APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1979, p. 109-116 Vol. 37, No. 1
0099-2240/79/01-0109/08$02.00/0

Effects of Glucose, pH, and Dissolved-Oxygen Tension on *Bacillus cereus* Growth and Permeability Factor Production in Batch Culture

W. M. SPIRA[1] AND G. J. SILVERMAN[2]*

Division of Geographic Medicine, The Johns Hopkins University School of Medicine, Baltimore City Hospitals, Baltimore, Maryland 21224,[1] and Food Microbiology Group, Food Sciences Laboratory, U.S. Army Natick Research and Development Command, Natick, Massachusetts 01760[2]

Received for publication 16 October 1978

The production of a *Bacillus cereus* enterotoxin, measured as rabbit skin permeability factor (PF), in response to differences in glucose availability, pH, and dissolved oxygen tension was studied in a 1-liter batch fermentor system. Glucose had to be present for toxigenesis to occur. In uncontrolled fermentation an increasing inhibition of PF production and growth occurred as pH dropped below 6.5. Optimum pH for toxigenesis was 7.0 to 7.5, and fermentations maintained at this level yielded 10- to 20-fold more PF than comparable uncontrolled fermentations. PF production was appreciably diminished at or below pH 6.0 and at or above pH 8.5. Peak PF titer was associated with a drop in acid output, and the titrant utilization profile could be used as an indication of this point. Productivity was greatest in the early exponential phase of growth and decreased to zero at the transition phase. Differences in dissolved oxygen tension affected both the maximum productivity early in the fermentation and the rate of its decrease as growth progressed. The optimum dissolved oxygen tension for toxigenesis was 0.002 atm, and the most rapid growth occurred at 0.10 atm. Productivity and growth were reduced under anerobic conditions, whereas a hyperoxic environment severely reduced productivity, but not growth. Postexponential-phase loss of toxic activity coincided with a rapid increase in cellular oxygen demand. Neither was inhibited by the presence of glucose. However, PF loss was completely prevented by stringent oxygen limitation. Extracellular proteolytic activity did not appear to be responsible for the loss of toxic activity.

Thus far, two distinct enterotoxic activities have been demonstrated in *Bacillus cereus* isolated from outbreaks of food poisoning. The first is one or more diarrheal factors which cause fluid accumulation in the ligated ileal loops of young rabbits (18), alteration in capillary permeability in rabbit skin (12), and diarrhea in rhesus monkeys (16). These activities appear to be the function of a single toxin or a group of closely related toxins. Turnbull (19) has recently shown that these activities may be mediated by the stimulation of the adenylate cyclase-cyclic AMP system. The second toxic activity has been demonstrated in strains of *B. cereus* isolated from outbreaks of nausea and vomiting resulting from the consumption of heavily contaminated cooked rice. This factor is capable of causing vomiting in rhesus monkeys, but has no apparent diarrheal activity (16).

An ileal loop-active enterotoxin was first described by Spira and Goepfert (17), and many of its toxic activities and biological charactertistics have been described previously (10, 12, 17, 18). The strain (B-4ac) used in the work reported here was used extensively in these earlier studies. All data published thus far on this strain indicate strongly that a single moiety is responsible for the variety of enterotoxic manifestations reported. In particular, a one-to-one relationship appears to exist between capillary permeability activity and the indication of fluid accumulation in the rabbit ileal loop (W. M. Spira, Ph.D. Thesis, University of Wisconsin, Madison, 1974). We have reconfirmed in our laboratory that this relationship holds good under a variety of environmental conditions (unpublished data) and consider the blueing activity seen in the rabbit skin test to be a function of ileal loop-active enterotoxin, at least in strain B-4ac. Since there is some question among investigators whether this relationship holds for other strains, however, we have limited ourselves to calling this skin activity "permeability factor" (PF) until the matter is publicly clarified.

Our primary purpose in this investigation has been to gather basic data on cellular response to the environment in terms of toxin production so we could project this knowledge to food systems. We have focused, in particular, on pH and dissolved oxygen tension (DOT). We were especially interested in the effect of DOT, since *B. cereus* is a facultative anaerobe and we expected differences in metabolism at different levels of DOT that might be reflected in differences by PF production. Harrison (14) has reviewed the close relationship of DOT to intracellular physiological state and points out that a series of DOT thresholds may exist for a particular organism in a given environment. Thus, observed alterations in cell physiology may be the result of accumulated changes to a series of thresholds rather than a single overall response to one threshold. A careful study of the relationship of PF secretion to DOT might discover such thresholds and establish a pattern for the regulation of PF production.

We have reported previously (4) that overall production of PF is best at low DOT and that this level did not coincide with the optimal DOT for growth. We have also reported on the need for pH and DOT control to increase PF yields in fermentor culture (W. M. Spira and G. J. Silverman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, P17, p. 189). Similar results have been reported by Glatz and Goepfert (11). This paper reports on the continuation of our studies on PF production using a 1-liter fermentor system, particularly as they relate to stages of growth in batch culture.

MATERIALS AND METHODS

Strain and medium. *B. cereus* B-4ac was used for all studies. Stock cultures were maintained in a freeze-dried state at 4°C. The growth medium consisted of 10 g of Hy-Case, S. F. (Sheffield), and 50 ml of yeast extract solution (Difco) (dialyzable fraction, standardized to 20.0 mg of Lowry protein per ml) in 400 ml of deionized water. This medium was autoclaved in the fermentor. To it was added 100 ml of filter-sterilized solution containing 5 g of glucose and 2.5 g of Na_2HPO_4, water, and 1.0 ml of filter-sterilized mineral salts solution (5.0% $MgSO_4$, 0.5% $MnSO_4$, and 0.5% iron citrate). The pH was adjusted to the desired value by the addition of 5 N NaOH.

Cultural methods. The fermentor system has been described previously (4). Control of DOT and pH was maintained within ±0.002 atm and ±0.1 pH unit, respectively. Anaerobic conditions were achieved with nitrogen sparging. The flow of titrant (5 N NaOH) and air/oxygen required to maintain the desired pH and DOT were monitored at 15-min intervals throughout the fermentation. When glucose concentration was maintained above depletion, sterile 40% glucose solution was added at the rate of 2.5 ml/ml of titrant used. The fermentor volume was monitored at 30-min intervals using previously calibrated measurements of medium height in the fermentor beaker. These readings were accurate to a volume of ±5%.

Measurements of the volumetric oxygen transfer coefficient during fermentations were performed using the method of Bandyopadhyay and Humphrey (2).

Assay procedures. Measurements of bacterial density, viable cell numbers, enterotoxin, and glucose concentrations were performed as described previously (4). PF activity is reported as PF units, which represent the dose required to elicit a standard area of blueing in the rabbit skin test. Details of this have been given previously (12). Values given are averages of two samples, each tested with six to eight replications.

Extracellular protein was determined by the method described by Carpenter and Silverman (3). No acid-precipitable material was present in the original medium.

Protease activity was determined with casein as the substrate using the procedure described by Cowman and Speck (6).

RESULTS

The effect of glucose and pH on growth and PF production. Figure 1 shows the effect of glucose on the growth of and PF production by *B. cereus* B-4ac. When the culture was grown in the medium with no added glucose (Fig. 1A), growth was very much slower than when glucose was present (Fig. 1B and C). The slight drop in pH in Fig. 1A was due to the small amount of fermentable carbohydrate present in the basal

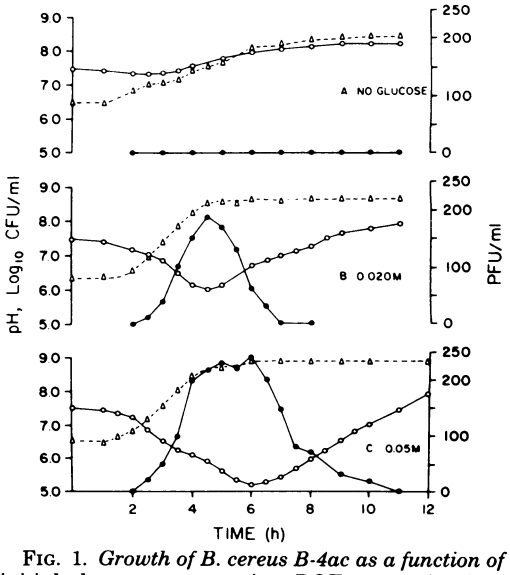

FIG. 1. *Growth of B. cereus B-4ac as a function of initial glucose concentration. DOT was maintained at 0.02 atm. Symbols: (△) growth; (○) pH change; (●) PF titer. PFU, PF units; CFU, colony-forming units.*

medium. Without added carbohydrate, no PF was detectable at any time during the fermentation. At an initial glucose concentration of 0.025 M (Fig. 1B), exponential growth was complete by 4.5 h, during which time the pH decreased to a minimum of 6.1. Glucose depletion occurred at this point, and the pH soon began to rise. Toxic activity increased throughout exponential growth, peaked at the point of glucose exhaustion, and then decreased as the pH rose.

The net loss of toxic activity that began at the time of glucose depletion occurred only in the presence of cells. Culture fluid collected at that time, filter-sterilized, and replaced in the fermentor under identical conditions as the original culture retained full toxic activity for several hours. We were unable to detect the activity of any protease in culture fluid during the first hour after depletion, though increasing activity was demonstrable after this time. Since PF is highly sensitive to the action of proteolytic enzymes (17), it appears that proteolytic turnover, if responsible, may be occurring at the cell surface under the conditions employed here. We have not investigated this aspect further as yet.

The addition of more glucose to the growth medium delayed depletion and also delayed the loss of PF activity, but did not cause any significant increase in maximum PF yield (Fig. 1C). Initial concentrations of glucose greater than 0.10 M resulted in complete inhibition of growth once the pH dropped below 5.0. For initial concentrations of glucose less than 0.025 M, PF production was directly related to glucose concentration.

Maintaining a constant pH during the course of fermentation proved to be an extremely important factor in boosting PF production. Table 1 shows that, under comparable fermentation conditions, cultures in which pH was maintained at 7.0 produced much more PF than those in which pH was uncontrolled. The magnitude of this increase was about 6-fold when the initial concentration was 0.025 M and 12-fold when the concentration was 0.20 M. When pH was controlled, growth and PF concentration were not limited by pH inhibition. They then reached levels related to initial glucose concentration up to 0.20 M. Above this, more glucose had no advantageous effect. The overall productivity in pH-controlled fermentations decreased about 50% with increasing glucose concentrations in the range studied. At all concentrations, however, pH control caused a significant increase in the efficiency of net PF units per cell. The ratio of PF units to exoprotein production was fairly constant when pH was not controlled. When pH was controlled, it was twofold higher at 0.025 M glucose and decreased with increasing glucose concentration.

The effect of the pH level at which the fermentation is maintained is shown in Table 2. Glucose concentration was kept at or above 0.02 M throughout the fermentation for each pH tested. Both growth and overall PF productivity were high within the pH range 6.5 to 8.0, with the best yields achieved in the range of 7.0 to 7.5. Growth and toxigenesis declined appreciably above pH 8.0 or below 6.5. The comparison of toxigenesis to total exoprotein production shows that PF production is much more sensitive to changes in pH than is exoprotein production as a whole. The sharp decrease in productivity below pH 6.5 explains the poor PF yields in fermentors run without pH control. In these runs, the pH dropped quickly to 6.5 or below,

TABLE 1. *PF yield from B. cereus B-4ac in response to initial glucose concentration in pH-controlled and uncontrolled fermentations*[a]

Initial glucose concn (M)	pH control[b]	Harvest time (h)[c]	Turbidity at harvest (Klett units)	PF units per ml	Overall productivity[d]	Exoprotein (mg/ml)	PF units per mg of exoprotein
None added	None (7.9)	8.00	235	<20	<0.011	0.008	<2,500
None added	7.0	8.00	255	<20	<0.010	0.009	<2,200
0.025	None (6.2)	4.50	310	180	0.13	0.012	15,000
0.025	7.0	2.50	225	1,150	2.04	0.036	32,000
0.050	None (5.7)	4.50	350	160	0.10	0.014	11,000
0.050	7.0	3.25	480	1,800	1.15	0.065	28,000
0.10	None (5.3)	4.50	365	210	0.13	0.014	15,000
0.10	7.0	3.75	750	2,300	0.82	0.10	23,000
0.20	None (5.0)	4.50	365	220	0.13	0.016	14,000
0.20	7.0	4.00	865	2,600	0.75	0.13	20,000

[a] DOT was maintained at 0.02 atm.

[b] Number in parentheses is pH at time of harvest. Controlled fermentations were maintained at pH 7.0.

[c] Harvest time is the point of maximum PF titer.

[d] Overall productivity is defined as measurable PF (PF units per ml) at harvest time divided by the turbidity (Klett units) and elapsed time (h).

and very little further PF production took place.

A typical fermentation with glucose maintenance and pH and DOT control is given in Fig. 2. Accumulation of PF was rapid and was complete after 4.0 h. The loss of toxic activity was equally rapid, and none was detectable at 6.5 h. This loss was more rapid than that seen in uncontrolled-pH fermentations even though detectable proteolytic activity in culture filtrates was delayed until well after most of the toxin loss had occurred. The upper curve in Fig. 2 demonstrates that productivity was highest at the earliest time at which toxin could be detected. It decreased thereafter as the culture proceeded to mid- and late-exponential growth. At a point in the transition phase, the productivity became negative.

TABLE 2. *Effect of controlled pH on growth and PF production by B. cereus B-4ac when glucose is not limiting*[a]

pH	Harvest time (h)	Turbidity at harvest (Klett units)	PF units per ml	Overall productivity	Exoprotein (mg/ml)	PF units per mg of exoprotein
5.0	8.75	325	<20	<0.007	0.007	<2,900
5.5	6.75	560	<20	<0.006	0.011	<1,800
6.0	5.50	745	200	0.049	0.062	3,200
6.5	4.50	795	1,620	0.45	0.11	15,000
7.0	4.25	820	2,700	0.73	0.12	22,000
7.5	4.00	835	2,700	0.80	0.14	19,000
8.0	4.50	805	2,400	0.67	0.11	22,000
8.5	6.25	640	540	0.14	0.043	13,000
9.0	8.25	310	<20	<0.007	0.006	3,300

[a] DOT was maintained at 0.02 atm.

A problem which occurs in the batch production of PF for use in other experiments is apparent from the data in Fig. 2. It is imperative that one be able to determine the point of maximum PF titer within a short time of its occurrence to prevent a significant loss of PF activity. In fermentations without pH control, the pH minimum is a very useful indicator of the proper harvest time. Even though toxigenesis may have ceased at some point prior to this, little if any degradation occurs before the pH begins to rise again. When pH is controlled, the "titrant utilization profile" provides an equally useful indicator of the proper harvest time. The most sensitive and useful plot is given in Fig. 3. Since the titration system employed with our fermentor operated intermittently, wide variations could

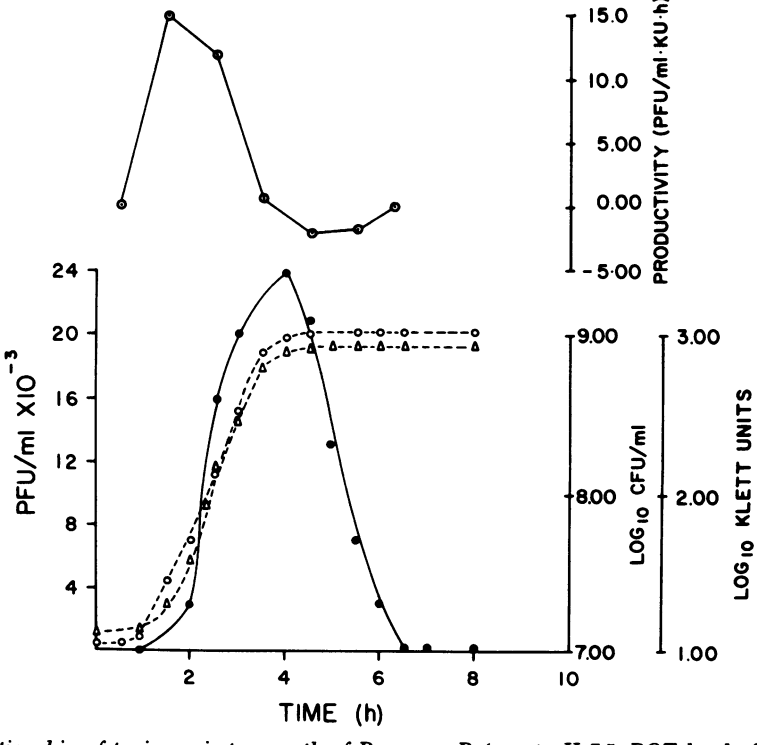

FIG. 2. *Relationship of toxigenesis to growth of B. cereus B-4ac at pH 7.5, DOT level of 0.02 atm, and glucose concentration of 0.020 to 0.050 M. Symbols:* (△) *turbidity;* (○) *colony-forming units (CFU) per ml;* (●) *PF titer; and* (◎) *productivity. PFU, PF units; KU, Klett units.*

occur in the volume of titrant added during each short time interval observed. To obtain a smooth curve, we first calculated the total volume of titrant used per elapsed time (N/t) as a function of elapsed time (t). The peak of PF activity is close to the point that $d(N/t)/dt = 0$. In practice, however, this point is difficult to calculate as the fermentation is progressing. Instead, we used the plot of $\Delta(N/t)/\Delta t$ versus t, which is easy to calculate and a very sensitive indicator of the proper harvest time. This plot yields a fairly stable curve for the latter stages of the fermentation (Fig. 3, top curve). As a working principle, we stopped the fermentation as soon as we determined two consecutive decreases in the value of $\Delta(N/t)/\Delta t$.

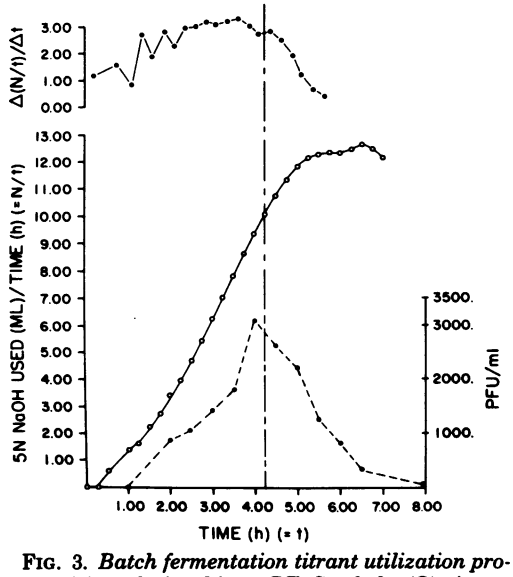

FIG. 3. *Batch fermentation titrant utilization profile and its relationship to PF. Symbols:* (○) *titrant utilization profile;* (●- - -●) *production/degradation. Top curve* (●—●) *indicates incremental rates of titrant utilization. Vertical broken line shows point at which fermentation would normally be stopped to recover PF. PFU, PF units.*

The effect of DOT on growth and PF production. Table 3 presents the overall response of PF production by *B. cereus* B-4ac to various levels of DOT. Maximum overall productivity was achieved when the DOT was maintained at 0.002 atm. The shortest generation time, however, was obtained in cultures grown at 0.10 atm. Both growth rate and PF production dropped fairly uniformly on both sides of their respective DOT optimums. Total exoprotein remained relatively constant in the aerobic cultures and was less affected by anaerobiosis than was PF production. Hyperoxic conditions had little or no effect on cell growth, but severely inhibited the production of PF.

The turbidity at time of harvest (i.e., maximum PF titer) was constant throughout the range of DOT studied, with the exception of the culture grown under anerobic conditions. The turbidity at harvest time represented 1×10^9 to 2×10^9 colony-forming units per ml under the cultural conditions used. A sharp rise and fall in PF titer, as noted in Fig. 2, was characteristic of all conditions but one (strongly oxygen limited) that support appreciable PF synthesis. We have previously described the stabilization of PF titer at very low oxygen tensions (4). Plots of productivity versus culture age, similar to that in Fig. 2, drawn from different DOTs vary little in shape. The inactivation phase was far less efficient than the maximum productive phase at all DOT levels studied.

In Fig. 4, productivity has been plotted against turbidity so that the effect of DOT alone can be compared. The ratio of productivities at 0.002 atm and 0.2 atm varied from 1.3 to 1.5 at mid-exponential phase to 7 to 8 during the later exponential-transition phase. Productivity end points occurred at somewhat lower cultural densities at higher DOT levels. Although this

TABLE 3. *PF production by B. cereus B-4ac at various levels of DOT (pH maintained at 7.5)*

DOT (atm)	Harvest time (h)	Generation time (min)	Turbidity at harvest (Klett units)	PF units per ml	Overall productivity	Exoprotein (mg/ml)	PF units per mg of exoprotein
0.40	3.75	31	905	400	0.12	0.08	5,000
0.20	3.50	27	910	1,800	0.57	0.10	18,000
0.15	3.50	26	885	1,800	0.58	0.13	14,000
0.10	3.25	24	895	2,400	0.83	0.13	18,000
0.05	3.50	26	910	2,700	0.85	0.17	16,000
0.02	3.75	29	925	3,300	0.95	0.14	24,000
0.005	3.75	32	930	3,500	1.00	0.12	29,000
0.002	4.25	36	930	4,100	1.04	0.13	31,000
ND[a]	4.50	39	895	3,200	0.79	0.16	20,000
Anaerobic	6.25	55	600	900	0.24	0.049	18,000

[a] ND, Not detectable, i.e., aeration was maintained at as great a rate as possible without raising the DOT to a detectable level.

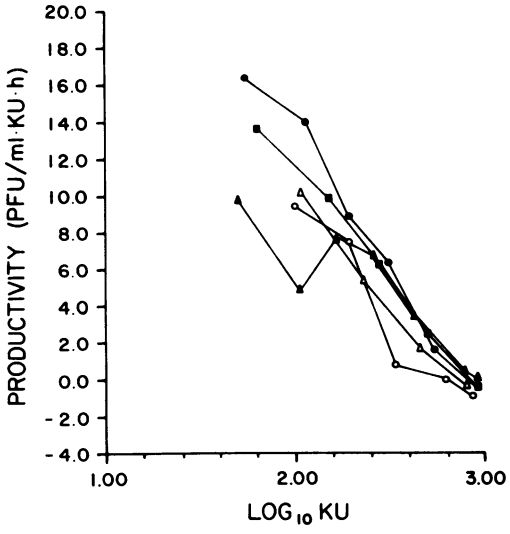

FIG. 4. *Positive incremental productivities of PF (PF units, PFU) at various DOTs as a function of culture density (Klett units, Ku). Symbols:* (○) *0.02 atm;* (△) *0.10 atm;* (■) *0.02 atm;* (●) *0.002 atm;* (▲) *not detectable. (See Table 3 for explanation.) pH was maintained at 7.5.*

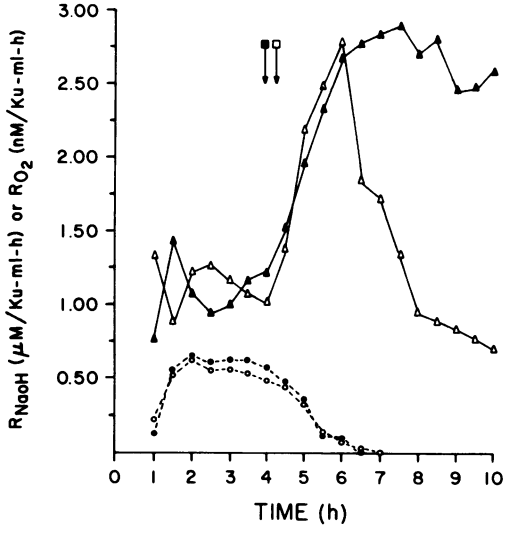

FIG. 5. *Titrant (R_{NaOH}) and oxygen (R_{O_2}) requirement profiles for fermentations with and without glucose maintenance. Symbols—R_{NaOH}: glucose maintained* (○); *glucose depleted* (●); *R_{O_2}: glucose maintained* (△); *glucose depleted* (▲); *arrows indicate point of maximum PF titer in each fermentation. Ku, Klett units.*

has a strong impact on final PF titer, it represents less than one generation difference between end points. When the fermentation was carried out under strongly oxygen-limited conditions, maximum productivity was much reduced from that in more aerobic cultures. As the fermentation progressed however, productivity decreased less rapidly, and the reverse relationship occurred in the latter part of exponential phase. Overall, however, the decrease in productivity was relatively constant with increasing culture density, regardless of oxygen concentration.

Figure 5 shows the amount of oxygen (R_{O_2}) and titrant (R_{NaOH}) per unit of cell time that must be supplied to maintain two fermentations within the desired limits of DOT and pH. We were unable to determine actual oxygen uptake rates, but the volumetric oxygen mass transfer rate (K_La, min^{-1}) was monitored during the course of several preliminary fermentations and did not change appreciably with increasing cell density. It increased from 1.2 to 1.4 during the course of growth. The increase in oxygen supply rate, thus, reflects mainly an increase in cellular requirements. Both fermentations were run at a DOT of 0.002 atm and a pH of 7.0. In one, glucose was depleted at 5 h, whereas the other maintained a concentration of between 0.005 and 0.010 M at all times. The exponential growth phase in each fermentation was marked by a high stable R_{NaOH} and low R_{O_2}, and decreasing R_{NaOH} coincided with PF loss in both cultures. At 6 h, however, R_{O_2} began to drop rapidly in the glucose-depleted culture, but remained high in the other. Microscopic examination from this point on revealed that a significant fraction of cells in the depleted culture proceeded to sporulate, whereas many in the nondepleted culture eventually lysed. Sporulating cells were only very rarely observed in the nondepleted culture. Shutting off the oxygen supply at the point of rising R_{O_2} prevented the further increase in culture turbidity, completely inhibited sporulation, and prevented the loss of toxic activity.

DISCUSSION

These studies on the production of *B. cereus* permeability factor demonstrate that toxigenesis is possible in a variety of environments, providing that basic nutritional requirements are met. Since PF production is enhanced at low levels of DOT, if toxigenesis does occur in foods, it is probably greatest at some point in the interior rather than at the surface.

Several distinct patterns exist with respect to PF production during the course of a batch fermentation. The level of DOT exerts a strong influence on the magnitude of toxigenesis at any given time during a fermentation. But a pattern of decreasing productivity and cessation of PF

synthesis as a function of culture density is also evident. This pattern is unaffected by manipulations of the organisms' available oxygen. We have been unable to demonstrate whether the pattern results from a generalized decrease in each cell's productivity or the result of a progressive increase in the fraction of nonproductive cells in the population. The latter view would fit more closely with Fraser and Baird's (9) observation that populations of *B. cereus* at various stages in the growth cycle are heterogeneous with respect to several distinct cell types. Increasing cultural density is marked by transitions in the ratios of these cell types rather than by intermediate stages in individual cells.

The observable loss of PF activity occurs at a point at which the culture appears to be in transition to the sporulation phase. We do not know, however, if the degradation of PF activity is occurring before this and is masked by production, or whether PF degradation occurs only after a new cellular physiological state has been established at this transition point. It seems fairly certain that the PF-degrading factor is not an extracellular protease of *B. cereus*, even though PF is sensitive to proteolytic degradation (18). The kinetics of toxin loss in glucose-depleted cultures is similar to that in cultures in which glucose is maintained. Loss of PF titer precedes the finding of proteolytic activity in culture filtrates. Also, PF titer is relatively stable in filtrates but is lost rapidly in the presence of cells. This suggests that a membrane-associated protease may be responsible.

The factor responsible presumably falls in the category Dancer and Mandelstam (7) describe as "biochemical events related temporally to sporulation but not dependent events or side events of the sporulation process," since toxin degradation still occurs under catabolite repression of sporulation. Sporulation in *B. cereus* and other *Bacillus* spp. appears to be under general catabolite control, and this has been shown to affect, as well, the production of metallo-protease and other inducible enzymes (1, 15). On the other hand, the production of another group of inducible enzymes related temporally to sporulation has been shown to be insensitive to the inhibition of sporulation (5, 7). This group includes α-amylase, histidase, and sucrase. Presumably, it also includes the PF-degrading factor. If not, this activity represents an extremely early event in the sporulation process.

The postexponential-phase loss of PF activity coincided with an increased cellular oxygen demand and could be prevented by restricting the supply of oxygen at this point. Increased respiratory activity is, thus, intimately related to PF loss. Felix and Lundgren (8) have observed a progressive increase in enzymes of electron transport and membrane cytochromes in *B. cereus* as cultures progress through the exponential phase, but before the transition to sporulation occurs. The respiratory apparatus is available for use when needed, with no significant lag period for macromolecular synthesis. It has long been known that the sporulation process coincides with a rapid increase in electron transport activity and a high cellular oxygen demand (13). As we have noted, the initial rise in R_{O_2} can occur even when glucose is still present, though it remains high only for glucose-depleted cultures in which sporulation does occur.

ACKNOWLEDGMENTS

W.M.S. gratefully acknowledges the Postdoctoral Research Associateship from the National Academy of Sciences, National Research Council, Washington, D.C., in support of a portion of this study.

We also thank T. J. Brown for conducting the biological assays.

LITERATURE CITED

1. **Aronson, S. I., N. Anaelo, and S. C. Holt.** 1971. Regulation of extracellular protease production in *Bacillus cereus* T: characterization of mutants producing altered amounts of protease. J. Bacteriol. **106**:1016–1025.
2. **Bandyopadhyay, B., and A. E. Humphrey.** 1967. Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems. Biotechnol. Bioeng. **9**:533–544.
3. **Carpenter, D. F., and G. J. Silverman.** 1974. Staphylococcal enterotoxin B and nuclease production under controlled dissolved oxygen conditions. Appl. Microbiol. **28**:628–637.
4. **Carpenter, D. F., W. M. Spira, and G. J. Silverman.** 1975. Effects of dissolved oxygen tension on bacterial enterotoxin production. Dev. Ind. Microbiol. **17**: 363–374.
5. **Coote, J. G.** 1974. Comparative studies on induction of sporulation and synthesis of inducible enzymes in *Bacillus subtilis*. J. Bacteriol. **120**:1102–1108.
6. **Cowman, R. A., and M. L. Speck.** 1967. Proteinase enzyme system of lactic streptococci. I. Isolation and partial characterization. Appl. Microbiol. **15**:851–856.
7. **Dancer, B. N., and J. Mandelstam.** 1975. Criteria for categorizing early biochemical events occurring during sporulation of *Bacillus cereus*. J. Bacteriol. **121**: 411–415.
8. **Felix, J. A., and D. G. Lundgren.** 1973. Election transport system associated with membranes of *Bacillus cereus* during vegetative growth and sporulation. J. Bacteriol. **115**:552–559.
9. **Fraser, D. K., and J. P. Baird.** 1968. Evolution of developmental stages in cultures of *Bacillus cereus*. J. Gen. Microbiol. **51**:393–402.
10. **Glatz, B. A., and J. M. Goepfert.** 1973. Extracellular factor synthesized by *Bacillus cereus* which evokes a dermal reaction in guinea pigs. Infect. Immun. **8**:25–29.
11. **Glatz, B. A., and J. M. Goepfert.** 1976. Defined conditions for synthesis of *Bacillus cereus* enterotoxin by fermentor-grown cultures. Appl. Environ. Microbiol. **32**:400–404.
12. **Glatz, B. A., W. M. Spira, and J. M. Goepfert.** 1974. Alteration of vascular permeability in rabbits by culture

filtrates of *Bacillus cereus* and related species. Infect. Immun. **10**:299–303.

13. **Goepfert, J. M., W. M. Spira, and H. U. Kim.** 1972. *Bacillus cereus*: food poisoning organism. A review. J. Milk Food Technol. **35**:213–227.
14. **Harrison, D. E. F.** 1972. Physiological effects of dissolved oxygen tension and redox potential on growing populations of microorganisms. J. Appl. Chem. Biotechnol. **22**:417–440.
15. **Levisohn, S., and A. I. Aronson.** 1967. Regulation of extracellular protease production in *Bacillus cereus*. J. Bacteriol. **93**:1023–1030.
16. **Melling, J., B. J. Capel, P. C. B. Turnbull, and R. J. Gilbert.** 1976. Identification of a novel enterotoxigenic activity associated with *Bacillus cereus*. J. Clin. Pathol. **29**:938–940.
17. **Spira, W. M., and J. M. Goepfert.** 1972. *Bacillus cereus*-induced fluid accumulation in rabbit ileal loops. Appl. Microbiol. **24**:341–348.
18. **Spira, W. M., and J. M. Goepfert.** 1975. Biological characteristics of an enterotoxin produced by *Bacillus cereus*. Can. J. Microbiol. **21**:1236–1246.
19. **Turnbull, P. C.** 1976. Studies on the production of enterotoxins by *Bacillus cereus*. J. Clin. Pathol. **29**:941–948.