THE EFFECT OF AGITATION ON THE BEHAVIOR OF COMPLETELY MIXED BACTERIAL SYSTEMS

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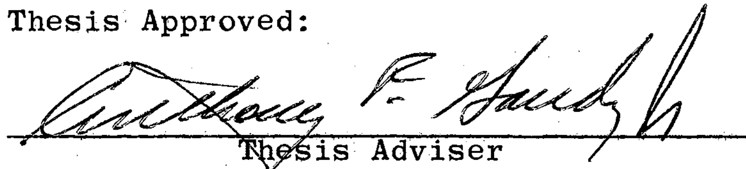

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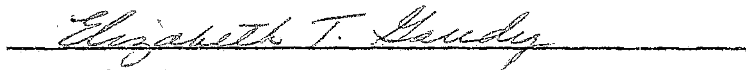

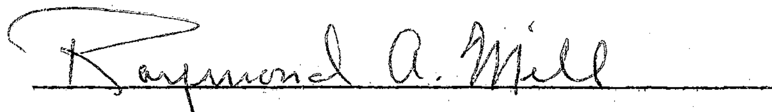

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CHAPTER I

INTRODUCTION

Since the initial development of the process in 1914 by Ardern and Lockett (1), activated sludge treatment has become one of the most widely used methods for the stabilization of domestic sewage and industrial wastes.

In its simplest form, activated sludge treatment consists of mixing the influent waste-water with a large inoculum of biologically active return solids, aeration of the resulting suspension (mixed liquor) for a period of time, and sedimentation of the suspension to allow solids-liquid separation. The aeration step provides a suitable environment for the aerobic growth of the microbiota contained in the return sludge which use the soluble and colloidal organic pollutants present in the influent waste as substrate. Sedimentation of the mixed liquor then allows for the removal and concentration of the highly flocculent suspended solids. The clarified supernatant may then be discharged to the receiving water course. The solids are removed from the underflow of the sedimentation basin; a portion is returned to the head of the plant for use as an inoculum, while the remainder is subject to disposal. Thus, the overall accomplishment of the acti-

vated sludge treatment is the removal of organic pollutants from the waste-water by biological oxidation and conversion to settleable cellular material.

The primary advantages of the activated sludge process are the high degree of treatment obtained--85 to 90% BOD removal being common--and the small land area required for the installations.

The disadvantages of the process are high power requirements, as compared with other methods of biological waste treatment, and operational difficulties. Activated sludge plants require a large expenditure of power to provide for aeration of the mixed liquor. Besselievere (2) states that about 30 horsepower are required per million gallons of waste-water to obtain satisfactory treatment. This, of course, represents a considerable operational cost. Another significant source of operational expense in activated sludge plants is treatment and disposal of the sludge produced. Although the actual figure varies widely, approximately 50% of the BOD removed will be converted to sludge. Thus, if an aeration basin receives an influent BOD of 200 mg/1, achieves a removal of 85%, has a sludge yield of 50%, and has a concentration of the solids during sedimentation to 2%, the plant will produce 2250 pounds of putrescible matter for each million gallons of waste-water treated. When it is considered that some activated sludge plants exceed a capacity of 100 million gallons/day, the magnitude of this problem becomes apparent.

Operational difficulties in activated sludge are many; two major and somewhat related types are shock loading and sludge bulking. Shock loading is defined as a sudden change in the composition or concentration of the organic material in the influent waste. If the organisms in the return sludge are unable to acclimate rapidly enough to the variation in the feed, disruption of the process may occur to the point that treatment efficiency is severely reduced. Bulking of activated sludge is another very serious operational problem. A bulking sludge is one which has lost the ability to settle and compact readily. This problem may be caused by a number of factors, including shock loading, and if severe can result in the loss of large amounts of solids in the effluent.

One very interesting aspect of the activated sludge process is that only a small amount of the total energy used in the aeration basin is needed to supply the necessary dissolved oxygen to maintain aerobic conditions (2). The great majority of the energy furnished is required to provide sufficient mixing to maintain the solids in the aeration basin in suspension. Although the very earliest activated sludge plants constructed used diffused air for aeration, the realization of the importance of agitation led many engineers to search for a more economical means of furnishing both the required dissolved oxygen and mixing. This search led to the development of mechanical aeration which was shown to achieve both the required aeration and

mixing with a smaller expenditure of power (3). In spite of the power savings realized with mechanical aeration, however, a great number of engineers continue to prefer diffused air aeration, citing the greater density of sludge obtained with this type of installation.

Although the provision of sufficient agitation has long been realized to be essential to proper operation of activated sludge plants (2), a review of the available literature indicated that very little is known about the effect of agitation on the growth and biochemical behavior of activated sludge systems. A number of studies have shown that agitation may affect the oxygen uptake and substrate utilization of microbiological systems but insufficient evidence was found to allow precise definition of the relationships which exist. In addition, no mechanism is currently proposed in the literature which adequately explains all of the effects observed.

This report gives the results of a study designed to measure the effect of agitation on the growth and biochemical behavior of microbial systems of the type encountered in biological waste treatment under both steady and transient state conditions. In addition, studies performed on pure culture systems to allow elucidation of the mechanisms of the relationships involved are included.

CHAPTER II

LITERATURE REVIEW

Dissolved Oxygen Requirements of Bacterial Systems

In an aerobic biological process such as activated sludge, it is apparent that oxygen must be made available to the respiring microorganisms if substrate removal is to proceed in a satisfactory manner. The question of just how much oxygen must be furnished to ensure growth and respiration at a maximum rate has been the subject of considerable research by both microbiologists and pollution control engineers. Phillips and Johnson (4) studied the aeration requirements of various bacteria and molds, and concluded that for the former no reduction in the viable count or cell mass yield occurred if the dissolved oxygen level was maintained above 0.01 to 0.02 atmospheres (0.4 to 0.8 mg/l). For molds the values were somewhat higher, ranging from 0.07 to 0.15 atmospheres (2.8 to 5.0 mg/l). The authors stated, however, that these latter values were probably caused by low D. O. pockets in their fermenter resulting from insufficient mixing.

Finn (5) noted that respiration becomes independent of oxygen tension in all microorganisms over a very low range of oxygen tensions, termed the critical D. O., with the exact value being a function of the particular species.

chosen.

Smith and Johnson (6) studied the effect of aeration rate on the viable count and mass yield of two strains of *Serratia marcescens* and found that both of these parameters varied as a direct function of the aeration efficiency up to an aeration rate of 9 mm O_2/min/liter (0.224 liters O_2min/liter at 30°C). Above this rate, no correlation between yield and rate of oxygen supply was found.

In studies of the growth of *Mycobacteria*, various workers (7)(8)(9) have observed the occurrence of linear growth in shake flask cultures. When growth of this type appeared, the yield of cells obtained varied as a direct function of the shaker rate. Volk and Myrick (9) attributed this type of growth and yield limitations observed in the shake cultures to oxygen limitation and not to the agitation *per se*.

A number of investigators have determined the dissolved oxygen tension at which the performance of activated sludge becomes independent of oxygen tension. Porges, Jasewicz and Hoover (10) found that for the oxidation of milk wastes the critical D. O. lay in the range from 0.35 to 0.50 mg/l. Wuhrman (11) and Oldshue (12) both investigated the critical D. O. for activated sludge oxidizing domestic sewage, and found values ranging from 0.5 to 2.5 mg/l. Eckenfelder and O'Conner (13) feel that from 0.2 to 0.5 mg/l of D. O. is sufficient, stating that in this range respiration rate becomes independent of dissolved oxygen.

Smith (14) found no effect on the oxygen uptake rate or BOD removal of activated sludge at oxygen tensions as low as 0.2 mg/l.

In their study of the effect of oxygen tension on BOD removal, Orford, Heukelekian, and Isenberg (15) observed that when their unit was operated for a period of time at a D. O. less than 0.5 mg/l a significant retardation of removal occurred. Gaudy and Turner (16), on the other hand, were able to demonstrate that short term reduction of the dissolved oxygen concentration below this level during quantitative shock loading does not seriously affect performance.

Heukelekian (17) studied the effect of dissolved oxygen on the numbers of organisms developing in a heterogeneous population growing on very dilute medium. He found that higher counts were obtained at D. O. levels of 2.5 and 15 mg/l than at the other D. O. values tested. The bimodal nature of the curve obtained was attributed to the development of different populations at varying dissolved oxygen levels.

Sufficient information now appears to be available on the effect of dissolved oxygen concentration on the metabolism of both pure cultures and heterogeneous populations to allow a number of important conclusions to be drawn. For all the bacterial systems studied to date, a definite oxygen tension exists above which no change in the respiration rate of the culture will be evidenced as the dissol-

ved oxygen concentration is further increased. The mass and viable count yields appear to be controlled in the same manner as the respiration rate, i.e., the yield is also independent of the dissolved oxygen concentration if the oxygen tension is above the critical level and is directly proportional to the rate of oxygenation below the critical D. O.

Heterogeneous populations are undoubtedly controlled in the same fashion as are pure cultures. However, if natural selection is allowed to occur during the experiment, as is the case when a very small initial inoculum is used, variation in the yield with D. O. caused by the development of different populations at different oxygen tensions may occur.

Mechanisms Proposed to Explain the Existence of the Critical D. O.

Winzler (18) compared the oxygen uptake of baker's yeast exposed to carbon monoxide with the uptake of cells respiring at very low oxygen tension and concluded that the critical D. O. results from saturation of the requisite enzyme surfaces with oxygen. Therefore, according to Winzler, the critical D. O. would be the oxygen tension of the bulk medium at which sufficient oxygen is being transferred into the cell to allow saturation of the respiratory enzymes. Johnson, et al. (19), working with luminous bacteria, further concluded that the rate of oxygen uptake above the critical D. O. is controlled by the rate at which

reduced substrate is furnished to the respiratory system.

Longmuir's study (20) on the respiration rate of several bacterial species at very low oxygen tensions caused him to disagree with the conclusions of Winzler and of Johnson. He found that respiration below the critical D. O. follows Michaelis-Menten type kinetics. He then determined the value of D. O. at which the oxygen uptake rate is equal to one-half of its maximum value ($K_M(O_2)$). This value was found to bear a definite relationship to the size of the cell. From these data, Longmuir concluded that oxygen transfer into the cell and not enzyme saturation was responsible for the critical D. O. Tsao and Kempe (21) in their study of *Pseudomonas ovalis* also concluded that the factor controlling the respiration rate above the critical D. O. was the resistance to transfer of oxygen across the barrier provided by the cell-liquid interface.

From the above discussion it may be seen that two distinct theories have been proposed to explain the existence of the critical D. O. The first of these theories implies that the critical D. O. is the point at which respiration rate ceases to be controlled by the rate of mass transfer of oxygen to the cell, and becomes dependent instead on the rate at which reduced substrate is furnished to the respiratory system. The second theory also requires that oxygen uptake rate below the critical D. O. be controlled by the rate of mass transfer of oxygen into the medium but states that above the critical D. O. respiration

rate becomes dependent instead upon the rate of transfer across the cell membrane. Since independence of oxygen tension is required in this latter transfer, it is necessary to further postulate the existence of an active transport mechanism for oxygen into the cell.

Mechanical versus Diffused Air Aeration

The first activated sludge plants constructed used air diffusers placed in the bottom of the aeration chamber to provide the oxygen necessary to maintain aerobic conditions (22). It was not long, however, before engineers realized that it might be more economical to provide the required oxygen by mechanical means. To this end three different types of devices were developed: the turbine mixer, the hydraulic injecter, and the brush aerator (23)(24). Following the development of these devices, a number of studies was published (3)(23)(25) showing that mechanical aeration gave more economical operation. Other investigations (26)(27), however, indicated that diffused air systems gave superior operation because the sludge produced was denser and gave fewer problems in settling. Also, in some studies it was concluded (28)(29) that the cost advantage of mechanical aeration devices was confined mainly to small treatment plants.

In the following years, both mechanical and diffused air plants continued to be built. Mechanical aeration was particularly popular in England (30) while diffused air was generally preferred in the United States. Development of

mechanical methods for aeration continued and many improved devices were introduced (30)(31). As a result of this continued development, mechanical aeration, particularly of the turbine variety, has again become popular in the United States. This has been especially true since recent studies (32) have shown that the sludge settling problems encountered in mechanical systems are caused primarily by gassification and destruction of the floc matrix and can be prevented simply by reducing the amount of mixing provided in the last few feet of the aeration basin.

The Effect of Agitation on Bacterial Systems

The first realization that agitation might affect the growth of bacterial cultures arose from the previously-mentioned studies on the effect of shaker rate on the growth of *Mycobacteria*. This early work illustrated the importance of the relationship between agitation rate and reaeration, and resulted in microbiologists becoming aware of the necessity of defining the reaeration kinetics of reactors.

Tsao and Kempe (21) and Bennet and Kempe (33), however, noted an additional effect of agitation in their study of the aerobic fermentation of glucose by *Pseudomonas ovalis*. These authors observed that the oxygen uptake of their system was a function of the agitation rate of their reactor even though the dissolved oxygen concentration remained above the critical level. Imhoff (34) also had observed very early that the oxygen uptake of a heterogen-

eous population in a Warburg respirometer increased with increasing shaking rate even though oxygen limitation was prevented by using oxygen instead of air as the aerating gas.

Pasveer (35) demonstrated that activated sludge systems were able to assimilate much higher loadings under conditions of high agitation without deterioration of effluent quality. Zahradka (36) further showed that under conditions of constant solids recycle and BOD loading, better treatment efficiency resulted if high agitation were provided. He concluded from these studies that the BOD load to an activated sludge unit could better be expressed in terms of pounds BOD/unit of turbulence than in pounds BOD/unit of mixed liquor suspended solids. In addition, Zahradka observed that agitation appeared to aid in the control of the growth of filamentous organisms in his units.

Fair, Gemmel, and Myrick (37) examined the effect of agitation on the growth and substrate utilization rates of batch activated sludge. These workers were able to find no differences in either of these parameters as the amount of agitation provided was increased.

The above discussion indicates that agitation may play an important role in the behavior of both pure culture and heterogeneous microbial systems. Oxygen uptake rate has been shown to increase with agitation in studies utilizing either pure cultures or heterogeneous populations. In addition, the permissible organic loadings to activated

sludge has been shown to be strongly influenced by the amount of mixing provided. It is, therefore, of definite interest to examine the mechanisms that have been proposed by the various workers to explain the influence of turbulence on bacterial systems.

Rincke (38) attributed the improved performance of activated sludge at high turbulences to improved frequency of contact between cells and substrate. Pasveer (35) (39) (40) (41), on the other hand, concluded that high turbulence results in the maintenance of a smaller floc which allows better penetration of oxygen and substrate. Zahradka (36) also concluded that smaller floc was responsible for the improved removals of BOD he observed at high turbulence.

Tsao and Kempe (21) from their studies of oxygen uptake by *Ps. ovalis* concluded that the resistance to the transfer of oxygen across the liquid-cell interface was the controlling factor in the rate of oxygen uptake in systems respiring at oxygen tensions above the critical D. O. They further hypothesized that the effect of agitation was to change the rate of transfer across the boundary imposed by the cell membrane. In order to demonstrate that this concept was not incompatible with the existence of the critical D. O., it was necessary to develop a model of oxygen transfer which was not a function of the oxygen tension of the medium. Using dimensional analysis, these authors developed a model which predicted that the oxygen uptake in their reactor would be a function of the agitator speed, the rate

of gas flow, the culture volume, and the fluid properties of the medium.

Swilley, Brant and Busch (42) agreed with the importance of transport across the cell membrane but, from consideration of mass transport, preferred a model which is dependent on the oxygen tension of the medium. It should be noted that this model is in direct conflict with the entire body of evidence available on the existence of the critical D.O. since it requires that the oxygen uptake be a function of the oxygen tension. In addition, in systems where the rate of respiration is limited by the oxygen tension, i.e., where the dissolved oxygen level falls below the critical, considerable amounts of data are available which indicate that respiration rate and yield are functions of the rate of mass transfer from the gas to the liquid, thereby making it unnecessary to invoke liquid-cell transport as a controlling factor.

The work of Winzler (18) and Johnson, et al. (19) does not agree with the model of Tsao and Kempe since, if the critical D. O. is attained when enzyme(s) are saturated with oxygen, no increase would be expected in the oxygen uptake as the resistance to transfer across the liquid-cell interface is lowered by increased agitation.

On the basis of the observation by Longmuir (20) that the $K_M(O_2)$ varies with organism size, Finn (5) considers the question of the mechanism for the critical D. O. to be unanswered for bacterial systems. Unfortunately, Longmuir

reported only the $K_M(O_2)$ and not the D. O. at which maximum uptake occurred. Thus, his data cannot be used as a conclusive refutation of Winzler's conclusions, since the $K_M(O_2)$ could vary with the resistance to transfer from the liquid into the cell without the oxygen tension at which maximum uptake occurs being affected.

CHAPTER III

THEORETICAL CONSIDERATIONS

Development of a Parameter to Measure Agitation

If a study of the effect of agitation on bacterial systems is to be useful in the waste treatment field, it is necessary that units for agitation which are applicable to all types of systems used in activated sludge plants for the provision of agitation and aeration be employed. Thus, a study based on the rate of agitation in a laboratory fermenter is, at best, of limited value to the waste treatment engineer who must deal with systems of varying geometry and with different methods of agitation. For this reason, the work herein reported is based on an energy function which accounts for both the method of addition of energy and the geometry of the unit in which the studies were conducted.

Camp and Stein (43) defined the mean temporal velocity gradient as the square root of the ratio of the power dissipated by agitation in a fluid system to the absolute viscosity of the fluid. Although originally developed for use in flocculation systems, this parameter is very convenient for determining the effect of agitation on biological systems because it is a function of the work done against

fluid shear and is thus applicable to all systems regardless of their geometry or the method of addition of energy used.

In their original paper, Camp and Stein (43) developed the equations for the velocity gradient of mechanically agitated systems. From a consideration of the shear on an elementary fluid particle in a stirred system, they were able to show that the power dissipated against fluid shear was related to the geometry of the system and the velocity of the agitator with respect to the fluid according to the relationship:

$$P = \frac{C_d A v^3}{2V} \qquad 1$$

where P is the power input, C_d is the drag coefficient of the agitator, A is the total agitator area, v is the linear velocity of the periphery of the agitator, and V is the volume of the reactor. Thus the velocity gradient, G, would be given by:

$$G = \sqrt{P/u} = \sqrt{\frac{C_d A v^3}{2Vu}} \qquad 2$$

where u is the absolute viscosity.

Fair and Geyer (44), using dimensional analysis, determined the work done by a bubble rising through a fluid and undergoing isothermal expansion. The power dissipated in this fashion is:

$$P = \frac{81.5\ Q_a \log \left[(H + 34)/34\right]}{2V} \qquad 3$$

where Q_a is the gas flow rate in cubic feet/min, and H is

the depth to the aerator in feet. Thus the velocity gradient for a system agitated by compressed air is given by:

$$G = \sqrt{\frac{81.5\ Q_a\ \log\left[(H + 34)/34\right]}{2uV}} \qquad 4$$

In systems using both compressed air and mechanical agitation, the total power dissipated can be estimated as the summation of the power dissipated by the gas bubbles and the mechanical stirring. Thus, the velocity gradient of a system of this type would be given by:

$$G = \sqrt{\frac{C_d A v^3 + 81.5\ Q_a\ \log\left[(H + 34)/34\right]}{2uV}} \qquad 5$$

It should be noted that the above formulation for velocity gradient is only one of several approaches that could have been used. Zahradka (36), for example, proposed a formulation for diffused air systems based on a number of factors including bubble size, type of movement, and distribution of the bubbles leaving the aerator. Unfortunately, the difficulty involved in evaluating some of the factors in his equation severely limits its utility.

Mechanism for the Effect of Turbulence on Biological Systems

In the previous chapter of this report the mechanisms postulated by the various workers to explain the effect of turbulence on activated sludge systems were presented. It is now worthwhile to examine the kinetics of steady-state, completely mixed bacterial systems and to assess the implications of each of the various mechanisms for the behavior

of a system of this type. Analysis of this kind should provide a means of testing the various hypotheses from the data gathered in this investigation.

In a completely mixed, bacterial system of the type employed in these studies, the kinetics of the reactions may be derived by examination of the differential rates of change of the cells and substrate passing through the system. These are given by:

$$dX/dt = D(X_o - X) + \mu X \qquad 6$$

and

$$dS/dt = D(S_o - S) - \mu X/Y \qquad 7$$

where μ is the first order growth rate constant, X is the cell mass per unit volume, X_o is the influent solids concentration, D is the dilute-out rate equal to the reciprocal of the detention time, $\bar{t}$, S_o is the concentration of the rate-limiting nutrient in the influent in the aerator, S is the concentration of the rate-limiting nutrient, Y is the cell yield, and dX/dt and dS/dt are, respectively, the rates of change of the cell and the substrate concentrations in the reactor.

If the system is at steady state and no solids recycle is employed, dX/dt, dS/dt, and X_o are equal to zero and the above expression simplifies to:

$$\mu = D \text{ and } Y = \frac{X}{(S_o - S)}$$

Further, the term $\mu X/Y$ or DX/Y represents the rate of substrate consumption in the reactor. This term is made up of

two parts, the rate at which substrate is utilized for the production of energy and the rate at which substrate is utilized for the synthesis of new cell mass. Since oxygen uptake rate is also a measure of energy production:

$$DX/Y = k_1U + DX \qquad 8$$

where U is the steady-state oxygen uptake rate and k_1 is the substrate conversion constant. From this relationship it may now be shown that the oxygen uptake rate of a steady-state, completely mixed system is given by:

$$U = \frac{DX(1/Y - 1)}{k_1} \qquad 9$$

If the relationship $X = Y(S_o - S)$ is now substituted into equation 9, the following is obtained:

$$U = \frac{D(S_o - S)(1 - Y)}{k_1} \qquad 10$$

If the Monod model of growth which states that:

$$D = \frac{\mu_m S}{S + K_S} \quad \text{or} \quad S = \frac{K_S D}{\mu_m - D}$$

where K_S is the Monod constant and μ_m is the maximum growth rate constant, is substituted for the reactor substrate concentration in equation 10, the following expression for the oxygen uptake results:

$$U = \frac{D\left[S_o - \frac{K_S D}{\mu_m - D}\right](1 - Y)}{k_1} \qquad 11$$

This latter equation allows expression of the oxygen uptake rate of a steady-state system in terms of constants

dependent only on the population contained in the reactor and the substrate used.

For systems of the above type it should now be possible to derive expressions for the effect of agitation on the substrate utilization and oxygen uptake rates based on the mechanisms proposed in the literature. Comparison of the experimental data obtained in this study with the theoretical relationships developed should then be useful in determining the mechanisms in operation.

Rincke (38) in his discussion of the work of Zahradka (36) attributed the improved substrate removal observed as well as the increased oxygen uptake seen by Imhoff (34) to improved contact between the cells, substrate, and oxygen as agitation was increased. This mechanism lends itself very nicely to kinetic evaluation if mixing is expressed in terms of velocity gradient. According to Fair and Geyer (44) the number of contacts per unit time between suspended particles per unit volume is given by:

$$N = \frac{n'n'' \, (d' + d'')^3 G}{6} \qquad 12$$

where N is the number of contacts, and n' and n'' are the numbers of each of two distinct types of particles having respective diameters of d' and d''.

Two cases should now be differentiated. The first is the case where contact between the oxygen present and the cells is rate limiting, i.e., the rate of substrate removal is controlled by the rate at which oxygen becomes available

to the respiratory chain. The second case is one in which the contact between the cells and substrate is controlling with the oxygen uptake rate then being controlled by the rate at which reduced substrate becomes available to the respiratory system.

In the first case, the rate of oxygen uptake in the system would be of the form:

$$U = f_o k_o N_o \qquad 13$$

where f_o is the fraction of the total collisions that result in uptake, k_o is the mass of a single molecule of oxygen, and N_o is the total number of contacts occurring between cells and oxygen molecules per unit time. Appendix D shows the reduction of equations 12 and 13 for a steady-state system assuming that the diameter of an oxygen molecule is negligible and that the mean diameter of the cells is two microns. This reduction results in the following expression:

$$U = 1.34 \times 10^{-6} X_n f_o GT \qquad 14$$

where T is the dissolved oxygen concentration in mg/l and X_n is the viable count in cells/ml. From this equation it may be seen that if contact between the cells and the oxygen is rate-limiting, the oxygen uptake will increase with both the velocity gradient and the oxygen tension. Thus, this mechanism may be tested experimentally simply by determining whether a relationship exists between the oxygen uptake and oxygen tension at a constant velocity

gradient. If no such relationship exists, then this mechanism cannot be controlling. In other words, if the concept of critical D. O. holds for reactors of the type under consideration here, then contact between the oxygen molecules and the cells present cannot be the controlling mechanism.

It should be pointed out that if a relationship does exist between the oxygen tension and the oxygen uptake, the above mechanism will not be proven since the existence of the proper kinetics does not ensure the operation of a given mechanism. Thus, the above analysis is satisfactory only as a negative proof.

In the second case, where contact between substrate and cells is considered controlling, the percentage of substrate removed may be shown to be of the form:

$$\frac{100(S_o - S)}{S_o} = 100(1-S/S_o) = 100\left\{1 - \left[\frac{D}{D+(8x10^{-11}X_n f_S G)}\right]\right\} \qquad 15$$

where f_s is the fraction of effective contacts between substrate and cells. In this relationship it may be seen that the percentage removal of substrate increases with increasing G. Thus, if the reactor is found to exhibit substrate removal which is independent of the velocity gradient, then this mechanism cannot be rate controlling.

One objection which may be advanced against the above analysis is the view held by some investigators (42) that a stagnation layer may exist around the bacterial cell

which might control oxygen transport. The effect of velocity gradient would then be to reduce the thickness of this boundary layer. However, if such a layer were to exist, oxygen transport across it would follow the law of mass transport, as proposed by Swilley et al. (42). This would again make oxygen uptake dependent on the extant D. O. and hence require violation of the concept of the critical D. O.

Pasveer (39), in his study of the effect of intense aeration on activated sludge, considered the effect of fluid shear on the floc particles contained in the mixed liquor. From this he concluded that the improved permissible loading obtained at high aeration rates was caused by decreased floc size which allowed better penetration of both substrate and oxygen to the organisms in the system. Two cases were distinguished in this study, one in which the entire floc is receiving oxygen and one where a portion of the floc particle is anaerobic. In the following analysis only the first case, that of the wholly aerobic floc, is considered, since the kinetics of the two cases are identical.

According to Pasveer, if the oxygen uptake of an activated sludge system is rate limited by the penetration of oxygen into the floc structure, the oxygen uptake per unit time is given by:

$$U = 4/3\ r^3\ k_1 S \left[\frac{4T_p + T_m}{5}\right] \qquad 16$$

where r is the floc radius, k_1 is the substrate conversion constant, T_p is the oxygen concentration at the center of the floc particle, and T_m is the oxygen concentration at the floc-water interface. Pasveer further stated that the factors T_m and T_p could be evaluated from the relationships:

$$T_m = T_p \left[\frac{15k' - 4k_1 Sr^2}{15k' + k_1 Sr^2} \right] \qquad 17$$

$$T_p = \left[\frac{T}{1 + \frac{2.5\ k_1 Sr}{15k' + k_1 Sr^2 \sqrt{k't'}}} \right] \qquad 18$$

where k' is the diffusion constant of oxygen in water and t' is the hypothetical time in seconds of subsistence of the static water-floc interfacial surface.

It may now be seen that if equations 16, 17, and 18 are combined under conditions of constant agitation, all of the above factors may be combined into a single constant. If this is done, an expression results of the form:

$$U = K''T \qquad 19$$

where K" represents the combined factors in equations 16, 17, and 18. Examination of equations 14 and 19 will show that with respect to oxygen tension the same kinetic behavior will exist for systems limited either by mixing or by oxygen diffusion into the floc particles. For this reason if the experimental results of this study show that the oxygen uptake is dependent on oxygen tension, no choice between these two mechanisms can be made on the basis of kinetic behavior. If, on the other hand, the system shows

no dependence of oxygen uptake on oxygen tension at constant agitation, both mechanisms can be rejected.

In his studies of the effect of agitation on the performance of activated sludge, Zahradka (36) observed that the predominating population in his units varied with the amount of mixing energy provided. Thus, it is conceivable that the improved substrate removal noted by this author, as well as the increased oxygen uptake noted by others at high stirring rates, could be caused by changes in microbial predominance.

Heukelekian (16) also observed variation in the viable count yield of a heterogeneous population as the amount of dissolved oxygen present was varied. Changes in the predominance of the population of the reactor, therefore, may occur in response to either changing oxygen tension or to changes in the amount of mixing energy provided. The primary point of interest here, however, is not whether such changes occur but whether or not they result in a change in oxygen uptake and the other parameters of a steady-state system which can be correlated with the amount of mixing provided. Because this mechanism does not lend itself to mathematical analysis, it was considered necessary to design a set of experiments capable of determining if predominance changes were responsible for the effects noted with increasing velocity gradient. It was decided that this goal could best be accomplished by eliminating the possibility of population variation entirely, through

the use of pure cultures, and then determining if the same relationships existed as in systems where the population was allowed to vary freely. If the same results were obtained in both the pure culture and the heterogeneous population systems, predominance changes could probably be eliminated as the primary mechanism in operation.

Another possible reason for the variation in oxygen uptake with agitation rate was proposed by Tsao and Kempe (20). These authors considered that the rate limiting step in oxygen uptake was the transfer of oxygen from the bulk medium to the interior of the cell. They then demonstrated that a dimensionally homogeneous model could be derived in which oxygen uptake in a resting batch culture was related to the physical characteristics of the system:

$$U = f\ (s,\ d,\ u',\ Q_a,\ R,\ H,\ A) \qquad 20$$

where s is the surface tension, d is the fluid density, u' is the kinematic viscosity, and R is the agitator speed in RPM.

If the velocity gradient is substituted into the above model, it may be greatly simplified while retaining dimensional homogeneity. Thus:

$$U = f'\ (s,\ G,\ u',\ H) \qquad 21$$

This latter equation states that the oxygen uptake rate is a function of the fluid properties of the medium and the velocity gradient and allows for variation in oxygen uptake with the velocity gradient without violation of the principle of critical D. O.

The model as stated in equation 21 applies only to non-growing, batch systems having a constant solids concentration. In steady-state systems where growth occurs, variation in the solids level maintained must be allowed for by inclusion in the model. If the fluid properties of a given system are assumed to be constant, the oxygen uptake should be expressible as:

$$U = f''(G, X) \qquad 22$$

Using dimensional analysis the following equation may now be derived:

$$U = C_1 X + C_2 GX \qquad 23$$

where C_1 is the oxygen uptake per unit solids at a velocity gradient of zero and C_2 is a dimensionless constant.

In a steady-state, completely mixed reactor the amount of substrate entering is constant so that any change in the rate of oxygen uptake must be accompanied by either a change in the substrate concentration maintained or in the yield of the organisms. If the substrate removal in the reactor is assumed to be independent of the velocity gradient and if the steady-state substrate balance, equation 8, is applied to the relationship shown in equation 23, the following expression results:

$$Y = \frac{D}{(C_1 + C_2 G) k_1 + D} \qquad 24$$

This equation states that at a given growth rate the yield of cells obtained should vary inversely with the velocity gradient. Alternately, the yield may be assumed to be

independent of the velocity gradient giving:

$$(S_o - S) = \frac{(C_1 + C_2G)k_1}{D(1 - Y)} \qquad 25$$

Thus, the model proposed by Tsao and Kempe provides for variation in either the cell mass yield or the substrate removed. Experimental testing of this model may now be seen to be a simple matter, since operation of a steady-state unit at variable velocity gradient should give an oxygen uptake which varies directly with velocity gradient and a yield which shows inverse correlation with velocity gradient or a substrate removal which increases with increasing turbulence.

The preceding discussion has shown that examination of the kinetic requirements imposed by most of the mechanisms proposed in the literature to explain the effect of mixing energy on the behavior of bacterial systems suggests approaches by which their validity may be experimentally determined. In only one case, that of population variation, was this method not applicable. It should again be emphasized that kinetic behavior cannot be used as a proof of mechanism but that failure of a system to follow the expected kinetics is a good indication that the mechanism selected is invalid.

The next section of this report will outline the methods by which the data necessary to test each of the mechanisms discussed in this chapter were obtained.

CHAPTER IV

MATERIALS AND METHODS

Experimental Unit

A 5-liter Microferm laboratory fermenter (New Brunswick Scientific Co.) was used throughout these studies. Aeration of the medium was provided by a single air outlet in the bottom of the culture vessel through which filtered compressed air or gas having a known oxygen content was passed. Although designed for batch operation, the unit was converted to continuous flow by the addition of an influent feed line and an inverted standpipe, positive suction, effluent line. Sigmamotor Model AL4E pumps were used on both the influent and effluent lines. A schematic diagram of the unit as modified is shown in Figure 1.

As the volume of the medium in the culture vessel was found to change with varying agitation rate, the volume actually contained during operation was determined for a number of agitation rates over the range to be used in the experiments. This was accomplished by filling the reactor with a premeasured amount of tap water, setting the agitation rate at the desired level, and pumping the excess water above the standpipe level into a graduated cylinder. The volume of the reactor under the test conditions could

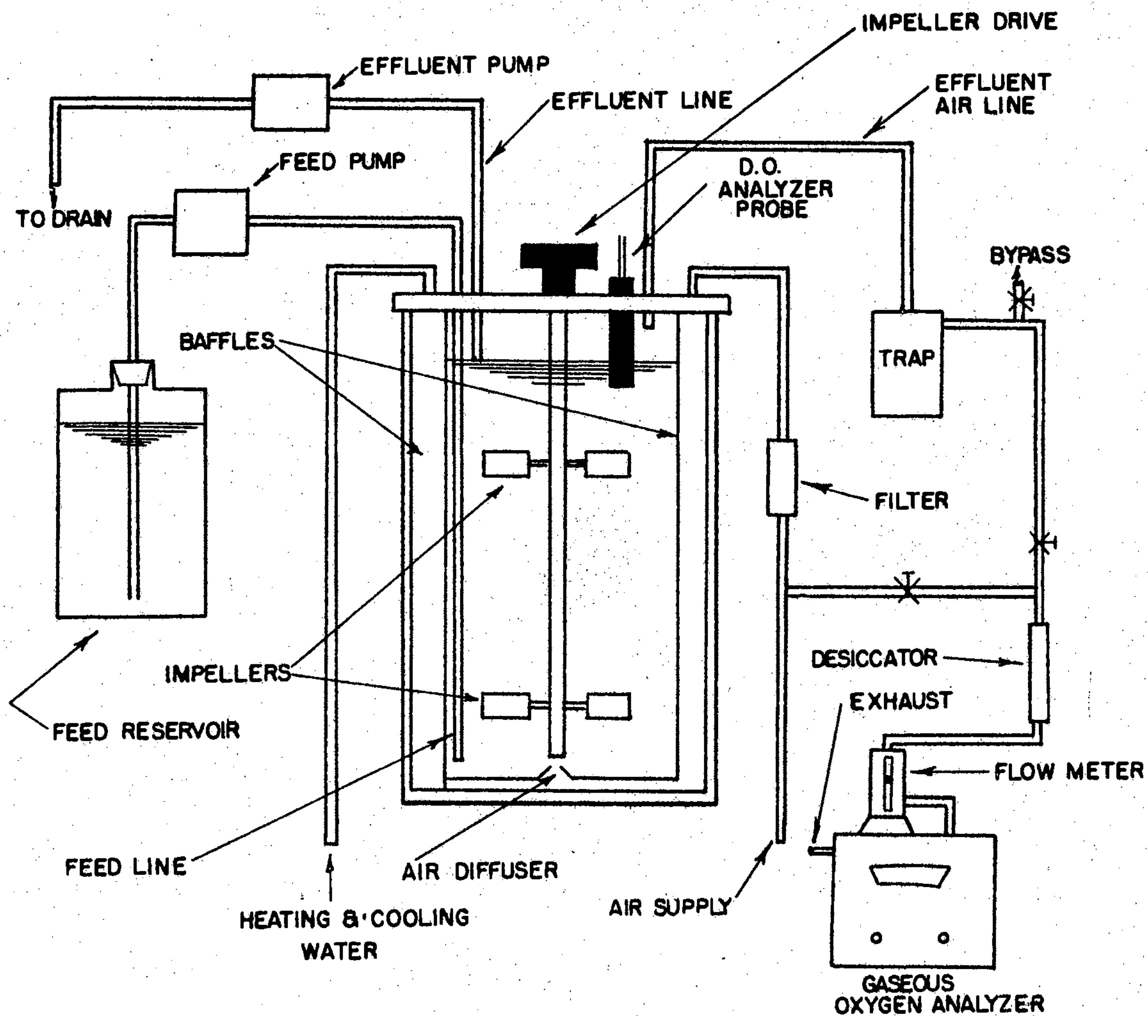


Figure 1 - Schematic Diagram of Experimental Unit.

then be determined by subtraction. The effect of agitation on the volume of medium maintained under aeration at an air flow rate of 1.0 l/min is shown in Figure 2. Once these data had been determined, it was possible to calculate the necessary flow rate to obtain a constant detention time so that all experiments could be conducted at a precisely determined growth rate.

In order to ensure that the unit was completely mixed at all agitation rates, dilute-out curves were determined for a series of agitation rates covering the entire range of velocity gradients to be used. These experiments were accomplished by filling the culture vessel with tap water containing phenol at 1000 mg/l COD and diluting out with tap water at a flow rate designed to give a detention time of eight hours. The effluent COD was then determined at appropriate times, and the dilute-out curves plotted. These data could then be compared statistically with the theoretical dilute-out equation assuming complete mixing:

$$\ln (S/S_o) = Dt \qquad 26$$

where D is the dilute-out rate. At all of the agitation rates examined the data agreed with the theoretical relationship at the 99% confidence level. The theoretical dilute-out for the reactor at the eight-hour detention time (D = 0.125) and the experimental points determined at four different agitation rates are shown in Figure 3.

Since the oxygen transfer rate would be expected to vary with the agitation rate of the medium, it was

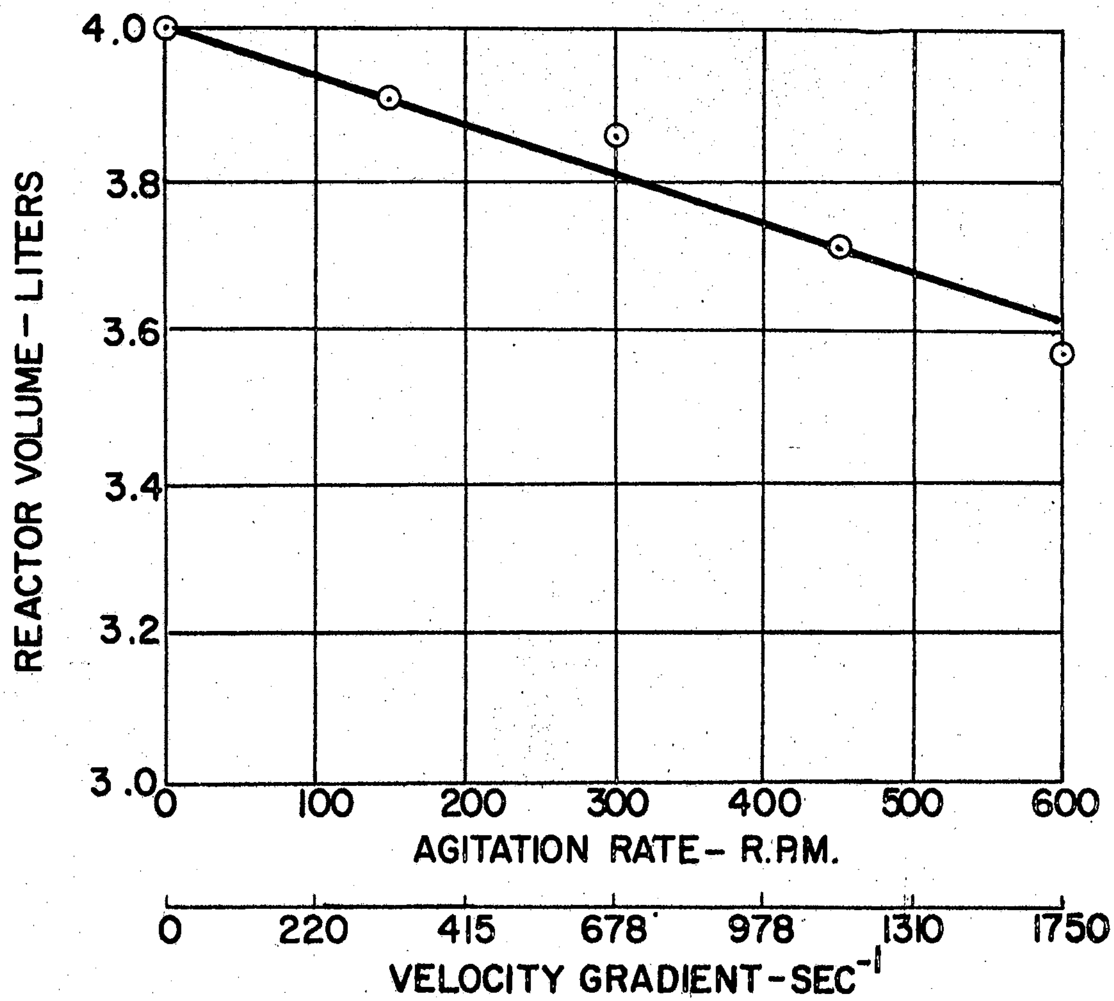


Figure 2 - Variation in Reactor Volume with Agitation at an Air Flow Rate of 1.0 1 min.

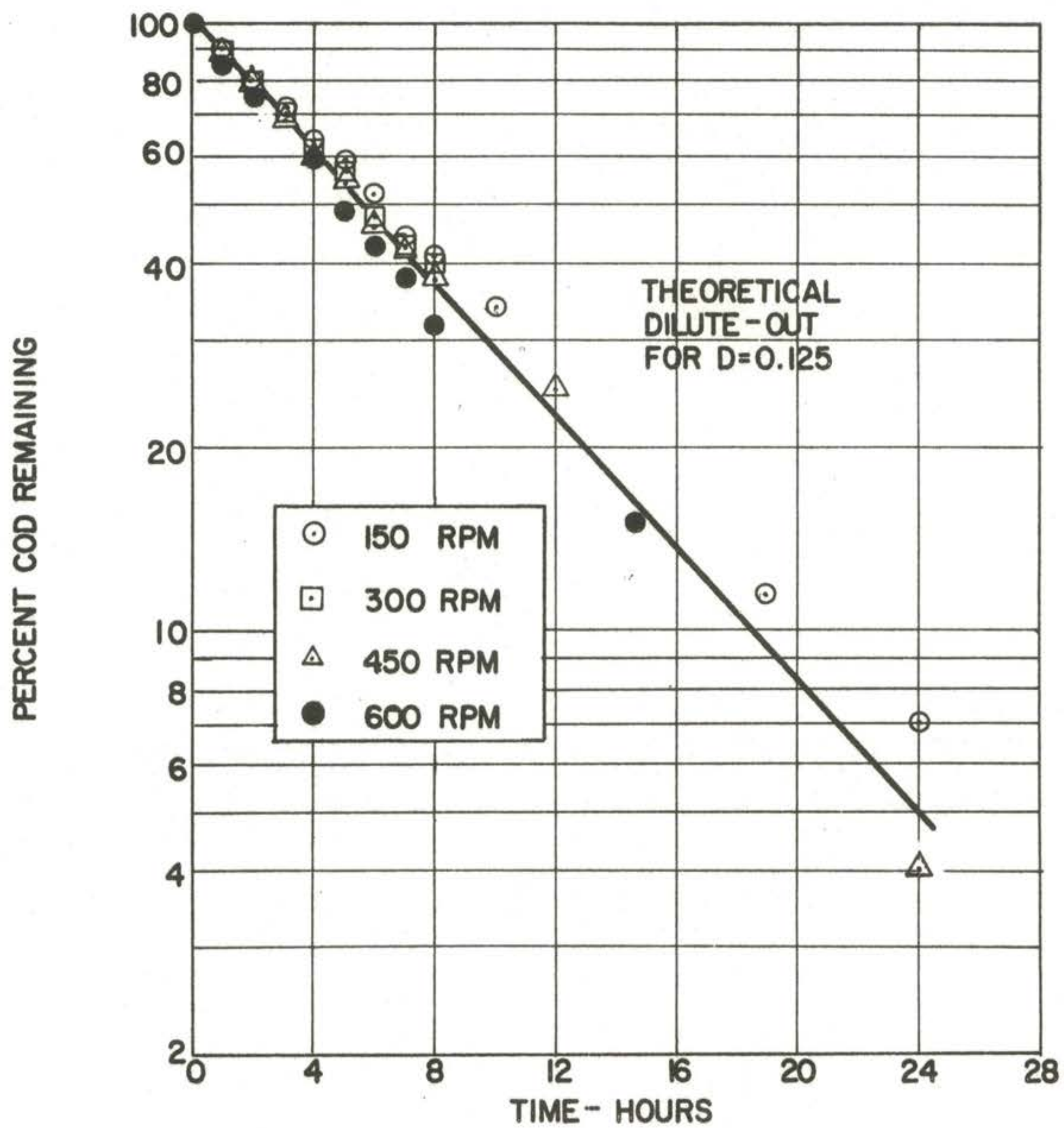


Figure 3 - Comparison of Experimental and Theoretical Dilute-Out Curves at a Dilution Rate of 0.125 Hrs^{-1}.

considered necessary to define this relationship for the experimental system employed. The rate of absorption of oxygen was measured at a number of agitation rates by stripping all of the D. O. from the medium with nitrogen and following the increase in dissolved oxygen concentration during reaeration at an air flow rate of 1.0 l/min. The reaeration coefficient, k_a, was calculated for each run by the method described by Isaacs and Gaudy (45). Regression analysis was then applied to the values obtained for each agitation rate to determine a functional relationship between agitation and k_a.

The velocity gradient provided to the medium by the reactor used in these studies, as defined by equations 2 and 3, is shown in Figure 4. In this figure, the dashed lines represent the individual components generated by diffused air aeration and by the agitator velocity. The solid line is a plot of the velocity gradient provided to the medium under the aeration conditions used in all of the experiments performed as a part of this work (1.0 l/min).

Bacterial Medium

The medium used in these experiments consisted of a glucose-mineral salts solution having the composition shown in Table I. The adequacy of the buffer system used was demonstrated by checking the pH of a number of samples during steady-state operation. These values were found to be 6.7 ± 0.1.

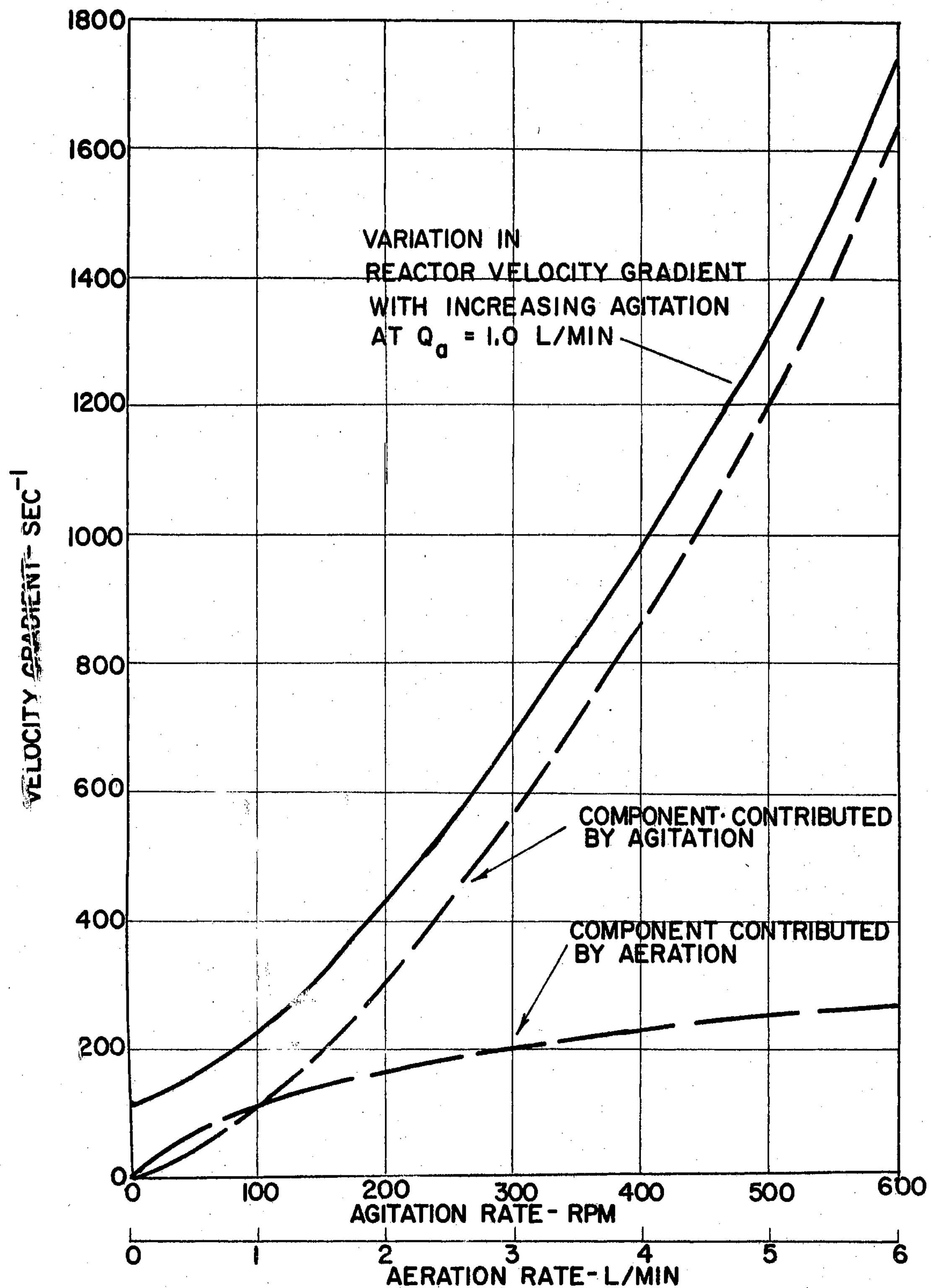


Figure 4 - Effect of Agitation and Air Flow Rate on Velocity Gradient in Experimental Reactor.

TABLE I

COMPOSITION OF MINIMAL MEDIUM

Component	Concentration
Glucose	1000 mg/l
$(NH_4)_2SO_4$	500 mg/l
$MgSO_4 \cdot 7H_2O$	200 mg/l
$MnSO_4 \cdot H_2O$	20 mg/l
$CaCl_2$	15 mg/l
$FeCl_3 \cdot 6H_2O$	1 mg/l
Tap Water	100 ml/l
1 M Phosphate Buffer	15 ml/l

Bacterial Populations Used

1. Heterogeneous Population

The experimental unit was started by filling the reactor vessel with the standard glucose medium and adding 100 ml of primary effluent from the Stillwater, Oklahoma, municipal waste treatment plant. Operation was then conducted in a batch manner for twenty-four hours at 30°C to allow the development of a vigorous microbial population. Pumping was then begun and the unit operated under continuous flow conditions for seventy-two hours before an experiment was started. During the last twenty-four hours

before sampling was begun and prior to the start of each subsequent run, the unit was checked for steady-state conditions by determining the optical density of a number of samples. In all cases the unit was found to achieve steady-state with respect to optical density within three detention times after a change in operating conditions.

At the conclusion of each run the flow rate and velocity gradient were changed to the next desired values and the unit was operated for another three detention times. Operation was then halted and the culture vessel inspected for growth adhering to the vessel or baffles. Any such growth was removed and operation continued for an additional three detention times. After all of the runs were performed at a given growth rate, the culture vessel was emptied and cleaned. The unit was then filled with fresh medium, re-inoculated, and operation conducted at the new growth rate as described above.

3. Pure Cultures

A pure culture isolate was obtained from the experimental unit during operation at a low agitation rate by streaking an effluent sample on nutrient agar (Difco). The streak plate was then incubated for twenty-four hours at 37°C. A single colony was picked from this plate and re-streaked on nutrient agar. This procedure was performed four times to ensure that the culture was pure. The organism obtained in this manner was found to be a small, gram-negative rod about two microns in length, which showed

the presence of a heavy capsular layer when grown on either glucose or nutrient agar. For convenience of discussion this organism is referred to in this report as isolate SE-4.

A second culture, consisting of Escherichia coli K-12, was obtained from Dr. Elizabeth T. Gaudy, of the Microbiology Department of Oklahoma State University.

Both cultures were maintained on agar slants stored at $4^{o}C$. Forty-eight hours before operation of the unit was to be started, 40 ml of sterile nutrient broth (Difco) were inoculated with the culture to be used and incubated on a shaker at 100 oscillations per minute at $25^{o}C$. The contents of this flask were then transferred aseptically to the reactor which had previously been filled with the standard medium, sterilized by autoclaving ($240^{o}F$--15psig), and allowed to cool. Operation of the unit was then conducted as described for the heterogeneous population except that cleaning of the reactor between each run was eliminated and aseptic techniques were followed throughout. During experimentation with the pure cultures, the unit was checked for contamination twenty-four hours before the start of each run. With E. coli this was accomplished by streaking an an effluent sample on E.M.B. agar (Difco) and observing the color of the colonies formed after twenty-four hours' incubation at $37^{o}C$. A metallic green colony is indicative of enteric organisms on this medium. With the sewage isolate, an effluent sample was streaked on nutrient agar, incubated as above, and observed for Gram-staining reaction,

gross morphology, and the ability to produce capsular material on nutrient agar.

Experimental Protocol

1. Studies at Varying Velocity Gradient

A total of twenty-six runs were conducted with the heterogeneous population at four different growth rates ranging from five to twelve hours. Compressed air was used as the aerating gas at a flow rate of 1.0 l/min. The velocity gradients employed ranged from 300 to 1750 sec^{-1}.

2. Studies at Varying Oxygen Tension

In order to assess the effect of dissolved oxygen tension on the growth of the cells in a steady-state reactor, a series of four runs was made at a detention time of eight hours, a velocity gradient of 1000 sec^{-1}, and dissolved oxygen concentrations ranging from 1.4 to 7.1 mg/l. The variation in steady-state D. O. was accomplished by varying the oxygen content of the aerating gas. Gas mixtures containing 5% oxygen and 95% nitrogen (D. O. = 1.4 mg/l), 10% oxygen and 90% nitrogen (D. O. = 3.6 mg/l), and 21% oxygen and 79% nitrogen (D. O. = 7.1 mg/l) were used.

3. Shock Load Studies

At the conclusion of certain of the runs performed at a dilution rate of 0.20 hrs^{-1} ($\bar{t}$ = 5 hours) the substrate concentration of the feed was changed from 1000 mg/l to 3000 mg/l. The other components of the medium were increased proportionally. Sampling was then continued for twenty-four hours to determine the effect of velocity

gradient on the response of the system to quantitative shock loading.

4. Pure Culture Studies

Five experimental runs were conducted with each of the two pure cultures described previously at a growth rate of 0.20 hr^{-1} and velocity gradients ranging from 300 to 1500 sec^{-1}. Compressed air was used as the aerating gas in all of these experiments. Because of the unusual nature of the data obtained from *E. coli* a sufficient number of additional experiments was conducted with this organism to ensure that the observed behavior was not an artifact of observation.

5. Sampling Procedures

All samples were obtained by collecting sufficient effluent for the required analyses in a graduated cylinder. In the studies using heterogeneous population, a minimum of six samples was collected over a period of three detention times. During the pure culture studies, a total of four samples was collected at each velocity gradient. In both cases the average value of all of the analyses for each parameter was taken to represent the steady-state value.

Analytical Methods

The biological solids level in the reactor was determined by the membrane filter technique (46). Substrate utilization was determined by analyzing the membrane filtrate for both COD (46) and total carbohydrate (47). The influent COD and glucose concentrations were also

determined so that percentage removals in terms of both parameters could be calculated.

Samples for cell composition analysis were taken by the method outlined by Gaudy (47). This resulted in a washed cell suspension having ten times the solids concentration of the culture sampled. One ml of this suspension was subjected to hot perchloric acid extraction according to the method of Burton (48) for subsequent nucleic acid analysis. The remainder (4 ml) was frozen and stored for carbohydrate and protein determination.

RNA analyses were performed on the perchloric acid extract according to the orcinol method reported by Ceriotti (49), except that extraction of the final colored product with isoamyl alcohol to achieve concentration of the color was eliminated as it was shown to be unnecessary with the concentrations found in the samples obtained.

DNA was determined on the perchloric acid extract by the diphenylamine reaction as given by Burton (48). Cellular carbohydrates and protein were determined on the washed cell suspension by the anthrone and biuret methods, respectively (47). The dissolved oxygen content of the medium was determined continuously during steady-state operation by a galvanic cell dissolved oxygen analyzer (Precision Scientific Co.) connected to a 10 mv recorder (E. H. Sargent Co.).

Oxygen uptake during steady-state growth was determined by measuring the difference in oxygen content of the

influent and effluent gas streams, in accordance with the manufacturers' recommendations (50) using a Beckman Model E2 gaseous oxygen analyzer. This instrument allowed the determination of the oxygen content of the gas stream with a precision of 0.01% in the range used. This difference was then converted to milligrams of oxygen consumed per hour, and the values divided by the volume of the medium in the fermenter under the conditions employed (Figure 2) to obtain the rate of oxygen uptake in mg/l/hr. An example of the method of calculation used and a table of values relating the instrument readings to the oxygen consumed are shown in Appendix C.

Viable count determinations during the pure culture studies were performed by spreading 0.1 ml of an appropriate dilution on a nutrient agar plate and incubating at 37°C for twenty-four hours. The number of colonies appearing was then counted, and the viable count in cells/ml calculated. A minimum of four replicate plates was made for each sample giving a total of sixteen observations of viable count during each run.

During the experiments performed with *E. coli* and the shock load studies, the presence of metabolic intermediates was detected in the effluent. In order to classify these materials, selected samples were subjected to organic acid analysis by vapor-phase chromatography according to the procedure of Krishnan.

CHAPTER V

RESULTS

Effect of Agitator Speed on Reaeration

The variation in oxygen transfer rate of the system as a function of the agitator speed is shown in Figure 5. As expected, the oxygen transfer rate constant, k_a, increased with increasing agitation of the medium. The least squares line of best fit for the data obtained on the unit employed is given by:

$$\log (100k_a) = 1.62 \log(R) - 2.58 \qquad 27$$

where R is the agitator speed in RPM.

Effect of Velocity Gradient on the Steady State Behavior of a Heterogeneous Population

In order to illustrate the nature and magnitude of the steady-state variation in the parameters assessed under constant operational conditions, the results of all analyses performed during a typical experimental run are shown in Figures 6 and 7. As previously noted, a single steady-state value was obtained by making a minimum of six observations of each parameter and calculating the average.

The average steady-state dissolved oxygen concentrations observed for the heterogeneous population experiments in which the agitation rate was varied are shown in

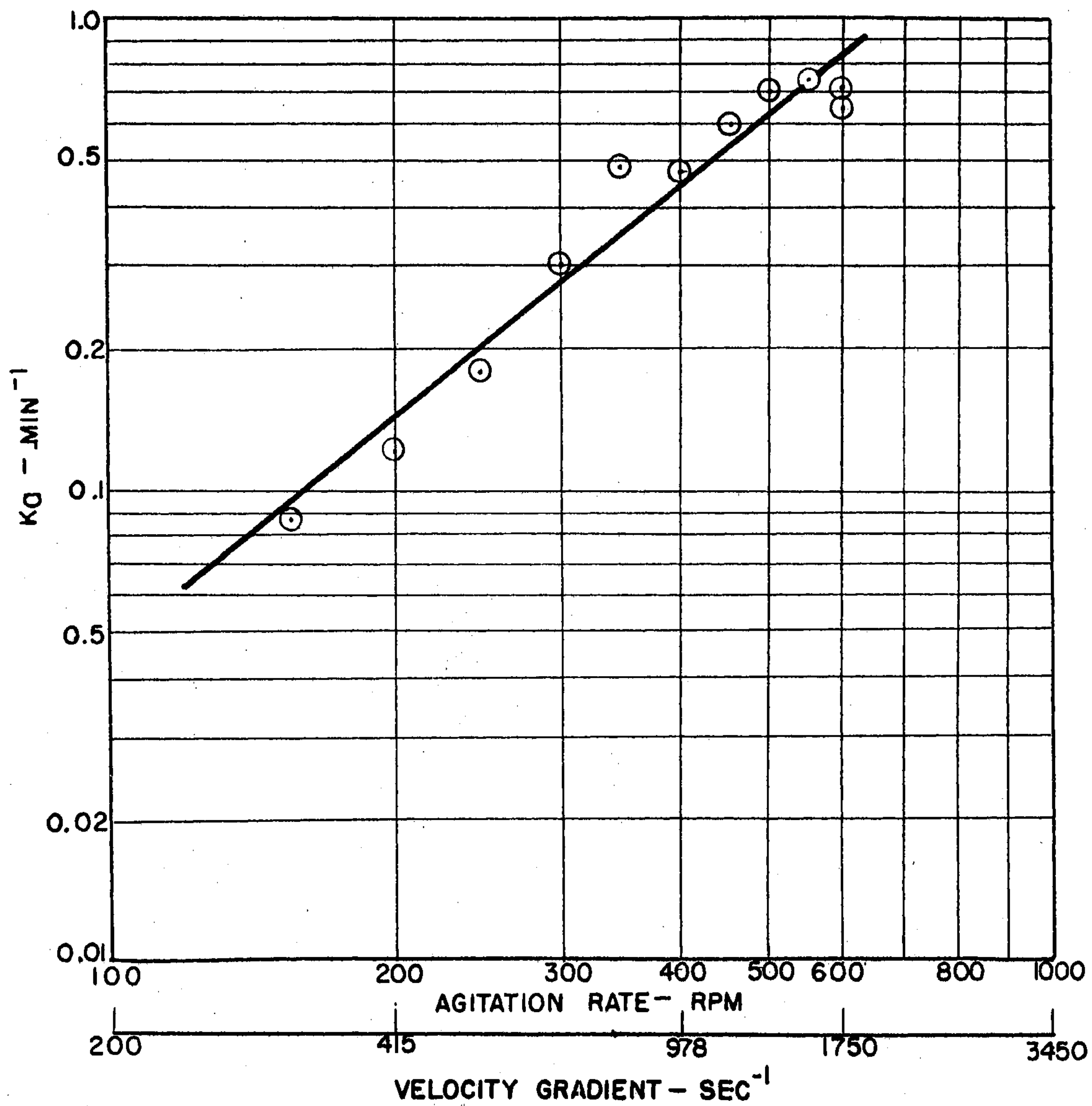


Figure 5 - Effect of Agitation on the Reaeration Coefficient of Experimental Reactor at an Air Flow Rate of 1.0 l/min.

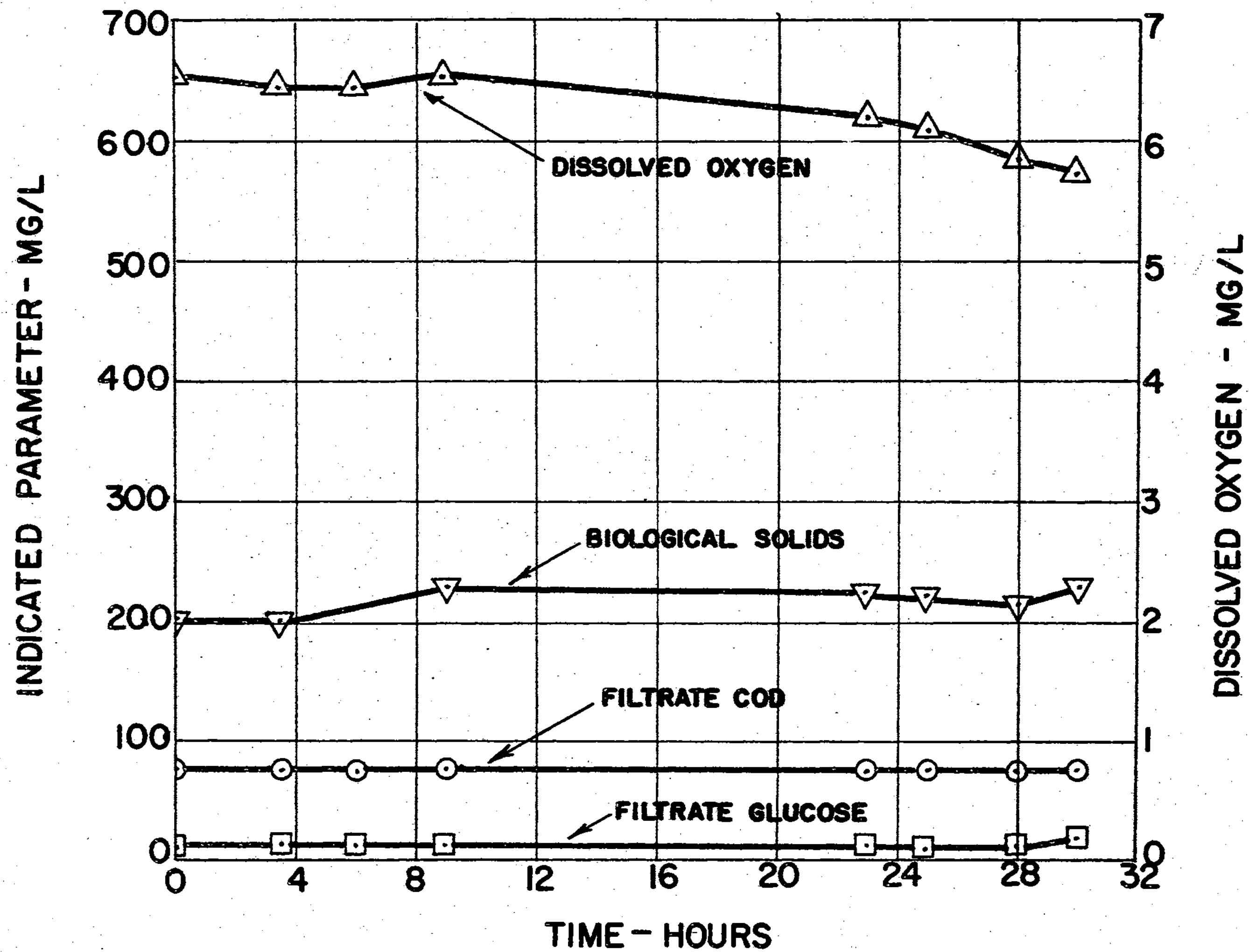


Figure 6 - Steady-State Parameters in the Completely Mixed Reactor during Growth of the Heterogeneous Population at a Velocity Gradient of 1000 Sec^{-1} and a Growth Rate of 0.125 Hrs^{-1}.

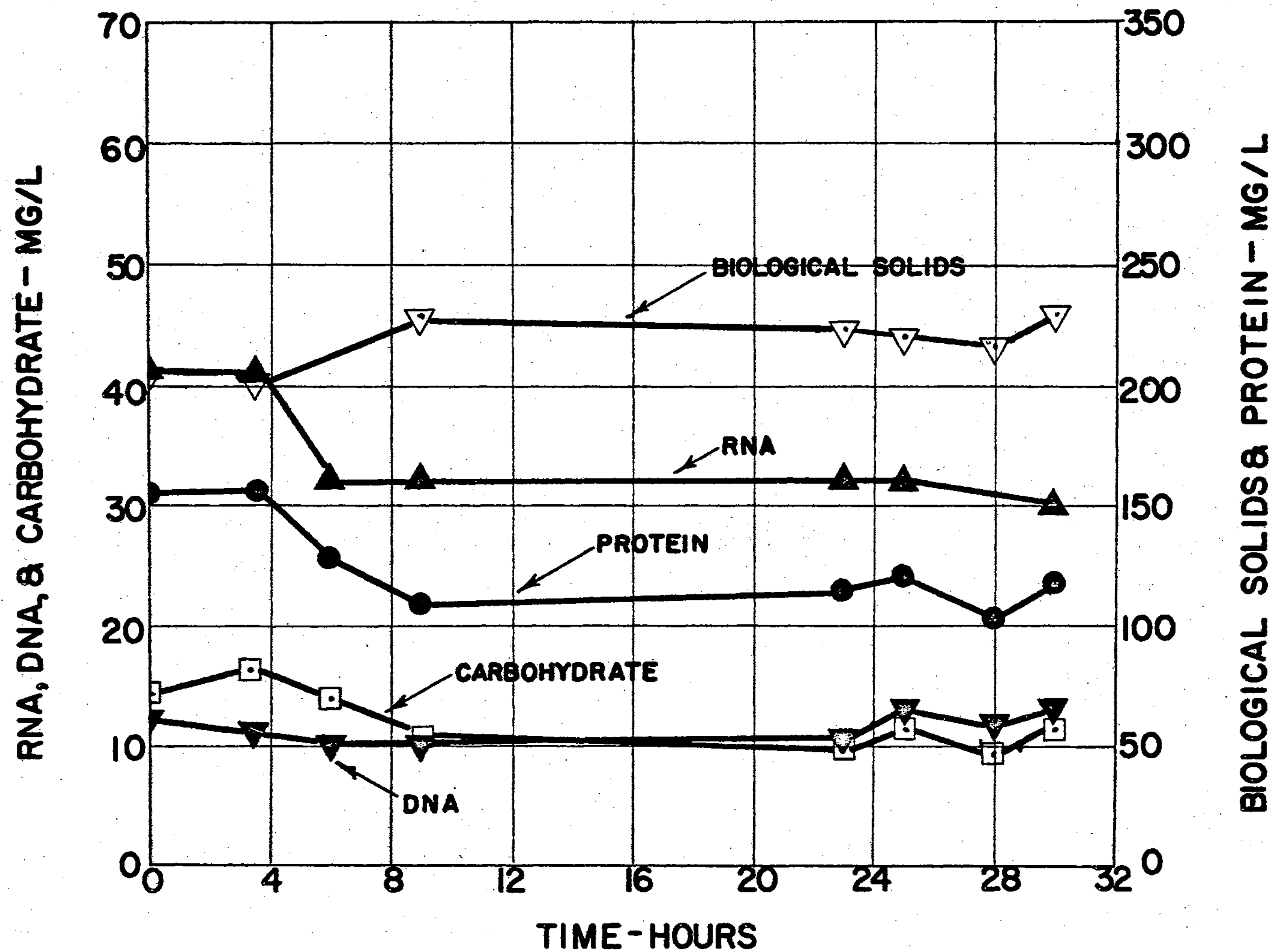


Figure 7 - Steady-State Concentration of Cell Components during Growth of the Heterogeneous Population at a Velocity Gradient of 1000 Sec^{-1} and a Growth Rate of 0.125 Hrs^{-1}.

Figure 8. Examination of this figure shows that the dissolved oxygen concentration maintained at steady-state increased with increasing velocity gradient. This result is to be expected because of the increase in k_a as a function of agitation. An interesting point about these data is that only at the lowest velocity gradient (300 sec^{-1}) was the effect of the growth rate of the system clearly reflected in the dissolved oxygen concentration. At the higher velocity gradients the dissolved oxygen concentration maintained appeared to be sufficiently high under all conditions to mask the effect of growth rate.

The results obtained from the cellular analyses performed on all of the experiments using heterogeneous population are shown in Figures 9 through 12. In order to obtain a better comparison of the behavior of the unit at the various velocity gradients, the concentrations of cellular material shown in these figures were divided by the difference between the influent and effluent COD to obtain the yield of all of the cellular parameters. Figure 13 is a plot of the calculated yield of biological solids at all of the growth rates examined. From this figure it may be seen that the biological solids yield at all of the growth rates tended to decrease as the velocity gradient was increased in the range from G = 300 sec^{-1} to 1000 sec^{-1}. In the range from 1000 sec^{-1} to 1200 sec^{-1}, a sharp increase in the yield occurred. Above G = 1200 sec^{-1} another decreasing trend was observed at three of the four

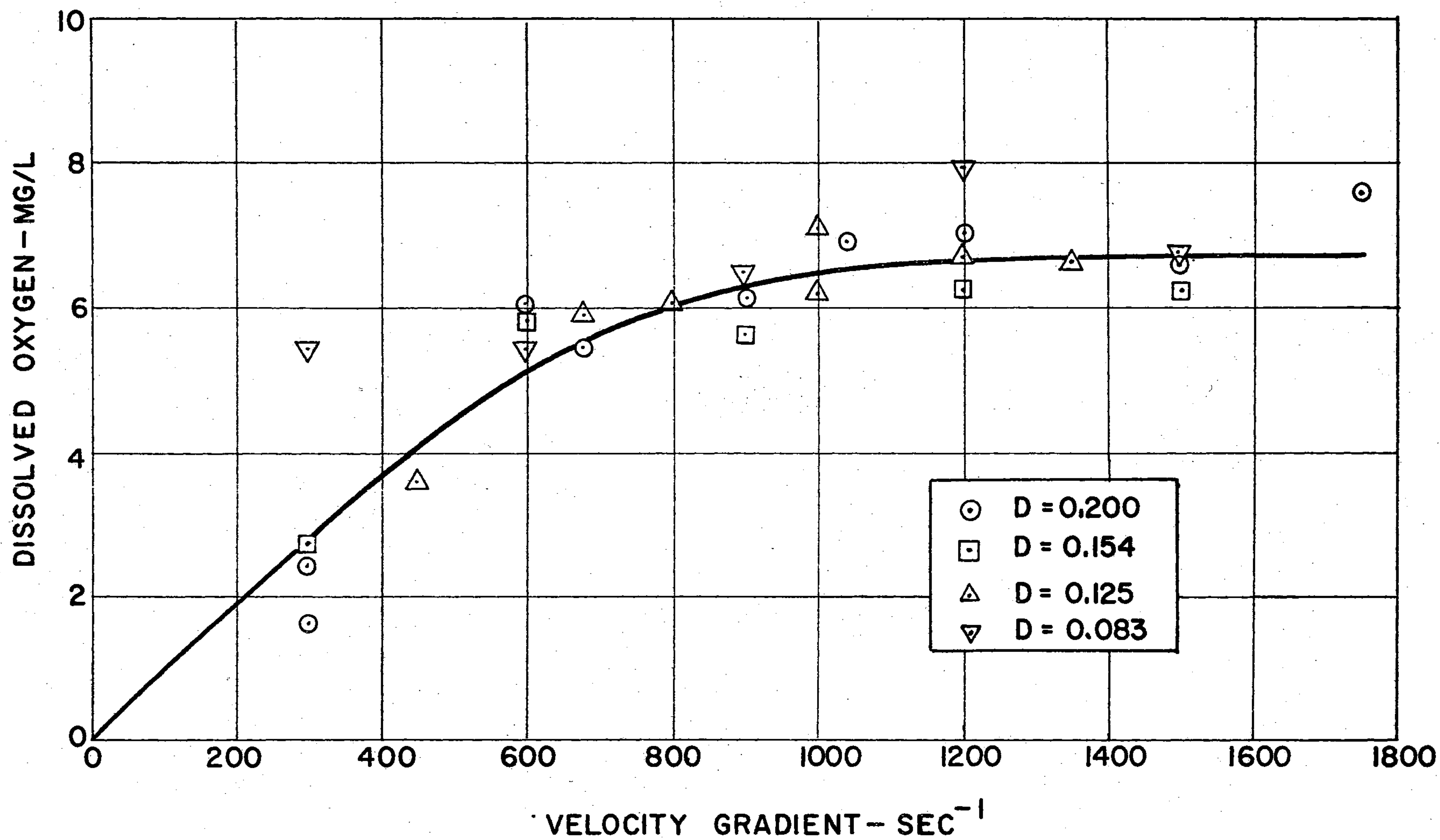


Figure 8 - Variation in Steady-State Oxygen Tension with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

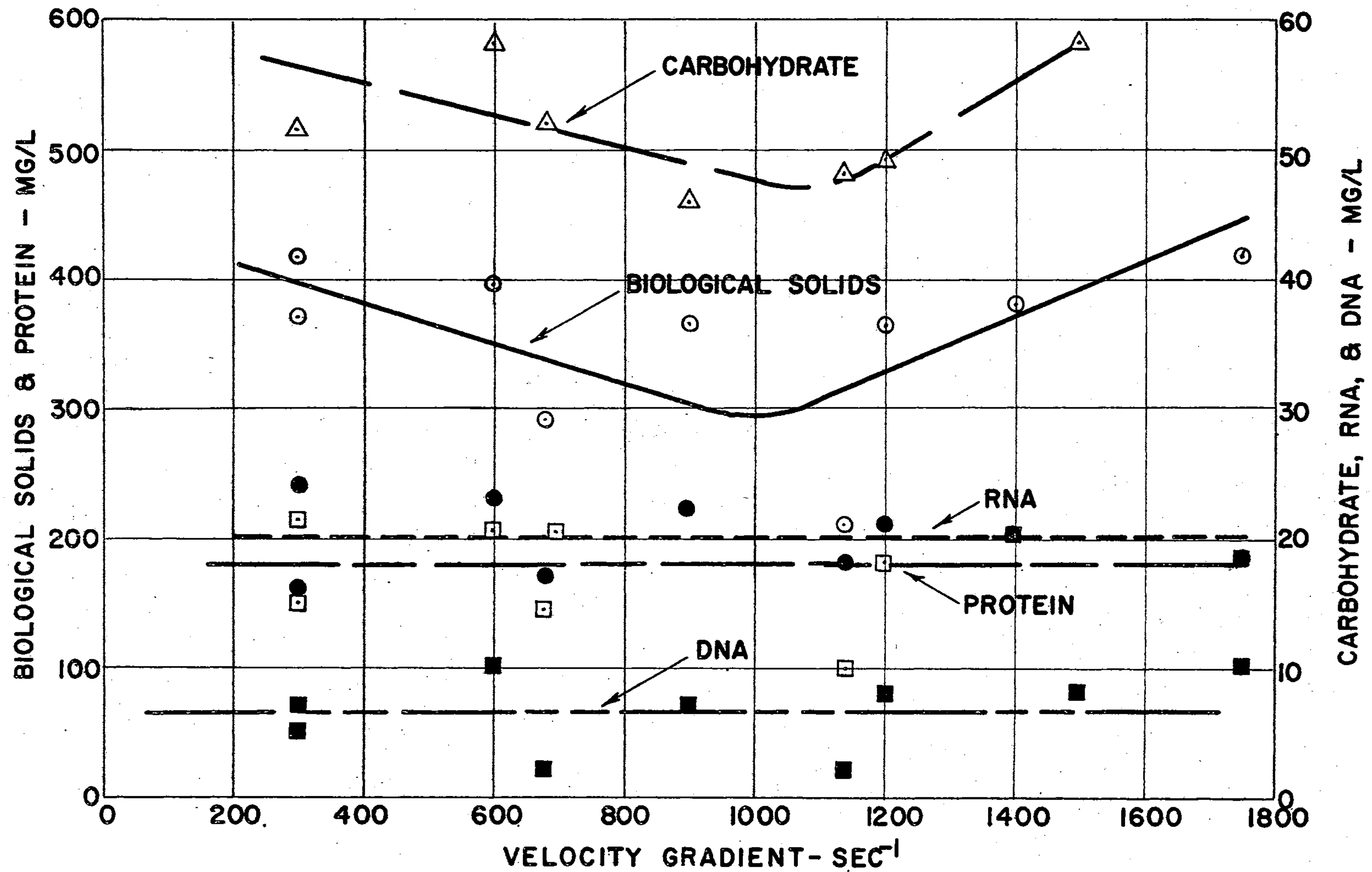


Figure 9 - Variation in Steady-State Levels of Biological Solids and Cell Components with Velocity Gradient during Growth of the Heterogeneous Population at a Growth Rate of 0.200 Hrs^{-1}.

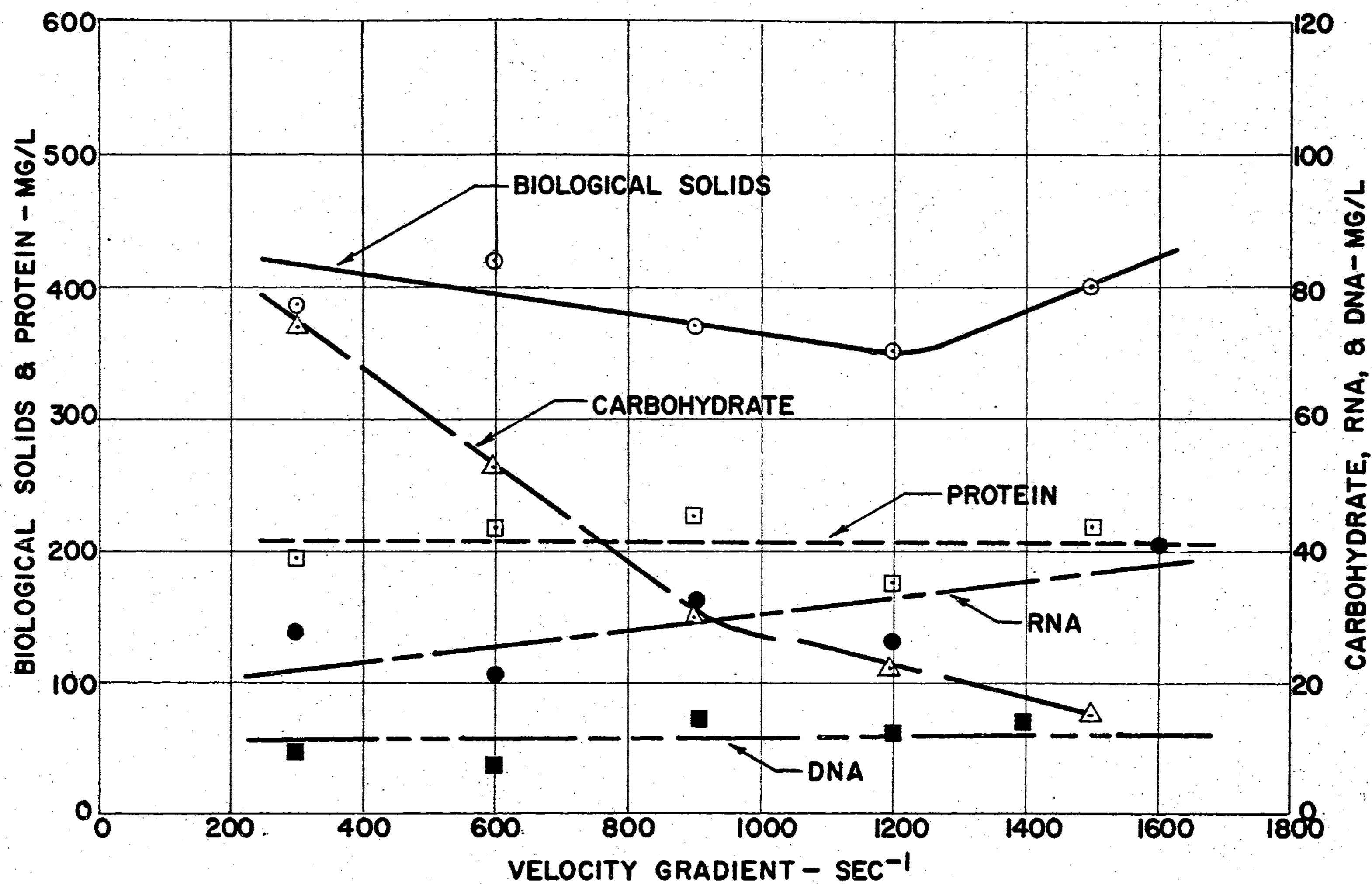


Figure 10 - Variation in Steady-State Levels of Biological Solids and Cell Components with Velocity Gradient during Growth of the Heterogeneous Population at a Growth Rate of 0.154 Hrs^{-1}.

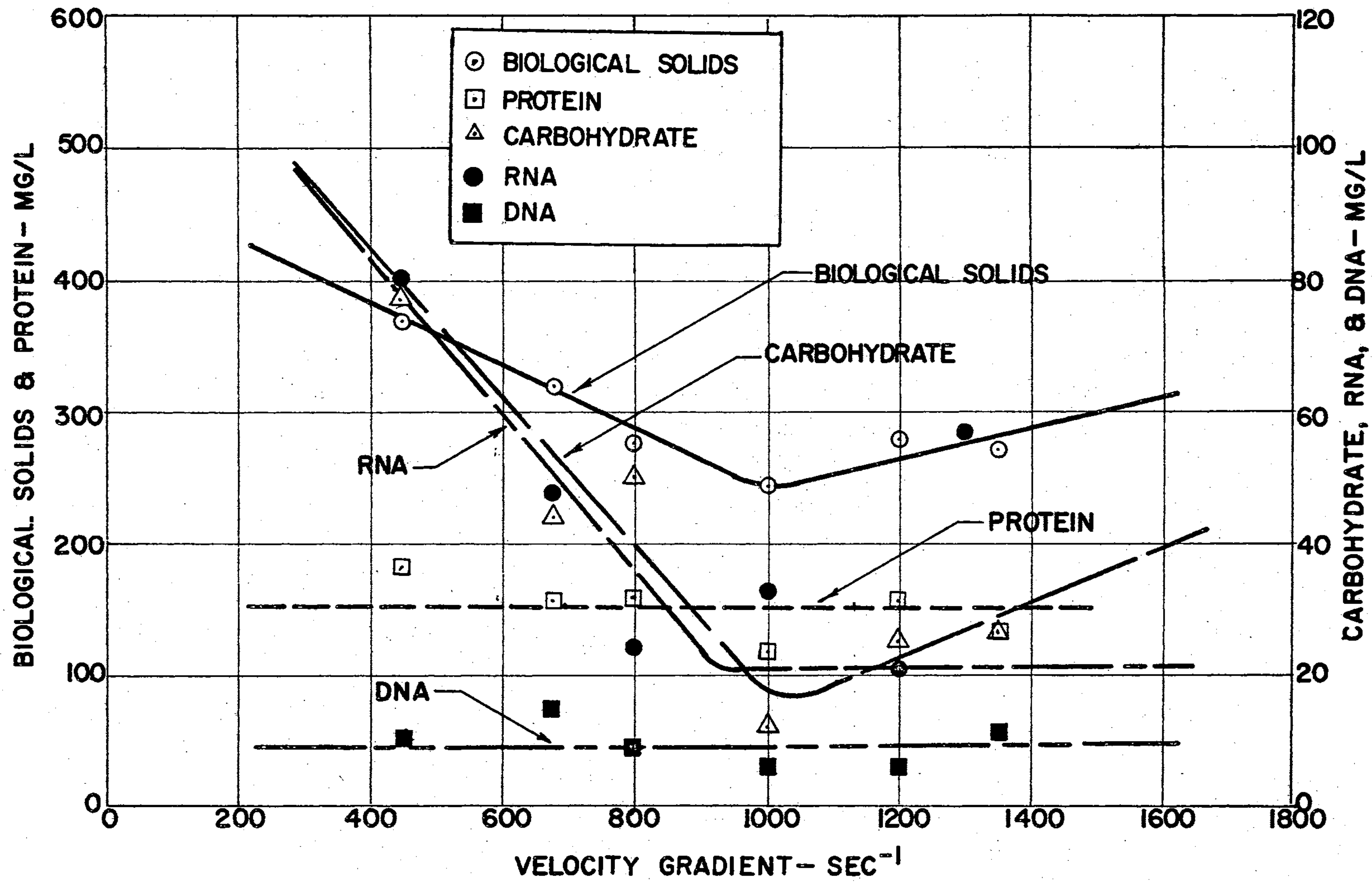


Figure 11 - Variation in Steady-State Levels of Biological Solids and Cell Components with Velocity Gradient during Growth of the Heterogeneous Population at a Growth Rate of 0.125 Hrs^{-1}.

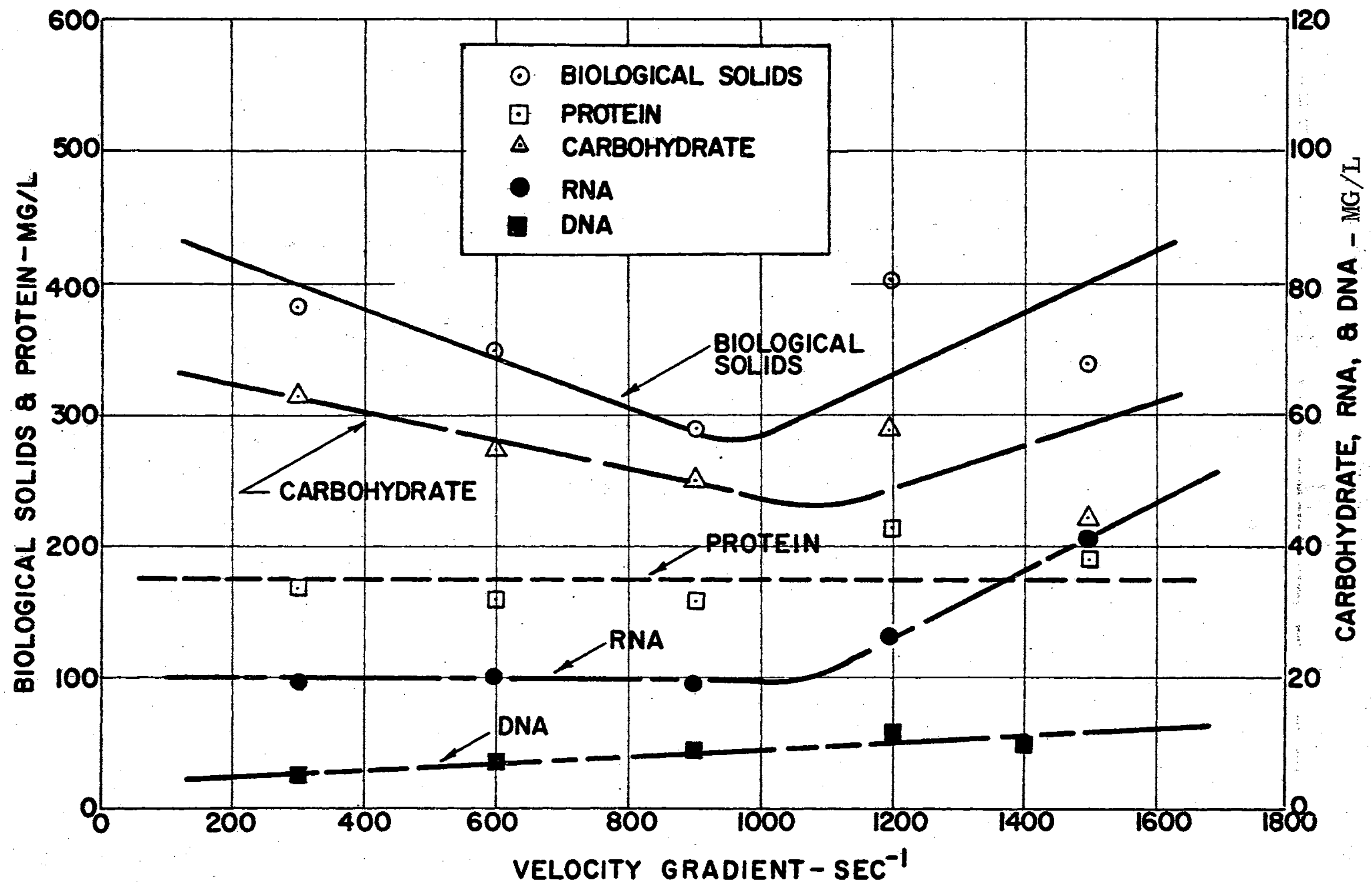


Figure 12 - Variation in Steady-State Levels of Biological Solids and Cell Components with Velocity Gradient during Growth of the Heterogeneous Population at a Growth Rate of 0.083 Hrs^{-1}.

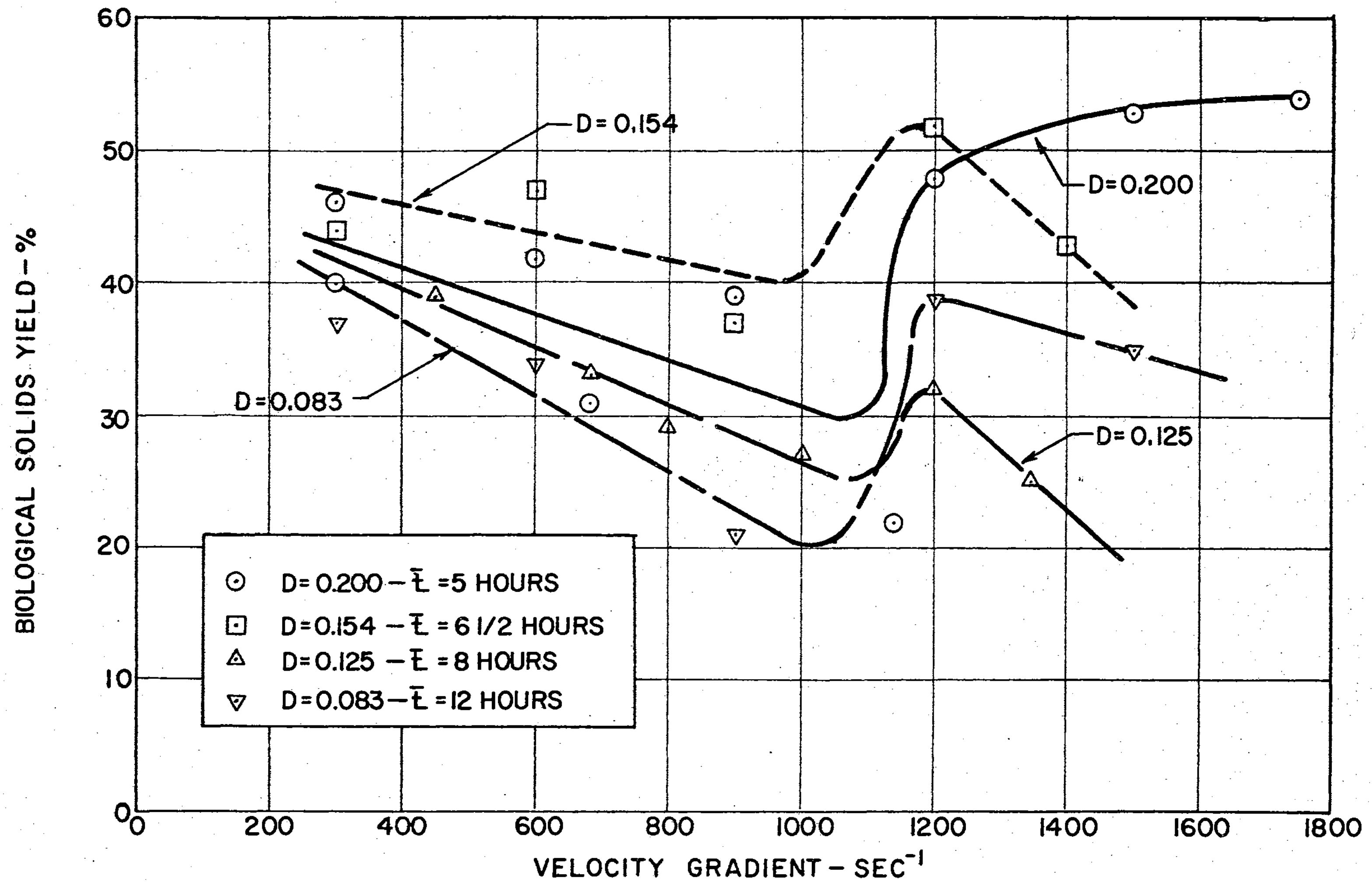


Figure 13 - Variation in the Steady-State Biological Solids Yield with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

growth rates employed as the velocity gradient was further increased.

Figure 14 shows the yield of cellular protein plotted versus the velocity gradient at all of the growth rates examined. It may be seen here that minor reductions were obtained at all of the detention times except $6\frac{1}{2}$ hours (D = 0.154) as the velocity gradient was increased from 300 sec^{-1} to 1000 sec^{-1}. Above this velocity gradient, the protein yield either maintained a constant value or increased with increasing turbulence.

The yield of cellular carbohydrate produced at the various energy levels is shown in Figure 15. This parameter may be seen to have been more strongly affected by the velocity gradient than either the biological solids or protein yields, with the relationship appearing to be of a similar nature to that observed with cellular protein.

The yields of RNA and DNA obtained at the various velocity gradients are shown in Figures 16 and 17, respectively. It may be seen from these figures that considerable variation in the yield of both nucleic acids occurred, and that no consistent trend could be discerned for these parameters at all of the growth rates examined.

The composition of the sludge obtained during operation at all of the detention times employed, expressed as percent of the biological solids, is shown in Figures 18 through 21. From these data it may be seen that the protein, RNA, and DNA contents of the sludge generally

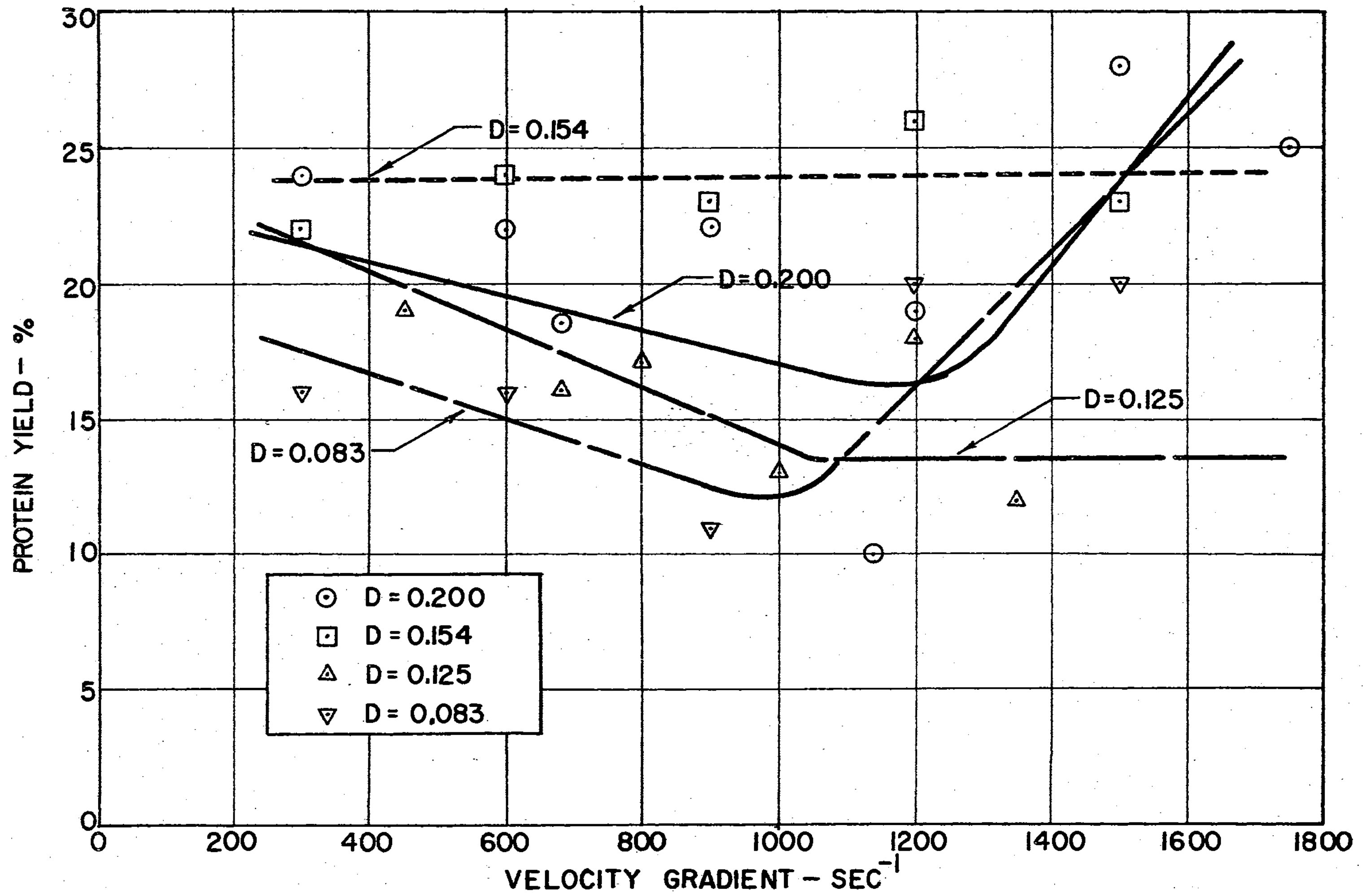


Figure 14 - Variation in the Steady-State Cellular Protein Yield with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

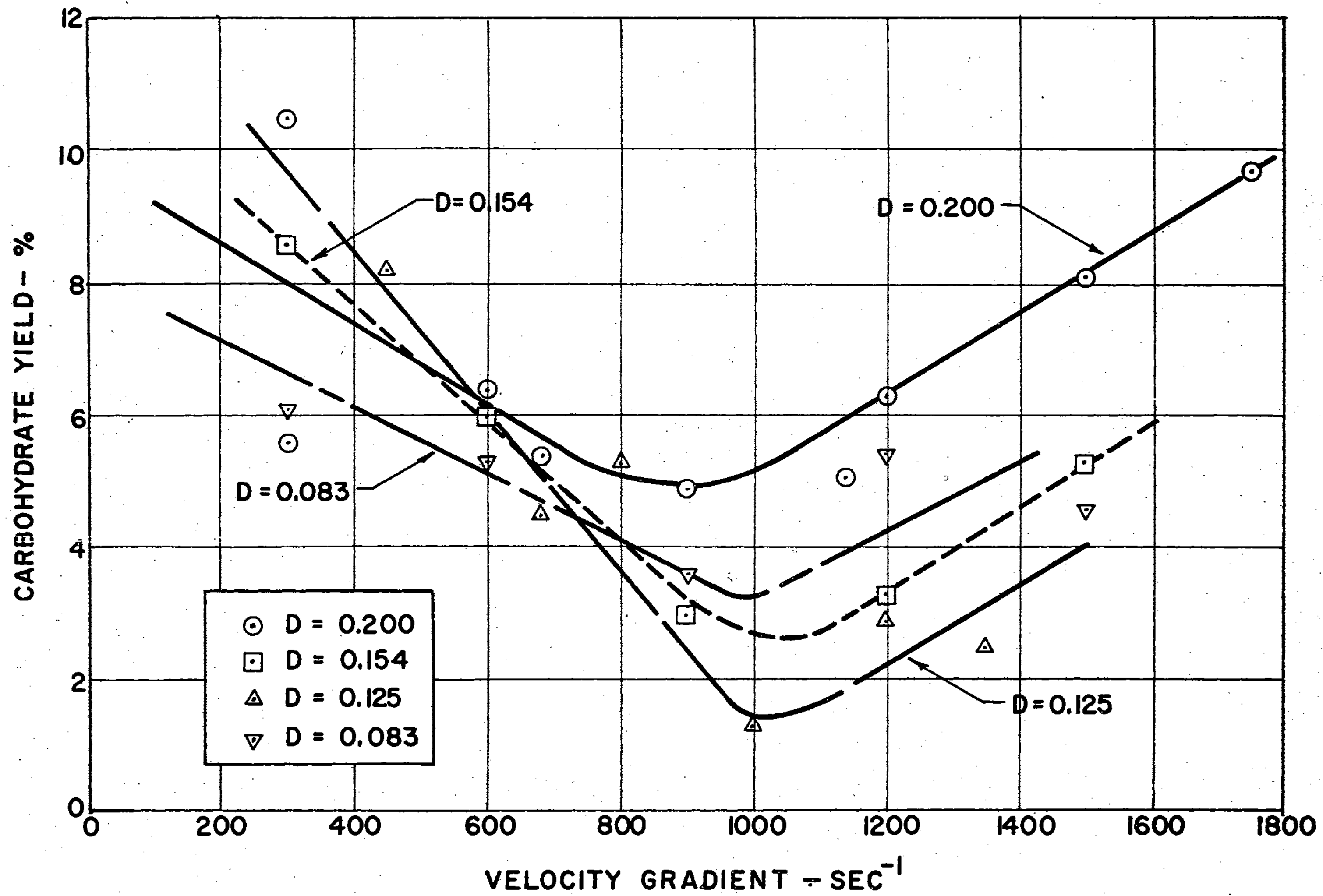


Figure 15 - Variation in the Steady-State Cellular Carbohydrate Yield with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

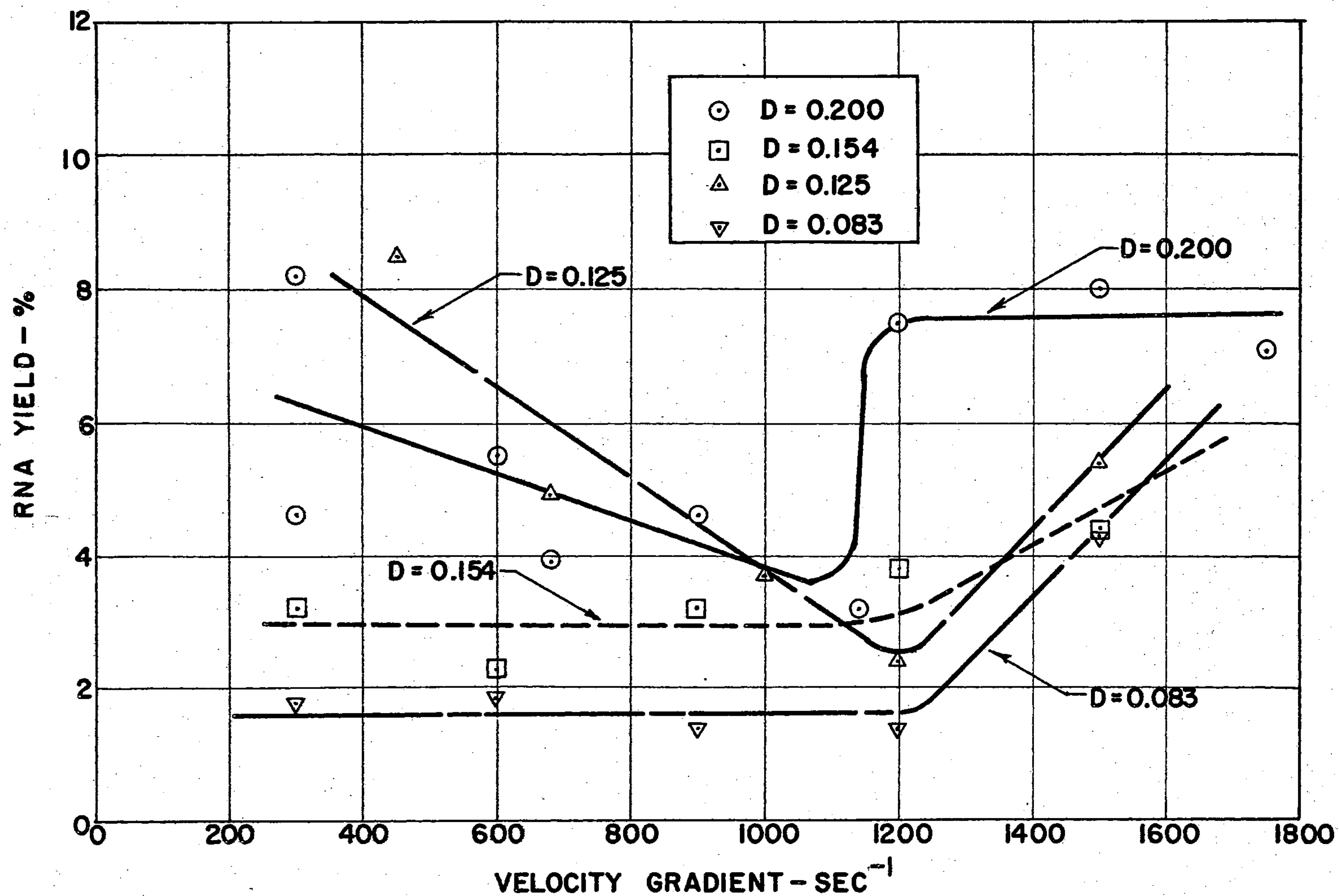


Figure 16 - Variation in the Steady-State Cellular RNA Yield with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

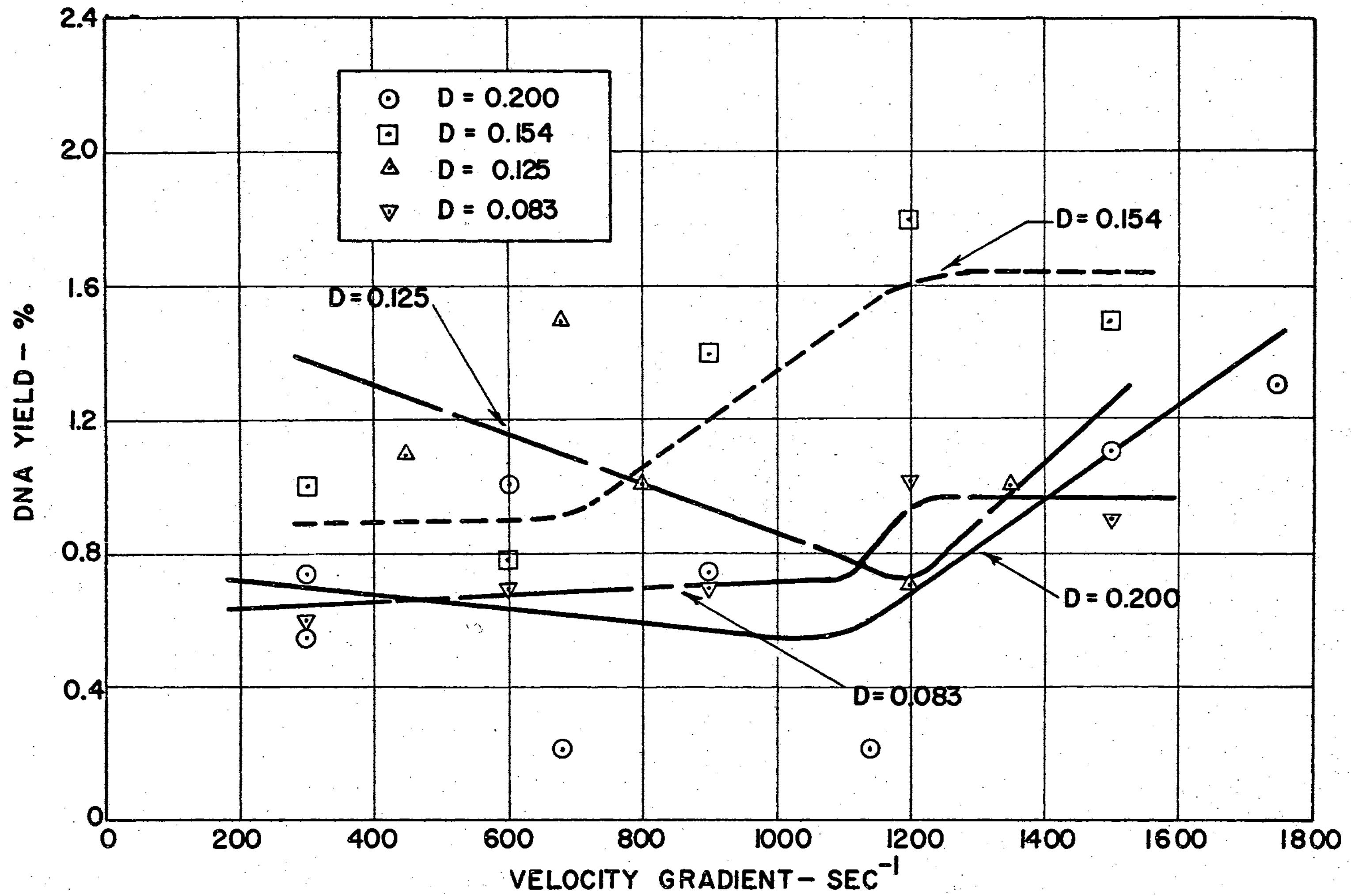


Figure 17 - Variation in the Steady-State Cellular DNA Yield with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

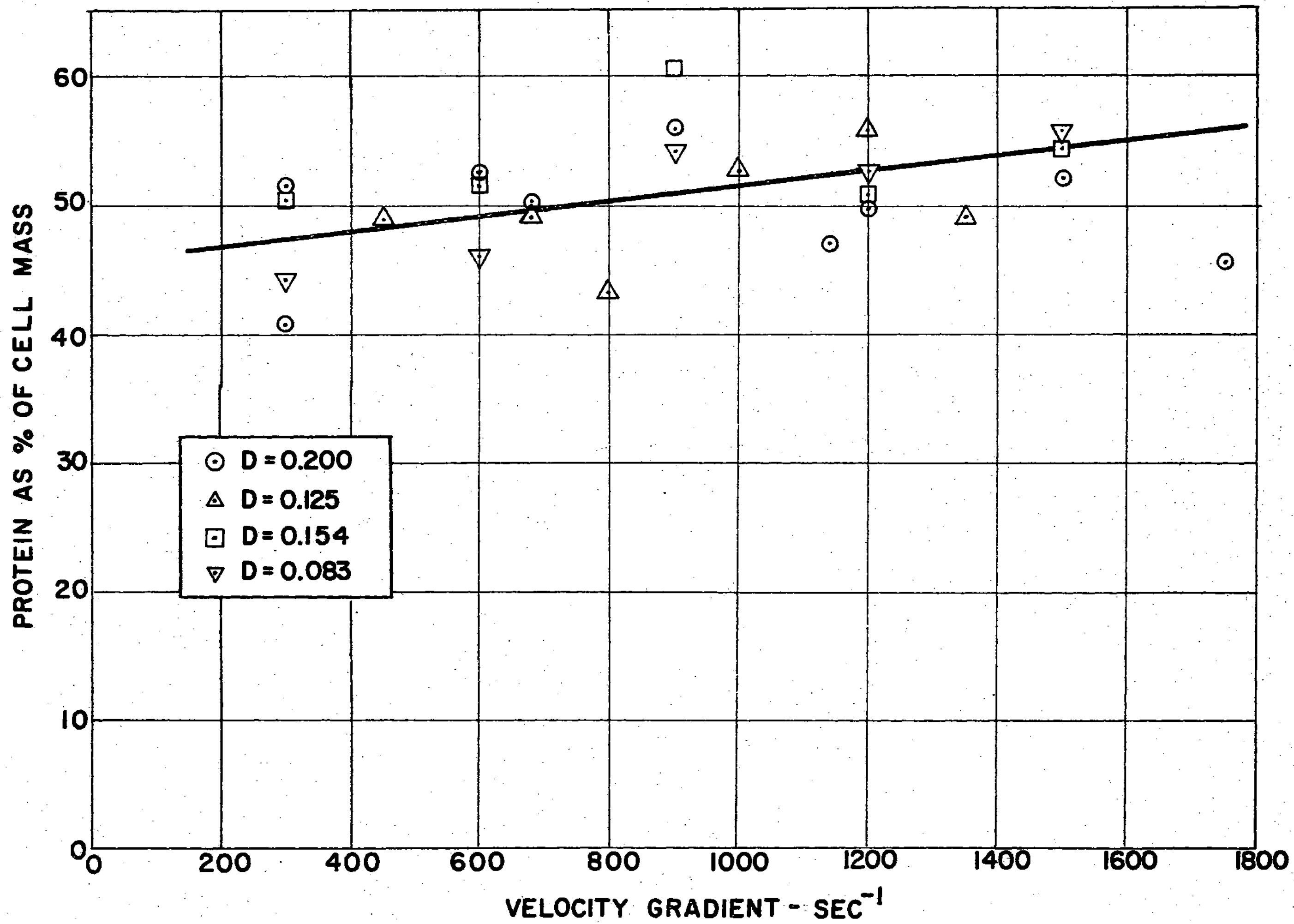


Figure 18 - Variation in the Steady-State Sludge Protein Content with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

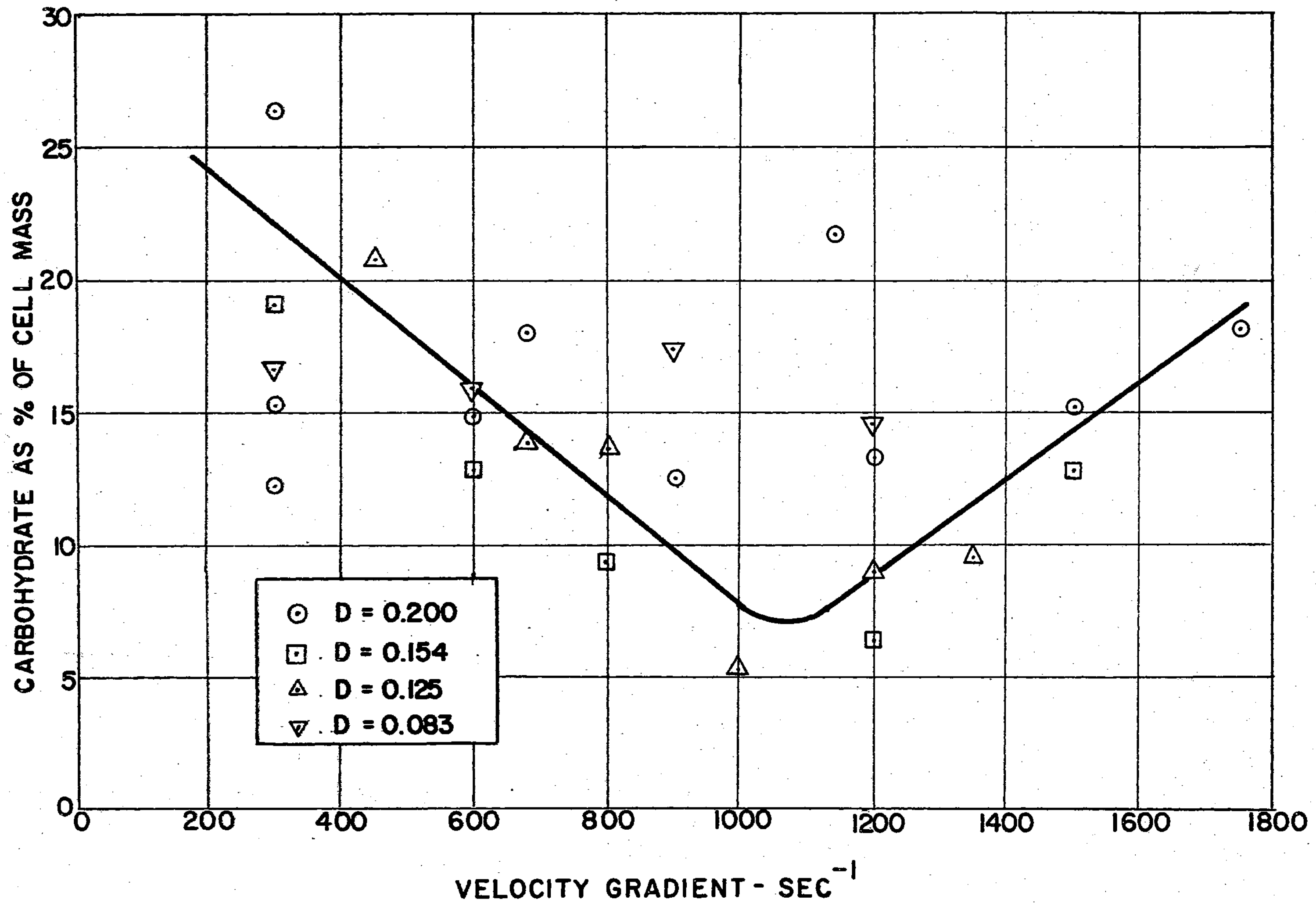


Figure 19 - Variation in the Steady-State Sludge Carbohydrate Content with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

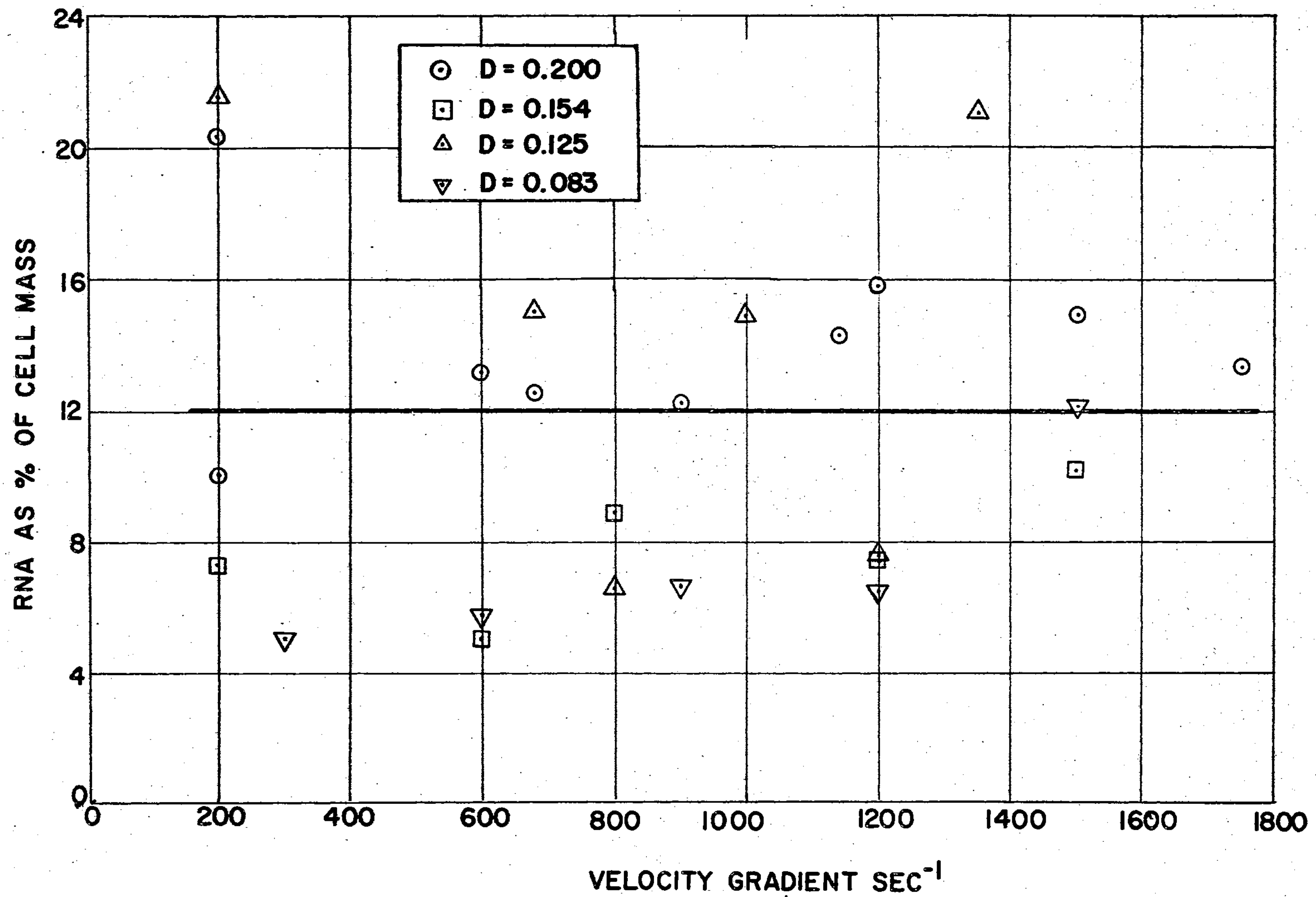


Figure 20 - Variation in the Steady-State Sludge RNA Content with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

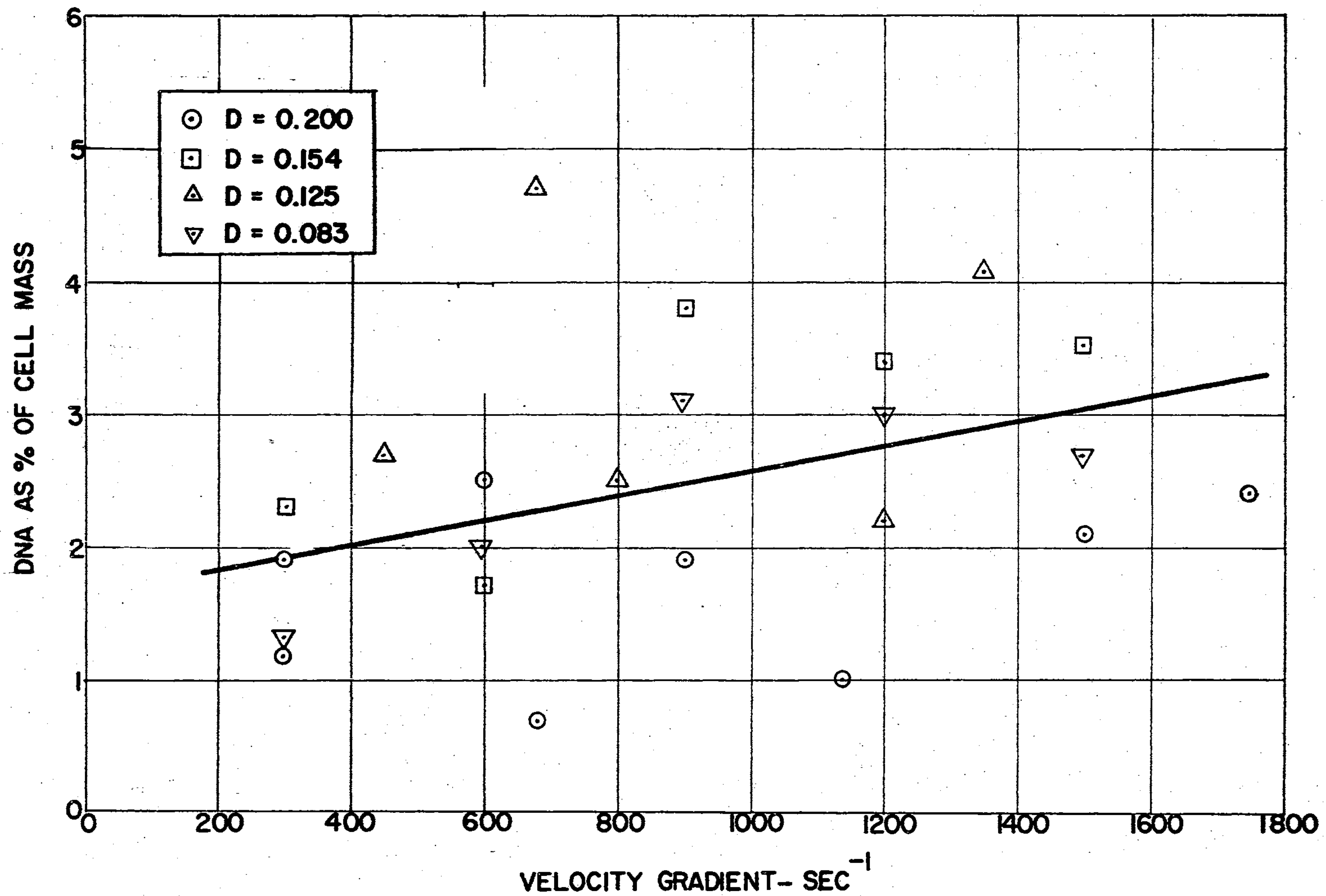


Figure 21 - Variation in the Steady-State Sludge DNA Content with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

increased with the velocity gradient while the carbohydrate content decreased up to a velocity gradient of 1000 sec^{-1}, increasing thereafter.

The steady-state oxygen uptake obtained during each experiment is shown plotted versus the velocity gradient in Figure 22. These data indicated that the oxygen uptake rate at steady-state tended to increase as a function of both growth rate and velocity gradient. This result would be expected from the previous discussion of the kinetics of oxygen uptake rate in steady-state systems.

A materials balance was made for all of the runs to obtain an indication of the validity of the relationship between the solids yield and velocity gradient. These data, expressed in terms of equivalent glucose, are shown in Table II. An average of 91.7% of the influent substrate was accounted for by the sum of respiration plus synthesis. This value compares favorably with materials balances reported for batch systems where the Warburg respirometer was used to determine oxygen uptake (52). Also included in Table II are the percentage removals of substrate calculated as both total COD and glucose (anthrone). These values show that no significant change in substrate removal occurred as the energy input was varied at any of the detention times employed.

In order to determine whether changes in predominance comparable to those observed by Zahradka (36) occurred in the system under study here, microscopic examination of

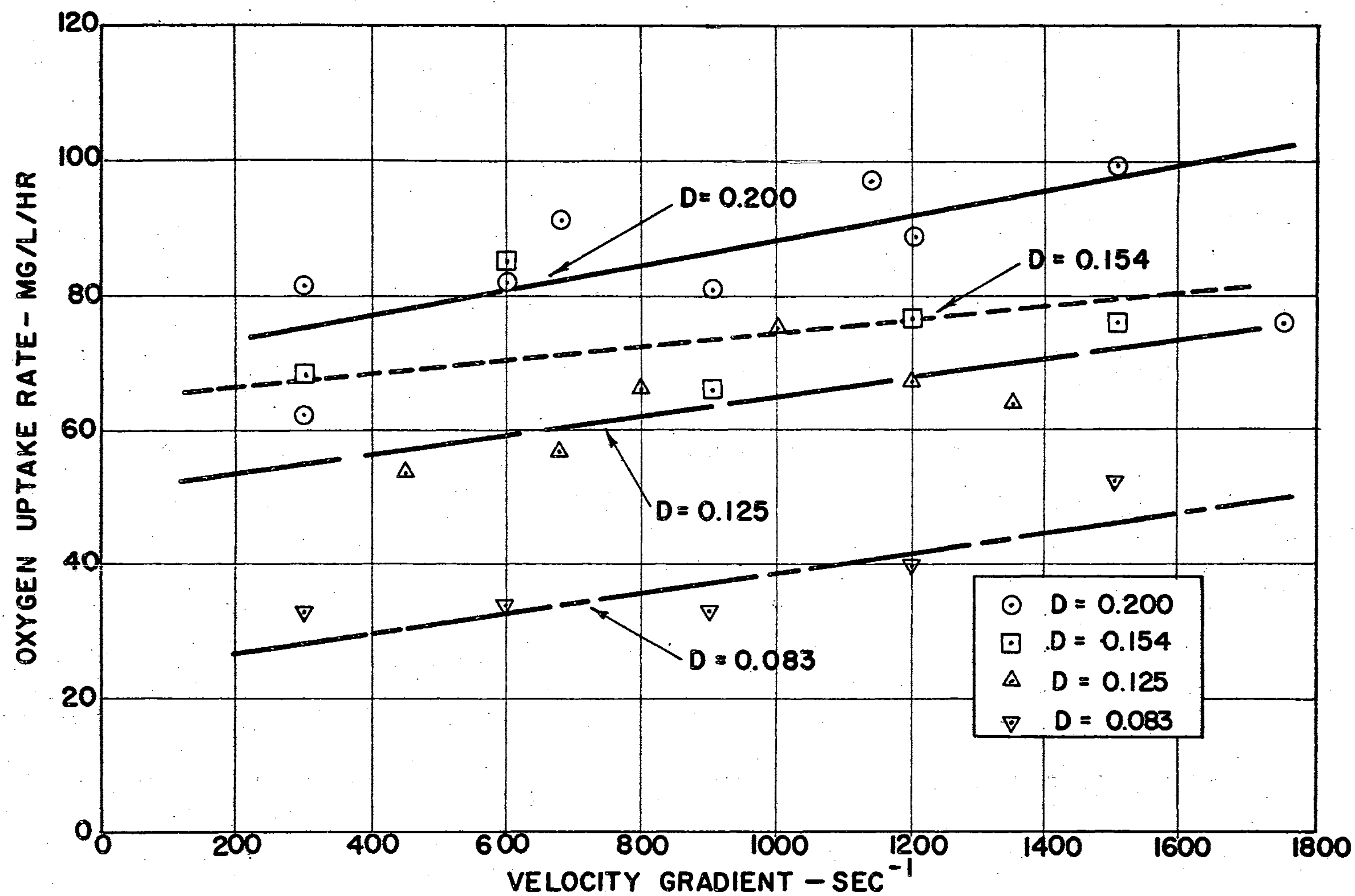


Figure 22 - Variation in the Steady-State Oxygen Uptake Rate with Velocity Gradient during Growth of the Heterogeneous Population at All Growth Rates.

TABLE II

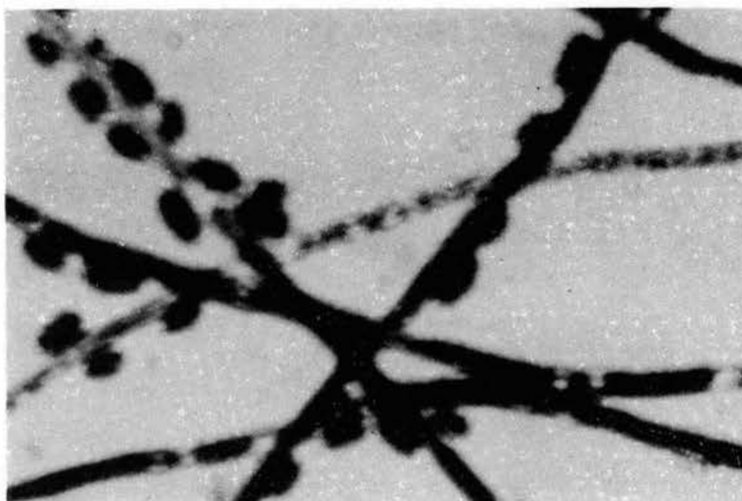
SUBSTRATE REMOVAL AND MATERIALS BALANCE FOR HETEROGENEOUS POPULATION AT VARIABLE VELOCITY GRADIENT

E	G	Reactor Volume	Feed Rate	COD Infl. mg/l	COD Eff. mg/l	COD mg/l	COD Consumed* mg/hr	Solids mg/l	Solids mg/hr	O_2 Uptake Rate mg/l/hr	O_2 Uptake Rate mg/hr*	Total Substrate Accounted for mg/hr	% Recovery	% Glucose Removed	% COD Removed
	300	3.91	0.78	626	52	574	422	372	290	61.1	225	515	122	99	91
	300	3.91	0.78	970	60	910	670	417	325	81.3	300	625	93	99	93
	600	3.82	0.76	970	31	939	673	393	299	81.9	295	594	88	99	96
	680	3.81	0.76	918	88	830	595	289	220	91.2	328	548	92	98	90
5	900	3.76	0.75	970	32	938	664	369	277	80.6	286	563	84	99	96
	1140	3.69	0.74	1020	83	937	654	209	155	97.2	338	493	75	99	91
	1200	3.69	0.74	820	44	776	542	367	272	89.0	310	582	107	98	94
	1500	3.64	0.73	780	65	715	492	381	278	98.9	340	618	126	98	91
	1750	3.60	0.72	860	77	783	532	420	302	76.7	260	562	106	93	91
	300	3.91	0.62	961	73	888	519	387	240	68.5	253	493	94	92	92
	600	3.82	0.61	961	65	896	516	421	257	85.6	308	565	109	93	93
6½	900	3.76	0.60	1085	92	993	562	371	223	66.2	235	458	81	92	91
	1200	3.69	0.59	728	52	676	376	349	206	77.1	268	474	126	93	92
	1500	3.64	0.58	992	49	943	516	403	234	68.5	235	469	91	95	95
	450	3.86	0.48	974	34	940	426	369	177	54.3	198	375	87	99	96
	680	3.81	0.48	1040	50	990	448	318	153	57.2	206	359	80	98	95
	800	3.78	0.47	961	28	933	413	275	129	66.3	236	365	88	98	97
	1000	3.71	0.46	970	75	895	388	244	112	75.8	265	377	97	99	92
8	1000	3.71	0.46	970	41	929	403	401	184	61.1	214	398	99	99	96
	1000	3.7.	0.46	1115	79	1036	450	353	162	58.2	204	366	81	98	93
	1200	3.69	0.46	890	18	872	395	279	128	67.6	235	363	91	99	98
	1350	3.67	0.46	1126	66	1060	470	270	124	64.5	223	347	74	99	94
	300	3.91	0.34	1090	52	1038	333	381	130	33.1	122	252	75	92	95
	600	3.82	0.33	1090	58	1032	321	349	115	34.1	123	238	74	95	95
12	900	3.76	0.32	1428	58	1370	414	289	92	33.5	119	211	50	96	96
	1200	3.69	0.32	1094	30	1064	321	403	129	40.1	140	269	83	97	97
	1500	3.64	0.31	980	25	955	279	338	105	52.8	181	286	102	97	97

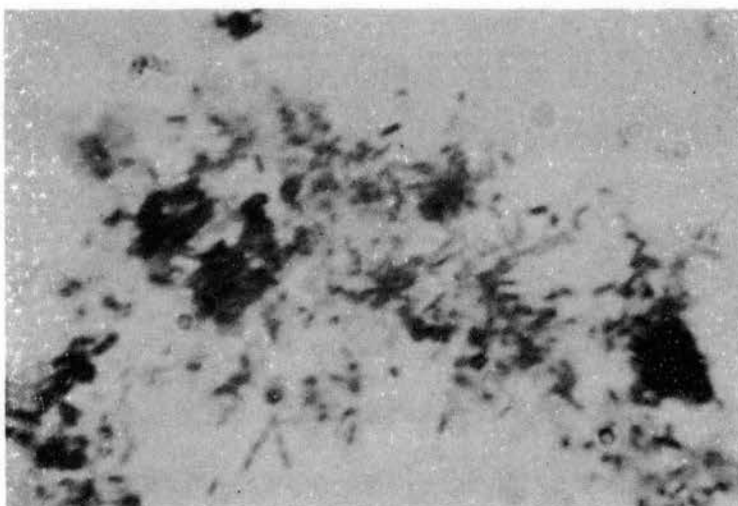
*Expressed as Equivalent Glucose

the sludge was conducted during each run. Only in the system having a detention time of eight hours was filamentous growth of the type observed by Zahradka encountered. At this detention time, filamentous organisms were found to be strongly predominant at a velocity gradient of 300 sec^{-1}. As the energy level was increased, the number of filamentous organisms occurring was found to decrease, until at a velocity gradient of 1000 sec^{-1} they were entirely absent. Figure 23 shows photomicrographs taken of the sludge during operation at 300 sec^{-1} and 1000 sec^{-1}.

At the other detention times studied, the predominating organisms at the low velocity gradients were small Gram-negative rods which showed large capsular layers. As the level of turbulence was increased, India ink examination of the sludge indicated that the number of cells having distinct capsular layers was diminished. Three possible explanations could be advanced to account for this observation. First, it is possible that the environment provided by the unit at high agitation rates was unfavorable for these organisms and being unable to compete successfully, they disappeared. Secondly, it is possible that no ecological changes occurred but that the cells were incapable of producing capsular material at the high velocity gradients. Thirdly, the high fluid shear caused by the high velocity gradients may have removed the exocellular polysaccharide from the vicinity of the cell as it was produced, preventing it from showing as a capsular



Photomicrograph of Sludge during Steady-State Operation at a Velocity Gradient of 300 sec^{-1} (x 2620)



Photomicrograph of Sludge during Steady-State Operation at a Velocity Gradient of 1000 sec^{-1} (x 2620)

Figure 23. Morphology of Sludge at Velocity Gradients of 300 and 1000 sec^{-1} and a Growth Rate of 0.125 Hrs^{-1}

layer in the microscopic observations. If this latter possibility were the case it is necessary that the material so produced acted as substrate for either the organism which produced it or for other cells present in the system, since it was not detected in either the cellular or filtrate analyses.

In order to determine if the system was capable of metabolizing the polysaccharide slime, samples of effluent obtained during growth at a detention time of five hours ($D = 0.200\ hrs^{-1}$) and a velocity gradient of 300 sec^{-1} were placed on a shaker and incubated at 25°C for thirty-six hours. Microscopic observation of the samples was conducted at the end of this incubation period, and it was found that the cellular capsule had indeed disappeared. In addition, anthrone analysis performed on the cells showed that the total carbohydrate content was reduced by 65% during the thirty-six hour period. Analysis of the membrane filtrate showed that no increase in the amount of soluble carbohydrate occurred. Thus, it was concluded that the microbiota present in the system were capable of metabolizing any exocellular polysaccharides which might have been produced at high velocity gradients and stripped from the cell.

Effect of Oxygen Tension on Steady-State Parameters

The average steady-state concentrations of biological solids, protein, carbohydrate, and nucleic acids determined under conditions of constant agitation and varying oxygen tension are shown in Figure 24. The yields calcu-

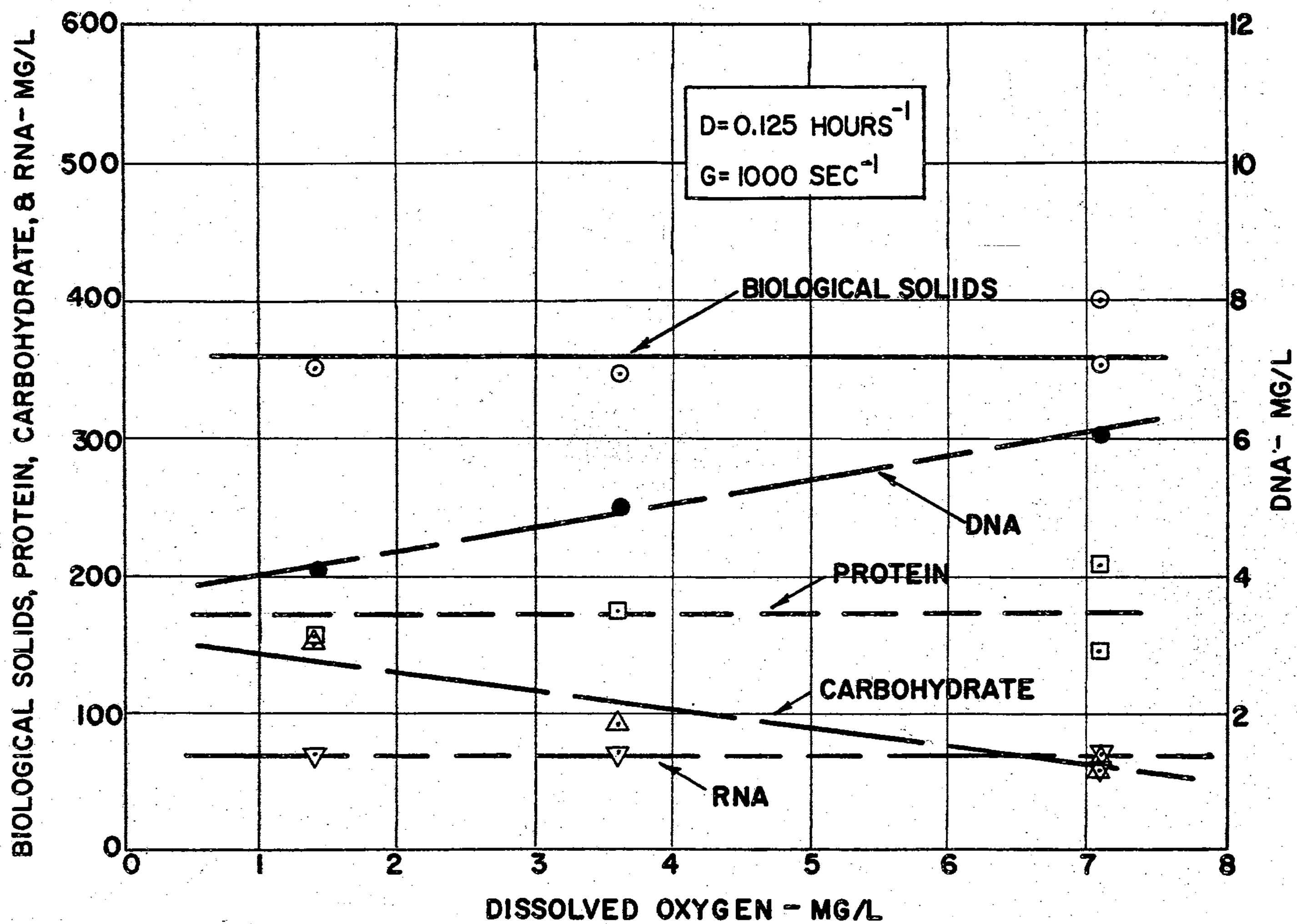


Figure 24 - Variation in the Steady-State Levels of Biological Solids and Cell Components with Oxygen Tension during Growth of the Heterogeneous Population at a Velocity Gradient of 1000 Sec^{-1} and a Growth Rate of 0.125 Hrs^{-1}.

lated from these data are shown in Figure 25. Examination of these figures indicates that no reduction in the biological solids yield occurred as the oxygen tension was varied from 1.5 to 7.0 mg/l. Although a reduction in the carbohydrate yield of comparable magnitude with that observed when the amount of agitation was varied occurred, the reduction was not reflected in the biological solids yield.

Figure 26 is a plot of the oxygen uptake rates determined for these runs. Here it may be seen that oxygen tension had no effect on the steady-state rate of oxygen uptake over the entire range studied.

Table III shows the materials balance and substrate removal data for these same runs. In this table it may be seen that only very minor reductions in the efficiency of substrate removal were observed as the dissolved oxygen concentration was varied.

Effect of Velocity Gradient on the Response of a Steady-State System to Quantitative Shock Loading

The response of the system to an increase in the influent substrate concentration from 1000 to 3000 mg/l of glucose during growth at a velocity gradient of 300 sec^{-1} and a detention time of five hours is shown in Figures 27, 28, and 29. Examination of the dissolved oxygen curve (Figure 27) shows that the oxygen tension of the medium was reduced to nearly zero within two hours after administration of the shock load. Both the solids and oxygen uptake responses definitely show the existence of oxygen

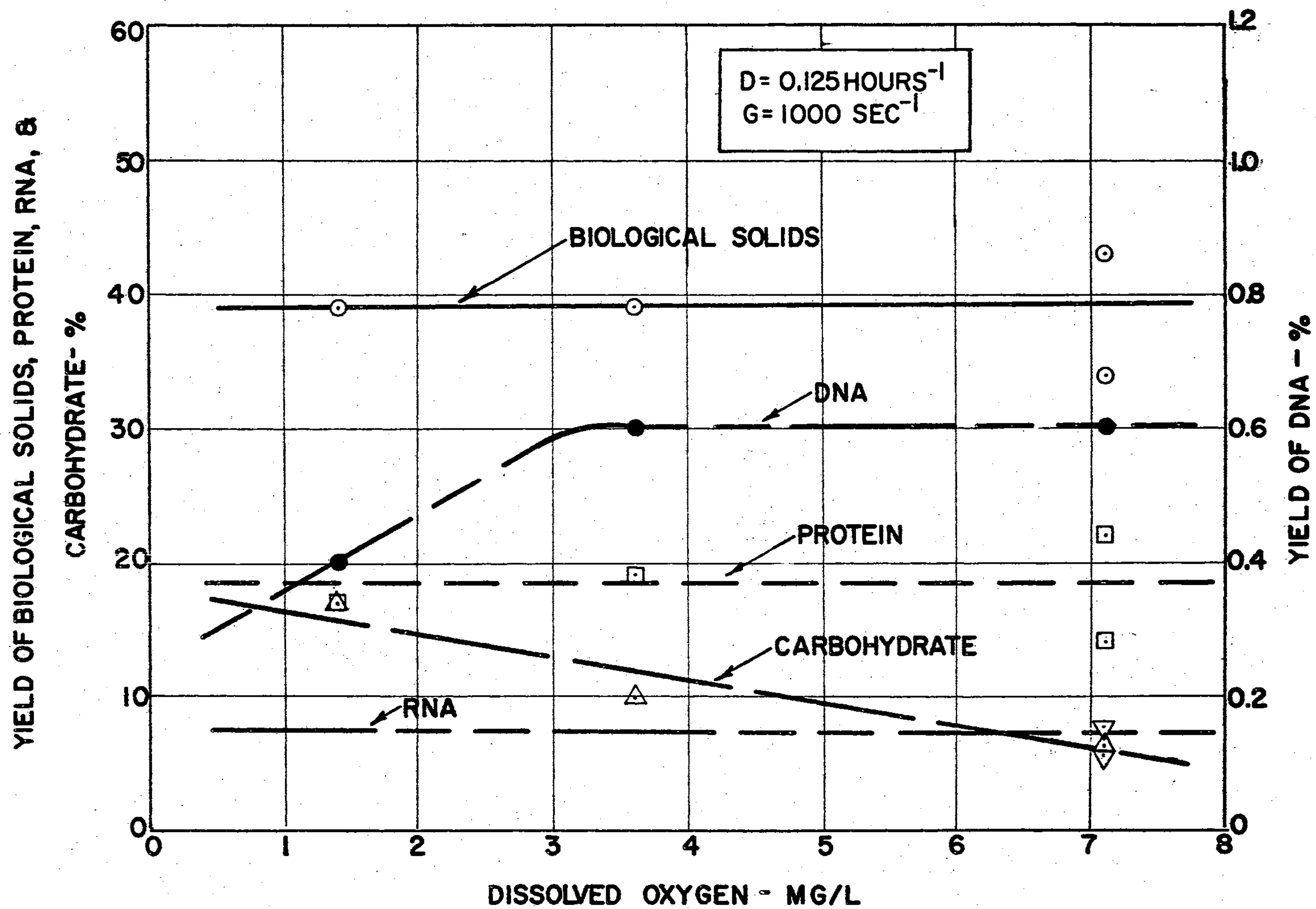


Figure 25 - Variation in the Steady-State Biological Solids and Cell Component Yields with Oxygen Tension during Growth of the Heterogeneous Population at a Velocity Gradient of 1000 Sec^{-1} and a Growth Rate of 0.125 Hrs^{-1}.

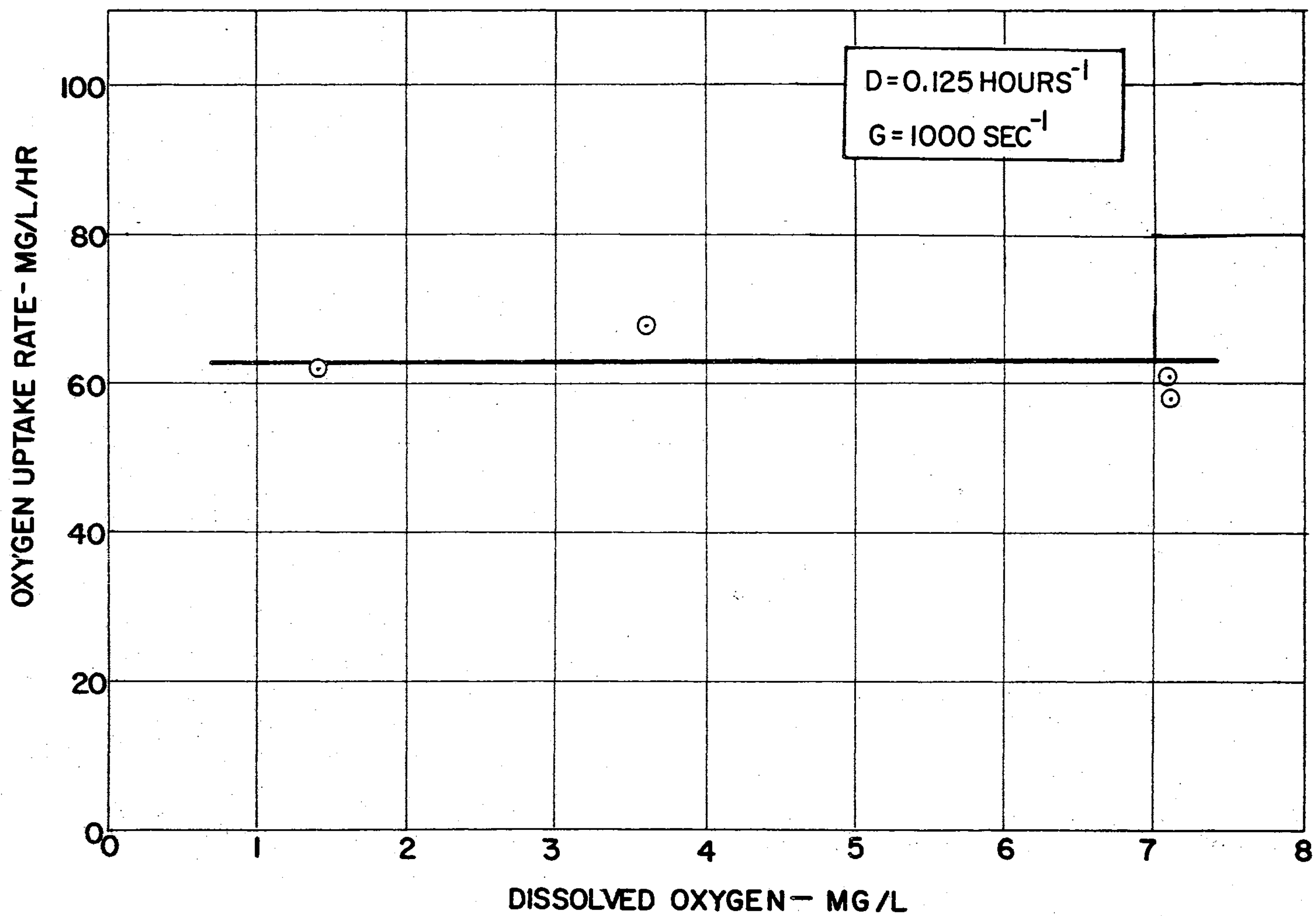


Figure 26 - Variation in Steady-State Oxygen Uptake Rate with Oxygen Tension during Growth of the Heterogeneous Population at a Velocity Gradient of 1000 Sec^{-1} and a Growth Rate of 0.125 Hrs^{-1}.

TABLE III

SUBSTRATE REMOVAL AND MATERIALS BALANCE FOR HETEROGENEOUS POPULATION AT VARYING DO

DO		COD			Solids		Oxygen Uptake Rate					
	Infl. mg/l	Eff. mg/l	mg/l	Con-sumed* mg/hr	mg/l	mg/hr	mg/l	mg/hr*	Total Substrate Accounted For mg/hr*	% Recovery	% Glucose Rem.	% COD Removed
7.1	970	41	929	403	401	184	61.1	214	398	99	99	96
7.3	1115	79	1036	450	353	162	58.2	204	366	81	98	93
3.6	980	85	895	388	346	159	69.7	244	403	104	97	91
1.4	991	101	890	386	350	161	62.4	219	380	98	97	90

*Expressed as Equivalent Glucose

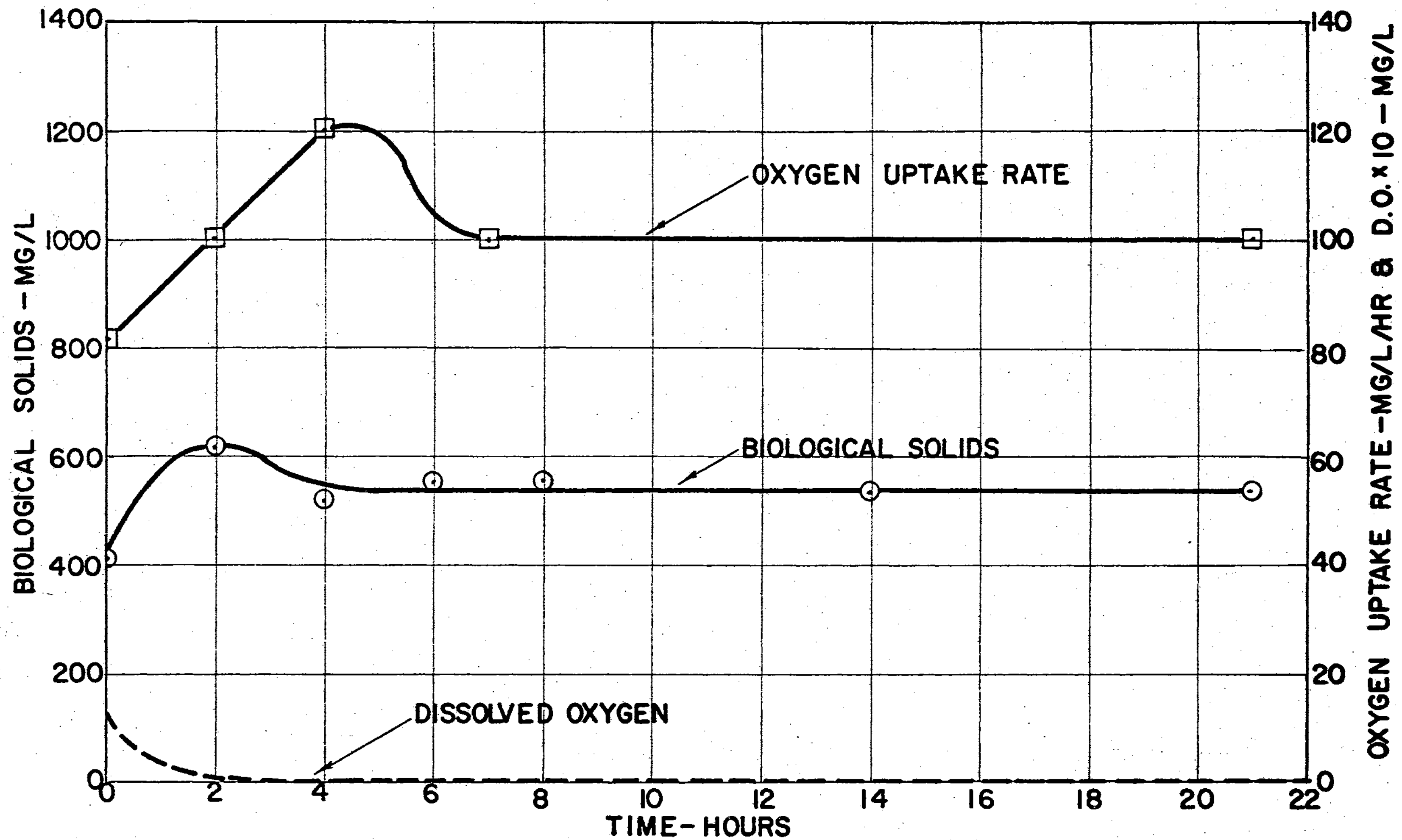


Figure 27 - Biological Solids, Oxygen Uptake Rate, and Dissolved Oxygen Response of the Heterogeneous Population to a Quantitative Shock Load at a Velocity Gradient of 300 Sec^{-1} and a Growth Rate of 0.200 Hrs^{-1}.

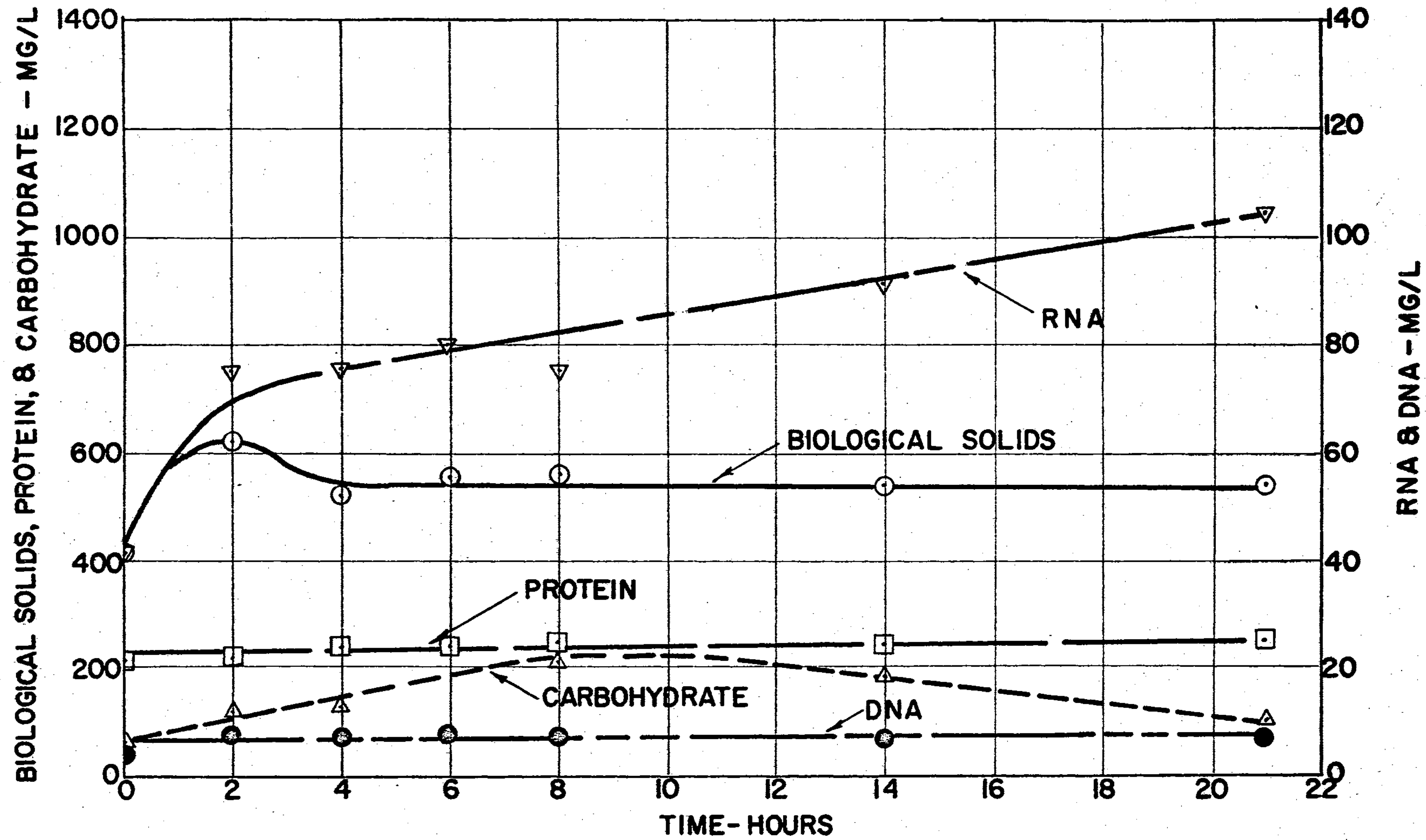


Figure 28 - Response of the Heterogeneous Population Cellular Components to a Quantitative Shock Load at a Velocity Gradient of 300 Sec^{-1} and a Growth Rate of 0.200 Hrs^{-1}.

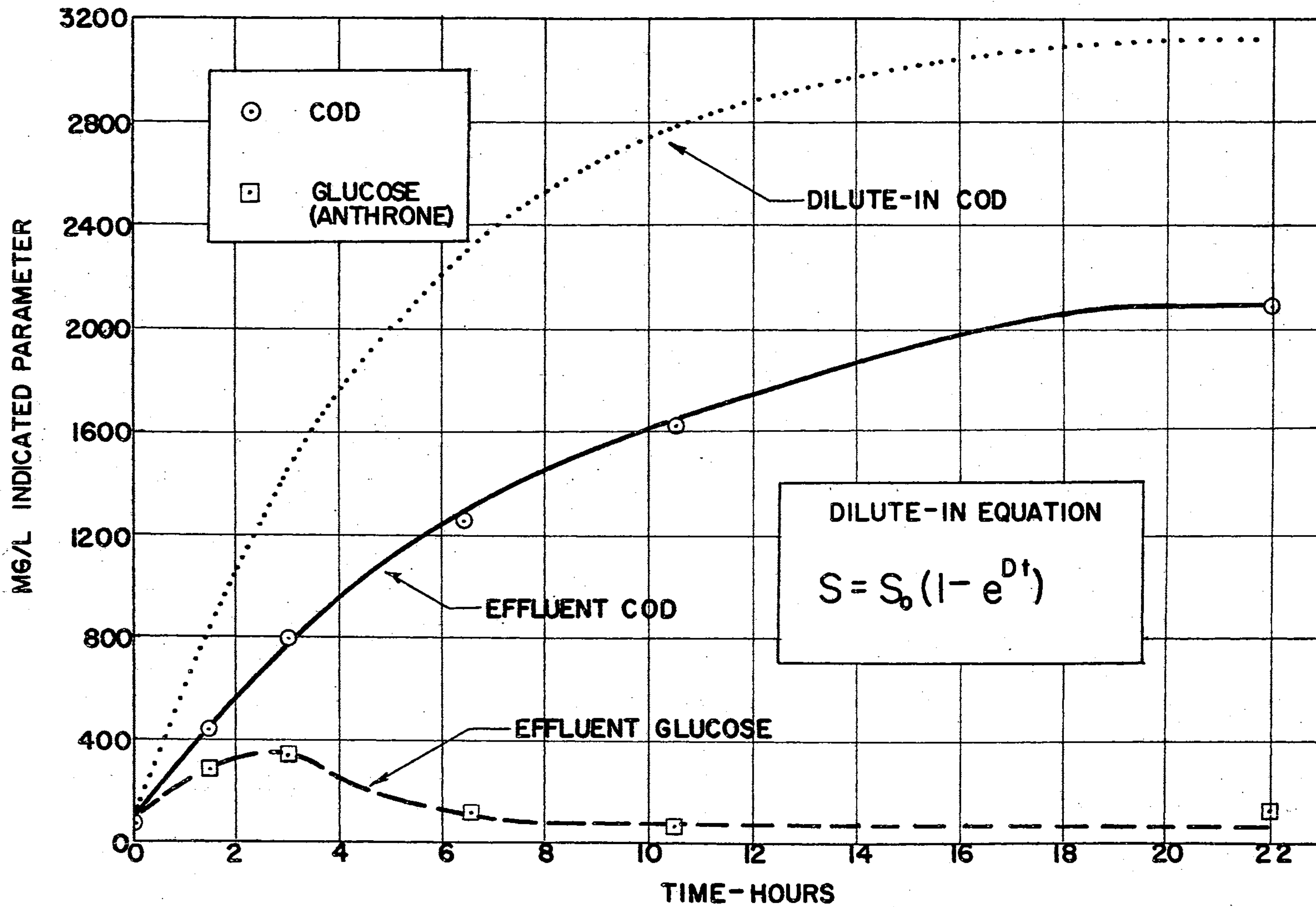


Figure 29. Substrate Response of the Heterogeneous Population to a Quantitative Shock Load at a Velocity Gradient of 300 Sec^{-1} and a Growth Rate of 0.200 Hrs^{-1}.

limitation. These parameters began a rapid increase immediately upon application of the change in the influent glucose concentration. The increase in biological solids concentration continued for approximately two hours until the oxygen demand increased to the point that the system became nearly devoid of dissolved oxygen. At this time, a reduction in the solids level occurred. After this slight retraction, the solids level achieved a new steady-state which was maintained throughout the remainder of the experiment. The behavior of the oxygen uptake rate was very similar to the biological solids response except that the initial increase continued for four hours after the imposition of the shock load. A decrease in the oxygen uptake rate then occurred and a new steady-state level was achieved after approximately six hours.

It is interesting to note that no significant change occurred in either the cellular protein or the DNA during the entire period of dilute-in of the substrate shock (Figure 28). The RNA level, on the other hand, increased rapidly during the first two hours followed by a retarded linear increase after the D. O. was exhausted. This increase in RNA is somewhat unexpected in light of the other cellular parameters and may have been caused by a change in the ecology of the system after onset of anaerobic conditions.

In Figure 29 the theoretical dilute-in curve of the glucose shock is shown along with the effluent COD and

glucose concentration observed. These curves show that while the response of the system in terms of glucose removal was quite successful in spite of the absence of significant amounts of dissolved oxygen, the COD removal efficiency was greatly reduced. Thus, the great majority of the organic material appearing in the effluent was in the form of metabolic intermediates and/or end products. In order to obtain some information on the nature of this COD, selected samples were subjected to vapor-phase chromatography. This analysis indicated that no volatile fatty acids were present. A trace of ethanol was detected by this method, but as its concentration was too low to allow quantification, it represented only a minor fraction of the COD present.

The dissolved oxygen responses to the same shock load at velocity gradients of 680, 1140, and 1750 sec^{-1} are shown in Figure 30. These data indicate that in all of these runs the reaeration coefficient of the system was sufficiently high to prevent the D. O. from dropping to a level at which an oxygen limitation might be expected.

Figure 31 is a plot of the solids level in the reactor for the same three runs. Although no significant difference existed between the solids development at these three energy levels, comparison of the data in this figure with Figure 27 shows that a drastic difference exists in the performance of oxygen-limited systems. The results of the protein determinations for these runs are shown in Figure 32.

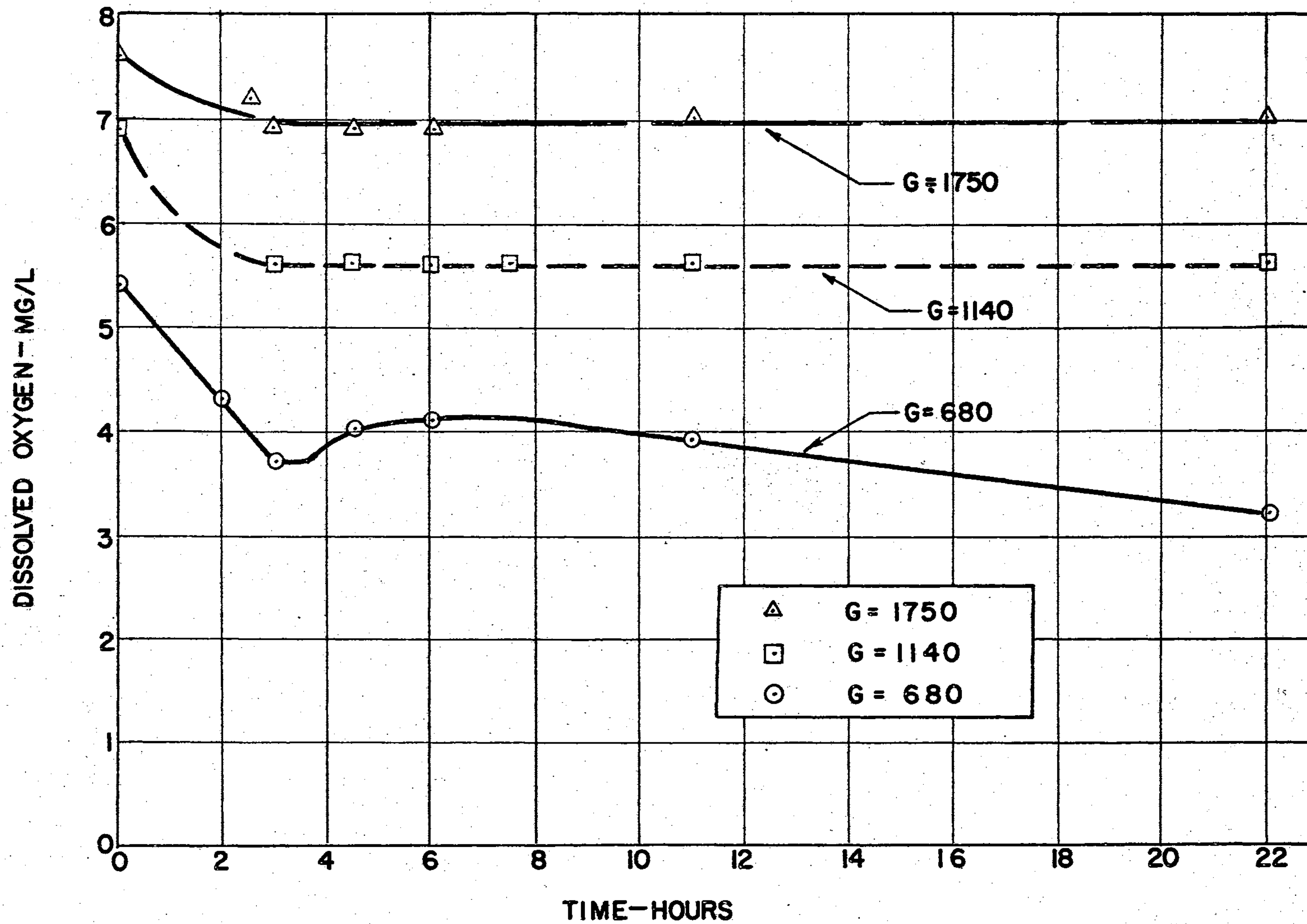


Figure 30 - Variation in the Oxygen Tension of the Completely Mixed System Containing the Heterogeneous Population during a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} at Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

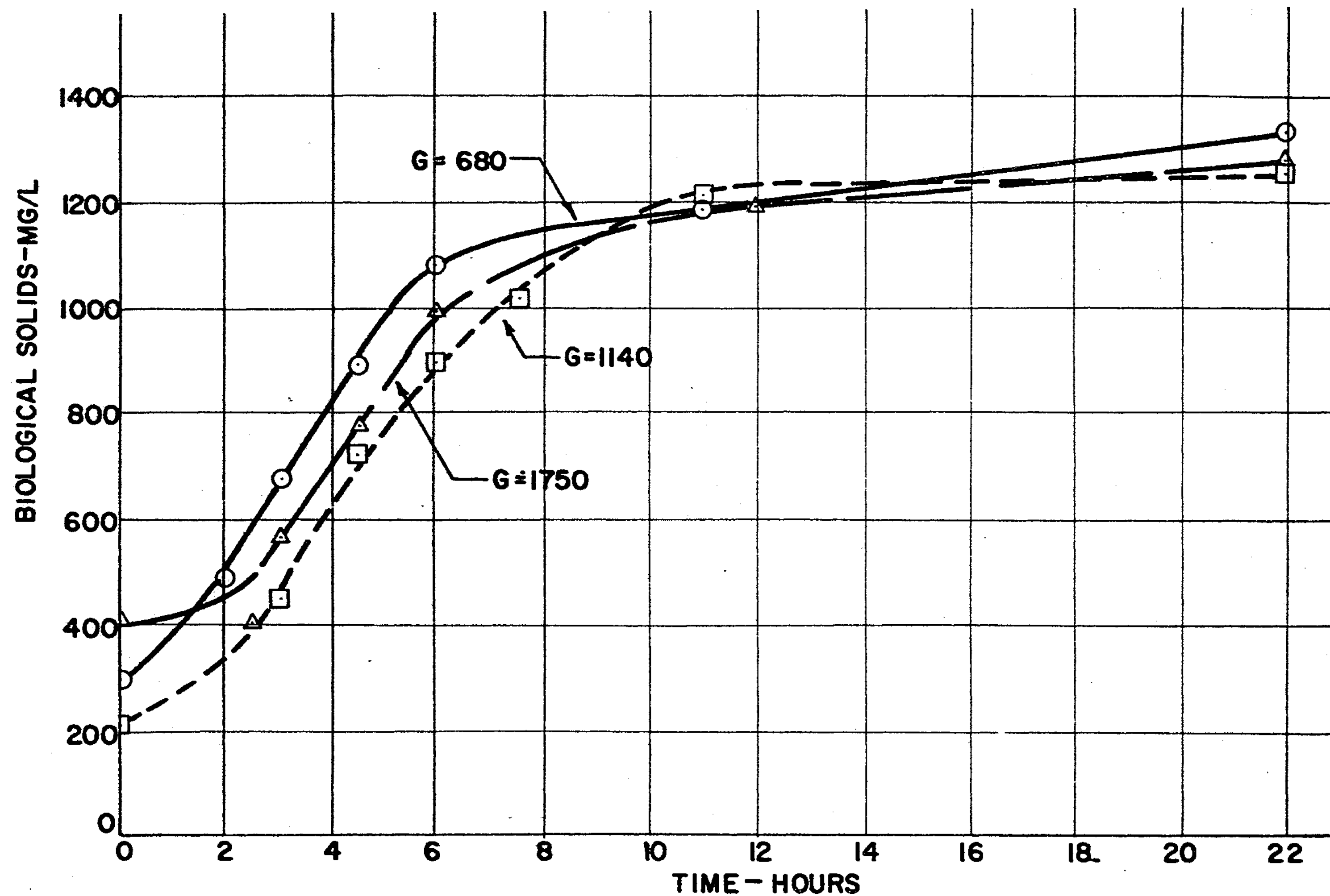


Figure 31 - Biological Solids Response of the Heterogeneous Population to a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} and Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

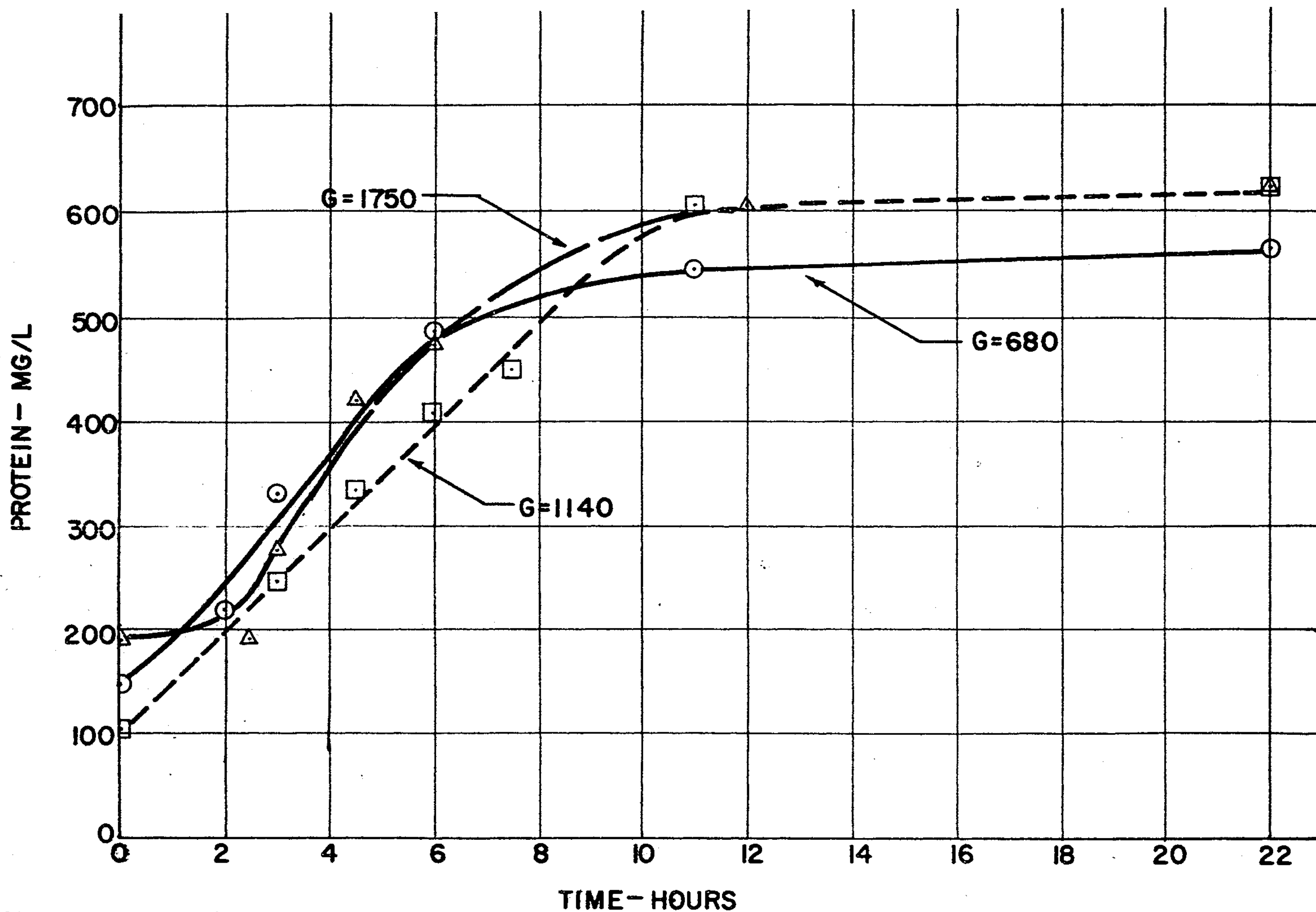


Figure 32 - Cellular Protein Response of the Heterogeneous Population to a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} and Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

These data again contrast strikingly with those shown in Figure 28, as the protein contained in the system increased by a factor of three during the course of the experiments.

The cellular carbohydrates and nucleic acid levels observed in the cells during the shock loads at all velocity gradients above 300 sec^{-1} are shown in Figures 33 and 34, respectively. The behavior of these parameters followed the same general pattern as did the biological solids and protein, i.e., approximately a three-fold increase in concentration occurred during the dilute-in period. One interesting observation concerning the response of DNA to the shock load is that a lag of approximately four hours was seen before any net increase in DNA occurred. This lag in DNA is probably analogous to the delay in DNA synthesis which occurs in batch growth during the lag phase. If so, it represents that period of time required for the population to initiate a change in growth rate in terms of viable count. This is not reflected in the biological solids data because the cells are able to incorporate considerable amounts of substrate during the lag phase without significant cell division.

Figure 35 represents the oxygen uptake rate observed during the shock loads. Again no differences between responses could be detected as the velocity gradient was varied from 680 to 1750 sec^{-1}.

The substrate responses of the shock load system at all velocity gradients above 300 sec^{-1} are shown in

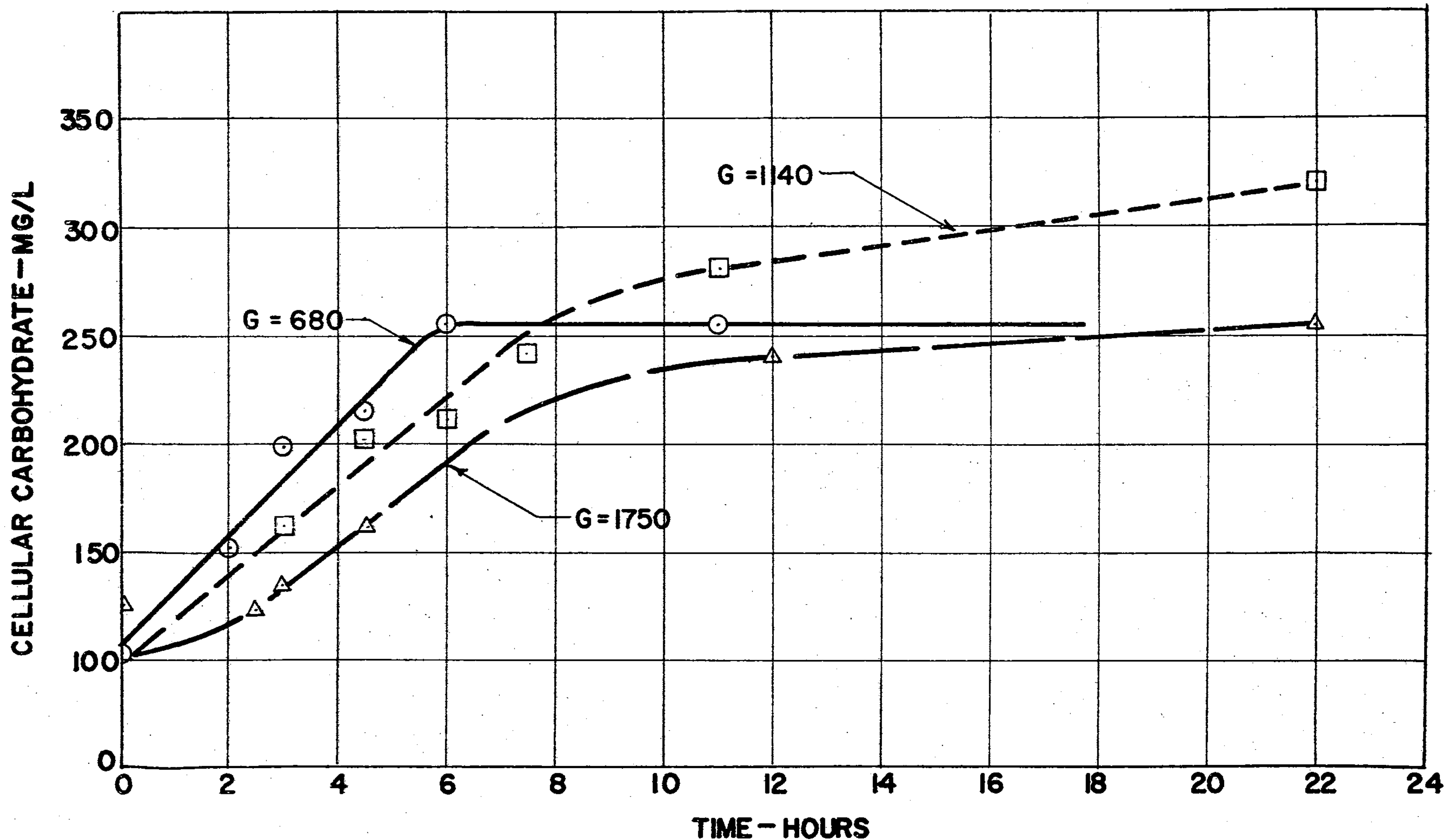


Figure 33 - Cellular Carbohydrate Response of the Heterogeneous Population to a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} and Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

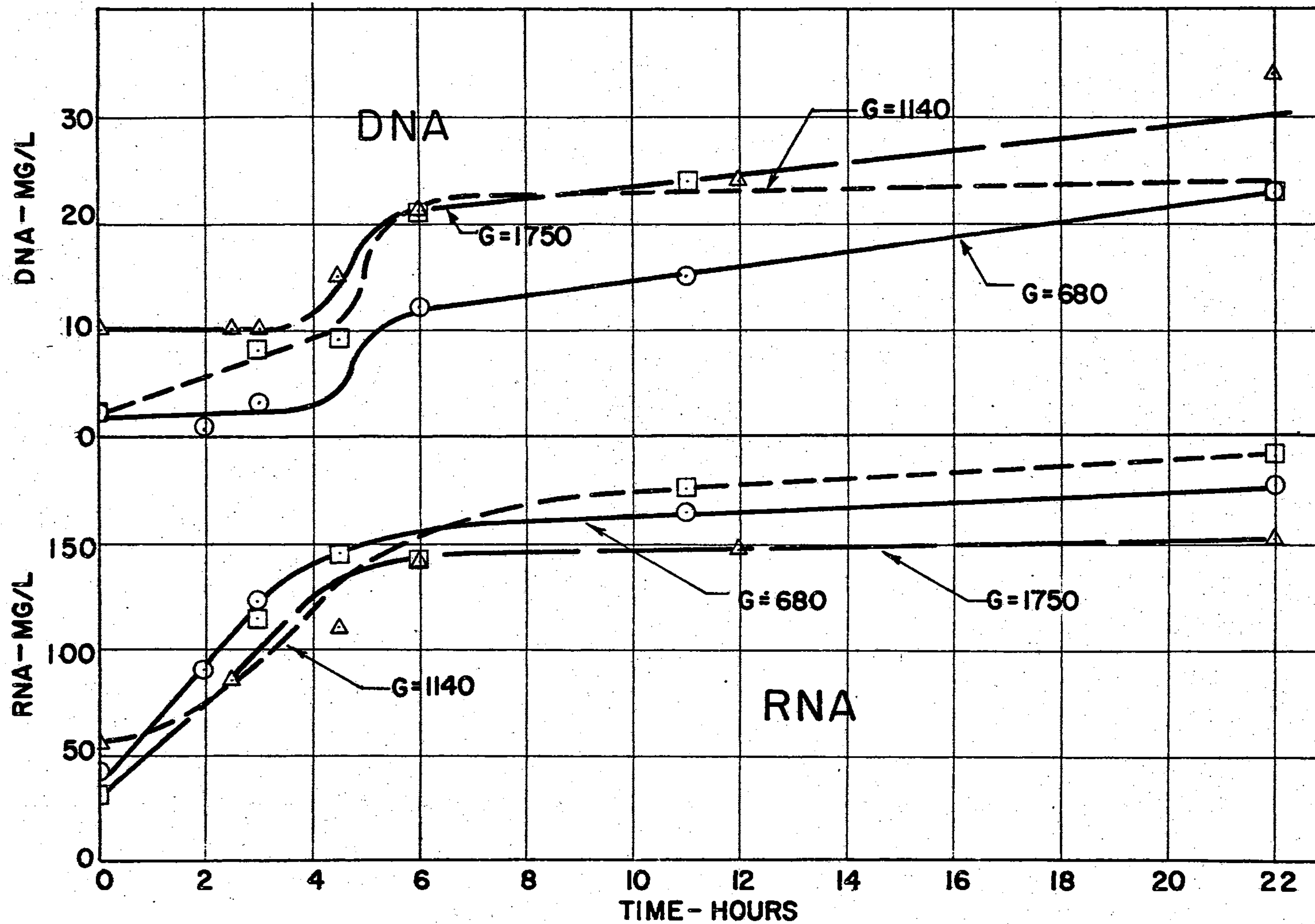


Figure 34 - Nucleic Acid Response of the Heterogeneous Population to a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} and Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

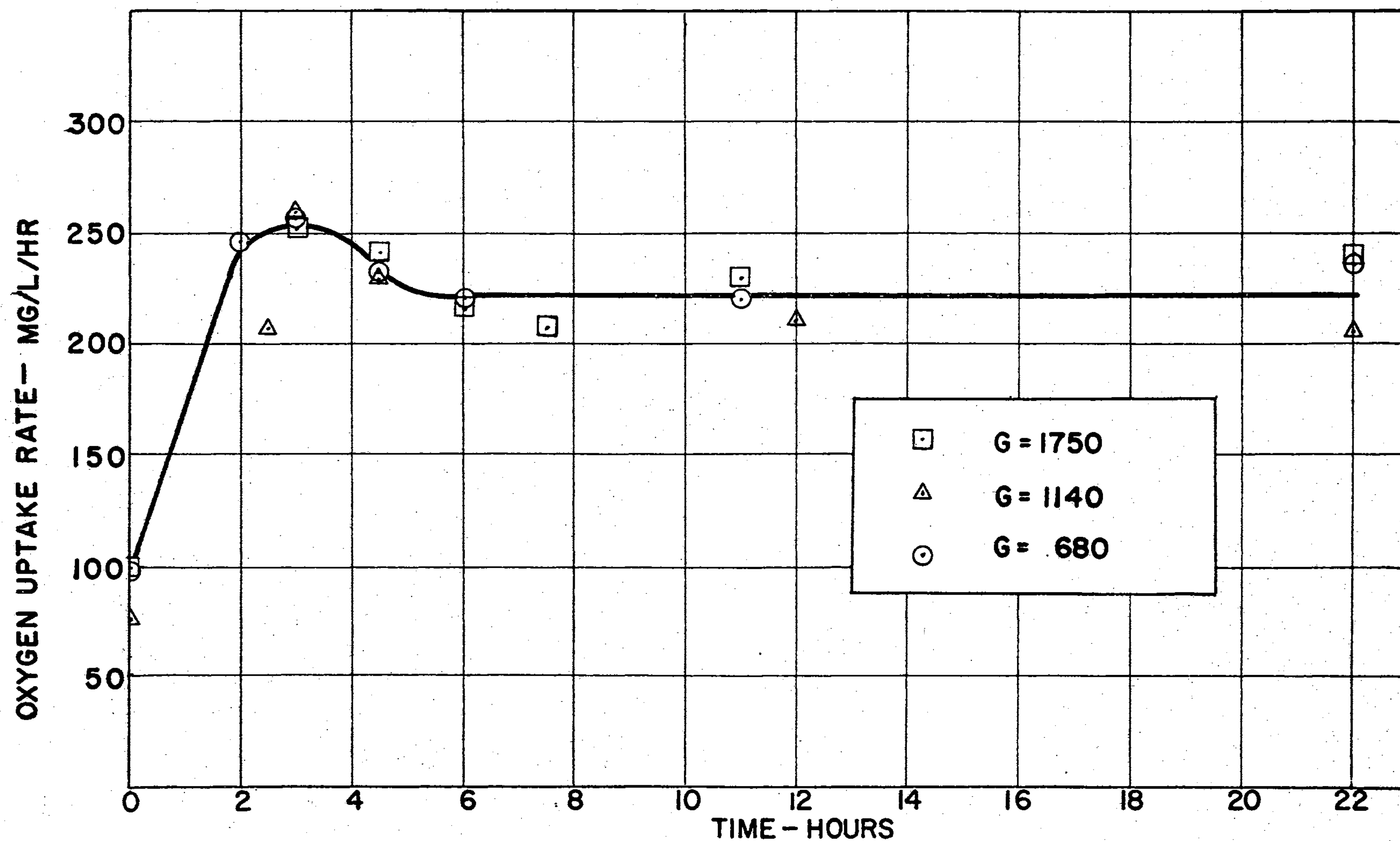


Figure 35 - Oxygen Uptake Response of the Heterogeneous Population to a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} and Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

Figure 36. Although no difference in the level of glucose (anthrone) was observed as a result of the amount of turbulence provided, the response in terms of COD removal may be seen to have been strongly influenced by the velocity gradient. Organic acid analysis by vapor-phase chromatography indicated that, in contrast to the run made at a velocity gradient of 300 sec^{-1}, approximately 80% of this non-carbohydrate COD was acetate.

Effect of Velocity Gradient on the Steady-State Behavior of Isolate SE-4

The biological solids and sludge composition data obtained from the runs performed with the pure culture isolate SE-4 at the various velocity gradients are shown in Figure 37. As was done with the heterogeneous population data, the yields of all of the cell components were calculated and are plotted versus the velocity gradient in Figure 38. The oxygen uptake rates observed at the various levels of mechanical energy input are shown in Figure 39. It may be seen from Figures 37, 38, and 39 that the behavior of the system closely paralleled that of the heterogeneous population at all velocity gradients below 1000 sec^{-1}. It should be noted that microscopic examination of this organism showed the existence of an extensive capsular layer at low velocity gradients. As the amount of agitation was increased, a reduction in the amount of the material was noted until at 1000 sec^{-1} the capsule was missing entirely. Above this energy level SE-4, unlike the

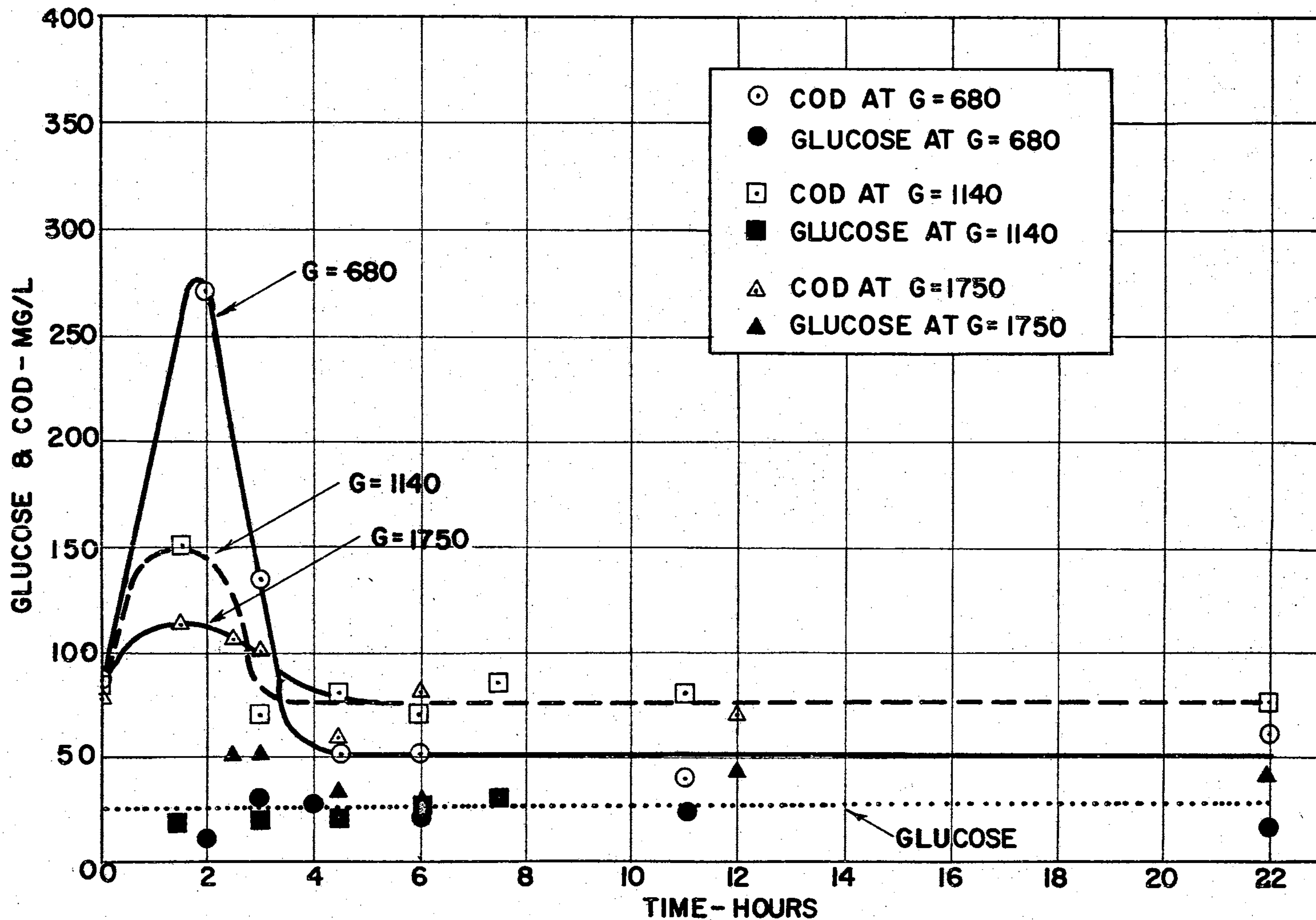


Figure 36 - Substrate Response of the Heterogeneous Population to a Quantitative Shock Load at a Growth Rate of 0.200 Hrs^{-1} and Velocity Gradients of 680, 1140, and 1750 Sec^{-1}.

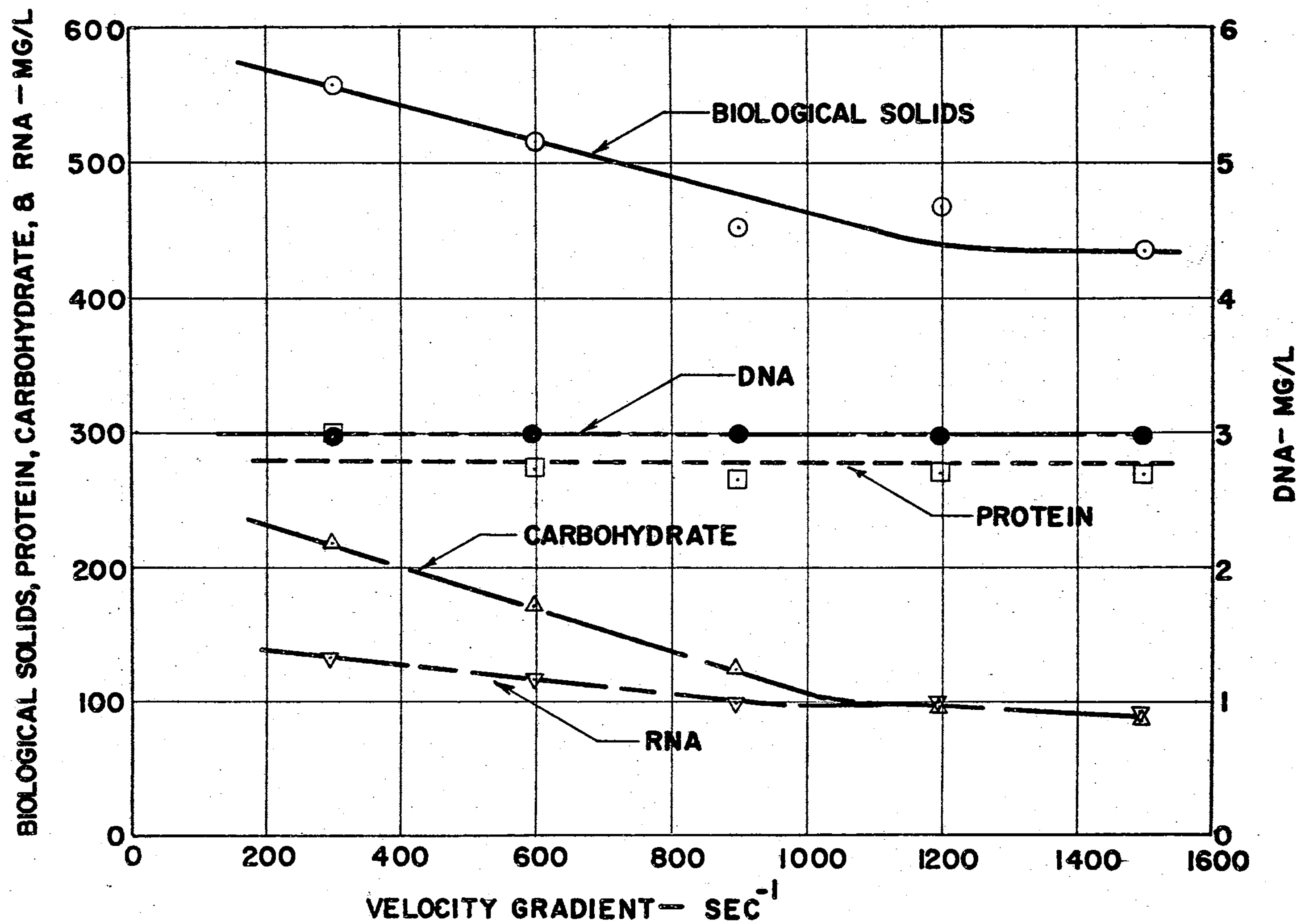


Figure 37 - Variation in the Steady-State Levels of Biological Solids and Cellular Components with Velocity Gradient during Growth of Isolate SE-4 at a Growth Rate of 0.200 Hrs^{-1}.

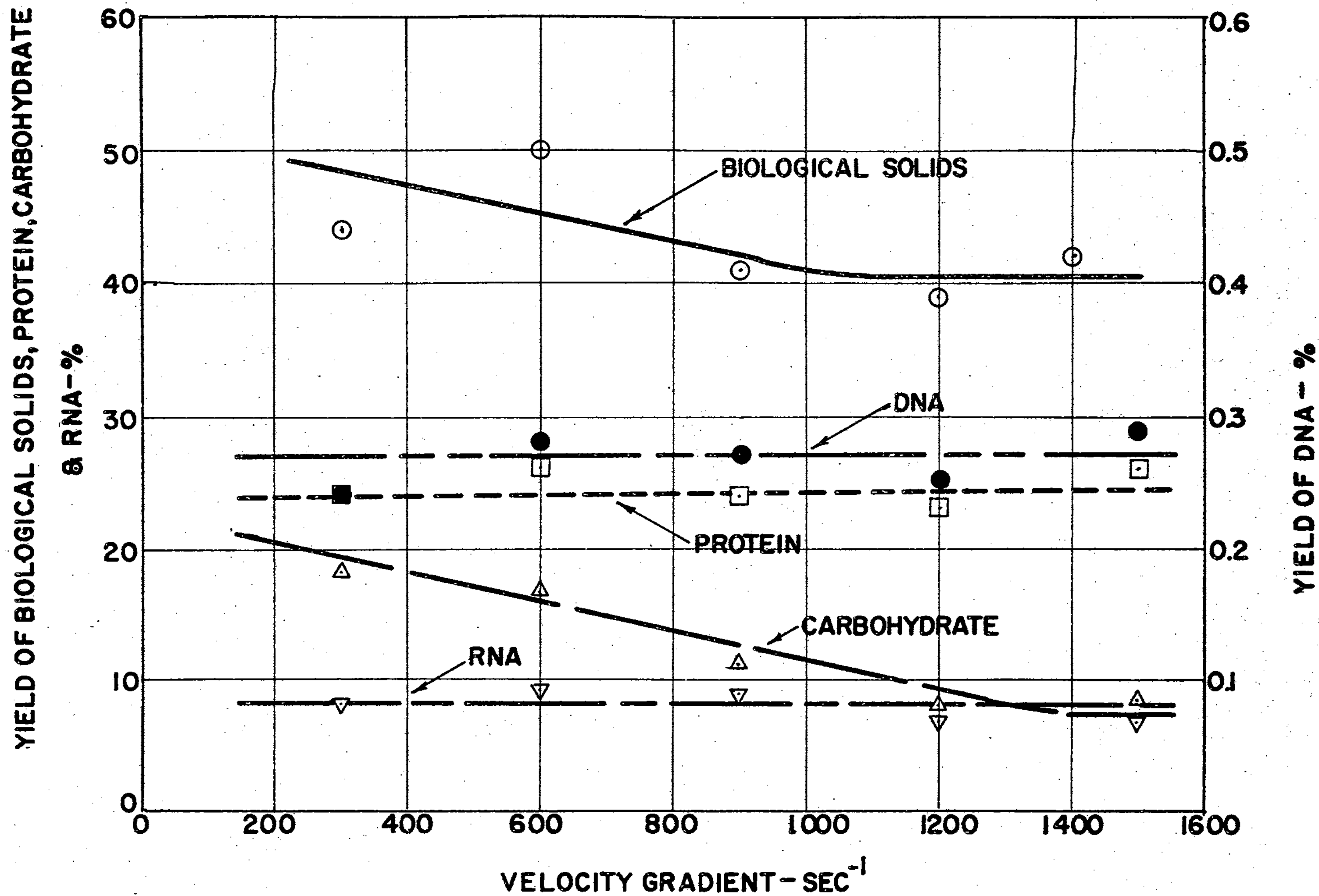


Figure 38 - Variation in the Steady-State Biological Solids and Cellular Component Yields with Velocity Gradient during Growth of Isolate SE-4 at a Growth Rate of 0.200 Hrs^{-1}.

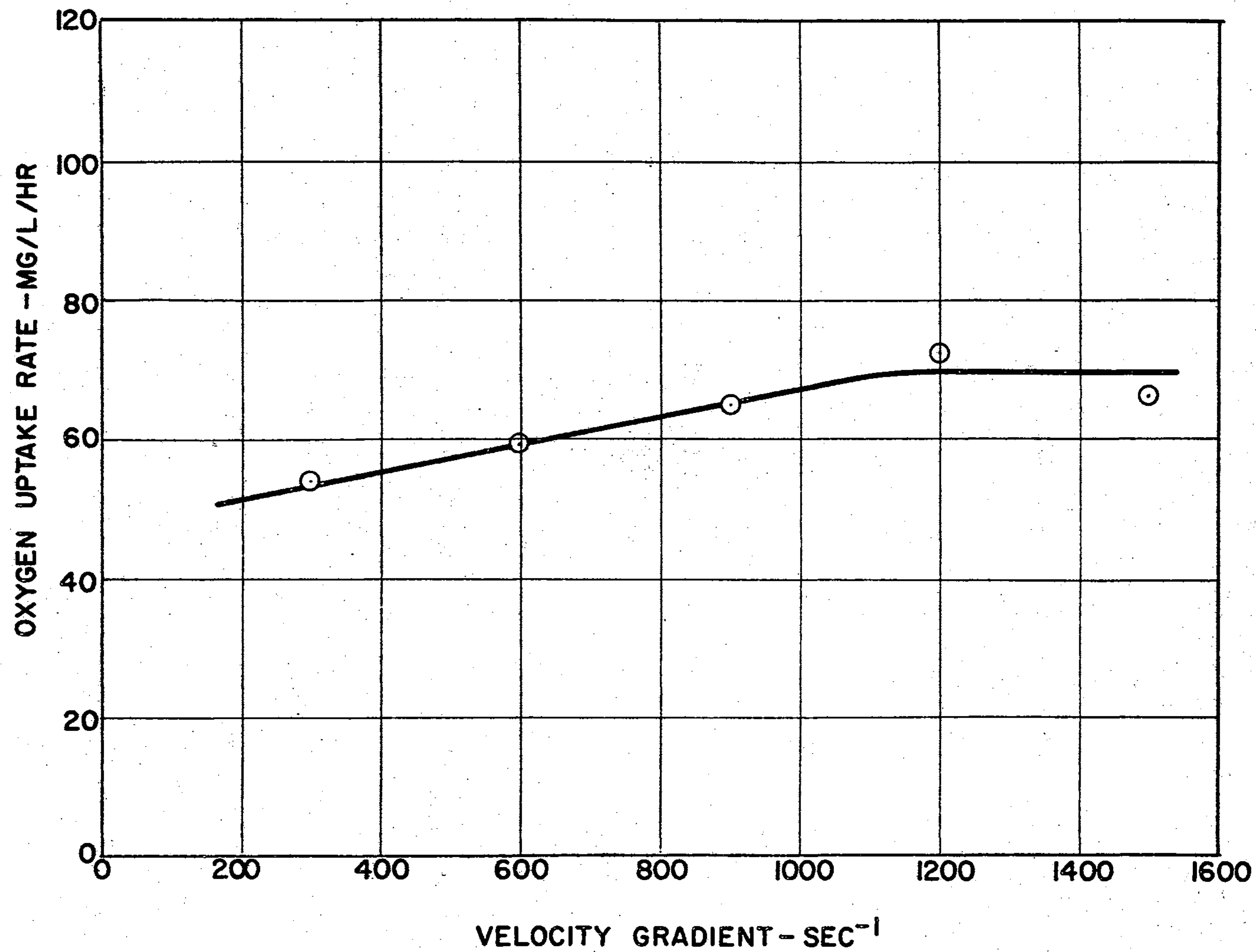


Figure 39 - Variation in the Steady-State Oxygen Uptake Rate with Velocity Gradient during Growth of Isolate SE-4 at a Growth Rate of 0.200 Hrs^{-1}.

heterogeneous population, showed a tendency for both the biological solids and carbohydrate yields to achieve and maintain a minimum value.

Table IV shows the substrate removal performance and materials balance data obtained for this system. Here again it may be seen that the reductions in solids yield were accomplished without any deterioration in effluent quality.

Also shown in Table IV are the average viable counts observed during steady-state growth at all of the velocity gradients employed. Although some scatter occurred in the viable count data, it may be concluded that the reduction in biological solids was not accomplished at the expense of the number of viable cells contained in the unit. The apparent correlation between the reduction in biological solids and carbohydrate yields and the disappearance of the capsular layer is quite interesting since it indicates that most of the loss of biological solids was probably accomplished at the expense of this material. Because studies of the heterogeneous population had shown that this system was capable of using the capsular material present during endogenous respiration, the question as to whether this material was not being produced at high velocity gradients or was being stripped off and reused was undecided. Since only an insignificant amount of carbohydrate was being released in the effluent in either system, it was obvious that if capsular material was being produced at high

TABLE IV

MATERIALS BALANCE, SUBSTRATE REMOVAL AND VIABLE COUNT DATA--ISOLATE SE-4

G	Reactor Volume	Feed Rate	COD Infl mg/l	COD Eff. mg/l	COD mg/l	COD Consumed* mg/hr	Solids mg/l	Solids mg/hr	Oxygen Uptake Rate mg/l/hr	Oxygen Uptake Rate mg/hr*	Total S Accounted For	% Rec.	% Gluc Rem.	% COD Rem.	Viable Count $x10^{-8}$
300	3.91	0.62	1302	47	1255	734	557	345	53.9	199	543	74	99	96	15.6
600	3.82	0.61	1089	50	1039	598	517	315	59.5	214	529	90	99	98	17.5
900	3.76	0.60	1160	43	1117	632	454	272	64.9	230	502	79	99	93	17.7
1200	3.69	0.59	1239	46	1193	664	469	277	72.5	252	529	80	99	98	15.3
1500	3.64	0.58	1097	108	1044	571	436	253	66.2	227	480	84	99	90	14.1

*Expressed as Equivalent Glucose

agitation rates, it was being reused by the cells as a carbon source. Therefore, a study was conducted with the pure culture to determine if it was capable of using its own capsular material during endogenous respiration. A series of sterile shaker flasks containing the standard minimal medium was inoculated with SE-4 and allowed to endogenate for seven days after maximum growth had been attained. Flasks were removed periodically for biological solids, cellular carbohydrate, and filtrate carbohydrate analyses. In addition, India ink slides were prepared to determine if capsular material was present. Throughout the period of observation, no reduction in the amount of capsule present as determined by either microscopic examination or by carbohydrate analysis was detected. From this it may be concluded that at high velocity gradients the capsular material was not produced by this organism. The excess substrate which was not then used for the production of slime provided an energy source for the increased oxygen uptake. This conclusion is probably valid for the heterogeneous population as well, since there is no reason to assume that the microbiota contained would be unable to consume the material at high but not at low velocity gradients.

Effect of Velocity Gradient on the Steady-State Behavior of Escherichia coli K-12

The concentrations of COD and glucose (anthrone) appearing in the effluent during steady-state operation

with *E. coli* at the various velocity gradients examined are shown in Figure 40. Because of the departure of these data from those obtained with other systems, three additional runs were made with this organism after a delay of approximately two months. These latter runs are illustrated in all of the figures concerning this organism by solid circles and squares, as opposed to open circles and squares for the original data points. It may be seen from Figure 40 that although the glucose removal of the unit remained quite high up to a velocity gradient of 900 sec^{-1}, the response in terms of COD was disrupted. At velocity gradients above 900 sec^{-1}, increased amounts of glucose also began to appear in the effluent. Figure 41 represents the difference between the total effluent COD and the glucose concentration expressed as equivalent COD. From this figure it may be seen that as the velocity gradient was increased, *E. coli* was unable to make efficient use of the substrate available and as a result excreted large amounts of intermediates into the medium. As the velocity gradient was further increased, the amount of these materials decreased somewhat with the additional COD being largely in the form of unmetabolized glucose. In order to determine the nature of this material, selected samples of effluent filtrate were subjected to analysis by vapor-phase chromatography. These analyses showed that 85% of the non-glucose COD in the effluent was in the form of acetate.

The oxygen uptake rate of this organism as a function

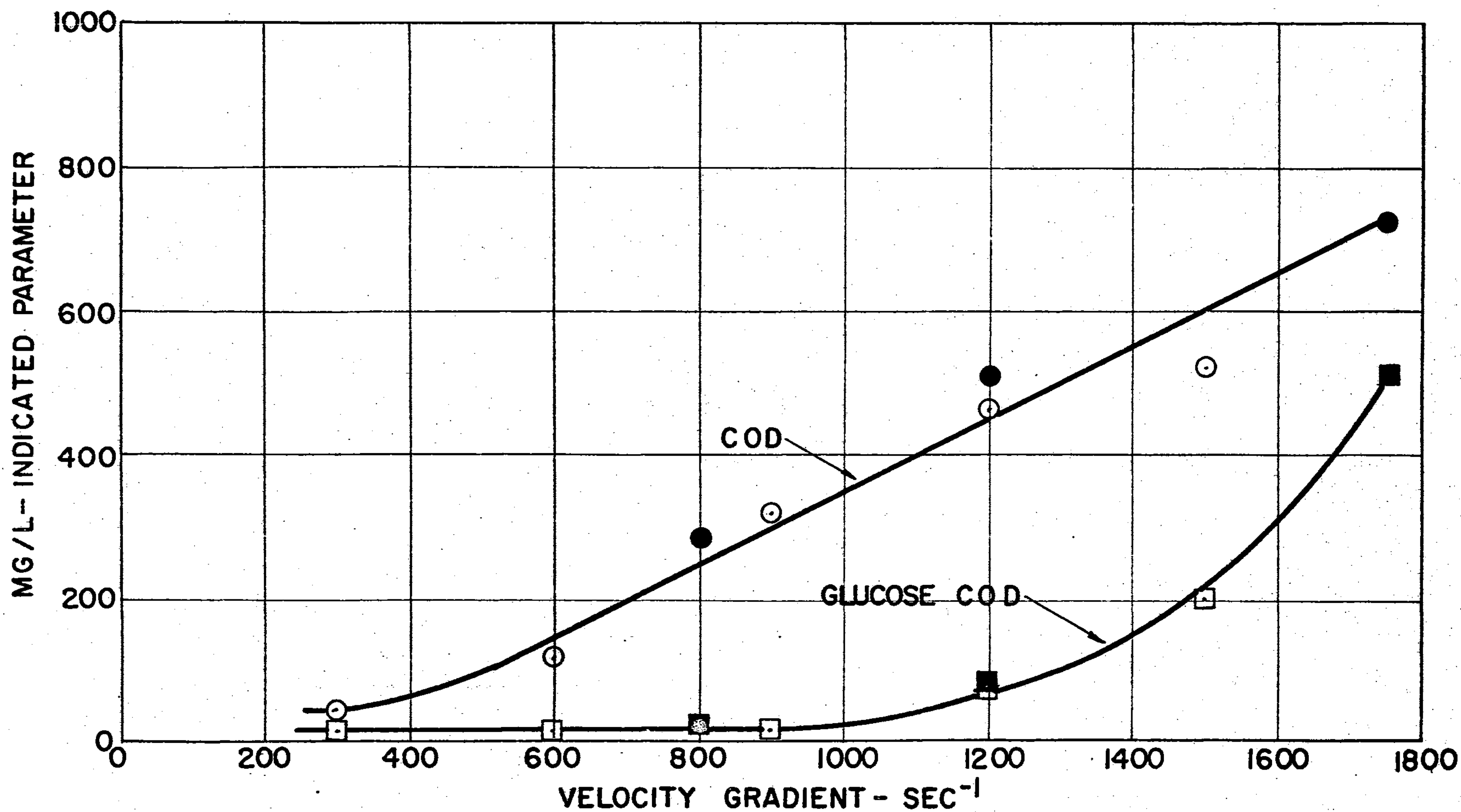


Figure 40 - Variation in the Steady State Substrate Levels with Velocity Gradient during Growth of E. coli K-12 at a Growth Rate of 0.200 Hrs^{-1}.

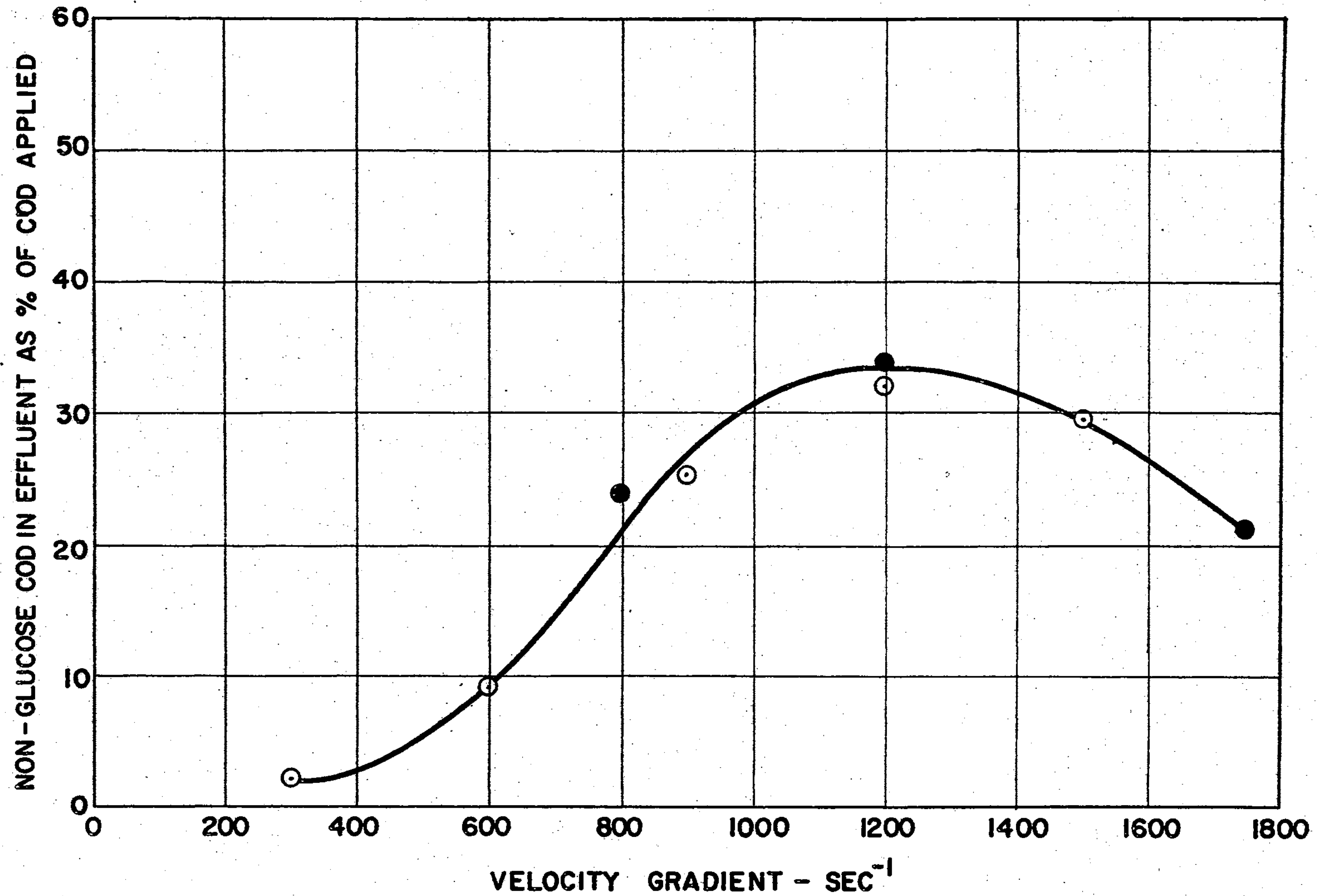


Figure 41 - Non-Glucose COD appearing in the Effluent during the Growth of E. coli K-12 at a Growth Rate of 0.200 Hrs^{-1} and Various Velocity Gradients.

of the velocity gradient (Figure 42) also shows a radical departure from that observed in the other systems. Here, the oxygen uptake rate above a velocity gradient of 800 sec^{-1} appeared to be an inverse function of the amount of agitation.

The results of the cell composition analyses performed at the various velocity gradients are shown in Figure 43. The yields calculated from these data are given in Figure 44. Unlike the other parameters, these data may be seen to be roughly equivalent to the behavior of the other systems.

The materials balances and viable count data for *E. coli* are shown in Table V. The viable counts observed at the various levels of turbulence illustrate another very interesting point about the behavior of *E. coli*. No change in the number of cells contained in the reactor occurred as the velocity gradient was increased from 300 to 900 sec^{-1}. Further increases in energy input, however, resulted in a sharp decline in the number of viable cells. The occurrence of this reduction correlated very well with the appearance in the effluent of increased amounts of glucose.

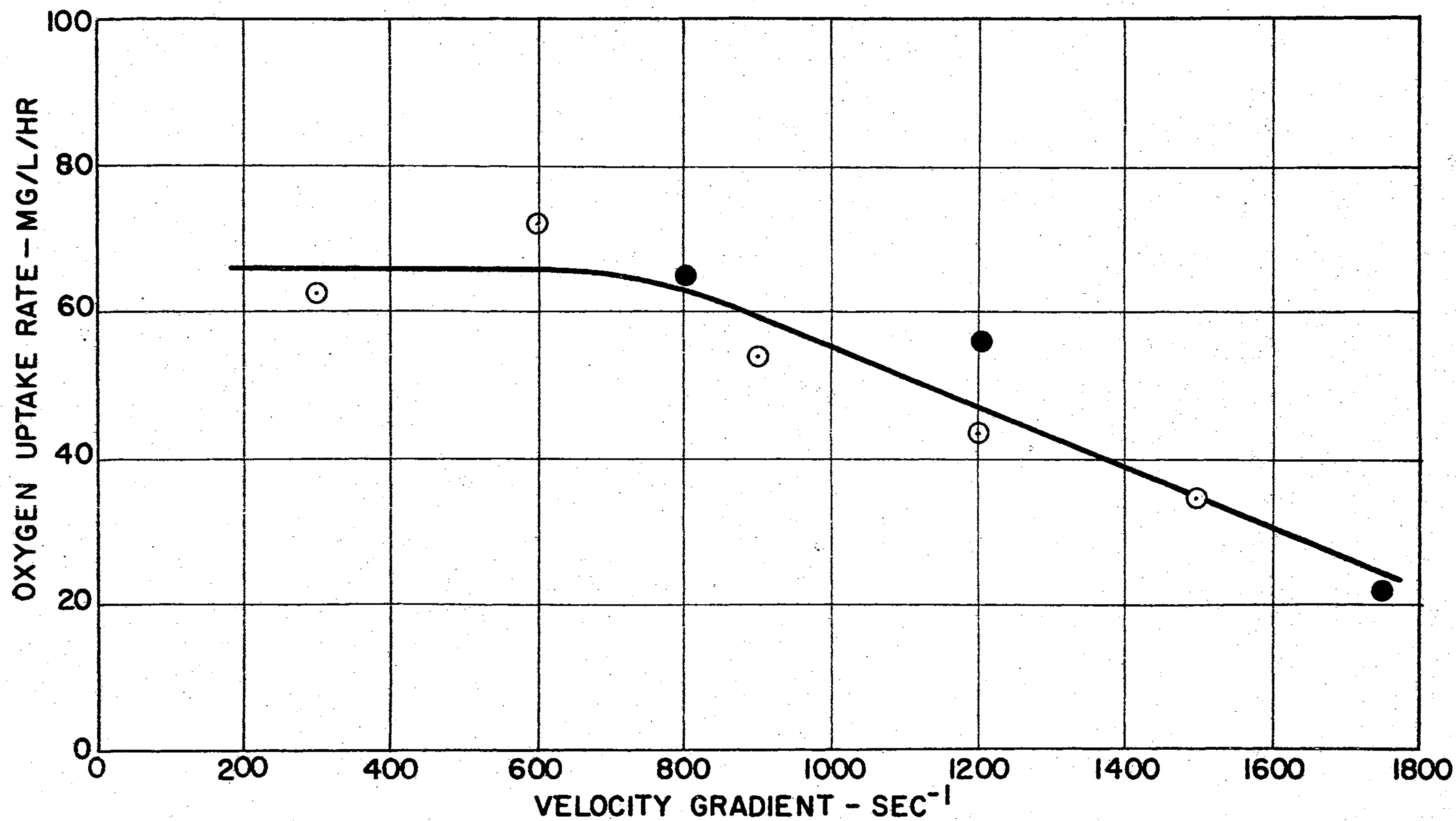


Figure 42 - Variation in the Steady State Oxygen Uptake Rate with Velocity Gradient during Growth of E. coli K-12 at a Growth Rate of 0.200 Hrs^{-1}.

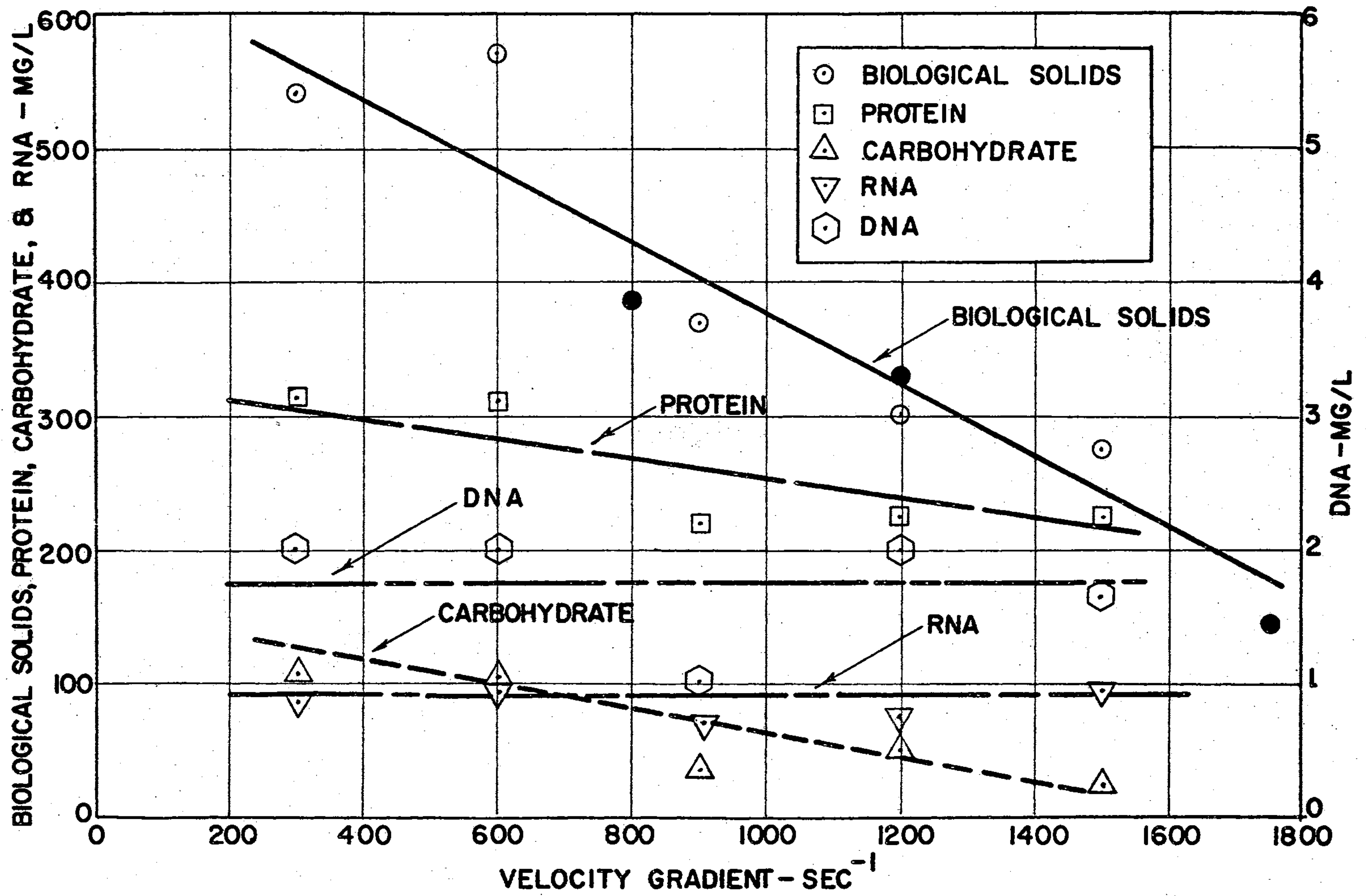


Figure 43 - Variation in the Steady-State Levels of Biological Solids and Cellular Components with Velocity Gradient during Growth of *E. coli* K-12 at a Growth Rate of 0.200 Hrs^{-1}.

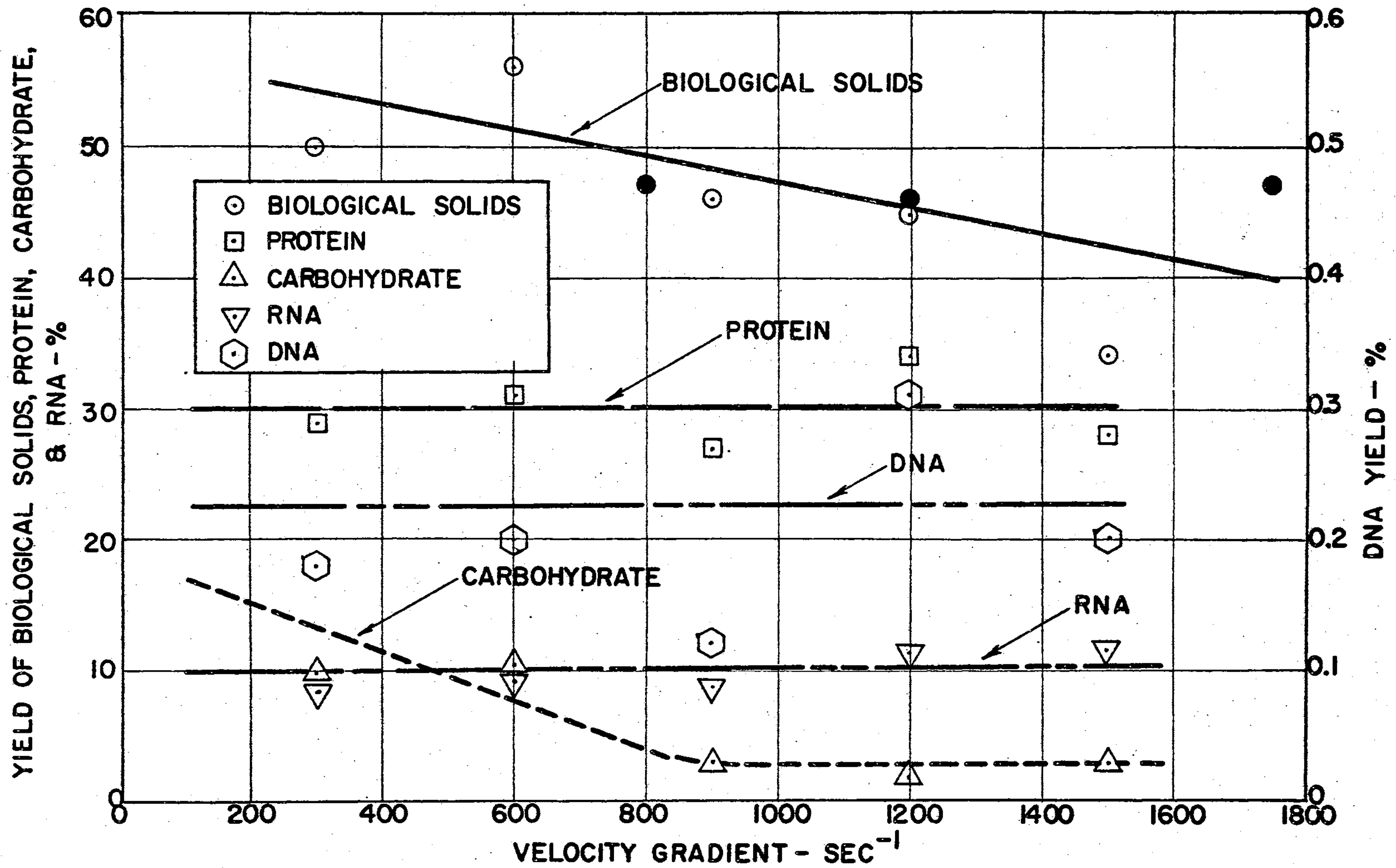


Figure 44 - Variation in the Steady-State Biological Solids and Cellular Component Yields with Velocity Gradient during Growth of E. coli K-12 at a Growth Rate of 0.200 Hrs^{-1}.

TABLE V

SUBSTRATE REMOVAL, MATERIALS BALANCES AND VIABLE COUNT DATA FOR *E. coli* K-12 AT VARIABLE VELOCITY GRADIENT

G	Reactor Volume L	Feed Rate L/hr	COD				Solids		Oxygen Uptake Rate		Total S* Recovered mg/hr	% S Rec.	Viable Count Data $\times 10^{-8}$
			Influent mg/l	Effluent mg/l	Δ mg/l	Consumed* mg/hr	Effluent mg/l	Produced mg/hr	mg/l/hr	mg/hr*			
300	3.91	0.78	1132	47	1085	798	540	421	62.4	230	651	82	14.0
600	3.82	0.78	1130	118	1012	775	570	445	71.7	258	703	91	12.8
800	3.78	0.76	1110	287	823	590	386	293	65.0	232	525	89	-
900	3.76	0.75	1128	324	804	569	370	276	53.7	190	466	58	11.6
1200	3.69	0.74	1112	465	647	452	300	222	43.4	151	373	83	12.6
1200	3.69	0.74	1255	512	743	519	334	247	56.0	195	442	85	-
1500	3.64	0.73	1325	524	801	552	275	201	34.4	118	319	58	8.5
1750	3.60	0.72	1026	728	298	202	141	102	20.5	70	172	58	-

*Expressed as equivalent glucose

CHAPTER VI

ANALYSIS OF RESULTS

In any study of the behavior of a heterogeneous bacterial population, consideration must be given to the inherent variability of the system. Rao and Gaudy (53) operated a batch activated sludge unit for an extended period of time and found that the biological solids yield varied by as much as 34% even though the experimental conditions were carefully controlled to maintain identical operating conditions. This variation in sludge yield was attributed to changes in microbial predominance.

Cassel, Sulzer, and Lamb (54) followed the population variance of a completely mixed, continuous flow bacterial system originating from a sewage seed and found that the population contained in their reactor followed a cyclic variation. In light of these studies it is apparent that considerable variation can occur in systems of the type under consideration here which are not controlled by any known external pressure, i.e., operational parameter. This being the case, it seems desirable that conclusions drawn on data from natural populations be based on statistically derived trends. In the present study it was decided that the significance of the variations observed in the experi-

mental data could best be determined through the use of three statistical methods: correlation analysis, regression, and analysis of variance.

Regression analysis provides a method by which an equation of best fit for a given set of data may be obtained. The technique consists simply of choosing a mathematical model which appears to fit the data adequately, expression of this model in linear form, and selection of the proper slope and intercept of the straight line form in such a manner that the summation of the variance of each point from the model is minimized. The technique does have considerable weakness in that it provides no means by which the investigator's judgement in selection of the initial model may be tested. In addition, least squares analysis does not provide a means of determining the probable significance of the expression obtained.

Fortunately, these deficiencies can be overcome through the use of analysis of variance. This technique provides a means by which the variability of the experimental data may be separated into components of random variation and model error. Analysis of variance also provides a means by which the significance of a least squares line of best fit may be measured. This is accomplished by comparing the slope and/or intercept of the linear form of the least squares equation along with their associated variances to a pre-selected value. Thus, the significance of the difference between a derived least squares slope and

a line of zero slope may be determined, giving an indication of whether or not a relationship between the dependent and independent variables actually exists. Alternately, a least squares slope may be compared with a theoretically predicted value to determine if departure from expected behavior is real or caused only by experimental error.

Application of the above techniques to the data obtained from the studies with heterogeneous populations should permit determination of the reality of the relationships observed and may also provide clues to the operative mechanism(s) by indicating the nature of the variation of the steady-state parameters with velocity gradient. All statistical analyses reported in this chapter were performed according to the methods outlined by Ostle (55).

Examination of the data obtained from the heterogeneous system during operation at varying velocity gradients (Figures 9-17) shows that of all the cellular parameters examined, only the biological solids and cellular carbohydrate yields demonstrated large, consistent trends. These two parameters showed a decreasing trend as the velocity gradient was increased from 300 to 1000 sec^{-1}. Above this value, there appeared to be a break in the biological solids-velocity gradient relationship. For example, at a detention time of five hours the biological solids yield at 1000 sec^{-1} was in the neighborhood of 40%, whereas at 1200 sec^{-1} a yield of 52% was observed. The cellular carbohydrate data, on the other hand, appeared to

decrease in a fairly regular manner with velocity gradient up to 1000 sec^{-1}, and to increase thereafter. Examination of the data obtained from the pure culture isolate SE-4 showed that no such break in the relationship between velocity gradient and the various parameters occurred in this system. Thus, the possibility that more than one factor was in operation in the heterogeneous population had to be considered. In any case, it is apparent that considerable difficulty would be encountered in an attempt to fit a single model to all of the data obtained. On this basis, the biological solids and cellular composition data were divided into two groups for statistical analysis. All data obtained at velocity gradients equal to or less than 1000 sec^{-1} were considered in the first group, while the second group consisted of all data obtained at velocity gradients above 1000 sec^{-1}.

In order to justify the above division of the data, it was considered necessary to establish that more meaningful relationships would be obtained by grouping the data in this fashion instead of treating it as a single body. Such justification was established through the use of correlation analysis. Table VI shows the correlation coefficients of the data obtained at each detention time, $\bar{t}$, when grouped as described above and when fitted to a single linear model. Also shown in Table VI are the correlation coefficients obtained by grouping all of the growth rates together. Although in some cases the

TABLE VI

LEAST SQUARES PARAMETERS AND CORRELATION COEFFICIENTS FOR RELATIONSHIP BETWEEN BIOLOGICAL SOLIDS YIELD AND VELOCITY GRADIENT - HETEROGENEOUS POPULATION

Correlation Coefficient			Intercept*		Slope**		Student's -T	
$\bar{t}$	G<1000	All Data	b_o	$S^2_{b_o}$	b_1	$S^2_{b_1}$	T'_{b_o}	T'_{b_1}
5	0.51	0.36	45.7	69.17	-1.09	1.91	-0.3	0.60
6½	0.68	0.09	49.7	180.7	-1.17	4.31	0.11	0.36
8	0.98	0.79	47.5	298.7	-2.25	5.18	0.02	0.15
12	0.94	0.02	46.9	639.8	-2.70	15.2	-0.05	0.20
Composite	0.65	0.05	48.2	-	-1.91	-	-	-

*b_o - Least Squares Intercept

$S^2_{b_o}$ - Variance of Intercept

**b_1 - Least Squares Slope

$S^2_{b_1}$ - Variance of Slope

correlation coefficients obtained were somewhat lower than is desirable, it is clear that division of the data provides a much better fit to a simple linear model.

In order to determine if a significant difference existed in the relationships between velocity gradient and biological solids yield at the various growth rates examined, the least squares line of best fit, assuming a linear model, was calculated for the data obtained at each detention time. The slope and intercept of the resulting least squares equations are shown in Table VI along with their associated variances. Also shown in Table VI are the slope and intercept of the composite line obtained by assuming that growth rate has no effect on the relationship between velocity gradient and biological solids yield. Since the differences between the least squares slope and the composite slope divided by the square root of the variance should be distributed according to Student's T, the significance of the difference between the slopes obtained at each growth rate and the composite slope may be determined. The last two columns in Table VI show the calculated values of these test statistics. Comparison of the T' values with a cumulative T' distribution table (55) indicates that none of the least squares equations obtained vary significantly from the composite equation. Also, as might be expected from kinetic considerations, calculation of the same test statistics for the intercepts also shows that no significant difference exists between the various growth rates.

It was also necessary to determine whether the relationship between velocity gradient and biological solids yield exhibited physical reality and whether the linear model chosen was appropriate. The first aspect, physical reality of the relationship, may be examined by preparing an analysis of variance such as that shown in Table VII and calculating the ratio of the variance contributed by the slope to the residual variance. This value is distributed according to an F-distribution and the significance of the slope of the line may be estimated by comparing the F-ratio to unity. For the biological solids yield versus velocity gradient data, the F-ratio is sufficiently large (107) to demonstrate significance at the 99% confidence level.

The adequacy of the linear model chosen was also determined from the values shown in Table VII. This was accomplished by computing the F-ratio of the term for lack of fit to the term for experimental error. It should be noted that experimental error, as used here, implies only the failure of the system to give identical behavior under identical conditions and therefore includes both population and analytical variation. Determination of the F-ratio for these data indicated that no real lack of fit exists in the linear model at the 99% confidence level. Therefore, the biological solids yield obtained at all velocity gradients below $G = 1000 \text{ sec}^{-1}$ may best be represented by the composite relationship of Figure 45.

TABLE VII

ANALYSIS OF VARIANCE FOR BIOLOGICAL SOLIDS YIELD DATA FROM HETEROGENEOUS POPULATION - G $\leq$ 1000 sec^{-1}

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	15	20,622	1,375	
Due to Intercept	1	19,874	19,874	
Due to Slope - Given Intercept	1	314	314	9.4*
Residual	13	434	33	
Experimental Error	8	332	42	
Lack of Fit	5	102	20	0.5**

*Magnitude indicates a significant difference between the slope of the least squares line and a line of zero slope (P = 0.99%)

**Magnitude indicates that no significant deviation exists from the linear model (P = 0.75%)

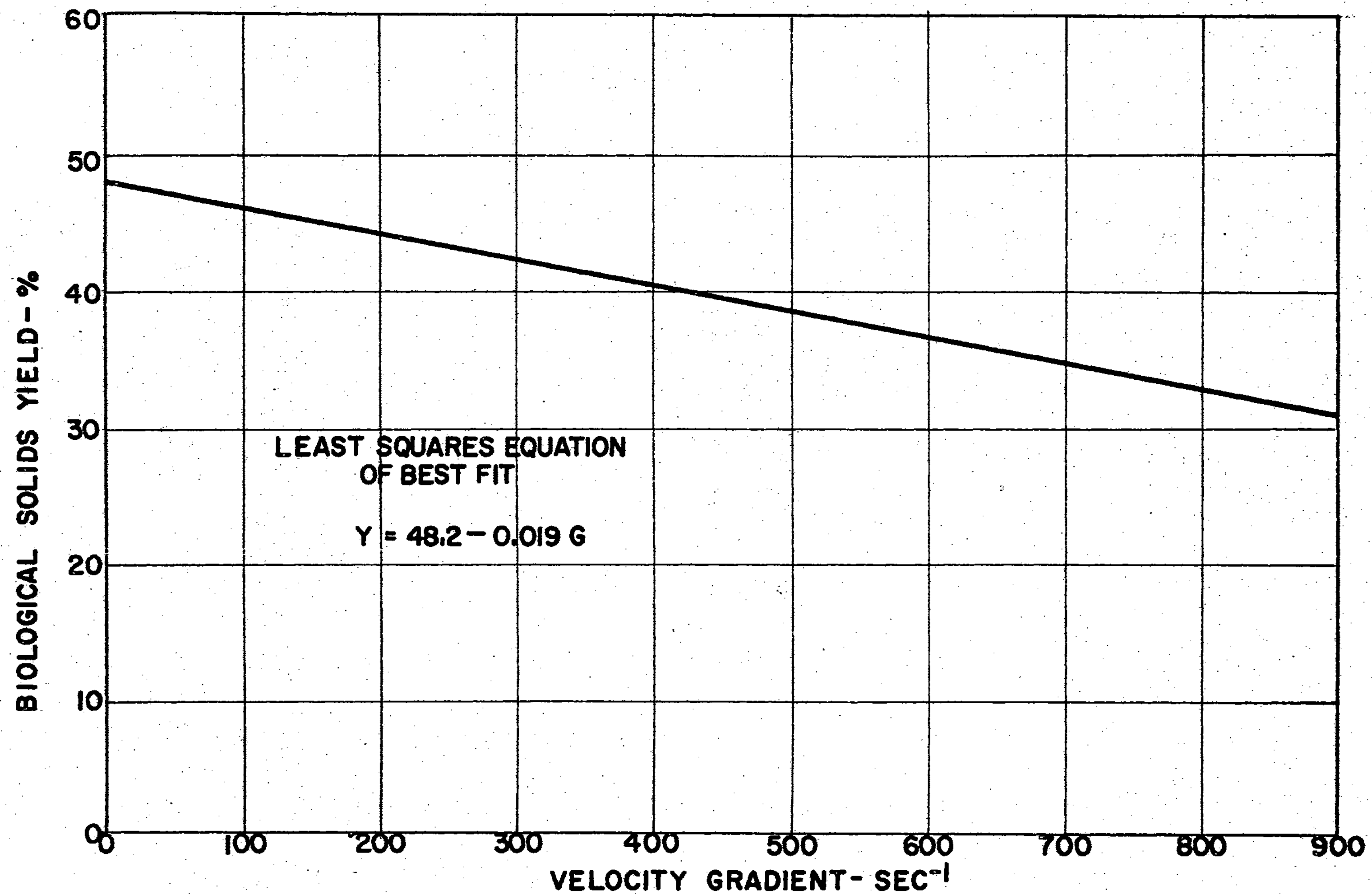


Figure 45 - Least Squares Line of Best Fit for the Biological Solids Yield versus Velocity Gradient for Heterogeneous Population Data at Velocity Gradients Less than 1000 Sec^{-1}.

Application of the same techniques of data analysis to the other cellular parameters which were followed in these experiments revealed additional information about the heterogeneous population system. First, the variations in the protein and RNA yields were shown not to be significant at any reasonable confidence level. Secondly, a small but real (P' = 0.95) increase in the DNA yield was observed as the amount of turbulence was increased from 300 to 1000 sec^{-1}. Thirdly, a rather large decrease in the amount of cellular carbohydrate occurred as the velocity gradient was increased (P' = 0.95) up to a velocity gradient of 1000 sec^{-1}. The linear model was again tested for lack of fit on these data, and all variance could be attributed to experimental error at the 99% confidence level. The least squares lines of best fit for the DNA and cellular carbohydrate yields are shown in Figure 46. Comparison of the relationships between the cellular carbohydrate and biological solids yields versus the velocity gradient indicated that approximately 75% of the reduction in biological solids is attributable to loss of carbohydrate.

Derivation of the least squares equation of best fit for the cell composition data obtained at velocity gradients in excess of 1000 sec^{-1} showed that the values obtained do in fact increase with the velocity gradient. However, a test for significance of these data shows that the relationship may equally well be expressed as a line of zero slope (P' = 0.75 - 0.95). Thus, the simplest

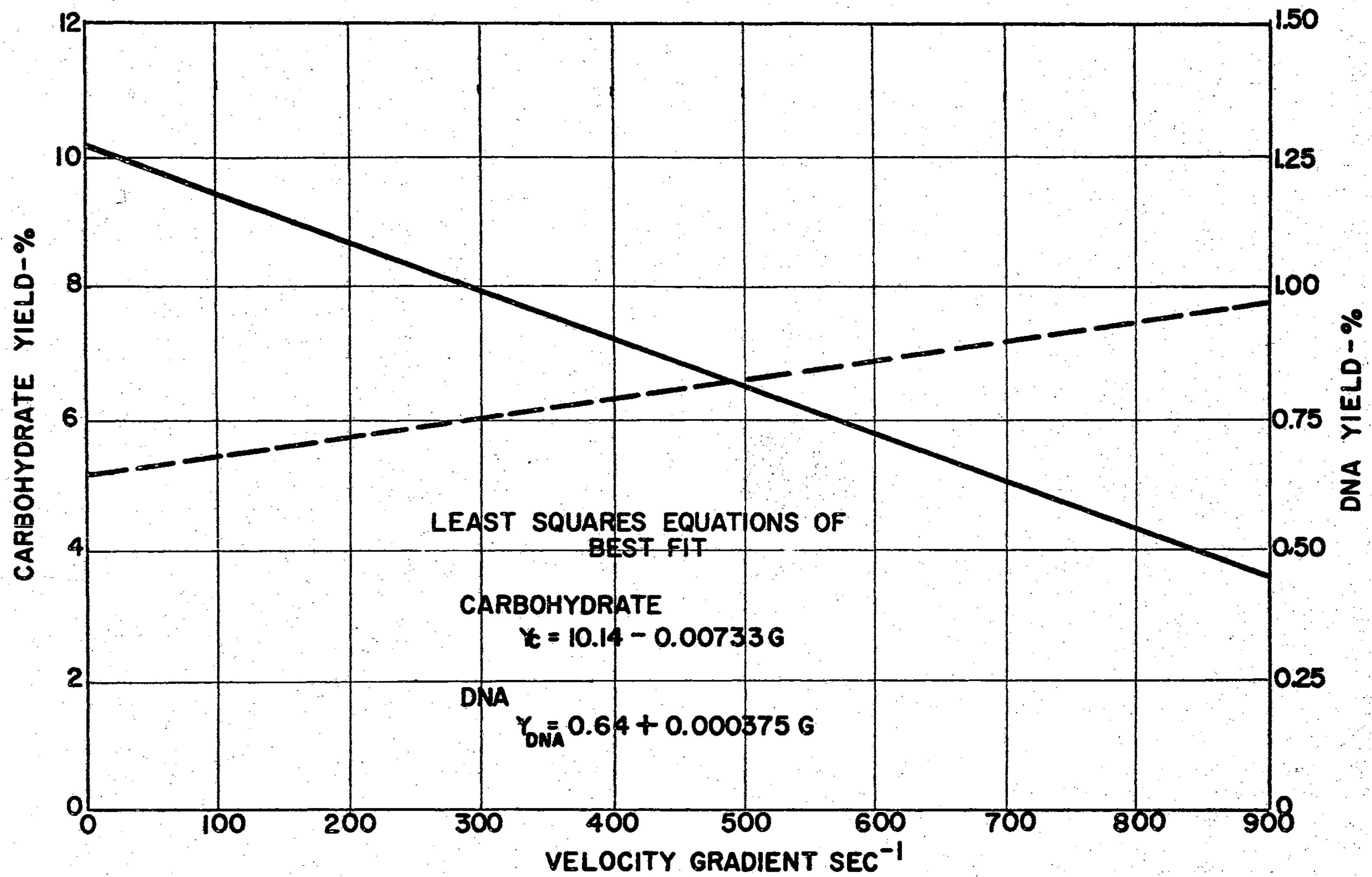


Figure 46 - Least Squares Lines of Best Fit for Cellular Carbohydrate and DNA Yields versus Velocity Gradient for Heterogeneous Population Data at Velocity Gradients Less than 1000 Sec^{-1}.

statistical representation of the biological solids and cell composition yields is that shown in Figure 47.

Application of least squares regression and analysis of variance to the oxygen uptake rate data obtained from the heterogeneous population studies (Figure 22) indicated that unlike the other parameters in this system, a significant difference existed in the intercept of the lines of best fit obtained at the various growth rates. No real difference, however, could be detected in the slopes of the lines. These results are not unexpected, since equation 11 shows that the growth rate of the system should affect the oxygen uptake rate independently of the velocity gradient. Figure 48 shows the least squares lines of best fit for the data using the slope of the composite curve and the intercept derived independently for each growth rate.

If the mechanisms presented earlier are now compared with the results of this study, considerable information on the causation of the effects which have been demonstrated can be obtained. These mechanisms are summarized as follows:

1. Contact between cells and dissolved oxygen controls oxygen uptake.

2. Contact between cells and substrate controls substrate utilization.

3. Floc size varies with the amount of fluid shear provided, thus improving uptake of both oxygen and substrate.

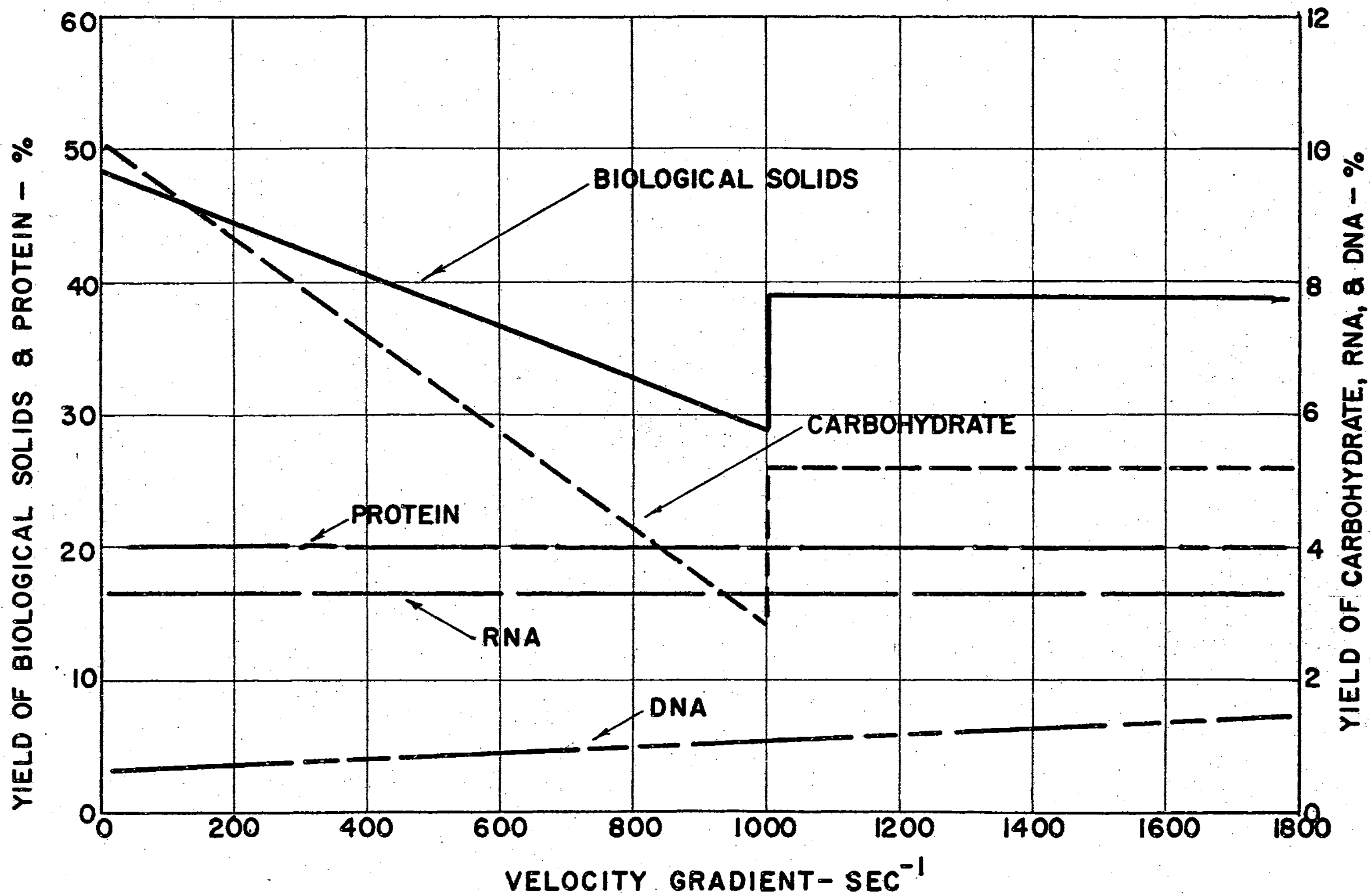


Figure 47 – Simplest Statistical Fit for Biological Solids and Cellular Composition Yields versus Velocity Gradient for Heterogeneous Population Data.

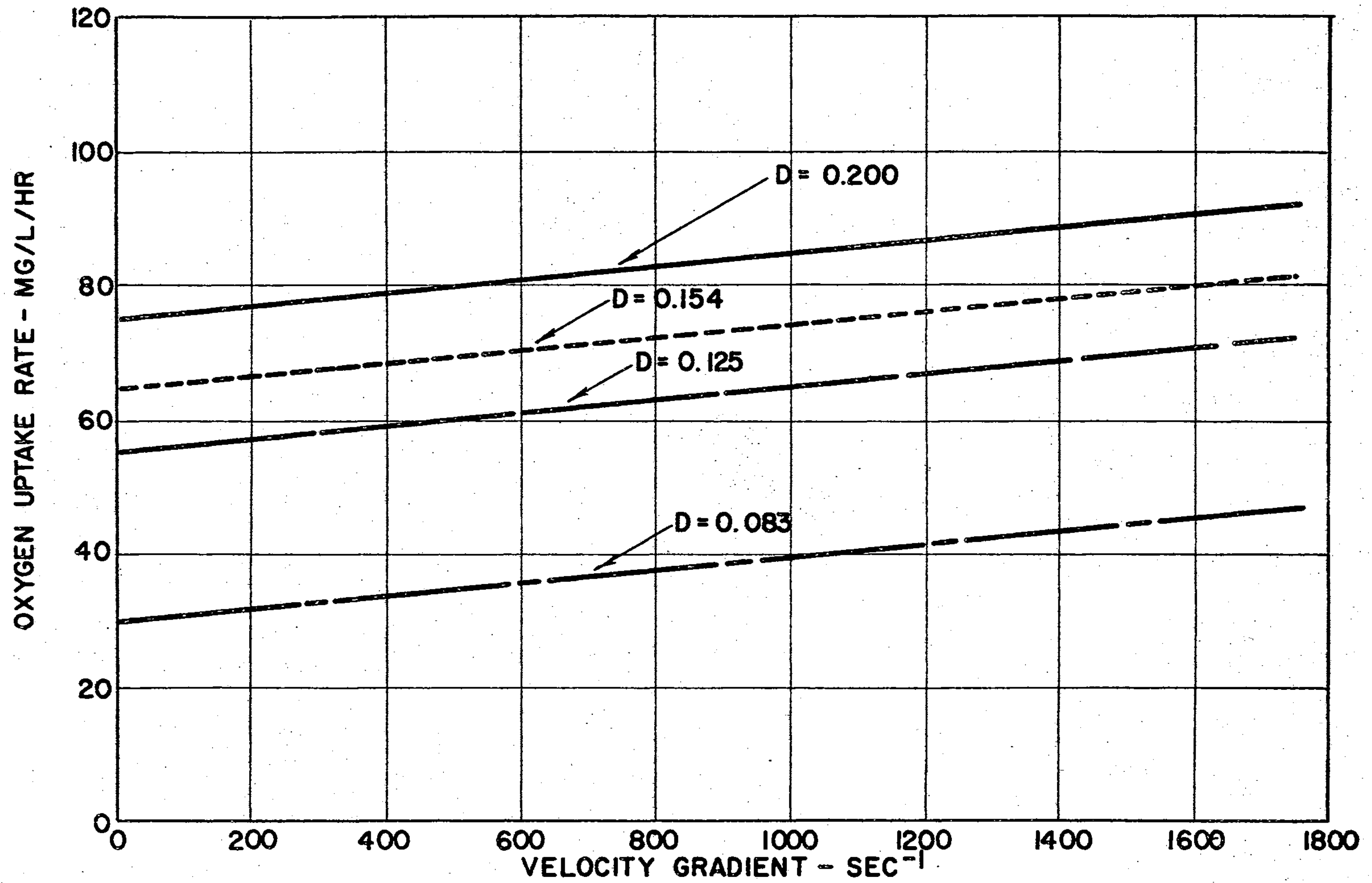


Figure 48 - Best Statistical Fit for Oxygen Uptake Rate versus Velocity Gradient for Heterogeneous Population Data.

4. Variations occur in the predominating organisms contained in the unit in response to changing fluid shear or dissolved oxygen, i.e., agitation and/or D. O. exert a selective pressure.

5. Transport of oxygen across the cell membrane controls oxygen uptake and the resistance to transfer is lowered as agitation is increased.

It will be recalled from equation 14 that if mechanism 1 controls, the oxygen uptake rate must then be a function of the dissolved oxygen concentration. Therefore, this mechanism may be easily tested by determining the significance of any relationship existing between the dissolved oxygen concentration and the oxygen uptake rate at a given velocity gradient. Application of analysis of variance to the data shown in Figure 26 shows that no such relationship existed. On this basis, mechanism 1 may be rejected.

Mechanism 2 has been shown to require that the uptake of substrate be a function of velocity gradient (equation 15). Inspection of Table II, however, indicates that this definitely is not the case, since no decline in substrate removal occurred as the amount of agitation was reduced. Therefore, it is felt that it is unlikely that mechanism 2 is rate-controlling in this system.

Mechanism 3, which considers oxygen transport into the floc particles present, has been shown in equations 16 through 19 to require that oxygen uptake rate again vary as a direct function of dissolved oxygen concentration.

Therefore, this mechanism may be eliminated from consideration on the same basis as mechanism 1.

If predominance changes are considered to be a possible factor in causing the decrease in yield with increasing amounts of mixing energy, it is obvious that if this were the only factor acting, the yield and oxygen uptake of a pure culture system should be unaffected by the amount of turbulence provided. Application of analysis of variance to the least squares line of best fit for the data shown in Figure 36 indicated, however, that the variation in yield with velocity gradient was significant for the SE-4 isolate. In addition, the oxygen uptake data obtained from this system exhibited the same type of variation with velocity gradient as did the data for the heterogeneous population. Therefore, although ecological considerations can never be eliminated entirely from data obtained from natural populations, it is highly unlikely that predominance changes alone were responsible for the oxygen uptake or biological solids yield variations observed with agitation at velocity gradients below $G = 1000\ sec^{-1}$.

Comparison of equation 24 with the data shown in Figures 13 and 38 indicates that mechanism 5 may provide an adequate kinetic description of the behavior of both the heterogeneous population and the pure culture isolate SE-4. This mechanism requires that the oxygen uptake of the system increase as the amount of agitation is increased because of additional oxygen transport across the cell

membrane. This is in turn reflected in a lower biological solids yield (according to equation 24) since any increase in the amount of substrate being oxidized must be accompanied by a decrease in the amount of cell synthesis. Unfortunately, examination of the oxygen uptake characteristics of the *E. coli* system (Figure 42) shows that the behavior of this system cannot be adequately explained on the basis of oxygen transfer across the cell membrane. From these data it appears that the metabolic efficiency of *E. coli* was an inverse function of the amount of mixing energy provided. It should be noted, however, that the highly unusual behavior of this system requires that it be viewed with caution. It is felt that, until additional data are obtained on other systems, mechanism 5 cannot be fully rejected from consideration.

CHAPTER VII

DISCUSSION

In the preceding chapters of this report, studies on the effects of agitation and dissolved oxygen tension on the behavior of completely mixed microbial systems have been described. It has been shown that none of the mechanisms proposed in the literature explains adequately the behavior of all of the systems studied. In this chapter the significance of these findings will be discussed along with other ways in which agitation might have affected behavior at both the ecological and biochemical levels.

Effect of Oxygen Tension on the Growth of Completely Mixed Systems

Swilley, Brant, and Busch (42) theorized that a stagnation layer might exist around the bacterial cell which could result in the rate of oxygen uptake being a function of the amount of dissolved oxygen present. Heukelekian (17) found that the dissolved oxygen level of a culture containing a heterogeneous population could affect the viable count yield by causing changes in the predominating organism. In addition, both of the mechanisms proposed by Rincke (38) and Pasveer (35)(39)(40)(41) to explain the effect of agitation on the behavior of activated sludge

processes required that the oxygen uptake rate of a bacterial system be a direct function of the oxygen tension. On the basis of these considerations it was thought necessary in the present study to determine whether the oxygen tension of a bacterial medium above the critical D. O. had an effect on the oxygen uptake or biological solids yield of this type of system. This was accomplished by operating a completely mixed, steady-state reactor containing a heterogeneous population under conditions of constant growth rate and agitation and varying the amount of oxygen contained in the aerating gas. These experiments clearly demonstrated that dissolved oxygen had no effect on either the oxygen uptake rate or the biological solids yield. In light of these findings and from the considerable body of information available attesting to the existence of a critical D. O. in diverse experimental systems, it may be concluded that stagnation layers are not an important factor in completely mixed systems. In addition, it is highly unlikely that the size of the floc particle or the amount of contact occurring between the cells and the oxygen present in the medium is limiting in systems having a velocity gradient equal to or above those employed in this study. Further, it is probable that the above statement may be extended to include most, if not all, aerobic microbial systems. Although there is a possibility that any of the above mechanisms could be rate controlling in systems receiving very small amounts of agitation, it is

unlikely that the organisms in such a system could be undergoing aerobic metabolism, since all currently used methods of aeration also involve agitation of the medium.

In spite of the absence of any correlation between the oxygen tension and the rate of oxygen uptake or the biological solids yield, there was a definite tendency for the amount of carbohydrate produced by the cells to decrease as the dissolved oxygen concentration was increased. This variation was of comparable magnitude to that seen, for the same change in dissolved oxygen, when the the amount of agitation provided to the system was varied. Since no change in the amount of cellular protein or nucleic acids was observed to accompany the decrease in cellular carbohydrate, it is probable that the cells grown at the high dissolved oxygen concentration contained more lipid than did those grown at low D. O.

It is conceivable that this variation in carbohydrate production arose as a direct result of oxygen tension on the metabolic fate of the substrate. However, in the absence of variation in any of the other cellular parameters examined and in light of the considerable body of evidence which indicates that the metabolism of bacteria is unaffected by variations in oxygen tension above the critical D. O., it is felt that such an explanation is unjustified. In any study of heterogeneous populations the possibility of changes in the predominating species present cannot be ignored as a causative factor for observations

of this type. Therefore, it is felt that additional data on the carbohydrate production of bacterial systems under conditions of varying oxygen tension is necessary before an explanation of the behavior of this parameter can be advanced.

One very important fact remains, however. The changes observed in the biological solids yield and the oxygen uptake rate accompanying changes in the amount of agitation did not occur when the dissolved oxygen concentration was varied independently. Therefore, it is clear that these variations arose as a result of agitation rather than oxygen tension.

The Effect of Agitation on the Growth of Completely Mixed Systems

The variation in biological solids yield with velocity gradient may be of considerable importance since, as has been pointed out, one of the largest economic problems associated with the operation of an activated sludge plant is the disposal of the considerable quantities of sludge produced. Thus, any process modification which can reduce sludge production without causing any deterioration in effluent quality is of considerable interest to the waste treatment engineer. The use of supplemental agitation provides a possible means by which this may be accomplished. Two problems arise, however, which must be considered before the use of mixing energy in this fashion can be recommended. The first is that the amount of agitation

provided must be carefully controlled in order that the system is operated in the area of maximum respiration and minimum synthesis. In the work reported here, minimum yields were obtained when the velocity gradient was approximately 1000 sec^{-1}. Unfortunately, there is no assurance that this figure will be optimal in every case. Therefore, considerably more experience is needed with different waste streams before it is possible to predict the optimum amount of energy that should be furnished to a given system. Secondly, the cost of furnishing this additional agitation must be carefully balanced against the cost of sludge disposal. Only after these data are available can the optimum design solution be obtained.

The results of the cell composition analysis indicated that the reduction in biological solids yield achieved as the velocity gradient was increased from 300 to 1000 sec^{-1} was accompanied by a nearly equal decrease in carbohydrate production. Although the same type of variation in carbohydrate was observed when only oxygen tension was varied, the absence of significant reduction in any of the other cellular components makes the conclusion that the decreased biological solids yield occurred primarily at the expense of cellular carbohydrate inescapable. Nor can the variations noted in yield and oxygen uptake rate be ascribed to predominance changes in the system since the same type of variation was observed to occur in the pure culture isolate SE-4 as in the heterogeneous populations. This

requires that an explanation for the decreasing yield as the velocity gradient was increased toward 1000 sec^{-1} be sought at the metabolic rather than the ecological level.

The same statement cannot be made about the behavior of the heterogeneous population at velocity gradients above 1000 sec^{-1}. In this case, the behavior of the heterogeneous population was not mirrored by the pure culture isolate and therefore could have been an ecological shift in response to an unfavorable environment.

Some evidence that high agitation may indeed be detrimental to the survival of certain organisms is shown by the behavior of the *E. coli* system. The results obtained here clearly show that increased agitation was harmful to efficient growth and substrate utilization. The failure of the SE-4 isolate to follow the same behavior indicates that either the sensitivity of *E. coli* to agitation was a special case or that resistance to the effect is specific for various organisms.

Although no unequivocal mechanism can be proposed at this time to explain the behavior of both the heterogeneous population and the pure culture systems, it is interesting to speculate, on the basis of the data obtained, concerning the effect of agitation on the metabolism of the organisms examined.

All of the data except that obtained on *E. coli* can be said to be compatible with the mechanism proposed by Tsao and Kempe (21). Therefore, if *E. coli* represents a special

case of some factor which was not operative in the other systems, then this mechanism explains adequately the effect of agitation on the oxygen uptake rate of the SE-4 isolate and the heterogeneous population at velocity gradients below 1000 sec^{-1}. The failure of the yield data to vary with the growth rate as predicted in equation 24 is not in serious disagreement with this view since the variation over the range of detention times studied is quite small as compared with the effect of velocity gradient.

Alternately, the behavior of the _E. coli_ system may be interpreted as an indication that the primary action of agitation is not the improvement of oxygen transfer across the cell membrane but that agitation acts by placing a greater challenge for survival on the cell. According to this view, the maximum energy at which _E. coli_ would be able to grow efficiently would be in the neighborhood of 600 sec^{-1}. At velocity gradients above this the cells were unable to utilize the substrate efficiently, and COD in the form of acetate was lost to the effluent. At velocity gradients above 900 sec^{-1} the cells were damaged to the point that the culture was no longer substrate-limited and free glucose began to appear in the effluent in significant quantities. The lowered oxygen uptake of this system, then, simply reflected the reduced substrate utilization as first acetate and then glucose was lost to the effluent.

If this view is correct, it must be assumed that the SE-4 isolate was better able to withstand the effects of

turbulence and was therefore capable of survival over the entire range tested. This organism, as well as the heterogeneous population, appeared to respond to the increased agitation by channeling greater amounts of substrate into energy production. This, in turn, led to greater rates of oxygen uptake. Since the total amount of exogenous substrate available was constant, the additional energy utilization was accomplished by lowered synthesis of non-essential carbohydrate which led to a lowered yield. The tendency for the oxygen uptake rate and yield of the SE-4 isolate to level out at high velocity gradients follows this view. At this point the level of non-essential carbohydrate was reduced to a minimum, as shown by the absence of capsular material, and any further increase in oxygen uptake rate would have had to be obtained at the expense of vital cellular components. If the above analysis is correct, as the velocity gradient was further increased the effluent quality of the SE-4 system should have begun a progressive deterioration. The substrate removal data for this organism (Table IV) provides some evidence that this may have been occurring at the highest velocity gradient examined as the substrate removal during this run was somewhat retarded.

It should be noted that if agitation does exert its effect by disruption of cell function, the release of COD by heterogeneous populations of the type encountered in waste treatment systems would not be expected to create

difficulties. This is true because the constant influx of new microorganisms would allow the development of a population which was well adapted to the conditions present.

The question now arises as to the need for additional oxidative utilization of substrate by the cells at high velocity gradients. Here there are two possibilities. If the effect of turbulence involved a reduction of the efficiency of oxidative phosphorylation, the cells would be required to channel more glucose into oxidative metabolism in order to attain the same amount of metabolic energy for synthesis of required cellular components. This would, at the same time, require an increase in the oxygen uptake rate of the system. An alternate possibility is that high turbulence in the medium makes it more difficult for the cells to maintain their integrity, thus requiring the expenditure of greater amounts of energy to maintain viability.

<u>Effect of Agitation on the Response of a Heterogeneous Population to Quantitative Shock Loading</u>

The results of the shock load study performed at a velocity gradient of 300 sec^{-1} provides an interesting documentation of the effect of a sustained quantitative shock load which results in oxygen limitation in a completely mixed, steady-state system. Although the substrate removal response of the system in terms of COD removal was severely impaired by oxygen limitation, the same was not true in terms of glucose. This illustrates a

very important point about oxygen-limited systems, namely, that their ability to utilize the primary substrate may not be impaired by anaerobiosis but, owing to the accumulation of fermentation products, the overall capacity of the system as a waste treatment unit is effectively destroyed.

The effect of oxygen limitation on the cellular composition may easily be ascertained by comparison of the data shown in Figure 27 with those shown in Figures 30 through 33. The retardation of synthesis of most of the cellular components under conditions of oxygen limitation represents another potential problem in the control of the effects of shock loading. If the amount of oxygen entering the system had been increased in order to offset the oxygen limitation at some time after the shock load had entered the unit, immediate improvement of treatment efficiency would most probably not have resulted. This is believed to be the case because the oxygen limitation which had already occurred would have prevented the normal increase in cell mass, i.e., number necessary to assimilate the additional substrate present. Also, time might be required in order for the existing cells to acclimate to the organic material accumulated during the anaerobic period. If such acclimation did not occur readily, treatment efficiency would not return to the pre-shock level until these materials were diluted out of the system.

The behavior of the substrate removal capacity as the velocity gradient was increased is of considerable

importance. Figure 36 indicates that the production of non-glucose COD during the early stages of the shock load was an inverse function of the amount of agitation. Unfortunately, this effect cannot be attributed solely to agitation on the basis of the data available, since the dissolved oxygen concentration also varied with velocity gradient (Figure 30). The results obtained do indicate, however, that mixing energy is a factor in the response of completely mixed systems to quantitative shock loading. Therefore, it is believed that additional investigation on the effect of agitation on the response of completely mixed systems to shock loading is warranted.

It is interesting to note that although the substrate removal characteristics of the non-oxygen-limited shock system varied with the velocity gradient, no significant difference could be detected in the oxygen uptake or cellular parameters. This is most probably caused by the brief period during which acetate was excreted. Even at the lowest velocity gradient used in these experiments (680 sec^{-1}) the production of acetate was limited to about three hours.

CHAPTER VIII

CONCLUSIONS

A study of the effect of agitation on the behavior of a heterogeneous population and two pure cultures under completely mixed, steady-state conditions allows the following conclusions to be drawn:

1. The solids yield, protein yield, and RNA yield of a heterogeneous population are not affected by changes in the dissolved oxygen concentration from 1.5 to 7.5 mg/l.

2. Oxygen uptake rate is not affected by changes in D. O. in this range.

3. Carbohydrate yield tends to be higher at low oxygen tension but this is not reflected in total solids yield. In addition, the amount of DNA produced appeared to be somewhat reduced at a D. O. of 1.5 mg/l.

4. No significant change in effluent quality occurred as a result of changes in D. O. in the above range for the dilution rates herein employed.

5. A significant decrease in solids yield occurred in all systems as the velocity gradient was increased from 300 to 1000 sec^{-1}. This reduction was accomplished with no deterioration in effluent quality in the heterogeneous population and one of the pure cultures.

6. Seventy-five per cent of the reduction in solids yield could be attributed to reduced carbohydrate synthesis in the heterogeneous population. No change in protein or RNA yield was observed to accompany the solids reduction for either the heterogeneous population or the sewage isolate. A minor increase in DNA yield was noted in the heterogeneous population as the velocity gradient was increased.

7. Oxygen uptake rates of the heterogeneous population and the sewage isolate were found to vary directly with the velocity gradient whereas a declining trend was observed in the oxygen uptake rate of _E. coli_ as the velocity gradient was increased.

8. All of the above effects except oxygen uptake rate were found to be independent of the growth rate (from $\bar{t}$ = 5 to 12 hours). In the case of the oxygen uptake rate, the slope of the least squares line of best fit was independent of the velocity gradient but the oxygen uptake at any given velocity gradient varied inversely with the detention time.

9. At velocity gradients above 1000 sec^{-1} a discontinuity existed in the solids and cell composition data obtained from the heterogeneous population. A linear model fits all of the cellular parameters above the 95% confidence level below G = 1000 sec^{-1}. Above this value no correlation was obtained.

10. Examination of the literature showed that no mechanism previously proposed served adequately to explain

the effects observed in this study.

11. The substrate removal capability of a completely mixed system containing a heterogeneous population during a quantitative shock load was found to be improved at high velocity gradients. No variation in the oxygen uptake or cell concentration was observed to accompany this variation.

CHAPTER IX

SUGGESTIONS FOR FUTURE WORK

Based on the findings of this investigation, the following suggestions are made for future work:

1. Information on the behavior of systems utilizing whole wastes is essential to demonstrate the generality of the findings of this work and the concepts thereby suggested. Such work would also provide information on the economic benefit of supplemental agitation.

2. Additional work on the effect of agitation on the behavior of pure culture systems would be of benefit in confirming the explanations proposed here for the effects of agitation.

3. More information on the effect of agitation and oxygen tension on the behavior of shock load systems is definitely needed. A thorough study of the problem of oxygen depletion of completely mixed systems during shock loading would provide valuable information on the operation and control of activated sludge plants. Additional work is also needed on the effect of agitation on the substrate removal characteristics of non-oxygen limited shock load systems.

4. Additional investigation of the behavior of

heterogeneous populations at very high velocity gradients (in excess of 1000 sec^{-1}) is needed to explain the unusual behavior of these systems.

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APPENDIX A

SUMMARY OF EXPERIMENTAL DATA

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TABLE VIII

DATA SUMMARY FROM VARIABLE AGITATION STUDIES--HETEROGENEOUS POPULATION

$\bar{t}$	G	Run No.	COD			Solids		Protein		Carbohydrate		RNA		DNA		Oxygen Uptake Rate m/L/hr 22
			Influent	Average Effluent	Difference	Mean 9-12*	Yield 13	Mean 9-12	Yield 14	Mean 9-12	Yield 15	Mean 9-12	Yield 16	Mean 9-12	Yield 17	
5	300	16	970	60	910	417	46	215	24	51	5.6	42	4.5	5	0.55	81.3
	300	24	982	52	930	372	40	152	16	98	10.5	76	8.2	7	0.75	61.1
	600	23	970	31	939	393	42	206	22	58	6.2	52	5.5	10	1.1	81.9
	680	17	918	88	930	289	31	145	16	52	5.4	36	3.9	2	0.21	91.2
	900	22	970	32	938	369	39	206	22	46	4.9	45	4.6	7	0.75	80.6
	1140	18	1020	83	937	209	22	98	10	48	5.1	30	3.2	2	0.21	97.2
	1200	21	820	44	776	367	48	182	19	49	6.3	58	7.5	8	1.0	89.0
	1500	20	780	65	715	381	53	198	28	58	8.1	57	8.0	8	1.1	98.9
	1750	19	860	77	783	420	54	192	25	76	9.7	56	7.1	10	1.3	76.7
6½	300	30	961	73	888	387	44	195	22	74	8.3	28	3.2	9	1.0	68.5
	600	31	961	65	896	421	47	217	24	54	6.0	21	2.3	7	0.78	85.6
	900	32	1085	92	993	371	37	226	23	30	3.0	32	3.2	14	1.4	66.2
	1200	33	728	52	676	349	52	176	26	22	3.3	26	3.8	12	1.7	77.1
	1500	34	992	49	943	403	43	219	23	51	5.3	41	4.4	14	1.5	68.5
8	450	11	974	34	940	369	39	181	19	77	8.2	80	8.5	10	1.1	54.3
	680	6	1040	50	990	318	33	156	16	44	4.5	48	4.9	15	1.5	57.2
	800	10	961	28	933	275	29	157	17	50	5.3	24	2.6	9	1.0	66.3
	1000	7	970	75	895	244	27	117	13	12	1.3	33	3.7	-	-	75.8
	1200	9	890	18	872	279	32	155	18	25	2.9	21	2.4	6	0.7	67.6
	1350	8	1126	66	1060	270	25	132	12	26	2.5	57	5.4	11	1.0	64.5
12	300	29	1090	52	1038	381	37	168	16	63	6.1	19	1.8	5	0.5	33.1
	600	28	1090	58	1032	349	34	160	16	55	5.3	20	1.9	7	0.7	34.1
	900	27	1428	58	1370	289	21	156	11	50	3.6	19	1.4	9	0.7	33.5
	1200	26	1094	30	1064	403	39	212	20	58	5.4	26	2.4	12	1.1	40.1
	1500	25	980	25	955	338	35	188	20	44	4.6	41	4.3	9	0.9	52.8

* Numbers refer to Figures in Which Data Appear

TABLE IX

DATA SUMMARY FROM VARIABLE D.O. STUDIES - HETEROGENEOUS POPULATION

Velocity Gradient = 1000 Sec^{-1}

D.O.	Run No.	COD			Solids		Protein		Carbohy.		RNA		DNA		Oxygen Uptake Rate mg/l/hr 26
		Influent	Effluent	Difference	Mean 24*	Yield 25	Mean 24	Yield 25	Mean 24	Yield 25	Mean 24	Yield 25	Mean 24	Yield 25	
7.1	12	970	41	929	401	43	207	22	57	6.1	69	7.4	6	0.6	61.1
7.1	13	1115	49	1036	353	34	144	14	62	6.0	60	5.8	6	0.6	58.2
3.6	14	980	85	895	346	39	173	19	93	10.4	69	7.7	5	0.6	68.7
1.4	15	991	101	890	350	39	155	17	152	17.0	67	7.5	4	0.4	61.7

*Numbers refer to figures in which data appear

TABLE X

DATA SUMMARY FROM VARIABLE AGITATION STUDIES - PURE CULTURES

Organism	G	Run No.	COD			Solids		Protein		Carbohy.		RNA		DNA		Oxygen Uptake Rate 39&42 mg/l/hr
			Influent mg/l	Mean 37&40 mg/l	Difference 41	Mean 37&40 mg/l	Yield 38&43	Mean 37&40 mg/l	Yield 38&43	Mean 37&40 mg/l	Yield 38&43	Mean 37&40 mg/l	Yield 38&43	Mean 37&40 mg/l	Yield 38&43	
E. coli K-12	300	37	1132	47	1085	540	50	313	28.8	109	10.0	87	8.0	2.0	0.18	6.24
	600	38	1130	118	1012	570	56	313	30.9	107	10.6	95	9.3	2.0	0.20	71.7
	900	39	1128	324	804	370	46	220	27.3	44	5.5	70	8.7	1.0	0.12	53.7
	1200	40	1112	465	647	300	46	225	34.0	50	7.7	75	11.6	2.0	0.31	56.0
	1500	41	1325	524	801	275	34	225	28.0	26	3.2	95	11.8	1.6	0.20	34.4
	800	47	1110	287	823	386	47	-	-	-	-	-	-	-	-	65.0
	1200	48	1255	512	743	334	45	-	-	-	-	-	-	-	-	56.0
	1750	49	1325	728	298	141	47	-	-	-	-	-	-	-	-	34.4
SE-4	300	42	1302	47	1255	557	44	299	23.8	220	17.5	133	10.6	3.0	0.24	53.9
	600	43	1089	50	1039	517	50	274	26.4	174	16.7	118	11.3	3.0	0.28	59.5
	900	44	1160	43	1117	454	41	266	23.8	125	11.2	101	9.0	3.0	0.27	64.9
	1200	45	1289	46	1193	469	39	272	22.8	98	8.2	100	8.4	3.0	0.25	72.5
	1500	46	1097	107	990	436	42	271	26.0	89	8.5	93	8.9	3.0	0.29	66.2

APPENDIX B

LIST OF SYMBOLS

A - agitator area

b_o - least squares intercept

b_1 - least squares slope

C_d - drag coefficient

C_1 - oxygen uptake at zero velocity gradient

D - dilution rate

d - fluid density

d' and d'' - diameter of particles in suspension

F - ratio following an F distribution

f_o - fraction of effective collisions between cells and oxygen molecules

f_s - fraction of effective collisions between cells and substrate molecules

G - velocity gradient

H - depth

K'' - a constant relating oxygen uptake rate to oxygen tension assuming diffusion into floc is rate controlling

K_s - Monod constant - substrate concentration at which $\mu = 0.5\mu_m$

k_1 - a constant relating substrate concentration to oxygen uptake

k_a - reaeration coefficient

k_o - unit mass of an oxygen molecule

k' - diffusion constant of oxygen in water

N - number of collisions occurring between two discrete classes of suspended particles per unit time per unit volume

N_o - number of collisions per unit time per unit volume occurring between cells and oxygen

N_s - number of collisions per unit time per unit volume occurring between cells and substrate

n' and n'' - concentrations of particles undergoing collision

P - power consumed

P' - probability level

Q_a - air flow rate

R - agitator speed

r - floc radius

S - concentration of rate-limiting nutrient

S_o - concentration of rate-limiting nutrient in feed

s - surface tension

$s_{b_o}^2$ - variance of least squares intercept

$s_{b_1}^2$ - variance of least squares slope

T - oxygen tension

T_m - oxygen concentration at floc-water interface

T_p - oxygen concentration at center of floc particle

T' - Student's T

t - time

t' - hypothetical time in seconds of subsistence of static water-floc interfacial surface

$\bar{t}$ - reactor detention time

U - oxygen uptake rate

u	- absolute viscosity
u'	- kinematic viscosity
V	- volume
v	- linear velocity of periphery of agitator blades
X	- reactor biological solids concentration
X_o	- feed biological solids concentration
X_n	- viable count in reactor
Y	- cell mass yield
Y_n	- viable count yield
μ	- first order growth rate constant
μ_m	- maximum first order growth rate constant

APPENDIX C

EXAMPLE CALCULATION OF OXYGEN UPTAKE

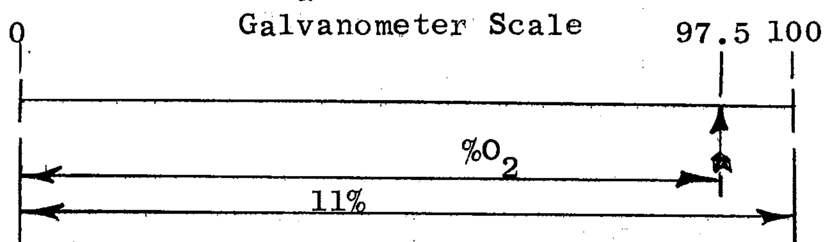
Instrument Range = 10 - 21%

Galvonmeter Reading = 97.5

Reactor Volume = 3.81 L

Aerating Gas - 21% Oxygen

Q_a = 1.0 L/min



$$\% \; O_2 = 10 + \frac{11 \times 97.5}{100} = 10 + 10.7 = 20.70\%$$

Difference between influent and effluent gas

= 21.00 - 20.70 = 0.3%

Density of oxygen at 0°C and 760 MM Hg = 1.429 gm/L

Density of oxygen at 30°C and 760 MM Hg = 1.287 gm/L

Weight of oxygen in one liter of air at 30°C and 760 MM Hg

= 0.21 x 1.287 = 0.27027 gm = 270.27 mg/l

Oxygen consumed mg/min = oxygen in - oxygen out

$$O_2 \text{ consumed} = 1287 \frac{\text{mg } O_2}{\text{L } O_2} \times 0.21 \frac{\text{L } O_2}{\text{L Air}} - 1287 \frac{\text{mg } O_2}{\text{L } O_2}$$

$$\times 0.207 \frac{\text{L } O_2}{\text{L Air}} \quad Q_a$$

$$O_2 \text{ consumed} = 1287 \; (0.21 - 0.207) Q_a = (1287 \times 0.3) Q_a = 3.86 Q_a$$

$$Q_a = 1.0$$

O_2 consumed = 3.86 mg/min

$$\text{Oxygen uptake mg/l/hr} = \frac{O_2 \text{ consumed/min} \times 50 \text{ min/hr}}{\text{Reactor Volume - L}}$$

$$= \frac{3.86 \times 60}{3.81}$$

Oxygen uptake rate = 50.8 mg/l/hr

TABLE XI

CONVERSION TABLE FOR BECKMAN MODEL E-2 GASEOUS OXYGEN ANALYZER*

Galvonmeter Reading % of Scale	Oxygen Consumed mg/min	Galvonmeter Reading % of Scale	Oxygen Consumed mg/min	Galvonmeter Reading % of Scale	Oxygen Consumed mg/min	Galvonmeter Reading % of Scale	Oxygen Consumed mg/min	Galvonmeter Reading % of Scale	Oxygen Consumed mg/min
		92.0	11.326	94.0	8.494	96.0	5.663	98.0	2.831
90.0	14.157	1	11.184	1	8.353	1	5.521	1	2.690
1	14.015	2	11.042	2	8.211	2	5.380	2	2.548
2	13.874	3	10.901	3	8.069	3	5.238	3	2.407
3	13.732	4	10.759	4	7.928	4	5.097	4	2.265
4	13.591	5	10.618	5	7.786	5	4.955	5	2.124
5	13.449	6	10.476	6	7.645	6	4.813	6	1.982
6	13.308	7	10.335	7	7.503	7	4.672	7	1.840
7	13.166	8	10.193	8	7.362	8	4.530	8	1.699
8	13.024	9	10.051	9	7.220	9	4.389	9	1.556
9	12.883	93.0	9.910	95.0	7.078	97.0	4.247	99.0	1.416
91.0	12.741	1	9.768	1	6.937	1	4.106	1	1.274
1	12.600	2	9.627	2	6.795	2	3.964	2	1.133
2	12.458	3	9.485	3	6.654	3	3.822	3	0.991
3	12.317	4	9.344	4	6.512	4	3.681	4	0.849
4	12.175	5	9.202	5	6.371	5	3.539	5	0.708
5	12.033	6	9.060	6	6.229	6	3.398	6	0.566
6	11.892	7	8.919	7	6.088	7	3.256	7	0.425
7	11.750	8	8.777	8	5.946	8	3.115	8	0.283
8	11.609	9	8.636	9	5.804	9	2.973	9	0.142
9	11.467								

*Range 10 - 21%, Qa = 1.0 L/min

APPENDIX D

DERIVATION OF FORMULATIONS FOR SUBSTRATE UTILIZATION AND OXYGEN UPTAKE RATE OF COMPLETELY MIXED REACTOR ASSUMING MIXING IS RATE-CONTROLLING

Case 1 - Assuming Contact between Oxygen and Cells is Rate-Controlling

$$U = f_oK_oN_o = \left[\frac{n'n''(d' + d'')^3}{6}\right]Gf_oK_o$$

$$n' = T \text{ mg/l} \times 10^{-3} \text{ gm/mg} \times \frac{1}{32} \frac{\text{mole}}{\text{gm}} \times 6.02 \times 10^{23}$$

$$n' = 1.88 \times 10^{19} \text{ T molecules/L}$$

$$(d' + d'')^3 = 8 \times 10^{-12} \text{ cm}^3 = 8 \times 10^{-15} \text{ L}$$

$$K_o = \frac{1}{6.02 \times 10^{23}} \times 32 \times 10^3 = 5.34 \times 10^{-19} \text{ mg/molecule}$$

$$U = \frac{1.88 \times 10^{19} \times X_n \times 10^3 \times 8 \times 10^{-15} \times 5.34 \times 10^{-19} Gf_o}{6}$$

$$U = 1.34 \times 10^{-11} f_oGX_nT$$

f_o = Fraction of Effective Collisions

n' = Number of oxygen Molecules/L

d' = Diameter of Oxygen Molecule = 0

d'' = Diameter of Cell = 2 Microns

Case 2 - Assuming Contact between Substrate and Cells is Rate-Controlling

$$D(S_o - S) = f_s K_s N_s = \left[\frac{n'n''(d' + d'')^3}{6}\right] G f_s K_s$$

$$(S_o - S) = \left[\frac{n'n''(d' + d'')^3}{6D}\right] G f_s K_s$$

$$K_s = \frac{1 \text{ mole}}{6.02 \times 10^{23} \text{ molecules}} \times \frac{180 \text{ gm}}{\text{mole}} \times 10^3 \frac{\text{mg}}{\text{mole}}$$

$$K_s = 2.99 \times 10^{-19} \frac{\text{mg}}{\text{molecule}}$$

$$(d' + d'')^3 = 8 \times 10^{-15} \text{ L}$$

n' = no. of substrate molecules/L
d' = diameter of substrate molecule = 0

$$n' = S/K_s$$

$$(S_o - S) = \left[\frac{S/K_s \times X_n \times 10^3 \times 8 \times 10^{-15}\ K_s}{6D/60}\right] f_s G$$

$$= 8 \times 10^{-11} \left[\frac{S\ X_n}{D}\right] f_s G$$

Let $M = 8 \times 10^{-11} \left[\frac{SX_n}{D}\right] f_s G$

$$\% \text{ Removal} = \left[\frac{100(S_o - S)}{S_o}\right] = 100(1 - S/S_o)$$

$$S_o - S = M \therefore S_o = S + M \therefore 1/S_o = \frac{1}{S + M}$$

$$S/S_o = \frac{S}{S + M} = \frac{1}{1 + M/S}$$

$$1 - S/S_o = 1 - \frac{1}{1 + M/S}$$

$$\% \text{ Removal} = 100 \quad 1 - \left[\frac{1}{1 + \frac{8 \times 10^{-11}\ SX_n f_s G}{SD}}\right]$$

$$\% \text{ Removal} = 100 \quad 1 - \left[\frac{1}{1 + \frac{8 \times 10^{-11}\ X_n f_s G}{D}}\right]$$

$$= 100 \quad 1 - \left[\frac{D}{D + (8 \times 10^{-11}\ X_n f_s G)}\right]$$

VITA

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Publications:

1. Grigoropoulos, S. G., and M. David Rickard, "Electrochemical Degradation of Alkyl Benzene Sulfonate." Presented at a meeting of the American Chemical Society in Cincinnati, Ohio, January, 1963.

2. Riley, W. H., and M. David Rickard, "Variation in Total RNA, DNA, and Protein during the Growth of <u>Aerobacter aerogenes</u>." Presented at a meeting of the Michigan Society for Microbiology, January, 1964.

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