Lactose Removal From Cheese Whey Using Saccharomyces Fragilis

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Story in Brief

The purpose was to determine whether yeast could be an effective means of removing lactose from whey. This could materially decrease the organic content, and thus the pollution potential of the whey. A lactose using yeast, *Saccharomyces fragilis* (NRRL-1156), was chosen for this study. The yeast was first cultured in small batches to determine optimum growth conditions, and then in larger batches to simulate commercial conditions.

This study indicated that cottage cheese whey was a good growth medium for the yeast. No additional salt or yeast extract was necessary, but added peptone did appear to accelerate yeast growth. Optimum conditions included a pH of 4.6-5.0, a temperature of 95-104°F, and an airflow rate of one volume per liter. A series of experiments showed that the yeast completely exhausted the lactose from whey in 7 to 9 hours, with an accompanying decrease in chemical oxygen demand of 60 percent or more.

Introduction

Recent statistics indicate that approximately 22 billion pounds of cheese whey are produced in this country annually. However, only one third of this whey is utilized. The remainder creates a monumental disposal problem since this volume of whey is roughly comparable to the sewage from a city of 210 million people (2). A large amount of oxygen is needed to degrade whey. In fact, one gallon requires the dissolved oxygen in over 4,500 gallons of fresh water for complete degradation (10). A large percentage of this oxygen is used by the lactose present in the whey. This amount of lactose, if dumped into a city's sewer, could completely overload the disposal system (16).

Actually, whey is a very good food—it retains up to 70 percent of the food value of the original milk (4). In addition to the lactose, the whey retains some of the protein and most of the calcium, riboflavin and minerals. The obvious solution would be to feed the whey to animals. However, most non-mammalians cannot utilize lactose, and ingesting any appreciable quantity will cause diarrhea (2). Even among mammals, the adults of the species often lose the ability to digest lactose. Swine, however, usually can tolerate lactose; but because of its high water content (90-93 percent). whey is expensive to haul; and if the swine are 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 before drying. However, the equipment necessary to do this is quite expensive, and an extremely large volume (100,000 lb/day) is required before the operation can be profitable (21). Spokesmen for the Federal Water Quality Administration have stressed the need for research into whey disposal problems and mentioned that many of the new whey disposal techniques are not economically applicable to the problems of the small cheese plant (2).

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

Literature

The research on yeast in general, as well as the lactose utilizing strains, has been reviewed in two recent books (11, 17). Lactose has long been used as a substrate for yeast. In the 1940's Graham, et al., (7) grew yeast in whey to increase its protein content and make it more useful as an animal supplement. They found that aerating the whey-yeast medium produced a higher cell yield. Others have stated that oxygen may be the single most limiting factor in determining yeast yields, and air flow rates ranging from ¼ to 4 volumes of air per minute have been recommended (2,6,14,15,20).

Early research of Porges and co-workers (15) showed that, of the several yeast strains tested, *Saccharomyces fragilis* was the most efficient in converting lactose to new cells. Since that time, the bulk of research done with whey has been done with some strain of this organism. It has been reported that carbon and nitrogen are present in yeast cells at about a 5:1 ratio. However, whey does not contain enough nitrogen to satisfy

this ratio (15). Although *Saccharomyces fragilis* can use lactose and lactic acid as carbon sources, it cannot use much of the nitrogen present in whey (19). Apparently it can use soluble nitrogen in the form of peptones or amino acids but will not break down the whey proteins for use as nitrogen sources (8). Therefore, most researchers have added supplemental nitrogen to whey to encourage yeast cell production (7,20).

Most authors stated that *S. fragilis* grows at temperatures ranging from 41°F through 116°F, and recommended an optimum of about 86°F (2,9,13,18). The literature also stated that *S. fragilis* grew over a wide pH range (from 3.0 to 8.0), and researchers have recommended optimum pH values of from 3.5 through 5.7 (3,13,18,20). When S. fragilis was grown in whey, an 85-90 percent reduction in organic matter was achieved in 8 hours providing the whey proteins were removed with the yeast cells (2,3).

Experimental Procedures

S. fragilis (NRRL-1156) was chosen for this study on the basis of literature recommendations. Optimum growth conditions were determined by incubating it first in 100-ml lots of artificial media and later in whey. Final trials used 1,000-ml lots of whey in order to more nearly simulate commercial conditions. Cell growth was determined by turbidity, with lactose and pH values being recorded during the course of all trials (12). On the 1,000-ml trials, the protein content and COD (Chemical Oxygen Demands) of the whey also were determined (1,5).

The amounts of yeast cells produced were determined by microscopic cell counts and by weighing. A constant air flow of at least one volume per minute was maintained through the media by means of submerged gas dispersion tubes, and temperatures were maintained by the use of heated water baths. The equipment was arranged so that up to 12 tubes of media could be incubated simultaneously under similar conditions of temperature and air flow.

The yeast mixture used as a "starter" for these trials was obtained by transferring yeast from a lactose-agar slant into 200-ml of broth containing 4 percent lactose, 2 percent peptone, and 0.1 percent yeast extract and incubating with aeration for 12-14 hours. After this period, the yeast was in the log phase of its growth cycle. A 10 percent inoculation of this "starter" was used for the growth studies.

Results and Discussion

S. fragilis used sucrose and glucose as well as lactose. However, the yeast seemed to grow somewhat better on lactose, with maximum growth when the media contained 4 percent of this sugar (Figure 1 and 2). Lactose is normally present in cheese whey at concentrations of 4-5 percent. Thus, it was concluded that *S. fragilis* would not require a sugar supplement when grown in whey, and the whey would not need to be diluted to achieve optimum yeast growth. 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 percent (Figure 3). Therefore, there did not seem to be any appreciable advantage in having more than a trace of this substance in the media.

When using peptone as the nitrogen source in a medium containing 4 percent lactose and 0.1 percent yeast extract, it was found that 2 percent peptone afforded maximum growth (Figure 4). Using a whey medium, attempts were made to replace the peptone with different nitrogen salts

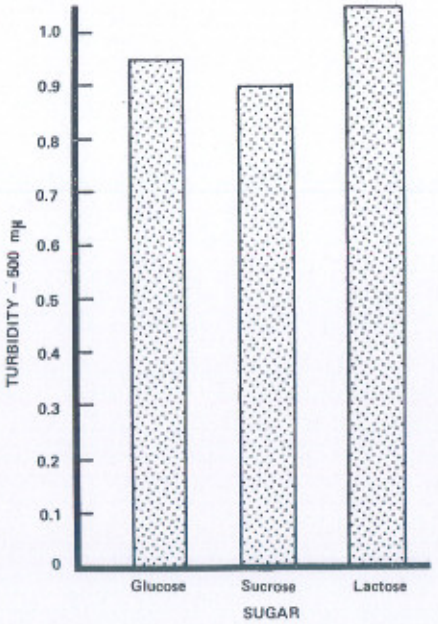

Figure 1. Growth of S. fragilis in artificial media containing 2 percent peptone, 0.1 percent yeast extract, and 2 percent glucose, sucrose, or lactose.

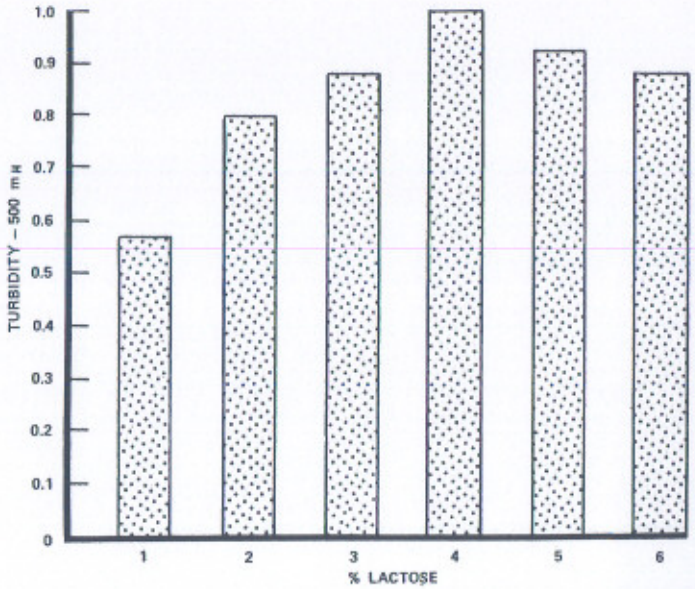

Figure 2. Growth of S. fragilis in artificial media containing 2 percent peptone, 0.1 percent yeast extract, and 1-6 percent lactose.

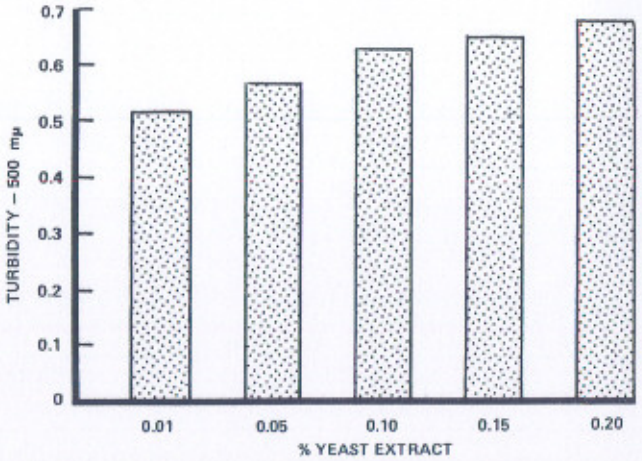

Figure 3. Growth of S. fragilis in artificial media containing 2 percent peptone, 2 percent lactose, and 0.01-0.20 percent yeast extract.

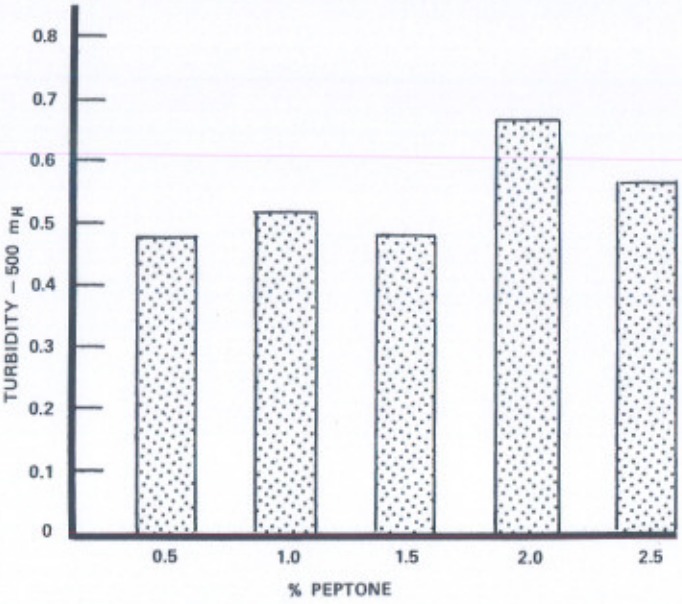

Figure 4. Growth of S. fragilis in artificial media containing 2 percent lactose, 0.1 percent yeast extract, and 0.5-2.5 percent peptone.

including ammonium sulfate, ammonium phosphate, and urea). However, none of these gave growth exceeding that of the peptone control, and many of them actually inhibited the yeast (Figure 5).

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 percent 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 improvement.

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 5.0 (Figure 6). 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 7).

An air flow of approximately 1,000 ml/min. (one volume per minute) was maintained throughout the growth period. When the air supply was increased, there was no appreciable increase in cell growth; but when

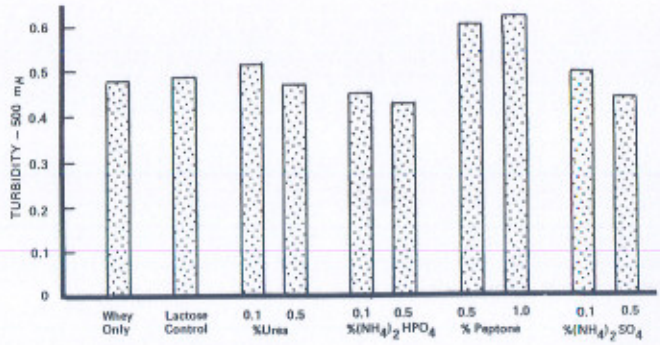

Figure 5. Growth of S. fragilis in whey containing various nitrogen compounds compared to controls of unenriched whey and artificial media containing 4 percent lactose, 2 percent peptone, and 0.1 percent yeast extract.

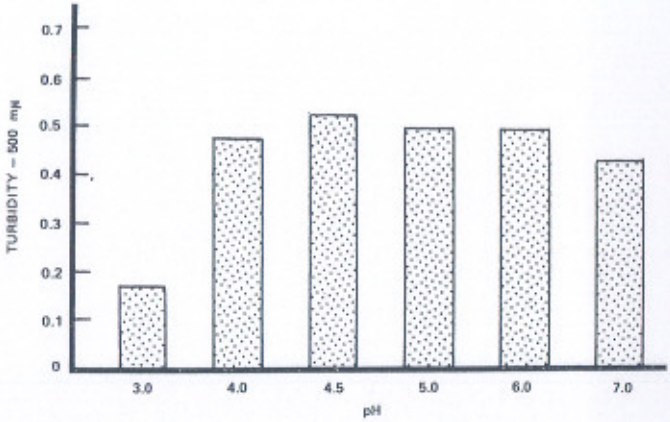

Figure 6. Growth of S. fragilis in whey at various pH values.

the supply was decreased to approximately 350 ml/min., a decrease in growth rate of almost 33 ⅓ percent was noted.

The data from a typical 1,000-ml trial using unenriched whey (Figure 8) show that 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

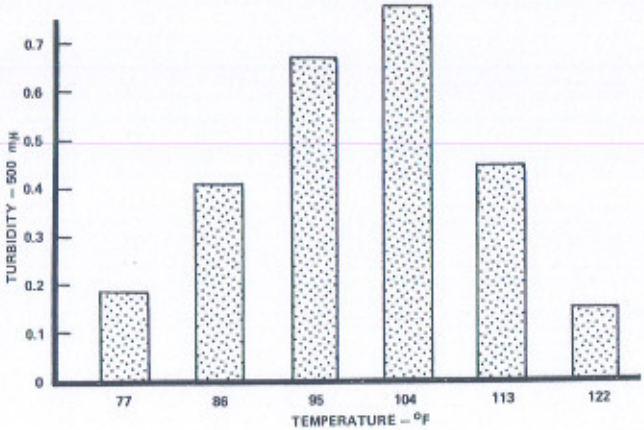

Figure 7. Growth of S. fragilis in whey at various temperatures.

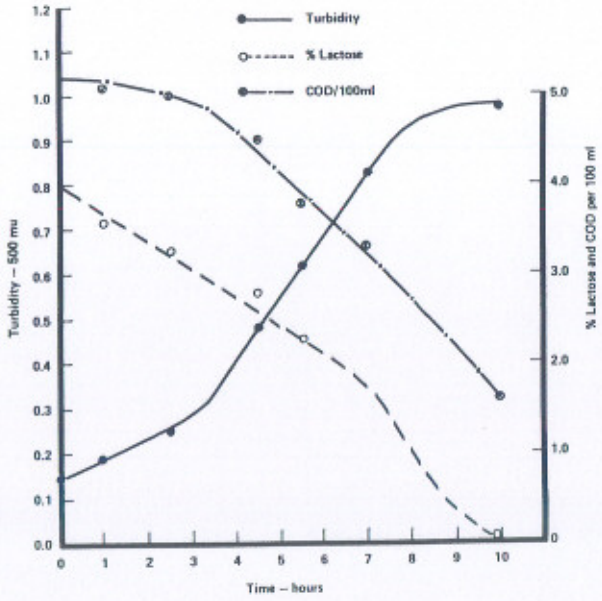

Figure 8. Growth of S. fragilis in whey with corresponding analysis of lactose and COD.

when the lactose was exhausted. This represented a reduction of more than 60 percent in COD. These data were representive of all 12 trials: *S fragilis* consistently exhausted the lactose from whey in 7-9 hours. During this time 0.3 to 0.4 grams of *S. fragilis* cells were produced in each 100 ml of whey. There was about 0.5 percent protein in the original whey, and the yeast used 20-40 percent of it; however, this probably represented the non-protein nitrogen in the whey, since *S. fragilis* cannot use nitrogen in the form of protein (19).

Theoretically, the carbon (from the lactose) in 100 ml of whey should have produced 2.5 grams of yeast cells. Yields of about 2.3 g/100 ml of whey, or 85 percent of this theoretical yield, have been reported (20). In an attempt to explain the apparent discrepancy between the literature and this study, trials were run using inorganic nitrogen, phosphate salts, and yeast extract, as recommended by the literature. These enrichments proved ineffective in the large batches just as they had in the 100-ml trials. In another trial, when 1 percent 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 1), but 0.7 percent of the protein was not used. 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., (20) 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 1, indicated possible increased yeast cell weights of 1.5 to 2.7 grams of cells per 100 ml if sufficient carbon and nitrogen were in the medium to support such growth. Thus, the results indicate that available nitrogen was the limiting factor in these yeast cell yields. Additional usable nitrogen probably would have resulted in greater increases in yeast cell weights, approaching the theoretical limit imposed by the amount of lactose.

Table 1. Growth of S. Fragilis in Whey[1] as Related to Lactose, Protein, and COD Analysis

Time	Turbidity	Cell Count	Lactose %	Protein %		Solids %		COD/ 100 ml
(Hours)	(Absorbance)	(X 10^6)	(Whey)	(Whey)	(Cells)	(Whey)	(Cells)	(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.

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 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.

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Heat Tapes—An Aid in Heat Detection

Milton Wells and Glenden Adams

Story in Brief

The use of artificial insemination continues to increase in both dairy and beef herds. It is estimated that approximately 30 percent of our dairy cattle and 2 percent of our beef cattle in Oklahoma were artfficially inseminated in 1971. It is expected that we will see increasing use of this beneficial technique in the next few years.