PROGRESS REPORT

to the

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

from

BIOENGINEERING RESEARCH FACILITY
Louisiana State University
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BACKGROUND INFORMATION

The LSU/MTF Bioengineering Research Facility has, for the past three years, been involved in the development of a process (and ancillary processing and analytical techniques) to produce bacterial single-cell protein of good nutritional quality from waste cellulose. A fermentation pilot plant and laboratory were developed and have been in operation for about two years. During this time we have proven that single-cell protein (SCP) could be produced from sugarcane bagasse--a typical agricultural cellulosic waste. Our efforts during the past year have been directed at the optimization and understanding of this process and its controlling variables. Both batch and continuous fermentation runs have been made under controlled conditions in the 535 liter pilot plant vessel and in the laboratory 14-liter fermenters.

To facilitate the optimization of this process, certain 'evaluation parameters' have been chosen to provide quantitative measurement of progress. The first six (of the eight) parameters are the critical dependent variables which control the last two--product cost and product quality.

These evaluation parameters are presented in Table 1. The explanations for the choice of these parameters is given in Appendix A. Table 2 is an evaluation of the status of each parameter compared to the goals set for the economical operation of the process. It can be seen that all goals have been achieved except for 'Continuous Productivity'. This value represents the experimentally verified productivity of the fermentation unit in continuous flow operation and is, in essence, the main evaluation parameter for economical operation of the progress. When this parameter

TABLE 1

PROJECT OBJECTIVE	EVALUATION PARAMETERS
TO DEVELOP A PROCESS TO ECONOMICALLY PRODUCE SINGLE-CELL PROTEIN OF GOOD NUTRITIONAL QUALITY FROM WASTE CELLULOSICS (SUGARCANE BAGASSE)	1. BATCH CELL CONCENTRATION AT END OF LOG PHASE GROWTH
	2. GROWTH RATE ON BAGASSE
	3. PRODUCTIVITY CALCULATED FROM BATCH CULTURE
	4. CELL YIELD ON SUBSTRATE
	5. SUBSTRATE UTILIZATION EFFICIENCY AT END OF LOG PHASE GROWTH
	6. CONTINUOUS PRODUCTIVITY
	7. PRODUCT QUALITY
	8. PRODUCT COST

TABLE 2

PROJECT EVALUATION CHART

EVALUATION PARAMETER	GOAL	CURRENT BEST LEVEL	CURRENT LEVEL GENERALLY ATTAINABLE		FURTHER WORK PLANNED
1. BATCH CELL CONCENTRATION IN L.P.G.	>10 g/l	10 g/l	4→6 g/l	YES	GAIN CONSISTENCY BY STANDARDIZATION
2. GROWTH RATE ON BAGASSE	>0.17 $hrs.^{-1}$	0.23 $hrs.^{-1}$	0.14 - 0.19 $hrs.^{-1}$	NO	
3. PRODUCTIVITY CALCULATED FROM BATCH	1.5 g/l·hr.	1.45 g/l·hr.	0.6 - 0.8 g/l·hr.	YES	(AS IN NO. 1)
4. CELL YIELD ON SUBSTRATE	0.5 gm/gm	0.5 gm/gm	0.4 - 0.5 gm/gm	NO	
5. SUBSTRATE UTILIZATION EFFICIENCY IN L.P.G.	>0.75	0.77	0.5 - 0.6	YES	STANDARDIZE BAGASSE TREATMENT METHODS
6. CONTINUOUS PRODUCTIVITY	1.5 g/l·hr.	0.43 g/l·hr.	INSUFFICIENT DATA	YES	IMPROVE OPERATIONAL METHODS AND UNDERSTANDING OF CULTURE DYNAMICS
7. PRODUCT QUALITY	50% PROTEIN	70% PROTEIN	50% - 60% PROTEIN	NO	
8. PRODUCT COST	10 - 14¢ LB.	-----	-----	YES	TO BE DEFINED AFTER SUCCESS WITH NO. 6

is improved to the level of its goal (1.5 grams of dry cells produced per liter of fermenter capacity per hour), the process will be considered functional.

Appendix B includes evaluation charts for each of the parameters and graphical presentations wherever appropriate. The definition of the effects of each controlling variable is evaluated for each parameter, and a systematic program of work to be done is presented.

CURRENT RESEARCH AND RESULTS

In addition to the pilot plant (535 liter) and laboratory (14 liter) cellulose fermentations that have been carried out, several pertinent problems were investigated to define the mechanisms and kinetics of the catabolic fermentation processes.

GROWTH EFFECTS OF TREATED BAGASSE SOLIDS AND SOLUBLES ON A MIXED CULTURE OF CELLULOMONAS AND ALCALIGENES[1]

The purpose of this set of experiments was to differentiate the growth of Cellulomonas and Alcaligenes on a substrate composed of solid and soluble fractions. The substrate is alkali-treated bagasse; the solid fraction is composed of that fraction which is insoluble after alkali treatment, whereas the soluble fraction is composed of that fraction which remains soluble after alkali treatment and subsequent neutralization.

It has been hypothesized that the Cellulomonas, which possesses a β-Glucanase enzyme, grows predominantly on the solid fraction and forms acidic products. Alcaligenes, on the other hand, possesses a β-Glucosidase enzyme and grows predominantly on the soluble fraction and forms basic products.

It is the aim of these experiments to prove the above hypothesis qualitatively and quantitatively. As an extension of this, it is desired to relate these results from batch processes to continuous cultures.

Procedure

The substrate used in this experiment was sugarcane bagasse, which was treated with 4% NaOH and heated at 170 F for one hour. This material was then blended in a Waring blender and filtered through a layer of "Birdseye". The material which passed through the "Birdseye" was collected and neutralized with HCl and shall henceforth be referred to as "soluble fraction". The soluble fraction was composed of 7.41 grams/liter of NaCl and 13.04 grams/liter of solubles other than NaCl. The DNS test at a 20:1 dilution yielded 1.70 grams/liter of reducing sugars, and the Phenol-Sulfuric Acid test at a 125:1 dilution yielded 4.125 grams/liter of soluble carbohydrates. The material which was retained in the "Birdseye" shall be called the "solid fraction". The solid fraction was washed repeatedly to insure all the solubles and salt had been washed away. The solid fraction had a moisture content of 79.3%. Both the solid fraction and the soluble fraction were stored at about 35 F until needed.

The first experiment was run using the above substrates, but merely mixing the relative proportions of the solid fraction and the soluble fraction. All runs were made in 500 ml. side-arm flasks which had a total of 250 ml. of media, and which had a total solid fraction plus soluble fraction concentration of 10.0 grams/liter. The substrate loading and the media for the first run is as follows:

Run No. 1

Flask No.	1,2	3,4	5,6	7,8	9,10
Solid fraction (gms/l)	10.0	9.0	8.0	7.0	6.0
Soluble fraction (gms/l) except NaCl	0.0	1.0	2.0	3.0	4.0
$(NH_4)_2SO_4$ (gms/l)	6.0	6.0	6.0	6.0	6.0
$MgSO_4 \cdot 7H_2O$ (gms/l)	0.4	0.4	0.4	0.4	0.4
$CaCl_2$ (gms/l)	0.1	0.1	0.1	0.1	0.1
NaCl (gms/l)	1.4	1.4	1.4	1.4	1.4
K_2HPO_4 (gms/l)	1.0	1.0	1.0	1.0	1.0
KH_2PO_4 (gms/l)	1.0	1.0	1.0	1.0	1.0
Yeast Extract (gms/l)	0.1	0.1	0.1	0.1	0.1
Trace Minerals (ml/l)	1.0	1.0	1.0	1.0	1.0

The flasks were then sterilized and inoculated with a mixed culture of Cellulomonas and Alcaligenes; however, the cells were spun down from the inoculum and resuspended in sterile water. The flasks were then inoculated with approximately the same quantity of both Cellulomonas and Alcaligenes. The second experiment will now be described, and the results will all be analyzed at the same time. The second experiment was the same as the first except that the solid and soluble concentrations were varied.

Run No. 2

Flask No.	1,2	3,4	5,6	7,8	9,10
Solid fraction (gms/1)	0.0	2.5	5.0	7.5	10.0
Soluble fraction (gms/1) except NaCl	10.0	7.5	5.0	2.5	0.0
$(NH_4)_2SO_4$ (gms/1)	6.0	6.0	6.0	6.0	6.0
$MgSO_4 \cdot 7H_2O$ (gms/1)	0.4	0.4	0.4	0.4	0.4
$CaCl_2$ (gms/1)	0.1	0.1	0.1	0.1	0.1
NaCl (gms/1)	5.5	5.5	5.5	5.5	5.5
K_2HPO_4 (gms/1)	4.45	4.45	4.45	4.45	4.45
KH_2PO_4 (gms/1)	3.40	3.40	3.40	3.40	3.40
Yeast Extract (gms/1)	1.0	1.0	1.0	1.0	1.0
Trace Minerals (ml/1)	1.0	1.0	1.0	1.0	1.0

The flasks were sterilized and inoculated in the same manner as the first run of this experiment.

An important tool in the differentiation of the two cell populations is the "Differential Plate Count" or the "Viable Plate Count". By performing a plate count with the organisms at a proper dilution, one can determine the number of viable or living organisms of each species.

Two types of agar are used in the preparation of the Petri dishes or plates. Cellobiose agar is specific for Alcaligenes; therefore, it gives the number of viable Alcaligenes at any given time during the fermentation.

Nutrient Broth Agar gives a total of the Alcaligenes and Cellulomonas which are viable at any given time. Therefore, the number of Cellulomonas can be determined by difference between the Nutrient Broth Agar and the Cellobiose Agar. The procedure for preparation of the agar and the plating technique is:

Nutrient Broth Agar -	Nutrient Broth	8.0 gms/1
/	Plain Agar	18.0 gms/1
	Yeast Extract	1.0 gms/1
Cellobiose Agar -	Cellobiose	1.0 gms/1
	Plain Agar	18.0 gms/1
	Nutrients	1N

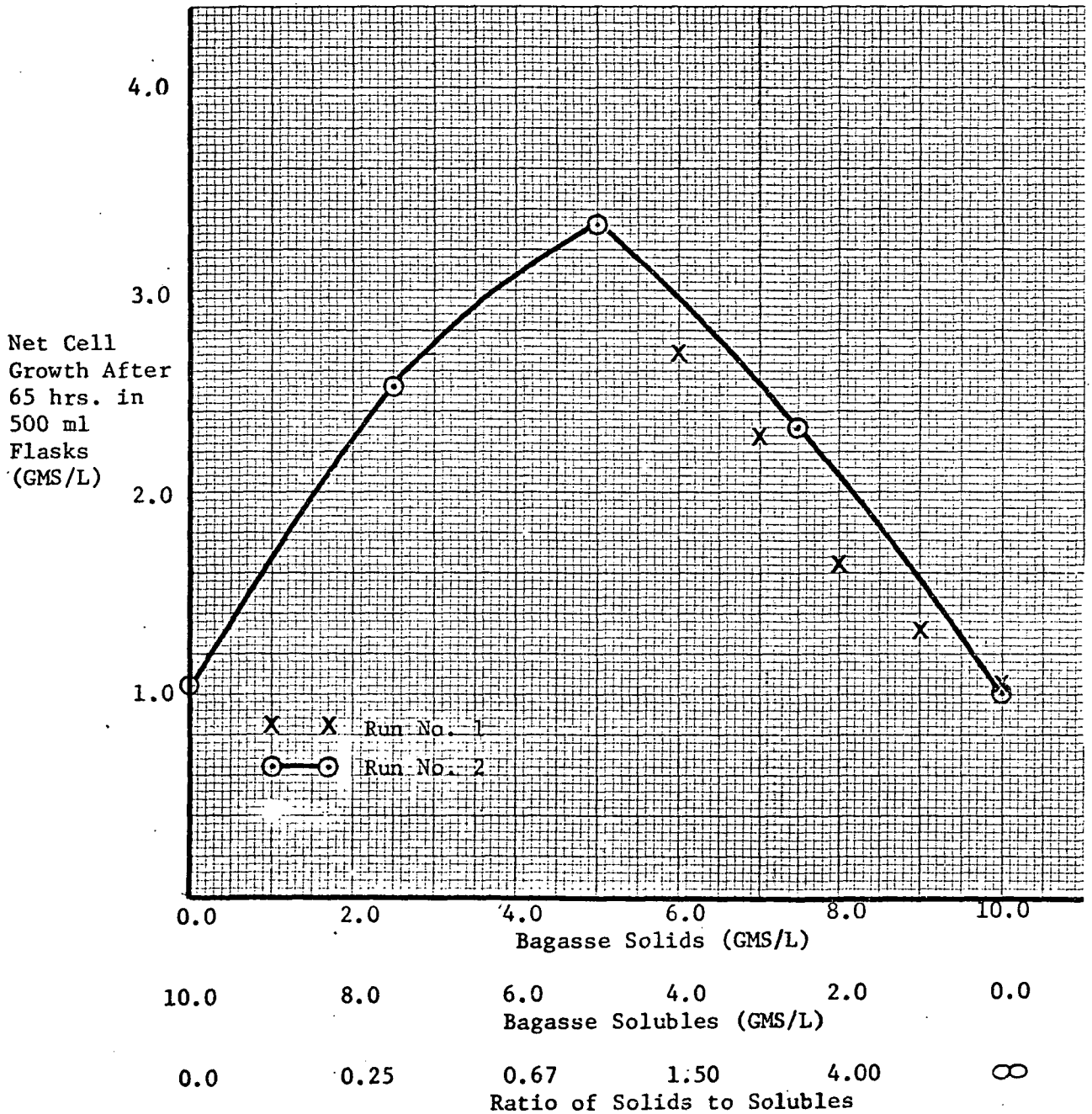
The agar must be mixed very well to insure that everything goes into solution, and should then be autoclaved at 15 psig for 15 minutes and stored.

If the agar is kept at 45 C, it will remain liquid and can be used. The plating technique used is the pour plate method. The sample of culture is diluted in Sterile 1N saline so that there are between 30 and 300 colonies on a plate. This assures a good statistical sample and accurate results. One milliliter of the diluted sample is placed in a petri dish, and about 15 ml. of agar are poured into the plate and mixed well. The plate is then incubated at 31 C for 48 hours and the colonies are counted.

Results and Discussion

Figure 1 illustrates net cell growth versus substrate loading. The data indicate that at both extremes; i.e., pure solid fraction and pure soluble fraction, growth is essentially the same. Based upon our postulate, these should correspond to cultures of pure Alcaligenes grown on pure solubles and pure Cellulomonas grown on pure solids. There should also be a

Figure 1. Net Cell Growth Versus Substrate Loading



respective increase and decrease in pH. Unfortunately, the plate counts did not yield any usable information other than the fact that the numbers gotten do not correspond to those one would expect in a mixed culture of Cellulomonas and Alcaligenes. This problem is being evaluated by the Microbiology Department. Figure 4, however, does indicate that the pH rises initially as the solubles are being utilized, and the pH decreases as the solids are being utilized. The only way to relate this to a particular microorganism, however, is by a plate count to determine the relative proportion of the two organisms. This will be accomplished in the near future.

Figures 1, 2, and 3 show that as we begin mixing the solid fraction and the soluble fraction the net cell growth increases. Maximum cell growth occurs at equal concentrations of pure solids and pure solubles. If one assumes that 1.0 gram of dry pure solid fraction yields 0.38 grams of cells, and 1.0 gram of soluble carbohydrates yields 0.50 grams of cells, then Figure 1 can be gotten from Figures 3 and 5. This cannot be done due primarily to the inadequacy of the test for soluble carbohydrates; i.e., the Phenol-Sulfuric Acid test. The test is not specific and many factors may interfere in readings. We are searching for a new test.

These data do indicate, however, that adding various proportions of soluble fraction to the solid fraction does enhance the net cell growth and also the percent and quantitative utilization of the substrate. The next step is to relate this to any continuous data available and to pick a few points and conduct the fermentation on a larger scale where growth can occur at a more optimum level.

Figure 2. Percent Bagasse Solids Digested Versus Substrate Loading

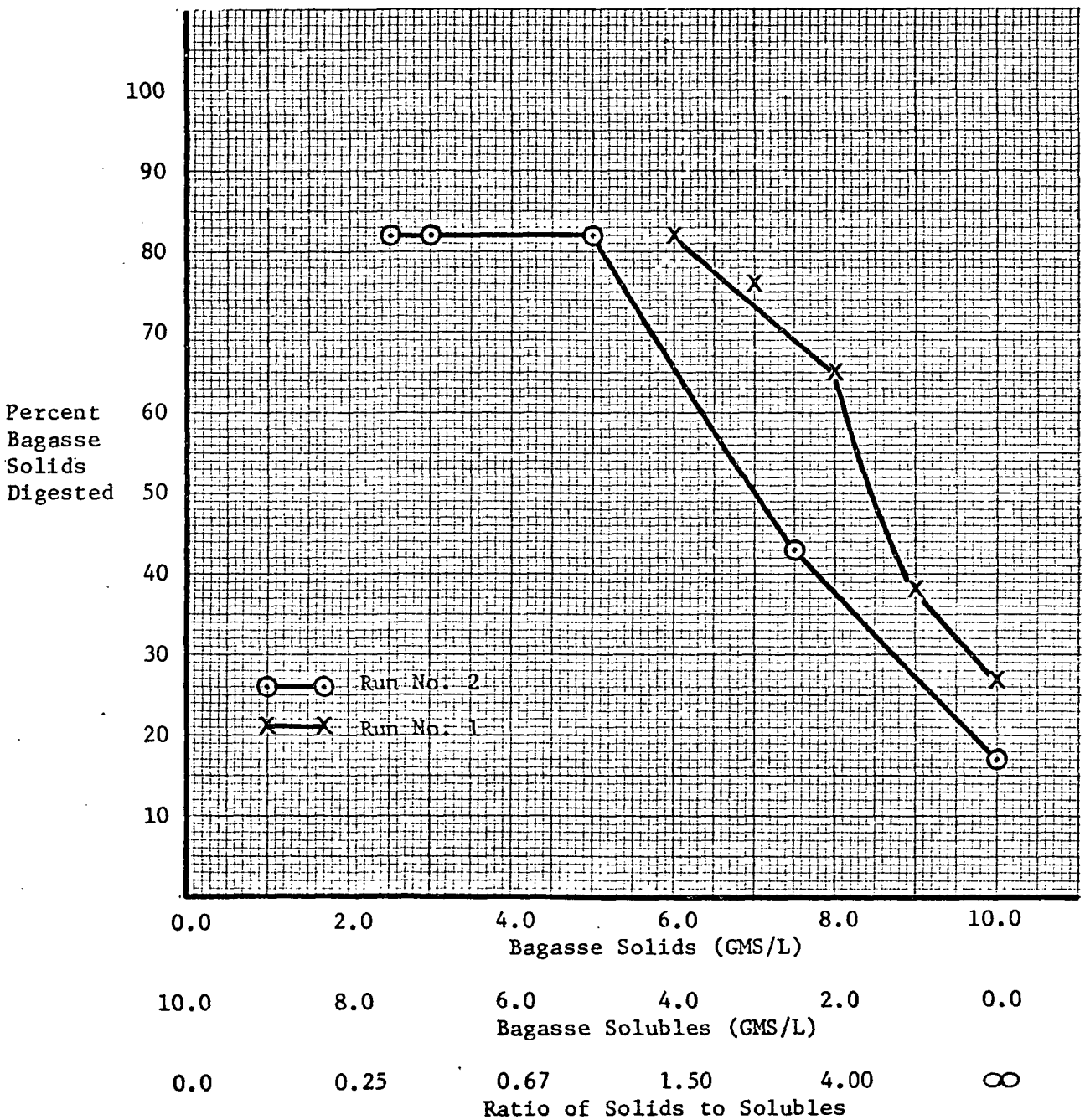


Figure 3. Bagasse Solids Digested and Bagasse Solubles Digested Versus Substrate Loading

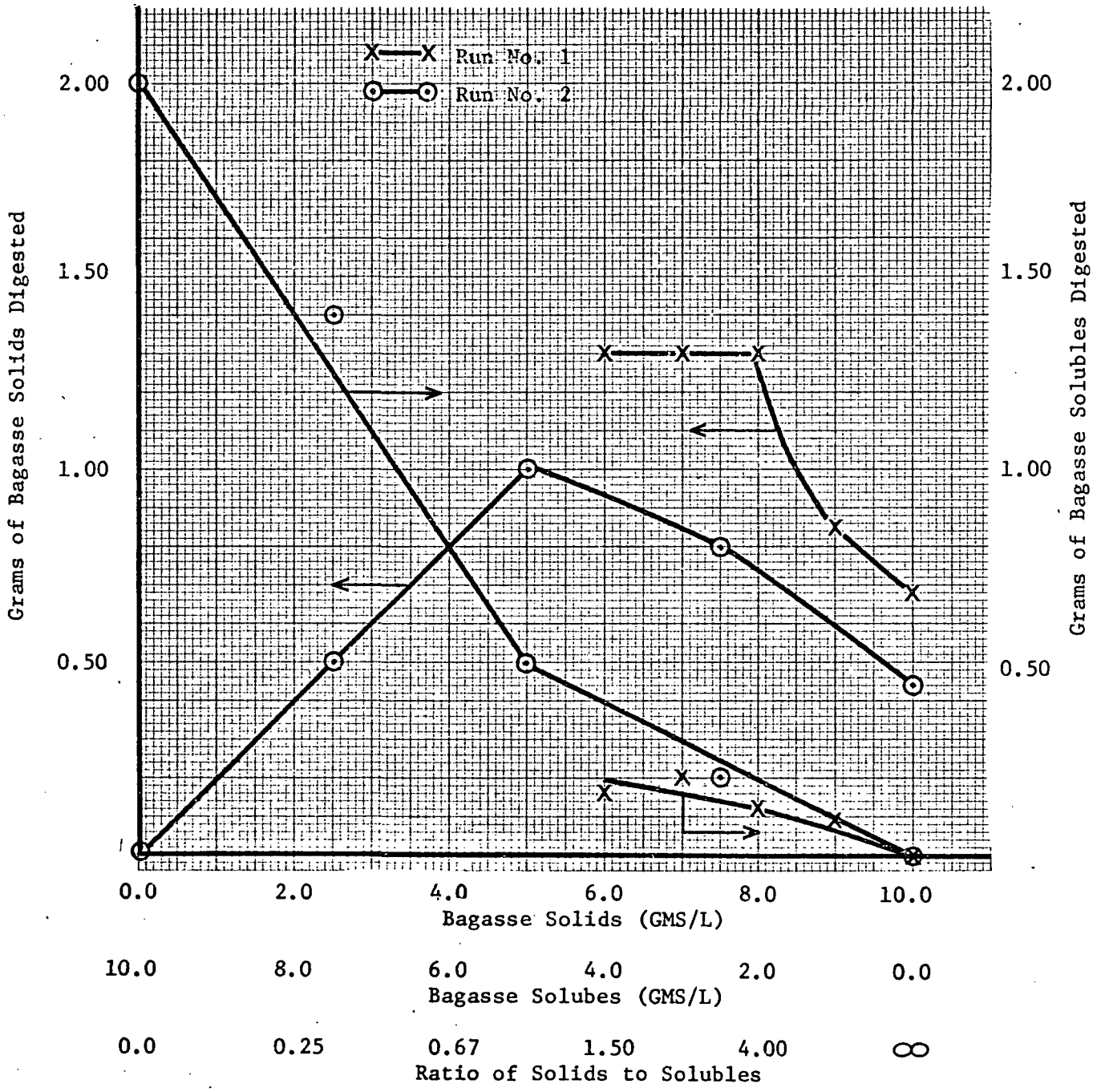
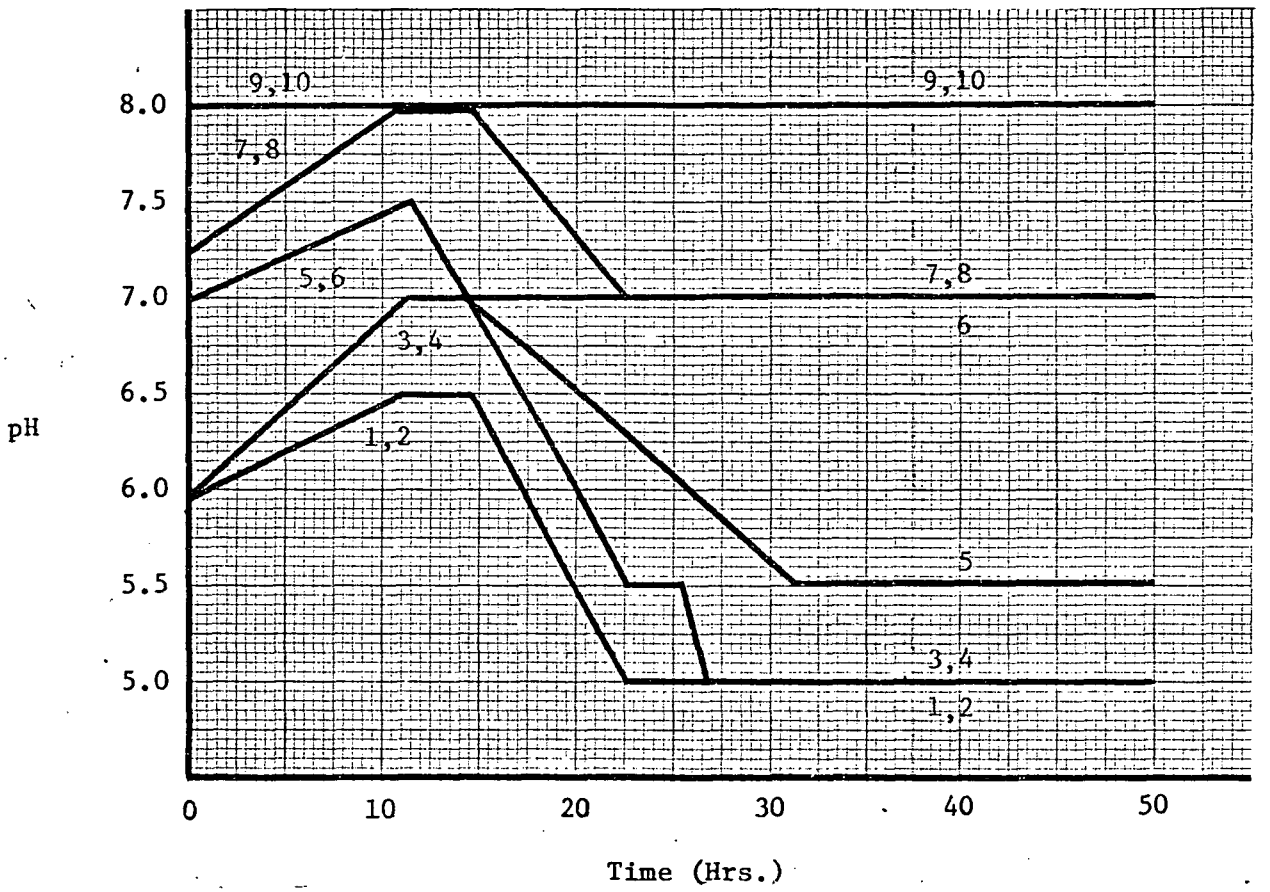


Figure 4. pH Versus Time for Run No. 2



Flask	1,2	3,4	5,6	7,8	9,10
Solids	10.0	7.5	5.0	2.5	0.0
Solubles	0.0	2.5	5.0	7.5	10.0

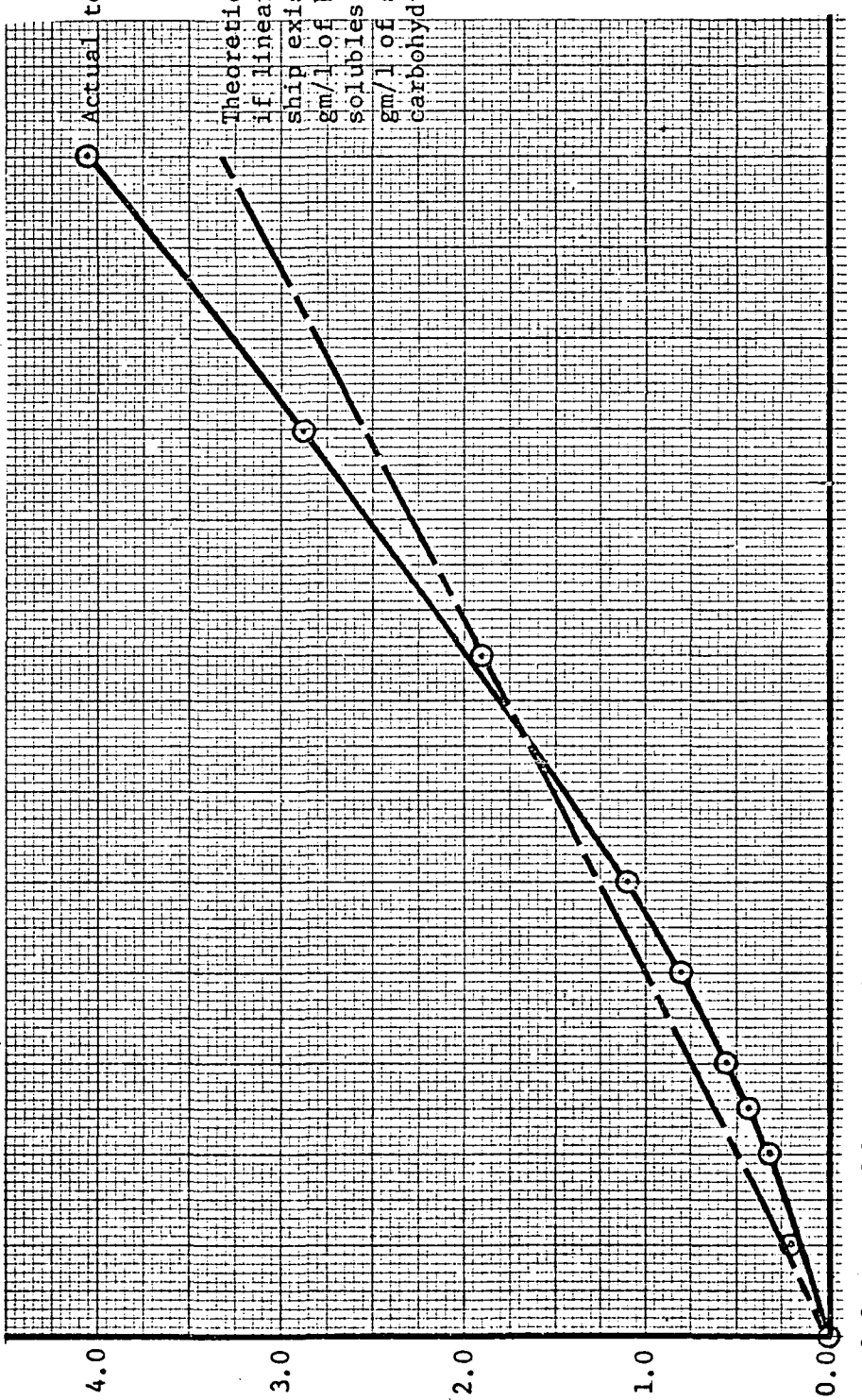


Figure 5. Soluble Carbohydrates (GMS/L)
Based Upon $\emptyset$ - H_2SO_4 Test

EFFECT OF DRYING AND BALL-MILLING ALKALI-TREATED BAGASSE AS A SUBSTRATE FOR MICROBIAL GROWTH[1]

The purpose of this experiment was to determine the effect on microbial growth of drying and ball-milling alkali-treated bagasse. Also, a comparison will be made of a fermentation run with continuous pH control and of one run with semi-continuous pH control.

It is believed that drying alkali-treated bagasse in an air oven will result in a re-crystallization of the cellulose. This would mean that the dried bagasse would be less susceptible to microbial degradation than the undried treated bagasse. If the dried bagasse were ball-milled, this should help to decrease the crystallinity and render the substrate more susceptible to microbial attack.

Continuous pH control should be more advantageous than semi-continuous control since the system will experience no large shocks due to deviation of the pH very far from 7.0.

Procedure

The bagasse to be used as substrate was prepared in three different ways:

1. Bagasse was treated with 4% NaOH at 170 F for one hour.
2. The bagasse from (1) was dried in an air oven at 90 C for 12 days.
3. The bagasse from (2) was ball-milled with 1/2-in. ball bearings for 48 hours.

The treated bagasse was loaded into a 7.0-liter fermenter which had semi-continuous pH control. The treated and dried bagasse was loaded into a 7.0-liter fermenter which had semi-continuous pH control. The treated, dried, and ball-milled bagasse was loaded at the same concentration into a 14.0-liter fermenter with pH control and into a 7.0-liter fermenter with semi-continuous pH control. The 7.0-liter fermenters were loaded with 25.0

grams/liter dry weight of substrate and 12.0 liters of media. The composition of the media is:

$(NH_4)SO_4$	6.0	grams/liter
KH_2PO_4	3.41	"
K_2HPO_4	4.35	"
$MgSO_4 \cdot 7H_2O$	0.4	"
$CaCl_2$,	0.1	"
Yeast Extract	0.1	"
NaCl	2.0	"
Trace Minerals	1.0	ml/liter
P-2000	1.0	"

The fermenters were sterilized and inoculated with a mixed culture of Cellulomonas and Alcaligenes which were grown on filter paper and 2% lactose, respectively. The inocula were in Log Phase Growth (LPG).

Results and Discussion

Results are shown on the growth curves in Figures 6, 7, 8, and 9 and Table 3. Based upon these data, it appears that the continuous pH control did not offer any advantage over the semi-continuous system. One way the low mass-doubling time of the 14-liter vessel can be explained is that the inoculum may not have been as healthy as the others. Although the treated bagasse has the lowest mass-doubling time in LPG, it did not have the greatest extent of growth. This may be somehow related to the phenomena in the growth curves which shows the treated bagasse culture leveling off rapidly after LPG, whereas the other cultures tail off gradually in a continuously increasing manner. The gradual decrease in growth rate and increase in extent of growth of the oven-dried material is probably due to

Figure 6. Treated, Dried, Ball-Milled Bagasse
Vessel No. 2 (14.0 liter)

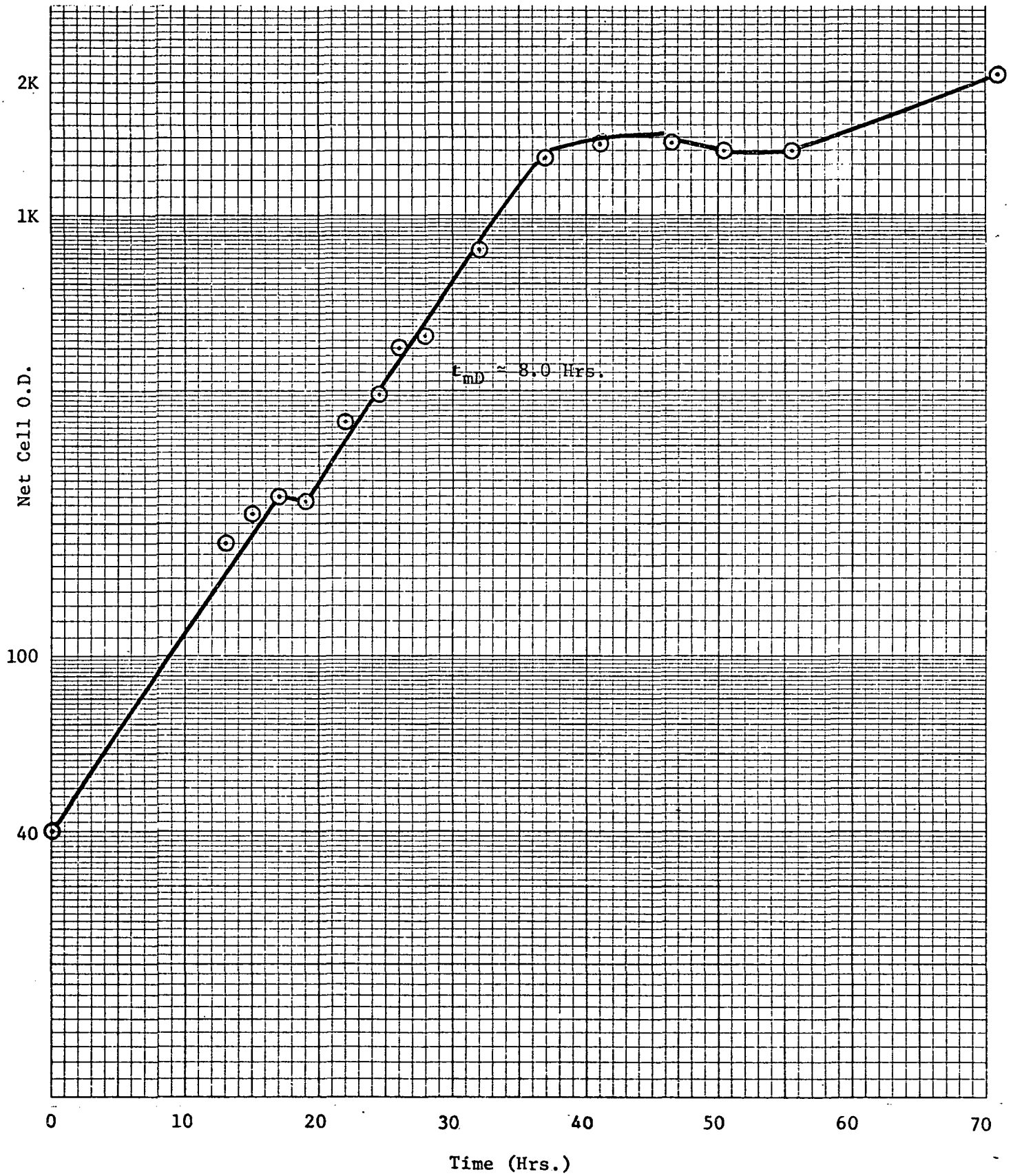


Figure 7. Treated, Dried, Ball-Milled Bagasse
Vessel No. 3 (7.0 liter)

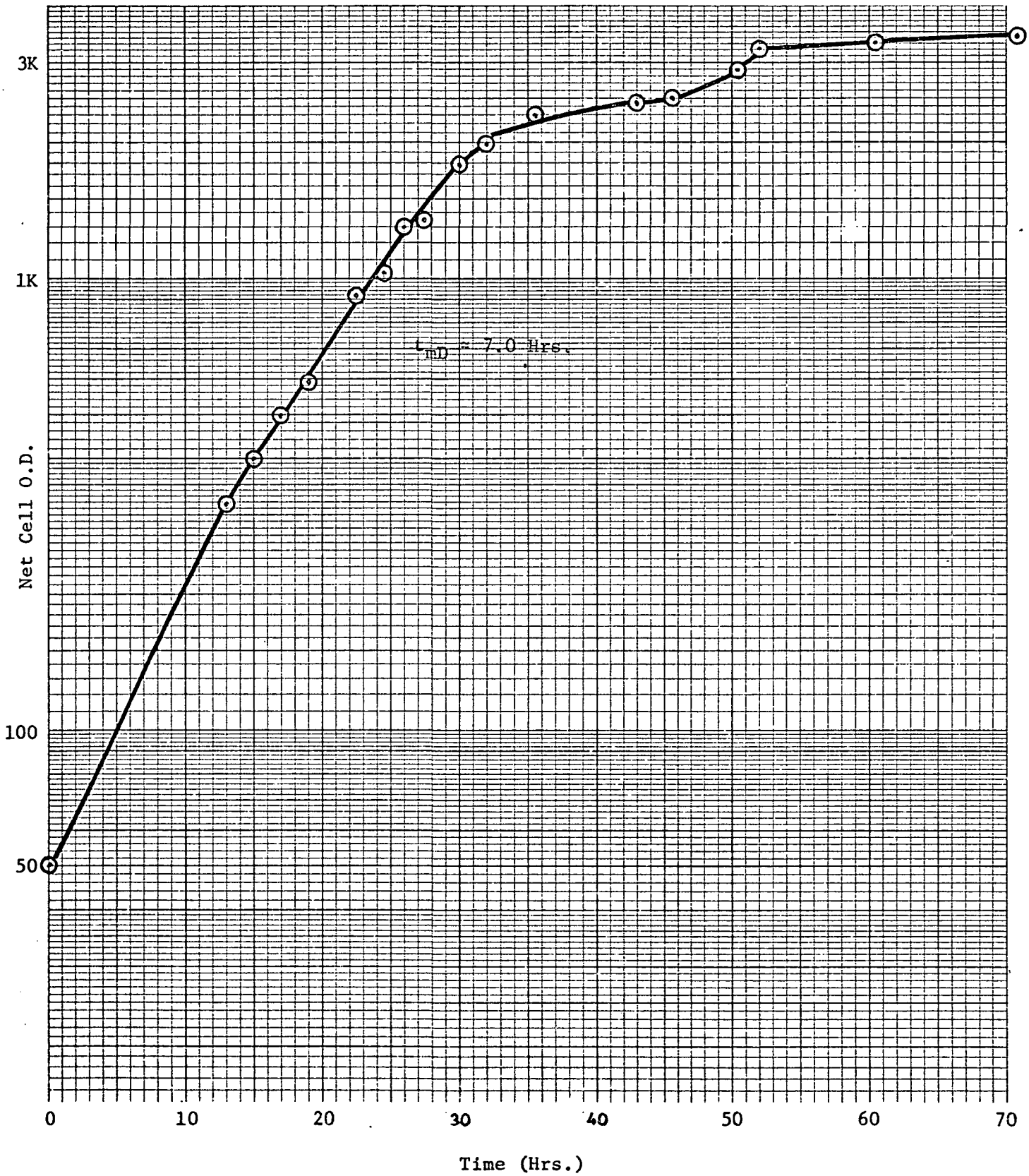


Figure 8. Treated, Dried Bagasse
Vessel No. 4 (7.0 liter)

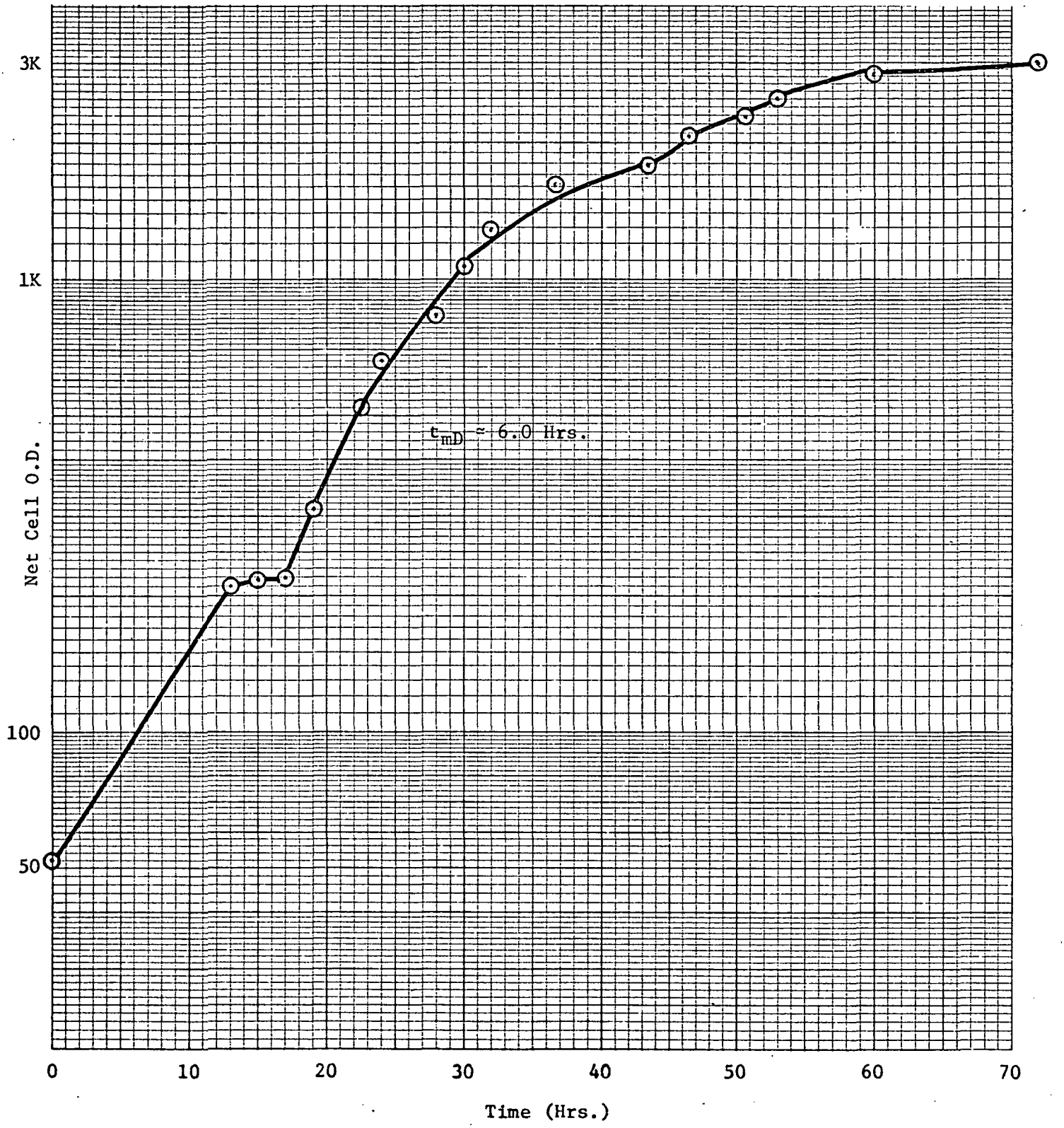


Figure 9. Treated Bagasse
Vessel No. 5 (7.0 liter)

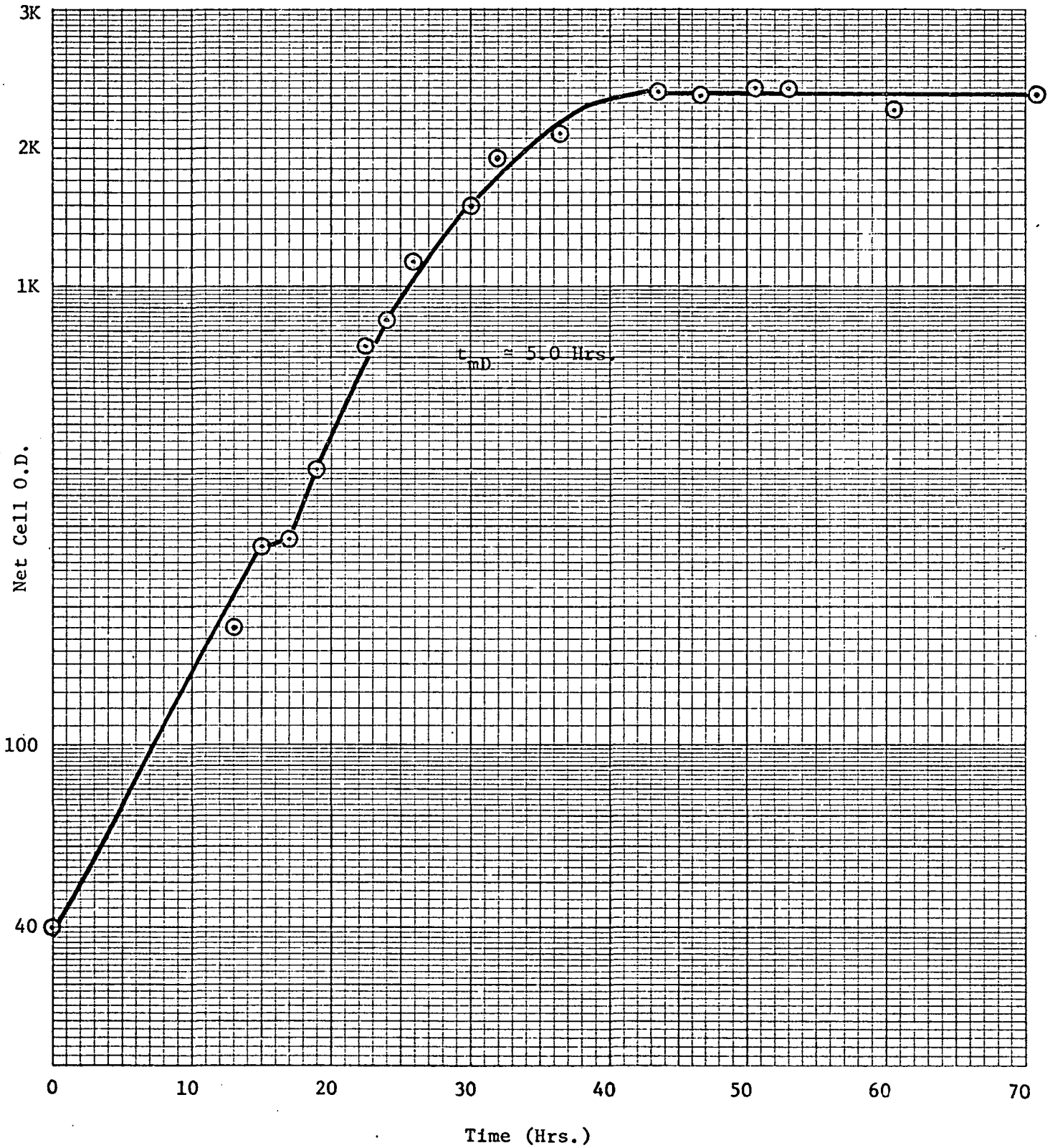


TABLE 3

CELL GROWTH DATA

Substrate	T	TD	TDBM	TDBM
Loading (gms/l)	25	25	25	25
Volume (L.)	6	6	6	12
pH control (C or S-C)	S-C	S-C	S-C	C
t_{mD} (hrs.)	5	6	7	8
KU at end of LPG	2000	1600	2100	1400
KU at end of 73 hrs.	2550	2950	3250	2150

T - Treated Bagasse
TD - Treated, Dried Bagasse
TDBM - Treated, Dried, Ball-milled Bagasse

decrystallization of some of the bagasse which had been crystallized in the oven. If the bagasse had been neutralized prior to placing in the oven, then the extent of crystallization would have been great. Since it was not neutralized, only a small amount of crystallization would have occurred.

EFFECT OF SUBSTRATE PARTICLE SIZE ON CELL GROWTH[1]

This experiment was conducted to determine if substrate particle size had an effect on the microbial utilization of the substrate. It was postulated that for a given mass of substrate, a decrease in the mean particle size would result in an increased total substrate surface area available for microbial attack. The particle size reduction would also decrease the degree of crystallinity of the substrate which would further enhance microbial attack. Since we believe microbial attack to be a surface phenomena, the

increase in total surface area and in the number of substrate particles should increase microbial utilization of the substrate.

Procedure

Bagasse was prepared for use in two different ways:

1. Treated, then ball-milled bagasse - Bagasse was treated with 3% NaOH, heated at 170 F for one hour, then neutralized with H_2SO_4 and dried in an oven at 90 C for 24 hours. It was then ball-milled for 48 hours. After milling, the bagasse was placed in a sieve shaker which contained sieves of 35, 80, 200, 230, and 325 mesh sizes. The corresponding sieve openings were 595, 177, 74, 63, and 44 microns. The bagasse was shaken for two hours, and the material which collected on each screen was used as substrate for that particular size range.
2. Bagasse was first ball-milled and separated into the same fractions as described above. Each fraction was then treated with 3% NaOH, heated at 170 F for one hour, and neutralized and used as substrate. Particle size ranges employed were:
   - Particles which passed through a 35-mesh screen but not a 80-mesh screen
   - Particles which passed through a 80-mesh screen but not a 200-mesh screen
   - Particles which passed through a 200-mesh screen but not a 230-mesh screen
   - Particles which passed through a 230-mesh screen but not a 325-mesh screen
   - Particles which passed through a 325-mesh screen.

The bagasse was loaded at 5.0 grams/liter dry weight into 500 ml. flasks which contained 250 ml. of the following media:

$(NH_4)_2SO_4$	6.0	grams/liter
$MgSO_4 \cdot 7H_2O$	0.4	"
$CaCl_2$	0.1	"
NaCl	1.0	"
KH_2PO_4	3.41	"
K_2HPO_4	4.35	"
Yeast Extract	0.1	"
Trace Minerals	1.0	ml/liter

The flasks were then sterilized and inoculated with a mixed culture of Cellulomonas and Alcaligenes which were grown on filter paper and 2% lactose respectively.

Results and Discussion

Results are presented in tabular form below.

Treated, then ball-milled bagasse -

Particle Size Range	Net Cell Growth (KU)	Unfiltered Net Growth
177 to 595 microns	190	790
74 to 177 microns	240	620
63 to 74 microns	190	520
44 to 63 microns	240	550
Less than 44 microns	180	500

Ball-milled, then treated bagasse -

Particle Size Range	Net Cell Growth (KU)	Unfiltered Net Growth
177 to 595 microns	270	380
74 to 177 microns	210	380
63 to 77 microns	210	390
44 to 63 microns	230	440
Less than 44 microns	240	400

Interpretation of the growth of the cultures based only on turbidometric measurements as above proved to be unsatisfactory. Even at the low substrate loading the small particles interfered with a proper determination of cell density. Since we do not have a quantitative chemical analysis for cell density at the present time, one will have to be devised before further work can be done with small particles of substrate. All the filters became plugged with substrate, thus making it impossible to measure the cell density. The Ninhydrin test, which is a quantitative test for protein, is being developed and will hopefully fill this technical void.

SELECTION OF A BUFFER SYSTEM - EFFECT OF VARIOUS CONCENTRATIONS OF PHOSPHATE BUFFER [2]

Selection of an appropriate and adequate buffer system to maintain pH within a range compatable to growth for shake flask cultures is a formidable problem. A phosphate buffer is a natural first choice since its pKa is near neutrality, and since the cells do require a small amount of phosphate for growth. A series of experiments were designed to determine the concentration of phosphate buffer that would best control changes in pH, and to determine at what concentrations phosphate inhibition would be in evidence.

Procedure

Media was prepared to contain:

$(NH_4)_2SO_4$	6.0 grams/liter
NaCl	1.0 "
$MgSO_4 \cdot 7H_2O$	0.4 "
$CaCl_2$	0.1 "
Yeast Extract	0.1 "
Trace Minerals	1.0 ml/liter
Glucose	3.0 grams/liter

Two hundred fifty ml aliquots of this media were put into 500 ml Belco sidearm flasks and prepared to contain the following concentrations of phosphate buffer.

Molarity of Buffer	gms. K_2HPO_4	gms. KH_2PO_4
0.015	1.3	1.02
0.05	4.45	3.40
0.1	8.70	6.80
0.2	17.4	13.6
0.4	34.8	27.2
0.5	43.5	34.0

All flasks were inoculated with three ml of Cellulomonas, and pH was taken at the beginning and end of the run. Cell growth was determined by increase in optical density with a Klett-Summerson colorimeter equipped with a red filter. Reducing sugars were measured at the beginning and end of the run using the 3,5 dinitrosalicylic acid (DNS) method.

It was noted that caramelization of the glucose increased with increased concentration of phosphate salt and that in no case did the amount of

reducing sugar indicated to be present using the DNS method correspond with the original substrate loading.

Results and Discussion

From the data presented in Table 4, it appears that the growth rate of Cellulomonas is inhibited at phosphate concentrations greater than 0.05 N. Growth rates at 0.015 N and 0.05 N are comparable; and though the pH at the higher concentration shows less of a decrease than at the lower concentrations, both final pH's are incompatable to maximum substrate utilization and cell growth.

The problem of pH control for bench-top fermentation or pilot plant scale fermentations is not a serious one. Addition of acid or base can be accomplished asceptically and mechanically so that the pH can be controlled within a very small range. However, the problem of maintaining optimum pH in a shake flask culture is a very real one. The measurement of pH at intervals, and the sporadic adjustment of pH, greatly enhances the opportunity for contamination not to mention the cellular trauma involved in abruptly adjusting the pH from 5.0 to 7.0.

The alternative to inadequate buffers at low phosphate levels and to inhibition at higher levels should reasonably be a switch to another buffer system that would provide a strongly buffered neutral solution and not inhibit the growth rate of Cellulomonas.

TABLE 4

Molarity of Buffer	pH		Reducing Sugar gm/l		Growth in Klett Units			
	0 hrs.	72 hrs.	0 hrs.	72 hrs.	0 hrs.	18 hrs.	23 hrs.	72 hrs.
0.015	6.8	4.2	1.95	0.31	4	200	220	230
0.015	6.8	4.1	1.85	0.52	5	200	220	240
0.05	6.8	5.0	1.85	0.1	5	130	211	260
0.05	6.8	5.0	2.05	0.09	8	145	210	253
0.1	6.8	5.8	3.05	0.1	3	22	52	240
0.1	6.8	5.9	1.95	0.1	6	41	91	255
0.2	6.8	6.0	1.85	0.16	4	8	27	219
0.2	6.8	6.0	1.85	0.16	2	39	70	100
0.4	6.8	6.4	1.70	0.49	5	5	14	90
0.4	6.8	6.5	1.85	0.89	2	4	9	64
0.5	6.8	6.8	1.80	0.94	2	4	4	4
0.5	6.8	6.8	1.75	0.94	3	4	4	4

REFERENCES

1. Erwin, George, unpublished data, 1971.

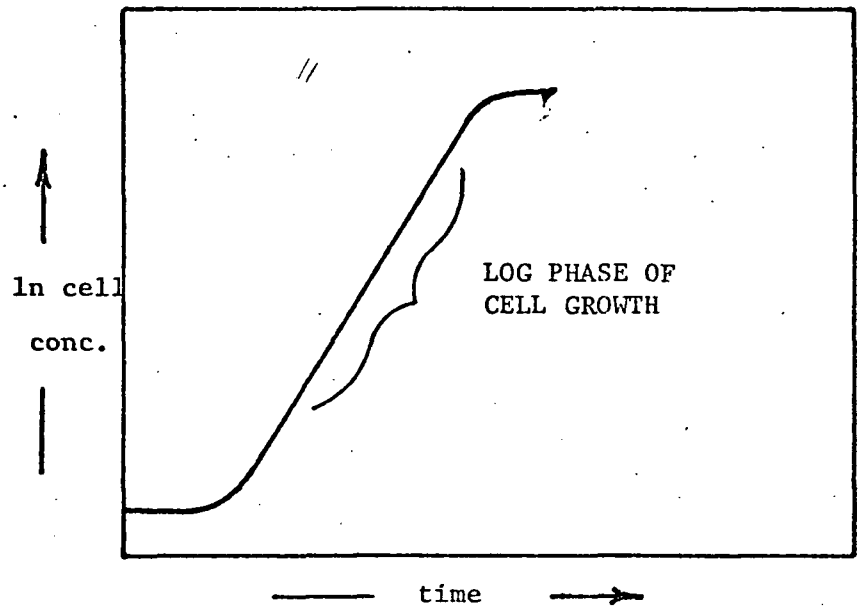
2. Little, Brenda, unpublished data, 1971.

APPENDIX A

APPENDIX A

EXPLANATIONS FOR CHOICE OF PROCESS EVALUATION PARAMETERS

1. Batch cell concentration at end of log phase growth - C_{LPG} (grams of dry cells/liter of culture)



   a. In log growth phase the mass of the cells of the culture is increasing by first-order kinetics--the cells are dividing by binary fission, and the culture is growing at its maximum growth rate.

   b. The end of the log phase of growth denotes that something is limiting the free growth of the culture.

   c. The C_{LPG}, therefore, gives us a measure of the extent of free growth possible for that culture under whatever conditions are imposed.

2. Growth Rate on Bagasse - μ_{LPG} (hrs^{-1}), (during log phase growth)

from $$\frac{dC}{dt} = \mu C; \quad \mu = \frac{0.69}{t_{MD}}$$

a. Gives the characteristic growth rate of the culture during the log phase under whatever conditions are imposed on the culture.

3. Productivity Calculated from a Batch Culture - $P_{C.B.}$ (grams dry cells/liter fermenter volume · hour)

$$P_{C.B.} = \mu_{LPG} \cdot C_{LPG}$$

a. Gives a measure of the performance of the particular batch culture if it were in steady-state continuous flow under the same conditions.

4. Cell Yield on Substrate - Y_{CH_2O} (grams dry cells/gram of bagasse fiber used)

$$\frac{dC}{dS} = Y$$

where; S = concentration of filterable solids

a. Has a usual maximum value of 0.50 for cells aerobically metabolizing carbohydrate.

b. Since not all bagasse is reported as filterable solids, this value does not represent the overall yield of cells on bagasse (which will be lower).

5. Substrate Utilization Efficiency at the End of Log Phase Growth - $\left(\frac{S_o - S}{S_o}\right)_{LPG}$ (non-dimensional)

where: S_o = grams dry filterable solids at start of culture

S = grams dry filterable solids at any time (in this case at end of LPG)

a. Gives a measure of the extent to which the substrate can be metabolized without limiting the free growth of the culture (assuming that nothing else is limiting culture growth).

b. Indicates the amount of the fiber that can be metabolized in a single pass through the fermenter.

6. Continuous Productivity - P, (grams of dry cells/liter of fermenter volume · hour)

$P = C \cdot D$ where

C = cell concentration in g/l in steady-state in a continuous culture

D = dilution rate in $hrs.^{-1}$

$\left(D = \frac{\text{FEED RATE}}{\text{FERM. VOL.}}\right)$ of the culture.

a. Experimental value for the productivity of the culture in steady-state continuous flow.

APPENDIX B

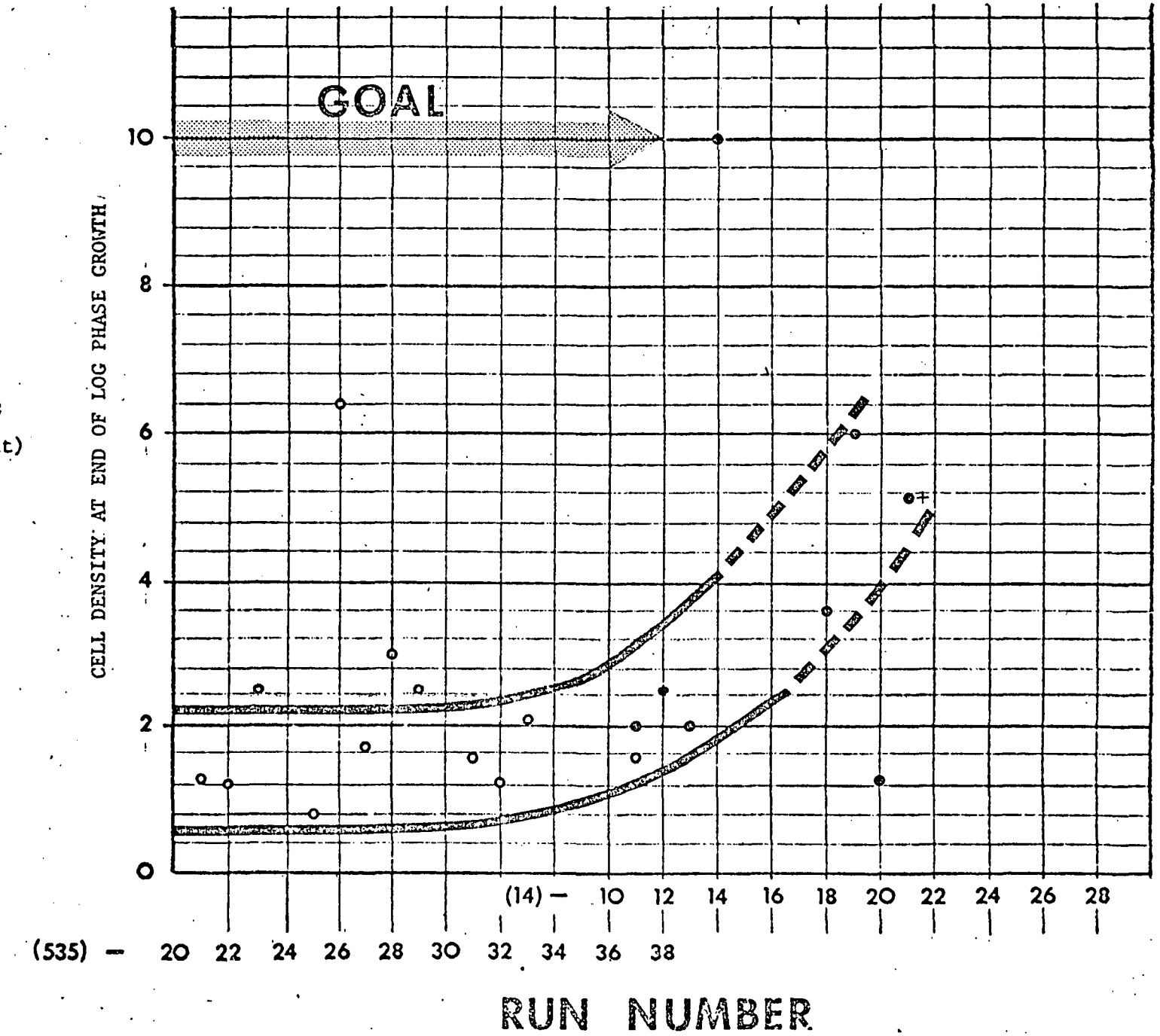
APPENDIX B

CONTROLLING VARIABLES - CHART 1

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED
1. BATCH CELL DENSITY IN LOG PHASE GROWTH	pH	GOOD	NO	
	TEMPERATURE	GOOD	NO	
	PREPARATION OF INOCULUM	FAIR	YES	NEEDS TO BE STANDARDIZED
	GROWTH EFFECTS OF SOLUBLE COMPONENTS OF TREATED BAGASSE	FAIR	YES	IDENTIFICATION AND EFFECTS OF COMPONENTS ON CULTURE DYNAMICS
	CELL POPULATION DYNAMICS	POOR	YES	DIFFERENTIAL COUNTS IN RELATION TO FEED; CULTURE AGE; CULTURE PHYSIOLOGY
	GROWTH EFFECTS OF SOLID FRACTION OF TREATED BAGASSE	FAIR	YES	CHARACTERIZATION TEST NEEDED
	INORGANIC NUTRIENTS	GOOD	NO	(CELL YIELDS ON N, O)
	GROWTH FACTORS	FAIR	NO	
	PRODUCTION AND ACTIVITY OF ENZYMES	FAIR	YES	INHIBITION AND REPRESSION BY FEED COMPONENTS; EFFECTS OF CELL AGE
	AERATION	FAIR	NO	
	AGITATION	FAIR	NO	

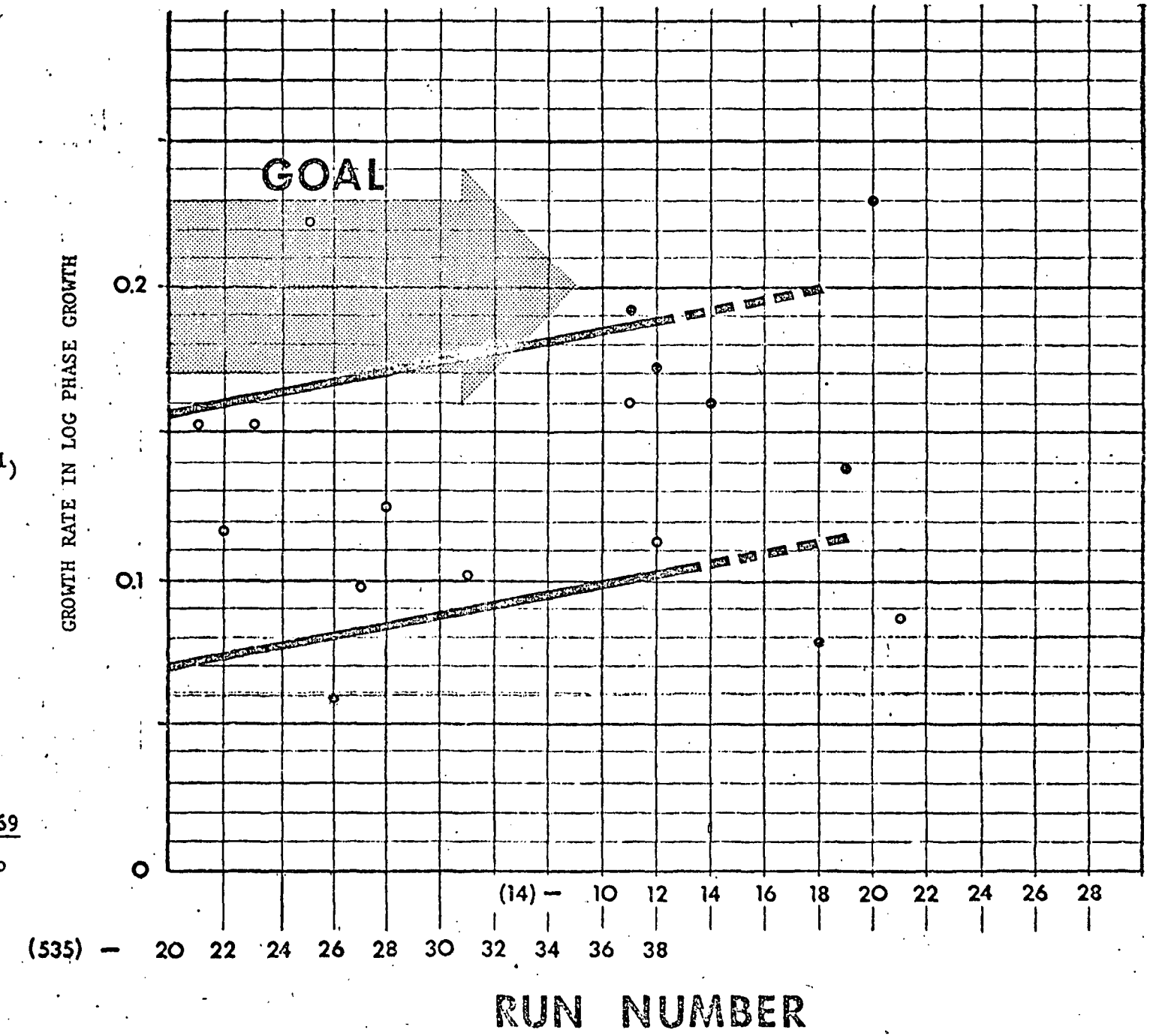
CONTROLLING VARIABLES - CHART 1 (Contd.)

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
1. BATCH CELL DENSITY IN LOG PHASE GROWTH	EFFECTS OF INITIAL SUBSTRATE MAKE-UP AND CONCENTRATION	FAIR	YES	CRITICAL INITIAL LEVELS OF SOLUBLES AND SOLIDS	


GOAL
CELL DENSITY AT END OF LOG PHASE GROWTH
C_{LPG} (gms/lit)
0
2
4
6
8
10
(14) — 10 12 14 16 18 20 22 24 26 28
(535) — 20 22 24 26 28 30 32 34 36 38
RUN NUMBER

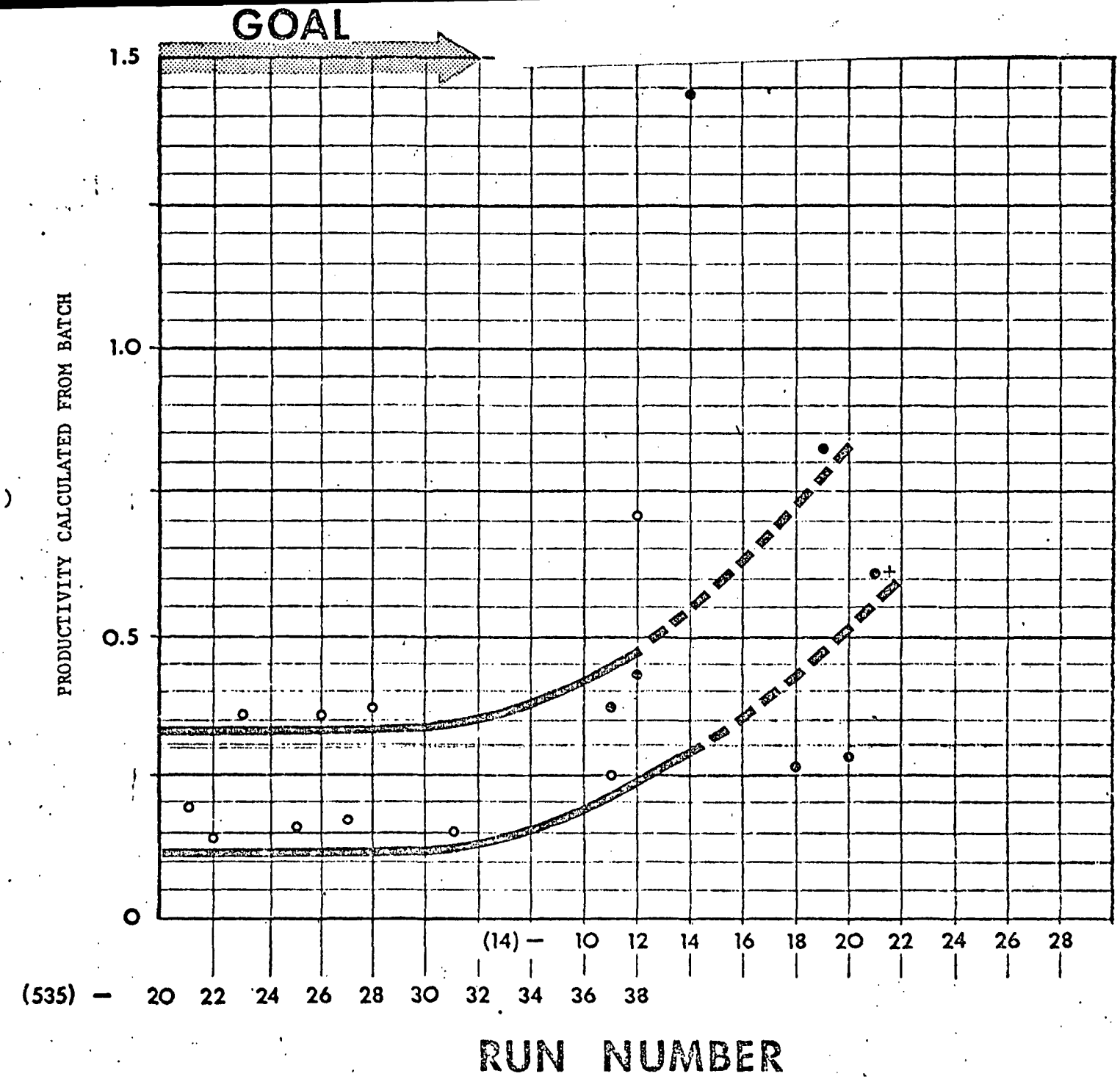
CONTROLLING VARIABLES - CHART 2

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
	CHARACTERISTIC GROWTH RATE OF ORGANISMS	GOOD	NO		
2. GROWTH RATE ON BAGASSE	pH	GOOD	NO		
	TEMPERATURE	GOOD	NO	//	
	EFFECTS OF SUBSTRATE TREATMENT	FAIR	YES	NEEDS TO BE QUANTITIZED	
	EFFECTS OF GROWTH FACTORS	FAIR	NO	(CRITICAL LEVELS NOT DETERMINED)	
	EFFECTS OF INORGANIC NUTRIENTS	GOOD	NO		


GOAL
GROWTH RATE IN LOG PHASE GROWTH
μ_{LPG} (hrs.$^{-1}$)
$\mu = \frac{0.69}{t_{mo}}$
0
0.1
0.2
(14) — 10 12 14 16 18 20 22 24 26 28
(535) — 20 22 24 26 28 30 32 34 36 38
RUN NUMBER

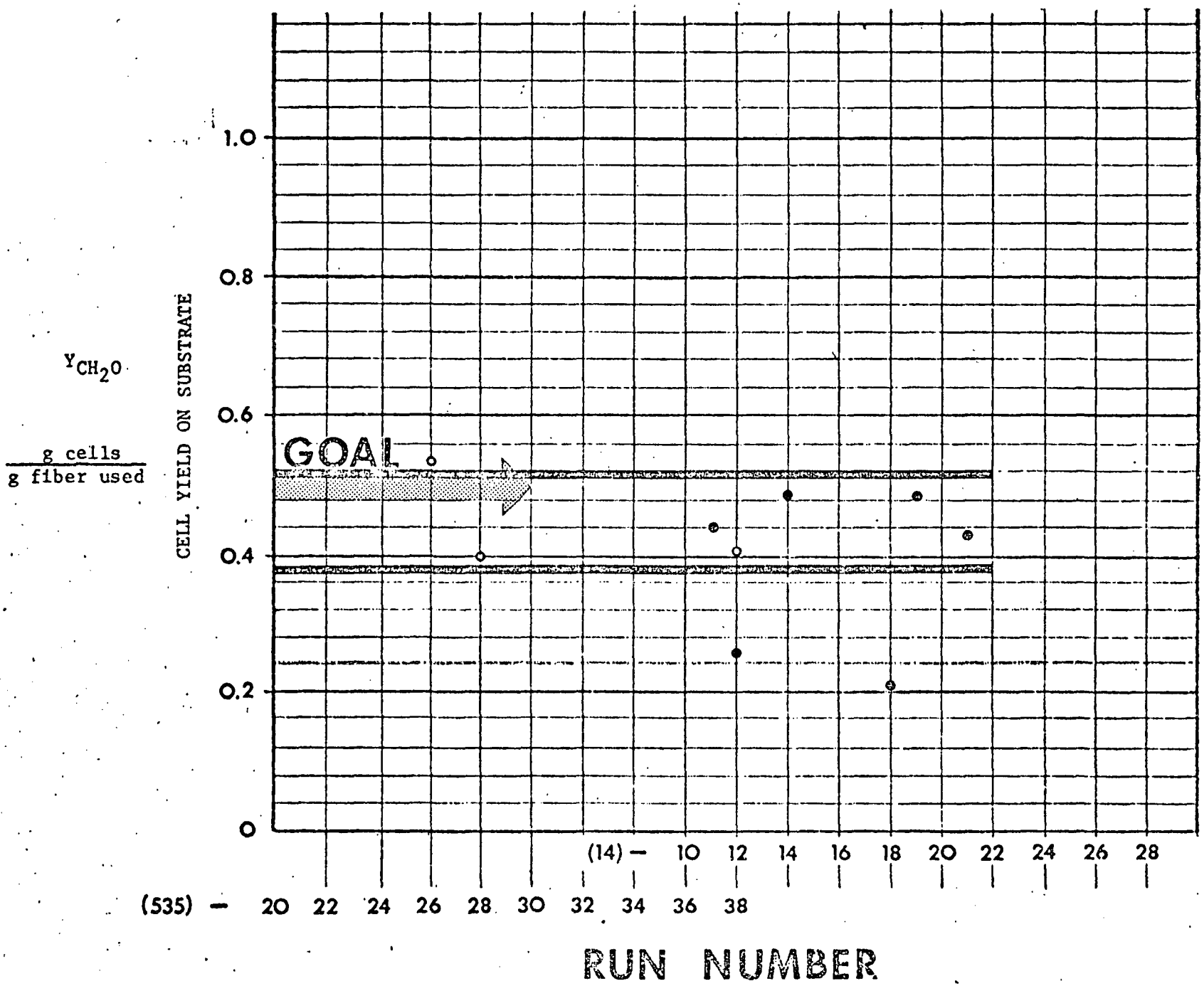
CONTROLLING VARIABLES - CHART 3

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
	BATCH CELL CONCENTRATION IN L.P.G.	(SEE EVALUATION PARAMETER NO. 1)			
	GROWTH RATE ON BAGASSE	(SEE EVALUATION PARAMETER NO. 2)			
3. PRODUCTIVITY CALCULATED FROM BATCH	COMPARISON WITH OTHERS	--			
	CONVERSION TO CONTINUOUS VALVES	FAIR	YES	MORE CONTINUOUS FLOW DATA NEEDED	


GOAL
1.5
1.0
0.5
0
PRODUCTIVITY CALCULATED FROM BATCH
$P_{C.B.}$
(gm/l·hr.)
(14) — 10 12 14 16 18 20 22 24 26 28
(535) — 20 22 24 26 28 30 32 34 36 38
RUN NUMBER

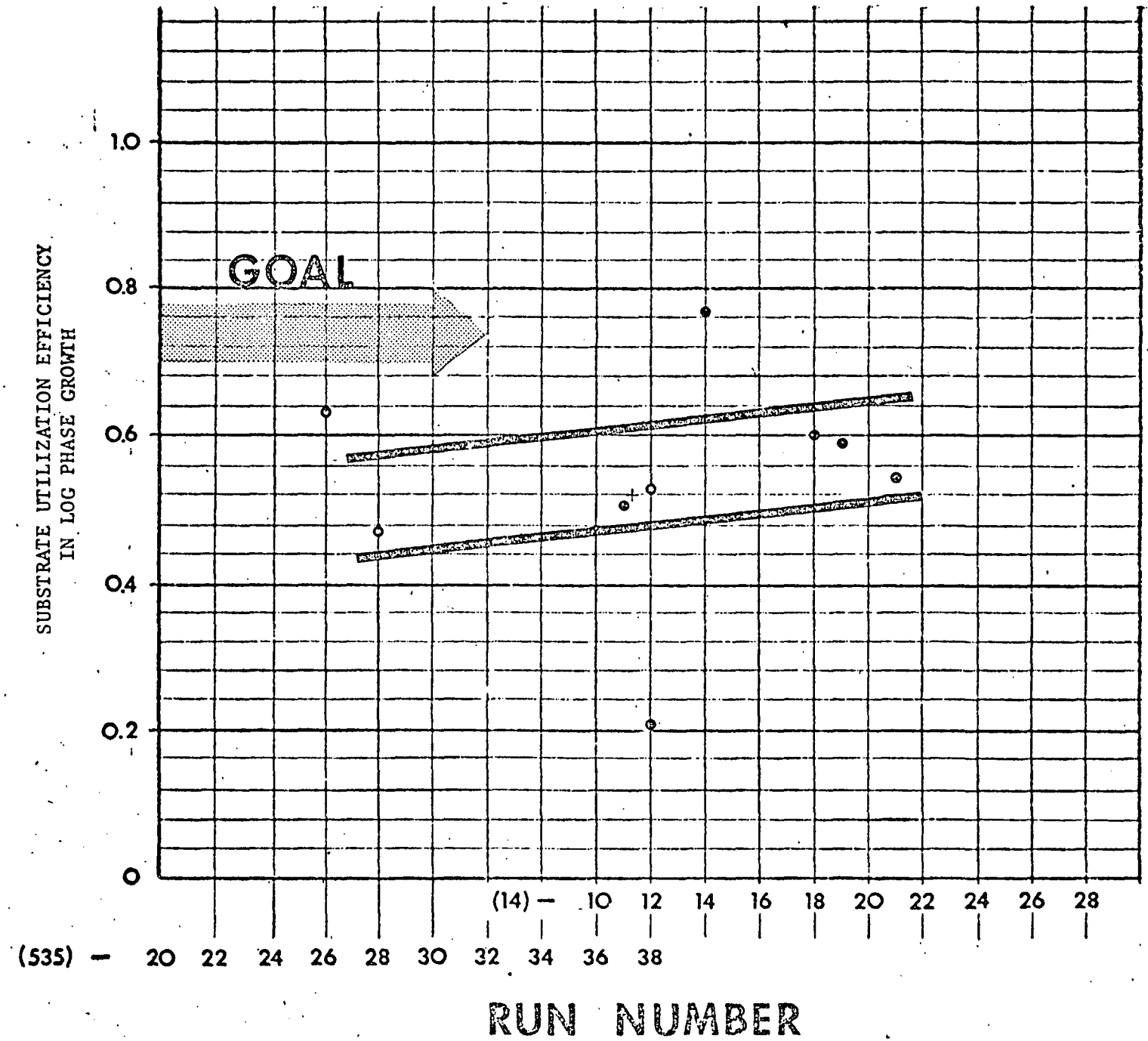
CONTROLLING VARIABLES - CHART 4

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
	MATERIAL BALANCES	GOOD	NO	(MONITORING ONLY)	
	YIELD ON SOLUBLES	FAIR	NO		
	YIELD ON SOLIDS	GOOD	NO		
4. CELL YIELD ON SUBSTRATE	EFFECTS OF CULTURE AGE	POOR	YES	CELL YIELD VERSUS CULTURE AGE AND DYNAMICS	


Y_{CH_2O}
g cells / g fiber used
CELL YIELD ON SUBSTRATE
1.0
0.8
0.6
0.4
0.2
0
GOAL
(14) — 10 12 14 16 18 20 22 24 26 28
(535) — 20 22 24 26 28 30 32 34 36 38
RUN NUMBER

CONTROLLING VARIABLES - CHART 5

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
	EFFECTS OF SEVERITY OF BAGASSE TREATMENT	FAIR	YES	STANDARDIZATION OF TREATMENT	
	FINDING A CHARACTERIZING ANALYSIS	POOR	YES	DEVELOPMENT OF A SIMPLE TEST TO PREDICT UTILIZATION	
	TRANSLATION OF BATCH TO CONTINUOUS UTILIZATION VALUES	POOR	YES	MORE CONTINUOUS DATA NEEDED	
5. SUBSTRATE UTILIZATION EFFICIENCY IN L.P.G.					


SUBSTRATE UTILIZATION EFFICIENCY
IN LOG PHASE GROWTH
$\frac{S_o - S}{S_o}$
GOAL
1.0
0.8
0.6
0.4
0.2
0
(535) — 20 22 24 26 28 30 32 34 36 38
(14) — 10 12 14 16 18 20 22 24 26 28
RUN NUMBER

CONTROLLING VARIABLES - CHART 6

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED
	CULTURE DYNAMICS AT START OF CONTINUOUS FEED	FAIR	YES	DIFFERENTIAL POPULATION; CULTURE METABOLIC ACTIVITY MATCHED TO FEED
	EFFECTS OF FEED MAKE-UP ON DYNAMICS	POOR	YES	DETERMINE EFFECTS OF SOLIDS/SOLUBLES LEVELS AND RATIOS ON POPULATION DYNAMICS
	INSTANTANEOUS MATERIAL BALANCES	FAIR	YES	ANALYTICAL TECHNIQUES O.K. NEED MORE CONTINUOUS RUN DATA
	PRODUCTIVITY OF FUNCTION OF DILUTION RATE	POOR	YES	NEED MORE DATA
6. CONTINUOUS PRODUCTIVITY				

CONTROLLING VARIABLES - CHART 7

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
	METHODS OF CELL HARVEST	FAIR	YES	(AFTER SUCCESSFUL CONT. RUNS)	
	CELL ANALYSIS	GOOD	NO		
	FEEDING STUDIES	GOOD	NO	//	
	PROTEIN PURIFICATION	FAIR	NO		
7. PRODUCT QUALITY					

CONTROLLING VARIABLES - CHART 8

EVALUATION PARAMETER	CONTROLLING VARIABLES	STATUS OF DEFINITION		FURTHER WORK PLANNED	
	DEFINITION OF FLOW PLAN	GOOD	YES	EQUIPMENT SIZING DEPENDS ON PRODUCTIVITY	
	OPERATING COSTS	FAIR	YES	ENERGY BALANCE CALCULATIONS	
	FIXED COSTS	FAIR	YES	EQUIPMENT CAPITAL COSTS	
8. PRODUCT COST					