LACTOSE REMOVAL FROM CHEESE WHEY USING *SACCHAROMYCES FRAGILIS* YEAST

By

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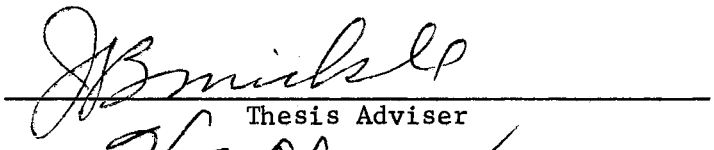

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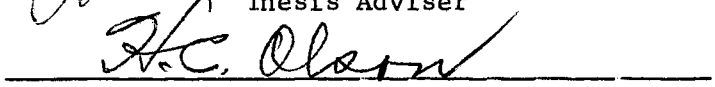

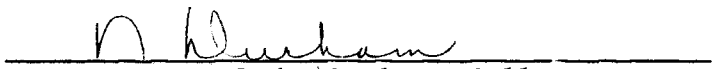

Dean of the Graduate College

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I wish to express my appreciation to Dr. James B. Mickle who made this research an invaluable learning experience, who let me make my own mistakes but patiently kept me from going too far down blind alleys; also to Mrs. Wanda Smith who showed by example how research is done, and to Mrs. Olive Pryor for her efficient management of the laboratory.

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CHAPTER I

INTRODUCTION

The disposal of cheese whey in the United States is a major pollution problem. Recent statistics indicate that approximately 22 billion pounds of whey are produced in this country annually. Only one third of this whey is utilized. The remainder, much of which comes from smaller cheese factories, is usually wasted. Not only is this a waste of a valuable food product, it creates a monumental disposal problem, since this volume of whey is roughly comparable to the sewage from a city of ten million people (2).

A large amount of oxygen is required to degrade whey. In fact, if one gallon of whey were dumped into a stream, the dissolved oxygen in over 4,500 gallons of unpolluted water would be required for its oxidation (13). A large percentage of the oxygen needed to biologically degrade whey is required for the milk sugar, lactose, which is present in the product. This lactose also accounts for some of whey's unique disposal problems. If whey were dumped into a city's sewer, the large oxygen demand could completely overload the system. Additionally, the average municipal sewage system does not have a high proportion of lactose-utilizing microorganisms necessary for efficient disposal of dairy wastes. Even specially designed dairy waste disposal systems often cannot handle a mixture as rich as whey (21).

Whey poured into streams or lakes causes pollution; whey poured

into evaporation lagoons causes odors. Whey can be spread on fields as fertilizer but should not be used in amounts that exceed the field's ability to absorb and use the fertilizer. Some manufacturers have dug holes to bury whey and have sprinkled it on dirt roads, but eventually the holes fill up and the roads draw flies. They have poured it down abandoned mines or pumped it into old oil wells until whey buildup in underground formations may even start to threaten fresh water strata--but the whey keeps right on coming. The Australian cheese industry built a pipeline system that can collect over a million gallons of whey daily and dump it several miles out in the ocean (4).

Actually, whey is a very good food--it retains up to 70% of the food value of the original milk (5). This includes most of the lactose, calcium, and riboflavin, some of the protein, plus other vitamins and minerals.

The obvious solution would be to feed the whey to animals. However, poultry and other non-mammalian animals are not adapted to properly utilize lactose; and ingesting it in any appreciable quantity will cause diarrhea and similar digestion problems (2). Even among mammals, the adults of the species, including man, often lose the ability to digest lactose. Most swine can tolerate fairly large amounts of lactose; but because of its high water content (90-93%), whey is expensive to haul; and if the pig pen is very far from the cheese plant, it usually is cheaper to buy feed than to haul whey.

Recent technological advances in whey concentration, i.e., reverse osmosis and ultrafiltration, have supplied a partial answer to the problem. Now whey can be separated into its various components--lactose, protein, minerals, etc.,--before drying. However, the

equipment necessary to do this is quite expensive, and an extremely large volume of whey is necessary before the operation can be profitable. Even then, markets for large quantities of dried whey must be found (30).

The protein fraction of whey does not present so great a disposal problem as does the lactose. There is about five times more lactose than protein in whey, and these whey proteins are readily decomposed by regular sewage microorganisms. When whey is processed rather than disposed of, the resulting proteins are easier to market than is the lactose fraction. The proteins in whey have a good balance of essential amino acids--better in fact than the whole milk. They, therefore, find a ready market once separated from the other components of whey. Pallansch (2) indicates some interesting possibilities for whey proteins as emulsifiers when removed in an undenatured form. Thus, it would appear that a process which could use the lactose while leaving the proteins intact could supply products for already developed markets.

Lacey and Rey (2), spokesmen for the Federal Water Quality Administration, recently stressed the need for research into whey disposal problems; but they said this research must be devoted to methods that could be carried from the laboratory into the "real world." They specifically mentioned that many of the new whey disposal techniques are not economically applicable to the problems of the small cheese plant. Most methods of whey disposal assume a volume of at least 100,000 pounds per day to justify the cost of drying and fractionating equipment. However, the large majority of cheese plants in this country produce much less than this amount. In fact Heinemann (2)

found that the average daily production per plant in 1968 was only 42,000 lbs. of whey.

The purpose of this study was to determine whether yeast would be an effective means of removing lactose from whey. This also would materially decrease the organic content of the remaining whey. A further purpose was to eliminate this lactose by a method that could be adapted to a small cheese operation.

CHAPTER II

REVIEW OF LITERATURE

Among the most current sources of information about growth conditions and characteristics of food yeasts were The Yeasts (23) and Single Cell Protein (14). These sources reviewed most of the recent work done with yeasts in general as well as much of the research on the lactose utilizing strains.

Yeast fermentation of whey. Milk sugar in whey has long been used as a substrate for yeast fermentation. In the 1940's, Rogossa and others (22, 29) grew yeast in whey producing ethanol anaerobically. Unfortunately, this did not prove to be an economical means of commercial alcohol production. Graham, et al., also grew yeast in whey, but their aim was to increase its protein content and thereby increase the use of waste whey as an animal feed supplement (9). They found that aerating the whey-yeast medium produced a higher yeast cell yield, because the yeast then could oxidize the lactose to CO_2 and new cells rather than fermenting the lactose anaerobically to alcohol as had been done by Rogossa (11, 22). In fact, Mayer (2) later stated that oxygen was probably the single most limiting factor in determining yeast yields and that air may be used at the rate of 6,000 cu. ft./min. in a 10,000-gal. culture of yeast. Other workers recommended air flow volumes ranging from 0.25 to 4.0 volumes of air per minute for oxidizing whey wastes (8, 19, 20, 28).

Nitrogen enrichment. Graham, et al., added supplemental nitrogen to whey to encourage yeast cell production (9). Wasserman, et al., found that adding phosphates and yeast extract, in addition to air and nitrogen, stimulated an even greater yeast cell yield (28). Wasserman's conclusions were based on the comparative amounts of yeast cell growth in the enriched whey medium compared to growth in plain (unenriched) whey at the end of a six-hour period. Growth was not allowed to continue until the lactose was completely exhausted in either medium. It has been reported that carbon and nitrogen are present in most yeast cells at about a five-to-one ratio. However, whey does not contain that much nitrogen (20). In addition, Saccharomyces fragilis (the most widely investigated lactose using strain of yeast) apparently could not even use all of the little nitrogen that was present in whey. Wasserman reported that, although yeast could use the lactose and lactic acid as a carbon source, it could not use all the nitrogen present (27). The yeast apparently could use soluble nitrogen in the form of peptones or amino acids and a little of the ammonium nitrogen. However, it could not break down the whey proteins (lactalbumins and lactoglobulins) so they could be used as a nitrogen source. Ingram (10) supported this conclusion and stated that yeasts as a group do not lyse proteins. He further implied that proteolytic activity of yeasts is associated with old age and ruptured or autolysed cells. Hoover (2) maintained that S. fragilis was "completely non-proteolytic."

Recommended yeast strain. Early yeast research of Porges and co-workers (20) showed that of several yeast strains tested, Saccharomyces fragilis was the most efficient when grown in whey in

terms of the rate at which it converted lactose to new cells. Since that time, the bulk of research done with whey has been done with some strain of S. fragilis.

Growth conditions for S. fragilis. Most authors stated that yeasts grew over a wide range of temperatures with an optimum ranging from 68° to 77°F (24). Phaff, et al., (18) reported growth of S. fragilis at 41°F through 116°F. Lodder and Kreger-Van Rig (12) stated that this yeast grew well at 113°F. In further studies with this yeast, most workers recommended a growth temperature of 86°F and used extensive cooling mechanisms to offset the heat produced by yeast fermentation (2). Wasserman (26) reported growth at 105°F but felt this higher temperature contributed to contamination problems.

The literature also stated that S. fragilis grew over a wide range of pH (from 3.0 to 8.0); however, it generally preferred slightly acid conditions (18, 24). As optimum, Wasserman, et al., reported pH values ranging from 5.0 to 5.7; but Amundson (3) recommended a pH of 3.5 Foaming and contamination were problems at the higher pH values and metal corrosion a problem at the lower pH.

Organic matter reduction and yeast protein quality. By fermenting whey with yeast, a substantial reduction in oxidizable organic matter was achieved. Amundson (3) reported a reduction of 85%, and Mayer (2) reported a 90% reduction in 8 hours providing the whey proteins were precipitated and removed with the yeast cells.

Wasserman (25) found that whey-grown yeast was higher in lysine than yeast grown in other media. Feeding tests using animals (rats, mink, dogs) showed whey-grown yeast was a valuable feed supplement. When whey proteins were harvested with the yeast cells, an even

higher quality protein supplement was obtained (2, 3, 6, 15). The dried yeast-plus-whey proteins mixture was composed of 50% protein and practically no lactose as compared to 13-14% protein and 73-75% lactose in dried whey (3). The whey proteins by themselves also found ready acceptance for a variety of purposes. Pallansch (16) reported that pure dried whey proteins were priced at one dollar per pound in 1970.

CHAPTER III

EXPERIMENTAL PROCEDURES

I. Preliminary Trials

Inoculum. Saccharomyces fragilis (NRRL-1156) was chosen for this study because of its ability to use lactose (20). This yeast also had been approved as a human food by the Food and Drug Administration (2). The original yeast strain was carried on agar slants composed of agar, peptone, and glucose (lactose later replaced the glucose). To prepare an inoculum, one loop of yeast from this slant was aseptically transferred into a flask of sterile medium composed of 2% lactose, 2% peptone, and 0.1% yeast extract. Air was bubbled through this medium by means of a glass tube connected to a small pump which gave a constant flow of air during incubation at 77°F. Turbidity measurements were used as an indication of cell growth. Figure 1, which is a typical growth curve, indicates that this yeast was in the 'log' growth phase when turbidity readings were between 0.30 and 0.65. Thus, inoculations into growth media were always made when the inoculum medium was within this interval to insure inoculation during the log growth phase. As the optimum growth conditions for S. fragilis became apparent from later experiments, the formula for the inoculum medium was changed to 4% lactose rather than 2%, and the incubation temperature was raised to 95°F.

Media. The maintenance of sterile conditions when growing S.

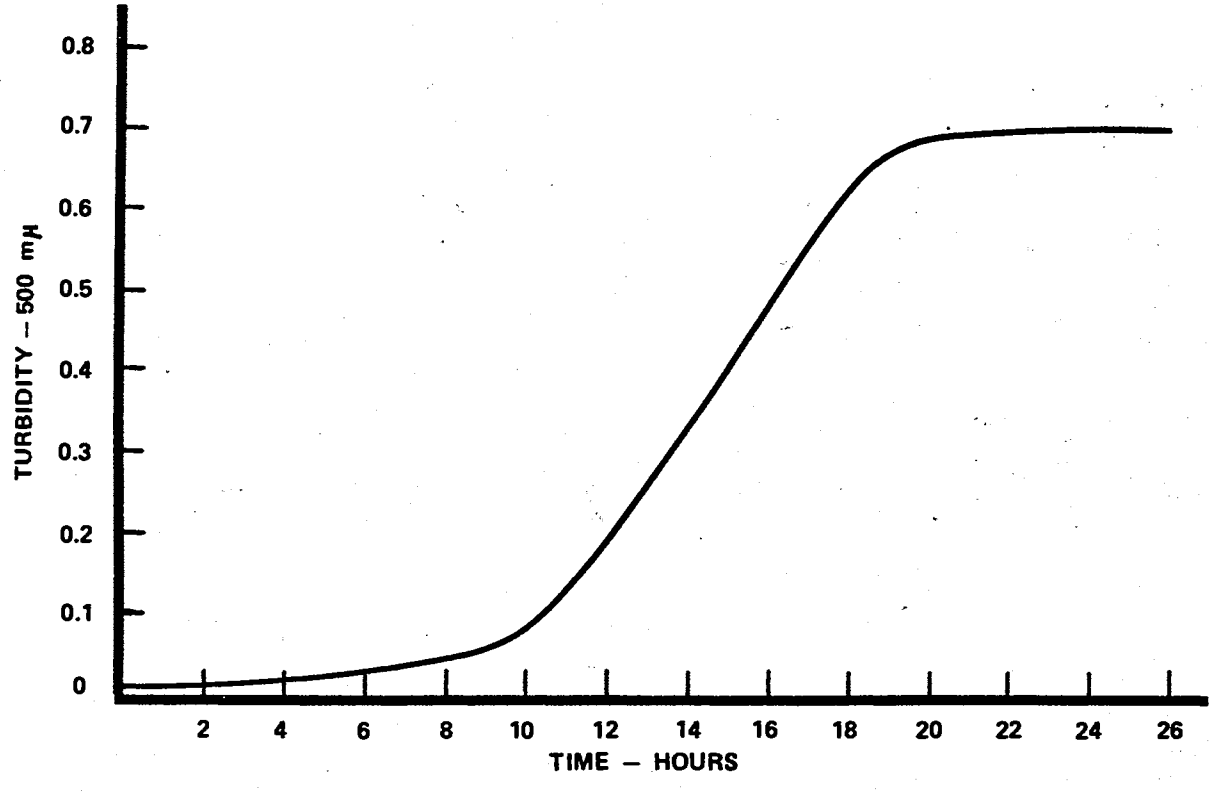

Figure 1. Turbidity of S. fragilis During Growth in Media Containing 2% Lactose, 2% Peptone, 0.1% Yeast Extract at 77°F

fragilis in cheese whey commercially would not be practical. Thus it was decided not to maintain sterile growth conditions in the laboratory. Instead, a large, actively-growing inoculum was used together with 'normal' cleanliness as might be practiced in a dairy plant. Later observations based on periodic 'streak plates' of the medium and microscopic observations while the yeast was actively growing demonstrated that no appreciable contamination occurred.

When artificial media were used, the ingredients included peptone, lactose, and yeast extract--all obtained from Difco Laboratories. In addition, various reagent-grade chemicals were used as sources of certain minerals. The whey which was used for media was acid cottage cheese whey (pH 4.6) obtained from the Oklahoma State University Creamery. The review of literature indicated that S. fragilis did not use whey proteins for growth. Thus, the whey was heated to 190°F to precipitate the proteins which were then removed by filtration. The clear, deproteinated whey was refrigerated until used. Removal of these proteins prior to using the whey decreased the initial turbidity of the whey and improved the accuracy of the optical density measurements used to follow the yeast's growth rate.

Apparatus. In preliminary work, the yeast was cultured in 38 X 300 mm Pyrex test tubes containing 100 ml media (Figure 2). Temperatures were controlled by placing these tubes in a heated water bath where the desired temperature was maintained, $\pm$ 5°F, by means of a hot plate. A 63 X 600 mm lucite cylinder was used to distribute air among the tubes. The air entered one end of this cylinder through rubber tubing connected to an air compressor. Excess air could be bled off at the other end of the lucite cylinder if

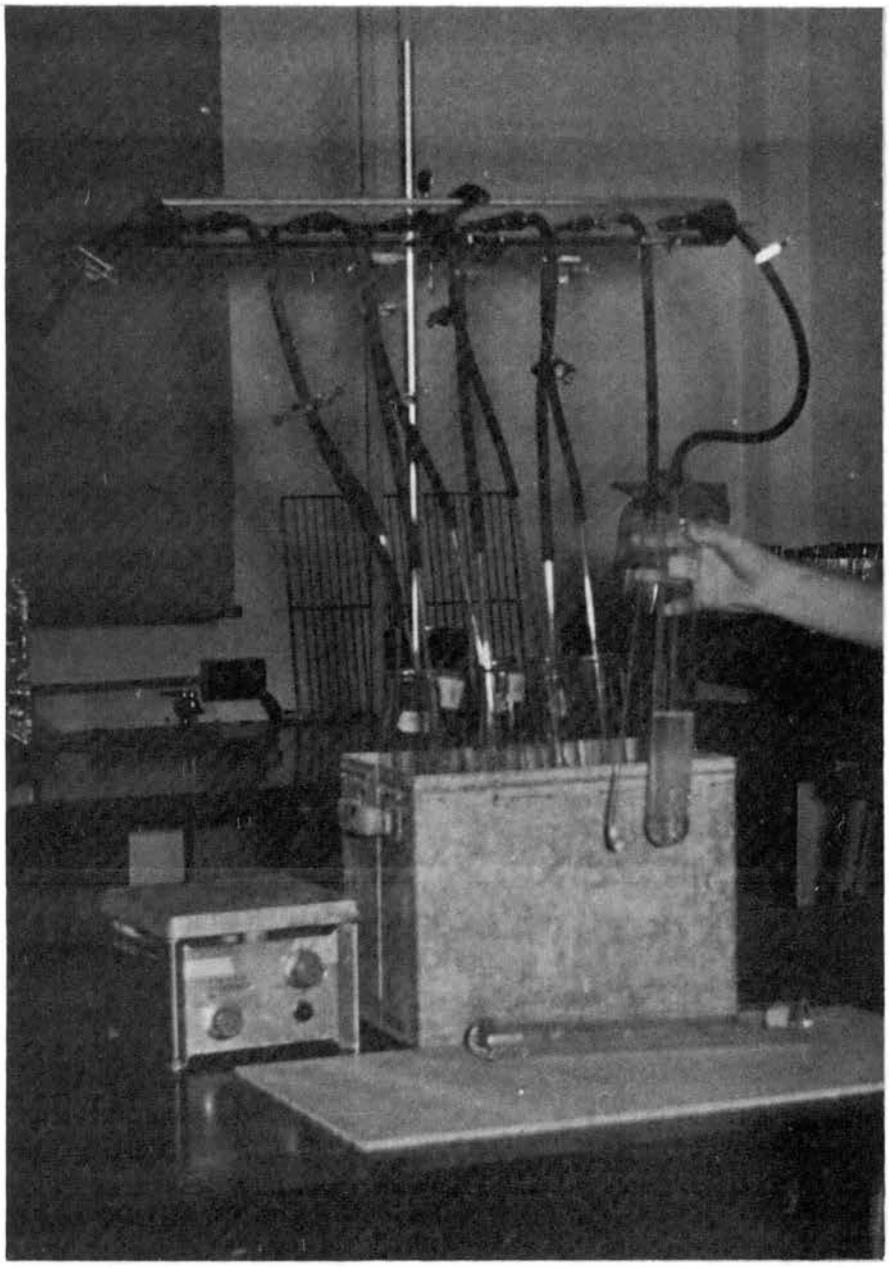

Figure 2. Aeration and Incubation Apparatus for Growing S. fragilis in 100 ml Media

necessary. Six 24 mm holes were drilled in each side of the lucite cylinder. These holes could be plugged with solid rubber stoppers or used as air jets by inserting one-hole stoppers which contained a short piece of glass tubing. Rubber hoses led from the glass tubing to the air dispersing rods which were used to bubble air through the media. The rate of air flow was regulated by means of screw clamps on the rubber tubing leading to each test tube. Rates were adjusted so that the air flow into each tube was approximately equal.

pH. To begin, the pH of the artificial media was adjusted to 6.0. It was soon discovered, however, that the pH of the media changed as the yeast grew, and was different at different stages of the growth cycle. To determine the effect of these changes, pH was sometimes adjusted with NaOH or HCl; pH measurements were made using a Beckman pH meter and an electrode. When using whey media, starting pH's were usually between 4.6 and 5.0 and not adjusted during growth.

Measurements. Growth of the yeast was followed with turbidity measurements. The samples of yeast and medium were diluted with 9 ml of distilled water, and absorbance readings were obtained in a Beckman Model B Spectrophotometer at 500 mu. One-ml aliquots were withdrawn from the media at regular intervals, and the lactose content determined using the procedure of Perry and Doan (17). The 1-ml aliquots usually were diluted with 9 ml of saturated picric acid, then allowed to stand at room temperature until measurements were made at a later date. Lactose percentages in experimental samples were obtained by referring to standard curves made from samples containing known amounts of reagent-grade lactose. Measurements of these standards were obtained at the same time as those of the unknown samples.

II. Large Batches

Inoculum and media preparation. After determining the optimum medium and growth conditions, larger batches of whey were inoculated with S. fragilis. The artificial media used in these large batches for inoculum preparation contained 4% lactose, 2% peptone, and 0.1% yeast extract--the formula which had been most satisfactory during preliminary trials. As in the preliminary 100-ml trials, the yeast for the inoculum was obtained from a slant of S. fragilis and grown in 200 ml of medium until an optical density of between 0.30 and 0.65 was obtained. At that time, a 10% inoculation into the growth medium was made, i.e., 100 ml of inoculum into 900 ml of growth medium to make a 1,000-ml total.

Air flow. These larger batches had two advantages over the smaller ones used in the preliminary work. First, the size of the batch (1,000 ml) was large enough to allow conditions of air circulation more nearly comparable to those in large vats which would be used under commercial conditions. The aeration was somewhat better than that obtained in the smaller preliminary batches since the fritted gas dispersion tubes (extra coarse size) used in the 1,000-ml tests produced finer, more widely dispersed air bubbles. These 1,000-ml batches were cultured in 63 X 900 mm cylinders (Figure 3). Air flow was regulated visually as in previous trials; but in all cases, the flow was more than 1,000 ml/min. This minimum figure was determined by measuring the time required to fill a balloon to a specified diameter and calculating the volume of air contained in the balloon.

Measurements. The larger volume of these 1,000-ml batches

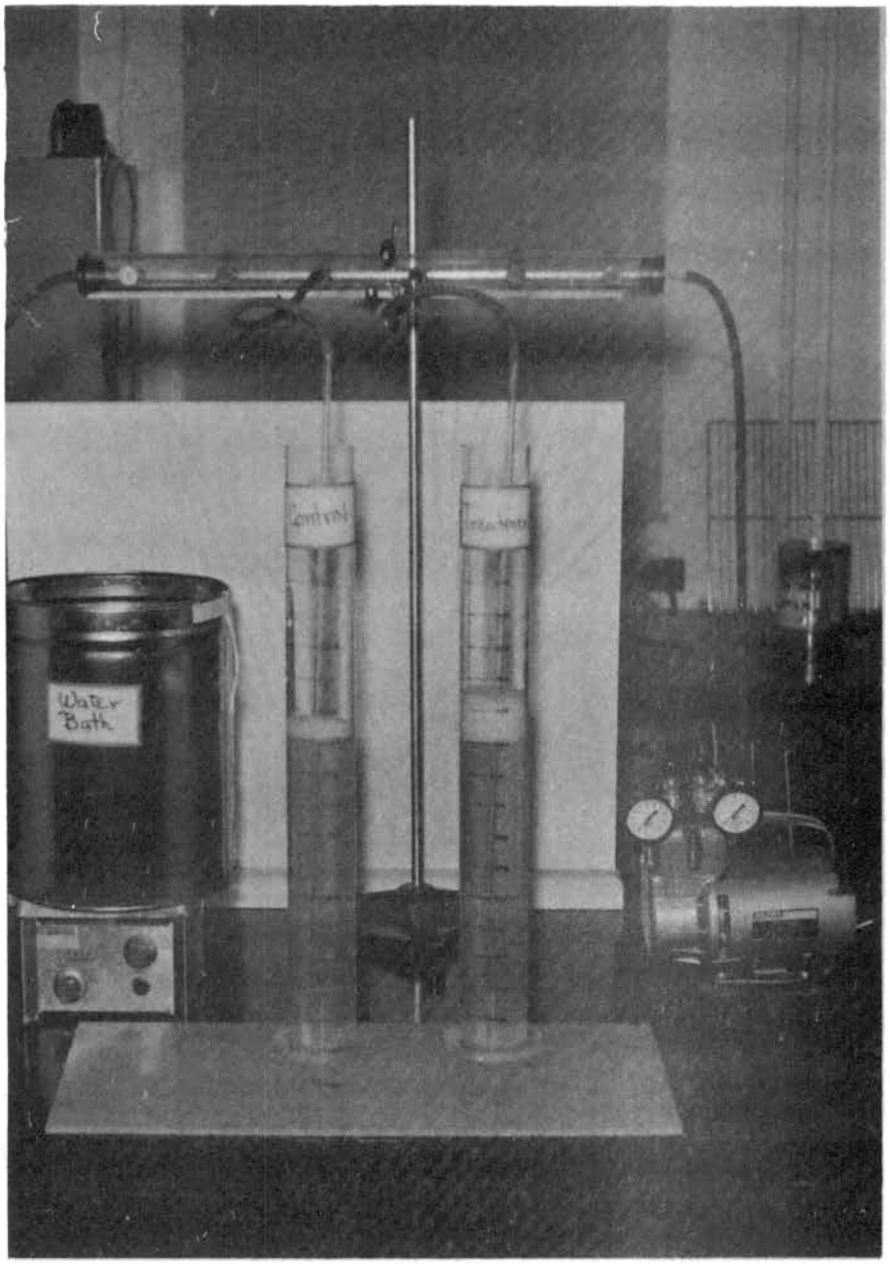

Figure 3. Aeration and Incubation Apparatus for Growing S. fragilis in 1,000 ml Media

provided a second advantage in that sizable samples could be withdrawn at regular intervals. Thus, in addition to measurements of turbidity, pH, and lactose obtained in the preliminary trials, the larger sample size allowed measurements of dry cell weight, protein, and Chemical Oxygen Demand (COD).

At first the dry cell weights were determined directly. A sample of cells-plus-whey was centrifuged for 5 minutes and rinsed and re-centrifuged twice. The cells were washed into tared pans and dried to a constant weight. Later it was decided that determining cell weights by difference was more accurate. The dried weight of 5 ml of whey-plus-yeast cells was compared with the dried weight of 5 ml centrifuged whey. The difference was assumed to be the actual cell weight. (These samples were taken in quadruplicate.) When the amount of cells was determined microscopically, a hemacytometer was used. Nitrogen determinations were made on a 5-ml sample using standard AOAC procedures (7). These nitrogen percentages were converted into protein by multiplying the results by 6.25. For COD tests, the method described in Agricultural Handbook 176 was used (1). The COD test was a means of determining chemically the amount of oxygen demanded for complete oxidation of the sample and was used as as estimate of BOD (Biochemical Oxygen Demand--the amount of oxygen required for biological decomposition of organic wastes, usually in a five-day period.) The BOD equalled about 60% COD according to Quirk and Hellman (21).

CHAPTER IV

RESULTS AND DISCUSSION

I. Preliminary Trials

Artificial media

Sugar requirements. S. fragilis utilized the disaccharides sucrose and lactose as readily as glucose (Figure 4). However, the yeast seemed to grow somewhat better on lactose. It showed good growth with lactose concentrations of 1-6% (Figure 5), but seemed to show maximum growth when the media contained 4% lactose. There was no apparent growth advantage in media containing more than that percentage. The sugar in cheese whey, lactose, normally is present in liquid whey at concentrations of 4-5%. Thus, it was concluded that S. fragilis should not require a sugar supplement when grown in whey and that the whey would not need to be diluted to achieve optimum growth.

Yeast extract. Using an artificial medium containing lactose and peptone, it was found that yeast extract furnished a necessary growth ingredient, but the biggest change in turbidity occurred between 0 and 0.01% (Figure 6). Thus, there did not seem to be any appreciable advantage in having more than a trace of this substance in the media. In later work a usage level of 0.1% was chosen; this rate agreed with Wasserman and co-workers' findings (28).

Nitrogen source. When using peptone as the nitrogen source in

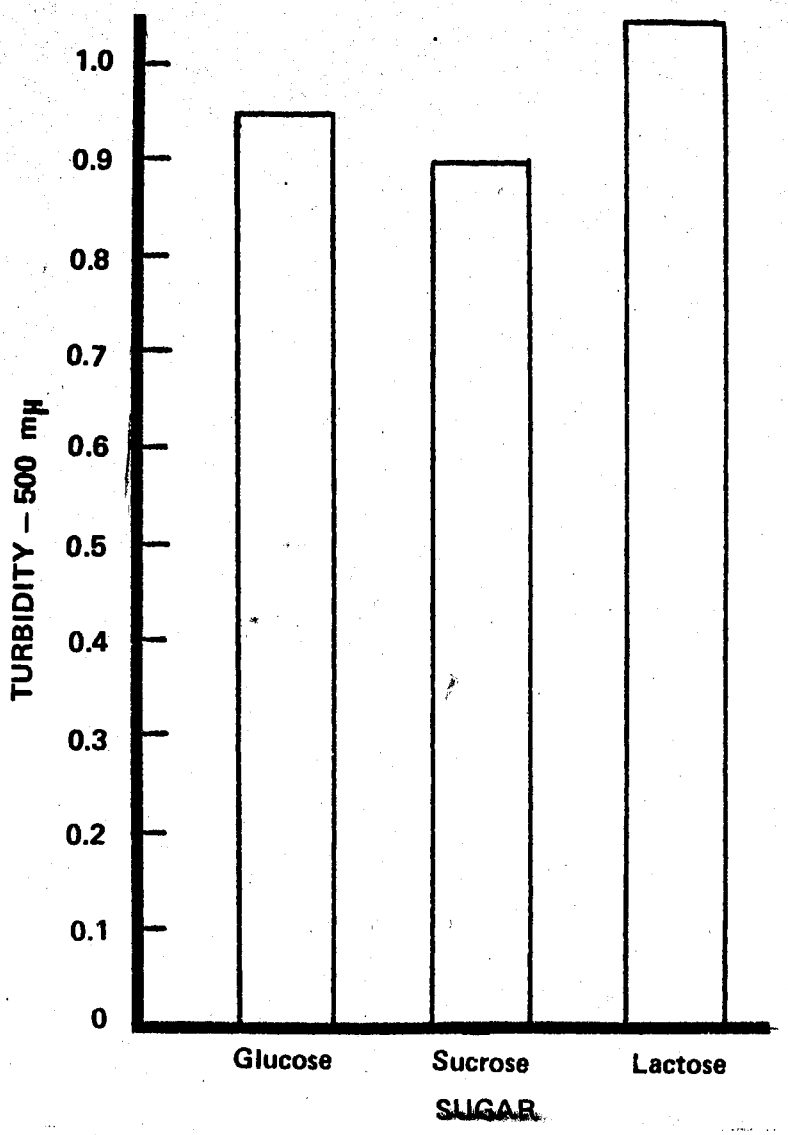

Figure 4. Turbidity of S. fragilis Grown in Artificial Media Containing 2% Peptone, 0.1% Yeast Extract, 2% Glucose, Sucrose, or Lactose

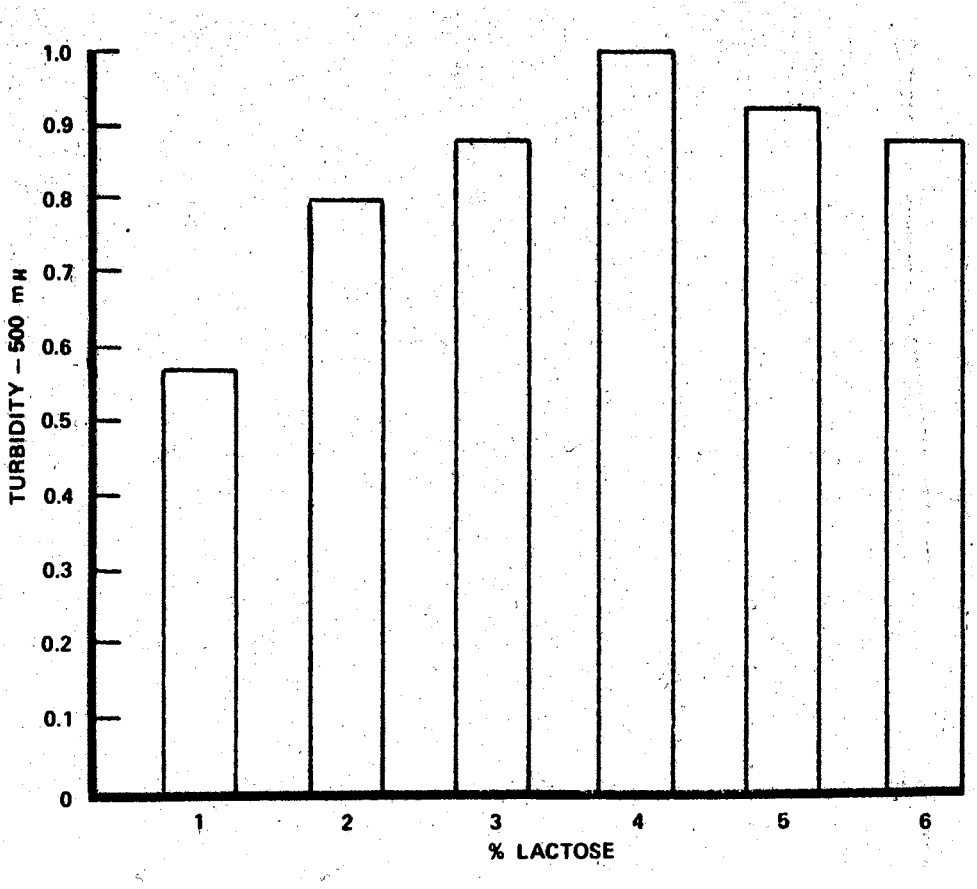

Figure 5. Turbidity of S. fragilis Grown in Artificial Media Containing 2% Peptone, 0.1% Yeast Extract, and 1 to 6% Lactose

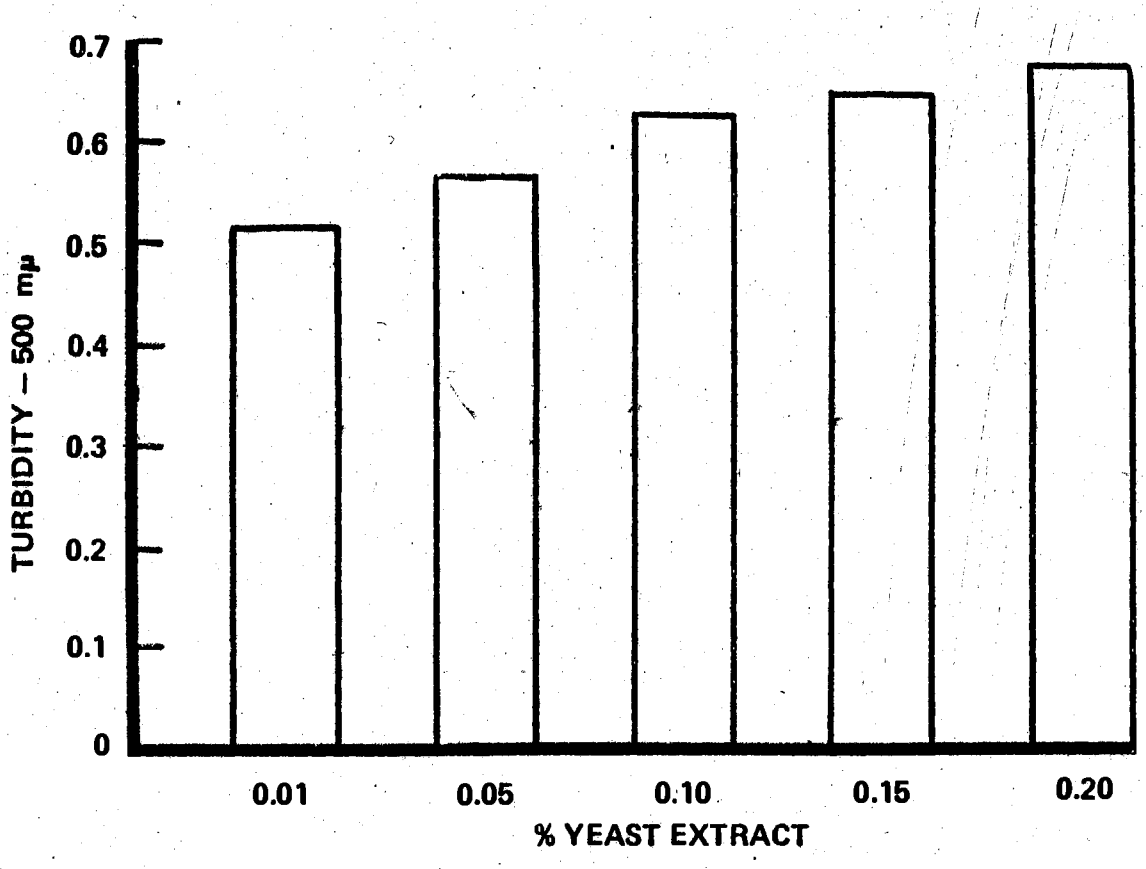

Figure 6. Turbidity of S. fragilis Grown in Artificial Media Containing 2% Peptone, 2% Lactose, 0.01 to 0.20% Yeast Extract

a medium containing 4% lactose and 0.1% yeast extract, it was found that 2% peptone afforded good growth. However, there was little apparent benefit in adding more than this amount (Figure 7). Attempts were made to replace the peptone with various amounts of different nitrogen salts (ranging from 0.1% to 5.0%). The salts tested included ammonium sulfate, ammonium phosphate, and urea. These salts were tested alone and in combination with various levels of potassium phosphate and yeast extract. None of these additives gave growth exceeding that of the peptone control, and many of them actually inhibited the yeast.

Whey as media

Experimental errors. Turbidity readings were accumulated from S. fragilis grown in similar media under similar conditions while the yeast was in the log growth phase. Statistical analysis of these data resulted in a standard deviation of 0.02. Thus at the 95% level of probability, experimental errors would account for deviations of 0.04 in turbidity readings above or below any given mean. For that reason, unless an experimental treatment gave turbidity readings at least 0.04 higher than the plain whey control, it was not considered to have caused a significant effect.

Plain vs. enriched whey. Growth of S. fragilis was studied in whey containing various nitrogen sources (Figure 8). These studies indicated that this yeast grew as well in undiluted, unenriched whey as it did in the best artificial medium tested (4% lactose, 2% peptone, 0.1% yeast extract). However, none of the nitrogen enrichments except peptone resulted in an appreciable increase in turbidity. Early studies using potassium phosphate, magnesium and iron sulfates,

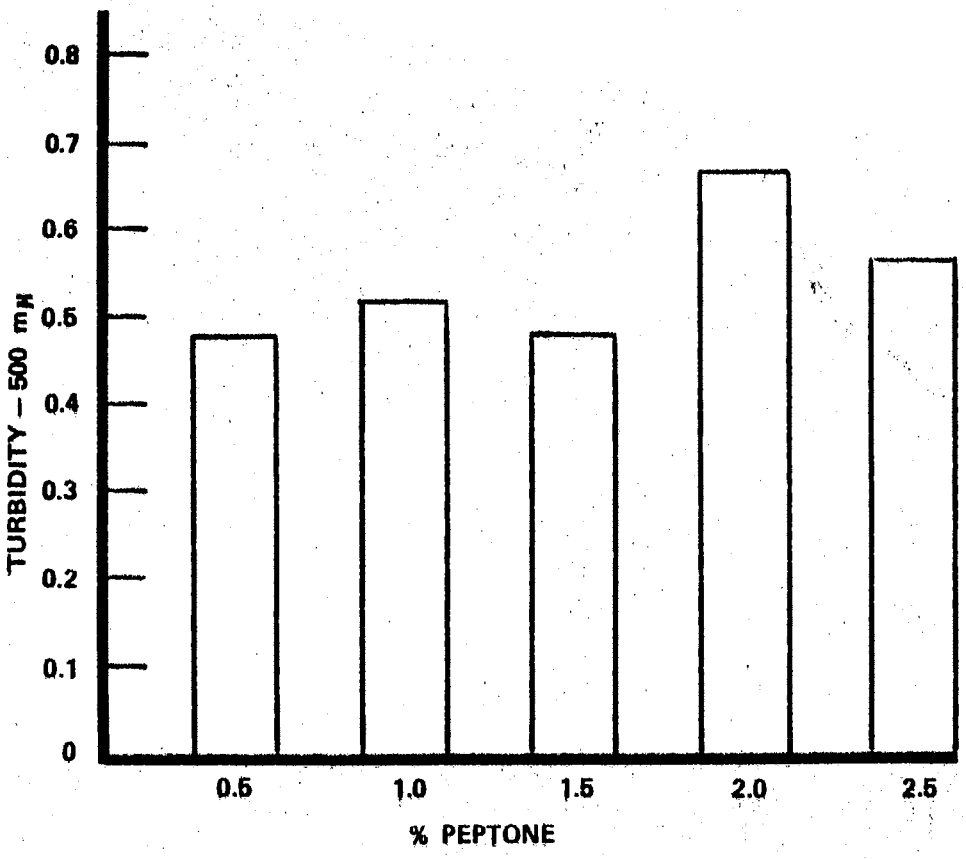

Figure 7. Turbidity of S. fragilis Grown in Artificial Media Containing 2% Lactose, 0.1% Yeast Extract, and 0.5 to 2.5% Peptone

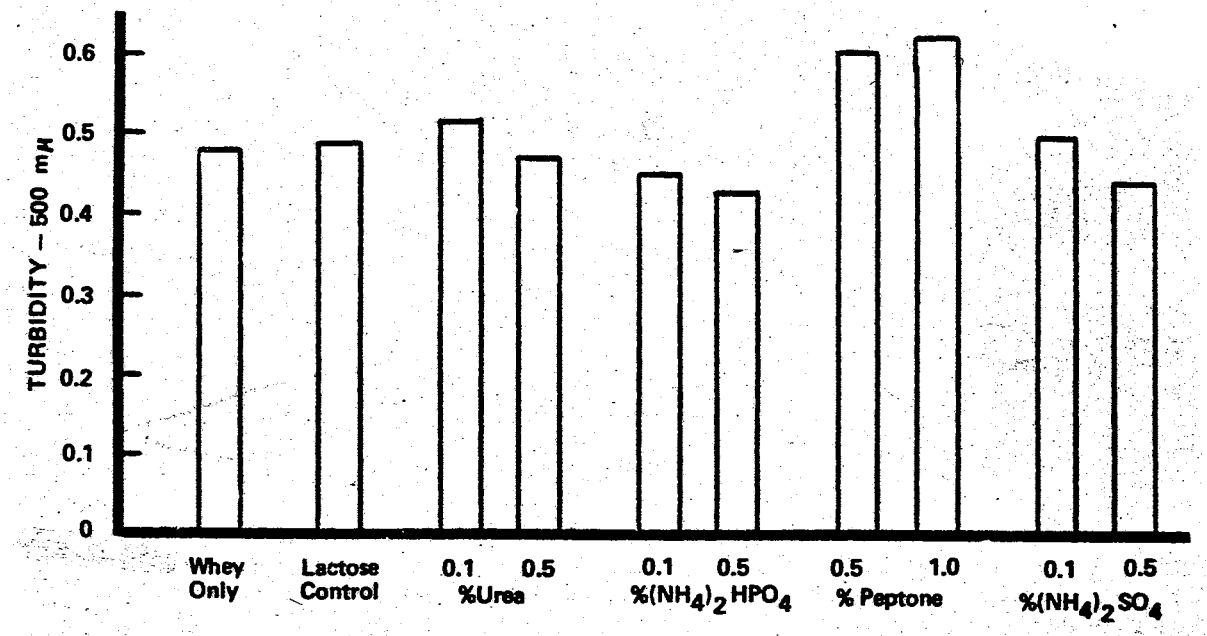

Figure 8. Turbidity of S. fragilis Grown in Whey Containing Various Nitrogen Compounds Compared to Controls of Unenriched Whey and Artificial Media Containing 4% Lactose, 2% Peptone, and 0.1% Yeast Extract

and nitric acid for nutrients did not appear to merit further exploration.

Temperature and pH. S. fragilis grew over a wide range of temperatures and pH values. The yeast showed growth at pH values below 3.0 and above 7.0. However, the optimum growth rates were observed between pH values of 4.0 and 6.0 (Figure 9). The yeast exhibited slow growth at temperatures below 86°F and little growth above 120°F, with the best temperature range being between 96 and 104°F (Figure 10).

II. Large Batches.

Air flow. An air flow of approximately 1,000 ml/min (one volume per minute) was maintained throughout the experimental period. When the air supply was increased, there was no appreciable increase in cell growth, but when the supply was decreased to approximately 350 ml/min., a decrease in growth rate of almost 33 1/3% was noted.

Lactose and COD reduction. The data from a typical 1,000-ml trial using unenriched whey are shown in Table I and graphed in Figure 11. It was evident that the turbidity readings increased as the lactose and COD decreased. Growth, as evidenced by turbidity readings, leveled off as the lactose in the whey was exhausted. The COD of the whey decreased to near 16,000 ppm (or 1.6 parts per 100 ml) and then also leveled off when the lactose was exhausted. This represented a reduction of more than 60% in COD even though a relatively high organic content still remained in the waste. These data (Table I and Figure 11) were representative of nearly all of the 1,000-ml trials using unenriched whey. S. fragilis consistently exhausted the lactose from the whey in 7-9 hours. During this time 0.3 to 0.4 grams

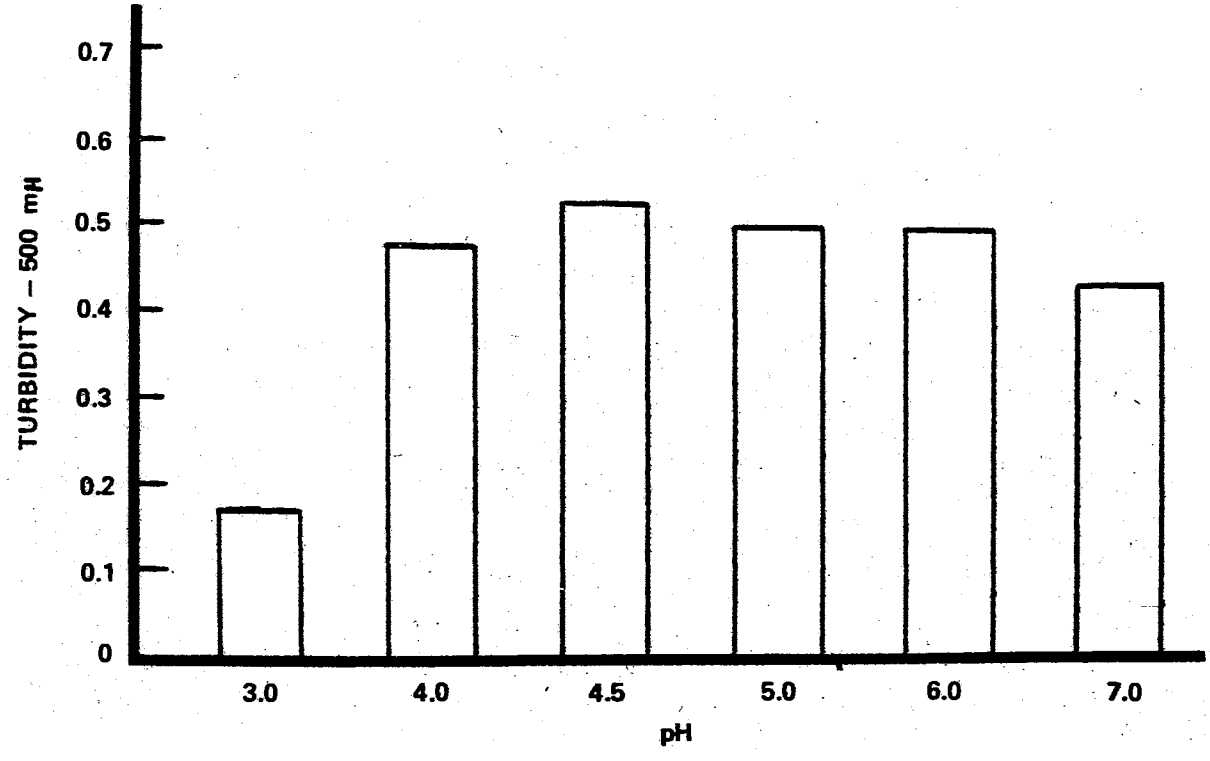

Figure 9. Turbidity of S. fragilis Grown in Whey at Various pH Values at 104°F

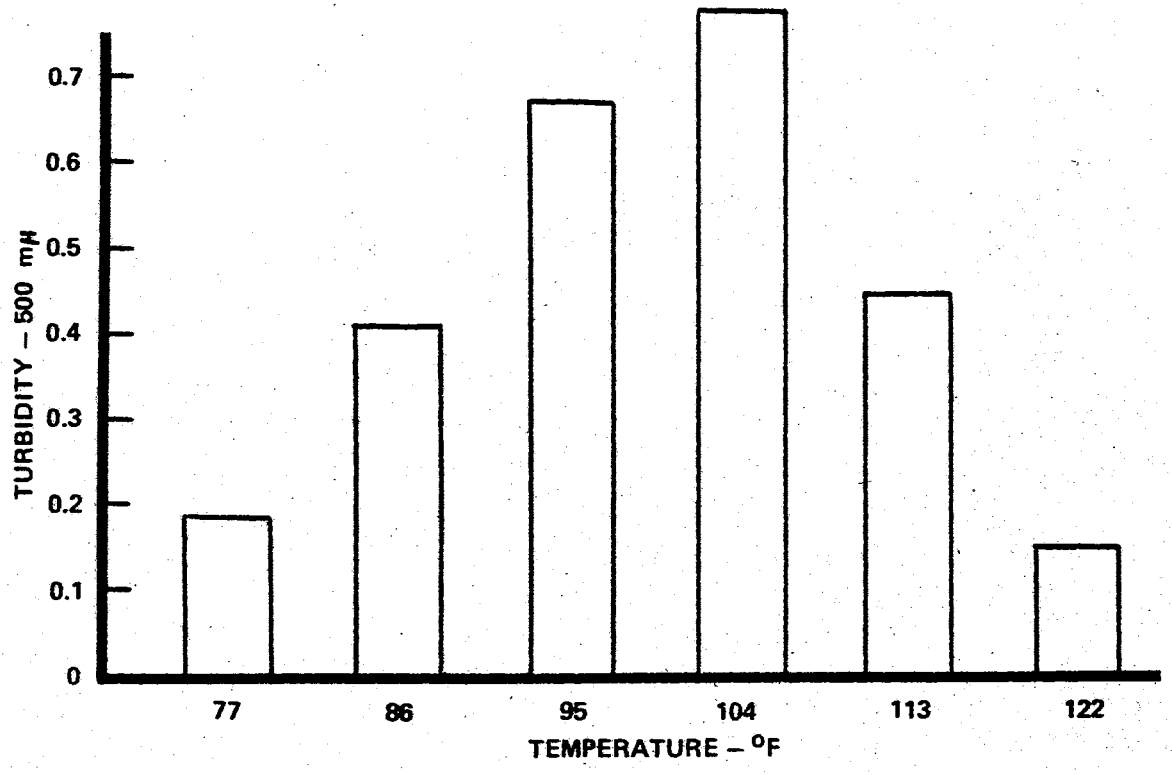

Figure 10. Turbidity of S. fragilis Grown in Whey at Various Temperatures at pH 5

TABLE I

GROWTH OF S. FRAGILIS IN WHEY AS RELATED TO ANALYSES OF LACTOSE AND COD IN THE WHEY

Hours	Turbidity	Lactose %	COD/100 ml
0.0	0.15	4.00	5.2
1.0	0.19	3.60	5.1
2.5	0.25	3.30	5.0
4.5	0.48	2.80	4.5
5.5	0.62	2.30	3.8
7.0	0.83	1.80	3.3
10.0	0.97	0.01	1.6

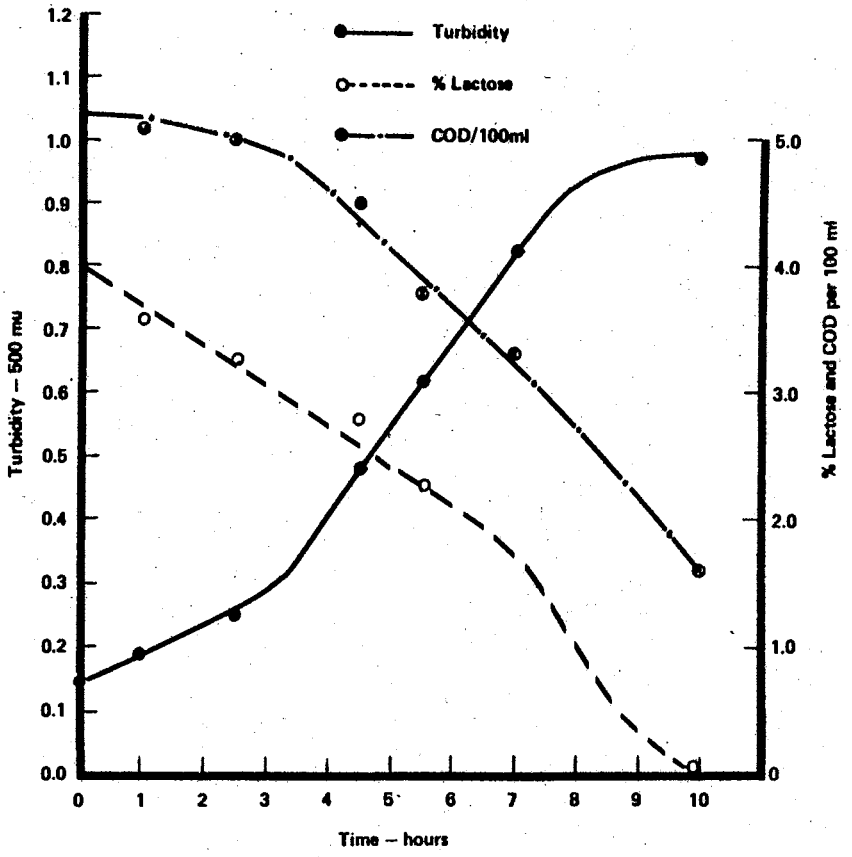

Figure 11. Turbidity of S. fragilis Grown in Whey with Corresponding Analysis of Lactose and COD at pH 5.0, 95°F

of S. fragilis cells were produced in each 100 ml of whey. There was about 0.5% protein in the clarified whey, and this yeast used 20-40% of it. However, the amount used probably represented non-protein nitrogen in the whey since S. fragilis cannot use nitrogen in the form of protein (27).

Yeast cell yields. Theoretically, the carbon (from the lactose) in 100 ml of this whey should have produced 2.5 grams of yeast cells. Yields of about 2.3 g/100 ml of whey, or 85% of this theoretical yield, have been reported (28). In an attempt to explain the apparent discrepancy between the literature and this study, trials were run using inorganic nitrogen and phosphate salts, recommended by the literature, and yeast extract. These enrichments proved ineffective in the large batches as they had in the 100-ml trials (Figure 8). When 1% peptone was added to the whey, a yeast cell weight of 0.5 grams per 100 ml of whey was obtained at the time the sugar was exhausted (Table II), but with 0.7% protein unused. This yeast cell weight of 0.5 grams per 100 ml corresponded to a count of 567 X 10^6 yeast cells per ml. Wasserman, et al., (28) using a very large inoculum (500 X 10^6) had final cell counts three to five times this high, which, when related to the data in Table II, would have indicated increased yeast cell weights towards 1.5 to 2.7 grams of cells per 100 ml would be possible if there were sufficient carbon and nitrogen in the medium to support such growth.

The results of the present study indicate that available nitrogen is the limiting factor in yeast cell yield. Additional usable nitrogen probably would have resulted in greater increases in yeast cell yields--thus approaching the theoretical limit imposed by the amount

TABLE II

GROWTH OF S. FRAGILIS IN WHEY[1] AS RELATED TO LACTOSE, PROTEIN, AND COD ANALYSIS

Time (Hours)	Turbidity (Absorbance)	Cell Count (X 10^6)	Lactose % (Whey)	Protein % (Whey)	Protein % (Cells)	Solids % (Whey)	Solids % (Cells)	COD/100 ml (Whey)
0	0.70	11	5.2	1.3	0.0	8.1	0.0	7.4
2	0.11	24	5.0	1.3	0.0	7.9	0.0	7.3
4	0.26	81	4.9	1.3	0.0	7.8	0.0	7.1
6	0.59	182	4.0	1.3	0.0	6.5	0.3	6.4
8	0.83	381	1.5	1.1	0.2	4.5	0.3	4.1
9	1.00	567	0.0	0.7	0.6	1.8	0.5	1.6

[1]Whey contained 1% added peptone.

of lactose.

However, the purpose of this work was to reduce the COD value of the whey as quickly as possible--not necessarily to produce yeast cells. Relatively small inoculums--as compared to the 500 X 10^6 inoculum used by Wasserman, et al.(28)--of S. fragilis consistently reduced the COD of the unenriched whey from over 50,000 to less than 20,000 ppm in about eight hours. Added nitrogen (to produce more cells) made the process more expensive. In addition, the extra nitrogen did not appreciably shorten the time needed to exhaust the lactose from the whey; and most of this extra nitrogen remained in the whey increasing the disposal problem.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine whether yeast would be an effective means of removing lactose from whey. This also would materially decrease the organic content of the remaining whey. A further purpose was to eliminate this lactose by a method that could be adapted to a small cheese operation. S. fragilis (NRRL-1156) was chosen for this study on the basis of literature recommendations. The yeast was first cultured in small batches to determine optimum growth conditions. Later it was grown in larger batches of whey to simulate commercial conditions. Lactose, protein, cell growth, Chemical Oxygen Demand (COD), and pH were measured during the growth of the yeast.

This study indicated that a good artificial medium for the growth of S. fragilis was composed of 4% lactose, 2% peptone, and 0.1% yeast extract. Optimum growth conditions were a pH of 4.6 to 5 with a temperature of about 100°F and an adequate air flow (approximately one volume per minute). None of the inorganic nitrogen salts tested replaced peptone as a nitrogen source. Cottage cheese whey in 100-ml lots was as good a growth medium for the yeast as was the artificial medium. No additional salt or yeast extract was necessary for growth. Peptone added to the whey did appear to accelerate yeast growth. These determinations were made on the basis of increased turbidity and sugar utilization. Trials performed in 1,000-ml batches of whey under

similar conditions of pH, temperature, and air flow tended to confirm the conclusions drawn using 100-ml batches of whey. Kjeldahl determinations indicated that the yeast used only 0.1 to 0.2% protein, but almost completely exhausted the lactose in approximately 7 to 9 hours, with an accompanying COD decrease of about 60%. At the time the sugar was depleted, a cell weight of about 0.4 grams per 100 ml whey was noted. No problems with contamination by other microorganisms were encountered, although sterile conditions were not maintained.

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