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Title SOME ASPECTS OF THE BIOLOGICAL METABOLISM OF A PARTIALLY COLLOIDAL SUBSTRATE

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An investigation was conducted to ascertain if certain concepts of microbial metabolism, derived from studies using simple soluble substrates, are applicable to the biological assimilation of complex, partially colloidal suspensions. A mixture containing one gram of Carnation dry-skim milk is considered to be a complex, partially colloidal substrate and was used as the medium for analysis throughout this inquiry.

The metabolism of the milk suspension was followed by means of a biological oxygen demand test (BOD test). The test was performed in such a manner that the point of maximum net cell growth was observed on the BOD curve through the occurrence of an inflection point called a plateau.

Experimental evidence shows that at the plateau 43 percent of the ultimate BOD of the milk suspension has been exerted through respiration and the remaining 57 percent is accounted for as new cell

growth.

Formulations were derived in the text showing that the biological oxygen demand encountered during the respiration phase of assimilation is directly related to the chemical oxygen demand of the milk suspension. These formulations when combined with experimental evidence yield the equation:

$$\text{Plateau BOD} = 0.49 \text{ COD},$$

where the Plateau BOD is the amount of oxygen (mg/l) removed from solution at the plateau, and COD is the initial chemical oxygen demand of the milk waste in mg./l.

Two activity indices were developed and used to determine plateau characteristics:

1. The slope of the endogenous curve of the seed organisms used in the BOD test,

and

2. an activity coefficient defined as the slope of the endogenous curve times 10^3 divided by the initial cell concentration of the BOD bottles.

It was shown that these activity indices are very closely related to the geometry of BOD curves.

Experimental evidence was recorded which shows that, under

the constraints of this study, at least ten hours of metabolism time were required before a plateau could appear. It was also noted that the time required for maximum cell growth to occur is related to the logarithm of the slope of the seed organisms' endogenous curve.

Relative activity coefficients multiplied by the slope of the seed organisms' endogenous curve were calculated. When this product was plotted against the time required for plateau appearance it was found that the product is optimum at about $-0.01 \frac{mg\,O_2}{l}$ per hour.

SOME ASPECTS OF THE BIOLOGICAL METABOLISM OF A PARTIALLY COLLOIDAL SUBSTRATE

by

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I. INTRODUCTION

The most frequently used tool in determining the pollution potential of a waste is the biological oxygen demand (BOD) test. This test is used as a basis for regulatory action, as a parameter in research, and as a basis for designing waste treatment works.

The test consists of bringing into contact a waste, or organic suspension, and bacteria. The microorganisms metabolize the organic molecules and in so doing remove dissolved oxygen from solution. The course of oxygen removal can be monitored and when plotted against time is referred to as a BOD curve.

BOD curves developed in the laboratory are frequently used as a basis for predicting variations in dissolved oxygen concentration, over a period of time, due to the metabolism of organic matter. Although a great deal of work has been done to insure the introduction of minimum error in relating BOD data to field conditions there is still apprehension about the use of such data. Hoover, Jasewicz and Porges have implied the questionable state of the arts by saying, "no one appears to consider it (the BOD test) adequately understood or well adapted to his work" (15).

Most researchers have explained the BOD test by investigating the reaction of the microbial environment to the presence of soluble

organic substrates. Although it is true that soluble organic substrates are frequently encountered in the field of water pollution it is also possible that paramount problems might stem from the presence of suspensions which contain, in part, collodial materials.

The impetus for this thesis is to determine whether or not some insight into the application of the BOD test might be gained by investigating certain components of the test during the microbial assimilation of a mixture of colloidal and soluble materials. A 0.1 percent solution of Carnation dry-skim milk contains both soluble and collodial materials; for this reason its biological degradation has been selected as an area of study.

The following introductory material discusses the biological metabolism of soluble organic substrates. The topics are selected in order to present a basis for understanding the principles behind the BOD test and to point out that the majority of research concerned with the fundamentals of metabolism has been conducted using soluble substrates.

In particular it is explained that biodegradable organic molecules are removed from the aquatic environment through respiration or synthesis followed by auto-oxidation. This removal process involves both the creation of new cells and the release and distribution of molecular energy.

Several pages of the introduction are devoted to describing

coupled reactions and the means by which part of the energy released during oxidation is stored for use during assimilation or other cellular functions.

It is noted that two schools of thought exist regarding the relationship between cell growth and the substrate's free-energy of oxidation:

1. The fraction of ultimate BOD going to cell synthesis varies with the chemical nature of the substrate,

and

2. the fraction of ultimate BOD going to cell synthesis does not vary with the chemical nature of the substrate.

Busch's work (2, 3, 21) is evaluated and shows that under some conditions a plateau in the BOD curve can be observed. It is at this point that cell growth reaches a maximum.

The section concludes by describing several theories concerning the existence of plateaus.

The Aerobic Decomposition of Organic Matter

When bacteria come in contact with suspended and dissolved organic matter the result is frequently a metabolism which produces as end products new cell growth, carbon dioxide, and water. This biological process is well documented and may be represented qualitatively by Equation 1.

$$\text{organic substrate} + O_2 \xrightarrow{\text{cells}} CO_2 + H_2O + \text{new cells} \qquad (1)$$

Because the oxidation process is an inter-cellular phenomenon, organic molecules must pass from suspension through the cell wall and into the cytoplasm before metabolism can occur. Small molecules can pass directly through the cell membrane, but before large molecules proceed into the cytoplasm they are temporarily adsorbed onto the cell wall and enzymatically cleaved into smaller organic units. Once the molecule enters the cytoplasm it becomes inter-locked with an inter-cellular enzyme and is trapped in the cell.

The metabolism of an organic compound can proceed either through respiration or through synthesis (See Figure 1). The cellular protoplasm formed during synthesis is, in itself, not a stable end product, but is eventually subjected to a catabolism called endogenous respiration.

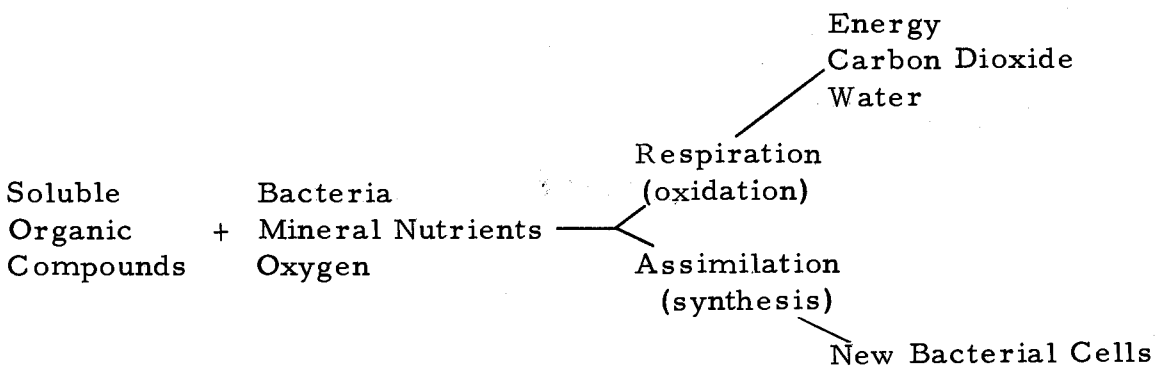


Figure 1. The relationship of respiration to assimilation during the microbial decomposition of organic matter.

Synthesis

McCabe (7, p. 265) has developed the mathematics of cell growth relative to the biological oxidation process. The classical growth curve used in his discussion is shown in Figure 2.

This growth curve depicts how bacterial numbers will increase with time during the assimilation of a single external carbon source. It does not necessarily represent growth in a system metabolizing a variety of substrates in the presence of both primary and secondary microbial organisms (protozoa).

It is possible to discuss the growth curve of Figure 2 in mathematical terms if the following notations are recognized.

At the beginning of the oxidation period let:

S_o = the initial cell concentration, mg/l,

L_o = the concentration of degradable organic matter initially present expressed as mg/l of biological oxygen demand.

At any time "t" let:

S = the cell concentration present, mg/l,

$\Delta S = S - S_o$ the increase in cell concentration, mg/l,

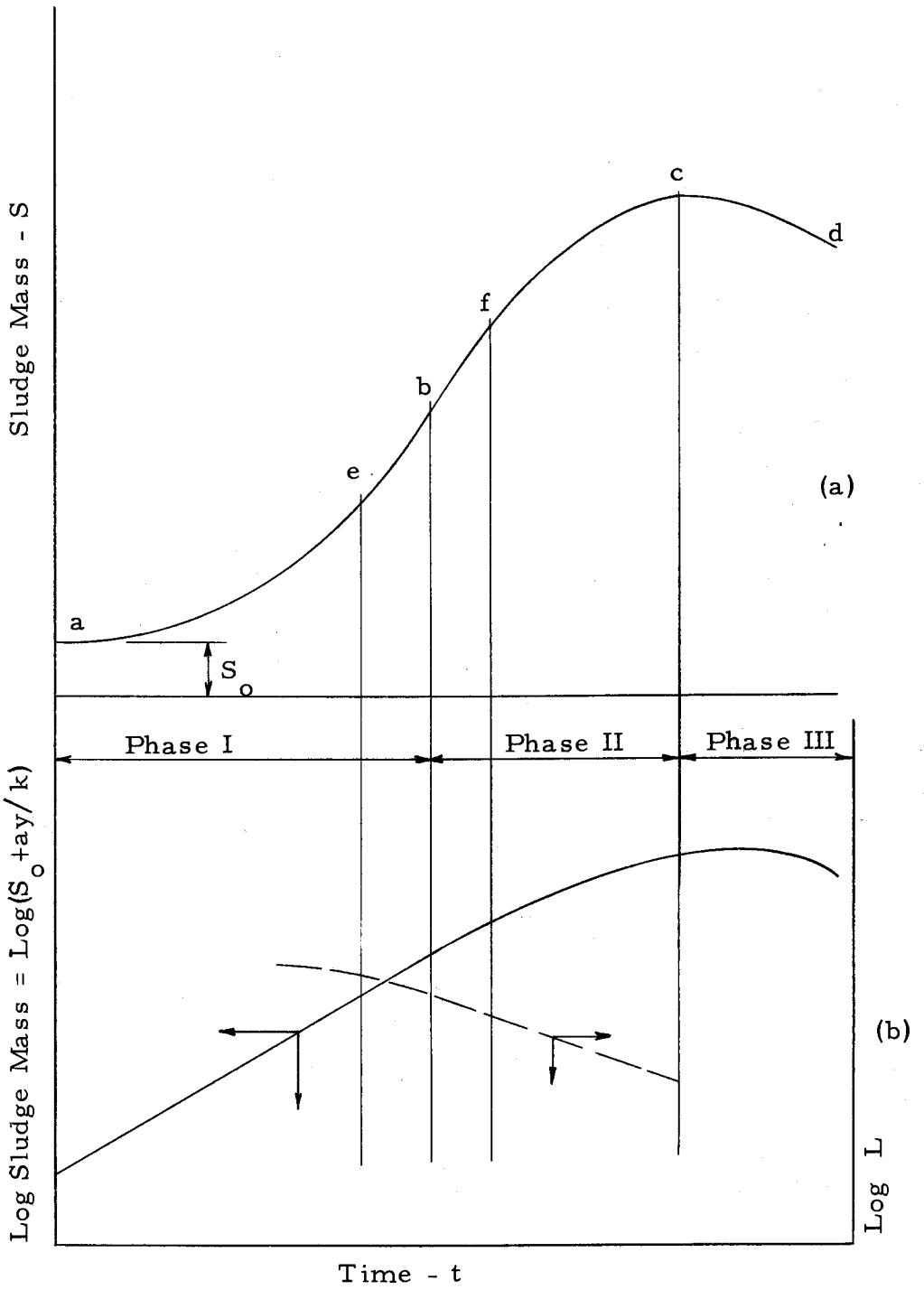


Figure 2. Classical growth curve for bacteria metabolizing a single external carbon source. Phase I - Constant growth rate; Phase II - Declining growth rate; Phase III - Auto-oxidation.

L = the concentration of organic matter remaining expressed as mg/l of BOD,

y = the BOD removed, mg/l,

y_i = the BOD removed by biosorption, mg/l,

$y-y_i$ = the BOD removed by assimilation for respiration and synthesis,

a = the fraction of BOD removed which is synthesized to cells,

k = the BOD of 1 mg of cell tissue, in mg/l per mg of cells.

If it is assumed that the organic substrate does not adhere to the cell wall prior to passing into the cytoplasm (no biosorption), then the following assumptions can be made: $y_i=0$, $ay/k = \Delta S$, and $S = S_0 + \Delta S = S_o + ay/k$, and the growth curve can be discussed mathematically as follows.

Logarithmic growth phase. Phase I of the bacterial growth curve is referred to in the literature as the logarithmic growth phase. In this portion of the curve, cell multiplication is assumed to be limited only by the generation time of the bacteria. In essence this means the growth rate is a constant and can be expressed by the differential equation:

$$ds/dt = d(S_o + ay/k)/dt = K_1 S . \quad (2)$$

This formulation assumes that the entire cell mass is in a state of active growth.

Equation 2 can be integrated:

$$L_n\left(\frac{S_o + ay/k}{S_o}\right) = K_1 t \quad (3)$$

or expressed in common logs:

$$\text{Log}\left(\frac{S_o + ay/k}{S_o}\right) = k_1 t . \quad (4)$$

This formulation is represented graphically in Figure 2b. It is seen that k_1, the growth rate constant, is the slope of the linear portion of the growth line shown in Phase I.

Arithmetic growth phases. Section e-f of the growth curve is frequently considered to be linear. Therefore, it is a zero order reaction and the rate of change of growth with time can be expressed as:

$$dS/dt = K . \quad (5)$$

It is further recognized that:

$$dS = \frac{a}{k} dy \quad (6)$$

where

$$dy = dL, \tag{7}$$

and hence the BOD removal can be expressed as

$$dL/dt = \frac{k}{a} K = K_2 . \tag{8}$$

Declining growth phase. Phase II of the growth curve is referred to as the declining growth phase. This portion of the curve is not controlled by generation time, but rather it is strongly influenced by the decreasing amount of BOD remaining in solution. Growth becomes limited at a transition point, see point "b" in Figure 2, after which the growth rate, and as a consequence the BOD removal rate, becomes proportional to the remaining BOD. This is written in differential form as:

$$-dL/dt = K_3 L , \tag{9}$$

which integrates to

$$Ln(L/L_o) = -K_3 t . \tag{10}$$

Equation 10 is plotted as a dashed line in Figure 2b. The plot is expressed in common logarithms and the slope of the dashed line is equal to k_3 when Equation 10 is expressed in common logs.

While Equation 10 does describe the course of BOD removal during the oxidation of simple substrates within Phase II, it does not account for removal rates encountered in heterogeneous mixtures. Such mixtures usually yield a progression of decreasing rate constants as the less readily assimilated substances are removed first. The problem of progressive K_2 values has been considered by Eckenfelder (7, p. 277).

Oxidation

The BOD of a waste not only changes form by being reconstituted as new cell growth, but it is also removed from the system as CO_2 and H_2O. The amount of energy released during oxidation is of major importance to the microbial environment, because it is through a proper balancing of energy changes that cells metabolize degradable substrates and thereby increase their cell mass.

Free-energy. The energy released during oxidation is often referred to as the free-energy of oxidation.

Fruton and Simmonds (9, p. 219) attempt to give a qualitative and physical meaning to free-energy from an inorganic view point. They suggest that a non-biochemical reaction such as:

$$Zn + Hg_2SO_4 + 7H_2O \rightarrow ZnSO_4 \cdot 7H_2O + 2Hg + Heat \qquad (11)$$

will release 82,000 cal. of heat, if placed in a calorimeter (i.e. $\Delta H = -82,000$ cal.). Enthalpy (H) is the heat content of a system and is defined as:

$$H = E + pv, \tag{12}$$

where

E = internal energy

p = pressure

v = volume.

If the reaction of Equation 11 is allowed to proceed reversibly in an electric cell, 66,000 cal. of electrical energy would be realized. There is then a difference between the change in enthalpy of a system undergoing a non-reversible reaction, and the amount of work which may be obtained from a reversible reaction. This realization leads directly to the definition of free-energy change (ΔF).

ΔF = The maximum useful work that can be obtained from a chemical reaction by operating it in a perfectly reversible manner, at constant temperature and pressure (e.g. the 66,000 cal. of the above example).

The unavailable energy ($\Delta H - \Delta F$), (or, as in the example, 16,000 cal.) is regarded as an entropy change (ΔS) directly influenced

by the temperature of the environment. In general the free-energy equation can be expressed as:

$$\Delta F = \Delta H - T\,\Delta S. \tag{13}$$

Fruton and Simmonds also consider the reversible reaction:

$$aA + bB \rightleftarrows cC + dD \tag{14}$$

and present the free-energy equation as:

$$\Delta F = -RT(\text{Ln}\,K) + RT\,\text{Ln}\,\frac{(C)^c\,(D)^d}{(A)^a\,(B)^b} \tag{15}$$

where the capital letters denote the chemical species, and the lower case letters denote the number of moles. The K term is the equilibrium constant, i. e. the ratio of the product of the activities of C and D to the product of the activities of A and B at equilibrium. The last term in the equation includes the reactants (A and B) and the products (C and D) at any activity values. An important special case of this equation refers to the conversion of unit activities of A and B to unit activities of C and D; under these circumstances, the last term of the equation becomes equal to zero, and

$$\Delta F^o = -4.6\,T\,(\text{Log}\,K), \tag{16}$$

where ΔF^o = free energy of oxidation in standard state.

Lipman (18, p. 39) defines free-energy in terms of group

potential. A group potential is defined as a measure of the degree of activation of a group in a certain binding state comparing it to what might be called the ground state, or the free compound. In high energy phosphate bonding this would be a comparison between inorganic phosphate and ATP.

In the hydrolysis of ATP the standard free-energy equation can be expressed as:

$$\Delta F^{o} = -4.6\ T(\text{Log } K), \tag{17}$$

where $K = \frac{[ADP]\,[P]}{[ATP]}$. Equation 17 has been evaluated (9, p. 352), and in the hydrolysis of ATP to ADP, 12,000 cal. of energy per mole of ATP is released.

Lipman expands Equation 17 for obtaining ΔF under non-standard conditions:

$$\Delta F = \Delta F^{o} - 1400\ \text{Log}\frac{(ATP)}{(ADP)} + 1400\ \text{Log}\,(P) - 1400\ \text{Log}\,(1 + K/H^{+}) \tag{18}$$

This equation is derived for a temperature of 30°C and implies that the free-energy of ATP hydrolysis is pH dependent.

Lipman goes on to say (9, p. 352),

> I find it quite significant and, as far as I can see, rather unappreciated that the amount of energy derivable from the system depends importantly on the ATP/ADP ratio, the phospho-dephospho quotient, which is analogous to the dependence of O/R potential on the O/R ratio, or of hydrogen ion potential on the buffer quotient.

Coupled reactions. Fruton and Simmonds (9, p. 225) illustrate the relationship between standard free-energy changes and the microbial environment by considering the enzymatic dissociation of fumarate ion into malate ion (See Figure 3).

$$\text{fumarate ion} + H_2O \rightleftharpoons \text{malate ion}$$

or

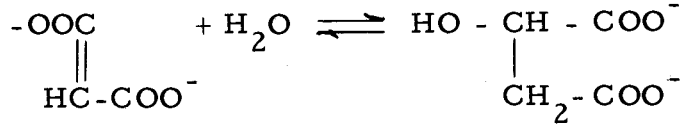


Figure 3. The enzymatic dissociation of fumarate ion into malate ion. The reaction will not proceed in the absence of the enzyme fumarase. At equilibrium the concentration of fumarate : malate is 4. 03 : 1.

If one sets the initial activity of water at one, and assumes the ratio of the activity coefficients of malate and fumarate to be unity, the equilibrium constant is calculated thus: (assume the initial concentration of fumarate is 0. 1 M)

$$K = \frac{(\text{malate}^{=})}{(\text{fumarate}^{=})(H_2O)} = \frac{(0.0801)}{(0.0199)(1)} = 4.03$$

and

$$\Delta F_{298}^{o} = -4.6\,T(\text{Log } K) = -825 \text{ cal.},$$

which is a measure of the useful work available from the conversion

of fumarate and H_2O at unit activities to a unit activity of malate at 25 °C.

In biochemical systems the product of one reaction is frequently the reactant for another reaction. For example consider the reverse of the fumarate-malate reaction:

$$\text{malate}^{=} + 825 \text{ cal.} \rightarrow \text{fumarate}^{=} + H_2O. \qquad (19)$$

If there is an enzymatic system present that is capable of removing fumarate with a large negative ΔF^{o} value, then the reaction would be driven to the right. In essence this would amount to the coupling of an exergonic reaction to an endergonic reaction.

An example of such a process can be shown by considering the reaction of fumarate and ammonium ion to form aspartate in the presence of the enzyme aspartase. At pH 7.4 and 37 °C the free-energy change of unit activities of fumarate and ammonium ions to unit activities of aspartate is -3720 cal. At 37 °C the free-energy change in the conversion of malate to fumarate and water is +700 cal. In a coupled reaction catalyzed by fumarase and aspartase there would be a net free-energy change of:

$$\Delta F_{310}^{o} = -3720 + (+700) = -3200 \text{ cal.} \qquad (20)$$

If the proper enzyme systems are present, some of this net free-energy

can be made available for cellular functions.

Energy and Cell Growth

The energy for cell synthesis is obtained from respiration when carbon-hydrogen or carbon-carbon bonds are split during metabolism. When the molecule is cleaved, some of the released energy is lost as heat and some may be transferred to molecules such as ATP, which possess high energy storage mechanisms, and subsequently used in the production of new cells.

It is, therefore, not surprising that the formation of protoplasm (i. e. new cells) from a given metabolic system has been postulated as being related to the energy reactions which take place during the biological degradation of organic matter.

Protoplasm Independent of Available Energy

McKinney says (7, p. 7), "Quantitatively speaking, a given quantity of organic matter of a fixed energy content can produce only a fixed amount of protoplasm and will consistently produce that amount of protoplasm." McKinney further states (7, p. 10), "two-thirds of the ultimate BOD of any waste will be converted to microbial protoplasm providing the ulitmate BOD is carbonaceous and does not include nitrification." Under these limitations the two-thirds ultimate BOD conversion merely indicates that for a given energy content a

constant proportion of the energy will be expended to form a unit of cellular protoplasm.

McKinney points out that Englebrecht and Symons (8, 27) agree with him and show data indicating that two-thirds of the ultimate BOD goes to new protoplasm.

Protoplasm Dependent on Available Energy

Servizi and Bogan (23, 24, 25) agree with McKinney and claim evidence showing that cell production is directly related to the theoretical free-energy of oxidation possessed by biologically degradable compounds. If this is true, then, because the free-energy of oxidation varies among compounds, it is impossible for the fraction of BOD going to synthesis to be a constant. Unless, of course, BOD and ΔF^{o} are directly related, but this has never been shown to be true.

Servizi and Bogan (23, 24, 25) have discussed metabolism and cell growth in relationship to free-energy of oxidation. Their literature review shows two conflicting viewpoints pertaining to sludge production and the metabolism of substrates:

1. The fraction of ultimate BOD going to cell synthesis varies with the chemical nature of the substrate,

and

2. the fraction of ultimate BOD going to cell synthesis does

not vary with the chemical nature of the substrate.

Servizi and Bogan (23, p. 23) postulated that since free-energy is a measure of the useful work which a process may do, the quantity of cell tissue synthesized (useful work done) per unit of substrate utilized should be proportional to the free-energy of substrate oxidation. That is:

$$Y = k_1 N_{ATP}, \tag{21}$$

where

Y = synthesis in grams of cell tissue per mole of substrate utilized,

k_1 = a constant having units gram per mole of ATP,

N_{ATP} = energy made available during the metabolism of one mole of substrate expressed as moles of ATP.

The investigators made calculations and performed experiments verifying that Equation 22 holds for many soluble substrates.

$$N_{ATP} = -k_2 \Delta F^{o}_{ox} \tag{22}$$

where

N_{ATP} = moles of ATP per mole of substrate,

k_2 = constant of proportionality, moles of ATP per k-cal.,

ΔF^{o}_{ox} = free-energy of oxidation in standard state, in k-cal. per mole.

By combining Equations 21 and 22 the hypothesis can be stated as:

$$Y = -k_1 k_2 \Delta F^{o}_{ox} \quad \text{for single substrates} \tag{23}$$

or

$$Y = -k_1 k_2 \frac{\Sigma M \Delta F^{o}_{ox}}{\Sigma M} \quad \text{for multiple substrates.} \tag{24}$$

Further support is given this hypothesis when it is recognized that the metabolism of most organics may be thought of as requiring one or more of three main functional steps (See Figure 4). Phase I is concerned with bringing an organic into the main stream of metabolism and generally results in the release of a small part of the substrate free-energy. Phase II is largely a further conversion of the substrate into one of a few intermediates and also accounts for a relatively small portion of the intitial substrate free-energy. Phase III is the terminal oxidation step, and it is here that most of the substrate free-energy is released. Thus it can be seen that most of the free-energy released during catabolism is associated with the metabolism of a relatively few intermediates, (Phase II) which are common to most organics.

This then is the point on which McKinney and Servizi disagree.

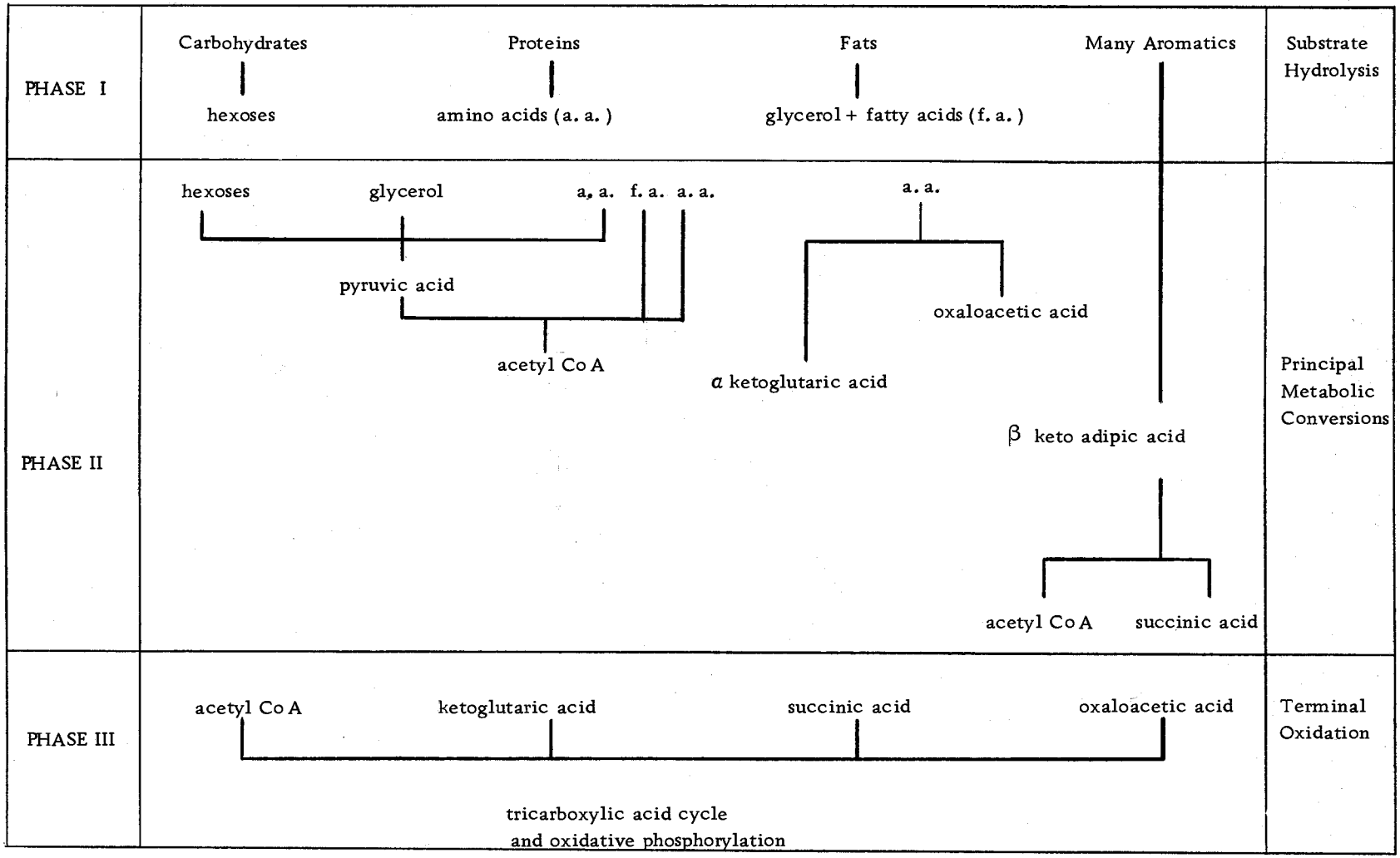


Figure 4. Major metabolic phases after Krebs [a] and Ubreit [b].

a. Krebs, H. A. Some aspects of the energy transformation in living matter. British Medical Bulletin. Vol. 9, 1953. p. 97.

b. Umbrecht, W. W. Metabolic maps. Vol. II, Burgess Publishing Co., Minneapolis. Minn., 1960.

Servizi maintains, see (1) above, that acetyl CoA, ketoglutaric acid, succinic acid and oxaloacetic acid all produce different quantities of ΔF^{o} when metabolized, and that the fraction of ΔF^{o} going to protoplasm formation is substrate dependent, but directly related to the free-energy of oxidation of the compound. On the other hand, McKinney believes, see (2) above, that even though different amounts of free-energy are relaeased during the metabolism of the components shown in Phase III, the fraction of energy going into protoplasm is independent of the free-energy change, i.e. it is a constant.

Servizi and Bogan investigated their hypothesis using soluble carbon substrates (25). Their work shows that cell synthesis is directly proportional to the change in free-energy of the substrate. Synthesis was also found to be proportional to substrate COD and can be defined by the equation,

$$Y = K\ COD \tag{25}$$

where K is substrate dependent and may vary from about 0.35 to 0.39. They show further that the BOD of a soluble organic waste is related to the COD by an equation of the form:

$$BOD = (1 - K\frac{COD}{C})\ COD \tag{26}$$

where

K = a substrate dependent constant of the order 0.016,

C = moles of carbon per mole of substrate.

Servizi's data also show that the sludge yield coefficient based upon substrate BOD is not a constant. It was noted that about half of the ATP produced appears to be used in synthesizing complex cell components from the substrate. The remaining ATP is believed expended on the other functions necessary for cell growth.

Implication of Plateau BOD

At the same time that Servizi was exploring the relationship between protoplasm production and free-energy changes, Busch and others (2, 3, 21) were investigating the possibility of predicting ultimate BOD from the point on the BOD curve where maximum cell production occurs. The projects of both investigations employed simple soluble organic compounds.

Busch was able to show that the point of maximum synthesis can be observed in the BOD curve by a temporary decrease in the rate of oxygen uptake. Busch termed this lag phase "the plateau". Other investigators (11, 17) have also demonstrated this existence of lags in oxygen uptake during metabolism.

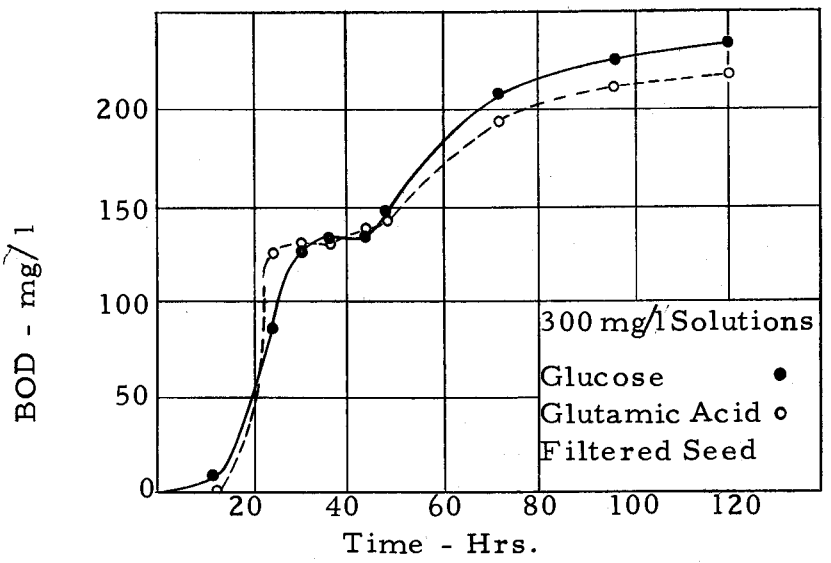


Figure 5. Typical BOD curves exhibiting plateau (2).

Characteristics of the Plateau

Gaudy and others (11) investigated the biological degradation of a variety of soluble substrates using several different organisms. His work shows that at the plateau 30 to 40 percent of the complete chemical oxidation demand has been removed. The length of the plateau is variable, but the number of viable organisms is always a maximum during this period. The rapid oxygen uptake encountered during the first phase of the BOD curve corresponds to rapid bacterial multiplication. The second stage of oxygen uptake corresponds to bacterial die-off.

Busch (2) presents evidence which indicates that the discernibility of the plateau is a function of the ratio of primary to secondary organisms (protozoa) initially present. He shows that filtered seed

(i. e. elimination of secondary growth) delineates the plateau much more clearly than unfiltered seed. He further shows that by increasing the seed concentration the plateau occurs sooner and obtains the same level of oxygen depletion. However, there is a limit to how soon the plateau can be reached by increasing seed concentration. Beyond this maximum seed concentration, undesirable oxygen depletions take place in the blanks, and no apparent decrease in the time required to reach the plateau is obtained. Busch concluded from his work that within the usual limits of seed concentration employed in the BOD test, the existence of the plateau is not dependent on seed concentration.

Existence Theories

Busch (2) feels that the existence of the plateau is attributed to a rapid oxygen depletion due to bacterial metabolism of the substrate with a second, less pronounced oxygen uptake due to the assimilation of bacteria by secondary organisms, and endogenous respiration. Busch's work shows that when the ratio of organisms to predators approaches 1. 0, synergistic activities may produce the 1st order equation commonly used in BOD formulations. A ratio of 1. 0 is common for stale sewage.

Wilson and Harrison (29) have objected to Busch's conclusion that the rise in the second stage represents the joint effects of endogenous respiration and predator activity. To test Busch's hypothesis

they performed an experiment using a pure bacterial culture and phthalic acid as substrate. In their experiments oxygen uptake was measured on two systems containing different amounts of cell inoculum. They felt from their results that it was "apparent that the magnitude of the second stage of oxygen uptake depends solely upon the number of cells present" (29).

McWhorter and Heukelekian (20) also have investigated the occurrence of the plateau in oxygen uptake measured in Warburg respirometers. They varied substrate concentration (100, 500, 1,000, 2,000, mg/l glucose), and used 0.5 ml settled sewage as seed. Potassium nitrate was used as a nitrogen source. They found that the lag between phases (the plateau) was shorter for the 500 mg/l glucose than for the 100 mg/l concentration. The lag did not appear in the 1,000 and 2,000 mg/l systems. For the two systems exhibiting a plateau, they observed that while the increase in substrate concentration increased oxygen uptake, the accumulated uptake at the plateau expressed on a relative basis (percent theoretical) was the same for both systems. They also investigated the effect of predators and concluded that the disappearance of the plateau with increasing substrate concentrations could not be solely attributed to changes in the predator:bacteria ratio, since O_2-uptake curves for sonicated and normal seeds did not diverge until 45 percent of the theoretical demand had been expressed. This was above the plateau value which both they and

Busch had obtained.

Gaudy (11) gives experimental evidence that the second stage of oxygen uptake can be due to other than the presence of protozoa. Evidence is also submitted which refutes Wilson's and Harrison's claim that the magnitude of the second stage is directly proportional to initial concentration of bacteria. In fact Gaudy's data show that the magnitude of the second stage was greater in the system containing fewer viable bacteria.

Gaudy's work has led to the establishment of four possible theories concerning the existence of a plateau.

1. In a heterogenous cell system, after reaching the stage of maximum growth (cell production or a combination of cell production and storage) the predominating cells would begin to die and release cellular components which may become food for other species of cells. Indeed, the bacterial cells themselves may become food for predator organisms. Time may be required before these secondary predominants can grow to such numbers that their growth is expressed in a measurable oxygen uptake. Such a sequential predominance of species may lead to two autocatalytic curves; the end of the first phase and the beginning of the second may form the plateau.

2. After plateau attainment, cells may die and release material. There may be no change in predominance, but because of the different structural configuration of the original substrate and the released cellular components, an acclimation period is required to synthesize the new enzymes needed to metabolize the new and exogenous substrates. If enough cellular components are released, an after growth may occur. On the other hand, the released components may not be of sufficient quantity to support much new growth, and the die-off in total population continues. It is also possible that the released components may provide enough energy to maintain the viable integrity of the cells and cell die-off proceeds more slowly.

Theories one and two differ only in that the secondary stage of oxygen uptake is caused by a new predominance in one, and by the same population with a required acclimation period in two. The fact that the plateau is short in some cases and long in others can arise by variations in (a) time for acclimation, and (b) time to gain enough population to be expressed in O_2 uptake.

3. After reaching maximum population density the cells do not die in the sense they no longer can respire, but they lose their ability to replicate. An acclimation period is needed

before cellular components formerly synthesized in replication processes can be broken down to supply energy. Another possibility within the scope of Theory 3 is that an acclimation period is needed before the cells can use "storage products" for energy. In this case the length of the plateau and indeed its existence in such systems would depend on the nature of the storage products and the induction time, if required, for producing the necessary catabolic enzymes. Reactions of this type might provide an explanation for the delayed onset of endogenous respiration.

4. During rapid metabolism of some compounds in the 1st stage of oxygen uptake, the cells may release intermediates into the medium rather than channeling all such compounds into storage products. In such cases time may be required for induction of enzymes to initiate metabolism of the new exogenous compounds. Time for induction of transport catalysts also may be required to bring these new components to existing enzyme systems within the cell. Since there no longer is enough substrate to support growth, the cells begin to die off.

All four theories are alike in that the plateau is considered to be caused by a change from metabolism of the original exogenous

source to the metabolism of new carbon sources produced by the cell.

In accord with the introductory statements the remainder of this paper is concerned with evaluating certain aspects of the metabolism of a substrate which is not completely soluble. The test substrate is a simulated milk waste and its metabolism will be studied in light of the material presented in the introduction of this paper.

II. NATURE AND SCOPE

It is the purpose of this study to relate first principles to the biological metabolism of a complex substrate containing both soluble and colloidal fractions. Specifically the investigation will attempt to differentiate between the respiration BOD and endogenous BOD of a simulated milk waste. If this can be done for a colloidal substrate, as it has been done for soluble substrates, then efforts will be made to predict the magnitude and the time of occurrence of the respiration BOD by evaluating substrate concentration in terms of its chemical oxygen demand.

There are four areas of exploration essential to the completion of the thesis:

1. Busch and Myrick (2, 3, 4, 21) have shown that BOD progression in many soluble substrates is a two phase phenomenon. The two phases are separated by a plateau possessing two unique properties: (a) The BOD at the plateau is reproducible for a given substrate concentration, and (b) the maximum net cell growth is present during this plateau period. In essence their work provides a graphical means of recognizing the point at which the BOD of the substrate has been divided into respiration

and synthesis.

The first stage of experimentation will be to employ a BOD test to determine whether or not a plateau can be discerned during the biological oxidation of the simulated milk waste, a waste which is not completely soluble. An attempt will be made to recognize the existence of any factors which might be sensitive to the establishment or clarity of plateaus for non-soluble substrates.

2. After it is apparent that a reproducible plateau can be established, experimental evidence will be collected to determine whether or not a "true" plateau has been established. A "true" plateau exists if all or most of the initial substrate COD, i. e. the biodegradable fraction, can be accounted for as respiration (Plateau BOD), or new cell growth.

3. Once the existence of the plateau has been determined an analysis will be made to decide to what degree and to what magnitude the substrate BOD is partitioned into synthesis (f_s) and respiration or oxidation (f_o).

4. The fourth stage of this study will be an analysis of the relationship between initial substrate COD, f_o, f_s, and Plateau BOD.

III. EXPERIMENTAL FACILITIES

Analytical Methods

Biological Oxygen Demand

The procedure for setting up BOD's is:

1. Place an amount of filtered endogenous seed into 18 liters of dilution water and aerate for at least 15 minutes. The dilution water must be preheated to the test temperature prior to the injection of seed. (When preheating was not done undesirable lags in the development of the BOD curve were noted.)

2. After the aeration period 23 BOD bottles were filled and used for determining a seed correction.

3. The seeded dilution water was withdrawn to the ten liter mark. A sample of this withdrawn water was taken to determine initial seed concentrations.

4. One-hundred milliliters of milk solution was then added to the remaining ten liters of dilution of water. After hand mixing the substrate BOD bottles were filled.

5. All BOD bottles were incubated at 29°C.

Periodically the BOD bottles were tested for dissolved oxygen concentrations. When the plateau was established three BOD bottles containing milk solution were emptied into a one liter container and sampled for final seed concentration.

Dissolved Oxygen Determinations

The sodium-azide modification of the Winkler dissolved oxygen test was used throughout this investigation. The procedure is outlined in Standard Methods (1, pp. 309-311).

Chemical Oxygen Demand

The chemical oxygen demand of the milk waste was determined by the procedure outlined in Standard Methods (1, pp. 399-402). No silver sulfate catalyst was required.

Reflux time was examined and it was found that a two hour digestion period was needed for reliable results.

Bio-mass Concentrations

Cell mass concentrations present in a solution at any time "t" were considered to be numerically equal to the total solids concentration of a filtered portion of the solution. Solids were obtained

by passing the solution through type HA millipore filters (diameter = 47 m.m., pore size = 0.45 microns).

Pretreatment of millipore filters. The millipore filters used for growth determinations were dried for one hour at 103°C prior to taring. This was accomplished by drying each filter in a weighing bottle. The bottles were capped with ground glass stoppers and stored in a desiccator for at least six hours before taring.

Filtering apparatus. In order to obtain measurable quantities of bacterial mass, high pressure filtration was used. This was made possible by connecting a stainless pressure filter holder (Millipore Cat. N. XX40 047 00) in series with the laboratory air supply and a Sioux pressure regulator. Pressure variations from 0 psi to 95 psi were possible.

Filtering procedure. The following procedure was followed in determining all seed concentrations.

1. Two tared bottles were removed from the desiccator for each determination; tests were run using three replicas.

2. The filters were taken from the flasks, wetted, and placed in series in the filtering apparatus.

3. After filtering a quantity of the cell suspension, the filters

were separated, replaced in their respective weighing bottles, and dried for three hours at 103°C.

4. The flasks were capped and again desiccated for at least six hours prior to final weighing.

It was necessary to use two filters in series in order to account for possible weight changes in the filters due to glycerol leaching. Busch (4) has discussed this problem in detail.

Weighing. All weighings pertaining to the determination of cell mass were made under contract to the Forage Analytical Service, a non-profit division of the Agricultural Chemistry Department.

Weighings were made to five places; however, the fifth place was considered to be an estimated value.

Weight recordings. Table 1 shows the standard form used in recording flask weights and the determination of cell concentrations. Letters indicate computational procedure.

Net cell growth was determined by subtracting a weighted average of the entries on line 12 from a weighted average of the entries on line 24 and multiplying this difference by the dilution factor 10^2.

Table 1. Format of Form Used to Record Information Necessary in Determining Cell Growth.

Measurement	Replicas		
	1	2	3
1. Vol. (l)	V		
2. Bottom Filter	No.	No.	No.
3. Final Wt. (g)	A		
4. Initial Wt. (g)	B		
5. Moisture Corr. (g)	B-A		
6. Top Filter	No.	No.	No.
7. Final Wt. (g)	C		
8. Initial Wt. (g)	D		
9. Wt. of Solids and Moisture (g)	C-D		
10. Moisture Corr. (g)	B-A		
11. Wt. Solids (g)	(C-D)+(B-A)		
12. Initial Solids Conc. (mg/l)	$\frac{(C-D)+(B-A) \times 10^3}{V}$		
13. Vol. (l)	V'		
14. Bottom Filter	No.	No.	No.
15. Final Wt. (g)	A'		
16. Initial Wt. (g)	B'		
17. Moisture Corr. (g)	B'-A'		
18. Top Filter	No.	No.	No.
19. Final Wt. (g)	C'		
20. Initial Wt. (g)	D'		
21. Wt. of Solids and Moisture (g)	C'-D'		
22. Moisture Corr. (g)	B'-A'		
23. Wt. Solids (g)	(C'-D')+(B'-A')		
24. Final Solids Conc. (mg/l)	$\frac{(C'-D')+(B'-A') \times 10^3}{V'}$		

Laboratory Procedures

Seed Development

This section discusses the procedures used to maintain a source of seed organisms for use during the study.

Source. Bacteria used to initiate the experimental program of this investigation were obtained from the influent of the Corvallis Sewage Treatment Plant.

To insure a high ratio of bacteria to predator organisms (protozoans) the sampled influent was filtered through Whatman No. 2 filter paper before use in the laboratory. Several samples of the filtrate were observed under the microscope and no predator organisms were apparent. This means of visual inspection was maintained throughout the investigation and at no time was the presence of predator organisms observed.

This filtrate was used to begin a cycling process which provided seed during the investigation. The method of cycling will become apparent as the remainder of this section is read.

Growth medium. The best medium for growing the desired seed organisms was found to consist of one liter of distilled water, 50 milliliters of pH 7 phosphate buffer (14.3 g of KH_2PO_4 and 68.8 g

of K_2HPO_4 per liter of distilled water) and one gram of Carnation dry-skim milk. When large quantities of seed organisms were needed 1.5 grams of milk was used. This solution was thoroughly mixed before being used as a growth medium.

A silicone defoamer was used to suppress slight and infrequent foaming.

Maintenance. Each time new cells were required a liter of growth medium was inoculated with 25 milliliters of filtered endogenous seed. (Initially the growth medium was inoculated with filtered influent from the treatment plant.) This mixture of bacteria and growth medium was maintained at a temperature of 29° C.

The air was saturated with water and dispersed into the medium through a coarse diffusion stone. The rate of discharge was sufficiently high to keep the cell mass in suspension.

When the growth medium contained one gram of skim milk per liter of solution, a 12 hour aeration period was considered adequate for obtaining maximum cell growth, whereas when the growth medium contained 1.5 grams of milk per liter of solution, a 24 hour aeration period was used.

These aeration times were determined by following the biological growth turbidimetrically. Percent transmittance was obtained with a Beckman D. B. Spectrophotometer. The 3.0 ml. vials were used

with distilled water as a blank. The slit width was 0.20 mm and the optimum wave length was established at 525 milli-microns.

Harvesting. At the end of the growth period cells were removed from suspension by centrifuging.

The laboratory centrifuge had a 12 inch diameter head and was capable of spinning the entire liter of cell growth at one time.

Several centrifugal speeds and spin times were investigated. No quantitative data were taken, but visual inspection with a microscope indicated optimum results were attained at a rotation speed of 10,000 rpm and a spin time of 15 minutes.

After centrifuging, the suspended cells were affixed to the bottom of the centrifuge vials in a mass generally referred to as a pellet. It was possible to pour off the supernatant without substantially disturbing the pellet.

Washing. The pellet was resuspended by adding approximately 15 mls. of standard dilution water (1. p. 319) to the vial and repeatedly discharging the water through a 5 ml. pipet against the pellet until it was completely resuspended.

The resuspended cells were diluted to 250 mls. with standard dilution water and filtered through Whatman No. 2 filter paper. The filtrate was kept under aeration in the 29°C incubator until required for seeding. This suspended cell mass is referred to throughout the

text as endogenous seed.

Filtration was used to remove clumped bacterial masses and predator organisms which may have entered the system.

Seeded Dilution Water

After at least a 12 hour aeration period the endogenous seed was removed from the 29°C incubator and again filtered through Whatman No. 2 filter paper. Following filtration some of the filtrate would be placed under aeration in dilution water. This solution was called seeded dilution water.

The dilution water was always preheated to approximately 29°C before the endogenous seed was introduced. Preheating was necessary to prevent lag periods in the microbial degradation of the substrate. Tests run where the dilution water had been kept at 20°C produced an eight hour lag in bacterial activity for BOD tests run at 29°C.

Storage

Several times during the test period it was necessary to shut down experimental activities. When this was done the endogenous seed was stored at 4°C. There were four such periods: 12 hours, 34.5 hours, 103 hours, and 243.5 hours.

At the end of the storage period a small quantity of the stored

endogenous seed was inoculated into growth medium and aerated for 12 hours.

After one growth cycle had been completed the endogenous seed responded to testing with no apparent ill effects due to cold storage.

It was noted that immediately after periods of storage a bright red culture dominated the microbial mass. After four to eight growth cycles the red culture was no longer visible. The only inference derived from this is that the experimental results were in no way affected by changes in microbial composition.

Simulating a Milk Waste

Porges and Hoover, (22, p. 318) reviewed the literature on milk waste composition and reported that:

> The composition of dairy wastes varies in total solids from less than 1 percent to more than 4 percent of that of milk (1), depending on waste-saving practices advocated by Trebler and Harding (11). The total solids in milk have been reported as 125,000 p.p.m. by Eldridge (2) and 131,500 p.p.m. by Bloodgood (1); hence a waste containing 1 percent milk would have about 1,300 p.p.m. total solids, almost all of which are organic solids. The constituents of whole milk vary within wide limits (1) (4); Table I gives the approximate average composition (11). The fat is rapidly coagulated during aeration, and the constituents left are mainly protein and lactose. It was decided, therefore, that a solution of dried skim milk would be suitable for preliminary surveys of the activities of various microorganisms.

Table I. Average Composition of Milk and Dried Skim Milk.

Constituent	Whole Milk (%)	Dried Skim Milk (%)	Solution Containing 1% Milk (p.p.m.)	Solution Containing 0.1% Dried Skim Milk
Fat	3.9	0.9	390	9
Protein	3.2	36.9	320	369
Lactose	5.1	50.5	510	505
Ash	0.7	8.1	70	81
Total Solids	12.9	96.4	1,290	964
Organic Solids	12.2	88.3	1,220	883

Table II. Comparison of Oxygen Demands of Solutions Determined Chemically and Biologically.

Type of Solution	C.O.D. (p.p.m.) Total	C.O.D. (p.p.m.) 68%	B.O.D. (p.p.m.) 20-day	B.O.D. (p.p.m.) 5-day
Skim Milk	1,052	715	1,056	636
Lactose	516	351	519	431
Casein	614	412	639	327

Table I includes the average composition of dried skim milk and a synthetic dairy waste prepared by dissolving 1 gm of this product in 1 l. of water. It will be noted that the fat-free organic solids in the milk waste and in the skim milk preparation are 830 p.p.m. for the former and 874 p.p.m. for the latter.

Porges and Hoover (22, p. 319) show: "... Table II gives results obtained in the laboratory on freshly prepared solutions..."

The Carnation Company (5, p. 2) has published a laboratory analysis of Carnation instant non-fat dry milk. Their results are

recorded in Table 2.

Table 2. Analysis and Composition of Carnation Instant Non-fat Dry Milk.

Constituent	Percent by Dry Weight
Protein	36.5
Carbohydrate	51.2
Fat	0.8
Mineral Salts	8.0
Moisture	3.5

The composition of Carnation's instant non-fat dry milk is very close to the average composition of dried skim milk reported in the literature of Porges and Hoover. Because of this similarity, the Carnation product was selected as a source for simulating the milk waste.

Dry milk preparation. A box of Carnation instant non-fat dry milk was brought to the laboratory. Fifty grams of the milk was pulverized with a mortar and pestle, and dried for six hours at 90°C. The dried milk was then placed in a glass jar with a screw-type cover. This preparation was the source for the simulated milk waste throughout the study.

Milk solution. To simulate the milk waste for test purposes, 0.2 grams of the dry milk preparation was dissolved in 200 mls. of distilled water. A mag-mix gently stirred this solution for ten

minutes before the mixture was subjected to chemical and biological oxygen demand tests.

IV. RESULTS AND DISCUSSION

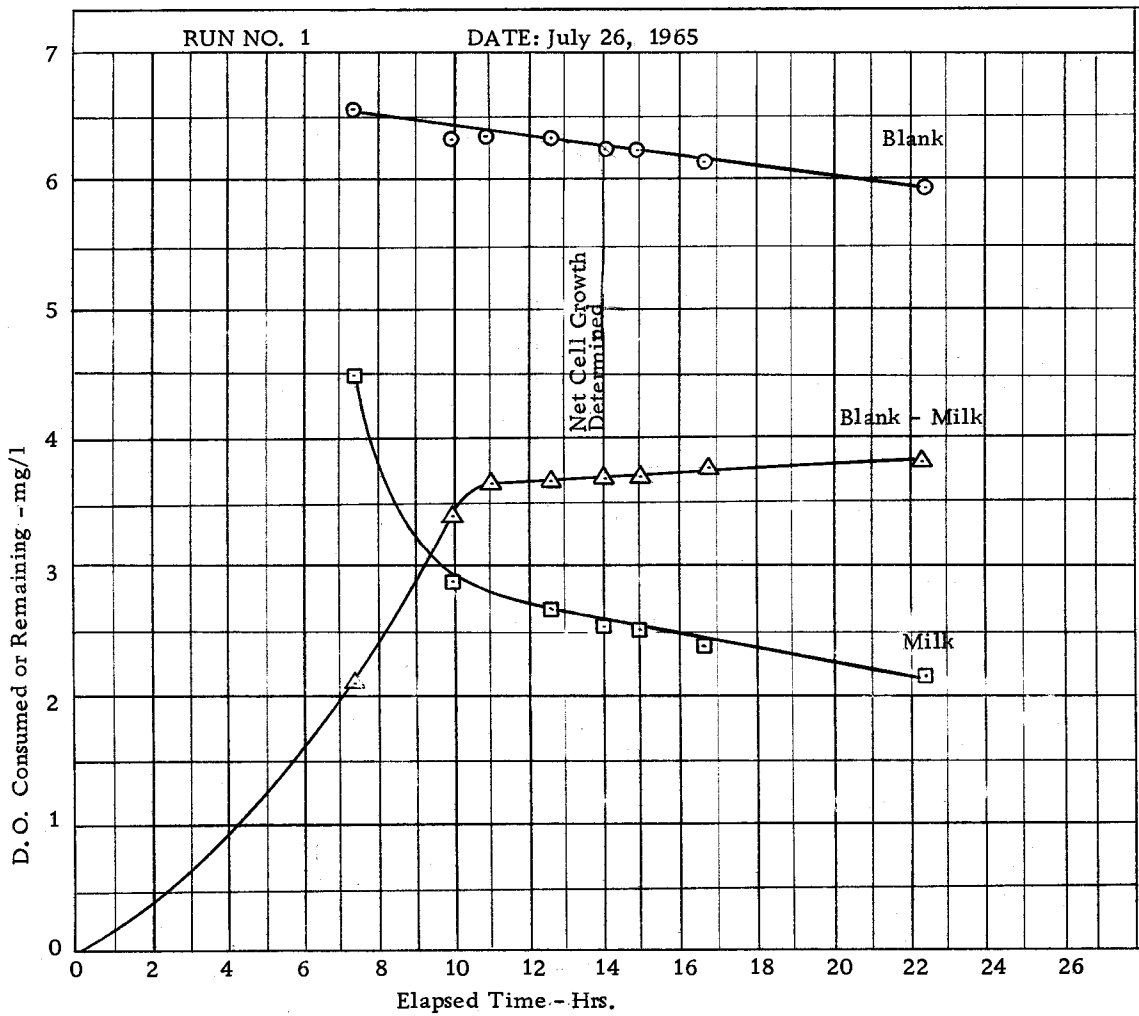
Metabolic Oxygen

Dissolved oxygen removed during substrate oxidation was determined by the sodium-azide modification of the Winkler test. The data are tabulated and presented graphically in the Appendix. A typical compilation of the data is shown in Figure 6.

In the tabulation "Blank" refers to the oxygen removed from solution in the BOD bottles containing only seed organisms. The column headed "Milk" pertains to dissolved oxygen removed in BOD bottles containing both seed organisms and milk substrate. Cell growth data are indicative of the microbial concentration present at the beginning of the test and at the point where the biodegradable portion of the substrate was assumed to be removed. Net cell growth is the difference between initial and plateau microbe concentrations. This concentration difference is attributed to the bacterial growth which takes place during metabolism.

A graphical presentation of the dissolved oxygen data is included in each figure. The "Blank-Milk" curve, subsequently referred to as "B-M", can be converted to a BOD curve by multiplying DO values by the dilution factor 10^2.

It is observed from Figures 11 through 27 that all of the



EXPERIMENTAL DATA

Dissolved Oxygen Data

t Hrs.	D. O. — mg/l Blk	D. O. — mg/l Milk	D. O. Blk-D.O. Milk mg/l
6.75	6.55	4.45	2.10
10.00	6.30	2.83	3.47
11.00	6.35	2.70	3.65
12.75	6.30	2.67	3.63
14.00	6.25	2.50	3.75
15.00	6.25	2.50	3.75
16.75	6.15	2.37	3.77
22.50	5.95	2.15	3.80

Cell Growth Data in mg/l

Final Concentration	933
Initial Concentration	567
Net Cell Growth	366

Figure 6. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.

"B-M" curves, or BOD curves, progress in the same general manner. It is of particular interest to note that in the case of each curve $\frac{d(DO)_{B-M}}{dt}$ approaches an undefined, but negligible value somewhere between 10 and 30 hours. If this is a plateau, it corresponds with Busch's work (2, 3, 4) where plateaus for soluble substrates were found to occur in less than 48 hours.

Plateau Existence

A BOD plateau is defined graphically, or mathematically, as an inflection point in the expression $\frac{d(DO)_{B-M}}{dt}$. In a physiological sense a plateau is said to exist only if an accounting can be made between the ultimate BOD of a substrate, and the synthesis and oxidation components existing at the plateau.

This requirement can be stated as:

$$\frac{\text{PLATEAU BOD} + 1.42\ (\text{Net Cell Growth})}{COD_i} = 1.0 \qquad (27)$$

where

Plateau BOD = the BOD at the plateau expressed in mg/l,

Net Cell Growth = the increase in microbe concentration measured at the plateau in mg/l,

1.42 = a constant which converts mg/l of cell mass to its equivalent BOD in mg/l;

its magnitude is based on an analysis of the oxidation of cell material defined by the equation (6, p. 19):

$$(C_5H_7NO_2)_n + 5nO_2 \rightarrow 5nCO_2 + 2nH_2O) + nNH_3 - \Delta H,$$

COD_i = Chemical oxygen demand of the simulated milk waste, and is considered to be numerically equivalent to its ultimate BOD.

Therefore, if an inflection point is observed in the BOD curve, and if experiments conducted at this inflection point reduce the above equation to a value close to or equal to 1.0, then the existence of a plateau is implied.

Table 3 is a tabulation and analysis of BOD data recorded at the plateau. Table 4 is a similar analysis of COD determinations conducted on the simulated milk waste at the outset of each experiment.

The ultimate BOD of net solids is recorded in Table 5. The data analysis is presented in Table 6. In estimating the mean value of this data several data points have been eliminated:

Runs No. 1 through 3 are rejected because only four place weighing accuracy was used in solids determinations.

Table 3. Tabulation and Analysis of Plateau BOD Data.

Run No.	Plateau BOD mg/l	Statistics
1	375	S^2
2	420	$\Sigma(y-\bar{y})^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$
3	410	
4	345	
5	410	
6	400	$= 2,696,999 - 2,690,481$
7	390	
8	400	$= 6,518$
9	410	
10	418	$\Sigma(y-\bar{y})^2/(n-1) = 407$
11	365	
12	390	
13	415	
14	415	CV
15	400	$\frac{S}{\overline{BOD}} = \frac{20}{398} = 0.05$
16	395	
17	405	
Total	6763	
Mean	398	

Table 4. Tabulation and Analysis of COD Determinations Conducted on the Simulated Milk Waste at the Beginning of each Experiment.

Run No.	Plateau BOD mg/l	Statistics
1	1067	S^2
2	990	$\Sigma(y-\bar{y})^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$
3	1027	
4	1027	
5	1027	
6	1023	$= 17,880,369 - 17,872,927$
7	1033	
8	1059	$= 7,442$
9	980	
10	1045	$\Sigma(y-\bar{y})^2/(n-1) = 465$
11	1038	
12	1010	
13	1010	CV
14	1020	
15	1020	$\frac{S}{\overline{COD}} = \frac{21.6}{1,025} = 0.021$
16	1035	
17	1020	
Total	17,431	
Mean	1,025	

Table 5. Experimental Data Pertaining to Experiments Conducted on the Metabolic Destruction of a Simulated Milk Waste.

Run Number	BOD Reading at Net Cell Growth Determination mg/l	BOD at Plateau	Ultimate BOD of Net Cell Growth mg/l	COD of Milk Solution mg/l	Slope of Endogenous Curve mg/l per Hr.	Percent of BOD Synthesized f_s	% Oxidized f_o	Test Temperature C°
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	370	375	518	1067	-0.038	58.5	41.5	29.0
2	415	420	594	990	-0.043	59.0	41.0	28.2
3	410	410	902	1027	-0.054	68.0	32.0	30.1
4	335	345	416	1027	-0.016	57.0	43.0	29.8
5	410	410	527	1027	-0.034	56.5	43.5	29.7
6	400	400	530	1023	-0.061	56.5	43.5	29.0
7	385	390	534	1033	-0.026	57.5	42.5	29.8
8	400	400	580	1059	-0.056	59.0	41.0	28.8
9	360	410	484	980	-0.018	57.8	42.2	29.1
10	355	418	571	1045	-0.0114	61.5	38.5	29.7
11	350	365	551	1038	-0.0023	61.5	38.5	29.6
12	390	390	512	1010	-0.0039	58.0	42.0	29.3
13	400	415	889	1010	-0.0040	69.0	31.0	29.5
14	415	415	612	1020	-0.0120	60.0	40.0	29.2
15	425	400	339	1020	-0.027	48.0	52.0	29.1
16	425	395	373	1035	-0.041	46.0	54.0	30.4
17	405	405	452	1020	-0.043	57.0	43.0	29.1

Entries in columns (2) and (3) are taken from the data plotted in Figure 11 through Figure 27. The entries of column (4) are obtained by multiplying the net cell concentration (see Figure 11 through Figure 27) by 1.42 (6. p. 19). Column (5) is an arithmetic average of 3 COD tests performed during the particular experiment. Column (6) was obtained by running a linear regression analysis on the Blank data of Figure 11 through Figure 27. Column (7) was calculated as $\frac{(4)}{(3)+(4)}$ and column (8) was obtained from the relationship $f_s = 1 - f_o$. Column (9) is an average of at least six runnings taken during the test period.

Table 6. Tabulation and Statistical Analysis of the Ultimate BOD of the Increase of Cell Mass Acquired During the Metabolism of the Simulated Milk Waste.

Run No.	Ultimate BOD of Net Cell Growth mg/l	Statistics
4	416	S^2
5	527	$\Sigma(y-\bar{y})^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$
6	530	$= 3,542,205 - 3,323,269$
7	534	$= 218,936$
8	580	$\Sigma(y-\bar{y})^2/(n-1) = 19,903$
11	551	CV
12	512	$\frac{S}{\overline{BOD}} = \frac{141}{526} = 0.268$
13	889	
14	612	
15	339	
16	373	
17	452	
Total	6,315	
Mean	526	

Four place precision is not enough when dealing with cell mass weights of this magnitude.

Runs No. 9 and 10 are rejected because the point at which solids were determined does not correspond to the Plateau BOD.

A statistical analysis has been made to determine whether or not experimental data support the plateau requirements defined by Equation 27.

The hypothesis is that the ratios obtained by substituting experimental data into Equation 27 constitute a normal population with a mean equal to one. The alternate hypothesis, of course, is that the population mean is less than or greater than one. The only assumption made in the test is that the samples are random, and are drawn from a normal population. The statistical device selected for testing the hypothesis is the t-distribution with a 70 percent significance level and 11 degrees of freedom.

The test is presented in Table 7. The conclusion is that the experimental data support the hypothesis, i.e. a true plateau does exist for the bio-destruction of a simulated milk waste, and this plateau is discernible in the laboratory. (The statistics imply that if the hypothesis is rejected there is a 70 percent chance of committing a Type I error).

Table 7. A "t" Test Performed on Laboratory Data Gathered to Test the Hypothesis that a Plateau Does Exist During the Metabolism of a Simulated Milk Waste.

Run No.	$\frac{\text{(BOD-mg/l) + 1.42 (Net Cell Growth - mg/l)}}{\text{(COD - mg/l)}}$
4	0.74
5	0.91
6	0.91
7	0.90
8	0.93
11	0.88
12	0.89
13	1.29
14	1.24
15	0.73
16	0.74
17	0.84

"t" Test

$\bar{y} = 0.92$

$S^2 = 0.94$

$$"t" = \frac{0.92-1}{[0.94/12]^{1/2}} = -0.29$$

$-0.29 > -0.40$

accept

Partitioning of BOD

To determine how much BOD is synthesized and how much is oxidized "f_s" and "f_o" have been tabulated and recorded in Table 8. An arithmetic mean of the runs containing reliable solids data yields:

$$f_o = 43.0\%$$

and

$$f_s = 57.0\%.$$

Hoover (13, p. 171) has reported an f_s of 60 percent and an f_o of 40 percent for the assimilation of a similar milk waste by activated sludge.

Correlating Plateau BOD and Initial COD

Servizi and Bogan (23, 24, 25) have shown evidence that an increase in cell mass during metabolism of a substrate is directly proportional to the free-energy of that substrate. This same line of reasoning will hold true for a specific waste varying only in concentration. That is, unless other parameters are limiting, the increase in cell mass experienced during the metabolism of a waste will be directly proportional to its free-energy concentration or to any parameter indicating the relative presence of biodegradable substrate.

Table 8. Time at which the Plateau Becomes Discernible and the Corresponding Activity Indices.

Run No.	The Time at which $\frac{d(DO)_{B-M}}{dt}$ becomes negligible - Hrs.	Slope of Endogenous Curve mg O_2/Hr.	Activity Coefficients $(\frac{\text{mg } O_2}{\text{Hr.}})/\text{mg of cells}) \times 10^3$
1	11.0	-0.038	-0.067
2	12.0	-0.043	-0.028
3	10.5	-0.054	-0.049
4	13.0	-0.016	-0.031
5	15.5	-0.034	-0.028
6	12.0	-0.061	-0.053
7	17.0	-0.026	-0.021
8	15.0	-0.056	-0.037
9	26.0	-0.018	-0.034
10	25.0	-0.0114	-0.034
11	30.0	-0.0023	-0.024
12	33.0	-0.0039	-0.017
13	25.0	-0.0040	-0.026
14	21.0	-0.0120	-0.027
15	14.0	-0.027	-0.010
16	10.0	-0.041	-0.020
17	12.0	-0.043	-0.018

When the metabolism of milk wastes is being considered, chemical oxygen demands are indicative of milk waste concentration. Therefore,

$$Ymax = K_1(COD_i), \tag{28}$$

where

Ymax = the maximum increase in cell mass occurring at the plateau,

COD_i = the initial chemical oxygen demand of the milk waste,

K_1 = a constant of proportionality which converts mg/l of COD to mg/l of microbial solids.

Substituting into Equation 28 the mean values for COD_i and Ymax, see Tables 4 and 6, the constant K_1 is calculated to be:

$$K_1 = \frac{526/1.42}{1025} = 0.36. \tag{29}$$

This means that for every mg/l of milk COD that is metabolized, 0.36 mg/l of new cell growth will be developed. It is interesting to note that the value of K_1 obtained in this study is in agreement with Servizi's work on soluble substrates (See Equation 25).

In the previous section a "t" test was used to show that at the plateau it is highly probable that all, or almost all, of the initial COD is accounted for in either synthesis or oxidation. This is expressed

mathematically as:

$$\Delta COD_i = COD_i - COD_{pl}, \tag{30}$$

where

ΔCOD_i = the chemical oxygen demand expressed in synthesis or oxidation in mg/l,

COD_i = the initial COD of the milk solution in mg/l,

COD_{pl} = the chemical oxygen demand of the milk solution, in mg/l, which can not be accounted for in terms of synthesis or oxidation.

The implication of the "t" test is that, for the simulated milk waste,

$$\Delta COD_i = COD_i. \tag{31}$$

It has been shown that the biodegradable substrate at the plateau is accounted for in terms of either oxidation (i.e. Plateau BOD) or an increase in cell mass. As this is true, then f_o, the fraction of COD_i initially oxidized, and f_s, the fraction of COD_i initially synthesized are related as:

$$f_s = 1 - f_o, \tag{32}$$

where

$$f_o = \frac{\text{Plateau BOD}}{\Delta COD_i} \tag{33}$$

and

$$f_s = \frac{\text{Ultimate BOD of the Net Cell Mass}}{\Delta COD_i} . \tag{34}$$

Using these relationships it is possible to develop a dependence between BOD_{pl}, i.e. the BOD measured at the plateau, and COD_i. By definition,

$$BOD_{pl} = f_o(\Delta COD_i) = (1 - f_s)(\Delta COD_i) . \tag{35}$$

Substituting Equation 34 for f_s,

$$BOD_{pl} = (1 - \frac{1.42\ Ymax}{\Delta COD_i})\ \Delta COD_i . \tag{36}$$

Replace Ymax with Equation 28,

$$BOD = (1 - \frac{(1.42)(0.36)(COD_i)}{\Delta COD_i})\ \Delta COD_i . \tag{37}$$

Substitute Equation 31 for ΔCOD_i,

$$BOD_{pl} = (1 - \frac{(1.42)(0.36)(COD_i)}{COD_i})\ COD_i , \tag{38}$$

which reduces to

$$BOD_{pl} = (1 - 0.51)COD_i = 0.49\ COD_i . \tag{39}$$

Activity Indices

This section discusses the time of plateau occurrence, plateau clarity and plateau variability in terms of two activity indices: (1) the slope of the endogenous curve, and (2) an activity parameter (a.c.), where

a.c. = the slope of the endogenous curve times 10^3 divided by the initial cell concentration in mg/l, i.e.

$$\frac{mgO_2}{1\ Hr.} \times 10^3 \frac{1}{mg\ cells} = \frac{mgO_2}{Hr.} \times 10^3 \text{ per mg of cells.}$$

Negligible $\frac{d(DO)_{B-M}}{dt}$ and Time

The times at which the plateau became discernible and the corresponding activity indices of a.c. and endogenous slope, are recorded and tabulated for each run, see Table 8.

A plot of the a.c. against the time of plateau occurrence was made, but no pattern appeared evident. However, when the slope of the endogenous curve was plotted against occurrence time, a definite logarithmic relationship appeared (See Figure 7). The correlation coefficient of these variables is -0.90, which implies dependency.

Table 9 gives the necessary data for the linear regression analysis. (For ease of computation the endogenous slopes were

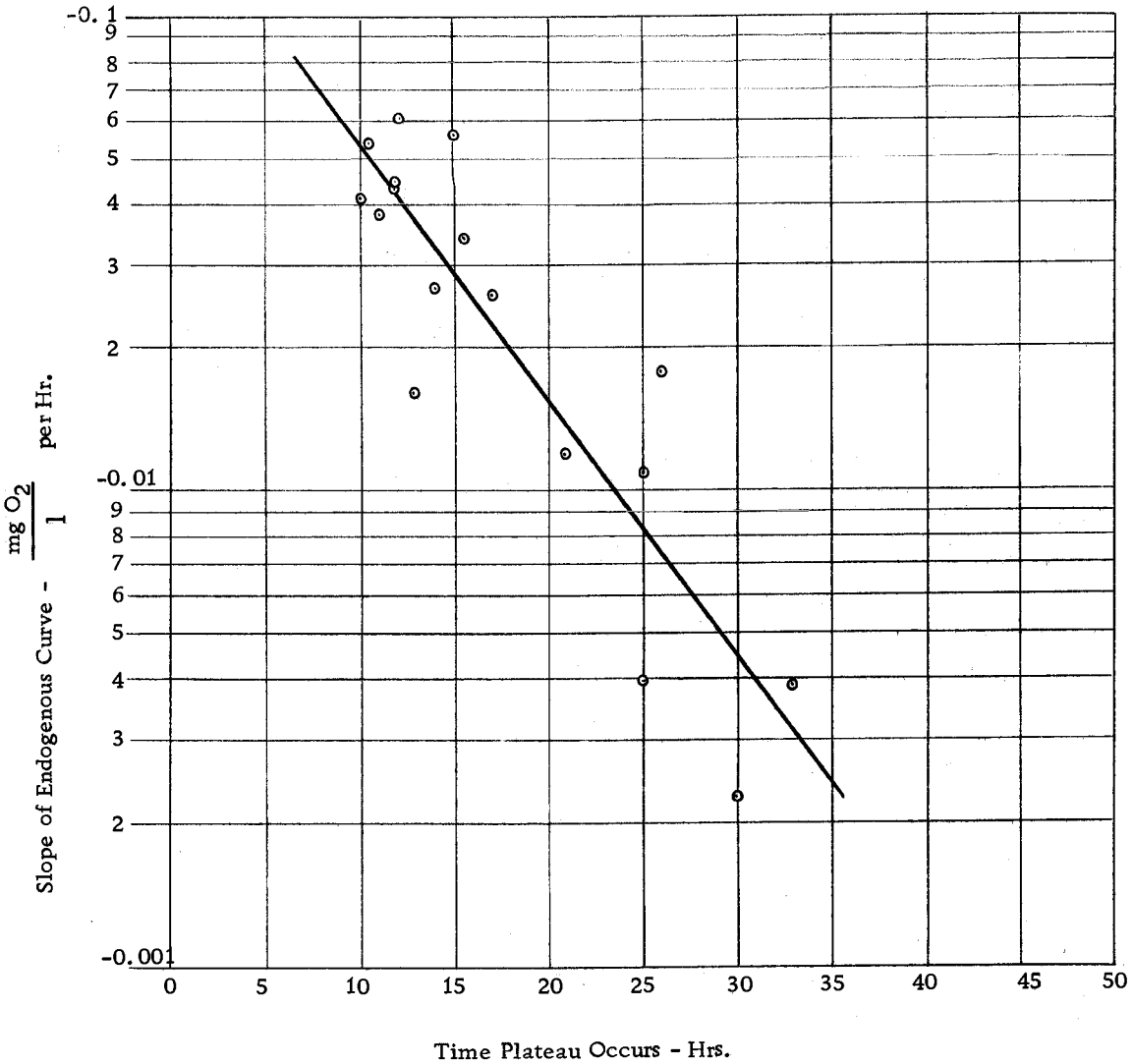


Figure 7. Linear regression analysis of the slope of the endogenous curve and the time at which the plateau occurs. Correlation coefficient = - 0.90.

Table 9. Linear Regression Analysis for Endogenous Slope vs Time of Plateau Occurrence.

n	17				
Σx	302			Σy	22.2538
$\bar{x}$	17.8			$\bar{y}$	1.3090
$(\Sigma x)^2$	91,204	$(\Sigma y)(\Sigma y)$	6,720.6476	$(\Sigma y)^2$	495.2316
$(\Sigma x)^2/n$	5,364.9	$(\Sigma x)(\Sigma y) \div n$	395.3322	$(\Sigma y)^2/n$	29.1313
Σx^2	6,238.5	$\Sigma(xy)$	349.1661	Σy^2	32.2250
SS_x	873.6	SP	-46.1661	SS_y	3.0937

Analysis

$SP/SS_x = -0.0528$

$y = -2.3090 - 0.0528\ (x-17.8)$

$r = SP/(SS_x SS_y)^{1/2} = -0.90$

x = Time of Plateau Occurrence

y = Log_{10} of Endogenous Slope

multiplied by -10^3 before performing the regression and subsequently returned to the original coordinate system in the analysis.)

Figure 7 might imply that the experimental evidence suggests the possibility of an instant plateau. This is, of course, impossible because time is required for substrate microbe contact, diffusion of the substrate into the cell, and the carrying out of the oxidation-reduction reactions during metabolism.

In order to determine what minimum time might be required for plateau attainment, relative activity coefficients have been calculated. This was done by assigning a value of 1.0 to the maximum a.c. value, i.e. the a.c. of Run No. 1. All other a.c.'s are expressed relative to an a.c. coefficient of 1.0. These values are given in Table 10.

Figure 8 is a graphical representation of Table 10. It appears that under the conditions of this study it would be unlikely to obtain plateaus in less then ten hours. It also appears that the product, (relative a.c.) x (endogenous slope), should be maintained at a minimum value of $-0.010 \frac{\text{mg } O_2}{1}$ per hour.

Plateau Clarity

The only apparently distinguishing feature about the clarity of the plateau is that concave and linear transitions seem to provide a sharper break than those curves whose initial geometry is convex.

Table 10. Relative Activity Coefficients, Corresponding Endogenous Slopes, and Time of Plateau Occurrence.

Run No.	Relative a.c.	Relative a.c x Endogenous Slope	Time at which Plateau Occurs
1	1	-0.038	11.0
2	0.42	-0.018	12.0
3	0.73	-0.039	10.5
4	0.46	-0.005	13.0
5	0.42	-0.014	15.5
6	0.79	-0.033	12.0
7	0.31	-0.008	17.0
8	0.55	-0.031	15.0
9	0.51	-0.009	26.0
10	0.51	-0.006	25.0
11	0.36	-0.008	30.0
12	0.25	-0.009	33.0
13	0.39	-0.0015	25.0
14	0.40	-0.0048	21.0
15	0.15	-0.004	14.0
16	0.30	-0.012	10.0
17	0.27	-0.012	12.0

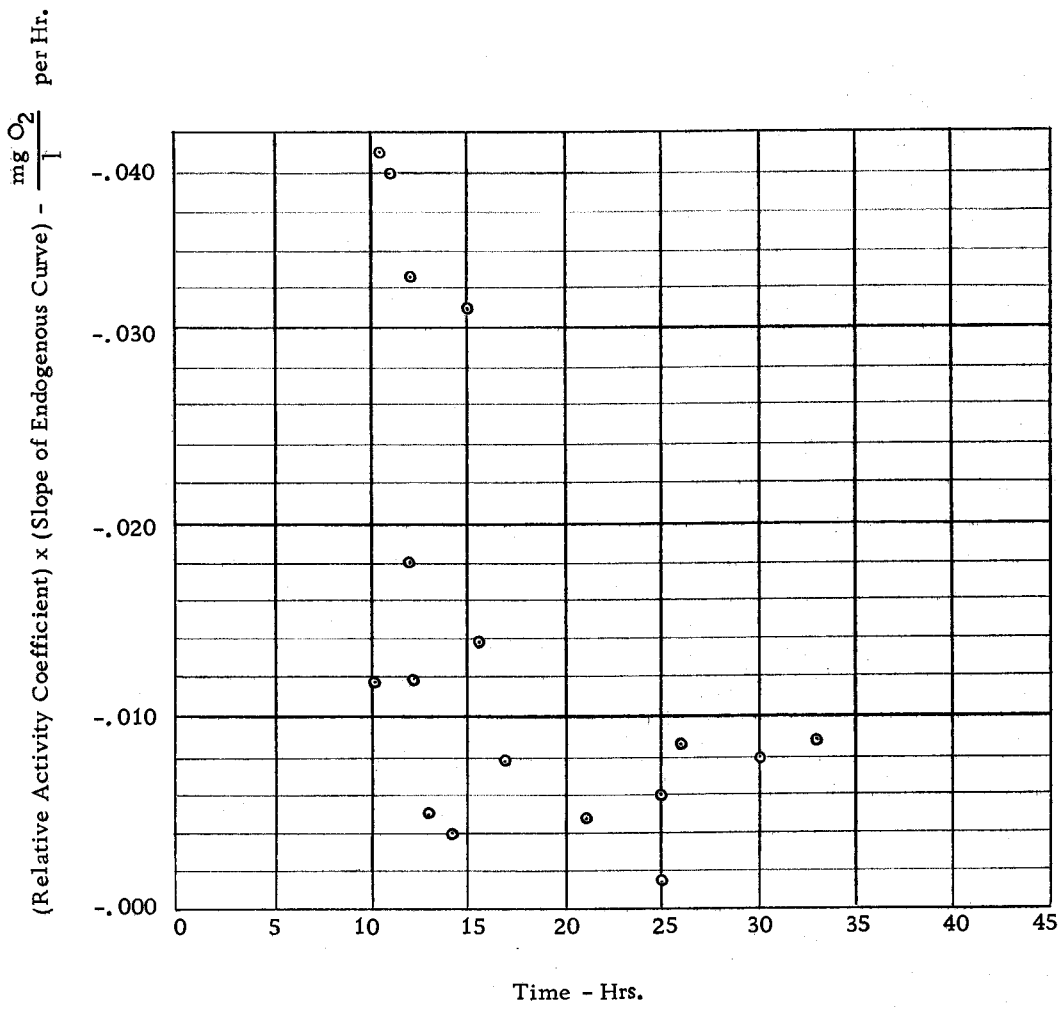


Figure 8. Time at which plateau begins compared to the slope of endogenous curves corrected for relative activity coefficients.

Plateau Variability

Although there is variability in the plateau BOD it is a relatively small amount (See Table 4). Therefore, the differences in plateau BOD are considered to be a result of the variation one would normally expect in an experiment of this type.

Geometry of BOD Curves

Figures 9 and 10 compare BOD curves with identical activity indices. There appears to be a high similarity between the geometry of a BOD curve and a given numerical value for its activity index.

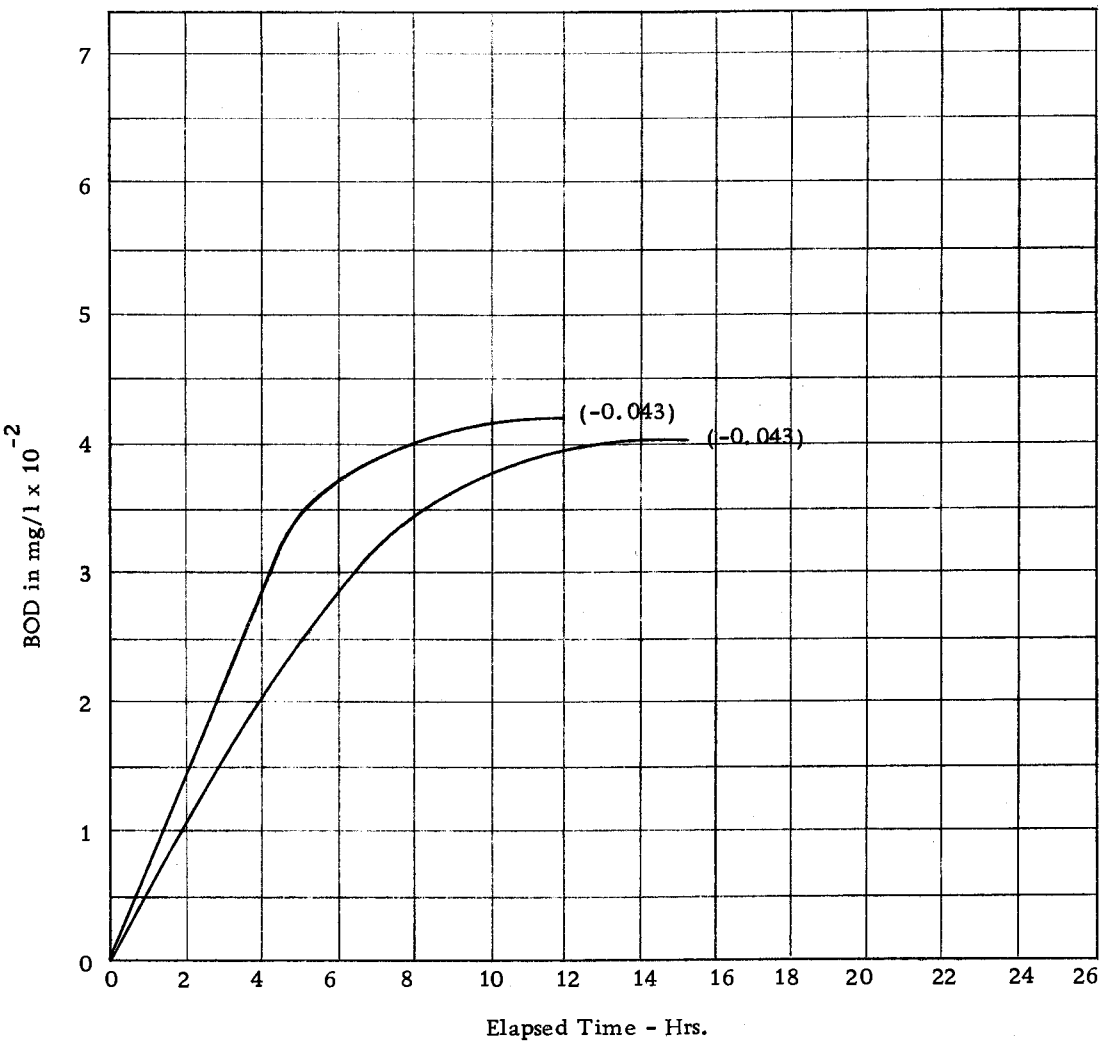


Figure 9. BOD progression as a function of the slope of endogenous curve. Endogenous slopes are shown in parenthesis.

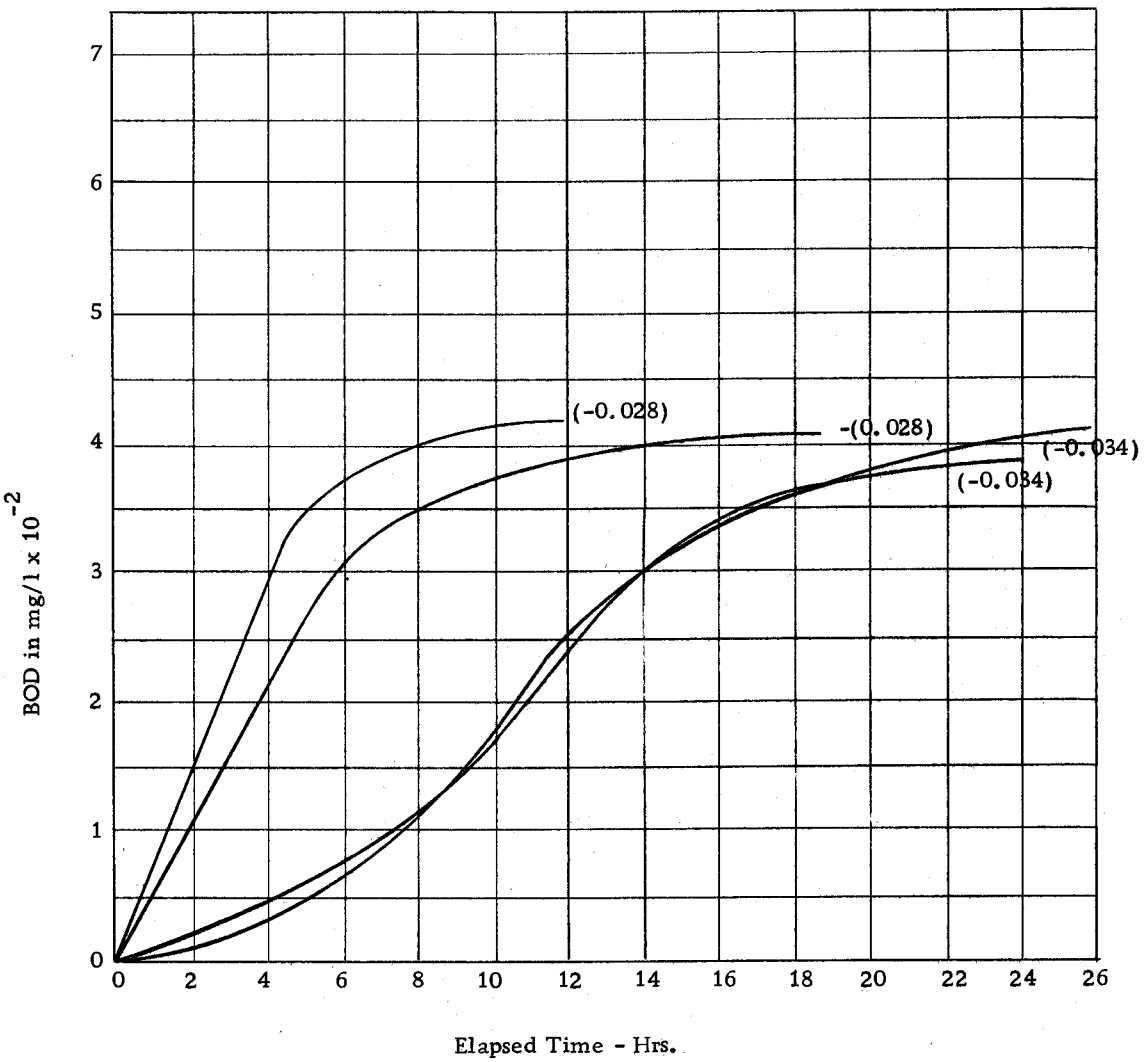


Figure 10. BOD progression as a function of activity coefficients. (a.c. is shown in parenthesis)

V. CONCLUSIONS

This study investigated certain aspects of the biological metabolism of a substrate containing both soluble and colloidal fractions. The following conclusions are referenced to the bacterial metabolism of a 0.1 percent solution of Carnation dry-skim milk at a temperature of 29°C.

1. Experimental evidence indicates that it is possible to observe a reproducible plateau BOD during the biological degradation of a colloidal substrate. A statistical analysis of the data demonstrated that the initial COD of the milk solution was accountable for in terms of plateau BOD, and the formation of new cell growth.

   The metabolism of a 0.1 percent solution of Carnation dry skim milk yielded a mean value of 526 mg/l of new cell growth (as BOD), and a plateau BOD of 398 mg/l. The average COD of the milk solution was 1,025 mg/l.

2. The fraction of the ultimate BOD oxidized (f_o) and the fraction synthesized (f_s) were determined to be:

$$f_o = 43.0\ \%$$

and

$$f_s = 57.0\ \% .$$

3. Mathematical formulations were developed relating plateau BOD and the COD of the milk solution. When these relationships were evaluated in terms of the experimental evidence, the following equation was obtained:

$$\text{Plateau BOD} = 0.49\ \text{COD}_i,$$

where

Plateau BOD = the amount of oxygen used during the respiration phase of metabolism,

COD_i = the initial chemical oxygen demand of the milk solution.

4. The time at which the plateau occurs appears to be related to the logarithmic slope of the endogenous curve. This is a bounded relationship in its lower limit, and it appears that at least ten hours are required to complete the respiration phase of metabolism.

5. BOD curves with identical activity indices were compared. In all cases the corresponding geometries were highly similar.

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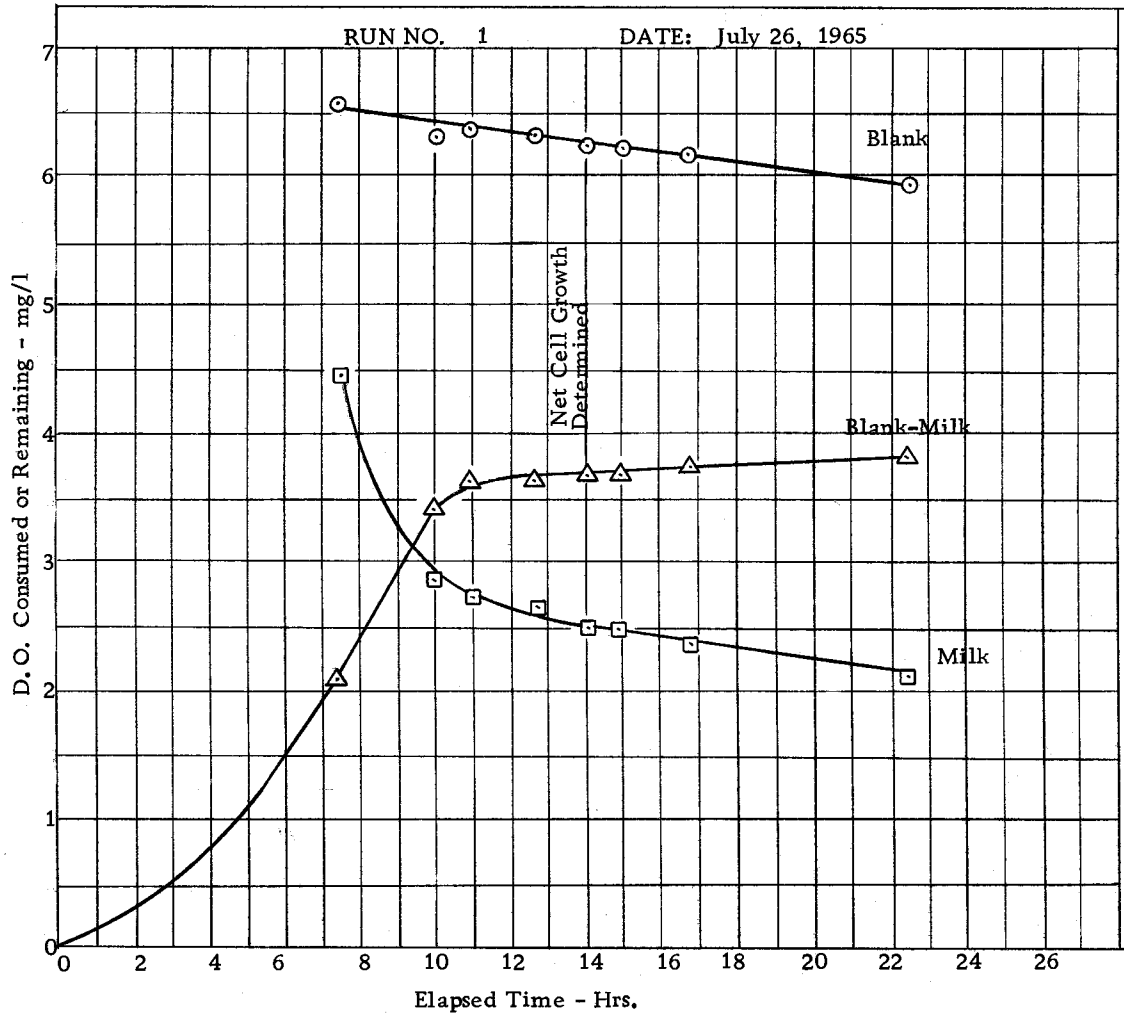
APPENDIX



EXPERIMENTAL DATA

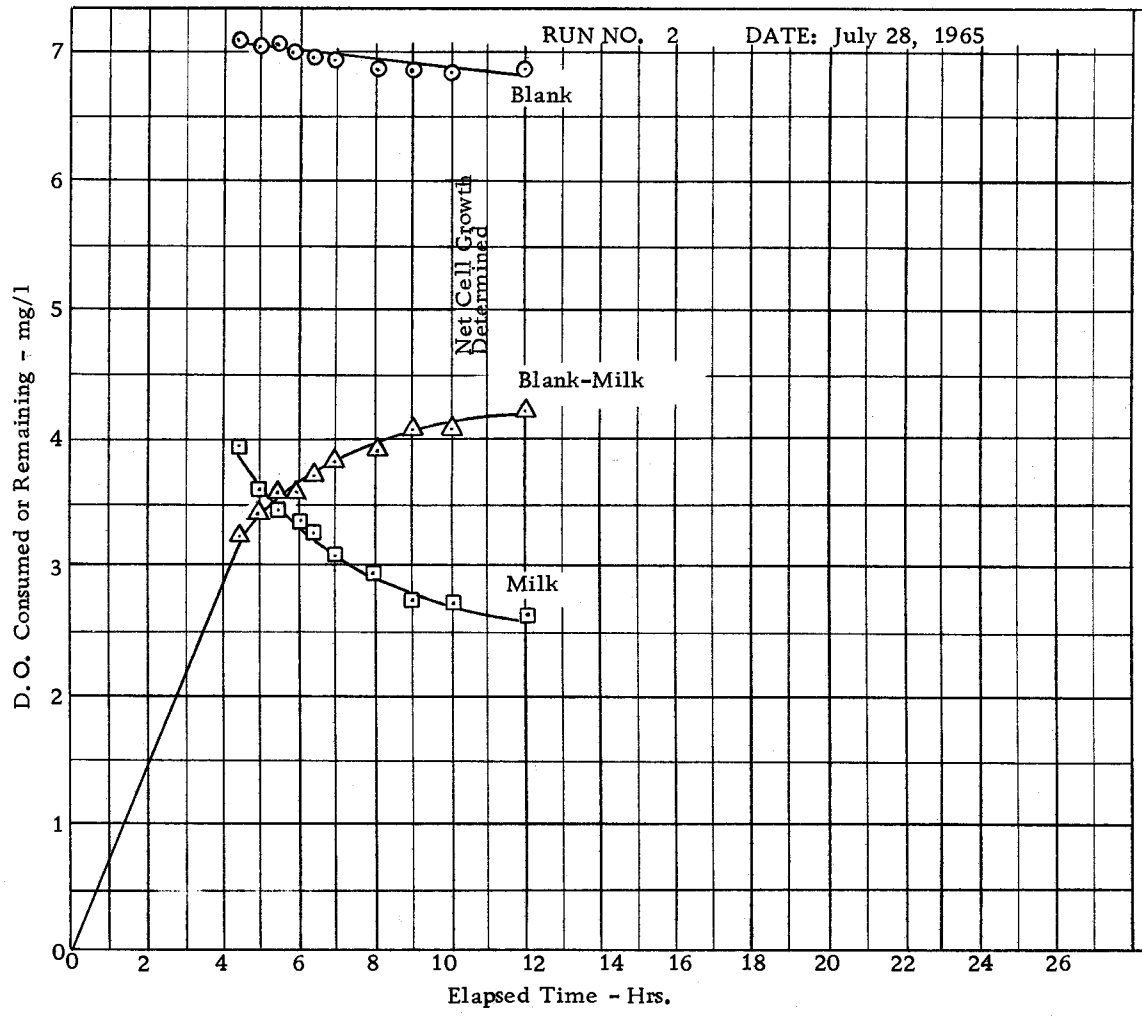
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D.O.Blk-D.O.Milk mg/l
6.75	6.55	4.45	2.10
10.00	6.30	2.83	3.47
11.00	6.35	2.70	3.65
12.75	6.30	2.67	3.63
14.00	6.25	2.50	3.75
15.00	6.25	2.50	3.75
16.75	6.15	2.37	3.77
22.50	5.95	2.15	3.80

Cell Growth Data in mg/l

Final Concentration	933
Initial Concentration	567
Net Cell Growth	366

Figure 11. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

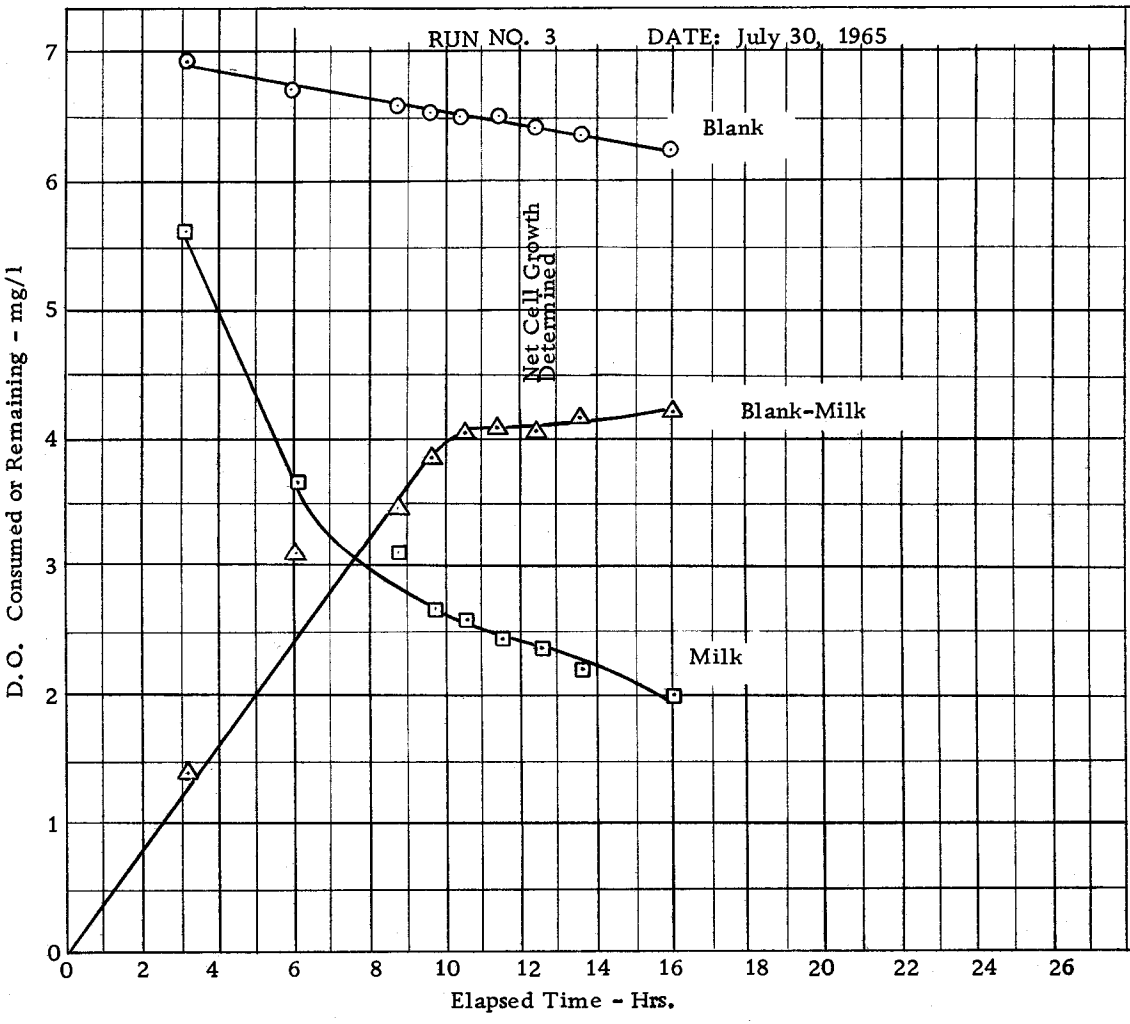
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D.O. Blk-D.O.Milk mg/l
4.50	7.15	3.90	3.25
5.00	7.05	3.62	3.43
5.50	7.05	3.45	3.60
6.00	7.00	3.35	3.65
6.50	6.97	3.25	3.72
7.00	6.95	3.10	3.85
8.00	6.87	2.97	3.90
9.00	6.82	2.72	4.10
10.00	6.80	2.72	4.08
12.00	6.85	2.65	4.20

Cell Growth Data in mg/l

Final Concentration	1950
Initial Concentration	1530
Net Cell Growth	420

Figure 12. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

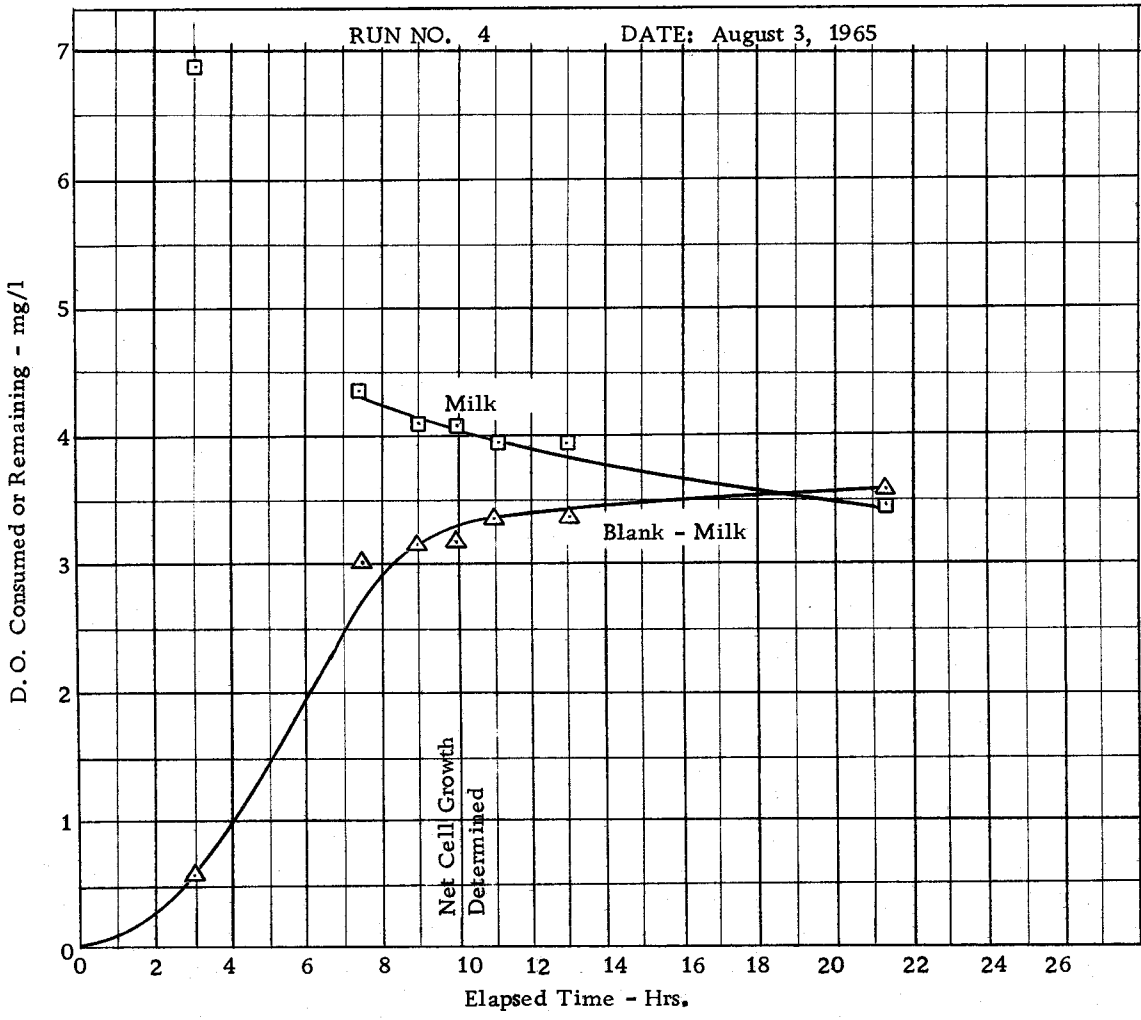
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D.O. Blk-D.O. Milk mg/l
3.25	6.95	5.65	1.35
6.00	6.70	3.62	3.08
8.75	6.60	3.12	3.48
9.67	6.50	2.70	3.80
10.50	6.50	2.57	4.03
11.50	6.50	2.42	4.08
12.50	6.40	2.35	4.05
13.67	6.35	2.17	4.18
16.00	6.25	2.00	4.25

Cell Growth Data in mg/l

Final Concentration	1750
Initial Concentration	1112
Net Cell Growth	638

Figure 13. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

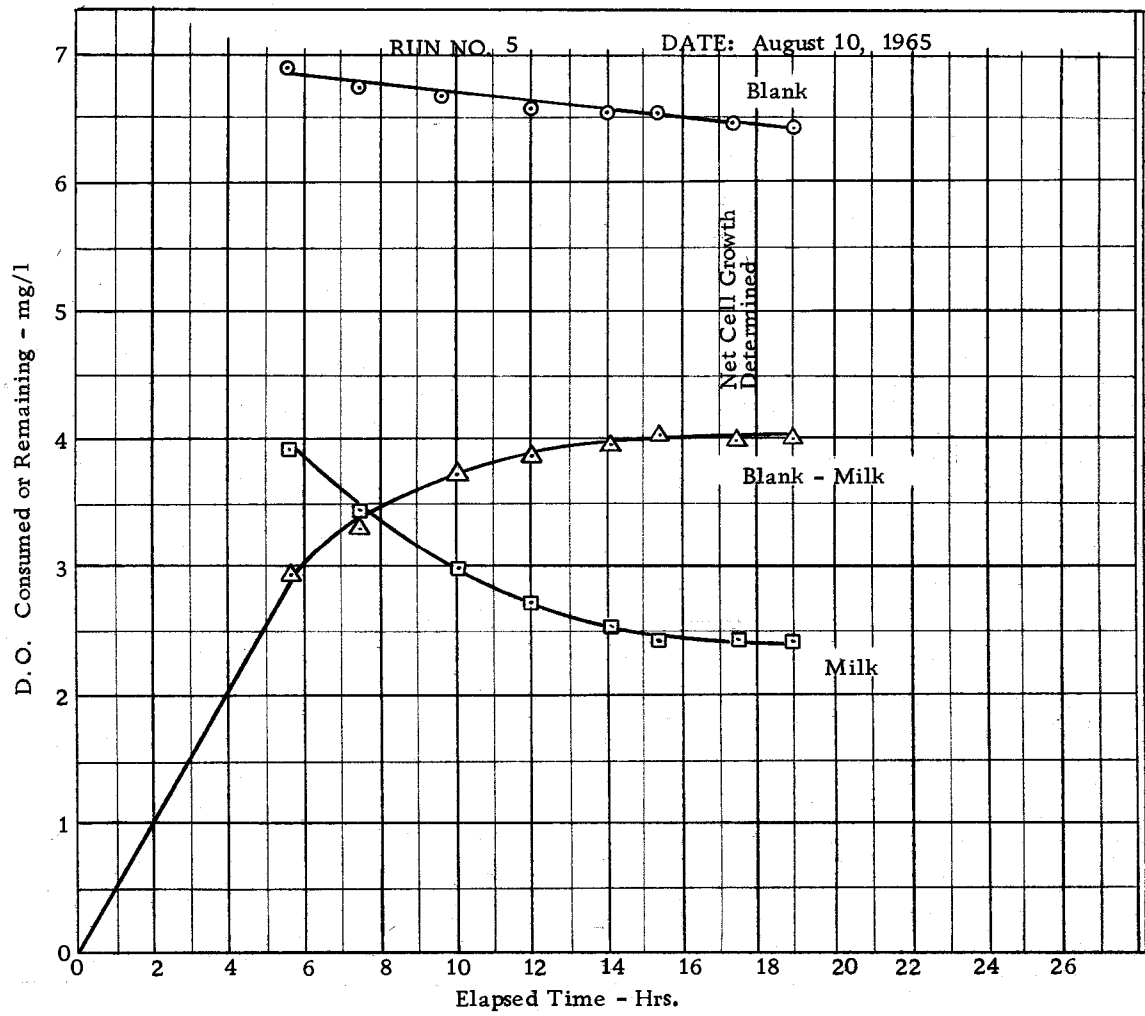
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D.O. Blk-D.O. Milk mg/l
3.00	7.45	6.82	0.63
7.50	7.35	4.35	3.00
9.00	7.25	4.10	3.15
10.00	7.25	4.10	3.15
11.20	7.27	3.95	3.32
13.00	7.25	3.90	3.35
21.50	7.05	3.45	3.60
29.67	7.02	2.97	4.05

Cell Growth Data in mg/l

Final Concentration	807
Initial Concentration	513
Net Cell Growth	294

Figure 14. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

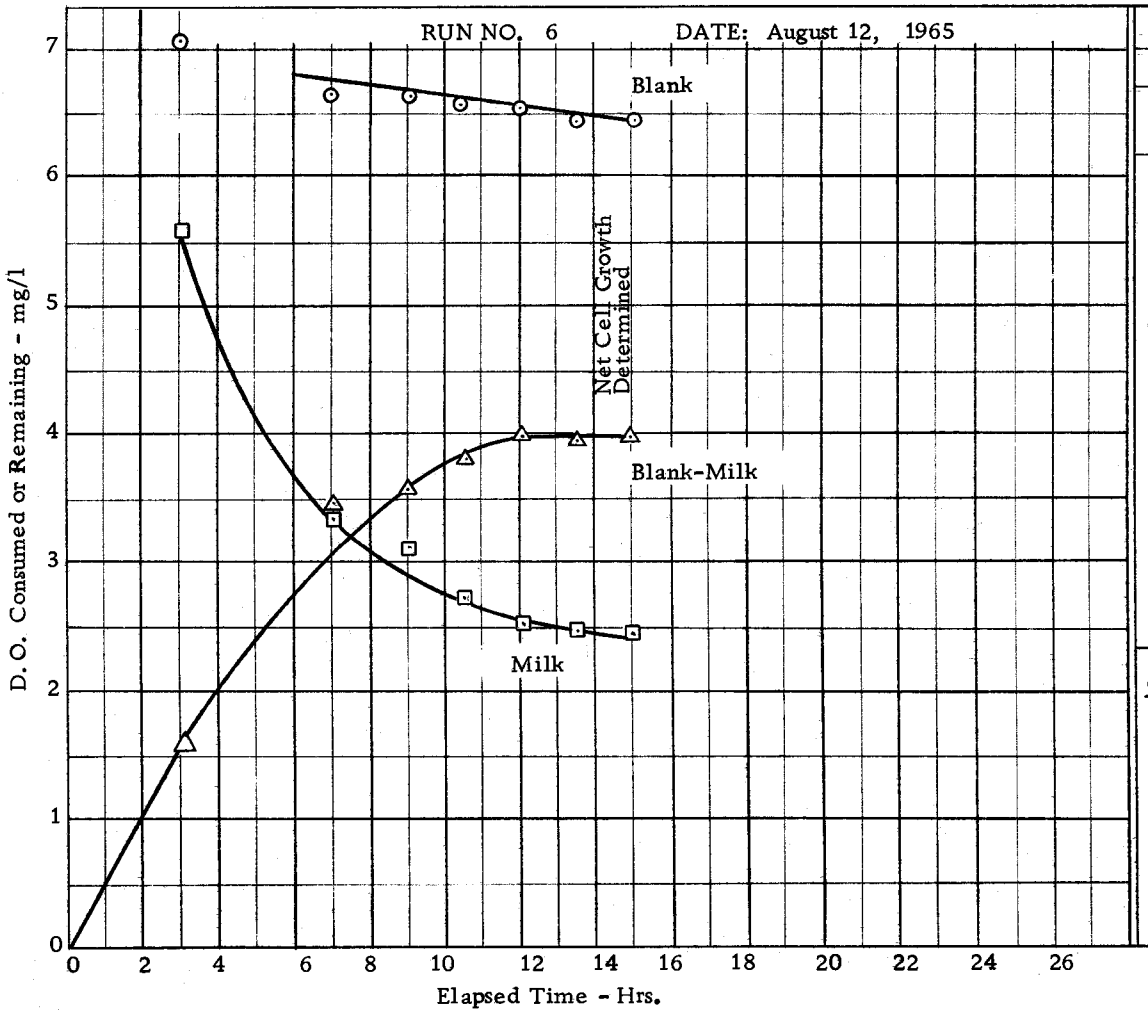
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D.O. Blk-D.O. Milk mg/l
5.75	6.85	3.90	2.95
7.50	6.75	3.40	3.35
10.00	6.70	3.00	3.70
12.00	6.57	2.72	3.85
14.00	6.52	2.60	3.92
15.50	6.52	2.47	4.05
17.50	6.45	2.45	4.00
19.00	6.40	2.40	4.00

Cell Growth Data in mg/l

Final Concentration	1309
Initial Concentration	936
Net Cell Growth	373

Figure 15. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

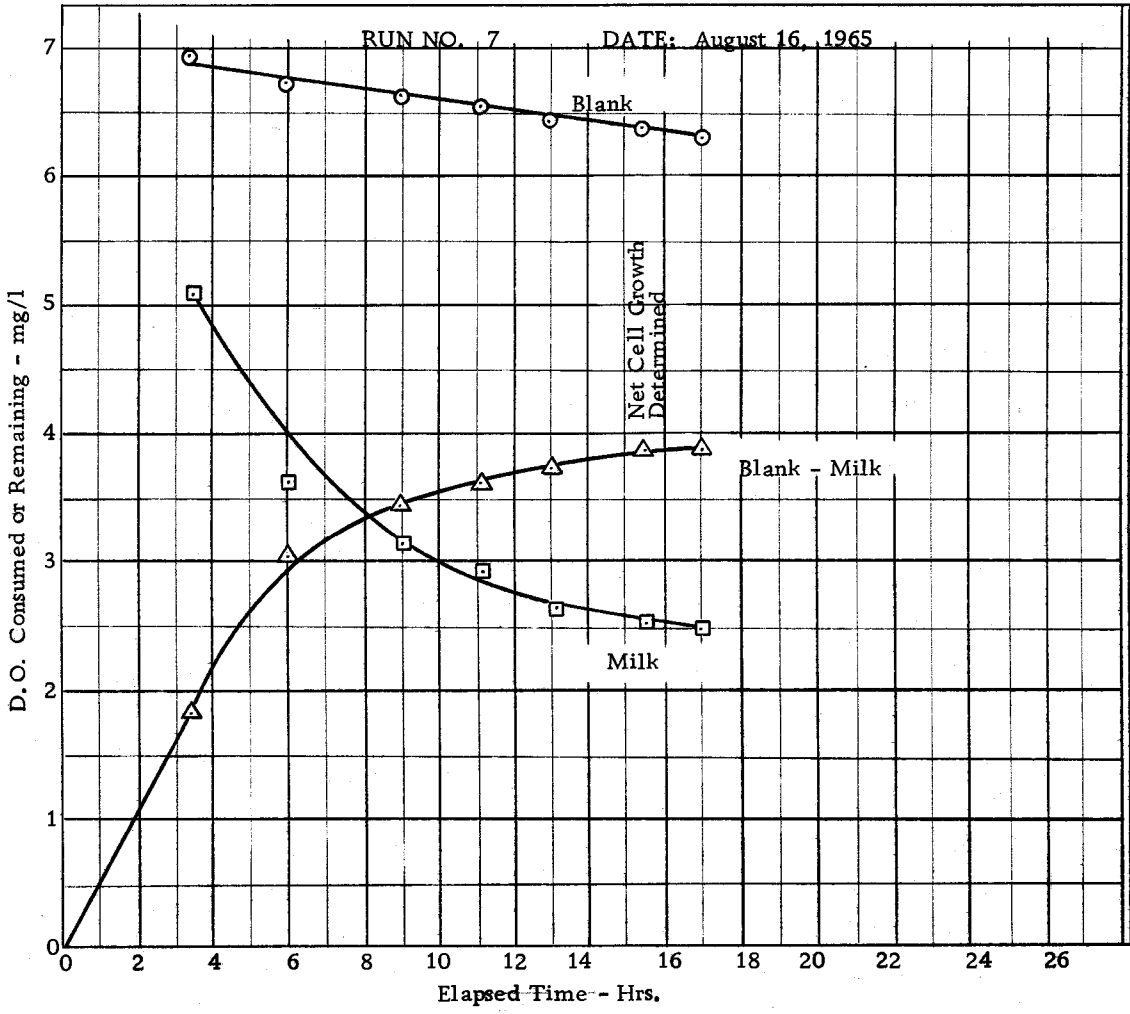
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D.O. Blk-D.O. Milk mg/l
3.00	7.02	5.60	1.62
7.00	6.70	3.30	3.40
9.00	6.70	3.10	3.60
10.50	6.60	2.77	3.83
12.00	6.55	2.55	4.00
13.50	6.47	2.50	3.97
15.00	6.45	2.45	4.00

Cell Growth Date in mg/l

Final Concentration	1530
Initial Concentration	1155
Net Cell Growth	375

Figure 16. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

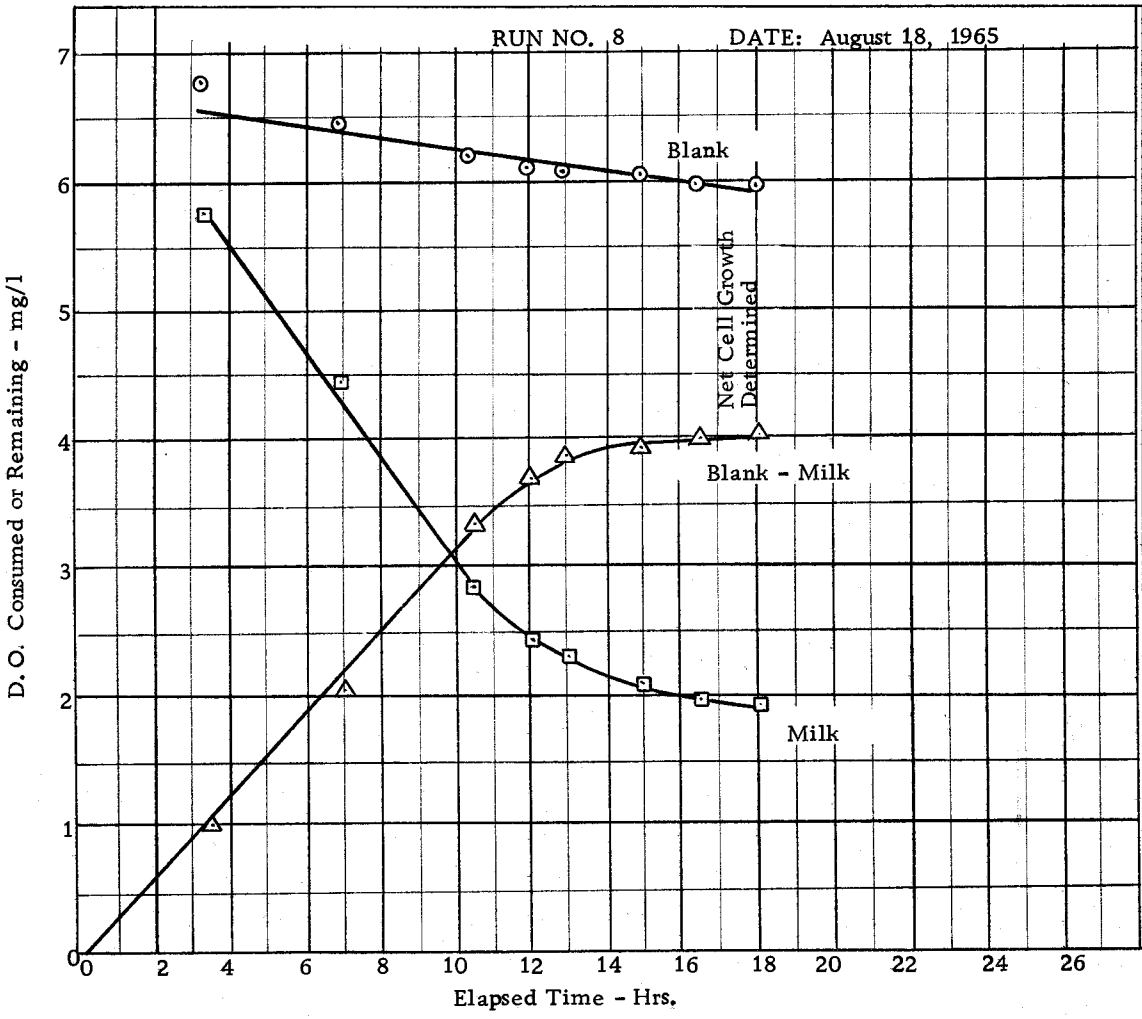
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D. O. Blk-D.O. Milk mg/l
3.50	6.90	5.10	1.80
6.00	6.75	3.65	3.10
9.00	6.67	3.20	3.47
11.25	6.52	2.87	3.65
13.00	6.42	2.67	3.75
15.50	6.35	2.55	3.80
17.00	6.30	2.50	3.80

Cell Growth Data in mg/l

Final Concentration	1600
Initial Concentration	1222
Net Cell Growth	378

Figure 17. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

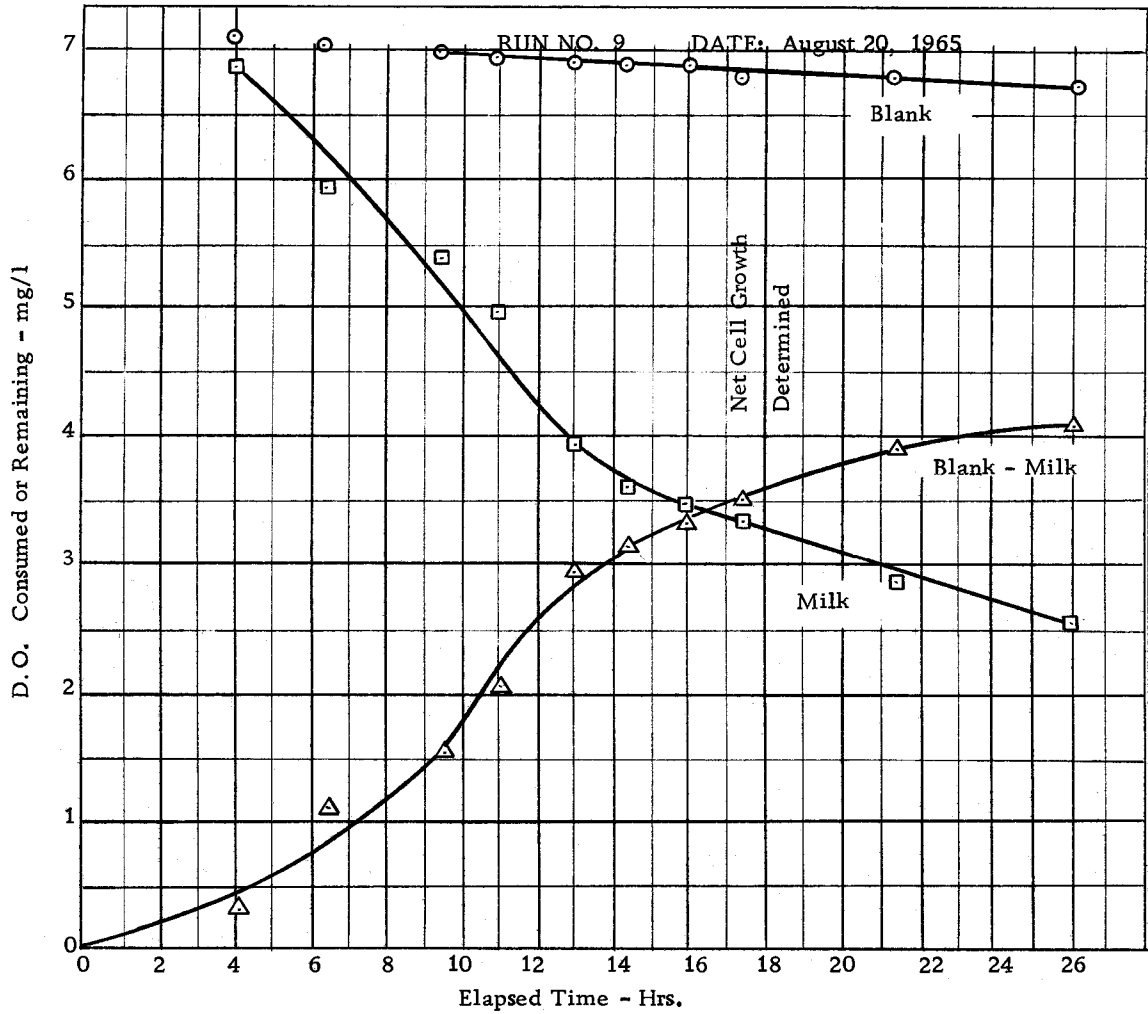
Dissolved Oxygen Data

t Hrs.	D. O. -mg/l Blk	Milk	D.O. Blk-D.O. Milk mg/l
3.50	6.75	5.75	1.00
7.00	6.45	4.40	2.05
10.50	6.22	2.85	3.37
12.00	6.15	2.45	3.70
13.00	6.10	2.30	3.80
15.00	6.02	2.10	3.92
16.50	5.95	1.95	4.00
18.00	5.95	1.90	4.05

Cell Growth Data in mg/l

Final Concentration	1925
Initial Concentration	1515
Net Cell Growth	410

Figure 18. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

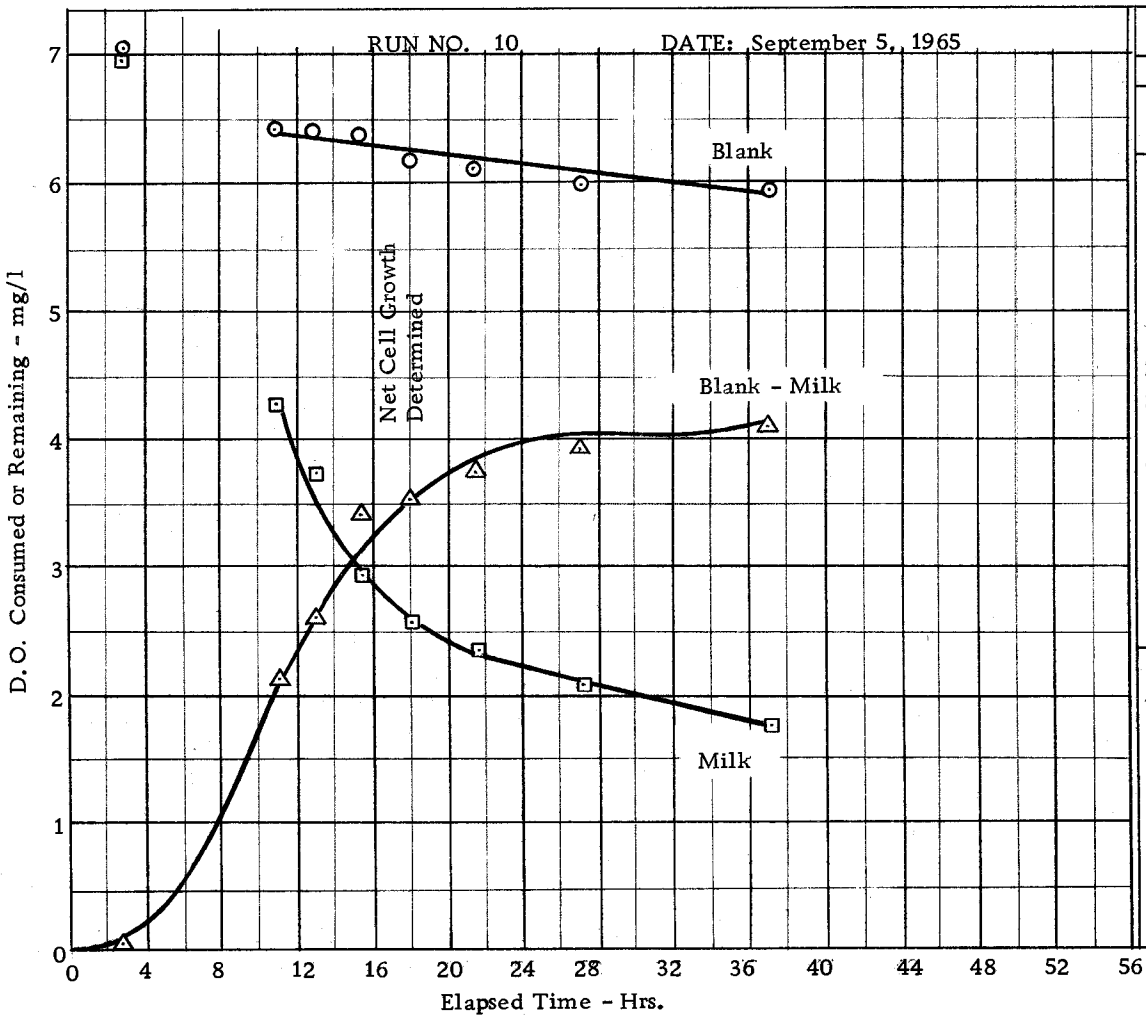
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	Milk	D.O. Blk-D.O. Milk mg/l
4.00	7.15	6.85	0.30
6.50	7.02	5.90	1.12
9.50	6.97	5.37	1.60
11.00	6.95	4.90	2.05
13.00	6.87	3.95	2.92
14.50	6.82	3.65	3.17
16.00	6.80	3.45	3.35
17.50	6.77	3.27	3.50
22.50	6.77	2.80	3.97
26.00	6.70	2.60	4.10

Cell Growth Data in mg/l

Final Concentration	870
Initial Concentration	528
Net Cell Growth	342

Figure 19. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

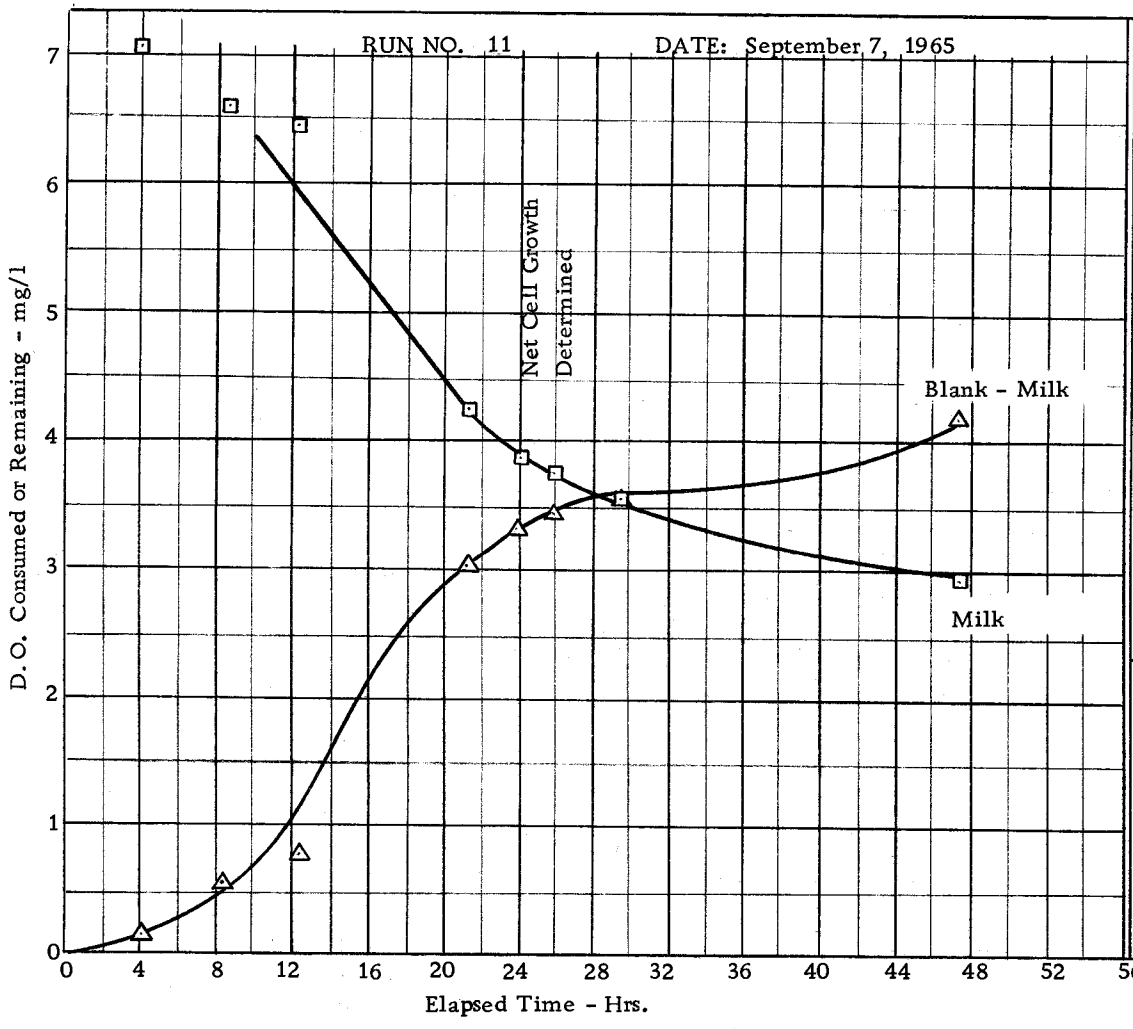
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	Milk	D.O. Blk-D.O.Milk mg/l
3.00	7.05	6.92	0.13
11.00	6.37	4.25	2.12
13.00	6.35	3.70	2.65
15.50	6.30	2.85	3.45
18.00	6.17	2.62	3.55
21.50	6.07	2.30	3.77
27.00	5.97	2.07	3.90
37.00	5.95	1.77	4.18
61.00	5.80	1.45	4.35

Cell Growth Data in mg/l

Final Concentration	738
Initial Concentration	334
Net Cell Growth	404

Figure 20. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

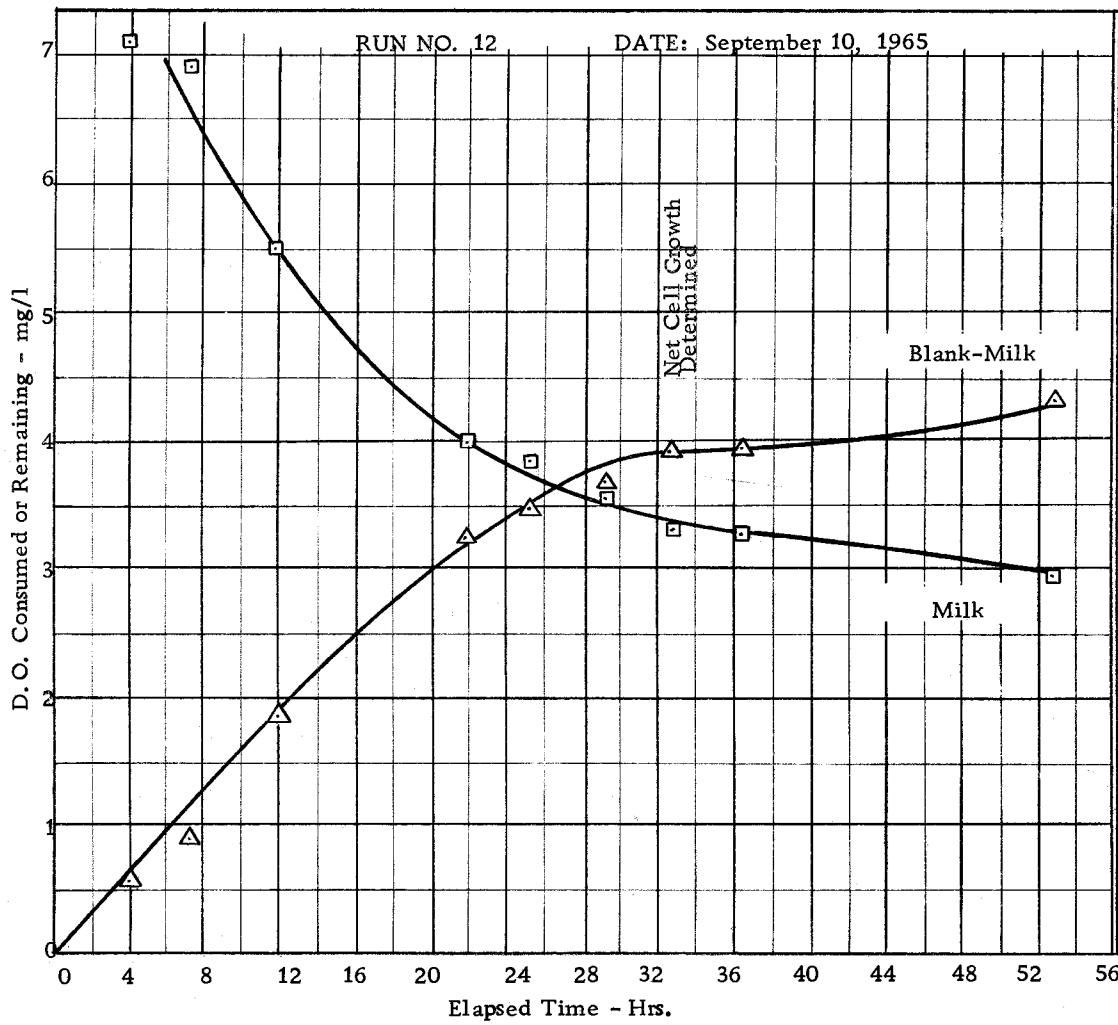
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D.O.Blk-D.O.Milk mg/l
4.00	7.25	7.10	0.15
8.50	7.20	6.62	0.58
12.50	7.20	6.45	0.75
21.50	7.25	4.20	3.05
24.00	7.20	3.85	3.35
26.00	7.17	3.75	3.42
29.50	7.10	3.55	3.55
47.00	7.15	2.90	4.25

Cell Growth Data in mg/l

Final Concentration	487
Initial Concentration	97
Net Cell Growth	390

Figure 21. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

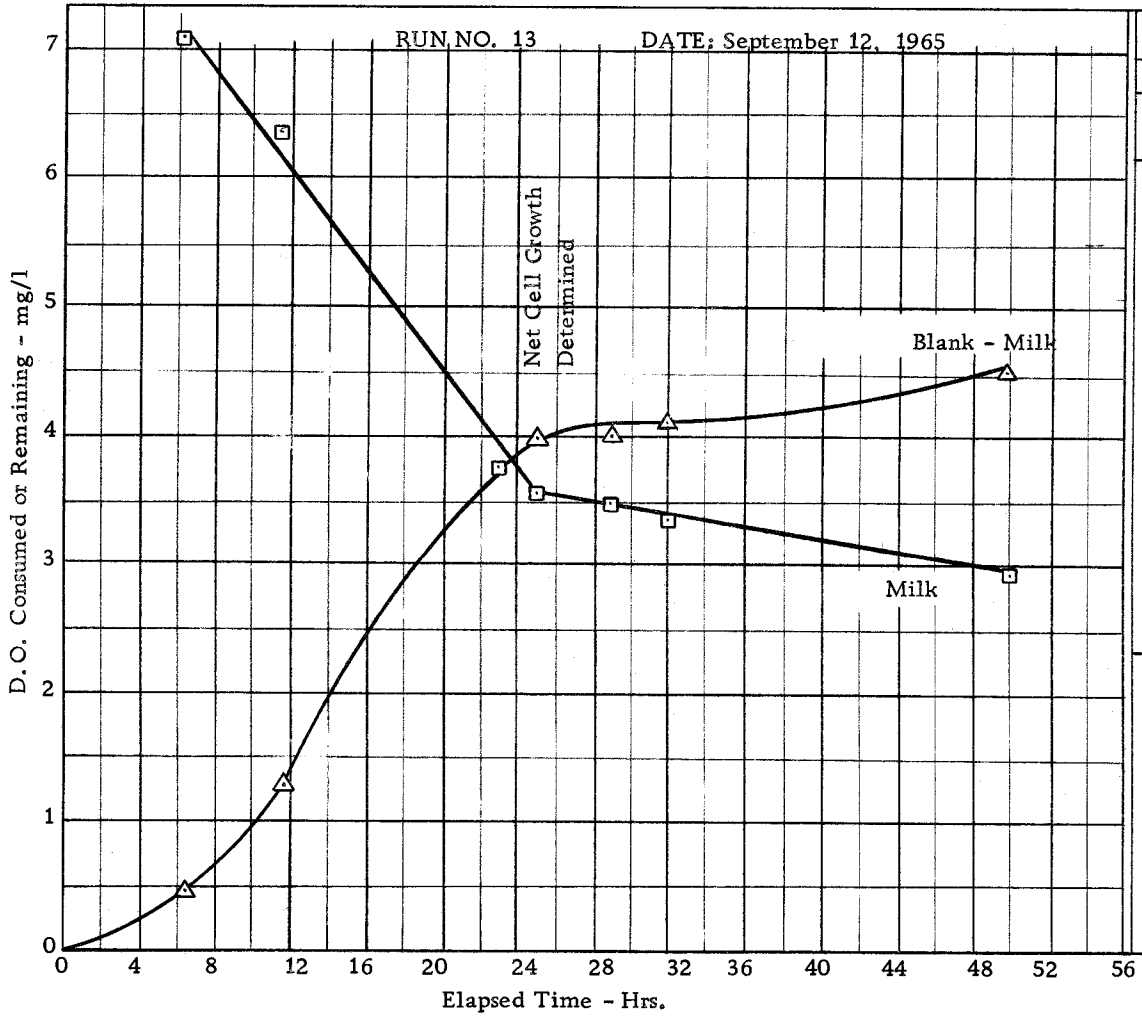
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D.O.Blk-D.O.Milk mg/l
4.00	7.45	7.17	0.28
7.75	7.30	6.90	0.40
12.00	7.35	5.50	1.85
22.00	7.25	4.00	3.25
25.50	7.32	3.85	3.47
29.50	7.25	3.55	3.70
33.00	7.25	3.32	3.93
36.50	7.22	3.32	3.90
53.00	7.20	2.92	4.28

Cell Growth Data in mg/l

Final Concentration	589
Initial Concentration	227
Net Cell Growth	362

Figure 22. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

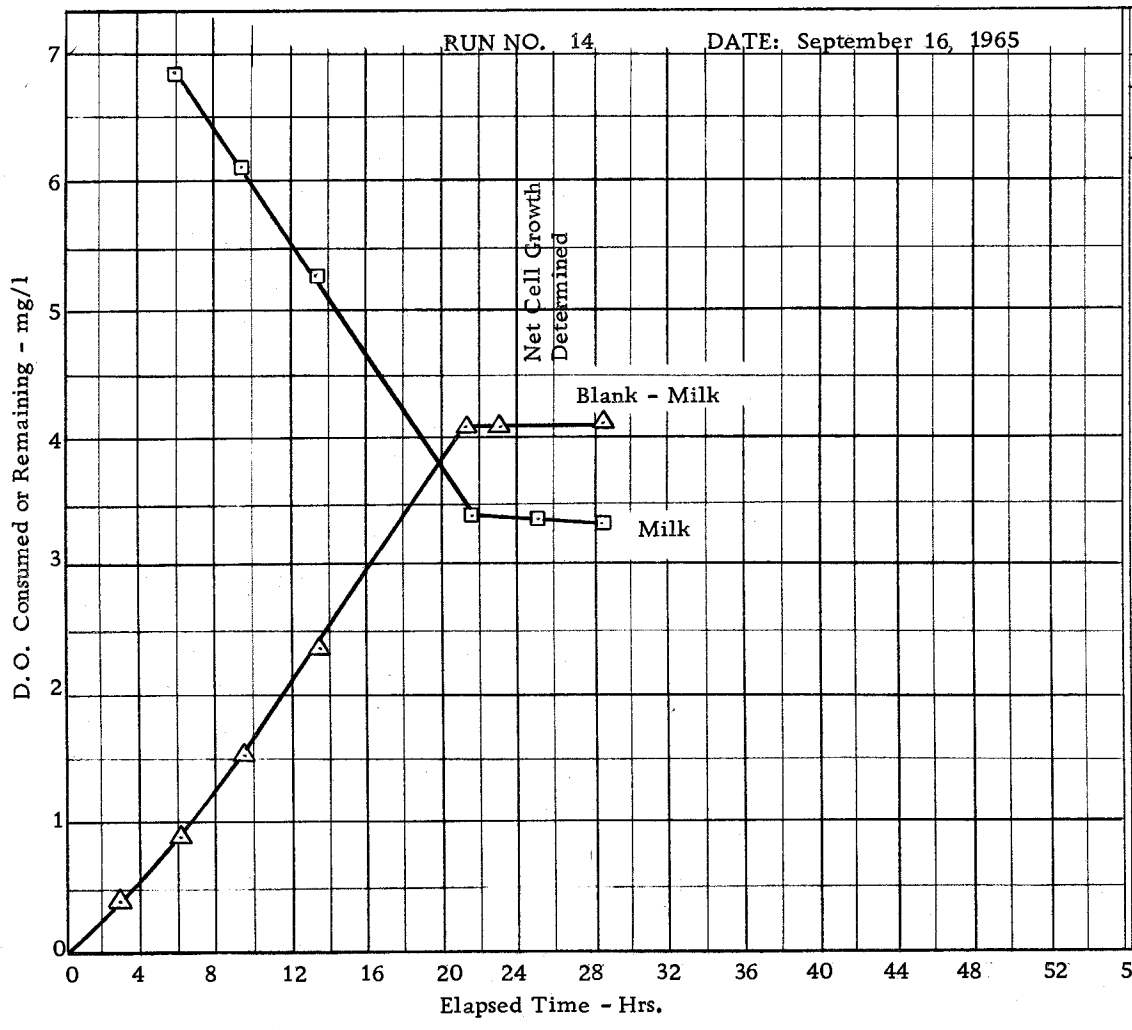
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D.O. Blk-D.O.Milk mg/l
6.50	7.60	7.10	0.50
11.75	7.62	6.30	1.32
23.00	7.50	3.75	3.75
25.00	7.55	3.55	4.00
29.00	7.55	3.50	4.05
32.00	7.50	3.35	4.15
50.00	7.45	2.90	4.55

Cell Growth Data in mg/l

Final Concentration	782
Initial Concentration	153
Net Cell Growth	629

Figure 23. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

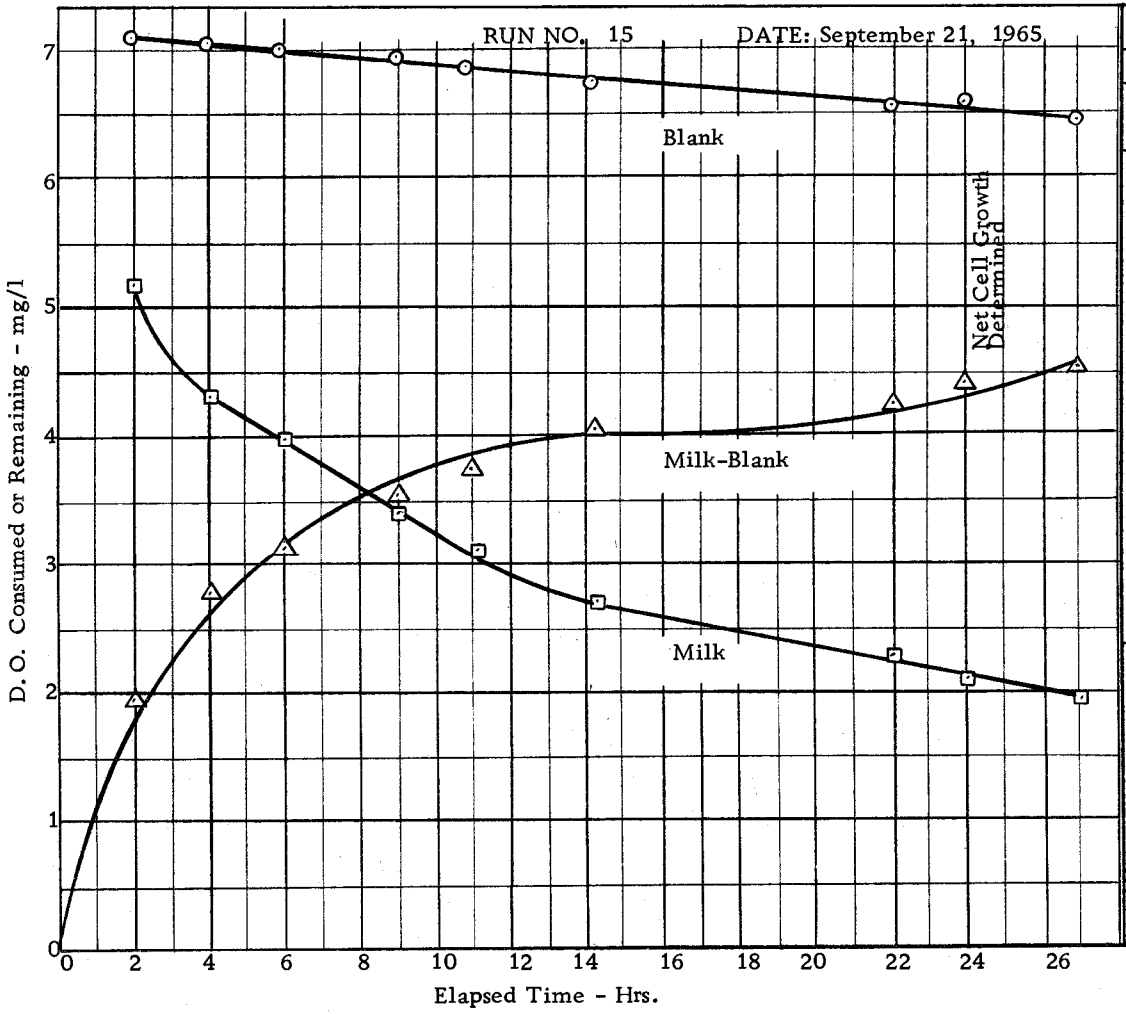
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D. O. Blk-D.O.Milk mg/l
3.00	7.75	7.30	0.45
6.00	7.75	6.85	0.90
9.50	7.65	6.12	1.53
13.50	7.55	5.25	2.30
21.75	7.55	3.37	4.18
25.00	7.45	3.35	4.10
28.50	7.50	3.30	4.20

Cell Growth Data in mg/l

Final Concentration	762
Initial Concentration	329
Net Cell Growth	433

Figure 24. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

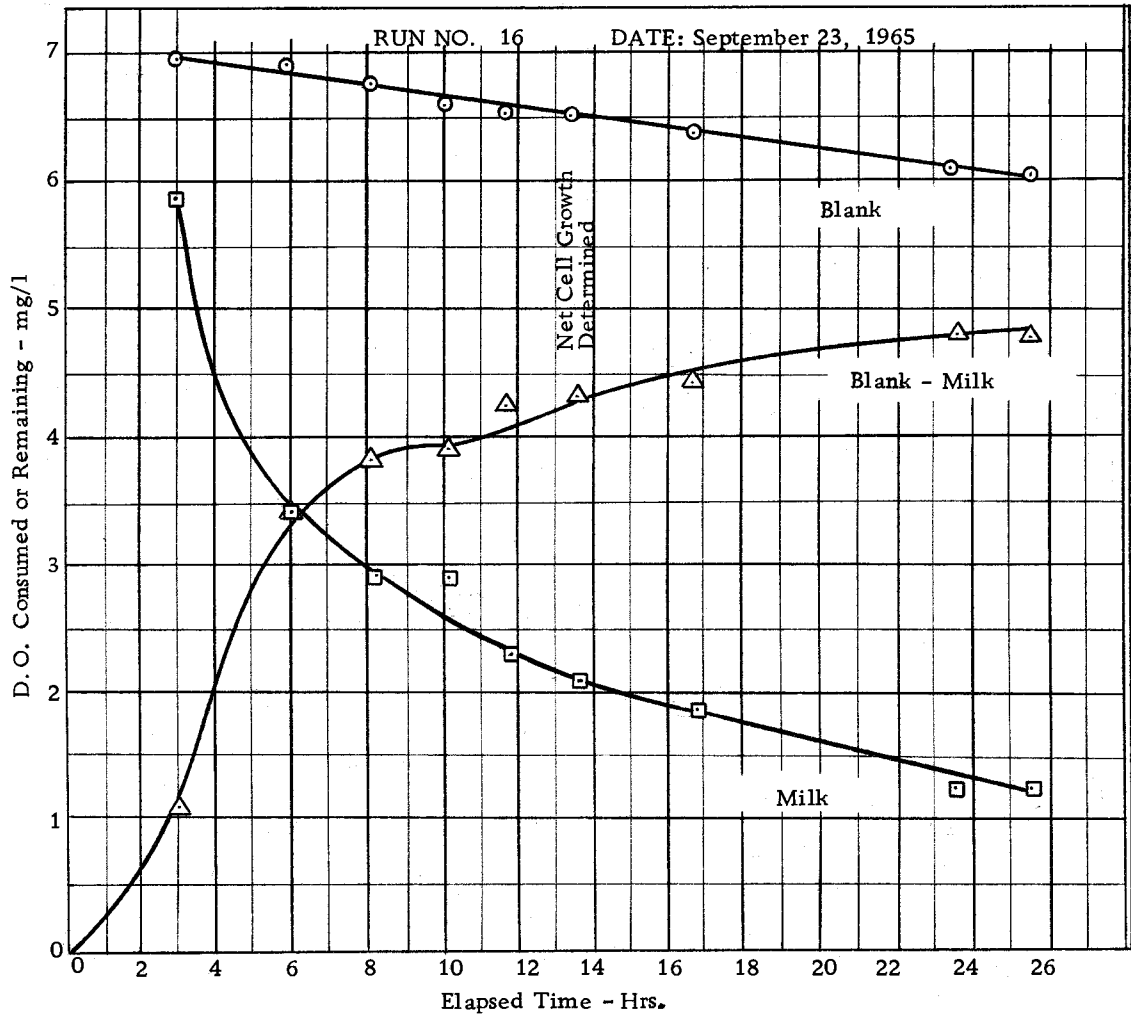
Dissolved Oxygen Data

t Hrs.	D.O. - mg/l Blk	D.O. - mg/l Milk	D.O. Blk-D.O.Milk mg/l
2.00	7.15	5.20	1.95
4.00	7.05	4.27	2.78
6.00	7.00	3.97	3.03
9.00	6.92	3.35	3.57
11.00	6.80	3.10	3.70
14.25	6.75	2.70	4.25
22.00	6.52	2.27	4.25
24.00	6.57	2.10	4.47
27.00	6.45	1.90	4.55

Cell Growth Date in mg/l

Final Concentration	2962
Initial Concentration	2722
Net Cell Growth	240

Figure 25. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

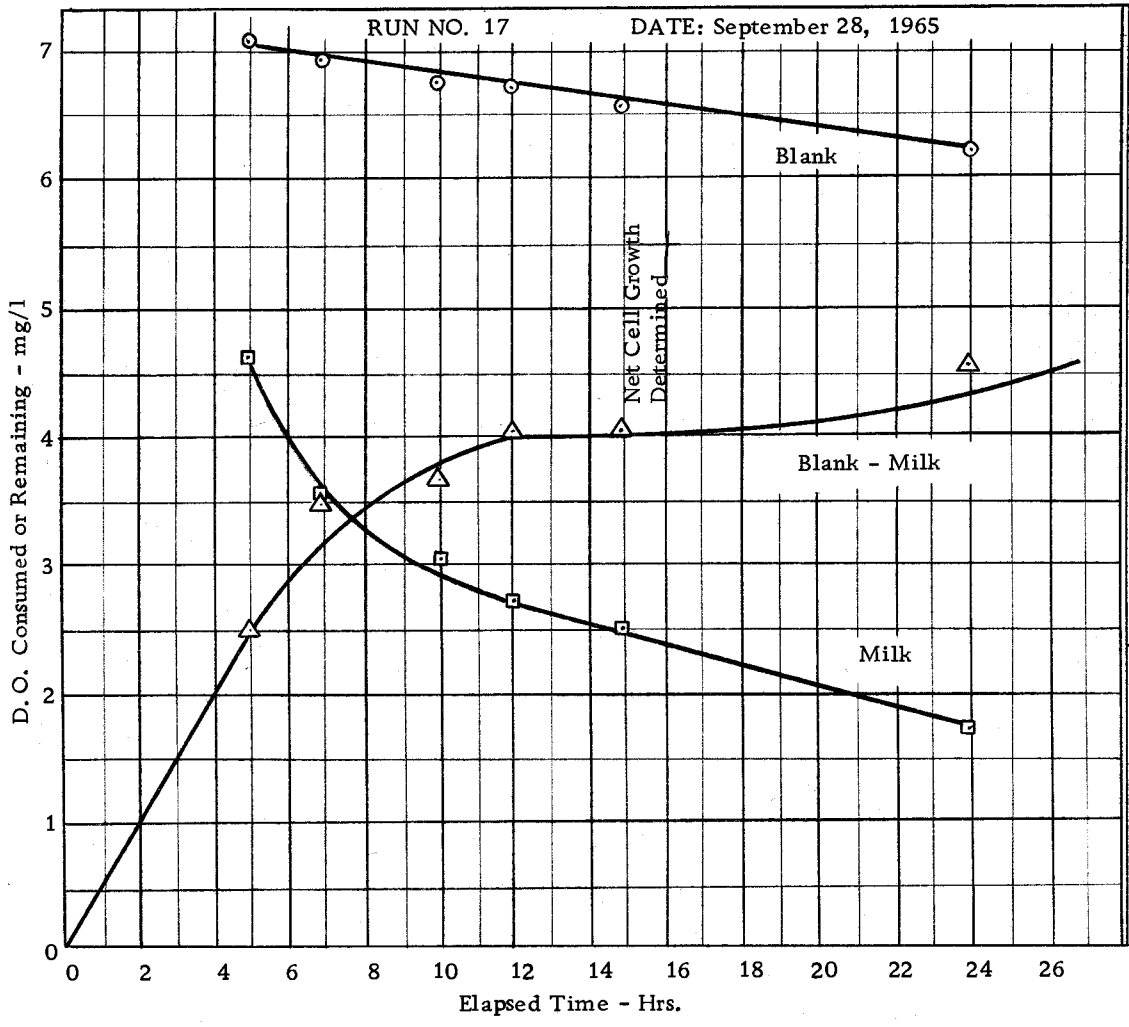
Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D.O. Blk - D.O. Milk mg/l
3.00	6.95	5.80	1.15
6.00	6.82	3.40	3.42
8.33	6.75	2.92	3.83
10.20	6.65	2.55	4.10
11.80	6.55	2.27	4.28
13.50	6.55	2.20	4.35
16.80	6.35	1.87	4.48
23.50	6.10	1.25	4.85
25.50	6.05	1.27	4.78

Cell Growth Data in mg/l

Final Concentration	2304
Initial Concentration	2037
Net Cell Growth	264

Figure 26. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.



EXPERIMENTAL DATA

Dissolved Oxygen Data

t Hrs.	D. O. - mg/l Blk	D. O. - mg/l Milk	D.O.Blk-D.O.Milk mg/l
4.00	7.10	4.65	2.45
7.00	6.92	3.52	3.40
10.00	6.75	3.07	3.68
12.00	6.70	2.70	4.00
15.00	6.52	2.50	4.02
24.00	6.22	1.70	4.52
28.50	6.00	1.45	4.55

Cell Growth Data in mg/l

Final Concentration	2650
Initial Concentration	2330
Net Cell Growth	320

Figure 27. Changes in dissolved oxygen concentrations with time during the metabolic destruction of a simulated milk waste.