STORAGE OF *KLUYVEROMYCES FRAGILIS* YEAST CULTURES FOR USE AS "STARTERS" IN COTTAGE CHEESE WHEY FERMENTATIONS

By

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Thesis Approved:

Thesis Adviser

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CHAPTER I

INTRODUCTION

Waste disposal is a major problem facing food processors at this time. Restrictions have been imposed by the Environmental Protection Agency and local governments as to the amount of solid sewage that can be disposed of down the drain. Food wastes,such as cottage cheese whey, contain concentrated waste solids that can overload a sewage plant if the waste volume is large enough. The result can mean a disrupted sewage system and possible stream pollution.

Calculations from the latest standards set by the Environmental Protection Agency show that the dairy industry can only put 0.1% of the solids in the sewer that originally entered the plant (11). This is a serious problem to cheese plants that must dispose of whey containing 3-5% solids, mostly in the form of lactose. Because lactose cannot be completely digested and utilized by most domestic animals, whey has limited usages as animal feed. Excess whey is being used to feed hogs and chickens, but has little market value due to its great bulk and low food value.

Whey has also been dumped in rural fields, but this eventually causes odors and pollution problems. Pretreatment of whey to reduce the Biological Oxygen Demand (BOD) is possible, but requires expensive filtering equipment that costs about $500,000. This is a great financial burden to small dairy plants. Many larger plants have been forced

to install their own sewage treatment facilities, often in the form of open lagoons, which can cause odor problems in populated areas (5).

One method of dealing with the problem of cheese whey studied at Oklahoma State University by Knight et al. (6) has been the fermentation of whey with the yeast Kluyveromyces fragilis (K. fragilis), previously called Saccharomyces fragilis (7). This method has two major advantages over the previously mentioned processes. It can economically lower the BOD of whey by removing the material which causes it. In addition, it requires only equipment already available in most dairy plants. The yeast also produces protein, valuable on today's market, from the relatively cheap whey lactose.

Work at Oklahoma State University has shown that whey fermentation with K. fragilis can remove up to 99% of the whey's BOD within 24 h. This fermentation process is now being scaled up to pilot plant size, revealing handling problems not encountered in the laboratory. One problem encountered was the difficulty in storing large quantities of inocula (starters) for long periods of time without destroying cell viability. Even in small dairy plants, starter volume may routinely be as high as twenty or thirty gallons, making frequent transfers impractical. The previous laboratory method of acquiring starter was to start from pure culture slants and successively transfer the cells into larger volumes of growth media until the proper volume was reached. This technique took several days and required considerable amounts of equipment and man hours.

A number of different ways to store yeasts are described in the literature, but most of these studies applied to other yeasts and very little work had been done with the storage of K. fragilis. In the

author's opinion, three methods seemed to offer a reasonable chance of being successful with this organism and involved only equipment and material available in most commercial dairy plants. These were "progressive transfer", freezing, and refrigeration. Progressive transfer involved using yeast produced from one fermentation as the starter for the next one. Freezing was done in 50% sucrose syrup to reduce the formation of ice crystals which often rupture cell walls. Refrigeration in 50% sucrose syrup was the third method studied. The purpose of this study was to determine which of these methods would work best to preserve a viable yeast starter for fermentation on a commercial "plant size" batch of whey.

CHAPTER II

REVIEW OF LITERATURE

The process of whey fermentation with K. fragilis has been well documented with several recent reviews on the subject (6, 9, 10). However, the problem of storing K. fragilis for long periods of time, while still retaining cell viability, has not been extensively studied. Cook (1) reported work done by Will in 1909 and Meisser in 1911 that involved storage of yeasts in a 10% sucrose solution at various temperatures up to 20 C. After extended storage (up to eight years), cultures stored in this manner were alive but grew very slowly. Cook also reported on a process of yeast storage in 10% lactose developed by Owen in 1949 and a method of storage in specially prepared media used by the British National Collection of Yeast Cultures. Reed and Peppler (9) reported that brewer's yeast kept in water for ten days at 1-2 C would begin autolysis and lose viability.

Some research on "quick freezing" of yeasts with liquid nitrogen has been done in the last few years. Rose and Harrison (10) related unpublished work by Burrows in which yeast was mixed with ethanol and frozen in liquid nitrogen to recover a high yield of viable cells. Tsuji (12) used liquid nitrogen to freeze S. carlsbergensis in several different media. His best results were 90% recovery of live cells from yeast grown on trypticase- soy medium until the cells reached stationary growth phase and then frozen in a vitamin B_6 basal assay medium.

Doebbler and Rinfret (2) sprayed S. cerevisiae onto liquid nitrogen with a twenty-six gauge needle to freeze the cells. They thawed the cells by stirring them into a 0.15 M aqueous solution of NaCl and reported a survival rate of 42±7%. Wellman and Stewart (14) employed liquid nitrogen to freeze three types of brewer's yeasts in six different media. Ten percent glycerol was the best medium with nearly 100% recovery of live cells for all three yeasts.

Lyophilizing (freeze-drying) has been a popular food-drying method in recent years and a number of researchers have tried to utilize it in yeast storage. Rose and Harrison (10) freeze-dried five different yeast cultures and reported different survival rates for each species. S. cerevisiae endured best with a 25% survival rate after twelve months of storage. Haynes et al. (4) stated that the viability of lyophilized yeasts are usually satisfactory, with rare failures in the genus Saccharomyces. Cook (1) reported that lyophilizing usually killed a large percentage of the yeast cultures.

Using yeast cells from one fermentation as the inoculum for the next fermentation (progressive transfer) is a method used extensively in the brewing industry. According to Reed and Peppler (9), this procedure is limited by microbial contamination, loss of viability, decreased fermenting ability, and changes in the flocculent properties of brewing yeasts. These authors stated that brewing yeasts are rarely used for more than five to ten successive fermentations. Wasserman et al. (13) did some research in this area using K. fragilis. After culturing this yeast through several transfers on whey agar, the culture was divided equally into two different fermenting broths of whey, one of which contained 0.1% yeast extract. After fermenting six hours,

the whey with yeast extract produced nearly twice as many cells, had 30% more dry weight, and used 60% more lactose. This work suggests the possibility that whey does not have sufficient nutrients for subsequent fermentations by one yeast culture over long periods of time.

A few other methods of yeast preservation have been investigated with varying degrees of success. Griffon (3) reported good survival and storage properties of yeast combined with starch, then dehydrated to a moisture content of 10%. Cook (1) mentioned a technique involving storage of yeast cultures under liquid paraffin. This method requires ½ inch of good quality paraffin over the culture and storage temperature of 18-20 C to achieve satisfactory results.

CHAPTER III

EXPERIMENTAL PROCEDURES

Stock cultures of K. fragilis (NRRL-Y1156) used in this research were carried on lactose agar slants composed of 2.5% agar,2.0% lactose, 1.0% peptone, and 0.1% yeast extract. The stock culture was transferred every 2-4 wk to keep it viable.

The cottage cheese whey used for the growth tests had a pH of 4.7 ± 0.1. Before use, it was heated to 190 C to precipitate the protein and filtered to remove large protein particles. This filtered whey was stored at -16 C until used. The equipment used for starter preparation was the same as that used later for growth trials. When checked at the beginning of the experiment, electric air pumps delivered approximately 1 vol air/vol media/min through plastic dispersion tubes. Temperatures of 35 C were maintained throughout all yeast fermentations. "Antifoam A Emulsion" (Sigma Chemical Co.) was used to prevent excess foaming.

After reviewing the literature, three methods of starter storage were chosen for study in this experiment. These three all involved equipment and techniques readily available in dairy plants. Materials such as liquid nitrogen had to be eliminated because it was not always easily available to the commercial dairy and equipment for handling it was not always obtainable. Glycerol and similar materials were not considered because it was questionable as to whether their presence in

a food fermentation would be desirable from a flavor standpoint and allowable by the health authorities.

For the frozen and refrigerated storage experiments, six lactose agar slants were inoculated from the K. fragilis stock culture and incubated 24 h at 35 C (Figure 1). Cells from these slants were divided equally and washed into each of two 250 ml flasks of sterile lactose broth (4.0% lactose, 2.0% peptone, and 0.1% yeast extract). The flasks were then incubated and aerated for 12 h. After this, the lactose broth was centrifuged to remove the yeast cells, and the cells from each 250 ml flask of lactose broth were then added to a 750 ml flask of filtered cottage cheese whey. These flasks were incubated and aerated until the lactose percentage began decreasing rapidly (6 h). Whey from each flask was centrifuged, the yeast cells removed, and 2 g aliquots of cells distributed into each of ten centrifuge tubes which contained 2 g of 50% sucrose syrup. This made duplicate sets of ten starter tubes for each experiment. Frozen starter tubes were stored at -16 C and refrigerated starters at 3 C. Yeasts for the progressive transfer starters were prepared in a similar manner, except that only two centrifuge tubes of yeast starter were preserved and stored at 3 C between each fermentation (Figure 2).

Analysis of starter performance was the same for all three experiments. Growth phases were identified from a "model curve" (Figure 3). A "control" growth trial (0 wk) was analyzed before actual storage took place. Growth trials for the frozen and refrigerated starter samples were then repeated every 2 wk during each storage experiment. These growth trials involved inoculating 2 g of duplicate starters into each of two 250 ml flasks of whey and fermenting for 8 h. Samples of the

fermentation broth were taken every 2 h, and cell counts were determined for each sample by plating on YM agar (2.5% agar, 1% dextrose, 0.5% peptone, 0.3% malt extract, and 0.3% yeast extract) (Tables I-III). The growth of cells to be used for progressive transfer were stopped at the end of 5 h in order to store rapidly growing cells for the next fermentation. Since different times (5 and 8 h) were used for growth, comparisons of lactose utilization for the three methods of storage were made after 4 h of growth. The percentage of lactose used in the whey was measured using picric acid (8) and expressed in the tables as the % lactose used by the organisms after each sampling period (Tables IV - VI). Cell survival rates were calculated by comparing the number of yeast cells present at 0 h for each sampling period to the number of cells originally present at 0 h and 0 wk.

CHAPTER IV

RESULTS AND DISCUSSION

Centrifuging the fermented cottage cheese whey to remove 2 g of K. fragilis cells for starter resulted in a 96% decreased in volume. The control growth curves (0 wk) for all three experiments had a 2 h lag phase, a 3-4 h log growth phase, and reached stationary growth after 6 h (Figures 5, 8, and 11). Lactose utilization after 4 h varied greatly in the control growth trials for each experiment (Figures 6, 9, and 12). The total percentage of lactose used in 4 h was 34% for frozen starter, 20% for refrigerated starter, and 78% for progressive transfer starter for the 0 wk controls.

K. fragilis frozen 2-6 wk at -16 C had 59 to 45% as many live cells as the control (Figure 4). The growth curves during this period of storage were similar to that of the control, but resulted in less total cell numbers after 8 h of growth (Figure 5 and Table I). This was also accompanied by a decrease in lactose consumption with 4 h lactose percentages decreasing from 25% at 2 wk to 11% at 6 wk (Figure 6 and Table IV). From 8-16 wk, survival rates decreased more slowly, reaching 29% after 16 wk. Extended lag phases (3 h) and log growth phases (5 h) were noted in growth trials during this period of storage. Stationary phases were not attained. Average lactose consumption fell to slightly below 10% and appeared to be stabilizing from 8-16 wk. Growth curves of K. fragilis that survived freezing were not seriously affected.

The limiting factor in frozen storage appeared to be the extensive death of cells due to freezing (41%). Loss of viability during storage did occur, but was at a much slower rate from 2-16 wk than from 0-2 wk. Sucrose syrup was probably instrumental in preventing the death of cells due to freezing (9).

K. fragilis starter refrigerated at 3 C had the most drastic drop in growth performance over its 16 wk storage period. After 2 wk, the starter survival rate had dropped to 82% and continued to decrease throughout storage, reaching 1-2% after 16 wk (Figure 7 and Table II). From 2-6 wk, lag phases were 2 h, but the slopes of log growth phases decreased and lengthened to over 6 h (Figure 8). In addition, the percentage of lactose used fell to near 10% (Figure 9 and Table V). From 8-12 wk, survival rates began falling below 25% and growth curves showed 4 h lag phases and depressed log growth phases. The lactose utilized averaged about 8% during these growth trials. From 14-16 wk, growth during the 8 h trials was minimal, with continuous lag phases. However, lactose was still being used at the average rate of about 8% for 4 h of growth.

From this data, it would seem advisable not to store refrigerated K. fragilis more than 6 wk and preferably no more than 2 wk. The author believes that K. fragilis may lose much of its activity at 3 C because of an accumulation of lethal waste products during storage.

Progressive transfer appeared to be the best method of maintaining K. fragilis starters. With this technique, there were no major deviations from the control growth curve during the 14 wk storage period, although lower 0 h cell counts than that of the control were observed for succeeding fermentations (Figure 10 and Table III).

Progressive transfer of K. fragilis in cottage cheese whey resulted in an active growth curve for each growth trial, with 2 h lag phases and with log growth phases that lasted for the duration of the 5 h growth periods (Figure II and Table III). However, lactose consumption decreased with successive transfers, reaching a level of 23% utilization for 4 h of growth after 14 wk (Figure 12 and Table VI). With frequent transfers of starter, contamination is possible but was rarely encountered during this study. This danger is minimal using cottage cheese whey media, since few organisms can thrive in the high acidity and lactose rich environment characteristic of cottage cheese whey. The few bacteria that can live in this environment usually convert lactose to lactic acid, which is utilized by K. fragilis. In addition, the contaminating bacteria usually have a long lag phase and an aversion to aeration, making it difficult for them to compete with an active K. fragilis culture under fermentation conditions.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of these experiments was to find economical methods of storing concentrated K. fragilis yeasts without impairing their performance as starters in whey fermentations. Two grams of active K. fragilis cells were centrifuged from the fermentation media (whey) after 4-6 h of growth, then diluted with 50% sucrose syrup. The volume of starter to be stored was reduced to 4% of its original volume. Three methods of storing K. fragilis concentrates were evaluated: freezing (-16 C), refrigeration (3 C), and removing cells from one fermentation to be used in the succeeding one ("progressive transfer").

Growth of the yeast cells stored by progressive transfer were the most active throughout the trial. Freezing the K. fragilis starters was satisfactory, but some cells were destroyed by freezing, so the starters' growth was slower than that of the progressive transfer method. Refrigerated starter showed marked decreases both in cell numbers and growth performance as storage time progressed. After 8 wk of storage, its growth was retarded to the point that this culture was unfit for use as a whey starter. However, refrigeration for a short time (up to 2 wk) had little effect on the culture's initial cell count and growth characteristics. From this study, it appeared that progressive transfer and freezing in sucrose syrup were satisfactory methods of storing a concentrated K. fragilis starter for 14-16 wk.

LITERATURE CITED

1. Cook, A. H. 1958. The Chemistry and Biology of Yeasts. Academic Press. N.Y.

2. Doebbler, G.F., and A.P. Rinfret. 1963. Survival of microorganisms after ultrarapid freezing and thawing. J. Bacteriol. 85:485.

3. Griffon, H. 1969. Method for producing active dried yeast. West German Patent Application 1-292-608. Abstract from Food Sci. and Technol. Abstracts. 2:919.

4. Haynes, W.C., L. J. Wickerham, and C.W. Hesseltine. 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. 3:361.

5. Knight, S., W. Smith, and J.B. Mickle. 1972 . Cheese whey disposal using Saccharomyces fragilis yeast. Cult. Dairy Prod. J. 17 (2):17.

6. Knight, S. 1969. Lactose removal from cheese whey using Saccharomyces fragilis yeast. M.S. Thesis. Oklahoma State University, Stillwater.

7. Lodder, J. and N.J. W. Kreger-Van Rij. 1970. The Yeasts: A Taxonomic Study. 2nd ed. John Wiley and Sons. N.Y.

8. Perry, N.A., and F.J. Doan. 1950. A picric acid method for the simultaneous determination of lactose and sucrose in dairy products. J. Dairy Sci. 33:176.

9. Reed, G., and H.J. Peppler. 1973. Yeast Technology. Avi Publishing Co. Westport, Conn.

10. Rose, A.H., and J.S. Harrison. 1969. The Yeasts. Vol. 3 Academic Press. N.Y.

11. Train, R.E., R.L. Sansom, A. Cywin, and R. Gregg. 1974. Development Document for Proposed Effluent Limitations Guidelines and New Source of Performance Standards for the Dairy Product Processing Points Source Category. U.S. Environmental Protection Agency. Washington, D.C.

12. Tsuji, K. 1966. Liquid nitrogen preservation of Saccharomyces carlsbergensis and its use in a rapid biological assay of vitamin B_6 (pyridoxine). Appl. Microbiol. 14:456.

13. Wasserman, A.E., W.J. Hopkins, and N. Porges. 1958. Whey utilization: Growth conditions for Saccharomyces fragilis. Sewage and Industrial Wastes. 30:913.

14. Wellman, A.M., and G. G. Stewart. 1973. Storage of brewing yeasts by liquid nitrogen refrigeration. Appl. Microbiol. 26:577.

APPENDIX

TABLE I

CELL COUNTS[a] OF K. FRAGILIS STARTER SAMPLES STORED AT -16 C FOR 16 WEEKS AND GROWN IN COTTAGE CHEESE WHEY FOR EIGHT HOURS EVERY TWO WEEKS[b]

	Weeks of Starter Storage [c]															
Hours of Growth	0		2		4		6		8		10		12		14		16	
0	86	91	53	52	59	50	42	37	49	44	28	21	29	31	23	22	27	24
2	129	122	86	90	99	66	42	40	47	34	33	36	33	73	34	28	33	37
4	247	220	183	185	154	150	100	107	135	133	108	77	75	89	90	73	64	88
6	297	305	219	222	314	294	179	160	256	245	223	179	172	171	189	171	150	201
8	314	317	255	252	288	303	200	265	291	297	265	272	221	255	209	228	217	241

[a]From YM agar pour plates.

[b]Inoculation concentration = 2 g starter/250 ml whey.

[c]Results are reported in duplicate as millions of yeasts/ml.

TABLE II

CELL COUNTS[a] OF *K. FRAGILIS* STARTER SAMPLES STORED AT 3 C FOR 16 WEEKS AND GROWN IN COTTAGE CHEESE WHEY FOR EIGHT HOURS EVERY TWO WEEKS[b]

Hours of Growth	Weeks of Starter Storage[c]															
	0		2		4		6		8		10		12		14		16	
0	75	55	52	54	37	27	44	49	20	12	10	12	8	2	<1	2	<1	<1
2	85	59	61	74	50	30	70	64	24	16	11	10	8	7	<1	2	<1	<1
4	186	179	154	146	120	92	117	95	34	78	15	18	15	13	<1	2	<1	<1
6	341	-	205	186	221	148	177	133	110	94	46	78	60	26	6	7	<1	5
8	345	347	292	252	299	218	288	223	208	202	124	145	118	76	7	15	2	26

[a]From YM agar pour plates.

[b]Inoculation concentration = 2 g starter/250 ml whey.

[c]Results are reported in duplicate as millions of yeasts/ml. When no colonies were observed on plate, it was reported as <1.

TABLE III

CELL COUNTS [a] OF *K. FRAGILIS* STARTER SAMPLES PROGRESSIVELY TRANSFERRED FOR 14 WEEKS AND GROWN IN COTTAGE CHEESE WHEY EVERY TWO WEEKS[b]

	WEEKS OF PROGRESSIVE TRANSFER[c]							
Hours of Growth	0	2	4	6	8	10	12	14
0	114	86 97	37 90	64 56	80 64	51 34	79 88	80 78
2	109	100 99	99 89	74 71	89 70	46 44	111 107	82 93
4	180	160 170	204 149	- 152	221 241	105 114	202 219	182 180
5	228	- 208	241 177	210 -	256 257	190 242	281 247	227 235

[a] From YM agar pour plates.

[b] Inoculation concentration = 2 g starter/250 ml whey.

[c] Results are reported in duplicate as millions of yeasts/ml.

TABLE IV

LACTOSE UTILIZATION[a] OF FROZEN K. FRAGILIS STARTERS IN COTTAGE CHEESE WHEY AFTER FOUR HOURS OF GROWTH IN THE WHEY[b]

Weeks of Storage	Lactose % in Media: Growth Time 0 h	Lactose % in Media: Growth Time 4 h	Difference	Average % Utilization
0	3.4	2.3	1.1	34
	3.5	2.2		
2	3.3	2.6	0.7	25
	3.4	2.5	0.9	
4	3.0	2.6	0.4	11
	3.4	3.1	0.3	
6	3.0	2.7	0.3	11
	3.5	3.2	0.3	
8	2.9	2.6	0.3	7
	2.9	2.8	0.1	
10	3.3	2.1	0.2	4
	3.2	3.1	0.1	
12	3.6	3.2	0.4	11
	3.4	3.1	0.3	
14	3.4	3.0	0.4	14
	3.4	2.9	0.5	
16	3.6	-	-	11
	4.0	3.5	0.5	

[a]Duplicate lactose determination with picric acid test (8).

[b]Inoculation concentration = 2 g starter/250 ml whey.

TABLE V

LACTOSE UTILIZATION[a] OF REFRIGERATED K. FRAGILIS STARTERS IN COTTAGE CHEESE WHEY AFTER FOUR HOURS OF GROWTH IN THE WHEY[b]

Weeks of Storage	Lactose % in Media: Growth Time 0 h	Lactose % in Media: Growth Time 4 h	Difference	Average % Utilization
0	3.3	-	-	20
	5.3	4.2	1.1	
2	2.9	2.3	0.6	21
	3.0	2.4	0.6	
4	3.5	3.1	0.4	7
	2.9	2.8	0.1	
6	2.7	2.2	0.5	13
	2.9	2.6	0.3	
8	3.7	-	-	4
	3.9	3.8	0.1	
10	3.4	3.0	0.4	14
	3.4	2.9	0.5	
12	3.1	2.9	0.2	7
	3.1	2.9	0.2	
14	3.6	-	-	14
	3.5	3.0	0.5	
16	3.7	3.6	0.1	3
	3.6	-	-	

[a] Duplicate lactose determination with picric acid test (8).

[b] Inoculation concentration = 2 g starter/250 ml whey.

TABLE VI

LACTOSE UTILIZATION[a] OF PROGRESSIVELY TRANSFERRED *K. FRAGILIS* STARTERS IN COTTAGE CHEESE WHEY AFTER FOUR HOURS OF GROWTH IN THE WHEY[b]

Weeks of Storage	Lactose % in Media: Growth Time 0 h	Lactose % in Media: Growth Time 4 h	Difference	Average % Utilization
0	2.6	0.6	2.0	78
2	2.5	1.2	1.3	49
	2.4	1.3	1.1	
4	4.2	2.9	1.3	35
	4.4	2.6	1.8	
6	3.4	2.2	1.2	39
	3.3	1.9	1.4	
8	2.7	1.6	1.1	43
	2.8	1.5	1.3	
10	3.1	2.8	0.3	9
	3.0	2.8	0.2	
12	3.1	2.4	0.7	29
	3.2	2.1	1.1	
14	2.7	2.3	0.4	23
	3.0	2.1	0.9	

[a] Duplicate lactose determination with picric acid test (8).

[b] Inoculation concentration = 2 g starter/250 ml whey.

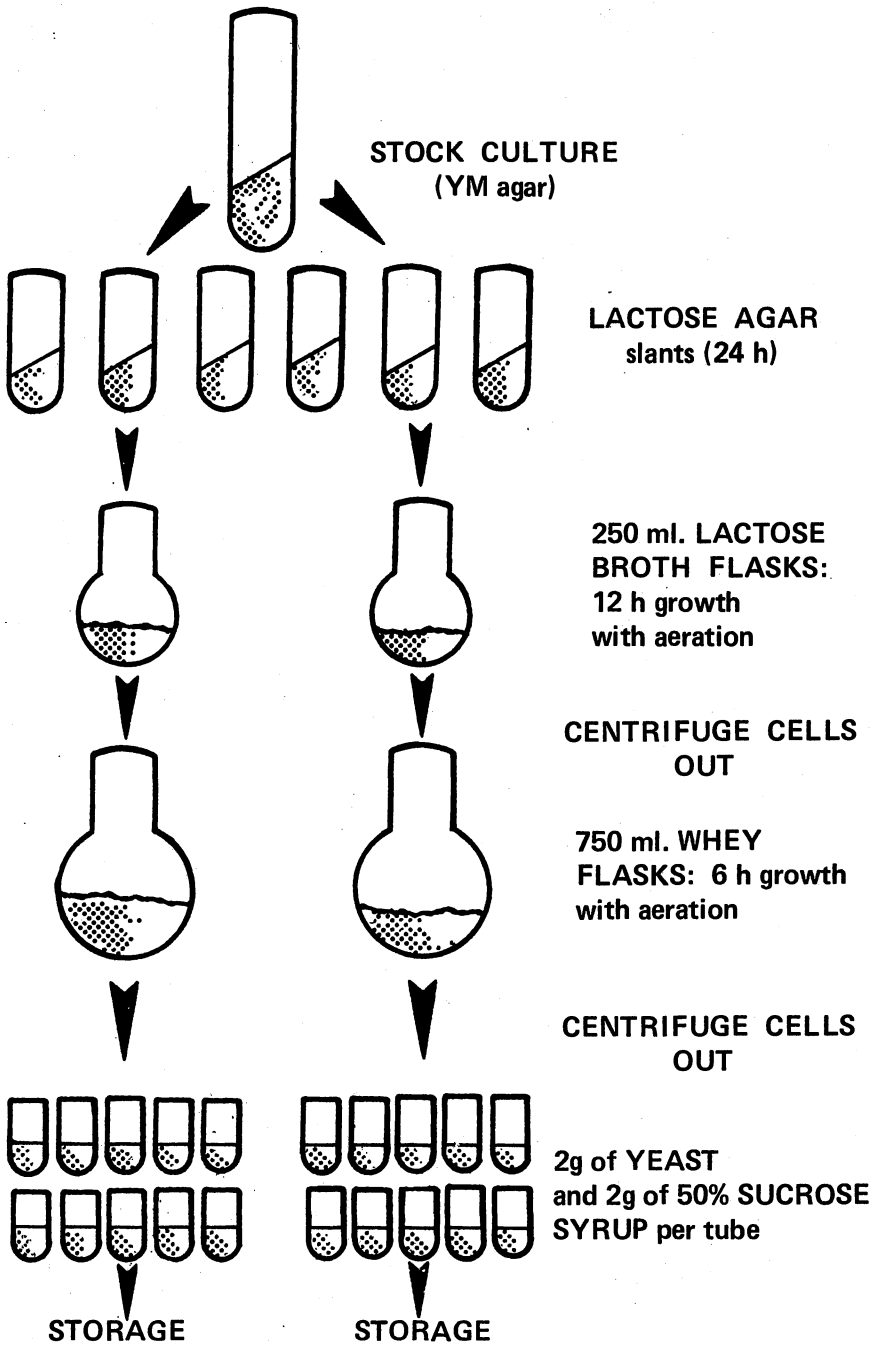

Figure 1. Method of Preparing K. fragilis Starters for Frozen and Refrigerated Storage

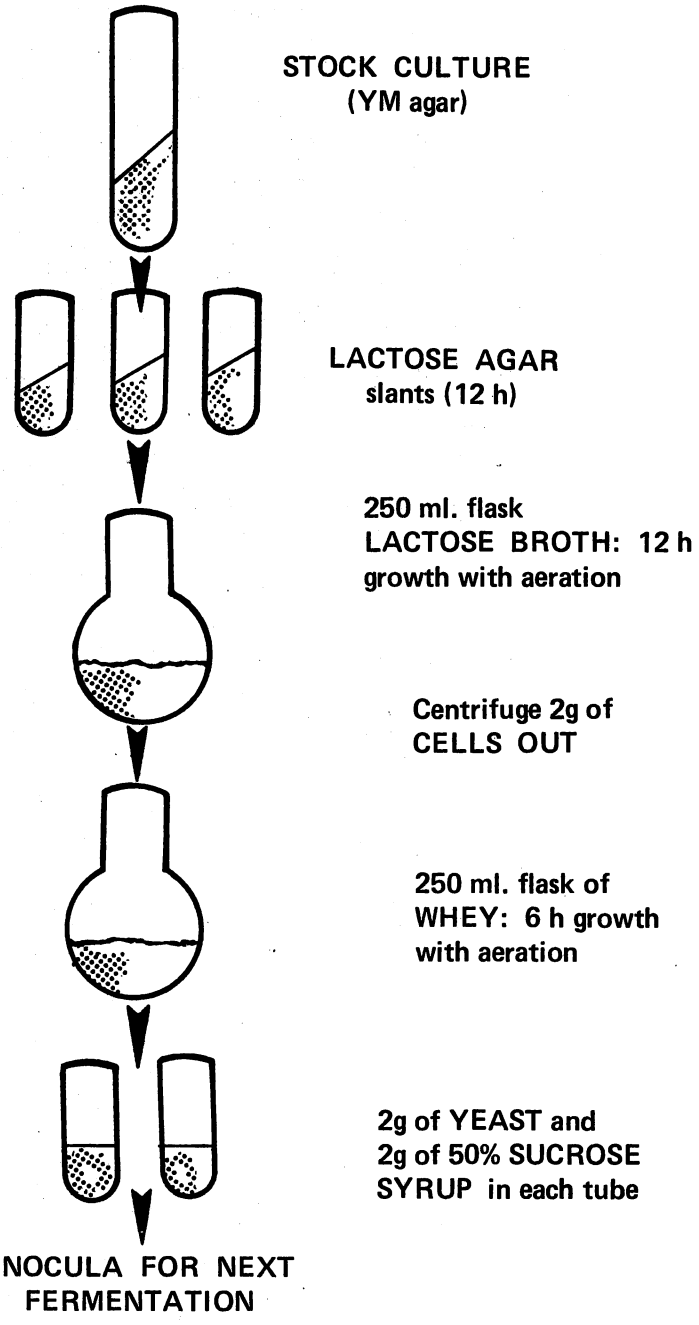

Figure 2. Method of Preparing K. fragilis Starters for Progressive Transfer

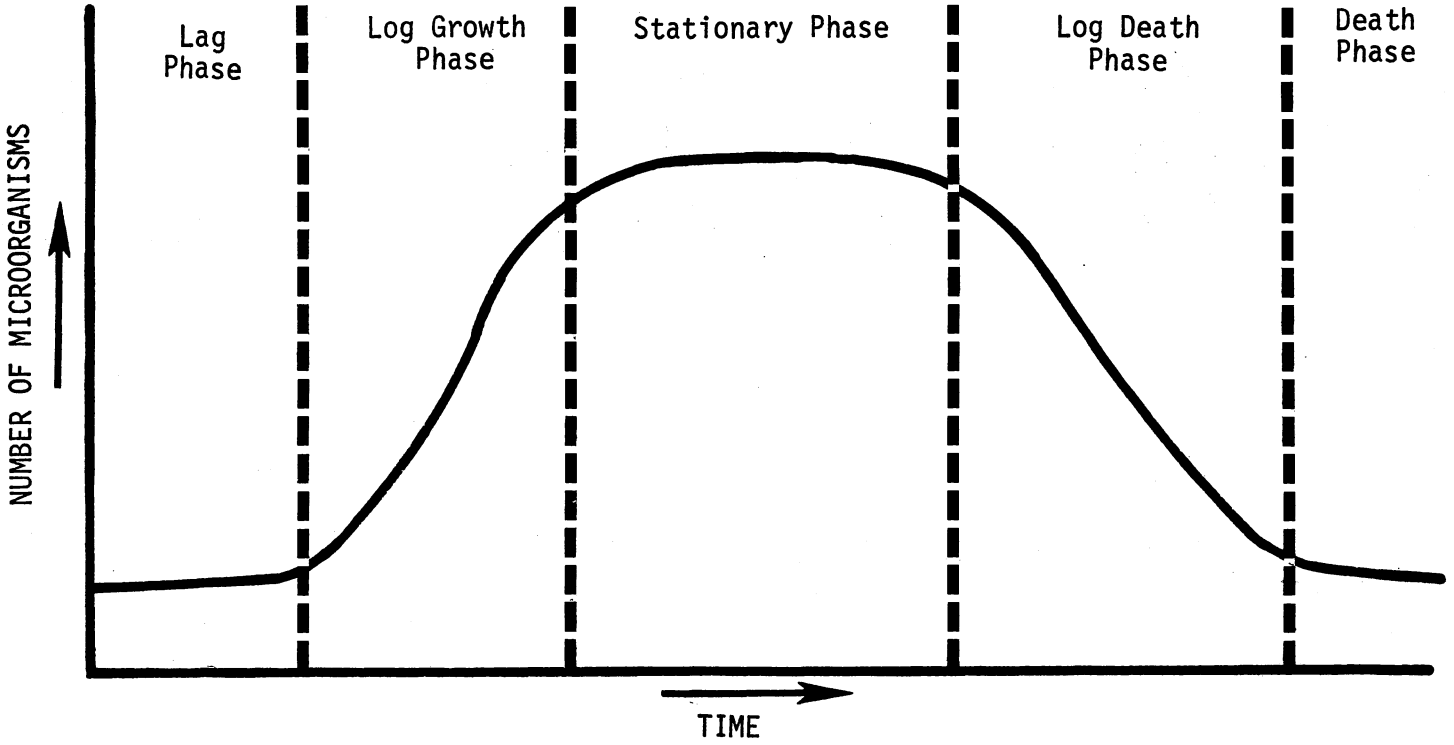

Figure 3. Model Growth Curve of Microorganisms

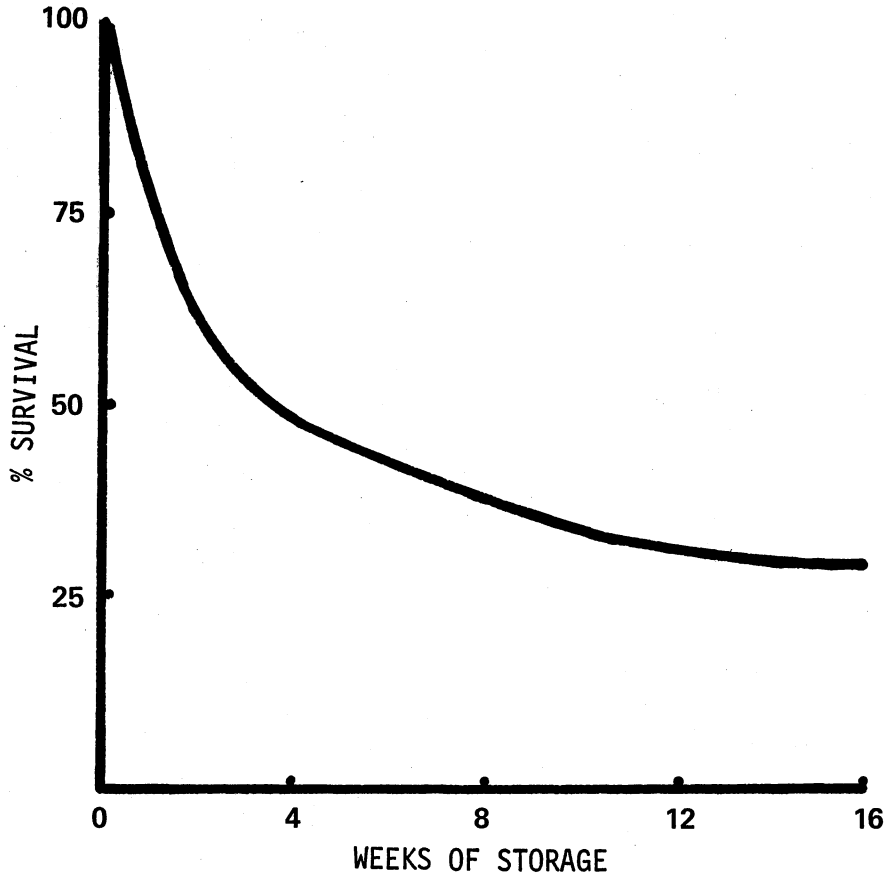

Figure 4. Percent Survival of Frozen K. fragilis in 50% Sucrose Syrup over a 16 wk Storage Period

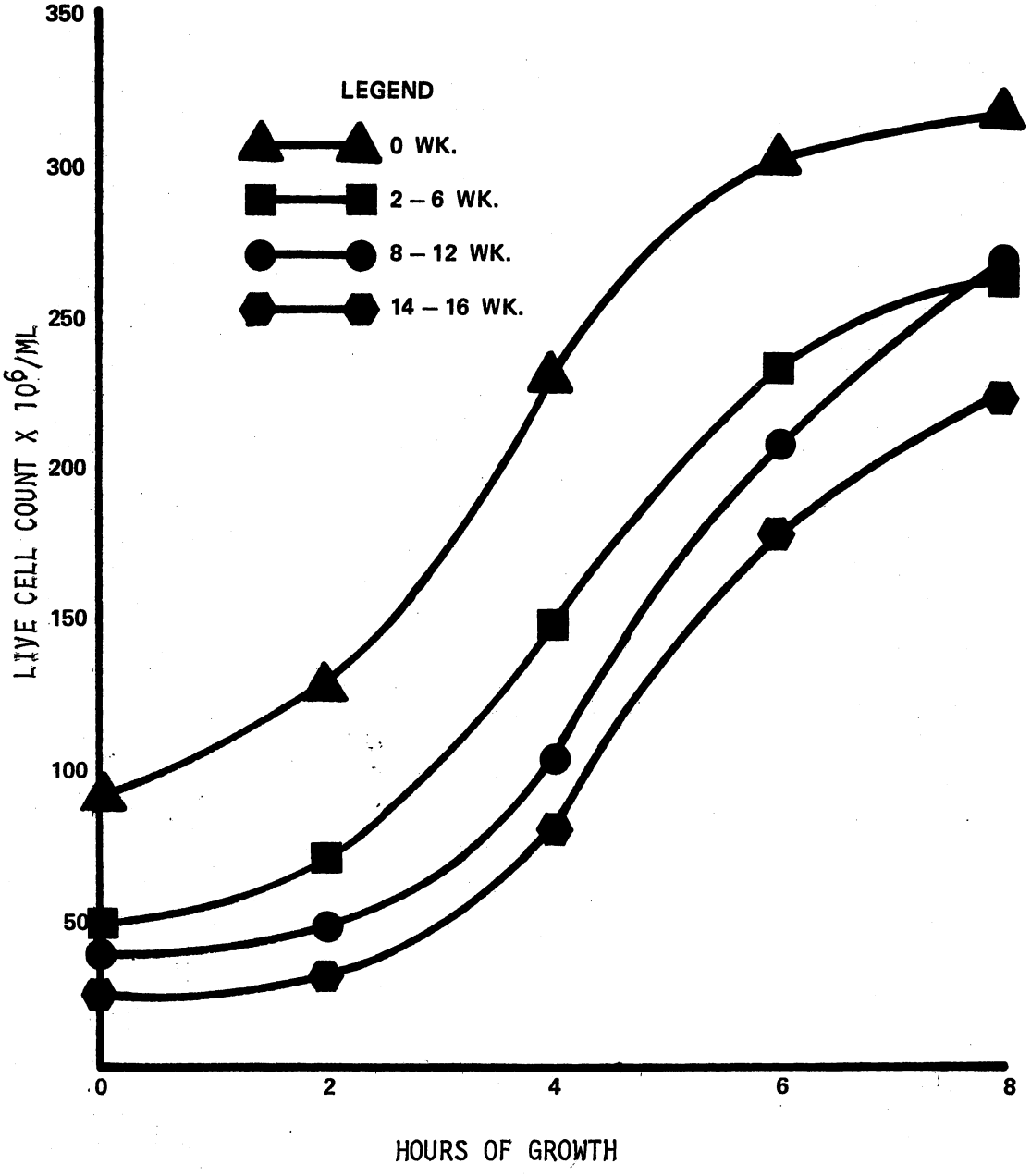

Figure 5. Growth Curves of Frozen K. fragilis Starters Inoculated at the Rate of 2g Starter/250 ml Whey

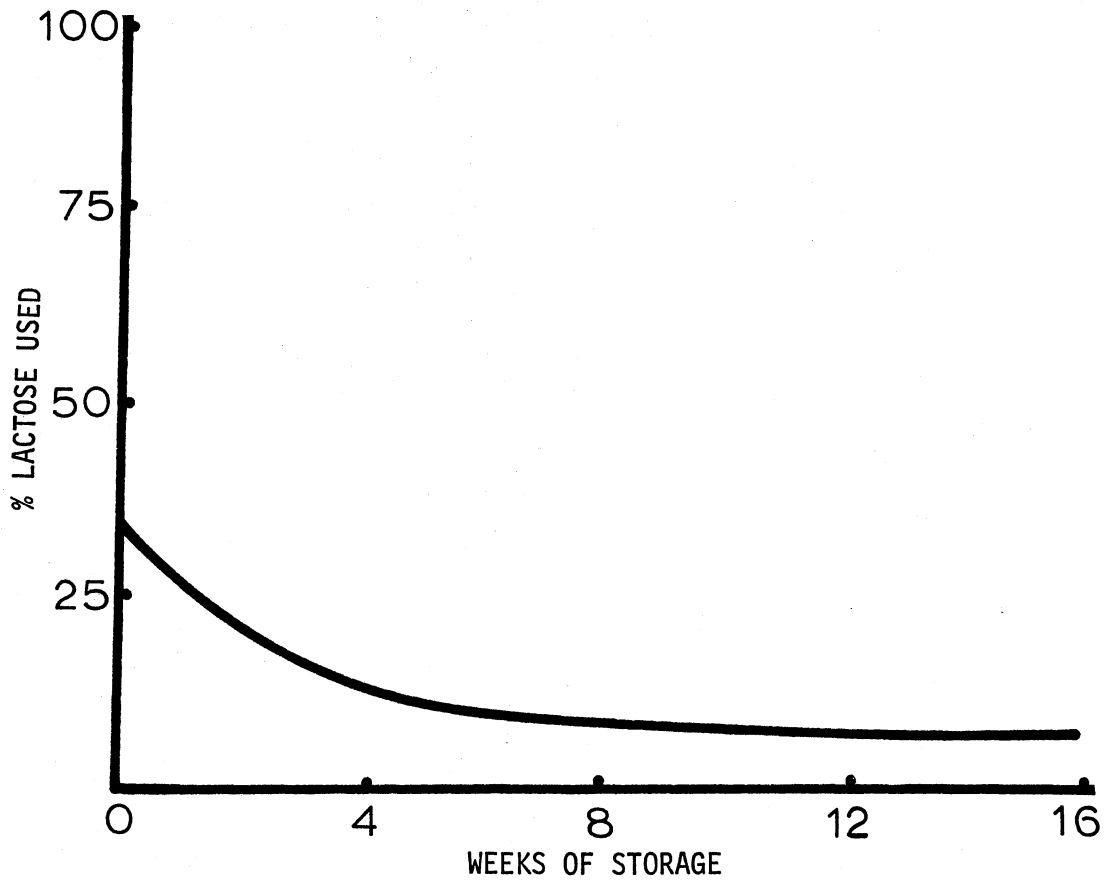

Figure 6. Percentage of Lactose in Whey Used after 4 h Fermentation by Frozen K. fragilis Starters

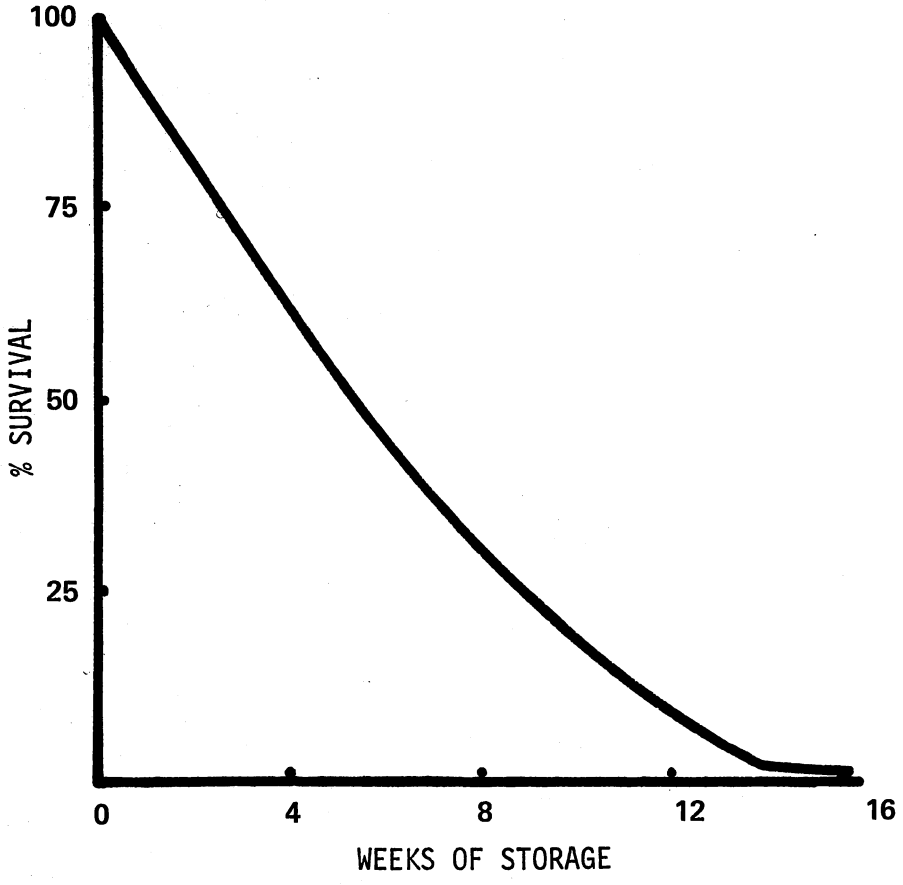

Figure 7. Percent Survival of Refrigerated *K. fragilis* in 50% Sucrose Syrup over a 16 wk Period

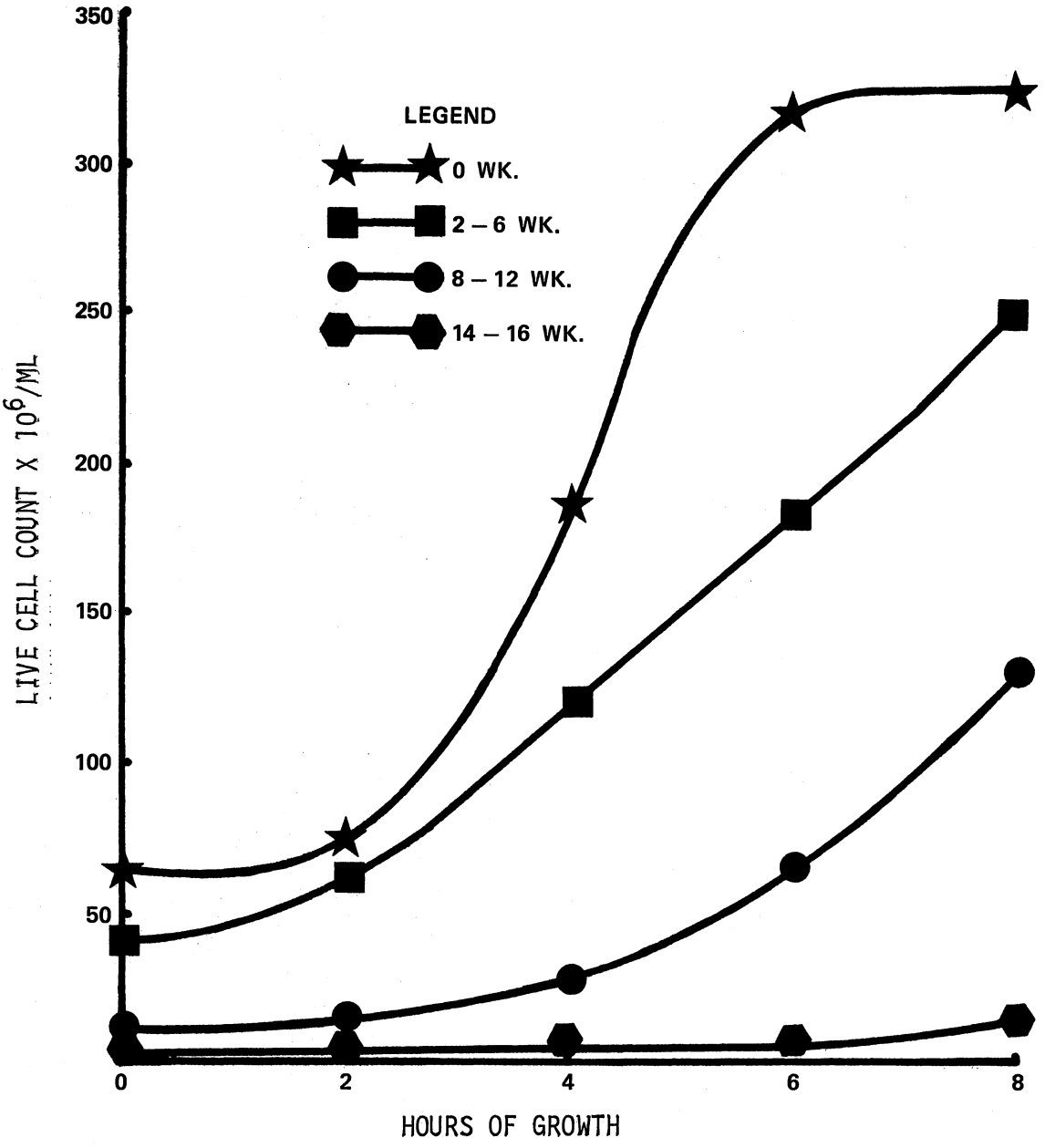

Figure 8. Growth Curves of Refrigerated K. fragilis Starters Inoculated at the Rate of 2g Starter/250 ml Whey

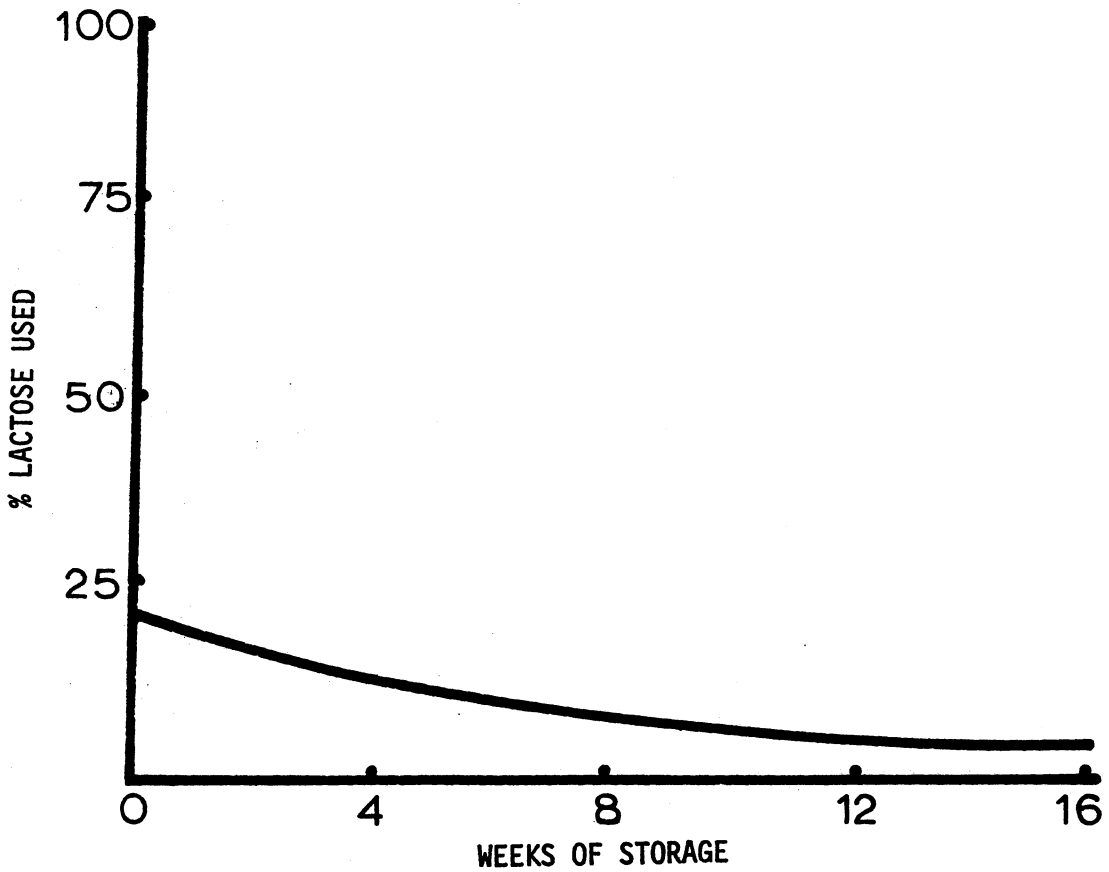

Figure 9. Percentage of Lactose in Whey Used after 4 h Fermentation by Refrigerated K. fragilis Starters

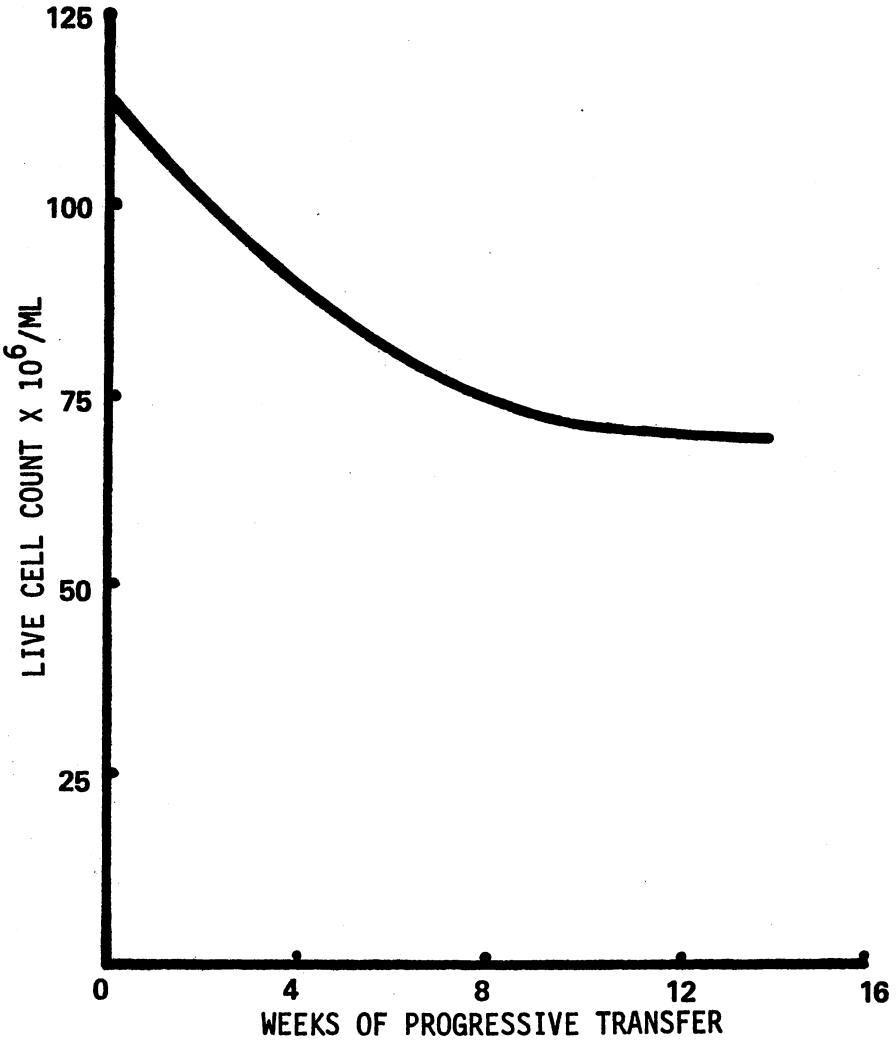

Figure 10. Concentration of Live K. fragilis in the Fermentation Media at the Beginning of each Fermentation over a 14 wk Period of Progressive Transfer

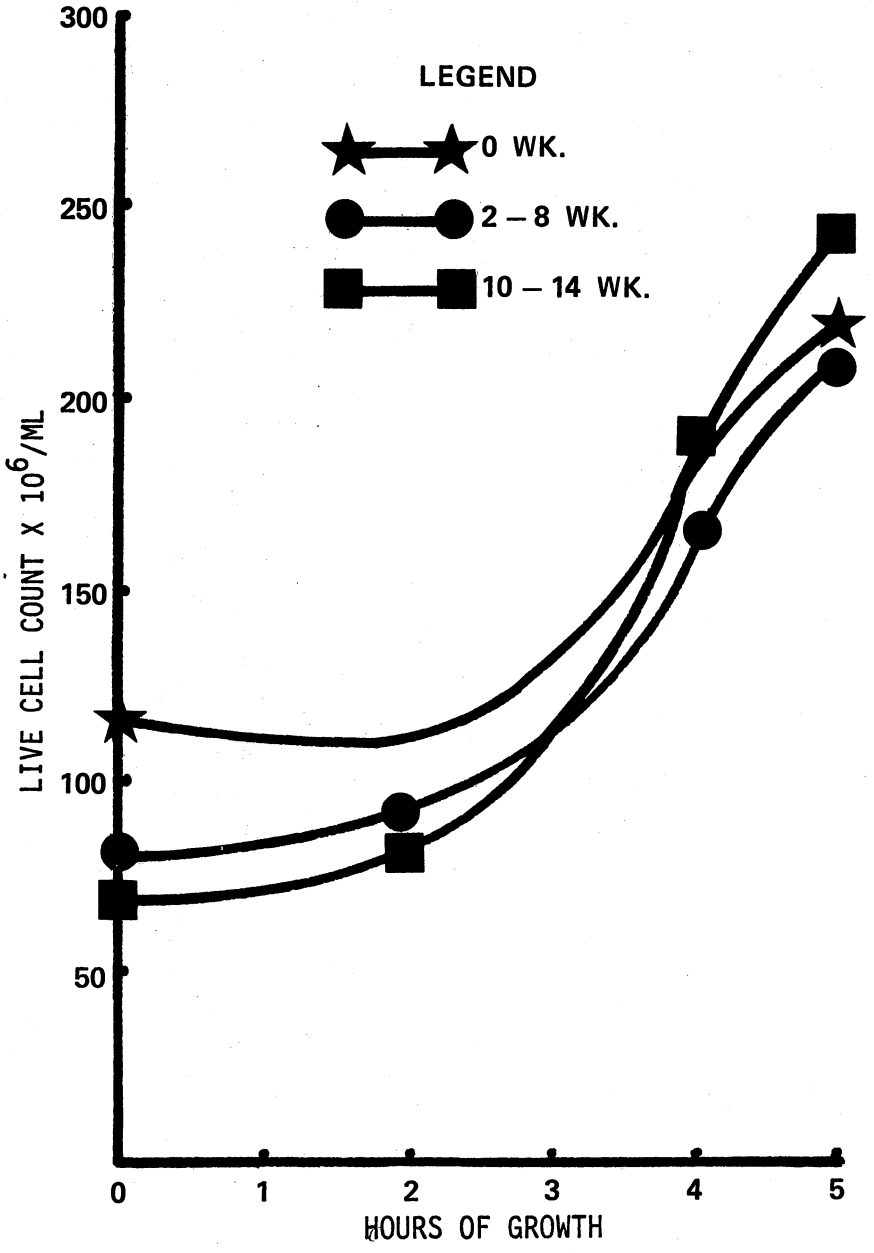

Figure 11. Growth Curves of Progressively Transferred K. fragilis Starters Inoculated at the Rate of 2g Starter/250 ml Whey

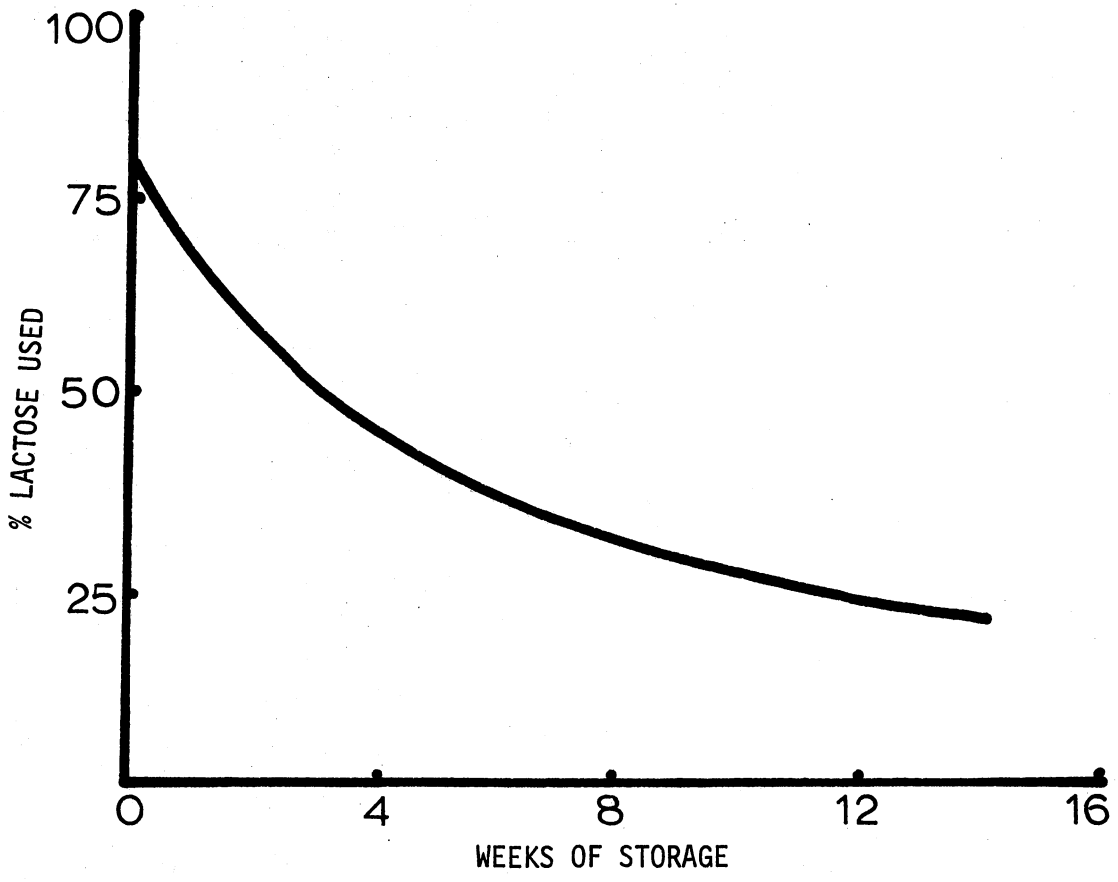

Figure 12. Percentage of Lactose in Whey after 4 h Fermentation by Progressively Transferred K. fragilis Starters

VITA

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Master of Science

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