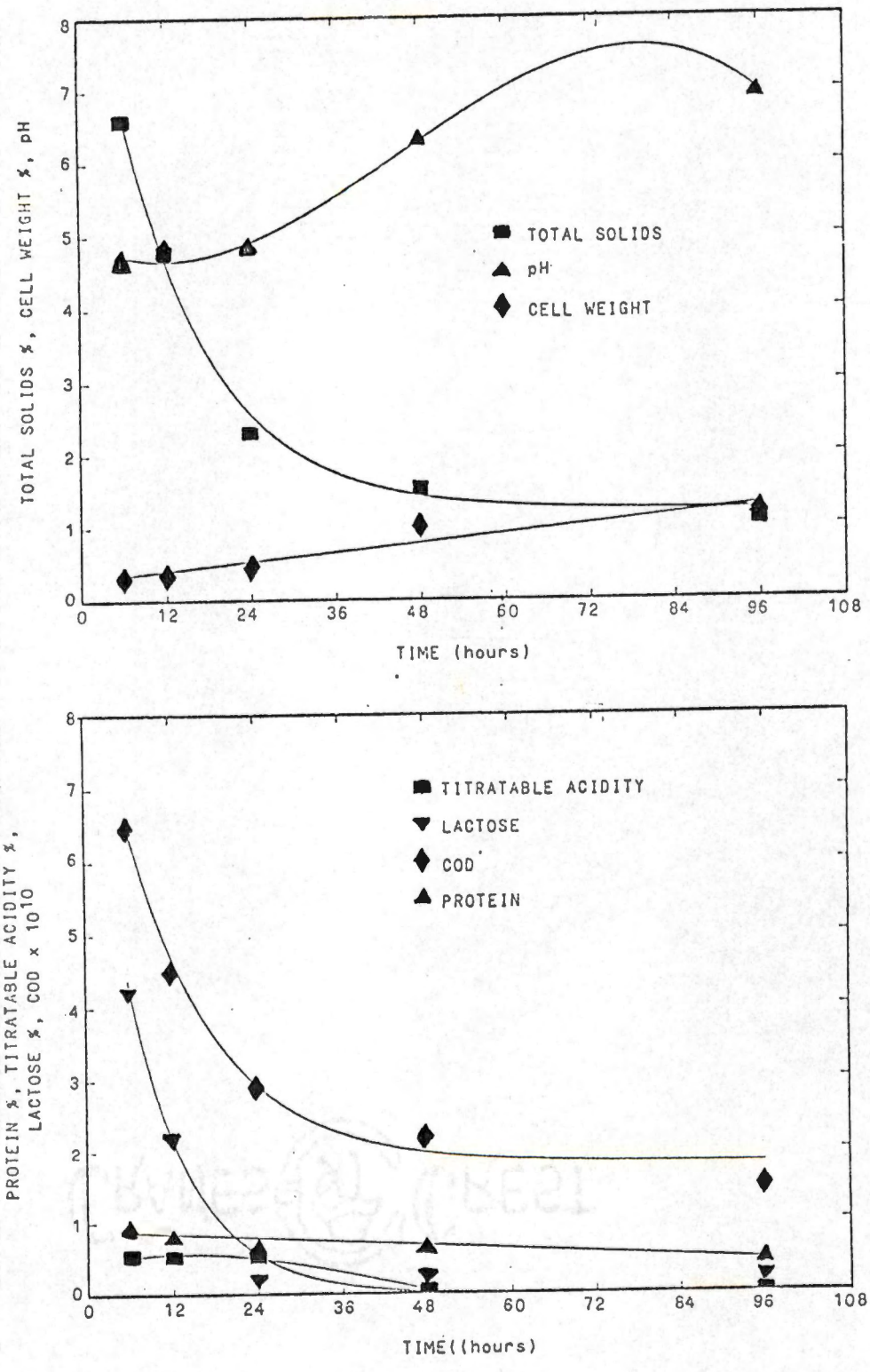



Figure 13. Variation Over Time of Seven Parameters with Culture Treatment 5.

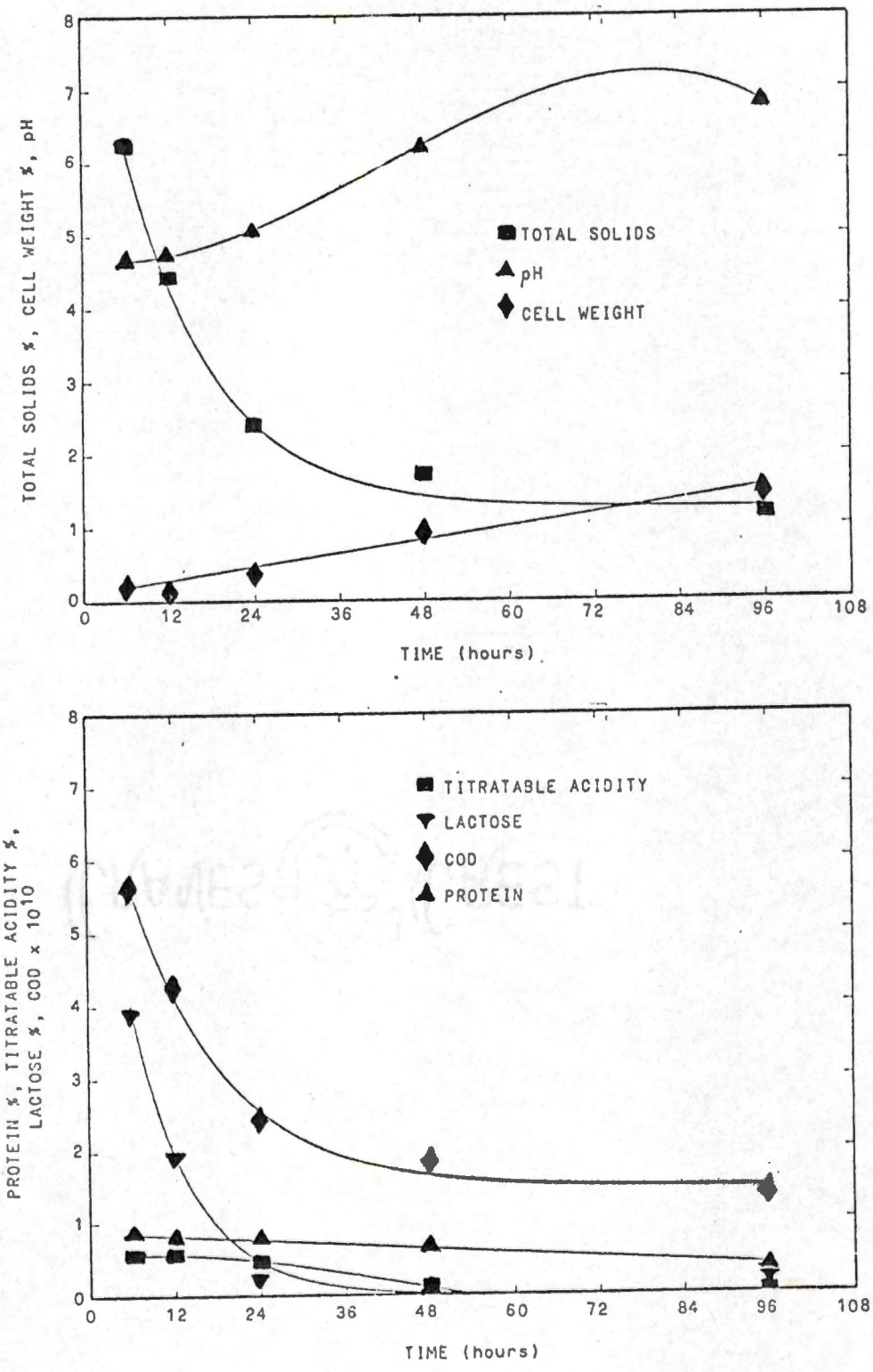



Figure 14. Variation Over Time of Seven Parameters with Culture Treatment 9.

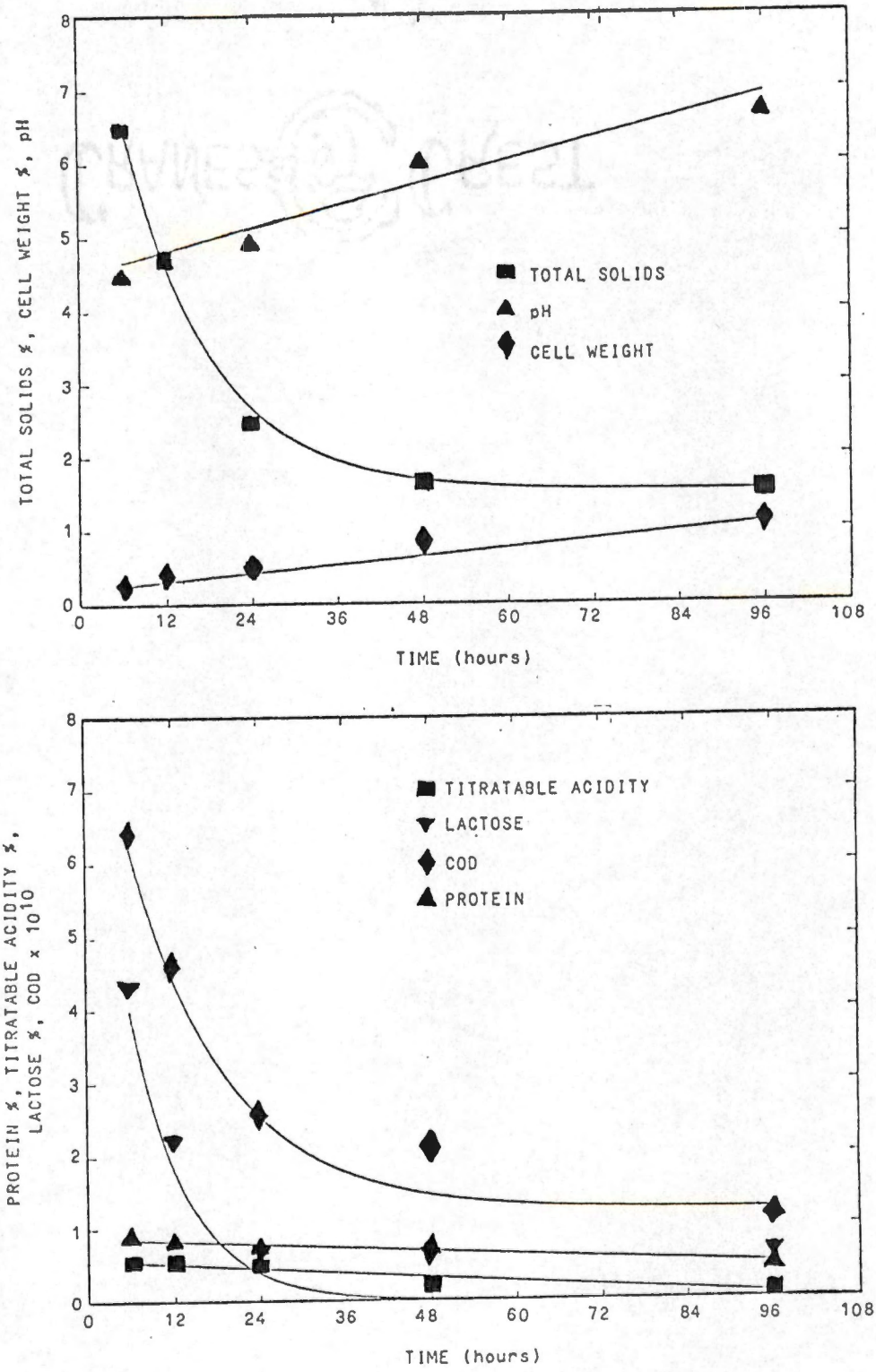



Figure 15. Variation Over Time of Seven Parameters with Culture Treatment 8.

of lactose. S, C and K continued to use total solids, protein and lactic acid throughout the fermentation. The COD of the whey was reduced by 80 percent by 96 hours. The "k" value for COD was also high which substantiates the rapid reduction in COD. Enhancement and compatability in growth of S, C and K was reflected in the increased COD reduction of culture treatment 8 compared to culture treatments 4, 6 and 14 which contained the organisms in paired combinations.

All four cultures, S, G, C and K, were combined in treatment 10 (Figure 16). Rapid utilization of lactose and total solids reflected in high "k" values (Table IV, Appendix) also caused a rapid reduction in COD. Although titratable acidity was only reduced to half its original value and the pH was slightly lower than 6, culture treatment 10 was the most efficient and successful treatment in reducing the COD of whey. Final COD was about 10,700 ppm. Even though individual components of whey were not removed from the whey to the extent demonstrated in culture treatment 9, it may be that a more complete assimilation occurred. It is possible that metabolites accumulated to a greater degree in culture treatment 9.

The fact that the culture treatments which included K best fit the exponential equations may be due to a short lag phase and rapid initiation of utilization of components in whey.

The feasibility of using simple methods of microbial assimilation to reduce the COD of cottage cheese whey appears to be substantiated by the results of this study. A lower COD might be possible with greater aeration than was provided in this study. It seems that accumulation of waste products prevents a greater reduction in COD.

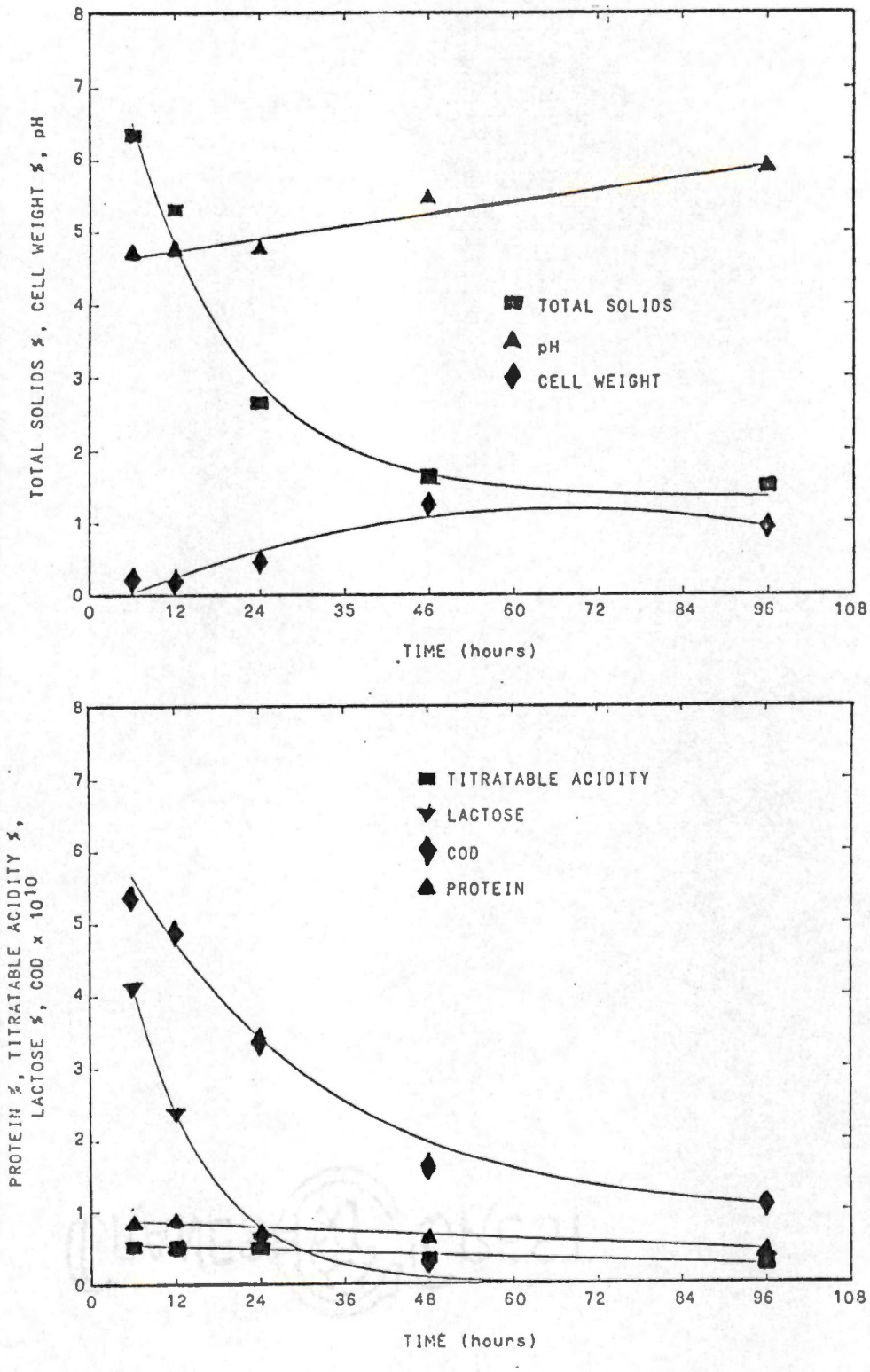



Figure 16. Variation Over Time of Seven Parameters with Culture Treatment 10.

It might prove better to add the cultures to the whey at different times in a stepwise fashion to decrease COD. Although lactose and lactic acid were readily assimilated, protein remains as a stumbling block in COD reduction. Further study into protein utilization by microorganisms is necessary. With some culture treatments, a shortening of the fermentation time from 96 hours would not be detrimental to reduction of the organic content of whey. Thus, with further study into the more successful culture treatments, microbial reduction of the chemical oxygen demand of cottage cheese whey may find application in dairy waste disposal.

# CHAPTER V

## SUMMARY

The purpose of this investigation was to study the microbial reduction of the Chemical Oxygen Demand (COD) and organic content of cottage cheese whey. Kluyveromyces fragilis was chosen for the study because it is a rapid lactose fermenting yeast. A Candida species which had previously demonstrated proteolytic capabilities was also chosen. Streptococcus faecalis subspecies liquefaciens has the ability to hydrolyze the proteins and ferment the lactose found in milk. Ability to neutralize acid brines demonstrated by Geotrichum candidum, made this species another selected microorganism. These four cultures were added to whey singly and in combinations of two, three and four making 15 different culture treatments.

A 10 percent inoculum of each of the culture treatments was added to 400 milliliters of whey. Culture treatments were incubated at 30°C. with aeration for 96 hours on a Burrell shaker. Samples of the culture treatments were analyzed at 6, 12, 24, 48 and 96 hours to follow the fate of total solids, cell weight, lactose, protein and titratable acidity present. COD and pH was also measured.

The change over time of the seven parameters was studied. Time was found to be highly significant for all parameters. Effect of culture treatments was significant on all parameters except protein which overall averaged 50 percent reduction. Several treatments showed an outstanding ability to utilize the organic contents of whey and thereby reduce the COD of the whey. Up to 96 percent of the lactose was

utilized. Typically titratable acidity was neutralized and pH approached 7. When these factors were combined with 50 percent utilization of protein, the COD was reduced by up to 82 percent. Culture treatments 5, 6, 8, 9, 10, and 12 reduced the COD to below 15,000 ppm. in 96 hours. Culture treatment 10 had the lowest COD at 48 hours among culture treatments which would make its use more advantageous when the time of fermentation was limited by practical demand in a dairy plant. Regression equations were derived which described the change over time of all seven parameters in the whey with each culture treatment.

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# APPENDIX

TABLE IV

EQUATIONS GIVING BEST FIT FOR THE VARIATION OVER TIME OF SEVEN MEASURED PARAMETERS FOR CULTURE TREATMENTS[1,2]

| | |
|---|---|
| **Treatment 1** | |
| Total solids (%) | $= 0.548362 + 6.757298e^{-0.01060788t}$ |
| pH | $= 4.3868 + 0.0791t - 0.000678t^2$ |
| Cell weight (%) | $= 0.2006 + 0.0141t - 0.000124t^2$ |
| Titratable acidity (%) | $= 0.6082 - 0.0193t + 0.000177t^2$ |
| Lactose (%) | $= 5.572763e^{-0.01090627t}$ |
| COD x $10^{10}$ | $= 1.5602000 + 5.139323e^{-0.0102109t}$ |
| Protein | $= 0.8427 - 0.00314t$ |
| **Treatment 2** | |
| Total solids (%) | $= 1.358998 + 8.268292e^{-0.07501598t}$ |
| pH | $= 4.1778 + 0.0542t - 0.000300t^2$ |
| Cell weight (%) | $= 0.1168 + 0.0116t$ |
| Titratable acidity (%) | $= 0.4560 + 0.0157t - 0.000770t^2 + 0.00000587t^3$ |
| Lactose (%) | $= 8.043595e^{-0.10124690t}$ |
| COD x $10^{10}$ | $= 1.572204 + 6.859498e^{-0.069484t}$ |
| Protein | $= 0.8928 - 0.0046t$ |
| **Treatment 3** | |
| Total solids (%) | $= 3.762311 + 4.041503e^{-0.03888516t}$ |
| pH | $= 5.0733 + 0.0223t$ |
| Cell weight(%) | = NONE |
| Titratable acidity (%) | $= 0.3901 - 0.0044t$ |

TABLE IV (continued)

| | |
|---|---|
| Treatment 3 (continued) | |
| Lactose (%) | $= 4.6376630e^{-0.00554511t}$ |
| COD x $10^{10}$ | $= 3.737062 + 2.881067e^{-0.09320046t}$ |
| Protein (%) | $= 0.8750 - 0.0036t$ |
| Treatment 4 | |
| Total solids (%) | $= 1.254148 + 9.033567e^{-0.09329946t}$ |
| pH | $= 4.3933 + 0.0164t + 0.0013t^2 - 0.0000122t^3$ |
| Cell weight (%) | $= 0.3287 + 0.0112t$ |
| Titratable acidity (%) | $= 0.6060 - 0.00068t - 0.00044t^2 + 0.000004t^3$ |
| Lactose (%) | $= 9.4186954e^{-0.13312397t}$ |
| COD x $10^{10}$ | $= 1.548472 + 7.587642e^{-0.07368300t}$ |
| Protein (%) | $= 0.8852 - 0.00482t$ |
| Treatment 5 | |
| Total solids (%) | $= 1.213833 + 8.734260e^{-0.07735367t}$ |
| pH | $= 4.9587 - 0.0534t + 0.0026t^2 - 0.000019t^2 - 0.000019t^3$ |
| Cell weight (%) | $= 0.2909 + 0.0104t$ |
| Titratable acidity (%) | $= 0.4297 + 0.0211t - 0.00094t^2 + 0.00000700t^3$ |
| Lactose (%) | $= 8.7337808e^{-0.11525858t}$ |
| COD x $10^{10}$ | $= 1.814681 + 7.456197e^{-0.07859199t}$ |
| Protein (%) | $= 0.8994 - 0.0047t$ |
| Treatment 6 | |
| Total solids (%) | $= 1.7572796 + 8.294048e^{-0.09355920t}$ |
| pH | $= 4.5868 + 0.0172t$ |

TABLE IV (continued)

| | |
|---|---|
| Treatment 6 (continued) | |
| Cell weight (%) | = NONE |
| Titratable acidity (%) | = $0.5669 - 0.0033t$ |
| Lactose (%) | = $7.5754490e^{-0.09697162t}$ |
| COD x $10^{10}$ | = $1.542897 + 6.320058e^{-0.06401315t}$ |
| Protein (%) | = $0.8527 - 0.0037t$ |
| Treatment 7 | |
| Total solids (%) | = $6.7682 - 0.0205t$ |
| pH | = $4.1518 + 0.0984t - 0.0007t^2$ |
| Cell weight (%) | = $0.2539 + 0.0051t$ |
| Titratable acidity (%) | = $0.69667 - 0.032801t + 0.000495t^2 - 0.00000238t^3$ |
| Lactose (%) | = $4.6534057e^{-0.00054965t}$ |
| COD x $10^{10}$ | = $6.292375e^{-0.014865t}$ |
| Protein (%) | = $0.8687 - 0.0046t$ |
| Treatment 8 | |
| Total solids (%) | = $1.516483 + 8.254702e^{-0.08162862t}$ |
| pH | = $4.5249 + 0.0251t$ |
| Cell weight (%) | = $0.2076 + 0.0092t$ |
| Titratable acidity (%) | = $0.5730 - 0.0052t$ |
| Lactose (%) | = $8.7110528e^{-0.13050945t}$ |
| COD x $10^{10}$ | = $1.250561 + 7.734571e^{-0.0742039t}$ |
| Protein (%) | = $0.8720 - 0.0040t$ |

TABLE IV (continued)

| | |
|---|---|
| **Treatment 9** | |
| Total solids (%) | $= 1.242791 + 7.960923e^{-0.07916601t}$ |
| pH | $= 4.703651 - 0.014240t + 0.001548t^2 - 0.0000122t^3$ |
| Cell weight (%) | $= 0.1222 + 0.01480t$ |
| Titratable acidity (%) | $= 0.515635 + 0.010786t - 0.000645t^2 + 0.00000498t^3$ |
| Lactose (%) | $= 8.2233997e^{-0.11998799t}$ |
| COD x $10^{10}$ | $= 1.4532313 + 6.5032487e^{-0.07358846t}$ |
| Protein (%) | $= 0.8780 - 0.0051t$ |
| **Treatment 10** | |
| Total solids (%) | $= 1.337750 + 7.67736e^{-0.06551210t}$ |
| pH | $= 4.5801 + 0.0140t$ |
| Cell weight (%) | $= 0.1969 + 0.0417t + 0.000312t^2$ |
| Titratable acidity (%) | $= 0.5529 - 0.0032t$ |
| Lactose (%) | $= 7.2565338e^{-0.09233810t}$ |
| COD x $10^{10}$ | $= 0.8764763 + 5.9035264e^{-0.03497314t}$ |
| Protein (%) | $= 0.8969 - 0.0046t$ |
| **Treatment 11** | |
| Total solids (%) | $= 0.802364 + 7.548882e^{-0.0232266t}$ |
| pH | $= 4.7129 + 0.0149t$ |
| Cell weight (%) | $= 0.2046 + 0.0054t$ |
| Titratable acidity (%) | $= 0.5014 - 0.0029t$ |
| Lactose (%) | $= 6.3602737e^{-0.03049502t}$ |

TABLE IV (continued)

| | |
|---|---|
| Treatment 11 (continued) | |
| COD x $10^{10}$ | $= -0.182881 + 7.7473689e^{-0.01883955t}$ |
| Protein (%) | $= 0.9053 - 0.0035t$ |
| Treatment 12 | |
| Total solids (%) | $= 0.124800 + 8.228392e^{-0.02411446t}$ |
| pH | $= 4.115794 + 0.1296532 - 0.003006t^2 + 0.00002018t^3$ |
| Titratable acidity (%) | $= 0.681746 - 0.031510t + 0.000712t^2 - 0.00000475t^3$ |
| Lactose (%) | $= 6.34838337e^{-0.03202557t}$ |
| COD x $10^{10}$ | $= -1.615836 + 8.51482e^{-0.08608576t}$ |
| Protein (%) | $= 0.9571 - 0.0051t$ |
| Treatment 13 | |
| Total solids (%) | $= 1.434378 + 8.557382e^{-0.08608576t}$ |
| pH | $= 4.751250 - 0.019665t + 0.0019069t^2 - 0.0000155t^3$ |
| Cell weight (%) | $= 0.2331 + 0.0692t$ |
| Titratable acidity (%) | $= 0.525714 + 0.012937t - 0.000782t^2 + 0.00000618t^3$ |
| Lactose (%) | $= 7.1737402e^{-0.10037908t}$ |
| COD x $10^{10}$ | $= 1.728157 + 7.856905e^{-0.07668377t}$ |
| Protein (%) | $= 0.8995 - 0.0041t$ |
| Treatment 14 | |
| Total solids (%) | $= 3.914799 + 3.259594e^{-0.01862060t}$ |
| pH | $= 4.2001 + 0.0958t - 0.000646t^2$ |

TABLE IV (continued)

| | |
|---|---|
| Treatment 14 (continued) | |
| Cell weight (%) | $= 0.1498 + 0.0099t$ |
| Titratable acidity (%) | $= 0.773968 - 0.042211t + 0.000728t^2 - 0.00000388t^3$ |
| Lactose (%) | $= 4.8189316e^{-0.00161866t}$ |
| COD x $10^{10}$ | $= 1.457963 + 5.325029e^{-0.00837494t}$ |
| Protein (%) | $= 0.9568 - 0.0135t + 0.0000826t^2$ |
| Treatment 15 | |
| Total solids (%) | $= 7.0772 - 0.0351t$ |
| pH | $= 4.4601 + 0.0204t$ |
| Cell weight (%) | $= 0.0812 + 0.0077t$ |
| Titratable acidity (%) | $= 0.6369 - 0.0040t$ |
| Lactose (%) | $= 5.0925826e^{-0.00938629t}$ |
| COD x $10^{10}$ | $= 6.642444e^{-0.032510t}$ |
| Protein (%) | $= 0.9881 - 0.01161t + 0.0000668t^2$ |

[1]t = time
[2]Number refers to Table III

# VITA

The author was born January 21, 1953 in Cincinnati, Ohio, to Mr. and Mrs. John Thomson. In 1970 she graduated from Oak Hills High School and in September entered the University of Tennessee at Knoxville. Completing her Bachelor of Arts in Microbiology in June, 1974, she graduated with honors. In August of the same year, she was employed as an Associate Control Biologist by Cutter Laboratories, Chattanooga, Tennessee. In September, 1975, she began a graduate program in the Department of Food Technology and Science at the University of Tennessee, Knoxville and in January, 1975, received a graduate research assistantship. Requirements for a Master of Science degree were completed in June, 1977.