Studies on Induction and Repression in Activated Sludge Systems

ANTHONY F. GAUDY, JR.

Division of Sanitary and Public Health Engineering, School of Civil Engineering, Oklahoma State University, Stillwater, Oklahoma

Received for publication December 20, 1961

ABSTRACT

GAUDY, A. F., JR. (Oklahoma State University, Stillwater). Studies on induction and repression in activated sludge systems. Appl. Microbiol. **10**:264–271. 1962.—Both repression and induction of substrate utilization have been the subject of many basic research investigations employing pure cultures. In this investigation these effects were studied using heterogeneous microbial populations prevalent in such biological treatment processes as activated sludge systems.

Diauxic substrate removal by activated sludge was observed in a multicomponent medium consisting of glucose and sorbitol. The sludge was acclimated solely to sorbitol; however, the presence of glucose blocked sorbitol removal until glucose was completely utilized. Both diphasic and triphasic oxygen utilization was shown for activated sludges metabolizing multicomponent synthetic wastes consisting of glucose, melibiose, and lactose. It appears from these studies that melibiose utilization was suppressed by the presence of glucose and, although melibiose induced acclimation to lactose, the presence of melibose suppressed lactose utilization. Studies were also conducted using glycogen and starch systems in which it was found that acclimation to either compound conferred immediate acclimation to the other. It was also found that loss of acclimation to lactose was a passive phenomenon and its kinetics could be predicted on the basis of simple diluting out of the enzyme(s) responsible for such acclimation.

Much basic research has been accomplished concerning bacterial acclimation and metabolism in multisubstrate media. Many of these findings, if of general occurrence in natural systems, have serious implications for biological waste treatment systems wherein multicomponent carbon sources as well as heterogeneous populations generally occur. A brief review of work reported in the basic literature will serve to delineate a type of phenomenon which has been studied in a number of pure culture systems and herein investigated using a heterogeneous population.

Monod (1949) reported diphasic growth of *Escherichia coli* and *Bacillus subtilis* in multisubstrate media; such growth of bacterial cultures is typified by the preferential use of one substrate when two or more substrates are present in the culture medium. This is not simply a case of more rapid acclimation to one or the other compound since organisms may be previously acclimated to a specific compound, yet when these cells are placed in a medium containing that compound and another (or others) to which they have not been previously acclimated, the new compound may be completely removed from the medium before the initiation of metabolism of the compound to which cells were acclimated.

Ravin (1952) has demonstrated diauxie in *Aerobacter aerogenes*. Glucose, in combination with compounds such as citrate, fumarate, glutamate, pyruvic acid, and glycerol, among others, yielded diauxic growth. From his studies using citrate$^+$ and citrate$^-$ mutants, Ravin concluded that mutation and selection were not involved in diauxie, but that it was due to a physiological adaptation by individual cells.

Using the same organism, Dagley and Dawes (1953) found, during diauxic growth on glucose and citrate of citrate-acclimated cells, that ability to respire citrate decreased rapidly during the first, or glucose, growth cycle. After glucose was exhausted from the medium an acclimation period was needed before growth on citrate was initiated.

Davis (1956) using *A. aerogenes* found that as little as 10 mg per liter of glucose in the medium completely blocked the growth response to citrate. Fructose and sorbitol also blocked citrate utilization but succinate, lactose, or xylose did not. Since citrate was known to be an intracellular intermediate (demonstrated with cell extracts), the logical conclusion in this case was that glucose interfered with the membrane transport mechanism (permeability).

Diauxic effects with *Pseudomonas aeruginosa* have been reported by Hamilton and Dawes (1959; 1960). However, in contrast to the results cited above, when citrate and glucose comprise the dual substrate medium, glucose metabolism is suppressed until the citrate concentration falls to a very low level. Preferential citrate utilization was also found for nonproliferating cell suspensions. In addition it was found that cells, grown on citrate, succinate, malate, fumerate, acetate, or peptone as sole carbon sources, exhibit a lag when transferred to glucose as a sole source of carbon. When cells were grown on glucose, high levels of such enzymes as glucose and gluconic dehydrogenases, hexokinase, and other enzymes of the Entner-Douderoff pathway were found. However, cells grown on the organic acids contained very low levels of these enzymes. This would seem to indicate an entire

shift of metabolic pathway. Studies using chloramphenicol indicated that acclimation to glucose involved a net synthesis of enzyme rather than activation of pre-existing enzymes.

This brief review serves to demonstrate a growing body of evidence supporting the concept that diphasic growth is the result of a general type of control mechanism in which various compounds (generally normal metabolites, but not restricted to such compounds) repress the formation of enzymes required to metabolize other compounds. Neither induction nor repression of enzyme formation is restricted solely to growing cultures. Mandelstam (1961) has shown that strains of *E. coli* deprived of an exogenous source of nitrogen can synthesize considerable amounts of β-galactosidase at the expense of endogenous protein; however, the rate of enzyme production is greatly curtailed. Under these conditions, a variety of compounds which can serve as an energy source to the cells suppress β-galactosidase formation.

The above studies employed pure cultures of microorganisms. However, diauxic effects are not a particular characteristic of any one species. If these effects are primarily controlled by substrate, rather than the response to various substrates of a few species, there is some basis for investigating the phenomenon using heterogeneous populations. Previous investigations using such populations supported this hypothesis (Gaudy, 1959) If such behavior is characteristic of natural populations, it could seriously affect the course of substrate removal during biological treatment of waste waters. Since waste streams exhibit a dynamic chemical composition, suppression of enzyme formation or function could prevent removal of new compounds in the incoming waste during the normal aeration or contact period.

Experiments were undertaken to explore these possibilities. Other acclimation phenomena of equal importance, such as the conferring of acclimation to one compound by acclimation to another and the rate of loss of acclimation, were also investigated using heterogeneous populations.

Materials and Methods

Growth was measured as increase in optical density at 540 mμ using the Evelyn[1] colorimeter and as increase in dry weight of cells using the membrane filter technique (Millipore,[2] HA 0.45 μ). Total substrate remaining in the medium was measured on the membrane filtrate using the chemical oxygen demand (COD) test (*Standard Methods for the Examination of Water, Sewage and Industrial Wastes*, APHA, 1960). Carbohydrate remaining in the medium was measured using the Anthrone test (Morris, 1948). Oxygen uptake was measured using the Warburg respirometer.

[1] Rubicon Co., Philadelphia, Pa.

[2] Millipore Filter Corporation, Bedford, Mass.

Since the development of populations and experimental techniques were varied for each study, these are described in the appropriate sections below.

Results

Diphasic substrate removal. The basal medium used for these studies contained (per liter): NH_4Cl, 500 mg; $MgSO_4 \cdot 7H_2O$, 500 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; $MnSO_4 \cdot H_2O$, 10 mg; $CaCl_2 \cdot 2H_2O$, 10 mg; 1 M potassium phosphate buffer (pH 7.0), 10 ml; tap water, 100 ml; distilled water to 1 liter. A mixed bacterial population was developed by adding 20 ml of settled sewage to 1 liter of the basal medium containing 1,000 mg of sorbitol per liter. This system was aerated for 12 hr, after which 20 ml of mixed liquor were transferred into 1 liter of fresh sorbitol medium. Successive transplanting of 20-ml portions of 12-hr mixed liquor over a 3-day period assured a thoroughly acclimated heterogenous population as adjudged by rapid growth during the aeration period. Microscopic examination revealed many morphologically different forms.

A portion of the biological sludge thus developed was harvested, washed three times in 0.05 M potassium phosphate buffer, and a small amount suspended in the basal medium. Fifty-milliliter portions of this suspension were placed in a series of flasks, and glucose and sorbitol were added to yield a final concentration of 300 mg of each per liter. The flasks were aerated on a reciprocal shaker at 30 C. At periodic intervals, flasks were removed and examined for cell growth and substrate removal.

At the beginning of the shaker study, Warburg flasks were also prepared containing the same cell suspension and the following carbon sources at the final concentrations indicated (per liter): glucose, 300 mg; sorbitol, 300 mg; glucose and sorbitol, 300 mg each. These flasks were used to measure oxygen utilization on the Warburg apparatus at 30 C.

The results of this study are shown in Fig. 1 and 2. Although the cells were thoroughly acclimated to sorbitol, it is seen in Fig. 1, using oxygen uptake as the parameter, that glucose was used at a faster rate than sorbitol when it was the sole source of carbon. When both substrates were present, the oxygen utilization was intermediate between the two single substrate curves. From these data alone, it cannot be concluded that substrate removal was diauxic.

Substrate removal and solids production for the two-substrate system are shown in Fig. 2. It is seen that the course of solids production was described equally well using either the membrane solids technique or optical density measurement; i.e., in the range of suspended solid concentrations encompassed in these studies, the two measurements were proportional. Both curves suggest diauxic or two-phase growth; however, this is not pronounced, there being only a slight lag between cycles.

The curve labeled *COD, glucose + sorbitol* shows the course of COD removal in the two-substrate system. The

theoretical COD of 300 mg of glucose plus 300 mg of sorbitol per liter is 663 mg. The actual initial COD obtained was slightly higher; this is primarily attributable to chlorides in the growth medium.

The curve labeled *COD, glucose* was obtained from measurements of the glucose concentration using the anthrone method since sorbitol does not react with anthrone. For convenience in plotting and comparing with the COD curve, glucose concentration was converted to COD using the stoichiometric relation:

$$\text{mg/liter glucose} \times \frac{192}{180} = \text{mg/liter COD, glucose}$$

The growth curves (*biological solids* and *optical density*) provide some indication that growth was diphasic. However, the major proof of the presence of the diauxic phenomenon is obtained from the COD removal curves. These curves show beyond doubt that the system was diauxic with respect to substrate removal. The results indicate that glucose was, within limits of measurement, totally removed before metabolism of sorbitol was begun. As would be expected, the slight diphasic tendency of the growth curves occurs in the region of glucose exhaustion. The lag period between growth cycles is fairly short, with sorbitol metabolism rapidly following the removal of glucose.

The basic significance of this experimentation is the demonstration of diauxic substrate removal with a heterogeneous population. The diauxic phenomenon would appear to be one of rather general occurrence. The results cannot be attributed simply to a higher rate of glucose metabolism since, if such were the case, diphasic growth would not be expected nor would all of one substrate be used before initiation of metabolism of the other to which the cells had been acclimated. It should be emphasized that no attempt was made to isolate various species from either the sewage seed or the sorbitol-grown sludge developed from this seed; indeed, simple microscopic observation provided definite assurance that the population was heterogeneous.

Induced acclimation. It is known that growth in the presence of some exogenous compounds can induce the synthesis of enzymes required to utilize others (Hogness, 1959). Thus *E. coli* grown on melibiose possesses β-galactosidase, an enzyme required for initial attack of lactose,

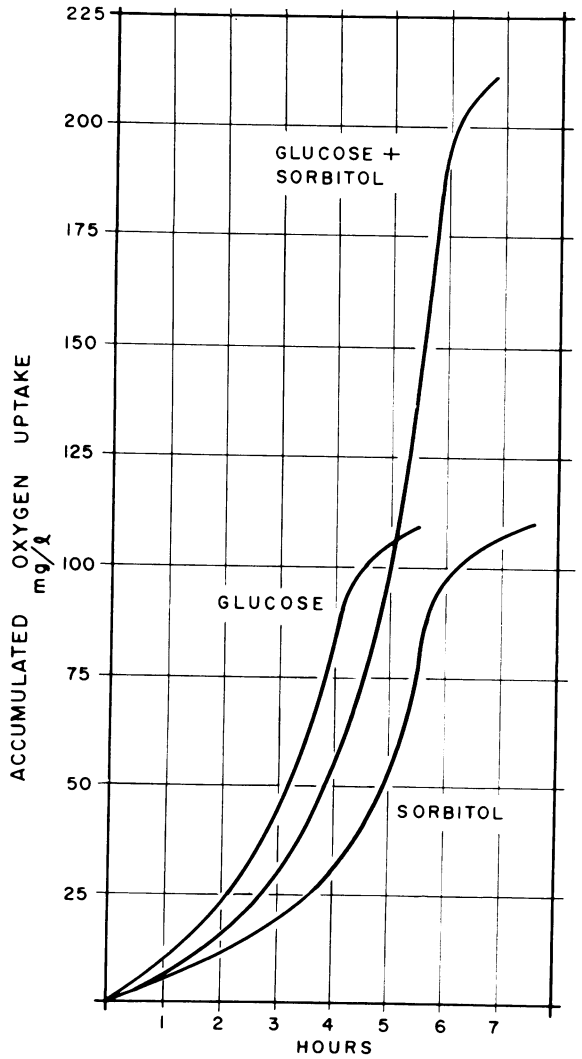

FIG. 1. *Oxygen uptake of sorbitol acclimated sludge on glucose, sorbitol, and combined substrates.*

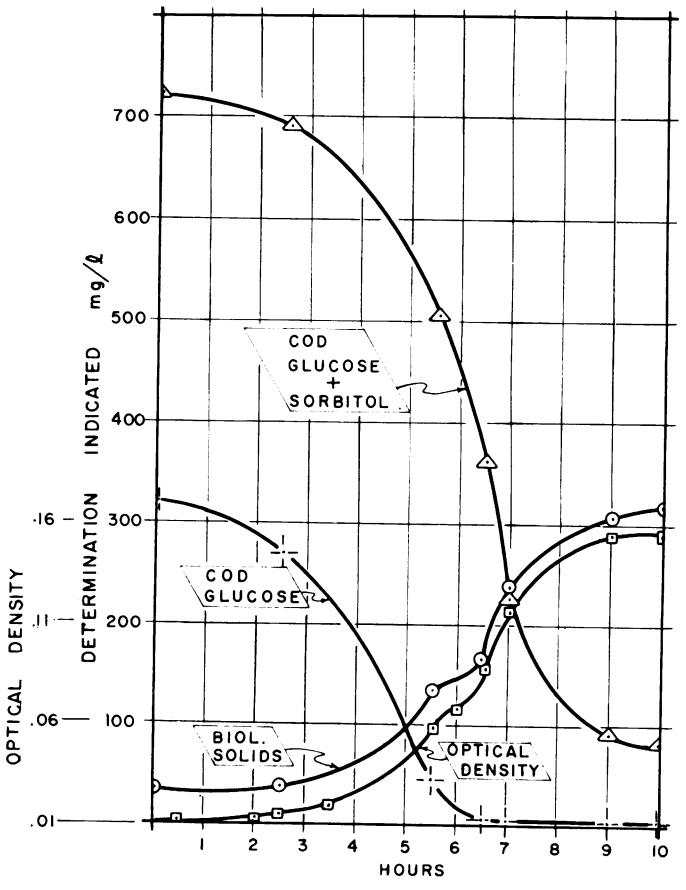

FIG. 2. *Substrate removal and growth in a multicomponent system; glucose, 300 mg per liter plus sorbitol, 300 mg per liter sludge acclimated to sorbitol.*

i.e., melibiose induces the production of β-galactosidase. In this case, although melibiose is not a substrate for the enzyme, it can be metabolized by the organism and serves as an energy source to the cell. Compounds which are not used as an energy source for the cell may also act as inducers of specific enzymes. In this case another compound must be present to supply the energy needed to synthesize enzyme. As in the case of diauxic growth, the induction by one waste water constituent of an enzyme critical to the initial attack of another compound could have important bearing on the course of waste purification. Accordingly, it was felt that initial investigation of the possibility of such occurrences in completely heterogeneous populations was warranted.

Cells used for these studies were harvested from a laboratory continuous flow activated sludge unit (Gaudy, Englebrecht, and De Moss, 1960) operating on a synthetic waste containing (per liter): NH_4Cl, 300 mg; $MgSO_4 \cdot 7H_2O$, 250 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; $MnSO_4 \cdot H_2O$, 10 mg; $CaCl_2 \cdot 2H_2O$, 10 mg; 1 M potassium phosphate buffer (pH 7.0), 10 ml; tap water, 100 ml; distilled water to 1 liter. Glucose was added to this basal medium at a final concentration of 1,000 mg per liter. After washing the cells in 0.05 M potassium phosphate buffer they were resuspended in the basal medium and portions placed in Warburg flasks along with the compound used as an inducer. After appropriate time intervals, as judged by oxygen uptake, the substrate used to test for induction was tipped in from the flask side arm. No data other than oxygen uptake were obtained in these studies, which involved the response to lactose after periods of incubation with melibiose. In these experiments temperature was maintained at 20 C.

Flasks were prepared containing the following substrates: glucose; lactose; melibiose; glucose plus melibiose; glucose plus melibiose, lactose tipped in at 4 hr; glucose plus melibiose, lactose tipped in at 10 hr; and glucose plus melibiose, lactose tipped in at 15.5 hr. The final concentration of each carbon source in the flask was 333 mg per liter.

The results of the experiment are shown in Fig. 3. The curves for all flasks except those containing lactose and melibiose alone were superimposable up to the 5-hr reading. Thereafter, the flask containing glucose alone showed a sharp break in oxygen uptake. The flask into which lactose had been tipped at 4 hr (*arrow no. 1*) continued to use oxygen at the same rate as the other three glucose-plus-melibiose flasks, i.e., all four curves were superimposable. At 10 hr, lactose was tipped into the second glucose-plus-melibiose flask (*arrow no. 2*). Oxygen utilization in all four systems continued to progress identically regardless of the introduction of lactose. After the 13th hr, the curve for the glucose-plus-melibiose control flask broke sharply, indicating exhaustion of both substrates. However, the flask into which lactose had been tipped at 10 hr continued to use oxygen at an increasing rate. The flask into which lactose had been tipped at 4 hr showed a slight break with the glucose-plus-melibiose control but, after 1 hr, continued to use oxygen at an increasing rate. At 15.5 hr (*arrow no. 3*), the last lactose tip-in was made. Response to lactose was rapid and the rate of oxygen utilization continually increased.

It is not proper to directly compare the lactose lag for the multiple substrate flasks with that for the flask containing lactose alone since the numbers of viable cells in the systems were continually increasing and the concentration of biological solids is expressed in the oxygen utilization. However, the lessening of the lag period after incubation with melibiose is unmistakable.

It is interesting to note the diphasic nature of the oxygen curve for the flask containing glucose plus melibiose into which no lactose was tipped. Since oxygen uptake is identical with that of the flask containing glucose alone up to 5 hr and since melibiose alone was used to some extent during the first 5 hr, it appears that the presence of glucose suppressed melibiose utilization. The adaptation to melibiose was under way, however, during the time glucose was being used since, upon exhaustion of the glucose,

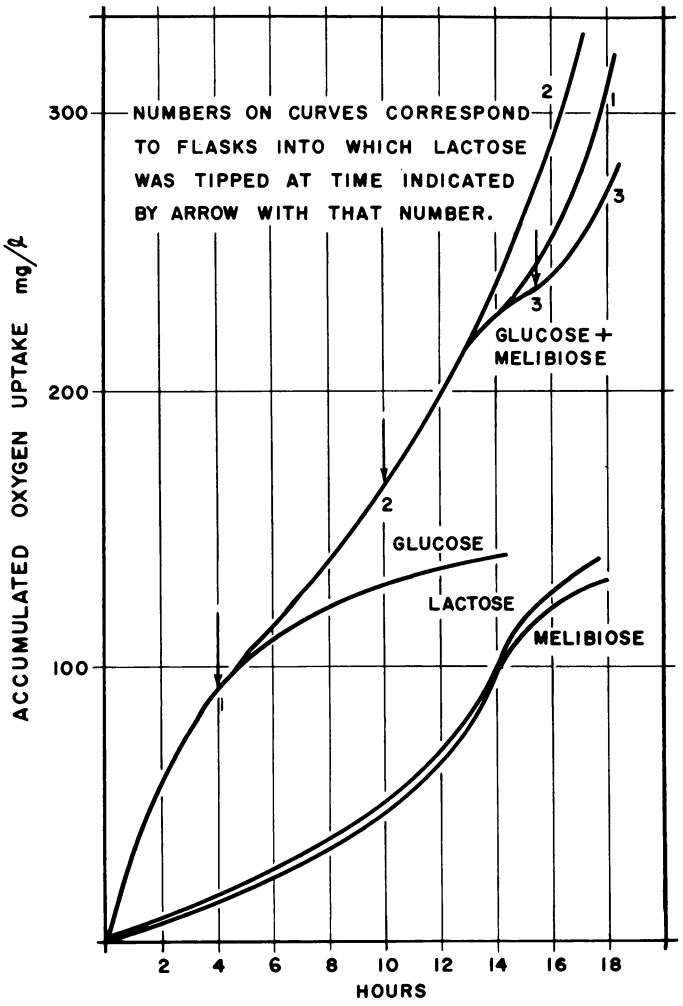

FIG. 3. *Induction of acclimation to lactose in a glucose-melibiose mixed substrate.*

the melibiose-glucose curve rose much more sharply than the curve for the flask containing melibiose alone. It seems apparent that the exogenous source of energy, represented by glucose, enhanced acclimation to melibiose but the presence of glucose caused diauxic growth. This is to say, expression of the acclimation may have been suppressed. It is also interesting to note that melibiose utilization suppressed lactose utilization. The induction occurred during the simultaneous presence of both substrates, as indicated by the nature of the curves after the glucose-melibiose substrate had been exhausted, but lactose was apparently not metabolized until the melibiose carbon source had been almost completely utilized. The time of lactose introduction was also important in the over-all behavior of these systems. When lactose was introduced before melibiose was actually being metabolized (*arrow no. 1*), the curve is triphasic; whereas, when it was introduced after melibiose utilization was well under way, the curve remains diphasic and continues on a course indicative of equal ability to use melibiose and lactose.

It is interesting to compare the various means of detecting diauxic substrate removal. It may be unequivocally detected by periodic measurement of the concentration of each substrate remaining in the medium or it may be indirectly observed by such effects of sequential substrate utilization as cell growth (optical density, cell weight, or viable count) and oxygen uptake. However, detection by measurement of its effect requires that there be a sufficient lag period between exhaustion of one substrate and initiation of metabolism of another compound. In some systems, for example the first set of experiments herein reported, (glucose-sorbitol at 30 C), detection would have been questionable as measured by the latter method. However, in the experiments described above (glucose-melibiose-lactose at 20 C), diphasic growth was clearly detected by oxygen uptake measurement alone. The above observations have provided significant direction for both basic and applied research presently under way in our laboratory pertaining to the conditions under which diphasic substrate removal may occur and under which it may be detected by measurements of its effects. In some respects, it is the effect rather than the gross cause which is of more immediate significance from an applied or engineering standpoint.

Multiple acclimation. In addition to induced acclimation, there would appear also a possibility of multiple acclimation; that is, acclimation to one compound may allow use of another simply because their related structure may allow use of both substrates by the same enzyme. Although much has been written about enzyme specificity, it is known that some enzymes exhibit multiple specificity, i.e., they may act, with varying rates, on more than one compound.

For example, starch and glycogen might be expected to exhibit, in some measure, this type of multiple acclimation since both consist predominantly of α 1 $\rightarrow$ 4 glucosidic bonds. This type of acclimation would seem important since initiation of glycogen utilization could occur not because starch induced enzyme(s) specifically required for glycogen metabolism, but because the enzyme(s) induced for starch could act upon glycogen; in such a case, no induction period may be needed to initiate metabolism of a new compound.

To investigate these aspects, the growth systems described below were prepared in Warburg flasks. All flasks were inoculated with washed suspensions of glucose-acclimated cells taken from a laboratory-scale continuous flow activated sludge unit previously cited. The basal medium in which the cells were suspended was the same as that used for the induced acclimation studies: glycogen; glycogen, glycogen tipped in 9 hr; glycogen, starch tipped in at 9 hr; starch; starch, starch tipped in at 9 hr; and starch, glycogen tipped in at 9 hr. The final concentration of each substrate in the flask was 333 mg per liter. Temperature was maintained at 20 C.

Oxygen uptake curves for glycogen and starch utilization are shown in Fig. 4 and 5, respectively. In Fig. 4, the lower curve depicts the course of glycogen oxidation by glucose-acclimated cells. To insure that the cells were acclimated, the systems were allowed to grow until the rate of oxygen utilization had passed its maximum before tipping in the second substrate (9 hr on the lower curve). To measure the oxygen uptake due solely to the tipped-in glycogen, accumulated oxygen uptake was calculated considering the tip-in time (9 hr) as zero time. This was done

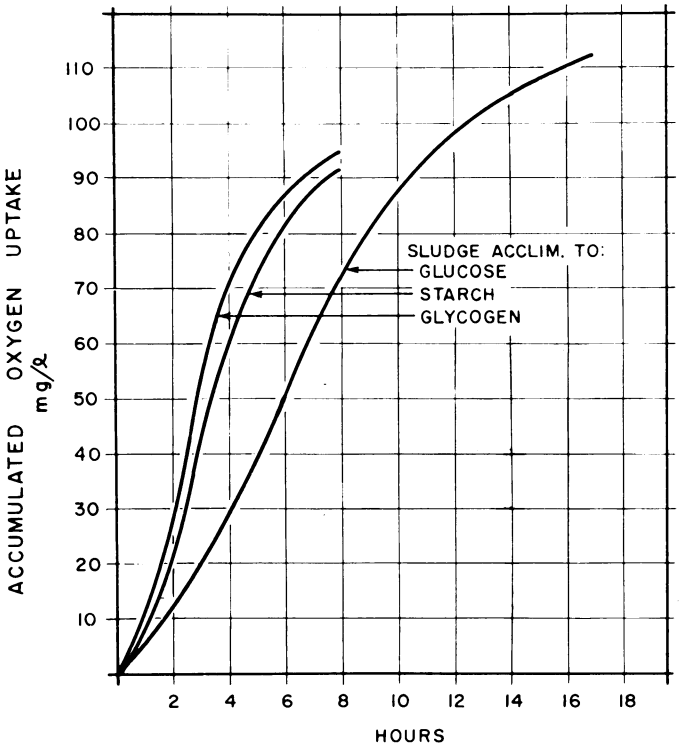

FIG. 4. *Utilization of glycogen by sludges acclimated to glucose, starch, and glycogen.*

simply by subtracting oxygen uptake due to residual substrate, either glycogen or starch. It was possible to make such a calculation since one flask of each system, to which no additional substrate was added, was retained as a control. It is emphasized that O_2 uptake of these glucose-grown cells was the same on starch and glycogen. To facilitate presentation of the results, the curves for oxygen utilization during glycogen metabolism after the 9-hr tip-in time were plotted from the zero time axis. Thus the two upper curves represent the O_2 uptake on glycogen by cells acclimated to glycogen and to starch. It is seen that acclimation to starch facilitates glycogen utilization. The same plotting technique was applied to starch-utilizing systems. These results are shown in Fig. 5. It is seen that acclimation to glycogen allows rapid utilization of starch.

Loss of acclimation. If the production of inducible enzyme is dependent upon the presence of an inducer, for example a substrate, it might be expected that a bacterial population could rapidly lose acclimation to a compound such as lactose upon its replacement by another substrate in the medium. If this situation occurred in heterogeneous cultures such as those in biological treatment units, it would be of considerable significance when the fluctuation in chemical composition of a waste stream is considered.

Experiments were designed, using lactose as the test substrate, to determine the rapidity of loss of acclimation under conditions similar to those in the activated sludge process. Glucose-grown cells were harvested from the continuous flow activated sludge unit and used as seed for the development of two batch activated sludges, one of which was acclimated to lactose and the other maintained on glucose. Both of these systems were operated by daily wasting one-third of the mixed liquor, centrifuging the remaining two-thirds, wasting one-third of the centrifuged supernatant, and adding two-thirds volume of fresh medium. The glucose system served as a control. After an acclimation period, the lactose-grown cells were transferred to glucose. The degree of deacclimation to lactose was measured as ability to respire lactose. This ability was tested daily until acclimation was returned to its basal rate for the system. The experimental design is shown in Fig. 6. All experiments were conducted at 25 C. The basal medium was the same as that used for the previous study. The concentration of carbon source in the batch systems was 2,000 mg per liter.

The control system, glucose-acclimated cells, exhibited a Q_{O_2} of only 20 toward lactose in contrast to a Q_{O_2} of 65 for the lactose-acclimated system. After changing the feed from lactose to glucose on day 4, the ability of the sludge to respire lactose returned in 6 days to that exhibited by the glucose-grown system.

The course of deacclimation is plotted in Fig. 7. The *dotted line* represents the theoretical loss of acclimation based upon the assumption that each day this loss should amount to one-third of the total remaining acquired acclimation. The rationale for this assumption is based upon the operational condition wherein one-third of the originally lactose-acclimated cells was removed each day. Since these systems were in solids balance, the daily

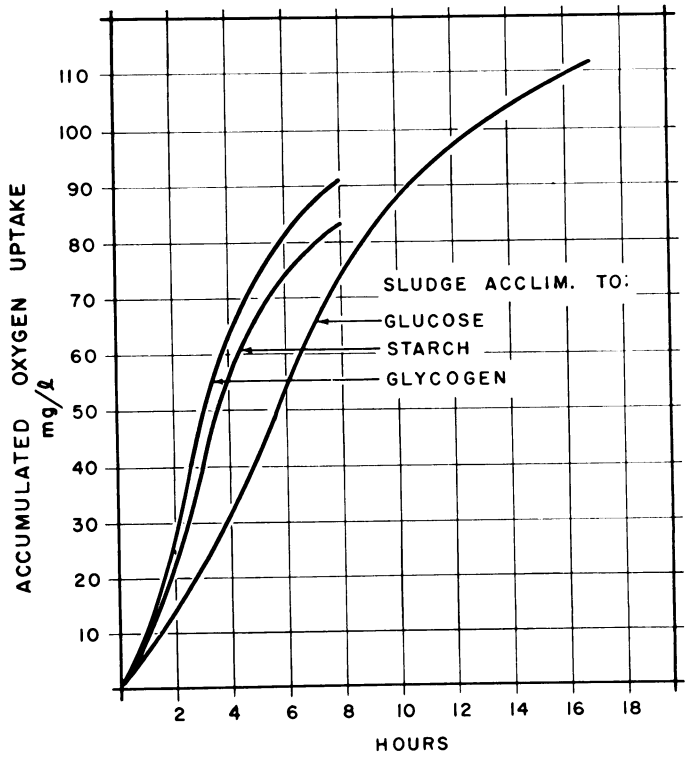

FIG. 5. *Utilization of starch by sludges acclimated to glucose, starch, and glycogen.*

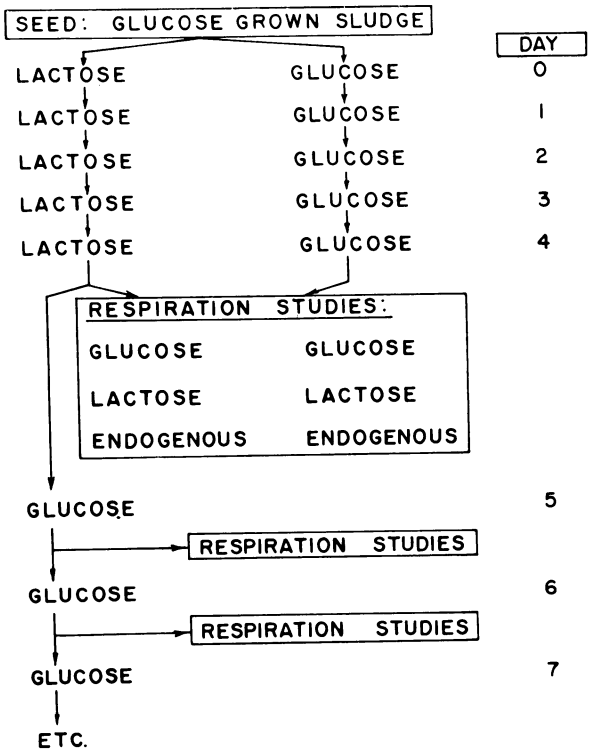

FIG. 6. *Enzyme diluation study*

replenishment of sludge consisting of cells devoid of β-galactosidase would be expected to lead to a one-third loss of acclimation each time the solids concentrations was rebalanced, in this instance, daily. With the exception of the observed 2-day Q_{O_2} for lactose, it is seen that the rate of loss predicted using the above assumption lies close to the observed loss.

The behavior of this system using a heterogeneous population appears to be consistent with conclusions drawn by workers in the biological sciences using pure cultures (Hogness, 1959). These studies indicate that the enzyme was not actively degraded in the absence of its inducer but was simply diluted out of the system.

Discussion and Summary

It is felt that the diphasic substrate removal studies using glucose and sorbitol are particularly significant because they clearly demonstrate, in a heterogeneous population, the diauxic phenomena cited in pure culture studies. It is also interesting to note that, although such a phenomenon was shown for the heterogeneous population, it could not be concluded from the oxygen uptake or the growth data (cell weight and optical density). Although there was a slight pause in both the optical density and cell weight curves, this could not be taken as conclusive evidence of diauxic growth. This result does, however, support the more concrete evidence provided by the substrate removal curves, which clearly show the removal of glucose before metabolism of sorbitol, although the population was acclimated to sorbitol. In some respects it is rather surprising, in a population consisting of many bacterial species, that the presence of one compound could completely block utilization of another to which the biological mass was acclimated. The results attest to the generality of diauxic phenomena. Since sorbitol was not used until the glucose was removed, it appears that the result observed was a response en masse of the entire population. It may be seen that changing chemical composition of a waste stream, in addition to using vital contact time for acclimation, in some cases may actually impede the use of a substrate to which the biological mass is already acclimated.

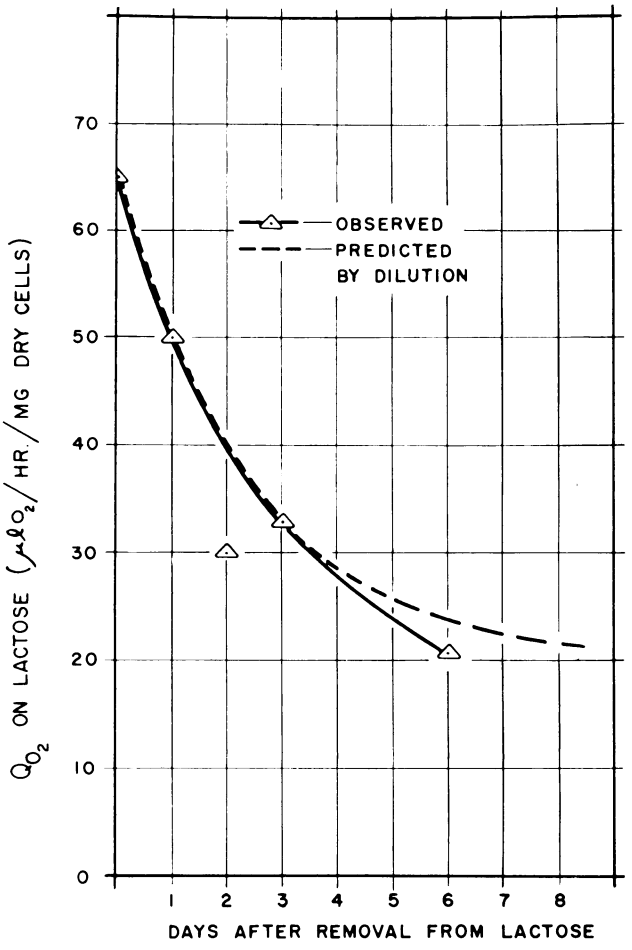

FIG. 7. *Loss of acclimation to lactose*

The induced acclimation studies using glucose, melibiose, and lactose also provide evidence of diauxic effects as well as induction of enzyme(s) required to initiate lactose metabolism. These cells were initially acclimated to glucose and, although the presence of glucose curtailed melibiose utilization, acclimation to melibiose must have begun during glucose utilization since only a slight lag was observed after the glucose curve broke off. Thus, a system may become poised for the rapid but sequential use of individual compounds in a multiple substrate medium. Again, in the induced acclimation study, although growth on melibiose induced acclimation to lactose, the system was diauxic for these substrates when lactose was introduced before expression of melibiose acclimation. When lactose was introduced during melibiose utilization there was no diauxic effect. From an applied standpoint, this result suggests that not only the occurrence of change in substrate components but the timing of such change may affect the response.

The results of the starch-glycogen studies indicate that less complicated polymers of the same structural units may be readily attacked by a sludge acclimated to the more complicated polymer as in the case of starch utilization by glycogen-acclimated sludge. In the reverse case, the attack was also easily initiated. It is emphasized that these were not in a strict sense multicomponent systems because most of the original substrate had been removed by the time the new substrate was introduced. It should also be noted that growth on glucose may supply the requisite enzymes for both starch and glycogen metabolism since similar polysaccharides may at some time in the growth cycle (particularly in the early phase) be internal substrates of the biological mass (Gaudy and Engelbrecht, 1960).

The passive nature of deacclimation, found for loss of acclimation to lactose, is deemed an asset in biological treatment since it indicates that the short periodic absence of a particular substrate in the waste stream may not necessitate an entire new cycle of acclimation. The

enzyme(s) responsible for initiation of metabolism of a substrate do not appear to be degraded in the absence of the substrate but are simply diluted out of the system. Although somewhat reassuring, this finding cannot be applied without due consideration of the particular treatment process in question. For example, under conditions wherein an abundant source of available nitrogen is present in the waste, the enzyme may be slowly diluted out as it was in these studies but, where the C:N ratio in the waste is high or when the external nitrogen source is not easily available, unused endogenous protein may serve as an available nitrogen source. Thus, in some waste treatment systems, the enzyme could be degraded.

It may be stated that findings of much of the research herein reported are in accord with conclusions of fairly recent basic research in the biological sciences using defined systems. That is to say, even though an entirely different experimental approach and design and quite different biological material were herein used, the same conclusions could be drawn. Although the phenomena investigated appear to hold for heterogeneous populations as well as in isolated pure culture studies, it is not yet known for what spectrum of population, substrates, and operational (experimental) conditions they are true; indeed, these are the subjects of continuing research. It is felt that the proof herein presented, that such an effect as diauxic substrate removal can occur in a heterogeneous population, represents a step forward in understanding the behavior of biological waste treatment processes, and serves as a useful adjunct to proving the universality of metabolic control processes which have been studied in various isolated and purified systems.

Acknowledgments

A portion of this work was conducted in the sanitary engineering laboratory of the University of Illinois and was partially supported by a fellowship grant from the Division of Research Grants, National Institutes of Health, U. S. Public Health Service. The author also wishes to express appreciation for the use of laboratory facilities to R. W. Wolfe and R. D. DeMoss, Microbiology Department, and D. Gottlieb, Plant Pathology Department, of the University of Illinois.

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