AN INVESTIGATION OF SUBSTRATE REMOVAL AND STORAGE IN THE ACTIVATED SLUDGE PROCESS

by

Steven Robert Hearne

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APPROVED:

E. W. Randall, Chairman

J. H. Sherrard

P. H. King

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I. INTRODUCTION

The principal types of wastewaters are municipal sewage, industrial discharges, agricultural runoff, and storm-water and urban runoff. There are many undesirable characteristics associated with these wastewaters. Soluble and suspended organics are subject to microbial degradation and, therefore, can result in dissolved oxygen depletions in streams or estuaries. These organics can also result in some taste and odor problems found in water supplies. Other undesirable characteristics include excessive nutrients, which may enhance the entrophication of lakes and also cause taste and odor problems, color, turbidity, and thermal pollution, which may result in a depletion of dissolved oxygen by lowering the saturation value of oxygen in water while increasing the rate of oxygen utilization by microorganisms. In most cases, wastewaters can be treated biologically.

The objectives of biological treatment of wastewater are to coagulate and remove the suspended solids and to stabilize the organic matter. Biological processes are efficient in removing organic substances that are soluble or colloidal in nature. One of the most frequently used biological processes is the activated sludge process, where the waste is stabilized biologically in a reactor under aerobic conditions and the resulting biological mass is separated from the liquid in a settling tank. A more thorough explanation of the process is presented in chapter two.

Several modifications have been made to the original design of the activated sludge process, and they include:

a. Extended aeration

b. High rate aeration

c. Complete mix aeration

d. Step aeration

e. Modified aeration

f. Contact Stabilization

The most controversial of these modifications is the contact stabilization process. The controversy arises from the various theories and process mechanisms that have been proposed in an attempt to explain how the process actually works. Contact stabilization consists of a contact tank with a short hydraulic detention time, followed by sedimentation and discharge of the effluent, with subsequent stabilization or reaeration of the activated sludge prior to return to the contact tank. Many operational and economical advantages have been realized by utilizing this process. In particular, it requires less aeration volume than most other activated sludge processes. The advantages and a more exact description of the process are discussed in chapter two. It is generally assumed that the process works because substrate is rapidly sorbed and/or stored by activated sludge flocs in the contact tank for later utilization by the floc bacteria in the stabilization tank.

The objective of this study was to investigate what effect the concentration of microorganisms would have on substrate removal,

microbial substrate storage, and oxygen utilization at a constant food-to-microorganism ratio. A series of batch experiments were conducted, under aerated and completely mixed conditions, using wastewaters of varying colloidal percentages and of varying complexities. It was felt that the results of the experiments might provide information of value towards the evaluation of current theories regarding substrate removal in the contact stabilization process.

II. LITERATURE REVIEW

The contact stabilization process is a modification to the conventional activated sludge process and was developed to take advantage of the sorptive properties of activated sludge. This process may be the most controversial of the various modifications. The controversy arises from the various mechanisms for removal of organic material from the wastewater that have been proposed for contact stabilization. A review of the literature was conducted and theoretical approaches to substrate removal and storage are presented. The operational concepts of the conventional activated sludge and the contact stabilization processes will be discussed. Also included in the review is a discussion on actual operating experience with contact stabilization and various design proposals and procedures.

THE CONVENTIONAL ACTIVATED SLUDGE PROCESS

The activated sludge process was developed in Manchester, England in 1913 by Ardern and Lockett (1), and was so named because it involved the production of an activated mass of microorganisms capable of aerobically stabilizing a waste. Their process consisted essentially of the aeration of a mixture of settled sewage with a bacteriologically active sludge followed by the separation of the sludge from the liquor. The effluent from the activated sludge process was found to be comparable to that obtained from a good percolating filter.

At the present time, the conventional activated sludge process consists of an aeration basin, a sedimentation tank, and a sludge recyle line. A schematic flow diagram of this process is shown in Figure 1. The aerobic environment necessary for complete stabilization of an organic waste is achieved by using diffused or mechanical aeration for a flow through period usually of from four to six hours.

The contents of the aeration basin which are primarily wastewater mixed with microbial masses, are termed the mixed liquor. The suspended solids concentration of the mixed liquor is usually maintained between 1,000 to 3,000 mg/liter. The biological mass, after leaving the aeration basin, is separated from the liquid in a sedimentation tank. A portion of the biological mass is recycled and the rest wasted to insure that the mass of microorganisms does not increase until the system could no longer contain them or maintain them in an aerobic environment. The flow of the returned sludge varies but usually ranges from 20 to 50 percent of the influent flow rate. The liquid supernatant is discharged as the final effluent.

The bacteria are the most important microorganisms in the activated sludge process because they are responsible for the decomposition of the organic material in the influent. In the aeration basin the bacteria utilize the organic waste matter to obtain energy for maintenance and synthesis of organic material into new cells. Many intermediate products are formed before the final end products of oxidation are obtained. Other microorganisms, predators such as

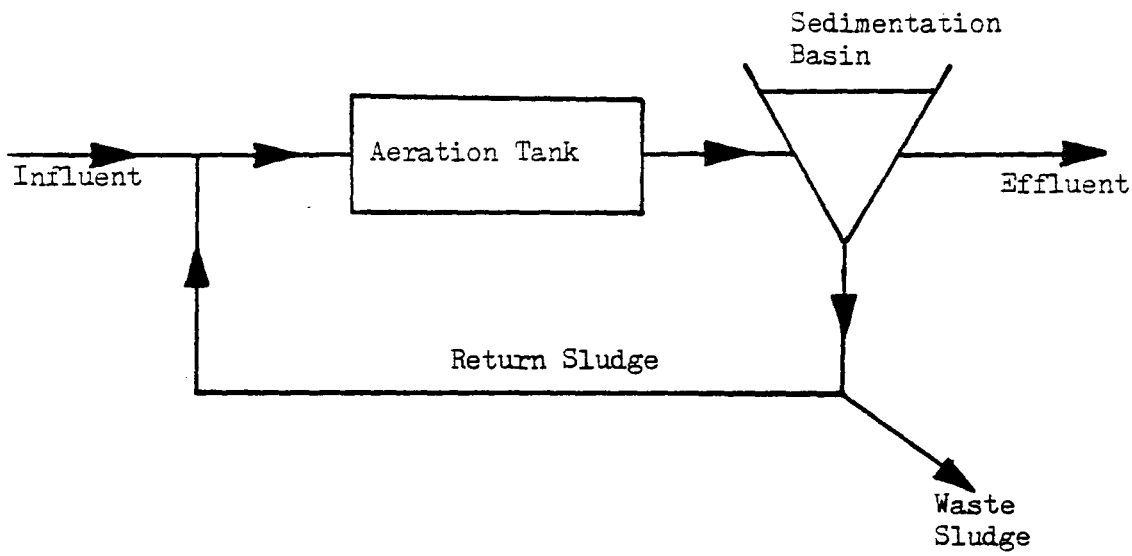

Figure 1. Flow scheme of conventional activated sludge plant.

protozoa and rotifers which act as effluent polishers, are also present in the activated sludge biomass. Protozoa consume bacteria that have not flocculated and rotifiers consume any small biological floc particles that have not settled. While bacterial decomposition of the organic waste is important, it is also important that a satisfactory floc be formed for efficient separation of the biological mass in the settling tank.

A number of techniques have been used in the design of the activated sludge process (2). An early design technique was based on the selection of an aeration detention time. For domestic wastewaters, aeration times of six to eight hours depending on flow rate, were established as standards for 90 percent removal of the 5-day biochemical oxygen demand (BOD). Organic loading per unit of aeration basin volume was later developed as a more basic design approach. Volumetric organic loadings in the range of 20 to 50 lb BOD/day/1000 cu. ft. of aeration basin volume were established to achieve 90 percent BOD removal. These design techniques did not relate to the fundamentals of the biological process because they were developed empirically.

A more fundamental approach to the design of an activated sludge process was developed based on the observation that the amount of biodegradable organics applied to a biological system affects the metabolic rate of the microorganisms. Therefore, a food to microorganism (F/M) ratio was established as the design parameter for the activated sludge process (3).

More recently, equations derived from microbial growth kinetics are being used in the design of biological treatment processes. Two major design approaches have been used to model the activated sludge process. One is based on the Monod (4) model of pure cultures in which biological growth rate and substrate concentration are related hyperbolically. The other approach is based on the investigations of Garrett and Sawyer (5) which suggest that the relation between the rate of growth and the remaining soluble biochemical oxygen demand (BOD) could be well represented by a discontinuous function. The rate of growth was found to be directly proportional to the concentration of organic substrate remaining up to a critical concentration above which it was constant and independent of the concentration of substrate. This type of relationship was in error only near the point of discontinuity. Lawrence and McCarty (6) have used the continuous model of Monod as the basis of their design approach while Eckenfelder (7,8) based his design approach on a discontinuous function similar to that proposed by Garrett and Sawyer.

In their design approach, Lawrence and McCarty developed general relationships which were applicable to a wide variety of bacterial mediated treatment processes by developing a unifying parameter termed mean cell residence time, θ_c. This parameter is especially useful because of its basic relationship to bacterial growth rate, and the relative ease with which it can be used in design calculations and in the control of biological treatment systems. The relationship

between biological growth and substrate utilization was formulated in two basic equations:

$$\frac{dX}{dt} = Y \frac{dS}{dt} - k_d X \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$\frac{dS}{dt} = \frac{kSX}{K_s + S} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where:

$\frac{dX}{dt}$ = net growth of microorganisms per unit volume of reactor, mass/volume-time

Y = growth yield coefficient, mass/mass

dS/dt = rate of microbial substrate utilization per unit volume, mass/volume-time

k_d = microorganism decay coefficient, time $^{-1}$

X = microbial mass concentration, mass/volume

k = maximum rate of substrate utilization per unit weight of microorganisms, time $^{-1}$

t = time

S = concentration of substrate surrounding the microorganisms, mass/volume

K_s = substrate concentration at which rate of substrate utilization per unit weight of microorganisms is one-half the maximum rate, mass/volume

The equations apply only to that portion of the waste that is soluble and biodegradable. Equation 1 describes the relationship between net rate of growth of microorganisms and rate of substrate utilization. Equation 2 relates the rate of substrate utilization both to the concentration of microorganisms in the reactor and to the concentration of substrate surrounding the organisms. Further

development of these equations results in relationships for determining the effluent waste concentration, microorganism concentration in the reactor, solids retention time, and the efficiency of the biolcgical treatment process. Other investigators have also demonstrated the importance of θ_c as the fundamental parameter for treatment plant design and operation (9). An alternative approach to the analysis of net growth accounts for the variation of yield by incorporating k_d into an observed yield coefficient (Y_{obs}) that varies with θ_c (10).

It has been observed that as the mean cell residence time increases there is an enhancement in the settling characteristics of the biological floc (11). This occurs because, as the mean age of the cells increases, the surface charge is reduced and the microorganisms start to produce extracellular polymers. They eventually become encapsulated in a slime layer which promotes the formation of floc particles that can be readily settled. Mean cell residence times of for 3 to 4 days or more are required to achieve effective settling of activated sludge treating domestic wastewater.

The design approach proposed by Eckenfelder is based on the following discontinuous function which relates the rate of substrate utilization to substrate surrounding the organisms and the concentration of microoganisms in the reactor

$$\frac{dS}{dt} = K X S \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

where

K = substrate removal rate constant time $^{-1}$

This equation suggests that the rate of substrate utilization per unit mass of microorganisms is directly proportional to the concentration of substrate remaining, and is therefore a first order reaction. At low organic substrate concentrations the rate of substrate removal, is in accordance with Equation 3 and, therefore, directly proportional to the concentration of microbial mass. However, Equation 3 becomes discontinuous at higher substrate concentrations until the rate of substrate removal becomes independent of the substrate concentration and only directly proportional to the concentration of microbial mass as shown by the following relationship

$$\frac{dS}{dt} = KX \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Stensel and Shell (2) compared the commonly used F/M design technique with the mean cell residence time (θ_c) design technique of Lawrence and McCarty. In a laboratory study, performed on an oil refinery wastewater to obtain design data, it was shown that the θ_c and F/M approaches are basically the same. The θ_c design technique, however, yielded operational and control advantages.

THE CONTACT STABILIZATION PROCESS

Contact stabilization as a modification to the activated sludge process has become widely used during the last few decades in the United States. It is only one of the many variations of sludge reaeration that have been employed under such names as bio-flocculation, biosorption, contact stabilization and sludge reaeration. The contact stabilization process was developed to take advantage of

the sorptive properties of activated sludge. A schematic diagram of this process is shown in Figure 2. The sequence of aeration-sedimentation-reaeration has been used as a secondary treatment process; however, current use is primarily in complete aerobic treatment without primary sedimentation.

There is considerable controversy regarding the actual mechanism of how the contact stabilization process operates. The most common explanation found in the literature is that organic removal occurs in two stages in an activated sludge process. The first is the sorptive phase which requires 20 to 40 minutes and consists of rapid uptake, or biosorption, of the soluble portion of the organic material, and the adsorption-entrapment of the colloidal organic fraction by the microbial flocs. The combined mechanisms result in a high degree of organic removal. In the second stage the adsorbed organics are metabolically assimilated. The contact stabilization process takes advantage of these two phases by separating them, allowing each phase to occur in a different tank. Raw wastewater is aerated for 30 to 90 minutes in the contact tank (aeration basin) and is then settled (sedimentation tank). The supernatant from the clarifier is the plant effluent, and the settled sludge is reaerated (reaeration basin) from 3 to 6 hours prior to mixing with raw influent in the contact tank. The mixed-liquor suspended solids concentrations for the contact and reaeration tanks are typically found to be 2,000 mg/liter and 6,000 mg/liter, respectively.

The contact stabilization process is thought to work best on colloidal wastes since the aeration period in the contact tank

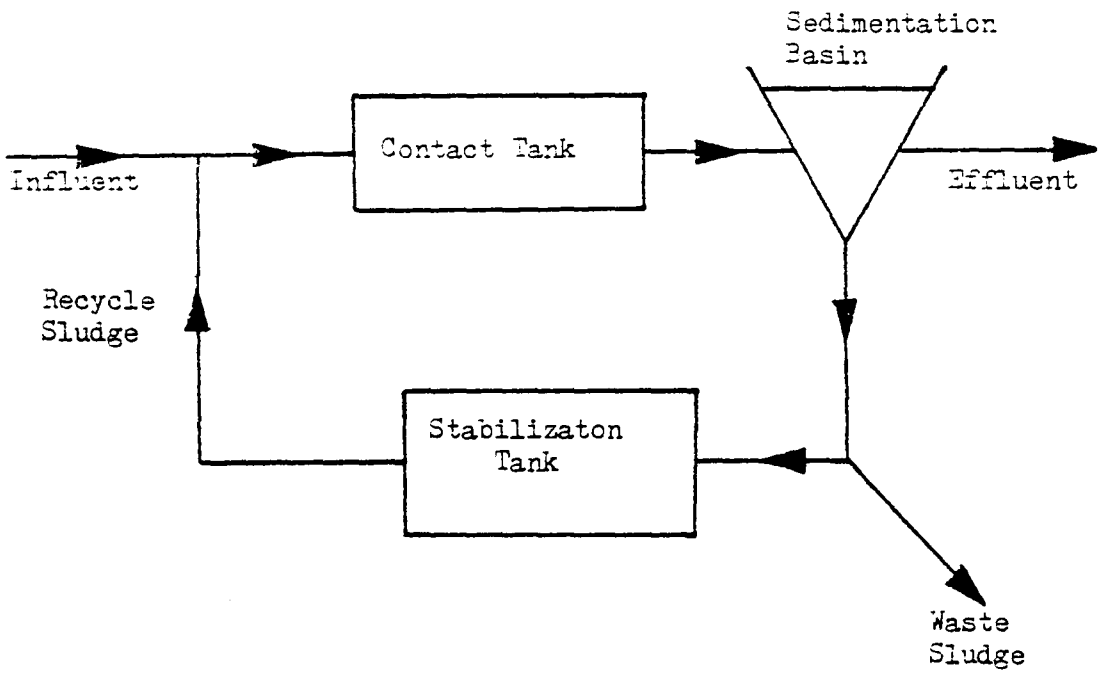

Figure 2. Flow scheme of contact stabilization activated sludge plant.

does not allow the activated sludge to absorb large quantities of soluble organics in the short contact period (3). Most wastes contain nonsoluble organic material ranging from colloids (10^{-6} to 10^{-3} mm in diameter) to particles greater than 1 mm in size, and the breakdown of these particles takes place after they have been adsorbed onto floc particles (12). Therefore, the best candidates for the contact stabilization process appear to be wastewaters containing very low soluble organic concentrations, such as certain industrial and municipal wastes. Colloids in domestic sewage have been estimated to represent as much as 52 percent of the BOD and 54 percent by weight of suspended solids. Industrial wastes from textile processes, paper and pulp mills, dairy plants and food processing industries are likely to have a high percentage of colloids (13).

The first full-scale activated sludge plants constructed in the United States were the North and South Side plants at Houston, Texas. Both plants utilized 33 percent of the aeration tank volume for sludge reaeration (14). Bioflocculation was developed in England in 1921. The first plant was placed into operation in 1930 and provided a 30 minute mixed liquor aeration time and an eight hour return sludge aeration time (15).

Ullrich and Smith (16) developed a similar type of process in the late 1940's while faced with expanding the existing Austin Sewage Treatment Plant in Texas. They proposed a short contact time of 15 to 20 minutes. In pilot plant studies of their biosorption

process, BOD and suspended solids removals of 85 to 95 percent were realized. It was believed that the biosorption process had certain advantages over the conventional activated sludge process. Less aeration tank capacity was required because the stabilization or reactivation was accomplished with the settled and concentrated sludge, and not with the mixed liquor. A minimum of plant and process adjustments was required for a wide range in raw sewage flows and loads. The process was also found to work well on raw sewage, eliminating the need for primary clarification and the associated costly disposal of raw sludge and the odors which are common to the operation of primary clarifiers.

Eckenfelder (17), working independently of Ullrich and Smith, also developed the biosorption process in the late 1940's. Pilot plant investigations of a cannery waste (tomato and apple processing wastes) demonstrated that BOD reductions of greater than 85 percent could be obtained. For a tomato waste the contact tank and reaeration tank detention times were 25 and 110 minutes respectively. In treating the apple waste a longer contact period was required to attain high process efficiency. The process exhibited an ability to sustain shock loads without serious detriment to the overall plant efficiency. It was also concluded that food processing wastes of variable BOD could be more economically treated by this process than by conventional activated sludge treatment. Based on these data a plant was constructed in 1952 to treat the wastes from the H. J. Heinz factory in Chambersburg, Pennsylvania (18).

The total aeration time required for good contact stabilization operation on domestic sewage is approximately the same as that required of the conventional activated sludge process (15). The newer modifications of sludge reaeration differ from the older processes primarily in the percentage of total aeration tank volume utilized for sludge reaeration. The older designs incorporating reaeration generally only allotted 10 percent or less of the total aeration volume for sludge reaeration. In current practice, the sludge reaeration volume constitutes about 68 to 70 percent of the total aeration volume (14).

THEORETICAL APPROACHES TO SUBSTRATE REMOVAL

There is considerable controversy regarding the actual mechanism which is involved in the cellular removal of organic material from wastewater. According to Borrough (19), two schools of thought have arisen from this controversy. The first school is based on an adsorption/absorption approach while the second school is based on an enzymatic approach. The second school of thought is really an extension of the first.

In the adsorption/absorption approach the wastewater mixes with activated biological sludge and the suspended solids are coagulated by and adsorbed onto the activated sludge cells. Concurrently, a large portion of the soluble organic matter is rapidly absorbed into and stored in the sludge cells as a reserve food supply. Aeration of the mixed liquor results in the synthesis of sludge, the production of carbon dioxide and water and, hence, the removal of further

dissolved biodegradable matter. The activated sludge process is represented by three phases as shown in Figure 3: the log growth phase, in which initial removal of BOD on contact of the waste with the activated sludge is achieved by storage within the cell; the steady state phase, in which BOD is removed in direct proportion to the biological sludge growth; and the endogenous phase, in which the biological cell material is oxidized by endogenous respiration. In the reaeration tank of the contact stabilization process absorption of the adsorbed organics, oxidation of the absorbed materials, and the stabilization of the sludge to ready it for a fresh load of wastewater were suggested to occur.

In the enzymatic approach the importance of enzymes in the removal of organic matter is stressed. Permease enzyme systems are thought to mediate the transport of exogenous soluble substrate into the cell where enzymatic reactions in metabolic pathways are completed for the synthesis of new cells. In addition extracellular hydrolases are secreted by the cells to hydrolyze long polymeric substrates into smaller units that can be transported into the cell by the permease system.

An enzyme can be defined as a protein of high molecular weight (from 10,000 to 1 million) that changes the rate of a chemical reaction but does not affect the nature of the final products (20). Acting as a catalyst, an enzyme is effective in small amounts, usually unchanged in the reaction, hastens the reaction to equilibrium without affecting the equilibrium of a reversible chemical equation,

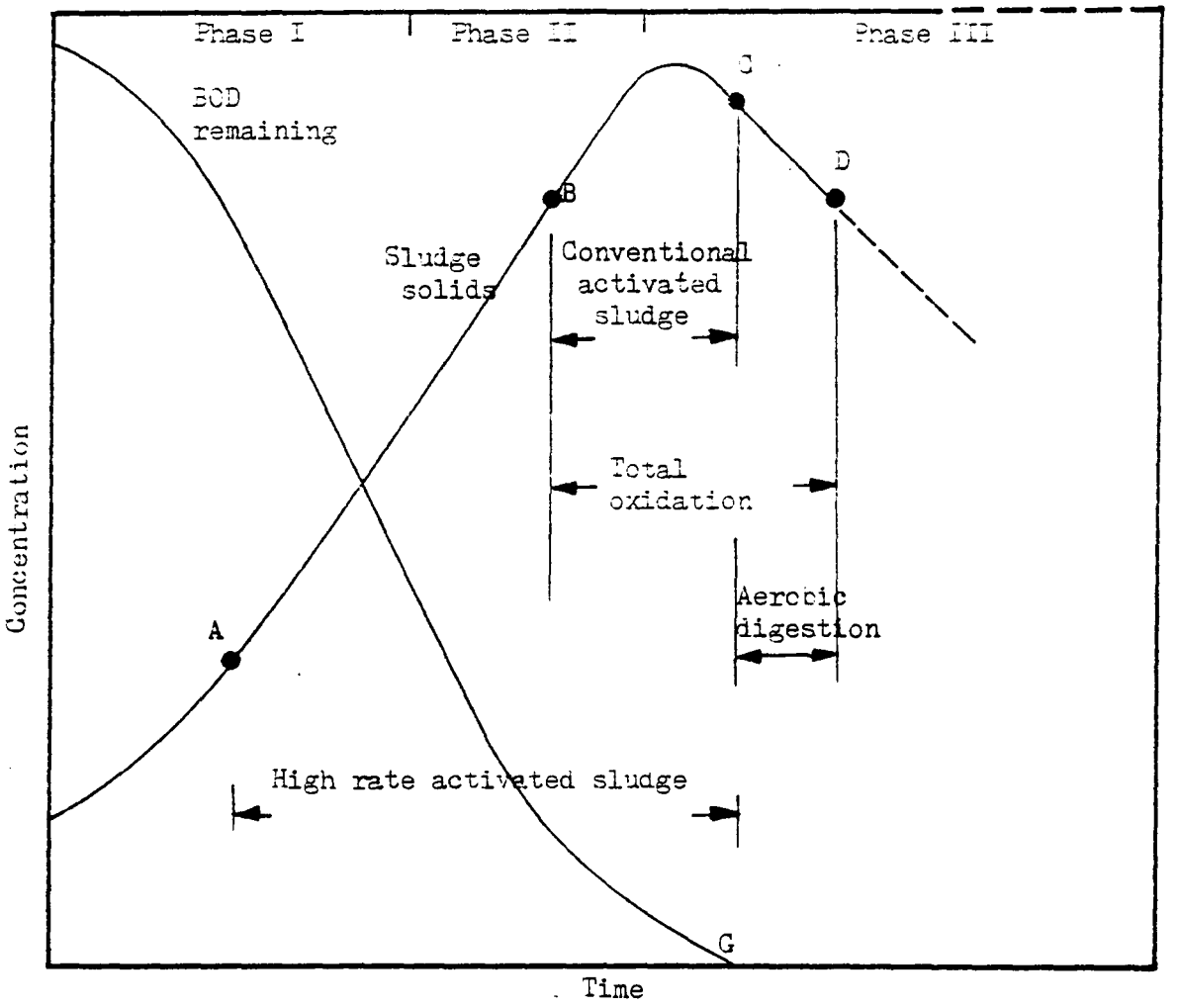

Figure 3. The three phases of the activated sludge process (after Borrough, 19).

and exhibits substrate specificity, which implies that the cell must produce a different enzyme for every substrate it uses. Exactly how an enzyme lowers the energy of activation of a chemical reaction is not known. An increase in temperature increases the rate of chemical reaction. However, at high temperatures the heat tends to destroy enzymes so that no further reactions can occur. Therefore, biological phenomena have optimum temperatures.

In metabolic processes, the substrate molecule must come in contact with a specific enzyme and, since the enzyme molecule is very large when compared to the substrate molecule and the substrate must combine with the enzyme at a precise spot on the enzyme surface, a relatively large number of substrate molecules are required to increase the probability of correct collision. An enzyme reaction can be represented by the following:

$$\underset{\text{enzyme}}{(E)} + \underset{\text{substrate}}{(S)} \longrightarrow \underset{\text{enzyme-substrate complex}}{(E)(S)} \longrightarrow \underset{\text{product}}{(P)} + \underset{\text{enzyme}}{(E)}$$

The enzyme, functioning as a catalyst, forms a complex with the substrate, which is then converted to a product and the original enzyme. The product may then be acted on by another enzyme. A sequence of such complexes and products may be formed before the final end product is produced. When the rate of reaction no longer changes with an increase in the substrate concentration, it can be assumed that the enzyme surface is saturated with substrate.

As previously stated, the exact mechanism of organic substrate removal from wastewater by activated sludge is not fully

understood but is believed to be delineated by an adsorption/absorption approach or an enzymatic approach. A review of the contributions of other investigators is presented in an attempt to support or refute these postulated approaches.

McKinney (3) states that early researchers, notably Smith (16) and Eckenfelder (17), observed an uptake and release phenomenon upon mixing raw sewage and activated sludge together in an aeration vessel. After removing samples at regular intervals for settling, and measurement of the BOD of the settled supernatant, a curve was constructed. This curve is shown in Figure 4. As can be seen, there is an immediate drop in soluble BOD followed by a rise, and then a second and final decrease. The contact stabilization process takes advantage of the high and immediate sorptive properties of the activated sludge by using a short contact tank hydraulic detention time that will still insure a good effluent. In the reaeration tank the adsorbed colloidal organic material is believed to undergo enzymatic solubilization resulting in an increase in the BOD, followed by another BOD reduction until all the biodegradable material has been utilized.

Weston and Eckenfelder (21) proposed that oxidative biological waste treatment is a threefold process consisting of initial high rate removal of BOD on contacting biologically active sludge, removal of BOD in direct proportion to biological cell growth, and oxidation of biological cell material with concurrent low-rate removal of BOD. In a later article, Eckenfelder and Weston (22) postulated

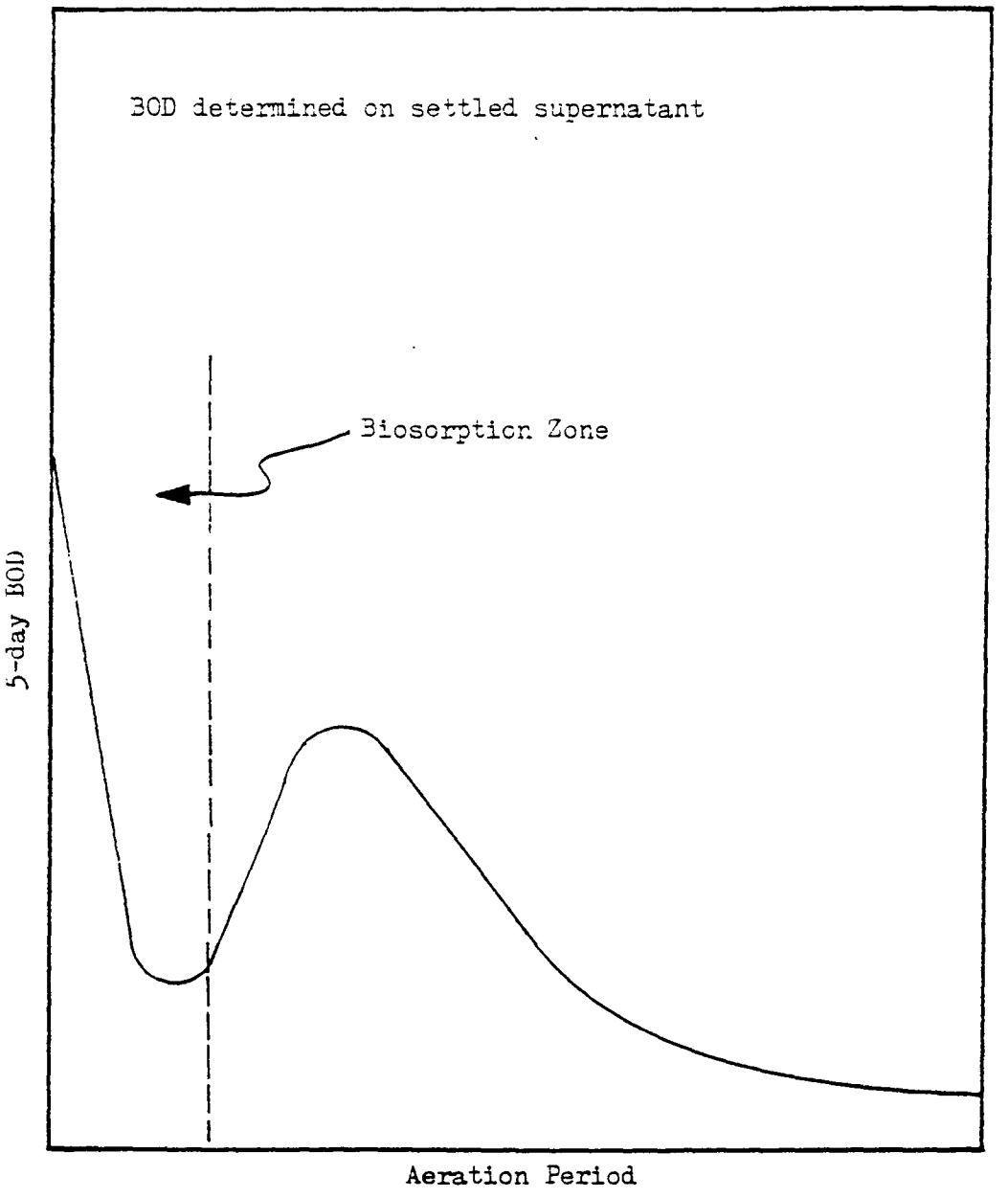

Figure 4. Variation of 5-day BOD of raw sewage-activated sludge mixture with aeration period (after McKinney, 3).

that soluble BOD, when removed from solution, reacts with enzymes associated with biologically active slimes and sludges. Various solubility and diffusional mechanisms take part in this reaction and the reaction rate can, therefore, be considered to be proportional to the concentration gradient across a membrane. Suspended or colloidal solids are removed by coagulation, entrainment, adsorption, and oxidation of components made soluble by enzymes. Eckenfelder and O'Connor (23) suggest that the state of the organic matter being removed will also influence the rate of BOD removal and oxidation in the activated sludge process. For example, sewage in which the organic matter is present in colloidal form is removed more rapidly from solution than a soluble organic substrate. Figure 5 shows relationships between BOD removals in the two phases of contact stabilization. This is supported by Zablatsky, Cornish and Adams (24) who stated that BOD in colloidal form was removed more quickly by the zoogleal masses moving through the solution than was BOD in the soluble form. However, stabilization of the colloidal BOD may take longer than for the soluble BOD.

When organic matter is removed from solution by microorganisms, two basic phenomena occur: oxygen is consumed by the organism for energy, and new cell mass is synthesized (7). The organisms also require energy for maintenance of cells and it is commonly assumed that they undergo auto-oxidation of their cellular mass for this purpose. These reactions can be illustrated by the following general equations:

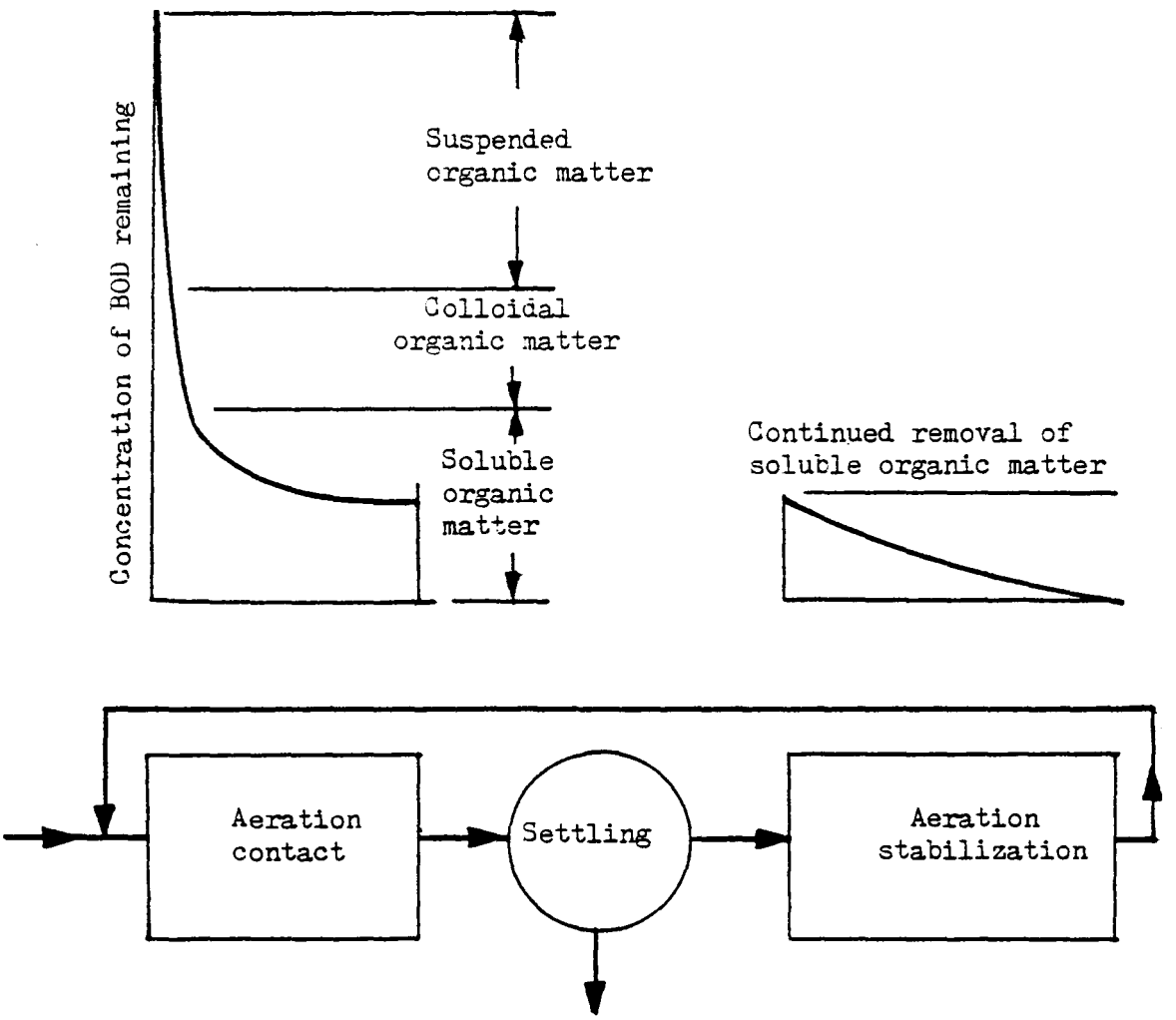

Figure 5. Schematic representation of the contact stabilization process (after Eckenfelder, 7).

Organic Matter Oxidation

$$C_x H_y O_z + (x + y/4 - z/2)\ O_2 \longrightarrow x\ CO_2 + y/2\ H_2O - \Delta H \quad \ldots(5)$$

Cell Material Synthesis

$$n(C_x H_y O_z) + n\ NH_3 + n(x + y/4 - z/2 - 5)\ O_2 \longrightarrow$$

$$(C_5 H_7 NO_2)_n + n(x-5)CO_2 + (n/2)(y-4)\ H_2O \pm \Delta H \quad \ldots(6)$$

Cell Material Oxidation

$$(C_5H_7NO_2)_n + 5nO_2 \longrightarrow 5nCO_2 + 2n\ H_2O + n\ NH_3 - \Delta H \quad \ldots(7)$$

where:

ΔH = heat of reaction

x, y, z = factors which may be positive or zero depending on the compound involved.

The empirical composition of the cells ($C_5H_7NO_2$) was arrived at by Porges, Jasewicz and Hoover (25) in their studies with 0.1 percent dried skim milk.

The volatile fraction of activated sludges will vary, depending on the nature and age of the sludge. Eckenfelder and Weston (22) have demonstrated that for a purely microbial sludge a volatile content of 90 percent can be expected. However, as the sludge solids are increased in a system (sludge age is increased) the volatile content will decrease because the increased endogenous respiration results in an increase of inorganic matter in the sludge mass, and, inert solids in the wastewater are trapped in the floc. The authors suggested that various mixed wastes have shown that 25 to 50 percent of the BOD removed is oxidized and the remainder synthesized to new sludge, neglecting endogenous respiration. It was also pointed out that sludge is destroyed by oxidation when the organic loading is insufficient to support active growth. Extensive endogenous

respiration will produce a sludge of low activity and reactive capacity. This was graphically depicted by Eckenfelder and O'Connor (23) and is presented in Figure 6.

Eckenfelder (7) states that BOD removal from a waste by a biological sludge may occur in two phases, an initial high removal of suspended, colloidal, and soluble BOD followed by a slow progressive removal of soluble BOD. The mechanisms responsible for the BOD removal are: removal of suspended matter by enmeshment in the biological floc which is rapid and dependent upon adequate-mixing of the waste with the sludge; removal of colloidal material by physicchemical adsorption on the biological floc; and biosorption of soluble organic matter by the microorganism. There is some doubt as to whether the latter mechanism is the result of enzymatic complexing or is a surface phenomenon and, therefore, whether the organic matter is held to the bacterial surface or is within the cell as a storage product, or both. All three mechanisms occur immediately on contact of the sludge with the waste although the colloidal and suspended material must undergo sequential breakdown to smaller molecules in order that it may be made available to the cell for synthesis. With respect to the contact stabilization process, the author states that effective removal in the contact tank requires sufficient active sludge to remove the colloidal and suspended matter and a portion of the soluble organics. The detention time in the reaeration tank must be sufficient to stabilize these organics. If it is insufficient, unoxidized organics will be carried back to the contact tank and the removal

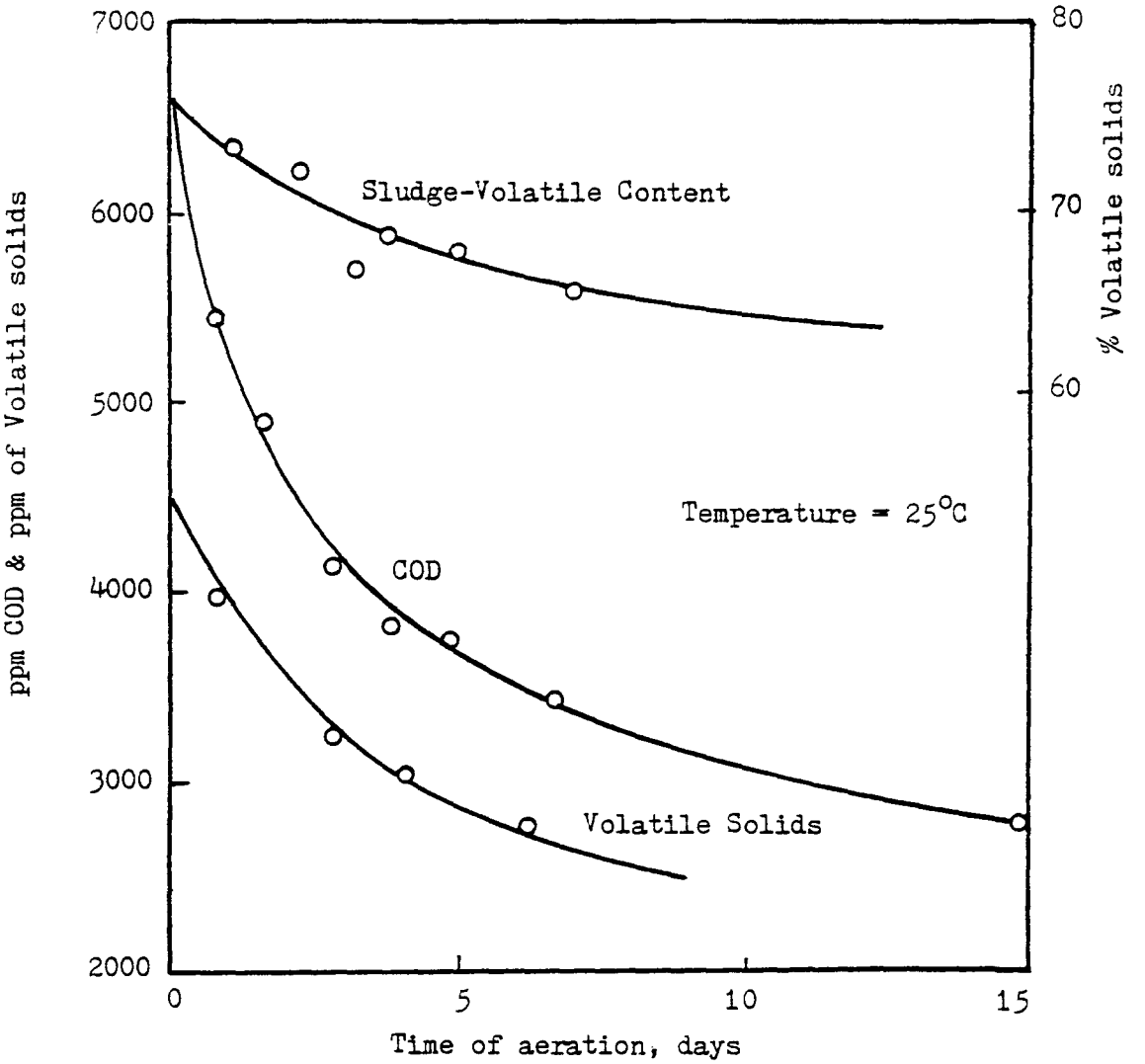

Figure 6. Endogenous respiration of activated sludge (after Eckenfelder and O'Connor, 23).

efficiency will be decreased. The sludge will lose its high initial removal capacity in the contact tank if the reaeration period is too long, because the sludge will undergo excessive oxidation. Increasing the detention time in the contact tank will increase the amount of soluble organics removed and decrease the required stabilization time. Too large a detention time in the contact tank negates the requirement for sludge stabilization by reaeration and the process essentially reduces to that of a conventional activated sludge process with sludge reaeration. Increasing the microbial mass also decreases the required aeration time because the organic loading per unit solids becomes less.

In a study by Porges, Jasewicz, and Hoover (26) on skim milk, an observed organic removal and storage ability indicated that cells loaded with glycogen and other storage products could be settled leaving a clear effluent. However, aeration of the sludge would have to continue to assure rapid oxidation of the stored COD. The depleted or stored cells could then be used again for further organic removal from the waste. This proposal is suggestive of the contact stabilization process.

In studies with soluble substrate of glucose, lactose, and α-methyl glucoside, Siddiqi, Engelbrecht, and Speece (27) investigated the kinetics of initial substrate removal. Their objectives were to determine the mechanism involved in the initial rapid removal of soluble organic substrate, and the change in sludge activity with stabilization time.

In their studies, oxidation and synthesis in an activated sludge system were believed to be performed by bacteria through the aid of enzymes. A schematic representation of a bacterial cell and its biochemical activities are shown in Figure 7. Exogenous soluble organic matter is transported by permeases into the bacterial cell. The intracellular enzyme systems can be divided into two broad functional categories: the hydrolases, and the synthesis and respiration enzyme systems. Not shown, in Figure 7, are the extracellular hydolases.

These enzyme systems may not be present in the cell at all times but may be produced by the cell in response to an extra cellular stimulus, such as by substrates. Such enzymes are termed inducible enzymes. Enzymes which are normal constituents of the cell are called constitutive enzymes.

In a conventional activated sludge system the biological mass is in contact with the substrate for a six to eight hour period, which is sufficient to allow the organisms to synthesize inducible enzymes. On the other hand, in the contact stabilization process the biological mass is in contact with the substrate for only a short period of from 0.5 to 1.0 hour. Therefore, for contact stabilization to operate correctly, the biological sludge should have a complete set of preformed enzyme systems at the time they are introduced to the substrate in the contact tank.

The enzymatic approach helps to explain why the organic removal rate appears to be independent of the organic substrate

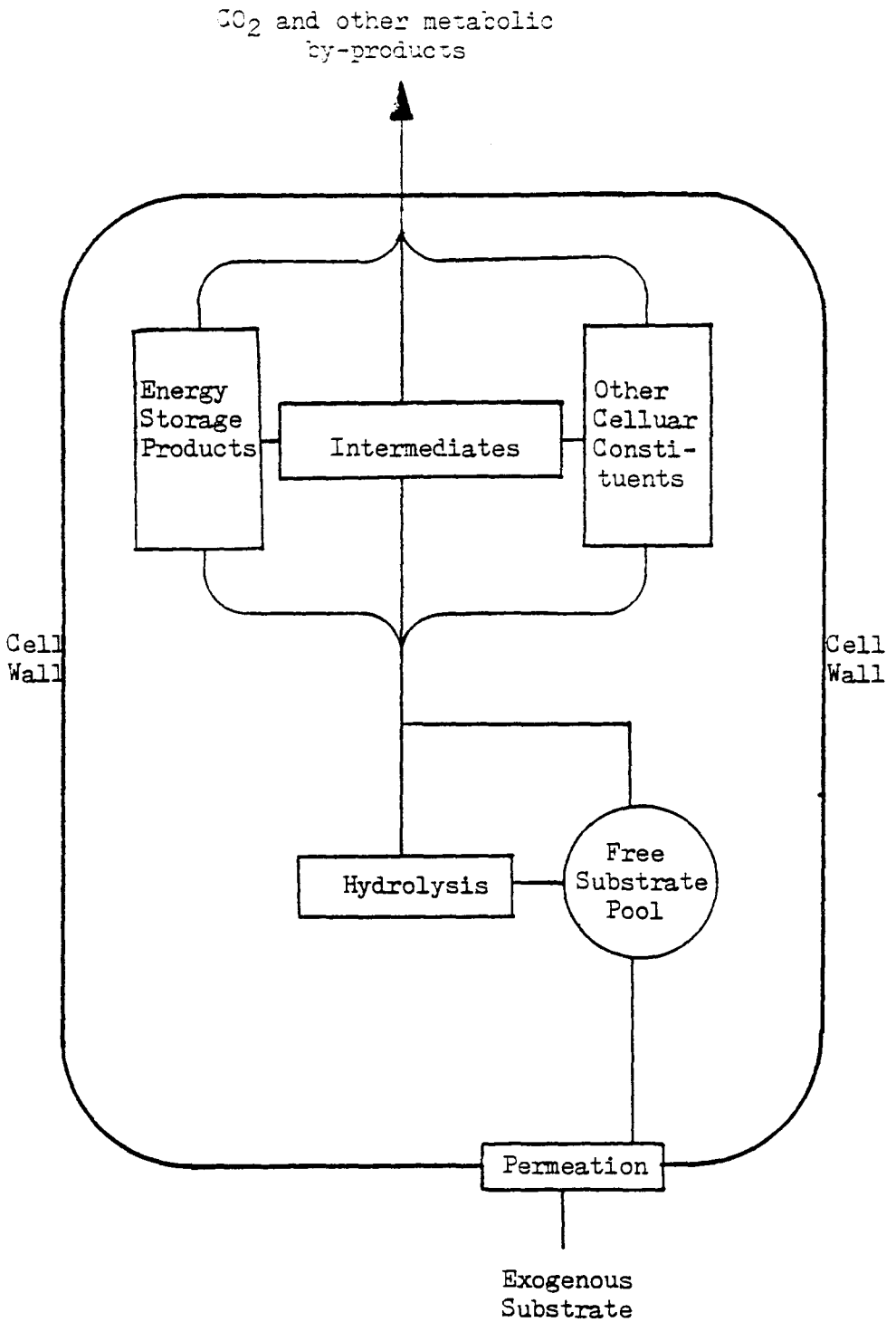

Figure 7. Schematic representation of a bacterial cell and its biochemical activities (after Siddiqui, et al., 27).

concentration at high organic substrate concentration, but proportional to the organic substrate concentration at low organic concentrations, as proposed by Eckenfelder (7). According to Figure 7, the exogenous soluble organic matter is transported to a substrate pool inside the cell by permease enzymes. Transport of substrate to this pool increases with an increase in substrate concentration until the transport enzymes become saturated. Subsequent utilization of the substrate from the inside pool by hydrolysis, as well as respiration and synthesis enzymes, allows for increased permeation of substrate into the cell. Therefore, the rate of removal can be saturated by increasing the F/M ratio.

An initial increase in sludge activity during stabilization has been recognized and attributed to the solubilization of entrapped suspended and colloidal matter and/or assimilation of intracellular stored organic matter, which makes the sludge again capable of rapidly removing additional organic matter. Siddiqi et al. (27) concluded that the initial increase in substrate removal capacity during the stabilization period was due to the production of synthesis and respiration enzyme systems. However, the loss in substrate removal capacity of sludges with prolonged stabilization was concluded to be due to inactivation of inducible enzyme systems.

In a discussion following Siddiqi's article, McCarty (28) demonstrated that their data can be reasonably represented by the substrate utilization equation (equation 2) proposed by Monod (4). McCarty questioned the need for a stabilization period for degradation

of sorbed soluble organic material since substrate metabolism can be obtained with short contact times. Therefore, if substrate metabolism approaches completion during the contact period, a stabilization period for degradation of sorbed material should not be required. However, it was suggested that in order to insure the greater sludge ages required for maintenance of a flocculent and well settling activated sludge, a stabilization period was still necessary. The results of the study indicated an advantage of the single-stage completely mixed activated sludge process, to that of the contact stabilization process. In the completely mixed system the sludge is always kept in contact with a small concentration of substrate, whereas in the contact stabilization process there is a starvation cycle and loss in the activity of inducible enzymes. McCarty stressed the economic advantages of contact stabilization because of a smaller aeration tank volume and a ready reserve which can be drawn upon in case of a toxic waste introduction into the contact tank.

In contrast, Weston (15) proposed that enzymes produced during the stabilization of mixed liquor in the conventional process may be sufficiently water soluble to be lost in the plant effluent. However, enzymes produced during the stabilization period of the contact stabilization process are reacted with incoming substrate and, thus, higher initial removal of substrate is conceivable.

Two studies were conducted by Smallwood (29) to determine whether organic material is removed from a substrate by adsorption

or whether the controlling mechanism is actually some other mechanism such as assimilation. In the first study, on a soluble organic material, a selective poisoning technique was employed using low concentrations of sodium azide, which is known to block assimilation processes while stimulating oxidation. The second study dealt with the fate of a complex colloidal organic material, consisting of algae that had been grown in an atmosphere of radioactive carbon dioxide, $C^{14}O_2$, and thus was uniformily tagged with C^{14}. He concluded from the studies that the significant and controlling mechanism in the removal of soluble organic substrate from sewage by the activated sludge process was assimilation and not adsorption while, for a colloidal organic substrate, the controlling mechanism was adsorption and not assimilation. The latter conclusion is questionable because the author admitted difficulties in measuring the C^{14} accurately. The author assumed that, because of the presence of 40 percent of the radioactivity in the supernatant liquid after 6 hours of aeration, the balance of the radioactivity was adsorbed onto the cells surface.

Banerji, Ewing, Engelbrecht and Speece (13), commenting on Smallwood's article, pointed out that the colloidal waste chosen was perhaps not representative of colloidal wastes and further stated there was a good possibility that the balance of the radioactivity had been incorporated into the cell protoplasm. Using potato starch as a colloidal organic substrate, they conducted studies to determine the starch removal pattern by activated sludge under various F/M ratios. An immediate uptake of starch on contact

with the sludge was observed and was referred to as adsorption. It was proposed that the amount of exoenzymes secreted by the cells into the medium was insignificant, which was contrary to the findings reported in the literature that α-amylases are liberated into the medium. A separate experiment on the starch degrading activity of cell wall fragments and whole cells verified their proposal. On the basis of their findings, they postulated that the mechanism of starch removal by activated sludge occurs as follows: adsorption of a portion of the starch on the sludge; degradation of the adsorbed starch by cell-wall-associated enzymes systems into smaller degradation products; utilization of the starch degradation products for assimilation; and adsorption of the starch in the medium onto the cell sites vacated by the degraded starch molecules, or degradation of the starch in the medium by small amounts of extracellular enzymes in the medium.

Utilizing starch as the only source of substrate carbon, Maier (30) sought to gain insight into the behavior of a typical colloidal waste matter. Two model reactor systems were used to determine where mass transfer limited the rate of substrate removal, and where cell metabolism limited the rate of utilization. A well-mixed batch reactor was used to simulate conditions of high rates of mass transfer and a film flow reactor was used to simulate the conditions of the slime layer found in a trickling filter. Results showed that mass transfer was a rate-controlling factor in the laminar flow reactor. High rates of mass transfer were obtained in

the well-mixed reaction vessel used to simulate activated sludge conditions, due to eddy current transport, and, as a result, mass transfer was generally not a limiting factor. Maier also suggested two mechanisms for colloid removal. The microorganisms either can evolve a mechanism for engulfing the molecule and then secrete the appropriate catabolic enzymes within the cell confines, or they can excrete the necessary enzymes into the surroundings to cause the solubilization reaction sequence to proceed outside the cell. The engulfing process or direct passage of large molecules into the cell is generally associated with higher forms of life than with bacteria, especially since the rigid wall of the bacteria would make it difficult to develop a mechanism for allowing large molecules to pass through the cell wall.

Jones and Brown (31) suggested that a solubility index (SI_o) may provide better insight into the nature of the waste being treated than the classical parameters, such as BOD_5 and solids determinations, which have been used to date. The solubility index was defined as the ratio of the soluble organic carbon divided by the total organic carbon, or the soluble COD divided by the total COD, or the soluble BOD divided by the total BOD. A synthetic waste of fish meal and glucose was used to obtain the desired range of solubility indexes. A definite relationship between percent COD removal and the ratio of soluble COD to total COD in the waste was observed and is shown in Figure 8. There was also observed a definite relationship between percent total solids removal and the ratio of dissolved

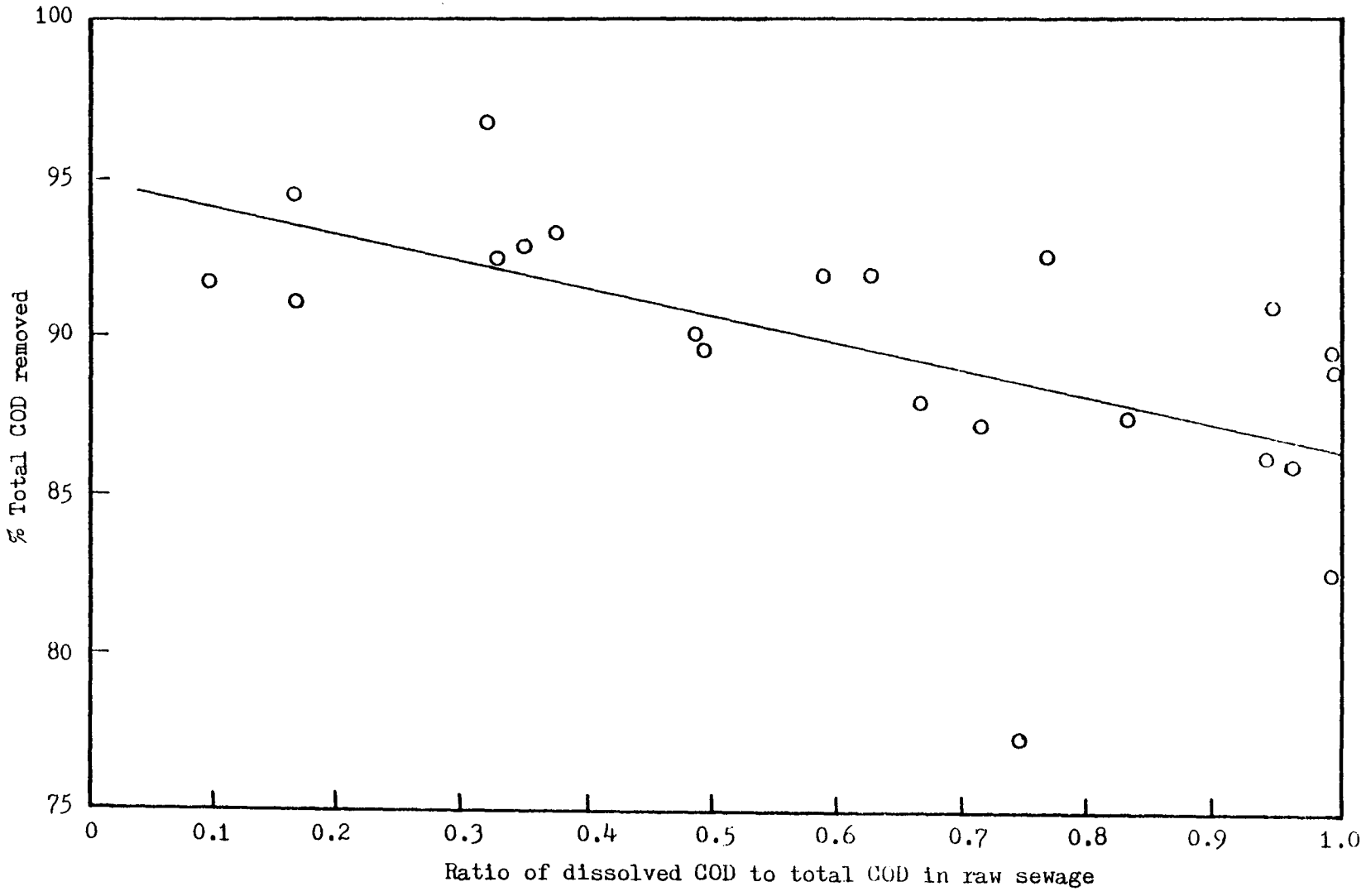

Figure 8. Relationship between the COD solubility index (SI_o) and the total COD removed (after Jones and Brown, 31).

solids to total solids as shown in Figure 9. These latter two curves suggest that a substantial amount of removal took place due to absorption. When the SI_o was 1 the COD removal ranged from 82 to 89 percent and, since adsorption could not have taken place in the absence of particulate matter, absorption must have been responsible for the entire removal. The study also indicated that a rapid increase in COD removal occurred as the MLSS was increased to about 3500 mg/liter, but little increase was accomplished for greater MLSS concentrations. Figure 10 depicts this and shows that below a MLSS of 3500 mg/liter the SI_o had a significant effect on the percent total COD removal. This data tends to confirm the statement by Zablatsky et al. (24) that removal of BOD in the soluble form may require a greater sludge concentration than does the BOD in the colloidal form.

In a later study, Jones (32) attempted to model the contact stabilization process and proposed several mechanisms for the removal of organic matter by a well conditioned activated sludge: absorption, adsorption, and simple physical entrapment. He also proposed that in a batch test run if activated sludge were mixed with raw domestic sewage, then aerated, and aliquots were withdrawn every 5 minutes, allowed to settle and the supernatant BOD evaluated, a response similar to Figure 11 would be expected. If most of the BOD were soluble (Solubility index $\rightarrow$ 1) the response would be similar to the other curve shown on Figure 11. In an actual batch experiment with an 80 percent colloidal waste, Jones suggested that the BOD data

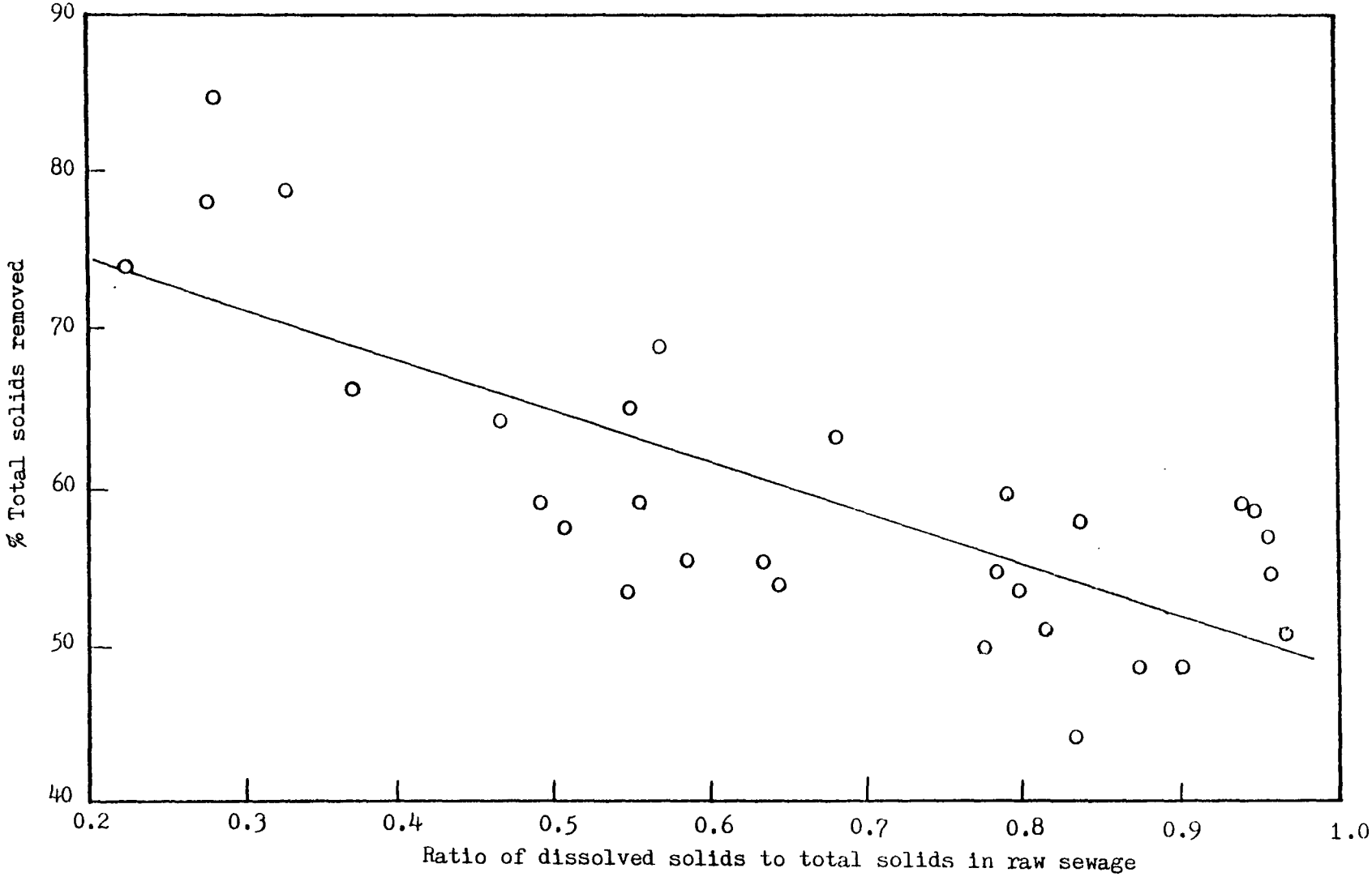

Figure 9. Relationship between the solids solubility index (SI_s) and the total solids removed (after Jones and Brown, 31).

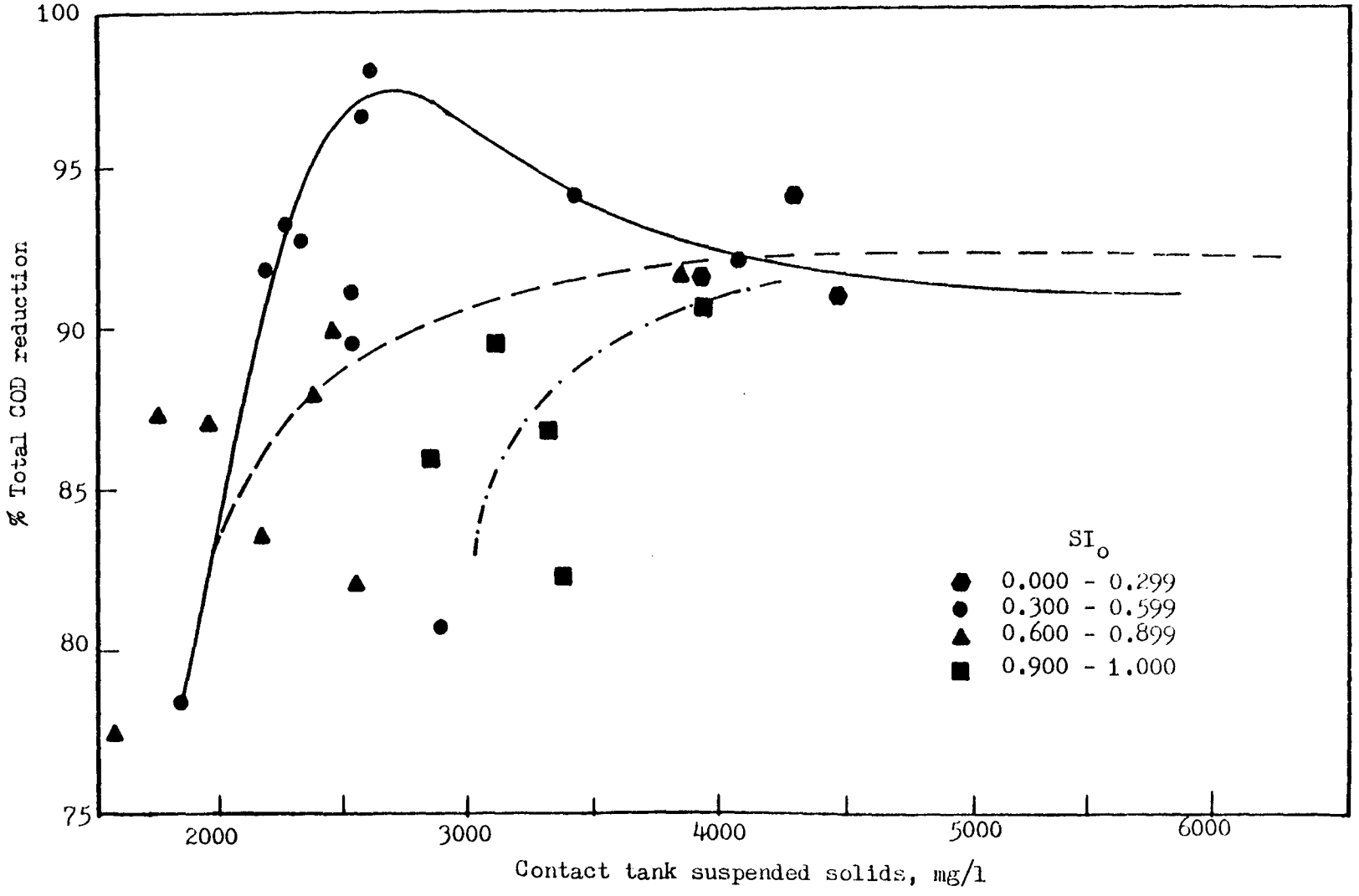

Figure 10. Relationship between COD removal and MLSS at a variety of organic solubility indices (after Jones and Brown, 31).

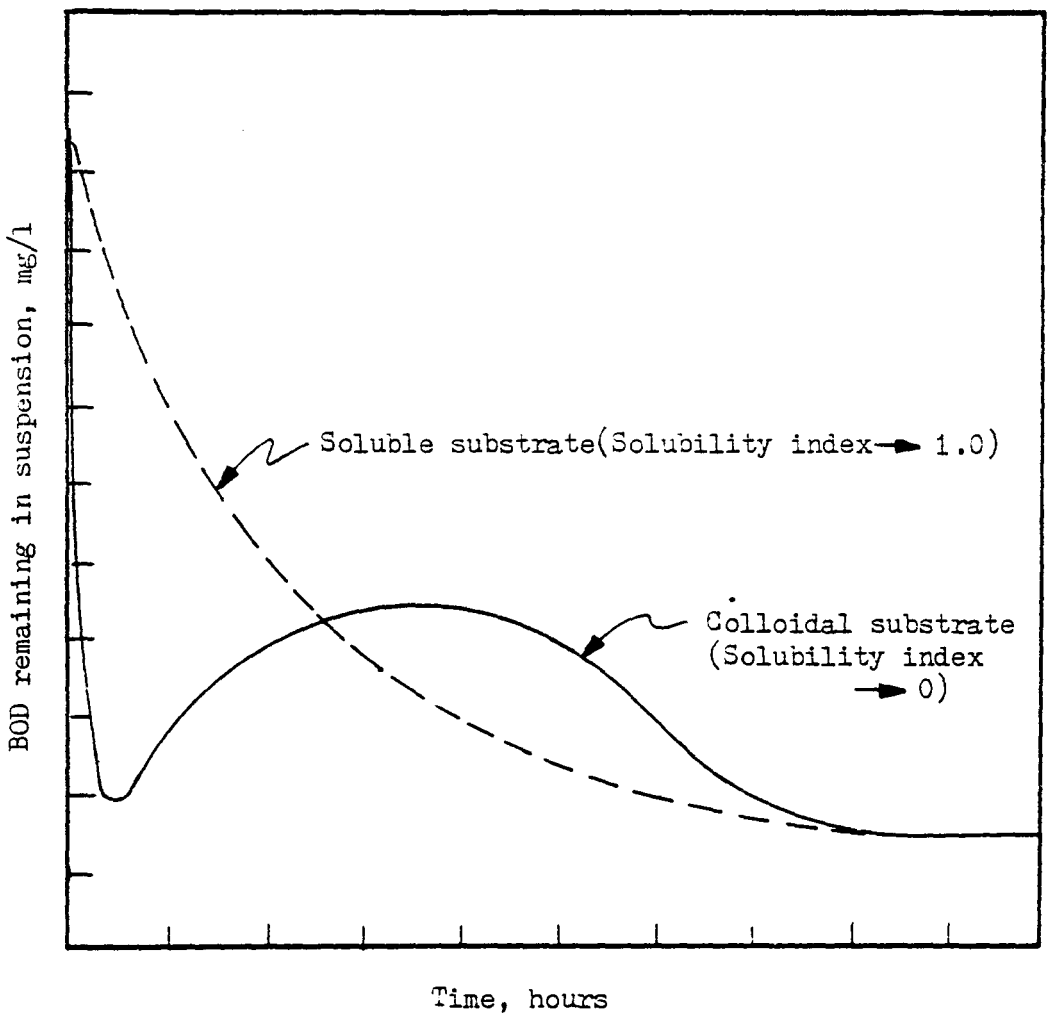

Figure 11. BOD removal in batch test with waste water containing primarily soluble or particulate BOD (after Jones, 32).

demonstrated an uptake and then a release of BOD as depicted in Figure 12. However, only one data point was observed to be truly suggestive of a BOD release and this point appears questionable, considering the accuracy of the BOD test as stated by Standard Methods (33).

McKinney (34) took a different point of view from his earlier text (3) by stating that, based on preliminary data, the contact stabilization process was not believed to function as originally theorized. He stated that current research suggested that all of the stabilization of organic matter occurred in the mixing tank and endogenous metabolism of the activated sludge occurred in the reaeration basin. Thus, there appeared to be a greater need for more oxygen in the mixing tank rather than in the reaeration tank. Failure to supply adequate oxygen in the mixing tank was pointed out as possibly being responsible for the decreased efficiency reported in some plants. However, as will be discussed later, the contact stabilization process as being applied presently only slightly resembles the system originally developed by early investigators (35).

Based on batch experiments, Bhatla, Stack, and Weston (36) suggested a method for evaluating BOD removal kinetics. In a batch biological oxidation system they suggested that after the addition of new food, the concentration of BOD moves in one direction as bioprecipitation, oxidation, and synthesis reactions remove BOD from the substrate. When the BOD has been substantially removed, there remains an essentially constant concentration of BOD, which

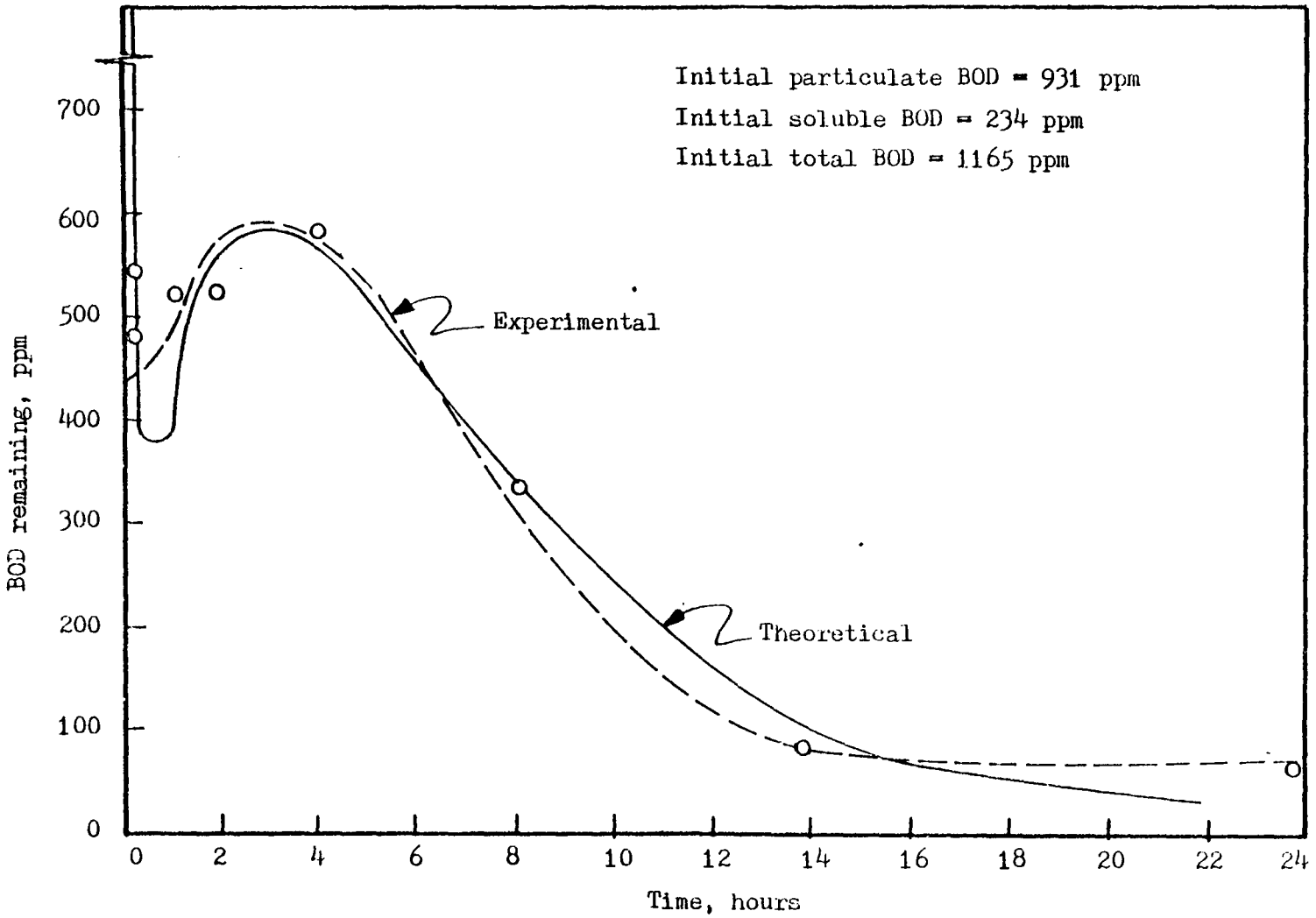

Figure 12. BOD remaining versus time for an industrial waste (after Jones, 32).

may decrease very slowly. A graphical model appears in Figure 13. A true process equilibrium exists where the bioprecipitation, oxidation, and synthesis reactions intersect with endogenous reactions. The process equilibrium points will follow the dashed curve in Figure 13 by keeping the F/M ratio constant and varying the initial BOD concentration. The BOD transfer rate can be calculated by the relationship

$$r_c = \frac{L_o - L_e}{L_e \; t_e} \quad \ldots\ldots\ldots\ldots (8)$$

where:

r_c = BOD transfer rate, time $^{-1}$

L_o = initial concentration of BOD, mass/volume

L_e = BOD concentration at substrate removal equilibrium, mass/volume

t_e = time at which equilibrium occurred, time

The authors suggested that a plot of the log of the BOD transfer rate versus the log of the organic loading determines the toxicity of the wastewater. This is shown in Figure 14. A slope of 45 degrees indicates that the mass of organisms used in calculating the loading ratio represents the transfer area. A slope exceeding 45 degrees is indicative of a toxic waste, because the transfer coefficient is decreasing significantly as the loading ratio is increased. The less than 45 degree slope is indicative of a normal and non-toxic wastewater.

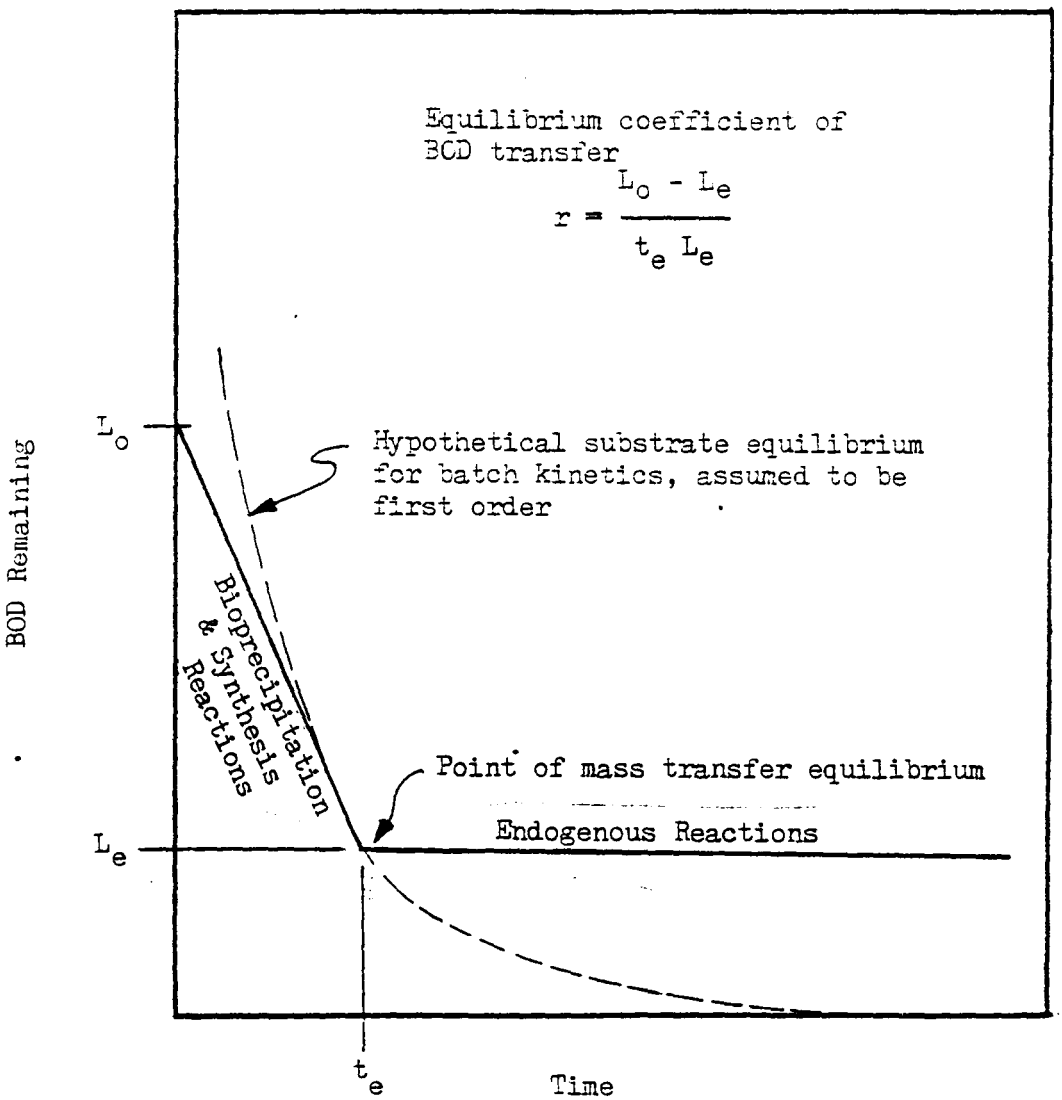

Figure 13. Graphical model of BOD transfer reaction in a batch system (after Bhatla, et al. 36).

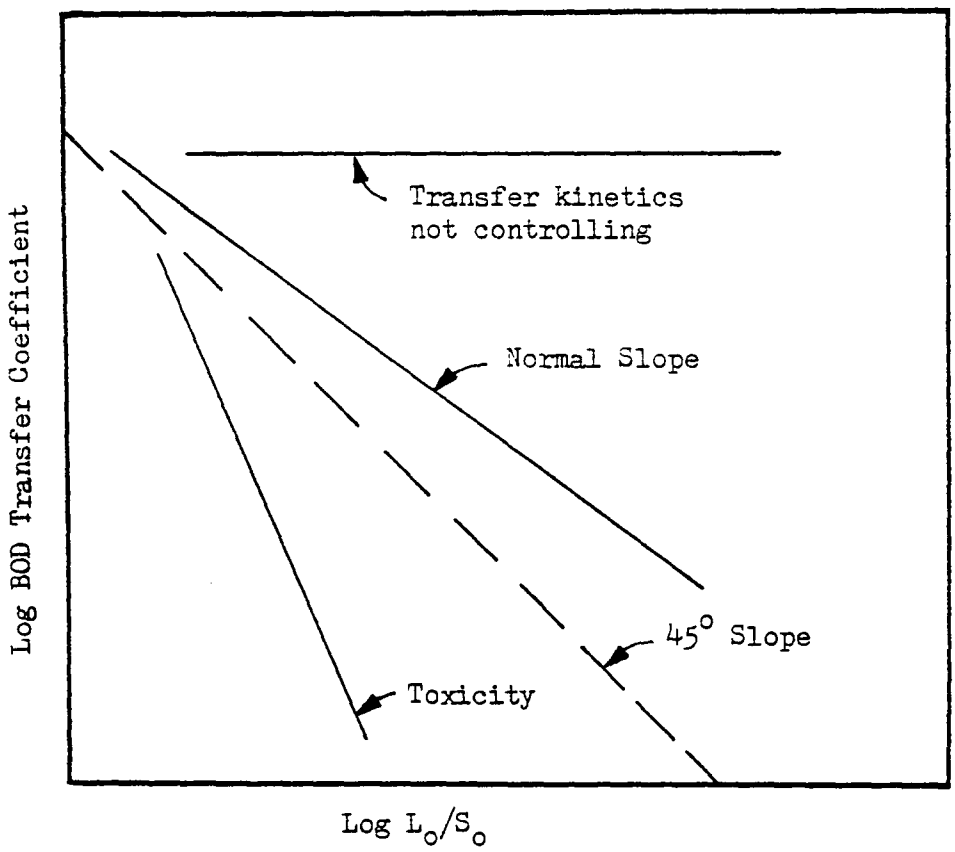

Figure 14. Typical correlations of BOD transfer coefficients with initial loading ratios (after Bhatla, et al., 36).

Kharajian (37), working with a colloidal yogurt substrate, applied the approach of Bhatla et al. (36) for toxicity and the results indicated that yogurt was a toxic substance to the microorganisms. Tests were run on BOD dilutions to determine if there was any toxicity in the waste and the results showed no indication of toxicity.

Bhatla et al. (36) suggested that for simple compounds which biodegrade readily, stabilization reactions occur almost as rapidly as BOD transfer occurs. More complex molecules may not degrade as easily. Transfer of these materials may occur by bioprecipitation, biosorption or other mechanisms. Stabilization rates as observed through oxygen uptake rates may proceed for some time after the BOD remaining indicates that BOD transfer has been accomplished. These relationships are illustrated in Figure 15. A stabilization rate is calculated in the same manner as the BOD transfer rate with the exception that the time at which equilibrium is reached is assumed to coincide with the time when oxygen uptake returns to the endogenous level. This relationship can be represented as follows:

$$r_s = \frac{L_o - L_e}{L_e \; t_s} \quad \ldots\ldots\ldots\ldots (9)$$

where:

r_s = rate of stabilization, time $^{-1}$

t_s = time when oxygen uptake returns to the endogenous level, time.

The total amount of oxygen utilized is assumed to be that necessary for endogeneous reactions plus that used in energy reactions. The area under the curve in Figure 16 represents the total quantity

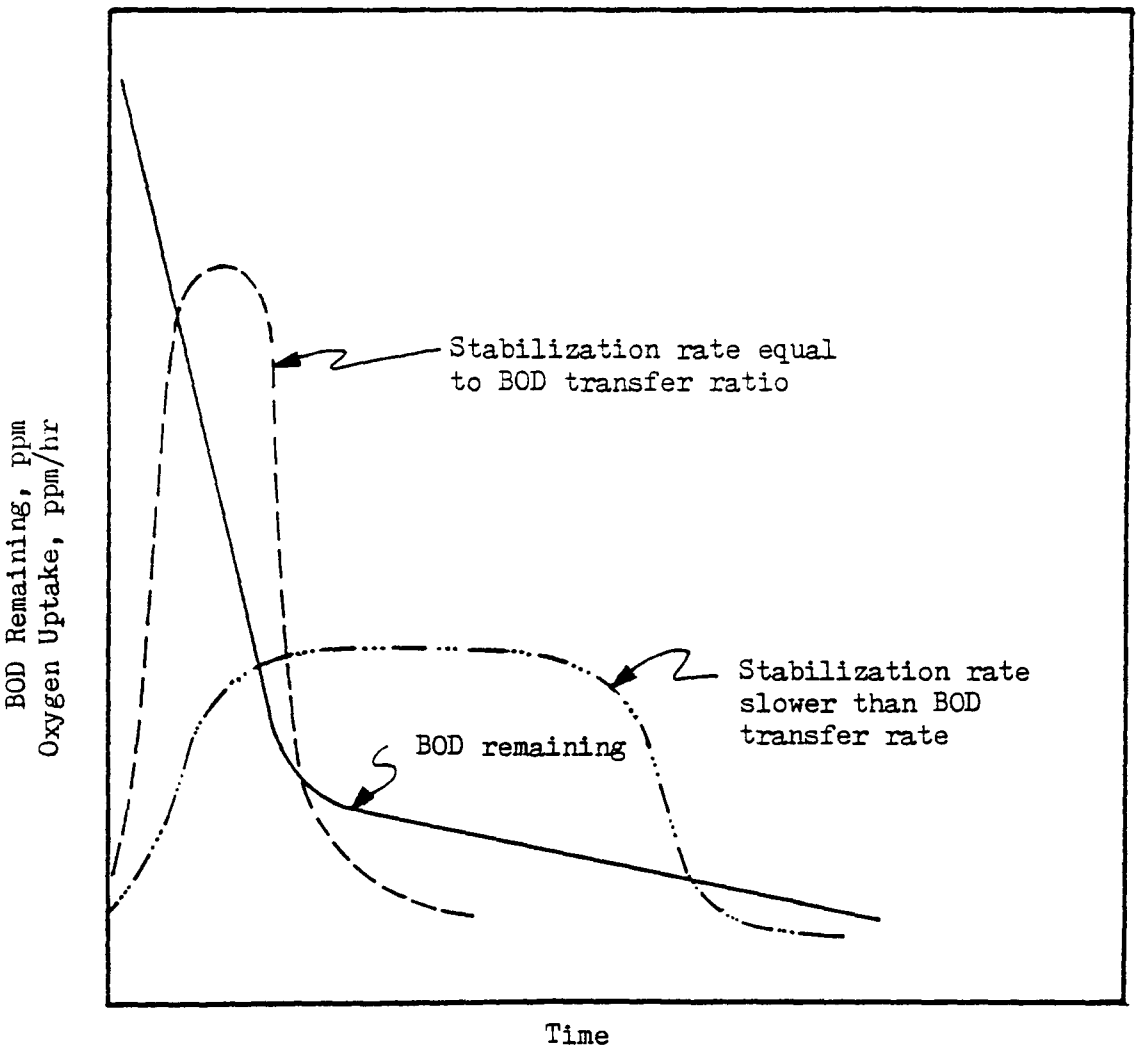

Figure 15. Typical relationship between batch kinetics of BOD transfer and stabilization reactions (after Bhatla, et al., 36).

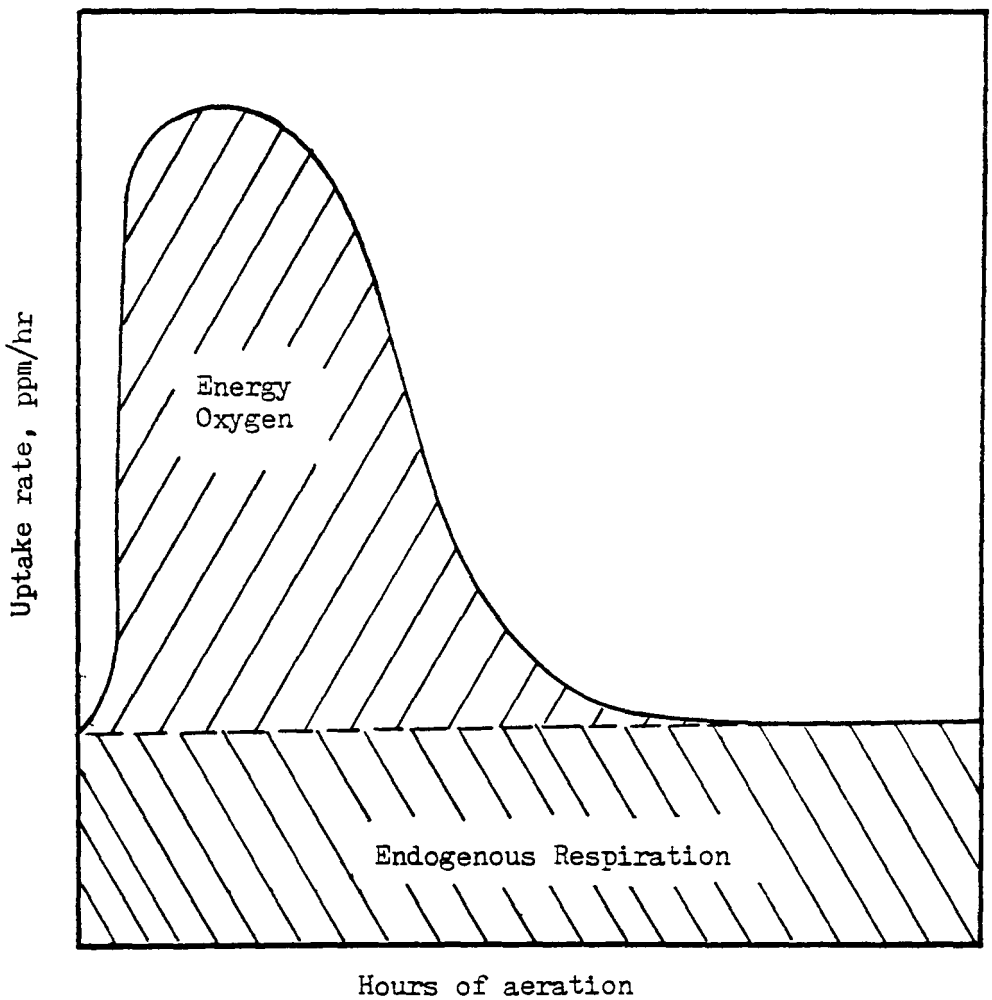

Figure 16. Consumption of oxygen by a batch biological oxidation system (after Bhatla, et al., 36).

of oxygen utilized. Bhatla et al. stated that energy oxygen is determined to be a definite quantity per quantity of COD removed, while endogenous requirements for oxygen are a definite quantity per unit mass of organisms present. However, in an actual tube run, Bhatla et al. observed an increase in the energy oxygen as the suspended solids increased and attributed this to organic material held in the biomass which had not completely biodegraded.

Armentrout (38), working with a domestic and two industrial wastes, found from his batch studies that the endogenous oxygen uptake per unit microorganism increased with increased F/M loadings for all three wastewaters. In contrast, the energy oxygen uptake per unit of COD utilized decreased as the F/M loading increased for all three wastewaters.

Kharajian (37), working with a colloidal yogurt substrate, observed an increase in total oxygen utilized per unit microorganisms as the F/M loading increased. The results also indicated that the endogenous oxygen uptake per unit microorganisms stayed relatively constant up to a loading of about 0.7 mg/l COD/mg/l MLSS. As the loading reached unity or greater the endogenous oxygen uptake increased sharply. The energy oxygen uptake per unit of COD utilized was also observed to remain constant until an F/M loading of one or greater was reached. At this point the energy oxygen uptake was found to decrease with an increase in the F/M loading.

Kharajian and Sherrard (39) performed batch type experiments over a wide spectrum of percentages of a colloidal material and initial loading conditions in order to determine if there was a substantial release of substrate after an initial rapid uptake. A basic nutrient solution was used and supplemented with differing portions of beef extract and yogurt. Their experiments demonstrated that no rapid uptake and subsequent release of organics occurred for the colloidal waste evaluated. Therefore, they concluded that not all colloidal wastewaters exhibit a rapid uptake, substantial release, and final drop in the concentration of organic material. They also concluded that simply because a wastewater contains colloidal organic material is no reason to specify a contact stabilization process.

Armentrout (38) also examined the uptake and release phenomenon postulated for the contact stabilization process. Batch experiments were conducted on four wastewaters: a synthetic non-colloidal wastewater, a domestic wastewater, a pulp and paper wastewater, and an acetate textile wastewater. The synthetic non-colloidal wastewater was run at one initial loading condition, with the other wastes run at three various loading conditions. The results of the experiments indicated that the wastewaters, for the most part, did not exhibit any significant uptake and release as a result of the colloidal content of the wastewaters.

In a study by Kharajian and Sherrard (40), a continuous flow bench-scale unit was operated under highly controlled conditions to investigate the parameters involved in the biodegradation of a

colloidal organic wastewater. The contact tank had an average hydraulic detention time of 2.2 hours and the stabilization tank had an average hydraulic detention time of 4.9 hours. A reproducible wastewater was used consisting of yogurt, beef extract and other nutrients at a specific colloidal percentage of 45 $\pm$ 3 percent based on the COD test. The mean cell residence time, θ_c, of the contact tank and the total system θ_c were varied. The results indicated high COD removals at low θ_c in the contact tank. High values of specific utilization were maintained in the contact tank because of the ability to recycle sludge with excellent sludge settling properties, and the low contact tank hydraulic detention time. A decrease in observed yield at lower θ_c values was found but was difficult to explain. An average of 82.8 percent of the microbial solids were present in the stabilization tank while 17.2 percent were present in the contact tank. An increase in MLSS with an increase in θ_c was also observed.

Kharajian and Sherrard (41) compared the process performance of both a completely mixed and a contact stabilization activated sludge process by performing a laboratory study in which both processes were operated side-by-side. There is considerable controversy as to how θ_c should be calculated. Many investigators believe that only the biomass in the aeration basin should be considered while others believe that total process system should be considered. Kharajian and Sherrard (41) compared the completely mixed and contact stabilization processes on a total system mean cell residence time basis.

The detention time in the contact tank and reaeration basin of the contact stabilization process was 2.2 and 4.9 hours respectively while the detention time in the completely mixed process was 5.9 hours. Organic constituents of the wastewater were beef extract and yogurt solution at a colloidal percentage of 45 ± 3% based on the COD test. When the completely mixed and contact stabilization processes were compared on a total system θ_c basis, the COD removal efficiency and specific utilization were found to be nearly equivalent and the maximum yield coefficient and the microorganism maintenance coefficient were also nearly equivalent. Other than hydraulic configuration, there was no difference in the performance of the two processes even though a substantial difference existed in the solids distribution between the contact and stabilization tanks of the contact stabilization process and the aeration and sedimentation basins of the completely mixed process.

MICROBIAL SUBSTRATE STORAGE

A portion of the assimilated organic matter which is removed from wastewater by activated sludge is believed to be stored by the microorganisms present in the sludge floc. It has also been assumed that exhaustion of this stored material must occur under prolonged aeration to insure that the sludge is in a suitable condition to be recycled. Two carbon and energy storage compounds, found in bacteria, have been identified and studied by microbiologists: glycogen, a glucose polymer, and poly-betahydroxybutyrate (PHB), a polymer of hydroxybutyric acid.

Glycogen is the polysaccharide of the animal body and has been referred to as animal starch. It resembles starch in appearance and has a molecular weight which may vary from one million to two million or more (42). Glycogen is similar to amylopectin in that it is a polysaccharide of D-glucose in α (1-4) linkage. However, it is more highly branched and a more compact molecule than amylopectin with branches occurring about every 8 to 12 glucose residues (43). A bacterial cell may be comprised of as much as 50 percent glycogen (25).

A viscous substance surrounds some bacterial cells forming a covering layer and the size of such a capsule is markedly influenced by the environment. The capsular substance is believed to be excreted from the cell and, because of its viscosity, is not readily diffused away. The capsule provides a protective covering and may also serve as a reservoir of stored food or a site for disposal of waste substances. The majority of the capsular material compounds are polysaccharides.

Within some cells, concentrated deposits of certain substances can be detected. Highly refractile globules occur in many bacteria, becoming more prominent as the cells age. They consist of granules of polysaccharides such as starch or glycogenlike compounds and granules of polymerized β-hydroxybutyric acid. These granules are not equally prominent in all bacterial species and their appearance is influenced by their environment as well as their age. It has generally been assumed that granules may serve the cell as a source of stored food, however, this is probably not their sole function (44).

Eckenfelder and Weston (22) postulated that the BOD which is removed immediately on contact of the sludge with the waste is stored in the cell for subsequent oxidation and synthesis. They defined storage as BOD which is removed from solution by sludges but which is not instantly metabolized. Immediately after aeration contact with the organic waste, they suggested that the sludge enters the active respiration storage phase and can be defined by the relationship

$$C_5H_7NO_2 + C_xH_yO_z$$

With continued aeration, the soluble BOD in the waste is removed by the sludge mass and then the microorganisms consume the stored material for metabolism and growth. After sufficient aeration to complete oxidation and synthesis of the BOD removed from the waste, the sludge is reduced to the endogenous form ($C_5H_7NO_2$).

Porges et al. (26) have suggested that the COD removed during purification serves many purposes. A part is utilized in cell synthesis, a part in the oxidation of organic matter, while the remaining portion is stored by the cell. In their study with skim milk it was determined that during the assimilative phase of growth a great deal of available COD was stored. The storage ability varied with the temperature, with lesser storage ability found at lower temperatures. At 30°C, in two hours, 1000 mg/l of sludge had removed 89 percent of 1125 mg/l available COD. Only 11 percent of the COD removed was oxidized to CO_2, 18 percent was converted to cell substance and 71 percent was stored. Glycogen was found to be the major storage

product of the sludge. The simple COD products removed from solution were converted to insoluble glycogen. When the stored material was calculated on a COD basis or a solids basis, a well aerated endogenous sludge was found to be able to store 50 percent of its own weight.

Walters, Engelbrecht, and Speece (45) defined a storage product as those cellular compounds which undergo rapid synthesis and rapid degradation upon exhaustion of the external substrate supply. Their study was concerned with determining the influence of the F/M ratio and substrate composition on the synthesis of storage products, and the influence of storage products on the ability of the activated sludge organisms to remove substrate. Throughout the study a stock unit, initially seeded with municipal activated sludge, was maintained on a once-a-day feeding schedule using a soluble substrate of yeast extract and glucose. Mixed liquor organisms to be used in each single experiment were removed 20 hr. after batch feeding, centrifuged, washed with a phosphate buffer solution and finally resuspended in a buffer solution. The organic substrate was then added and, during the ensuing aeration period, samples of the mixed liquor were removed for analyses. The variation in storage products was based on the protein content of the mixed liquor so that the change in storage products per cell could be estimated. Cellular protein is a stable component of the cell and the quantity of protein per cell does not usually change under different conditions. It was not considered desirable to express the variation in storage products by the change in the concentration of solids, since the

solid weight is not an accurate measure of the number of cells.

The variation in total carbohydrate content of the cells observed during metabolism of the substrate at four F/M ratios is depicted in Figure 17. There was an immediate high rate of increase in the carbohydrate of the cells in all units with the exception of the unit operating at the highest F/M. The rapid synthesis and subsequent rapid degradation suggested that much of the total carbohydrates of the cell were storage products. The variation in the other cellular storage product, PHB, is shown in Figure 18 and yielded similar results. The influence that the F/M ratio had on the maximum storage level in the cell is represented by Figure 19. The calculated maximum percentage of substrate COD that was channeled into each of the storage products is depicted in Figure 20. There was a decrease in the percentage of substrate used for carbohydrate synthesis at the higher F/M ratios and this may have occurred because the substrate was directed primarily into replicative pathways and not into storage pathways. The percentage of COD respired was calculated as the difference between the amount of substrate COD removed from solution and the increase in solids-COD.

To determine the change in the substrate removal capacity as the storage material underwent degradation during the stabilization period, a separate experiment was conducted. An acclimated sludge and a glucose and yeast extract substrate were aerated for 1.25 hours. This permitted the microorganisms to accumulate high storage levels of carbohydrate and PHB. The MLSS were then removed by

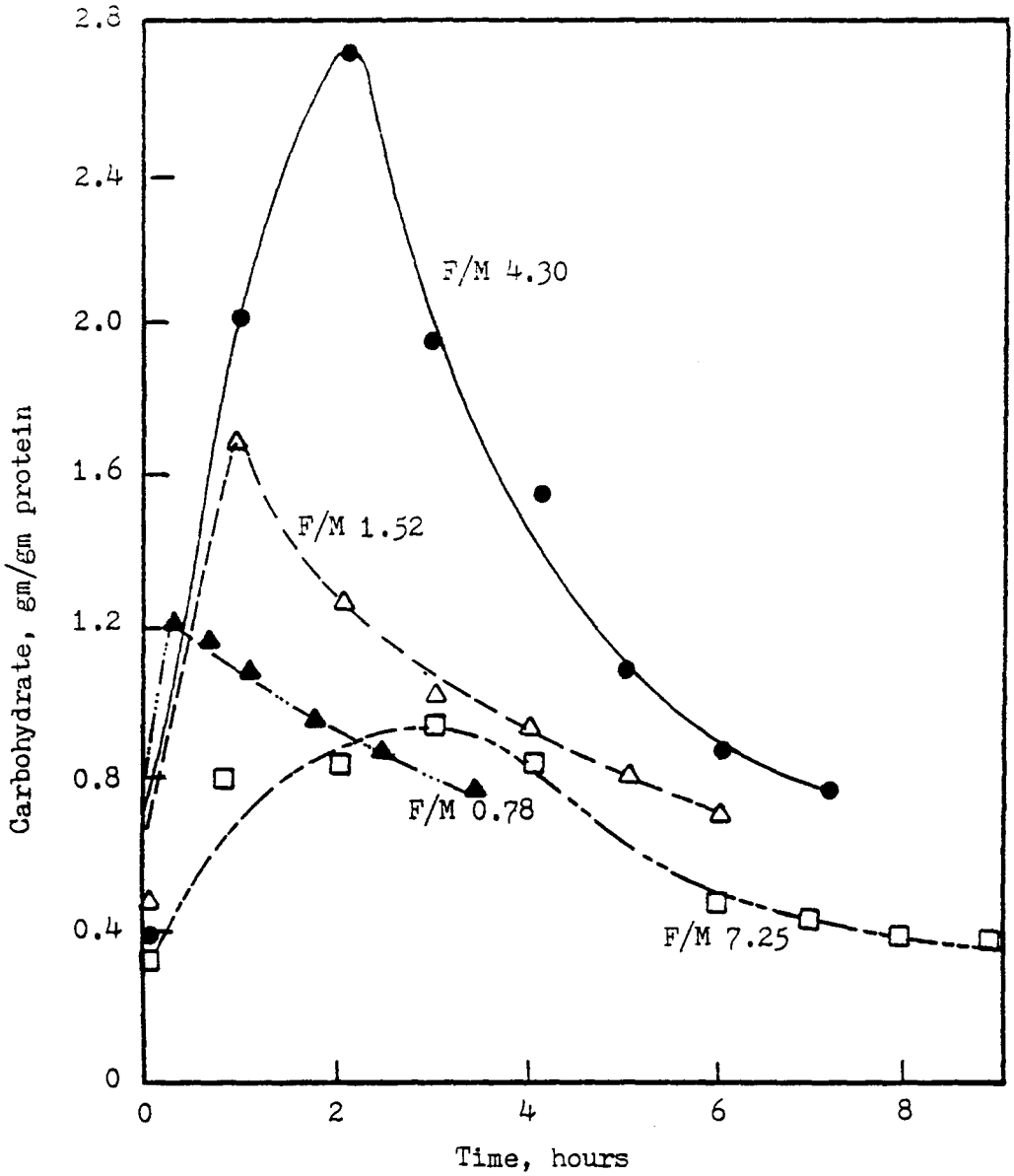

Figure 17. Variation in cellular carbohydrate during metabolism of substrate by activated sludge acclimated to F/M ratios indicated (after Walters, et al., 45).

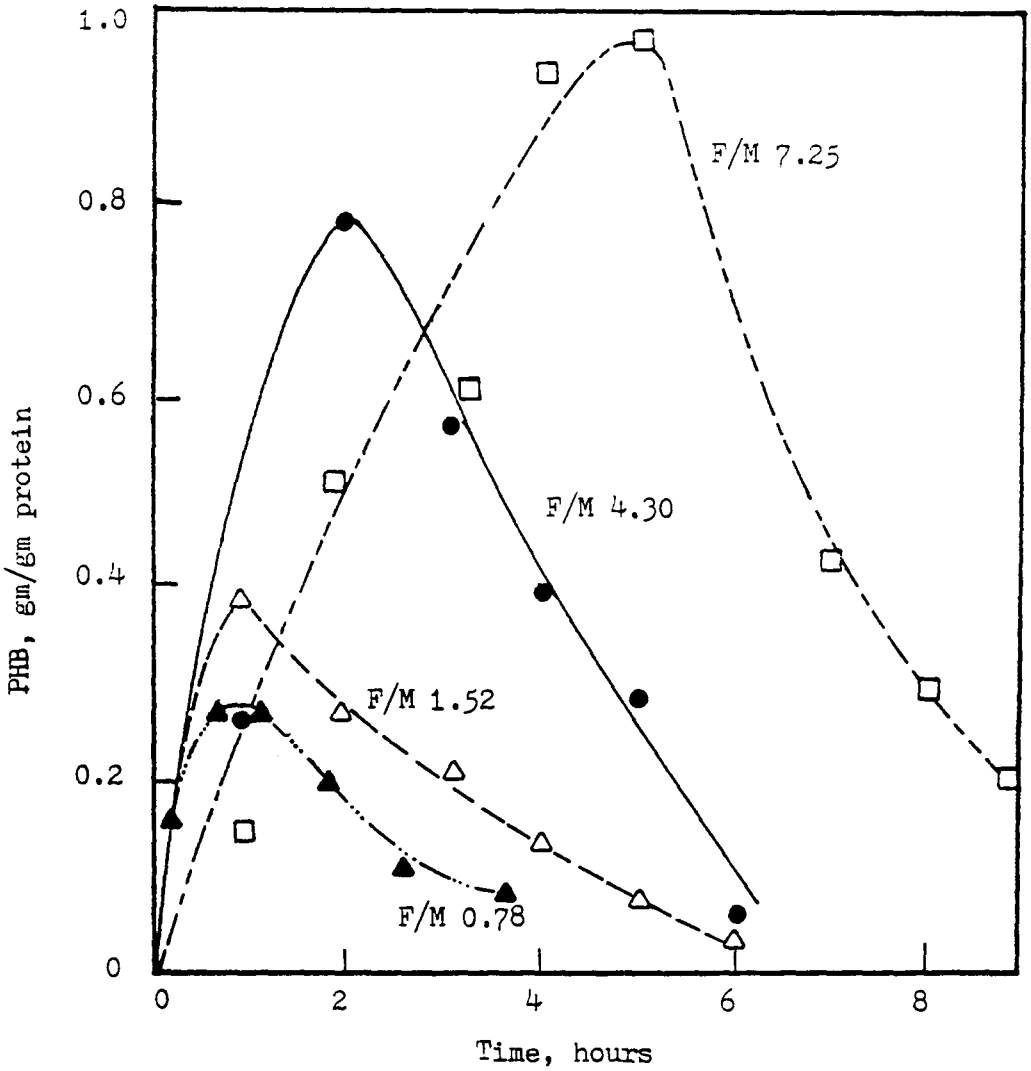

Figure 18. Variation in cellular PHB during metabolism of substrate by activated sludge acclimated to F/M ratios indicated (after Walters, et al., 45).

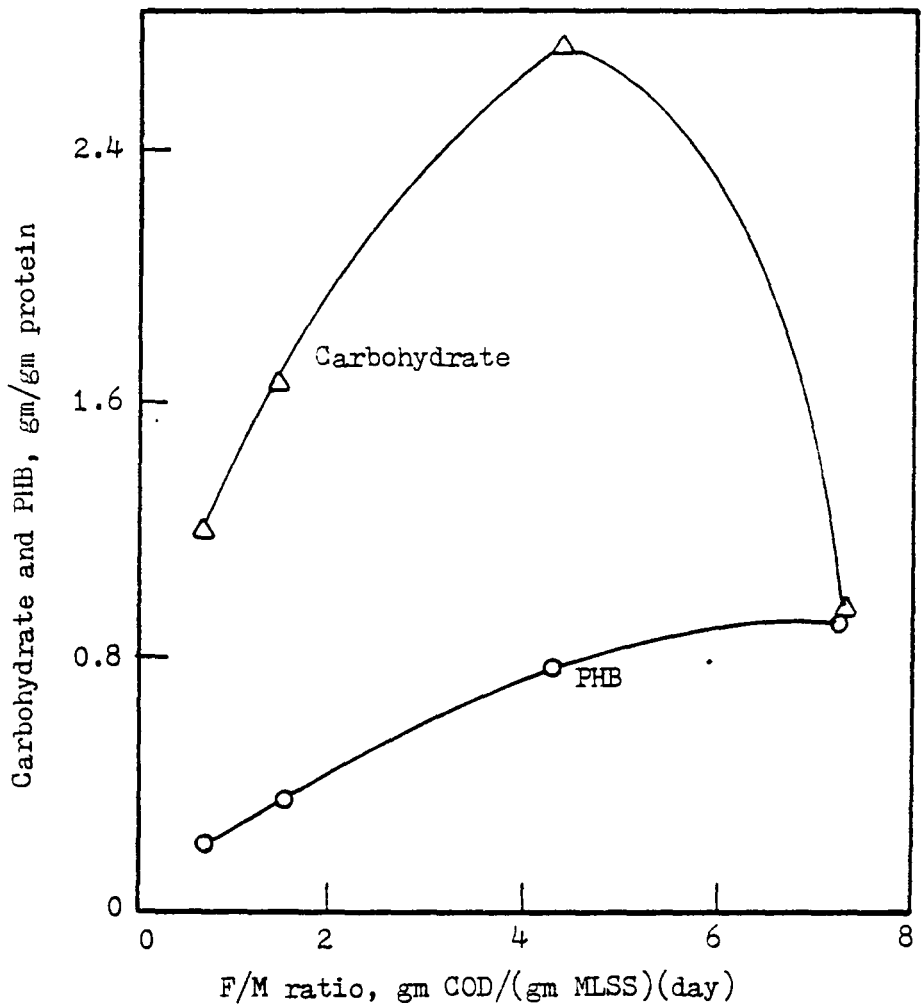

Figure 19. Influence of F/M ratio on the maximum amount of cellular carbohydrate and PHB stored by activated sludge microorganisms (after Walters, et al., 45).

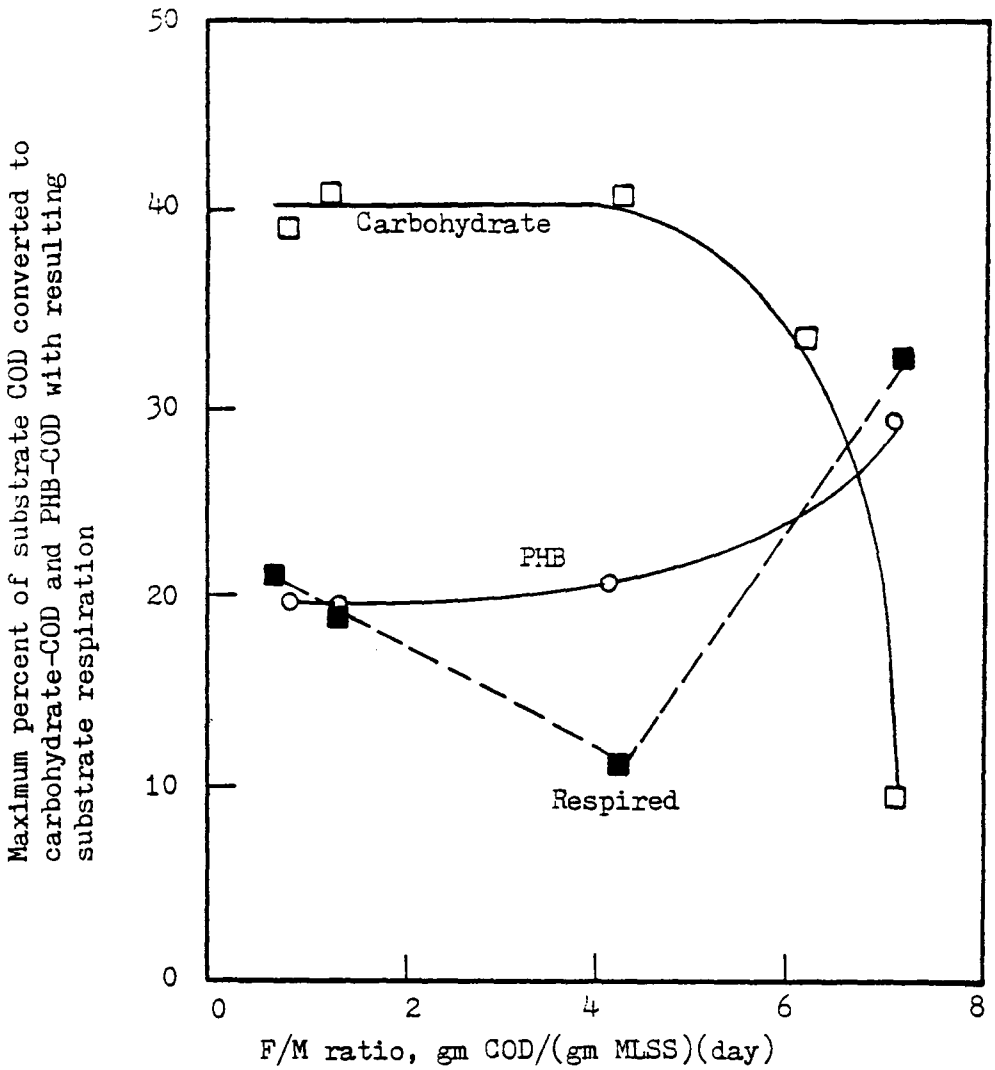

Figure 20. Influence of F/M ratio on the distribution of substrate into carbohydrate and PHB storage products and respiration (after Walters, et al., 45).

centrifugation, washed with a phosphate buffer solution and resuspended in tap water, and then aerated. At specific times, samples were removed and placed in a small aeration reactor and then fed the same concentration of organic substrate to which they were initially exposed. After an aeration period of 20 minutes the contents of the small reactor were centrifuged. The supernatant was filtered through a 0.45 μ filter and the pellet frozen until it could be analyzed. Carbohydrate, protein and PHB determinations were made on the pellet while the COD of the supernatant was determined. These analyses are graphically represented in Figure 21. It was observed that the activated sludge exhibited an increasing ability to remove substrate as the period of stabilization increased and as the stored material within the cell decreased during stabilization. When the carbohydrate and PHB content was at a minimum level in the cell, the amount of substrate removed by the cell was at a maximum, and as a result the authors suggested that the quantity of storage products in the cells may serve as one means for measuring the stabilization period. However, according to the authors, other operational characteristics, such as sludge settling, should not be neglected.

The storage function was also found to be specifically related to the type of synthetic waste being treated. For example, when units were acclimated to glucose, acetic acid and glutamic acid, only carbohydrate storage was observed and then only in the glucose-fed unit. No PHB storage was found.

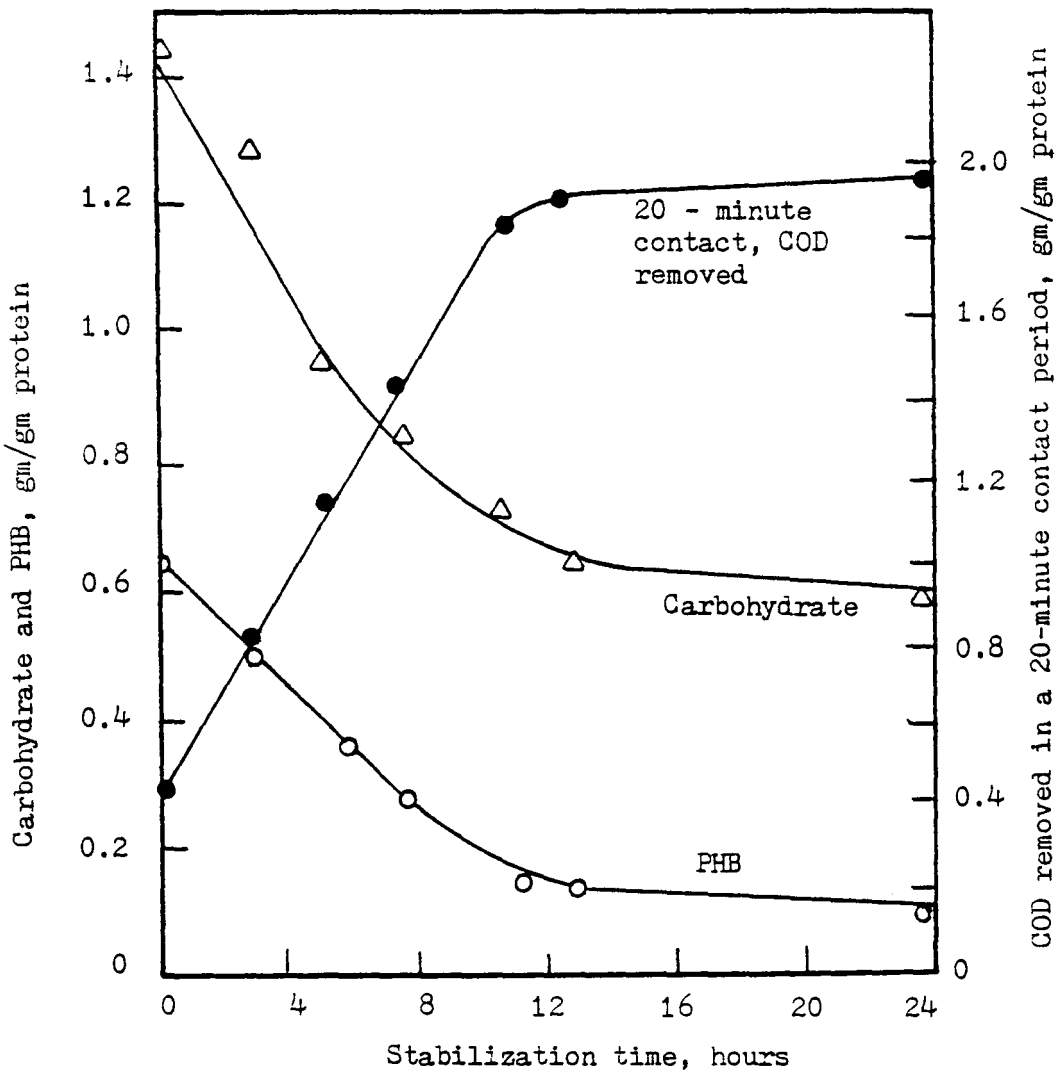

Figure 21. Variation in cellular carbohydrate, cellular PHB, and substrate-removal ability during activated sludge stabilization in a fill and draw unit (after Walters, et al., 45).

In concluding, the authors stated that substrate storage does not occur universally in all activated sludge systems but with a balanced source of organic materials, such as proteins, carbohydrates and lipids, one may expect to find cellular storage. The storage capability becomes more specific as the nature of the substrate becomes more specific and, with certain singular substrates, there may be no storage capability at all.

OPERATING EXPERIENCE WITH CONTACT STABILIZATION

Contact stabilization has been demonstrated to be economically attractive, with respect to a lower capital expenditure, because of the reduced aeration-tank volume requirements with little or no loss in treatment efficiency. As previously discussed, Ullrich and Smith (16) found that less aeration tank capacity was required because the real aeration was accomplished in the settled sludge and, therefore, rather than subjecting the entire waste flow to some required aeration period, only the return sludge was aerated for an extended period. Thus, aeration-tank volume requirements were reduced. In this respect a process modification may offer a relatively inexpensive alternative to needed plant expansions because of hydraulic or volumetric loadings. The authors also found that a minimum of plant and process adjustments were required for the diurnal flows and waste concentrations. Primary clarification was shown to be unnecessary and was eliminated, along with its accompanying odors.

Eckenfelder and Grich (18), after conducting pilot plant studies and two years of plant operation with the contact stabilization process, demonstrated that, in treating cannery wastes, BOD removal efficiencies of 80 to 90 percent could be achieved on wastes from a variety of products. The operational costs compared favorably to the conventional activated sludge process.

Zablatsky et al. (24), faced with a 100 percent increase in wastewater flow to the existing treatment facilities at Little Ferry, New Jersey, considered the possibility of contact stabilization in order to keep construction costs to a minimum. They concluded that the contact stabilization process could offer a satisfactory and economical solution to the problem of providing future plant facilities. The efficiency of the process was suggested to be largely dependent on the effectiveness of the stabilization process, the temperature of the sewage, and the uniformity of incoming BOD and suspended solids. Shock loads of industrial wastes had little effect on the process. Frothing, which had been a problem with a conventional activated sludge process was reduced to negligible amounts under the contact stabilization process.

A survey conducted by Haseltine (14) in 1961, compared plants practicing sludge reaeration with those using the conventional activated sludge process: The survey indicated that at BOD-to-solids loadings of 30 to 50 lb BOD/day/100 lb of sludge solids the two processes give substantially the same BOD reduction, while at BOD

loading of less than 25 to 30 lb BOD/day/100 lb. of sludge solids the BOD reduction for the reaeration process is two to four percent lower than that obtained by a conventional plant. Neither the sludge reaeration period nor the mixed liquor aeration period was found to be of any real significance. The author advised using an aeration time of greater than 30 minutes for the contact tank. Sludge reaeration periods were found to range from two to five hours except in designs where anaerobic supernatant was added directly to the stabilization tank, in which case, a reaeration period of 18 hours was suggested. In general, reaeration plants were operated at the same BOD-to-solids loadings as were conventional plants, achieved approximately the same degree of treatment, and required the same amount of air. Where sludge bulking was a problem at a conventional plant adoption of sludge reaeration resulted in easier operation, a higher average sludge density index (SDI), and less periodic fluctuation in the SDI value.

Grich (46) evaluated several existing waste treatment plants using the contact stabilization process and found several advantages in using this process rather than the conventional activated sludge process. The separation of the adsorption and oxidation phases into two units, as in contact stabilization, afforded greater flexibility because each phase could be independently controlled. The reservoir of sludge in the stabilization tank increased the plant's capability of handling shock loadings and, because of a reduced aeration volume, there was an economic savings in capital expenditure.

Weston (15) also found that the contact stabilization process can withstand wide variations in wastewater quality and has excellent recuperative capacity to toxic discharges because of the large supply of sludge in the stabilization tank. The process was also found to be economically advantageous only where initial removal of waste was a significant factor, and where the BOD of the waste was not sufficiently high that initial removal was relatively small. The settling properties of the sludge from the contact stabilization process were found to be better than for a conventional process, however, the difference did not appear to be so significantly great as to justify higher sedimentation tank overflow rates.

In a review of several existing small contact stabilization package plants, Dague, Elbert and Rockwell (35), have suggested that the method currently being applied to small plants only slightly resembles the system originally developed by earlier researchers (16,17). Changes introduced in applying the process to the treatment of low, widely varying wastewater flow volumes have resulted in less operational stability and significant increases in aeration-tank volume requirements when compared to the original concept. The current process also falls short in operational stability and process efficiency and it appears that modifications, other than contact stabilization, could provide increased process stability and efficiency at no increase in cost. Rather than use the original 15 to 30 minute contact time, current design standards require a three hour detention

time in the contact tank based on raw water flow. As previously discussed, McKinney (34) has indicated that in current systems all of the stabilization of organic matter in the raw waste occurs in the contact tank and, therefore, only endogenous respiration occurs in the reaeration zone. Thus, the BOD removal efficiency no longer depends only on the ability of the activated sludge to absorb pollutants from the raw waste, as with the original process, but rather on the ability to convert the waste organics into biological mass and efficient separation of the sludge by sedimentation.

Dague et al. (35) have suggested that a high F/M ratio in the contact tank will result in poor solids separation in the clarifier, and a decrease in the microbial population in the return sludge and, consequently, in the contact tank. Increasing the sludge recycle rate in an attempt to increase the solids concentration in the contact tank may do more harm than good because of greater turbulence in the clarifier. Thus, it was pointed out that the operator may be faced with a difficult situation. The authors concluded that the contact stabilization process as currently designed and applied to small systems is an unstable process and proposed employing the complete mixing or step-aeration flow modification to the conventional process, rather than using the contact stabilization process.

Jenkins and Orhon (47) observed that contact stabilization systems produced more readily settleable sludges than similarly operated conventional systems, based on continuous flow laboratory

activated sludge units and a pilot plant, both treating settled domestic sewage. These observations were explained as due to the bridging of particles of the floc by bacterial polymers of lytic origin. The authors suggested that the stabilization basin might be regarded as a lytic product, or flocculent, generator that enables lytic products to be partially retained in the system instead of, as in the case of a conventional system, being lost in the effluent. They found the COD removal efficiencies and effluent suspended solids concentrations of the conventional and contact stabilization processes to be comparable.

DESIGN OF THE CONTACT STABILIZATION PROCESS

In the past, many empirical approaches have been proposed for use in the design of a contact stabilization plant. Recently, many investigators have introduced more rational techniques. Some of the approaches that have been suggested will be discussed briefly.

Weston (15) used batch experiments to develop correlations of initial BOD removal, log growth phase removal, and equilibrium BOD remaining for different BOD-activated sludge suspended solids ratios. The BOD removal efficiency was estimated from graphs depicting percent removal of BOD with respect to time of contact. The contact aeration time design accounted for any initial removal of BOD and the time required for log growth removal to achieve the desired efficiency. The stabilization design time accounted for the time required to metabolize the waste matter associated with the activated

sludge remaining after contact aeration. The total aeration time was found to be substantially the same as that required of the conventional activated sludge process.

As was previously discussed in great detail, Bhatla et al. (36) developed a BOD transfer rate (Equation 3) by assuming that bioprecipitation, oxidation, and synthesis reactions cause the BOD concentration to approach the substrate equilibrium curve tangentially (Figure 13). A similar relationship was developed for the stabilization rate (Equation 9). From batch data they proposed that the kinetics of BOD transfer, the kinetics of stabilization of the transferred organic material, and the production of excess sludge could be determined. The stabilization kinetics were stated to be the controlling kinetics in a single tank because stabilization kinetics could not be faster than transfer kinetics. Bhatla et al. stressed the advantage in designing a contact stabilization process in which the contact tank provides adequate time for the desired BOD transfer, while the reaeration tank provides the time required for stabilization of the transferred materials. The COD removal (COD_r) and energy oxygen (O_e) were used to calculate the production of biological solids as follows:

$$\text{Cell Production} = \frac{COD_r - O_e}{1.42} \quad \ldots\ldots\ldots\ldots \quad (10)$$

The COD removed was assumed to be either oxidized to provide energy or synthesized to raw cell material. The proportionality constant of 1.42 was used in converting biological solids to equivalent COD.

Jones (32) has proposed that the soluble BOD and the insoluble BOD present in the contact tank of a contact stabilization plant are subjected to three different mechanisms of removal, all of which occur simultaneously, and will yield a response similar to that found in Figure 11. These three mechanisms and the accompanying reactions are as follows:

a. Adsorption and Physical Entrapment

The colloidal particulate BOD are adsorbed and trapped in flocs of the activated sludge. This reaction is assumed to occur almost instantaneously, obeying the first order differential equation:

$$P = P_o\, e^{-\alpha_1 t} \quad \ldots\ldots\ldots\ldots \quad (11)$$

where:

P = particulate BOD at time t, mass/volume

P_o = particualte BOD at time zero, mass/volume

α_1 = adsorption rate constant, time $^{-1}$

t= time

b. Absorption

The biodegradable soluble material in the waste is adsorbed and synthesized by microorganisms in the mixed liquor and would obey the same equation as the growth of microorganisms:

$$L = L_o e^{-\alpha 2 t}$$

where:

L = soluble BOD at time t, mass/volume

L_o = soluble BOD at time zero, mass/volume

α_2 = absorbtion rate constant, time $^{-1}$

c. Hydrolysis

The microorganisms secrete proteolytic and other lysing enzymes to render the particulate matter, that has been enmeshed and entrapped, soluble. It was assumed that hydrolysis proceeds at a rate which is a function of the concentration of material to be hydrolyzed and proceeds at the same rate of synthesis:

$$H = P_o - P_o e^{-\alpha 3 t} \quad \ldots\ldots\ldots\ldots \quad (13)$$

where:

H = material hydrolysed, mass/volume

α_3 = hydrolysis rate constant, time $^{-1}$

Jones concluded that the total BOD remaining could be adequately determined by the relationship.

$$\text{Total BOD remaining} = P + L + HA \quad \ldots\ldots \quad (14)$$

where:

HA = hydrolysis minus absorption, mass/volume

Jenkins and Orhon (47) have proposed an alternate mechanism for contact stabilization. They have assumed a growth/death rather than storage/metabolism mechanism by which a rapid growth of microorganisms occurs in the contact tank followed by microbial decay

in the stabilization tank. Their kinetic model made the simplifying assumption that only in the contact tank is cell growth significant while microbial decay predominates in the stabilization tank. Continuous flow laboratory activated sludge units and a pilot plant, both treating settled domestic sewage, were used in the conventional and contact stabilization modes. The stabilization basin mass decay rate was found to increase with increasing values of contact basin removal rates until a limiting value had been reached. This suggested that, for the contact stabilization process, high overall substrate removal rates could be achieved at significantly lower net growth rates than in conventional systems of the same COD removal rate. Also, the sludge production rate and the degree of nitrification, parameters which were directly proportional to the net growth rate, could be varied at a given substrate removal rate. Thus, it was concluded that the sludge production rate could be reduced, the loading at which nitrification could be achieved could be increased, and the sludge mass carried in the plant could be minimized because of its high viable organism content.

Gujer and Jenkins (48) described the contact stabilization process using four independent and major parameters: process loading, temperature, recycle ratio and sludge distribution between the reactors. In the contact stabilization process the recycle ratio was stated to be a more important process parameter than in the conventional process because it directly affected the hydraulic residence time distribution. For a very high recycle ratio the system

would approach a completely mixed reactor. Their study also showed that the recycle ratio does not have a significant influence on the removal of compounds that have a high removal rate, such as particulate COD, because the main removal mechanism involved was believed to be a result of a rapid physical reaction that occurs to virtual completion in the contact tank. The authors also introduced the parameter of oxygen equivalence (OE) and defined it as the mass of oxygen that must be consumed or liberated to oxidize or reduce a substance to a well defined redox level. An oxygen equivalence mass balance equation, applicable to all activated sludge process modifications, was proposed which could be used in the design and operation of these processes.

$$q = \mu_n i + r \quad \ldots\ldots\ldots\ldots \quad (15)$$

where:

q = rate of substrate removal, mass OE/mass VSS-day

μ_n = sludge production rate, mass VSS/mass VSS-day

i = conversion factor from VSS to OE, mass OE/VSS

r = Oxygen uptake rate (respiration rate), mass O_2/mass VSS-day

Equation 15 could be applied to single reactors as well as to processes with more than one reactor. The oxygen equivalent value was approximated by a combination of standard COD determinations and measurement of the oxidation state of nitrogen.

Benefield and Randall (49) developed design equations for the contact stabilization activated sludge process by using concepts

promulgated by Eckenfelder (8) and Lawrence and McCarty (6). In the development of their equations it was assumed that first-order kinetics were followed in the removal of both soluble and nonsettleable particulate substrate in the aeration tanks and that only soluble substrate was metabolized in the contact tank. It was further assumed that all substrate entering the stabilization tank was completely metabolized. Equations for the contact unit were developed from a materials balance for the net rate of change of microbial solids entering and leaving the unit. Similarly, equations were developed for the stabilization unit by utilizing a materials balance for the net rate of microbial solids entering and leaving the stabilization unit. A procedure for applying the design equations in the actual design of a contact stabilization activated sludge process was also outlined.

SUMMARY

The contact stabilization process has been demonstrated to have several advantages over the conventional activated sludge process. These include greater flexibility in control of the process because of the separation of the adsorption and oxidation processes, an increased capability in handling wide variations in wastewater quality and toxic discharges because of the reservoir of sludge contained in the stabilization tank, and better sludge settleability. One of the more important advantages is lower capital expenditure because of reduced aeration tank capacity, and this becomes an

important economic consideration when faced with future expansion of an existing treatment facility. The conventional and contact stabilization processes were found to be comparable with respect to COD removal efficiency and effluent suspended solids concentrations.

The various theories and process mechanisms underlying the contact stabilization process have been discussed. The phenomenon of "uptake and release," as observed by early researchers, is probably the most widely accepted theory regarding the removal of a colloidal organic substrate. However, no data were found that could adequately substantiate these findings. While the process mechanisms are not fully understood, the contact stabilization process has been shown to be an effective method of treatment for certain wastes, and many advantages can be realized in using the process.

III. MATERIALS AND METHODS

Batch experiments were conducted using three different wastewaters to determine the manner in which organic materials were removed from solution. The wastewaters studied were a domestic waste, a paper mill waste, and a food waste. A discussion of the experimental procedure, the characterization of the various wastewaters, and the analytical procedures used are presented in the following sections.

EXPERIMENTAL PROCEDURE

A series of three batch experiments was conducted for each of the aforementioned wastes. The food-to-microorganism (F/M) ratio for each series was kept constant. However, the mixed liquor concentration, for each of the experiments within the series, was varied in order to observe what effect the different microbial concentrations might have on organic removal and microbial substrate storage.

The F/M ratio used in each series of experiments was determined based on the organic concentration of the wastewater as measured by the chemical oxygen demand (COD) test and the highest mixed liquor concentration used as measured by volatile suspended solids. Standard Methods procedures were used (33). To insure that the remaining two batch runs in the series of experiments were conducted at the same F/M ratio, the amount of mixed liquor and wastewater added to the test reactor were appropriately adjusted.

During each series of experiments, a 10 liter batch tube reactor, containing a sludge acclimated to the wastewater being studied, was aerated and maintained using fill and draw techniques at an F/M ratio approximately equal to that which was to be used in the batch experiments. All batch experiments were conducted in a constant temperature room at 20° ± 2°C. The acclimated sludge maintained in the reactor, the wastewater being studied, the nutrient and buffer solutions added, and the distilled water used to dilute the wastewater to the desired concentration were allowed to equilibrate to this temperature before beginning an experiment.

Prior to each experimental run, the acclimated sludge in the above reactor was allowed to return to its endogenous level of respiration by continued aeration without the addition of any substrate for a period of 36 to 48 hours. An initial value of oxygen uptake was obtained. The contents of the reactor were then allowed to settle by discontinuing aeration. The supernatant was siphoned off and the volatile solids concentration of the settled sludge determined.

Based on the volatile solids concentration of the settled sludge, an appropriate settled sludge volume was added to another, smaller, tube reactor to provide the desired mass of mixed liquor volatile suspended solids for the run. This smaller batch reactor, in which the experiment was to be conducted, was to have a final total volume of four liters. To ensure the same F/M ratio at three different mixed liquor volatile suspended solids (MLVSS) concentrations, the concentration of wastewater also had to vary with each MLVSS concen-

tration. For the higher MLVSS concentration, the wastewater was applied at full strength, without diluting, to achieve the highest F/M ratio possible. At the lower two MLVSS concentrations, dilution of the wastewater was required and was accomplished using distilled water and appropriate amounts of nutrient and buffer solutions. A sufficient amount of wastewater was added to the batch reactor containing the settled sludge to bring the total volume of the test reactor to four liters.

The experimental run began immediately upon addition of the wastewater to the reactor, which already contained settled sludge under aerated and completely mixed conditions. Immediately after the contents had been completely mixed by aeration, a sample was withdrawn and the initial volatile suspended solids and pH of the mixed liquor was determined. Samples were then withdrawn at time intervals of 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240 minutes and thereafter at 60 minute intervals until the sludge in the reactor had returned to its endogenous respiration state as measured by a return to the oxygen uptake rate determined prior to the experiment.

The following analyses were conducted on the samples: oxygen uptake rate, COD, MLVSS, pH, protein concentration and carbohydrate concentration. Only the oxygen uptake rate was measured for each sampling interval. Portions of the sample were centrifuged and the resulting supernatant was filtered. COD determinations were performed on the supernatant and filtrate. Samples to be used in

determining the protein content of the sludge were also centrifuged. The supernatant was discarded and the remaining pellet was resuspended to its original volume with 1N NaOH solution. This suspension was then frozen until the analysis could be completed. A similar procedure was followed for the carbohydrate analysis except that a 0.1 M phosphate buffer was used instead of the 1N NaOH.

WASTEWATERS STUDIES

Domestic Waste

This type of waste was selected because it is generally thought to have a high colloidal content and is the wastewater on which many of the theories in the literature are based. The wastewater was obtained from the grit removal effluent of the Roanoke, Virginia water pollution control plant. Activated sludge was obtained from an extended aeration activated sludge plant serving a rest stop on Interstate 81 near Radford, Virginia. Although the sludge was already acclimated to a domestic type waste, further acclimation of the sludge was continued for five days in a 10 liter batch reactor using fill and draw techniques.

As previously mentioned, for each wastewater a series of three experimental batch runs was conducted. Every attempt was made to insure that the initial F/M ratio was constant for all three runs, while the mixed liquor concentration was varied. Mixed liquor volatile suspended solids concentrations of 1500, 1000, and 500 mg/l were used in this series of experiments.

Fresh wastewater was collected and characterized prior to each batch run. The characterization of the domestic wastewater is presented in Table I. The colloidal COD concentration was determined by subtracting the filtered COD concentration from the unfiltered or total COD concentration. Based on this determination the percent colloidal COD was calculated.

Paper Mill Waste

The wastewater used in this series of experiments was obtained from the flow to the complete mix activated sludge plant serving the city of Lynchburg, Virginia. This treatment facility has separate primary treatment for a wastewater discharged by a paper mill, also located in Lynchburg, that is operated by the Mead corporation. Primary treatment of the paper mill waste consists of grit removal, screening, and sedimentation. The clarified paper mill waste is then combined with the Lynchburg municipal waste and routed to biological aeration tanks for secondary treatment.

The paper mill waste used in these experiments was withdrawn after primary clarification. This waste was selected because of its complex nature and was thought to have a high COD concentration and colloidal content. The wastewater was stored at 4°C, but was allowed to rise to 20° prior to feeding it to the biological reactors.

Activated sludge was obtained from the aeration tanks at the Lynchburg treatment facility, and, therefore, was acclimated to a combination of the paper mill and municipal wastewaters. Acclimation

TABLE I Characterization of a Domestic Wastewater

Parameter	Conc. for run at MLVSS of 1500 mg/l	Conc. for run at MLVSS of 1000 mg/l	Conc. for run at MLVSS of 500 mg/l
Unfiltered COD	537 mg/l	549 mg/l	374 mg/l
Filtered COD	105 mg/l	123 mg/l	69 mg/l
% Colloidal COD	80%	78%	82%
Unfiltered BOD_5	245 mg/l	213 mg/l	178 mg/l
Filtered BOD_5	49 mg/l	32 mg/l	19 mg/l
Total Solids	951 mg/l	645 mg/l	562 mg/l
Total Volatile Solids	315 mg/l	313 mg/l	252 mg/l
Suspended Solids	300 mg/l	220 mg/l	183 mg/l
Volatile Suspended Solids	268 mg/l	180 mg/l	158 mg/l
pH	7.15	7.20	7.20

of the sludge to the full strength paper mill wastewater was accomplished in a 10 liter batch reactor. The sludge was initially fed a combined waste of papermill and municipal waste, in the same proportions to which it was accustomed. Then, over a 14 day period, the domestic waste was gradually phased out while the paper mill waste was increased. Nutrients normally supplied by the domestic waste were added to the feed as the domestic waste was phased out.

For this series of experiments mixed liquor volatile suspended solids concentrations of 2500, 1500, and 500 mg/l were used. Again, the F/M ratio for all three batch runs was kept constant. A characterization of the paper mill wastewater is presented in Table II. Although the colloidal content was not as high as expected, the waste was still thought to be of such a complex nature that a possible time lag might occur in the return of the oxygen uptake rate to its endogenous rate when compared to the time required for maximum organic removal. The pH of the wastewater was adjusted to approximately 7.0 using a NaOH solution, prior to adding it to the biological reactors.

Food Processing Waste

The final wastewater studied consisted of a food processing waste that was obtained from a secondary treatment facility which treated, solely, the wastewater discharged from the Morton Frozen Food plant located in Crozet, Virginia. The treatment scheme included dissolved air flotation for grease removal, an anerobic pond, two

TABLE II Characterization of a Paper Mill Wastewater

Parameter	Concentration
Unfiltered COD	1141 mg/l
Filtered COD	992 mg/l
% Colloidal COD	13%
Unfiltered BOD_5	664 mg/l
Filtered BOD_5	659 mg/l
Total Solids	1188 mg/l
Total Volatile Solids	570 mg/l
Suspended Solids	32 mg/l
Volatile Suspended Solids	26 mg/l
pH	5.30

trickling filters in series, and a contact stabilization activated sludge unit.

The wastewater used in these series of experiments was collected after the first trickling filter. Activated sludge was obtained from the stabilization tank of the contact stabilization unit. Although the sludge was already acclimated to this waste, further acclimation of the waste was continued for 7 days in a 10 liter batch reactor. Fill and draw techniques were used and nutrients (nitrogen and phosphorus) were added in excess to prevent the growth of filamentous microorganisms. The only deviation from the experimental procedure, as discussed in the Experimental Procedures section, dealt with the method of obtaining the initial oxygen uptake rate for the sludge treating this waste. With the other wastewaters, an initial value of oxygen uptake was obtained by removing sludge from a 10 liter batch reactor that had been permitted to return to the endogenous phase of respiration. This batch reactor was maintained at the same F/M ratio that was to be used in all three batch experiments of a series. However, the MLVSS of this reactor was not varied as were the MLVSS for each test reactor but contained the same MLVSS concentration as the highest MLVSS being studied in each series.

During the food processing waste experiments, the mixed liquor content of the 10 liter batch reactor was further adjusted to insure that the MLVSS concentration was approximately equal to the MLVSS concentration of the test reactor for each of the three batch experiments of the series.

For this series of experiments mixed liquor volatile suspended solids concentrations of 2500, 1500, and 500 mg/l were used. The F/M ratio for all three batch runs were the same. A characterization of the food processing waste is presented in Table III. The pH of the wastewater was adjusted to approximately 7.0, with a NaOH solution, prior to adding it to the biological reactors.

ANALYTICAL TECHNIQUES

Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined by the dichromate reflux method as described in Standard Methods (33). In determining the values of settled and filtered COD for the mixed liquor in the reactor at each time interval, a sample was centrifuged at 8,000 rpm for 75 seconds, using a Beckman Model J-21C Centrifuge. The supernatant was then poured off, leaving the pellet remaining in the bottom of the centrifuge tube. A COD determination was performed on the supernatant. The settled COD concentration was represented by this determination. A portion of the supernatant was then filtered through a 0.22 micron membrane filter manufactured by the Millipore Filter Corporation. A COD analysis was performed on the filtrate and this value was used to represent the soluble, or filtered, COD concentration of the sample. The filtered COD concentration for each wastewater was also found in a similar manner.

Solids Determinations

The total solids content was determined in the initial characterization of each wastewater by evaporating 100 mls of a well mixed

TABLE III Characterization of a Food Processing Wastewater

Parameter	Concentration
Unfiltered COD	1263 mg/l
Filtered COD	970 mg/l
% Colloidal COD	23 %
Unfiltered BOD_5	851 mg/l
Filtered BOD_5	774 mg/l
Total Solids	1127 mg/l
Total Volatile Solids	577 mg/l
Suspended Solids	73 mg/l
Volatile Suspended Solids	66 mg/l
pH	6.40

sample in a preweighed porcelain dish on a steam bath. The evaporated sample was then dried for at least one hour at 103°C. The volatile portion was determined by placing this dried residue in a 600°C muffle furnace for 20 minutes.

The total suspended solids of the wastewaters and the mixed liquor suspended solids of the reactors were evaluated in a similar manner. A well mixed sample was filtered through a Whatman Reeve Angel (934 AH) glass fiber filter, 5.5 cm in diameter, which had been placed on a membrane filter apparatus. The filter, with the residue, was then placed in its respective aluminum planchet, which had been previously weighed with the clean filter. The total suspended solids were determined by drying the filter and aluminum planchet in an oven at 103°C for one hour. The volatile suspended solids of the wastewater and the MLVSS of the reactor were determined by placing the above dried samples in a 600°C muffle furnace for 20 minutes. The procedures used for all solid determinations were in accordance with Standard Methods (33).

Oxygen Uptake

Oxygen uptake analyses were made using a YSI Model 54 A oxygen meter equipped with a self stirring dissolved oxygen (DO) probe. To determine the oxygen uptake rate at each sampling time interval, a standard 300 ml BOD bottle was filled with mixed liquor from the test reactor. The DO probe was then immediately inserted in the filled bottle and permitted to stabilize, and this stabilized position was considered as zero time. The DO concentration was

then monitored over a period of time and the data was plotted as DO concentration versus time. The slope of this straight line represented the rate of oxygen utilization by the microorganisms in the test reactor. A typical oxygen uptake curve is shown in Figure 22. The probe was calibrated for the barometric pressure and temperature of the constant temperature room.

Biochemical Oxygen Demand

Procedures used to determine the 5-day biochemical oxygen demand (BOD) of the various wastewaters were in accordance with Standard Methods (33). For each wastewater the infiltered and filtered 5-day BOD was determined. The filtered sample used in the BOD determination was obtained in the same manner as was the sample used for the filtered COD analyses.

Seeding of the paper mill and food processing wastes was necessary. The seed used was obtained from the supernatant of batch reactors with sludge acclimated to the wastewaters, that had been allowed to settle.

The 5-day BOD was determined by placing a known volume of the sample into a standard 300 ml BOD bottle and filling it with premixed oxygen saturated dilution water. The initial DO concentration and the DO concentration after a five day incubation period were evaluated using the DO meter and probe, described in the previous section. The DO meter was calibrated frequently employing the azide modification to the Winkler method, used in determing DO concentrations.

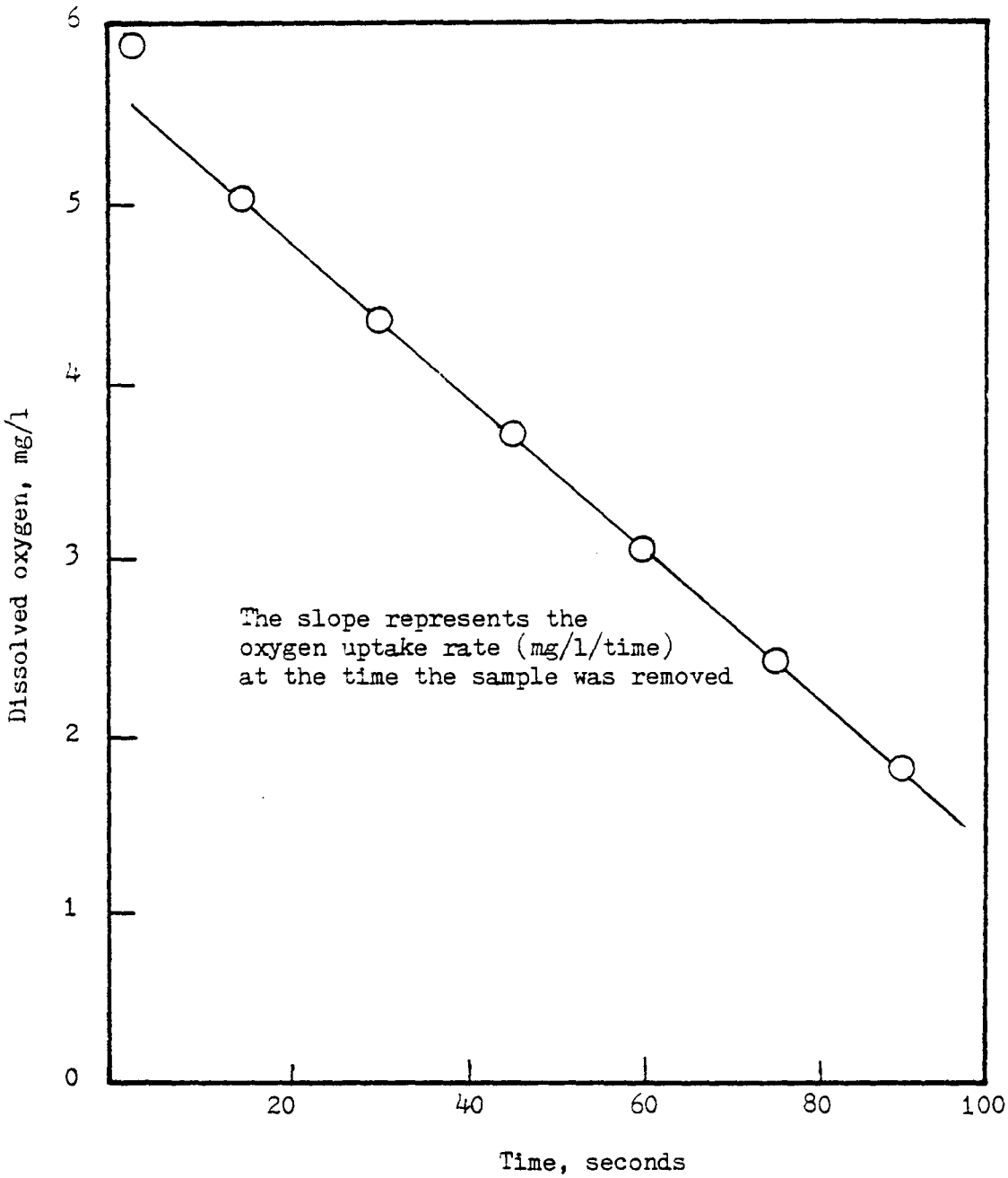

Figure 22. Typical oxygen uptake curve.

A separate series of seed dilutions and unseeded dilution water controls were also established.

Carbohydrate Analysis

Carbohydrate concentrations for the biological sludges were determined by the Anthrone method described by Ramanathan, Gaudy and Cook (50). The procedures used in preparing the sample for frozen storage, until the analysis could be completed, was discussed in the Experimental Procedure. As an additive comment, the sample was centrifuged at 8,000 rpm for three minutes to insure the formation of a stable pellet. Prior to the actual analysis, the frozen samples were allowed to reach room temperature.

The analysis consisted of placing 3 ml of appropriate dilutions of the sample to be tested and known concentrations of anhydrous dextrose in test tubes, cooling the contents of the tubes in ice water, injecting 9 ml of anthrone reagent, mixing, placing the tubes in boiling water for exactly 15 minutes, and cooling the content to room temperature. The absorbance of the samples and known standards was then measured using a Perkin-Elmer (Coleman 124) double bream spectrophotometer and a wave length of 540 mμ. The carbohydrate concentrations of the sludges were determined by comparison with known standards.

Protein Analysis

Protein concentrations for the biological sludges were determined using a method suggested by Lowry, Rosebrough, Farr and

Randall (51). The procedures used in preparing the sample for frozen storage were similar to those used in the carbohydrate analysis.

The analysis consisted of placing 1 ml of appropriate dilutions of sample to be tested and known concentrations of crystalline bovine serum albumin in test tubes, adding 5 ml freshly prepared alkaline copper solution (Lowry reagent) to each tube, and mixing. After 10 minutes 0.5 ml of Folin-Ciocaltean Phenol reagent was added and mixed, and 30 minutes were allowed for development. The absorbance of the samples and known standards was then determined using a Perkin-Elmer (Coleman 124) double beam spectrophotometer and a wavelength of 500 mμ. The protein concentrations of the sludges were determined by comparison with known standards.

pH Determinations

The pH of the wastewaters and mixed liquors was determined using a Fisher Accumet Model 230 pH/Ion meter. Prior to pH determinations, the meter was standardized against standard buffer solutions by Fisher Scientific.

IV. RESULTS

The purpose of this study was to investigate substrate removal and storage for three wastewaters. As discussed in the previous chapter, a series of three batch experiments (tube runs) were conducted for each wastewater. The food-to-microorganism ratio (F/M) was held constant for each series of experiments while the variable for each individual experiment was the mixed liquor volatile suspended solids concentration (MLVSS). The parameters monitored periodically during the experimental runs included settled COD, filtered COD, oxygen uptake, pH, MLVSS, protein concentration of the suspended solids, and carbohydrate concentration of the suspended solids. The wastewaters studied were a domestic waste, a paper mill waste and a food processing waste. The results obtained from each series of runs are presented in the following sections.

DOMESTIC WASTEWATER

The variations in COD, oxygen uptake, and mixed liquor volatile suspended solids with time for the series of experiments performed with the domestic wastewater, are presented in Figures 23 through 25. The initial loading, or F/M ration, calculated on a total COD basis, was 0.358 mg/l COD/mg/l MLVSS for all experiments conducted with the domestic wastewater.

Domestic wastewater has a high colloidal fraction and is, therefore, thought to be ideally suited for contact stabilization.

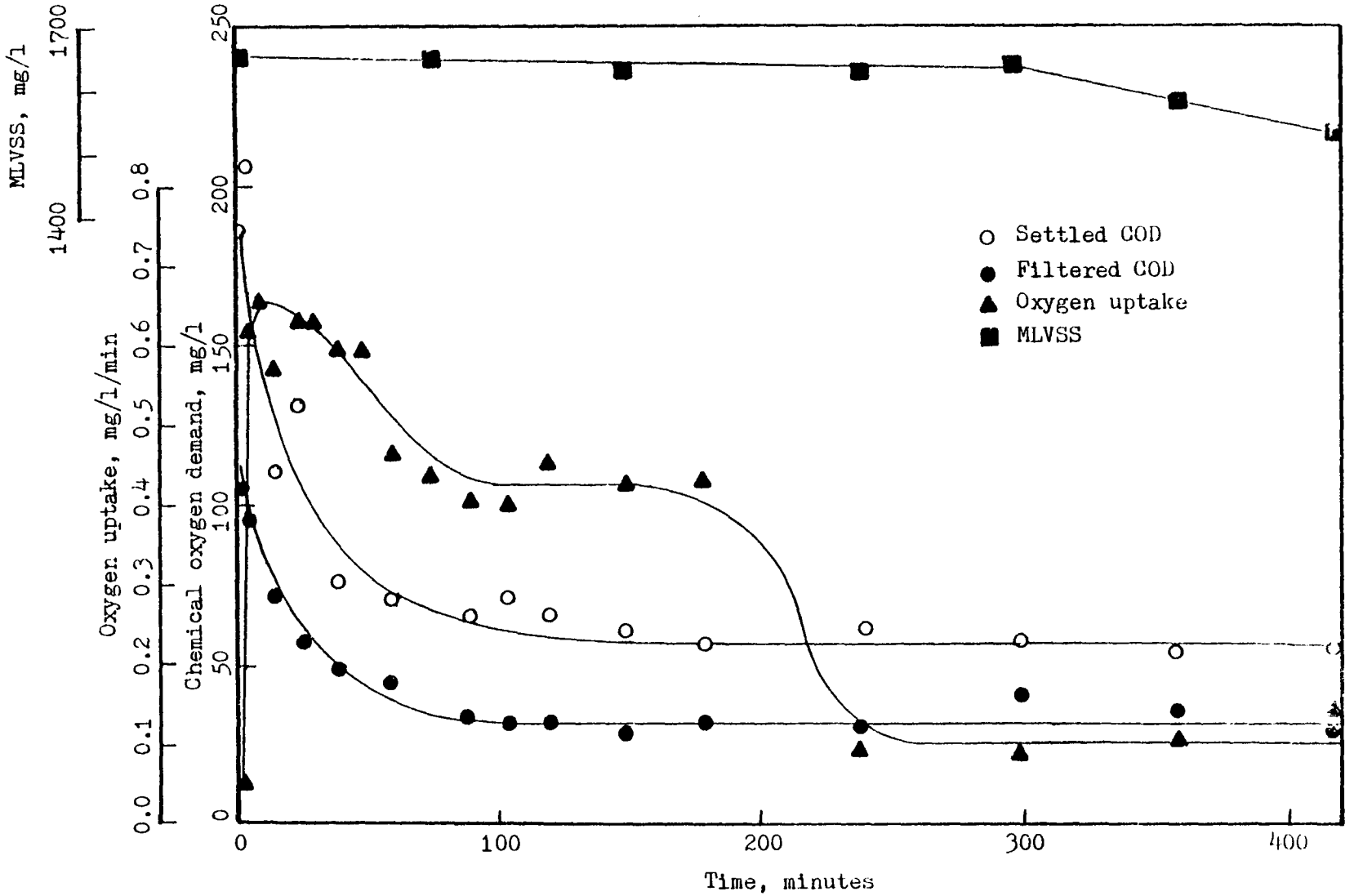

Figure 23. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the domestic wastewater at an initial loading of 0.358 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 1660 mg/l.

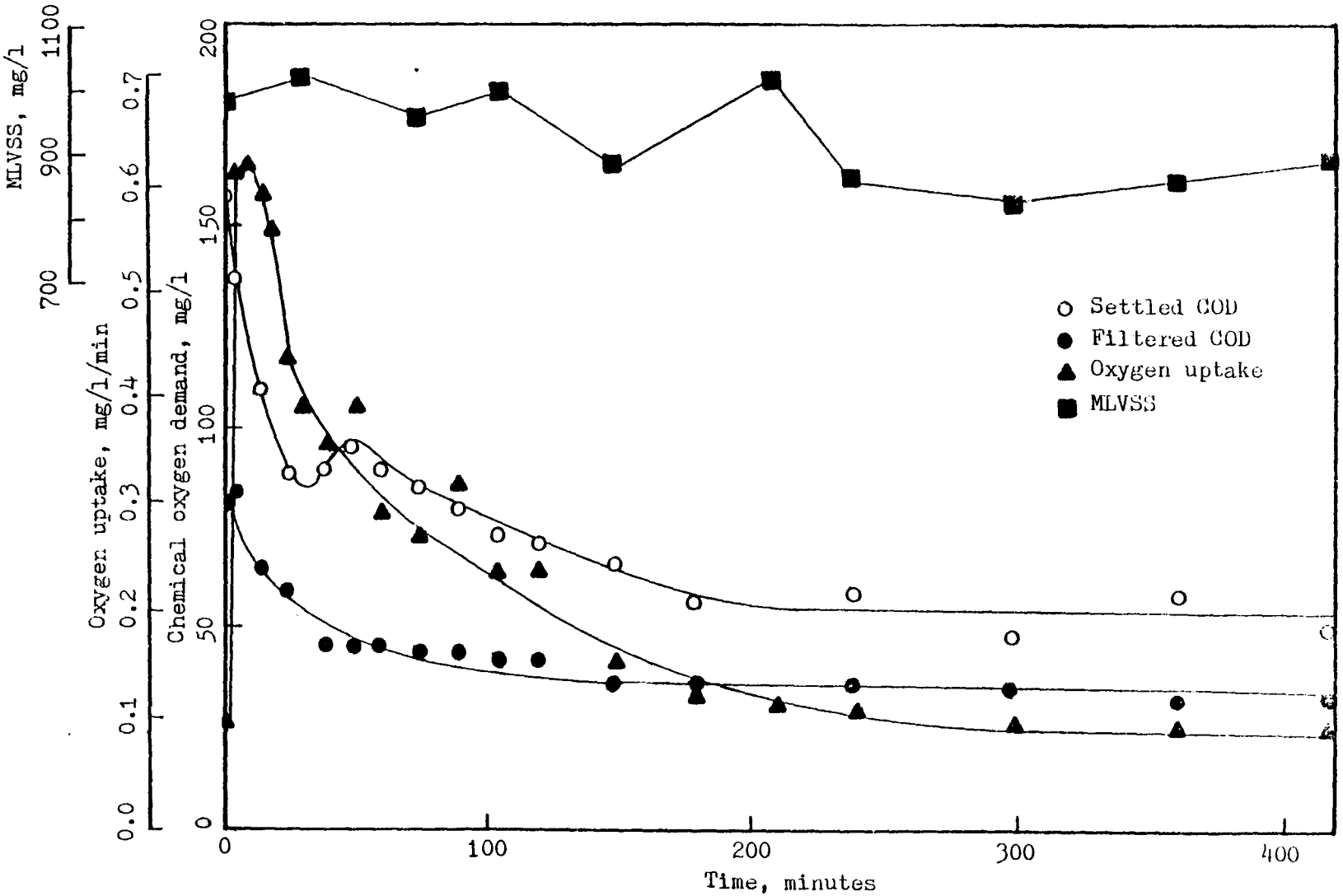

Figure 24. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the domestic wastewater at an initial loading of 0.358 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 990 mg/l.

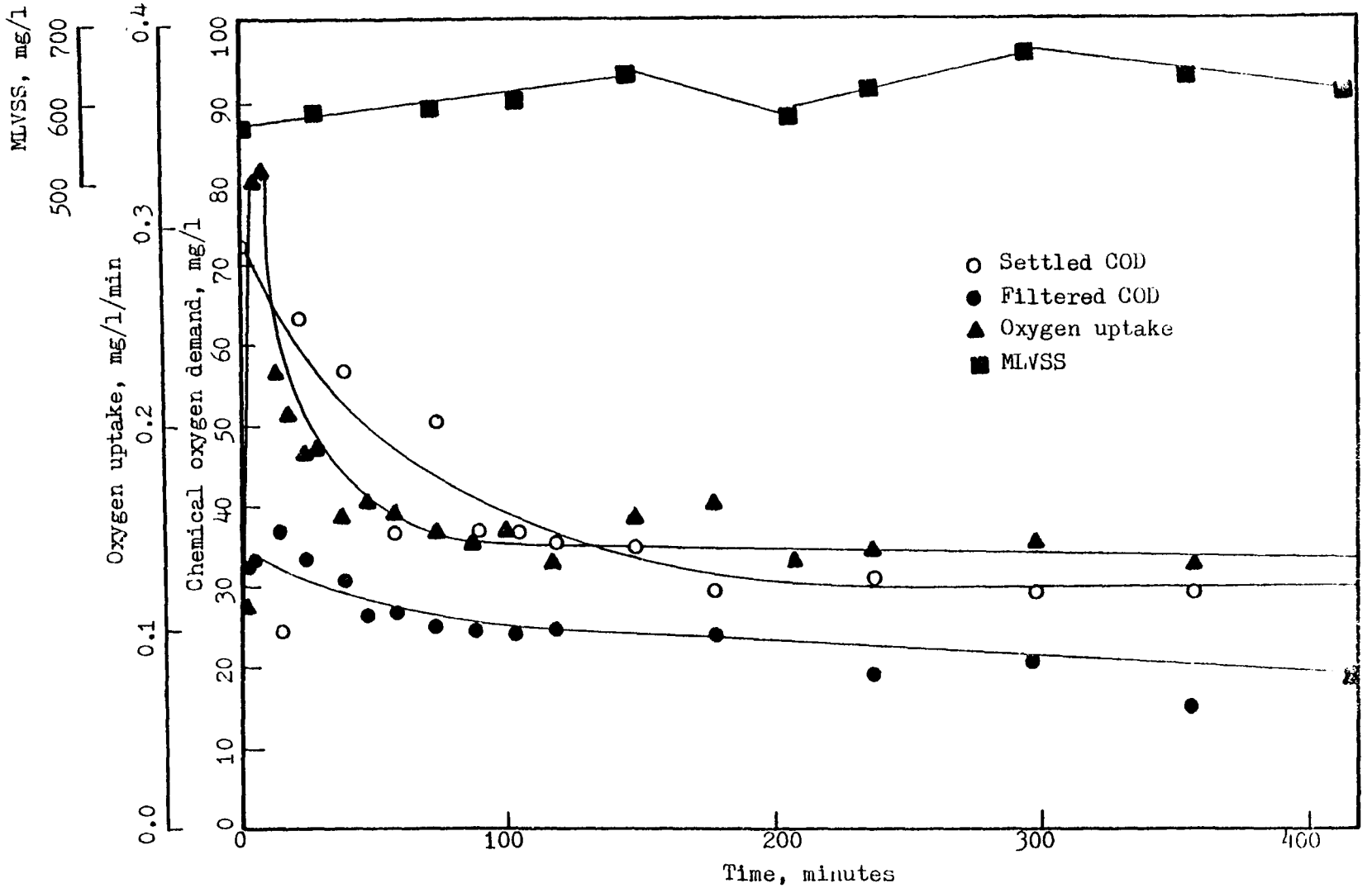

Figure 25. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the domestic wastewater at an initial loading of 0.358 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 570 mg/l.

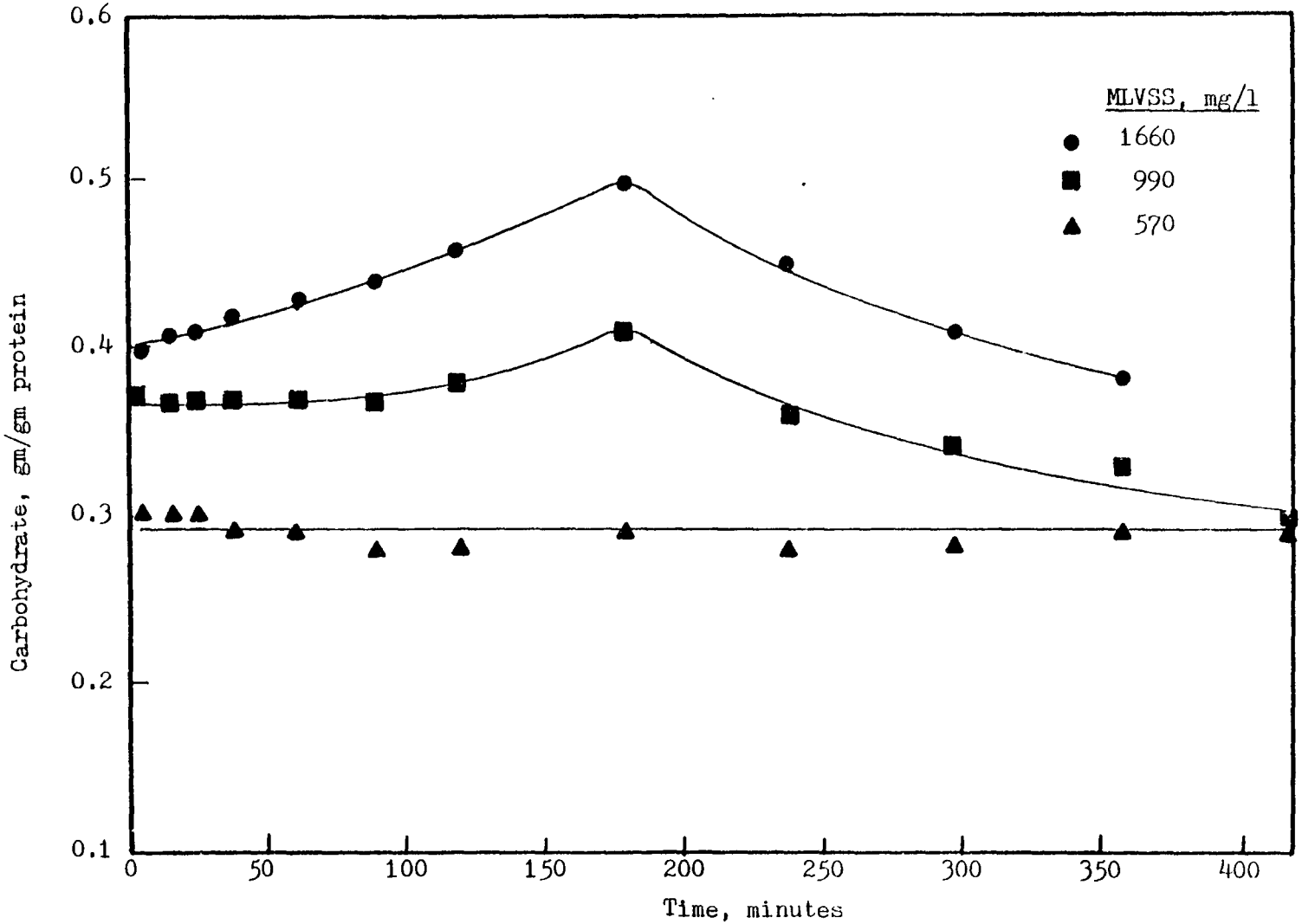

Figure 26. Variation in cellular carbohydrate during metabolism of domestic waste at the indicated MLVSS concentrations.

However, in Figure 23, where the initial MLVSS concentration was 1660 mg/l and the waste was 80 percent colloidal, there was no evidence of an uptake and release of organic substrate for either the settled or filtered COD curves. The percent COD removed during the batch run was found to be approximately 71 percent for both the settled and filtered COD curves. Oxygen uptake was observed to increase rapidly within the first 10 minutes of the experiment and then gradually decrease, leveling off and finally decreasing sharply to a constant value. Figure 23 also shows that oxygen uptake continued for some time after the concentration of filtered and settled COD remaining had reached minimum values, indicating that COD transfer had been accomplished.

Figure 24 depicts the results obtained for the second experimental run of the series, wherein an MLVSS concentration of 990 mg/l and a domestic wastewater that was 78 percent colloidal were used. No uptake and release of substrate was observed for the filtered COD curve; however, the settled COD curve did demonstrate a slight uptake and release. The percentage of released COD, when compared to the total settled COD removed, was determined to be 9 percent. This percentage is not very significant when compared to the total settled COD removed, the variability associated with the COD analysis ($\pm$ 6.5 percent using a synthetic waste with a concentration of 200 mg/l, (33)). Further, it was not supported by the filtered COD values. The COD removed for the filtered and settled COD curves was determined to be 59 and 65 percent, respectively. The oxygen uptake

rate was observed to peak rapidly and then decrease gradually to a constant endogenous value.

The data obtained for the third batch run with the domestic waste are represented in Figure 25. The MLVSS concentration was 570 mg/l. There was no rapid uptake or release of substrate observed. A wide scattering of the settled COD data was observed and it may have resulted from the use of an alternate procedure for low-COD analysis (COD values of less than 50 mg/l) or, possibly, from contamination of the glassware, or, contamination of air in the laboratory (33). The COD reduction was 42 and 58 percent for the filtered and settled COD curves, respectively. The oxygen uptake peaked rapidly and then quickly decreased to an endogenous oxygen uptake rate. The return to endogenous level was complete by the time the substrate had been transferred from the soluble phase, but the final endogenous level was somewhat higher than the initial endogenous level.

The pH variation of the mixed liquor with time for the series is not presented, however, for each experimental run there was a slight increase in pH with time. The variation of mixed liquor suspended solids with time is also depicted in Figures 23 through 25. In Figures 23 and 24 the MLVSS concentration remained fairly constant, however, there was a tendency for the MLVSS to decrease slightly toward the end of the two batch runs. There was an overall increase in the MLVSS concentration with time observed for run number three (Figure 25).

The variation in the cellular carbohydrate of the sludge during the metabolism of the domestic waste is shown in Figure 26 for the three experimental runs. The carbohydrate concentration of the sludge was expressed in terms of the protein concentration of the sludge so that the change in storage products per cell could be estimated. The carbohydrate content of the sludges, for both the 1660 and 990 mg/l MLVSS curves, gradually increased with time to a peak value at 180 minutes, and then decreased for the remainder of the run. The carbohydrate content of the 570 mg/l MLVSS curve remained fairly constant with time.

PAPER MILL WASTEWATER

The results obtained from the series of batch experiments using the paper mill waste are presented in Figures 27 through 30. As previously discussed the F/M ratio that was to be used for all three experiments of the series was to equal the F/M ratio determined for the batch run with the highest MLVSS concentration. It was assumed that the total COD of the paper mill wastewater would remain constant while stored at $4^{o}C$, and, therefore, the total COD value determined prior to the first experimental run was used when calculating wastewater requirements for the following two experimental runs. However, small differences from the initially determined COD value were found for the wastewater added to the units during the runs. Therefore, the actual F/M ratios for the last two experiments were found to vary somewhat from the F/M ratio used during the first

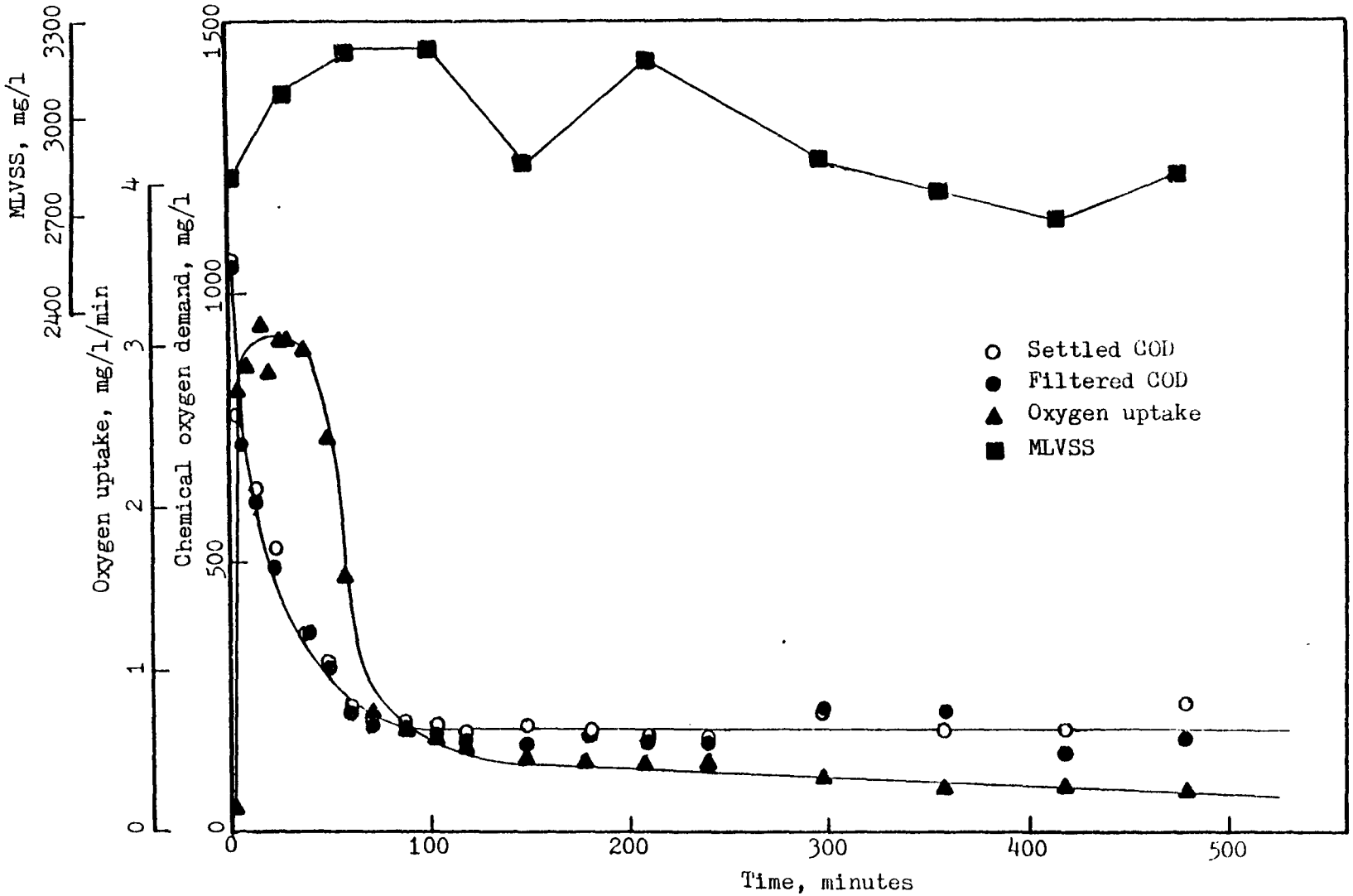

Figure 27. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the paper mill wastewater at an initial loading of 0.462 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 2825 mg/l.

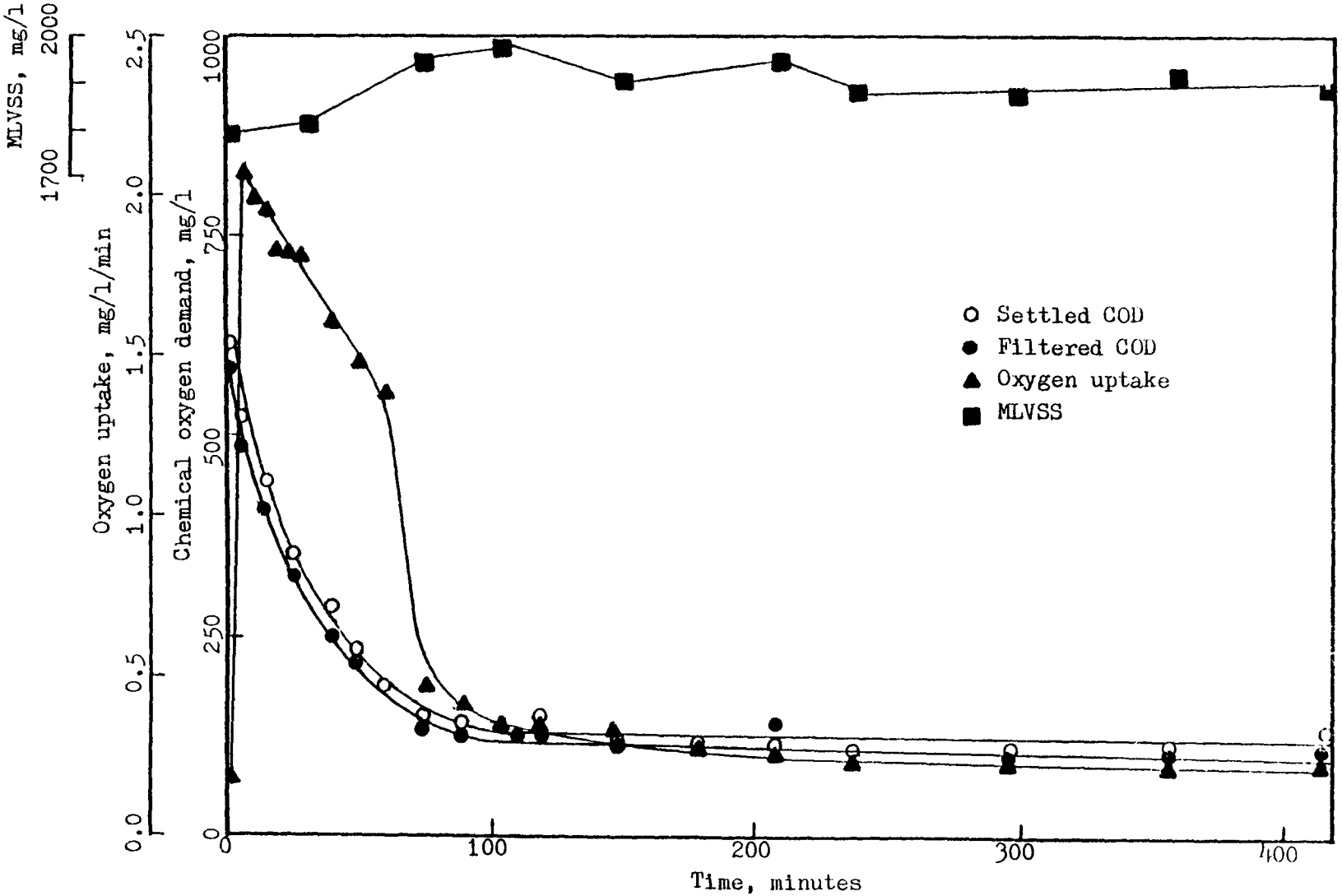

Figure 28. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the paper mill wastewater at an initial loading of 0.454 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 1800 mg/l.

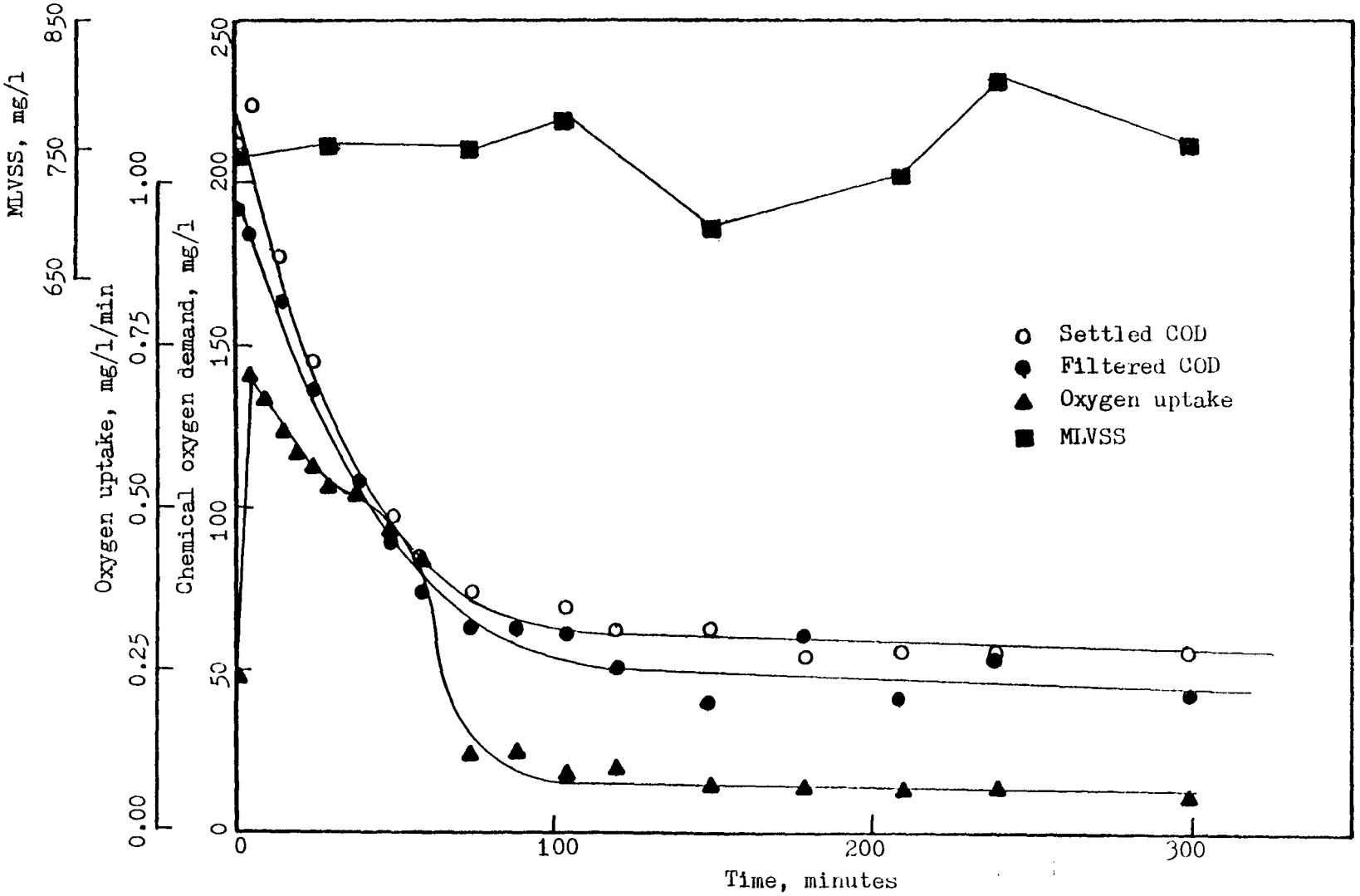

Figure 29. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the paper mill wastewater at an initial loading of 0.455 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 740 mg/l.

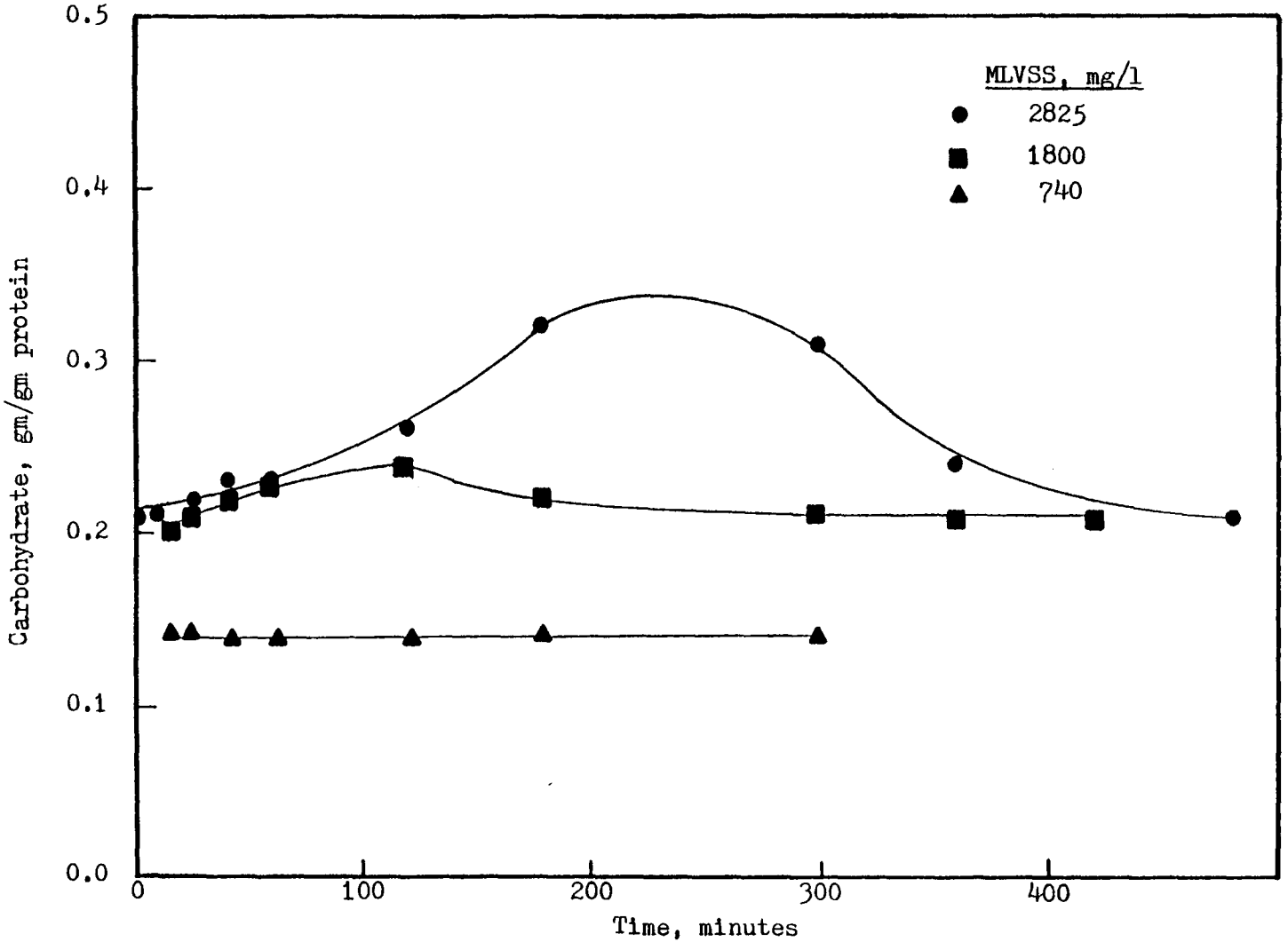

Figure 30. Variation in cellular carbohydrate during metabolism of paper mill waste at the indicated MLVSS concentrations.

batch run. The difference was small, however, with a drop from 0.462 to 0.454 and 0.455, respectively.

In Figure 27, at an initial MLVSS concentration of 2825 mg/l and an F/M ratio of 0.462 mg/l COD/mg/l MLVSS, the settled and filtered COD values for each time interval were found to be so nearly equal that a single curve was used to represent the variation of both COD values with time. The filtered and settled COD were observed to decrease rapidly and no uptake and release of substrate occurred, suggestive of the organic reduction observed for soluble substrates. As reported in the previous chapter, the paper mill waste was 13 percent colloidal. The total reduction in settled and filtered COD was found to be 83 percent. The oxygen uptake increased quite rapidly during the first few minutes of the run and then rapidly decreased, returning to, a relatively constant rate at 150 minutes and to the initial endogenous rate after 300 minutes into the experiment, while COD removal was completed at approximately 75 minutes into the experiment.

Figures 28 and 29 illustrate similar variations of filtered and settled COD with time for the last two experimental runs in the series. In both figures there is a rapid reduction in both settled and filtered COD, and a rapid increase and then decrease in oxygen uptake. No uptake and release in substrate was observed during either experiment, and no significant lag in the return of the oxygen uptake to a constant endogenous rate was observed for Figure 29. The oxygen uptake rate, as shown in Figure 28, returned

to the endogenous rate at 270 minutes into the experiment, while COD reduction was completed at approximately 90 minutes into the experiment. However, the oxygen rate at 100 minutes was only slightly higher than the endogenous level. A COD reduction of 80 percent for both the filtered COD and the settled COD was found for the 1800 mg/l initial MLVSS concentration (F/M loading of 0.454 mg/l COD/ mg/l MLVSS), while a COD reduction of 74 percent for the filtered COD and 72 percent for settled COD was observed for the 740 mg/l initial MLVSS concentration (F/M loading of 0.455 mg/l COD/mg/l MLVSS).

As discussed in Chapter three, the initial oxygen uptake values for the tube runs were obtained from a 10 liter batch reactor which was used as a reservoir for the microbial sludge to be used in the three experiments. The sludge in the reactor was maintained at a constant F/M ratio that was to be used throughout the experimental series. The reactor was not fed for a period of time sufficient to insure that the sludge was in the endogenous phase, prior to withdrawing an initial sample. The MLVSS concentration of this reactor, however, was held constant at the same concentration as that for the highest MLVSS concentration in the experimental series and was not varied as was the MLVSS concentration of the 4 liter test reactors. The more dilute sludge used during experiment 3 was probably responsible for the discrepancy observed in Figure 29 between the somewhat higher initial oxygen uptake rate and the final constant endogenous rate.

There was no significant change in the pH of the mixed liquor with time, for any of these batch runs. The variations of MLVSS with time, also presented in Figures 27 through 29, indicate a consistent pattern of increase and then a gradual decrease or leveling off.

The variation in the carbohydrate content of the sludge with time is shown in Figure 30. The curves for the 2825 mg/l and 1800 mg/l MLVSS concentrations reveal a gradual increase in the carbohydrate content of the sludge, followed by a gradual decrease with time. The carbohydrate content of the sludge for the 740 mg/l MLVSS curve remained constant throughout the run.

FOOD PROCESSING WASTEWATER

The variation in COD, oxygen uptake, and MLVSS with time for the series of three experiments conducted with the food processing waste are presented in Figures 31 through 34. The actual F/M ratios used for the last two batch experiments were found to vary slightly from the F/M ratio determined in the first batch run, for the same reasons discussed in the previous section.

In Figure 31, for an initial MLVSS concentration of 2560 mg/l and an F/M ratio of 0.512 mg/l COD/mg/l MLVSS, there was observed a rapid decrease in both the settled and filtered COD, resulting in a reduction of 88 percent of the settled COD and of the filtered COD. The oxygen uptake increased sharply during the first five minutes of the run, and then decreased sharply to a constant rate. There was no lag observed in the return of the

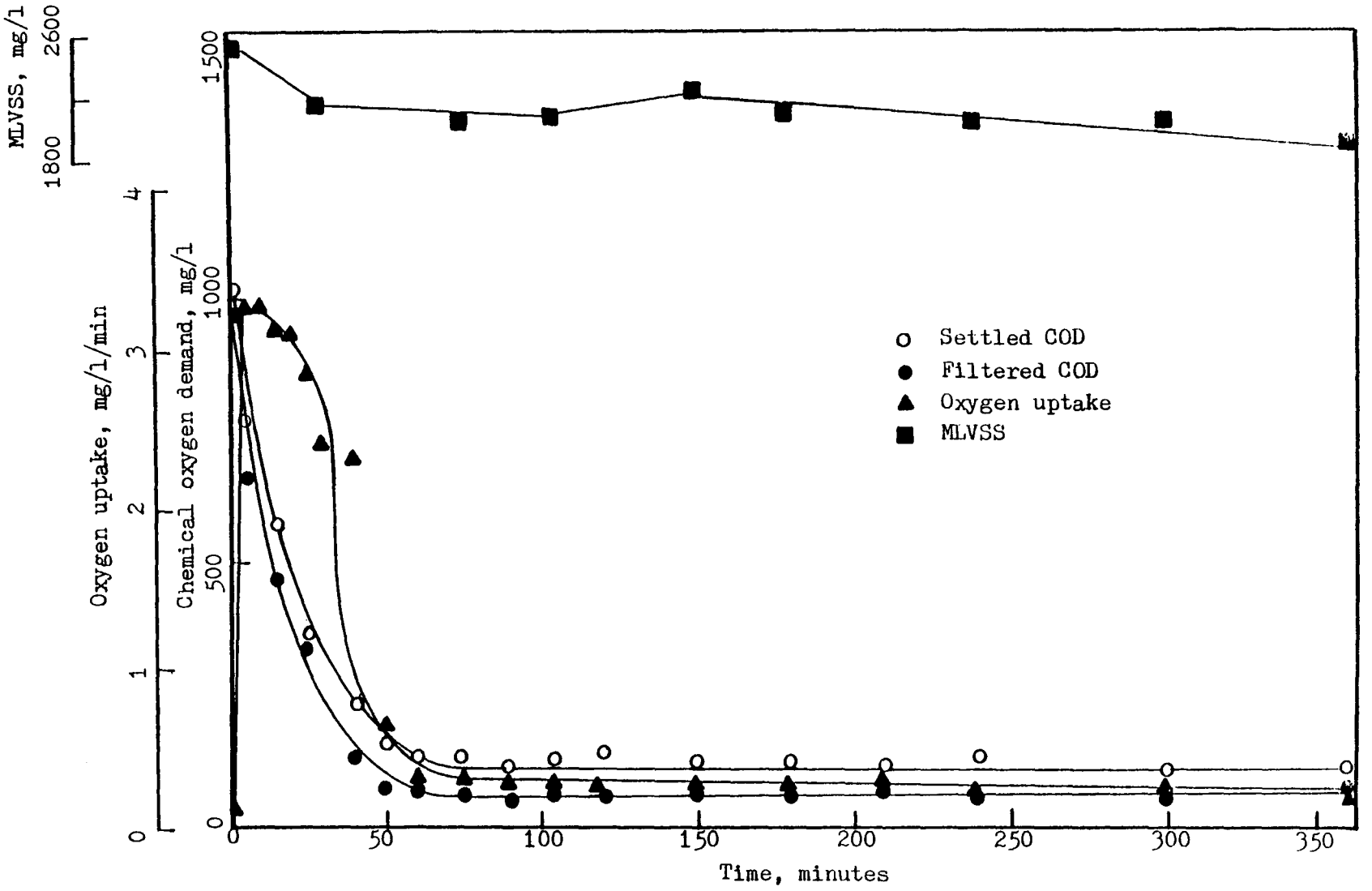

Figure 31. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the food processing wastewater at an initial loading of 0.512 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 2560 mg/l.

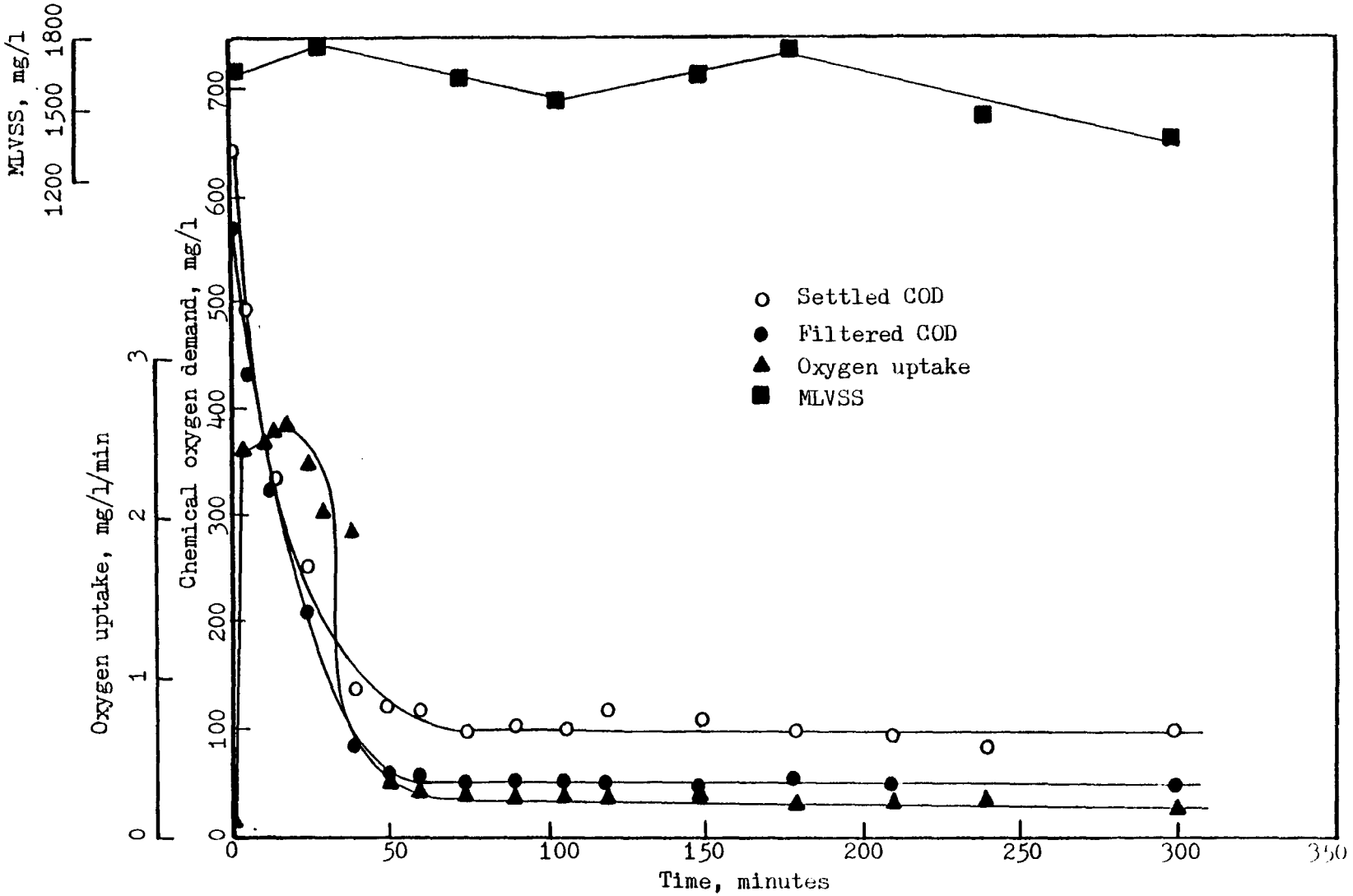

Figure 32. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the food processing wastewater at an initial loading of 0.487 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 1685 mg/l.

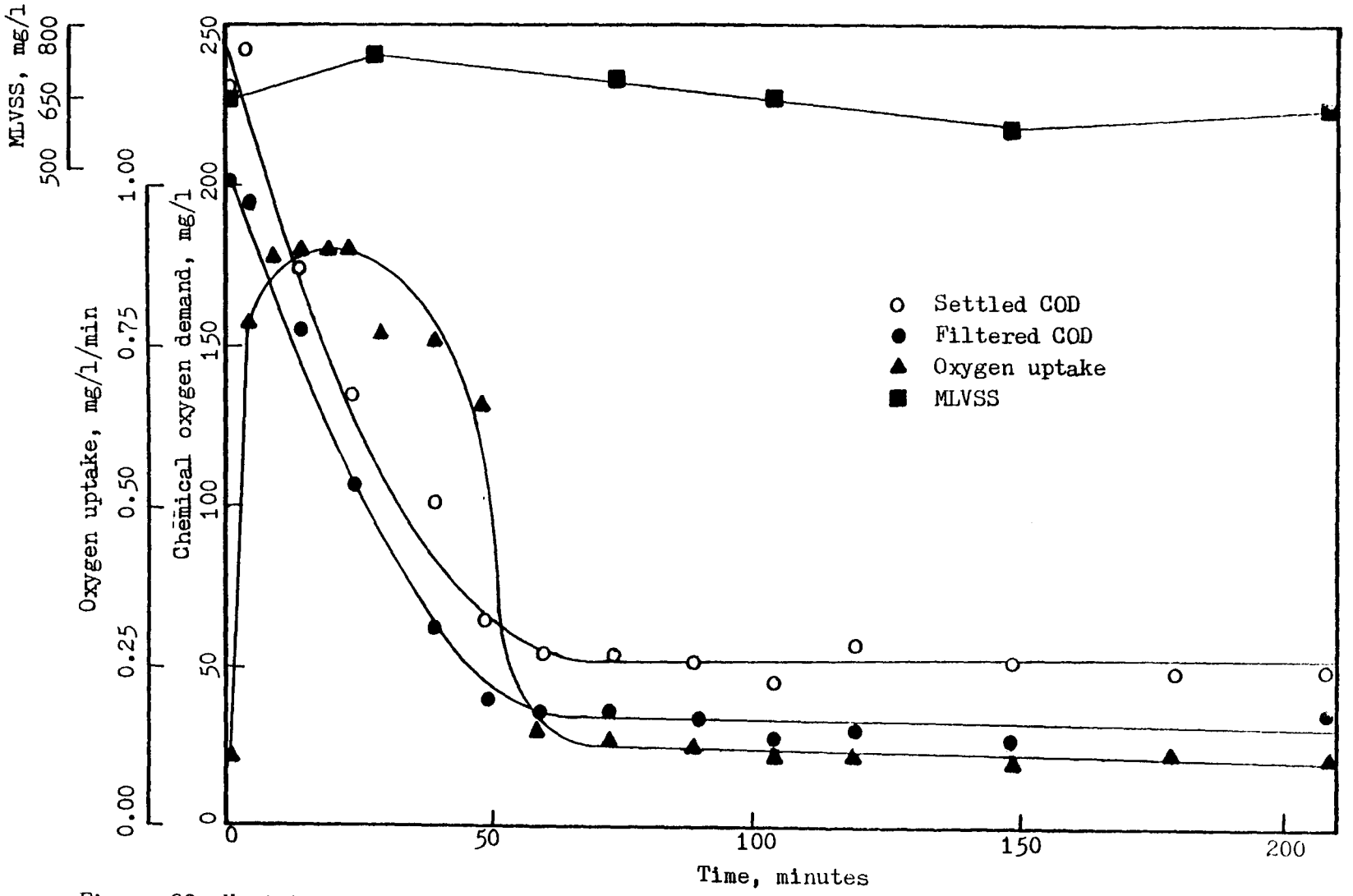

Figure 33. Variation of oxygen uptake, chemical oxygen demand, mixed liquor suspended solids with time for the food processing wastewater at an initial loading of 0.528 $\frac{\text{mg/l COD}}{\text{mg/l MLVSS}}$ and an initial MLVSS concentration of 640 mg/l.

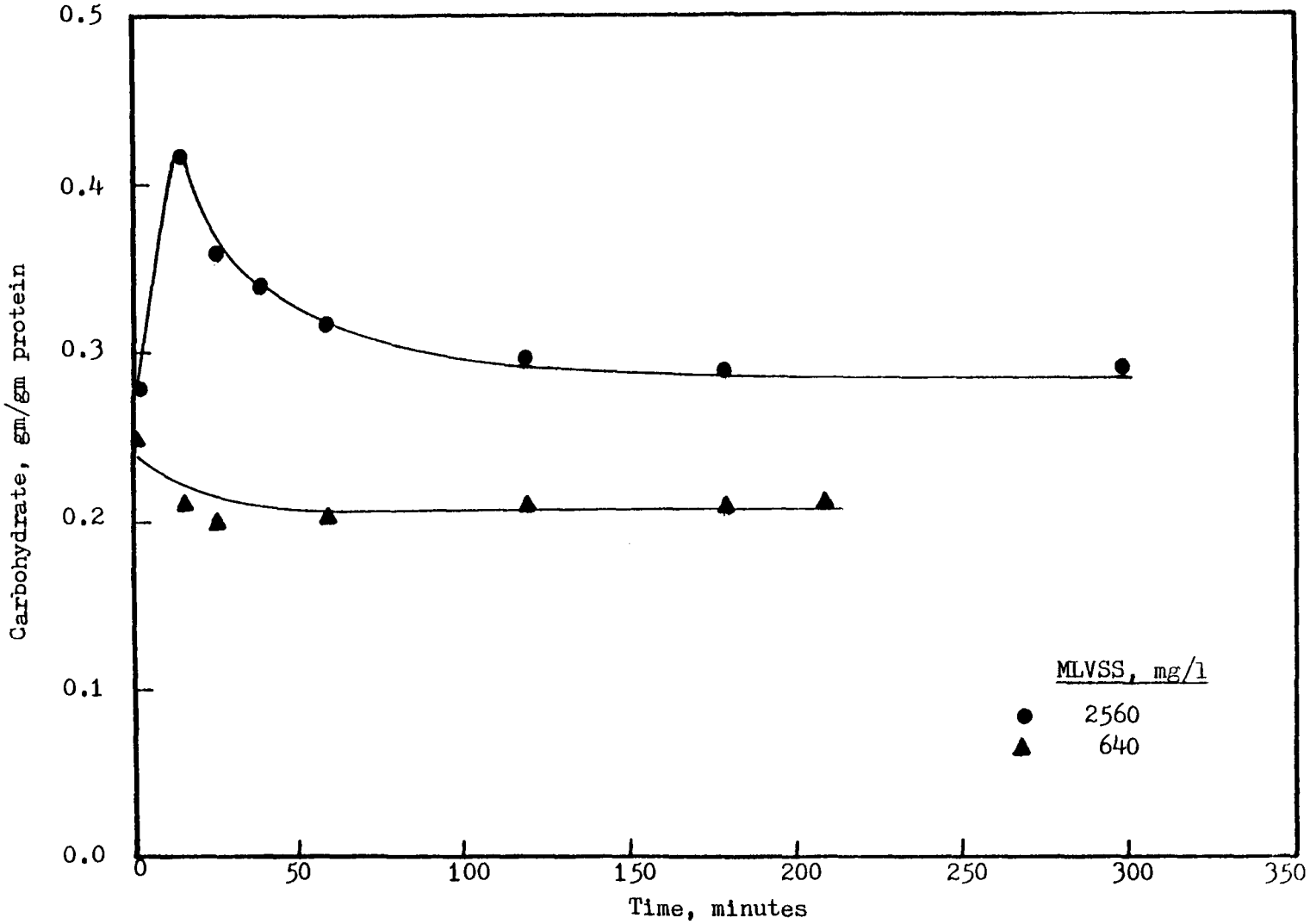

Figure 34. Variation in cellular carbohydrate during metabolism of food processing waste at the indicated MLVSS concentrations.

oxygen uptake to the constant endogenous rate when compared to the substrate removal curves.

Similar results were obtained for the experimental run conducted at an F/M ratio of 0.487 mg/l COD/mg/l MLVSS and an initial MLVSS concentration of 1685 mg/l, as presented in Figure 32. There was a reduction of 84 percent of the settled COD and 91 percent of the filtered COD.

Again, results similar to those obtained in the previous two batch runs were found using an initial MLVSS concentration of 640 mg/l and an F/M ratio of 0.528 mg/l COD/mg/l MLVSS. Reductions of 79 percent of the settled COD and 84 percent of the filtered COD were realized.

A very slight increase in pH with time was observed for the experimental series. The variations of MLVSS with time are also presented in Figures 31 through 33. In Figure 31 the MLVSS tends to decrease with time while Figures 32 and 33 indicate an initial increase and then a gradual decrease in the MLVSS concentration with time.

The variation of the carbohydrate content of the sludge with time for this experimental series is shown in Figure 34. A curve is not presented for the 1685 mg/l initial MLVSS concentration because of an experimental mishap associated only with certain protein and carbohydrate samples of this batch run. There was a rapid increase, followed by a rapid decrease, in the carbohydrate content of the sludge for the 2560 mg/l MLVSS concentration and the curve reached its peak at 15 minutes. The curve depicting the

variation of the cellular carbohydrate with time for the 640 mg/l MLVSS concentration, appears to decrease slightly during the first 20 minutes and then levels off for the remainder of the batch run.

The initial samples withdrawn for analysis in the experimental series, as was discussed in chapter three, were obtained from a 10 liter reactor which was used to maintain a reservoir of microbial sludge for the three experiments of the series and which had not been fed for a period of time sufficient to insure that the sludge was in the endogenous phase. Furthermore, the concentration of this 10 liter reactor was adjusted to the same MLVSS concentration that was to be used in each experiment. The F/M ratio was held constant for all reactors, throughout this experimental series.

Therefore, because of possible different and uncontrollable environmental conditions in the 10 liter reactor, the initial value for cellular carbohydrate for the 640 mg/l MLVSS concentration may be in error. The other values at this concentration suggest a constant relationship when the initial value is not considered. The final value of cellular carbohydrate should approach the initial value, as demonstrated for the 2560 mg/l MLVSS curve in Figure 34.

V. DISCUSSION

The contact stabilization process has been referred to as one of the most controversial of the activated sludge modifications because of the various theories that have been proposed to explain how the process works. Many investigators (32) have suggested that a wastewater containing a very low soluble organic concentration and demonstrating an initial uptake and subsequent release of organic substrate, followed by another uptake of organic substrate, is the best candidate for successful application of the contact stabilization process. The contact period would be that required for the initial uptake. Other researchers (36) recommend using contact stabilization for wastewaters demonstrating a high rate of organic substrate removal and a relatively low rate of stabilization of the sorbed and/or stored organic substrate. The period of stabilization required would be determined by the time it takes for the oxygen uptake rate to return to the endogenous level after substrate removal is complete.

The quantity of storage products in the cell has also been proposed as one means of measuring the required stabilization period (45). This potentially has practical applications, and is suggestive of the contact stabilization process because aeration of the cells must continue to insure substantial oxidation of the stored organics before the cells can be reused for further purification of the waste.

A series of batch experiments were conducted on three wastewaters of varying colloidal percentages and complexities. In the following

sections a discussion of the results obtained with respect to substrate removal, oxygen utilization, and microbial storage is presented.

SUBSTRATE REMOVAL

The slight uptake and release observed for the settled COD in Figure 24 was not considered significant compared to the total settled COD removed, the variability of the COD test, and lack of a similar effect for the filtered COD. No occurrence of an uptake and release phenomenon was found for any of the other experimental runs. From a practical standpoint, the uptake of organic substrate would have to be a very large percentage of the total biodegradable portion of the waste before utilization of the uptake and release phenomenon would be an effective method of treatment. The release would also have to be delayed sufficiently long enough to insure that release of the organic substrate would not occur while in the sedimentation tank of the contact stabilization process.

The filtered and settled COD removal rate constants were calculated for all experiments and are summarized in Table IV. There was an apparent increase in the filtered and settled COD removal rate constants with an increase in the mixed liquor volatile suspended solids concentrations (MLVSS). The percent COD reduction, for both filtered and settled COD, was also found to increase with an increase in MLVSS, as previously presented in chapter four.

TABLE IV Summary of operational parameters for the experimental runs.

Wastewater	Initial loading mg/l COD / mg/l MLVSS	MLVSS mg/l	Filtered COD[a] removal rate constant, r hour^{-1}	Settled COD[a] removal rate constant, r hour^{-1}
Domestic	0.358	1660	1.37	0.84
	0.358	990	0.51	0.59
	0.358	570	0.31	0.47
Paper Mill	0.462	2825	3.87	3.87
	0.454	1800	2.73	2.48
	0.455	740	1.51	1.53
Food Processing	0.512	2560	13.80	6.82
	0.487	1685	10.46	4.38
	0.528	640	5.06	3.69

[a]Removal rate constant was calculated by using the relationship: $r = \frac{L_o - L_e}{tL_e}$ (after Bhatla *et al.*, 36).

The food processing waste was found to have the highest removal rate constants and percent COD reductions for both the filtered and the settled COD. However, the actual relative increase in the removal rate constant with an increase in MLVSS did not vary substantially from one wastewater to another.

It appears that the ability of activated sludge mixed liquor to remove organic substrate is related to the concentration of microorganisms present, when the food-to-microorganism ratio is held constant. For the more colloidal wastewaters, this might be attributed to a higher incident of collision, physical impingement and enmeshment of organic matter resulting from formation of the larger biological flocs with the higher MLVSS concentrations. In the case of the paper mill wastewater, which was 13 percent colloidal, the increase in the COD removal rate constant may have been due, in part, to some of the aforementioned physical mechanisms. It is also possible that the ability of each microbe to transport substrate into the cell increased because of the production of more inducible enzymes within the cell that were capable ofutilizing and/or storing the substrate. The production of more inducible enzymes possibly could have occurred because the cells were exposed to higher substrate concentrations at the higher MLVSS concentrations, even though the overall food-to-microorganism ratio was held constant.

OXYGEN UTILIZATION

There were four cases in which oxygen uptake proceeded for a period of time after the filtered and settled COD remaining values

indicated that complete COD reduction had occurred. These are shown in Figures 23, 24, 27, and 28. The oxygen uptake curve in Figure 23 was the only curve that demonstrated a leveling off of oxygen uptake values for any extended period of time prior to returning to the endogenous phase. The lag or difference between the time that it took for the oxygen uptake to return to the endogenous rate and the time required for complete COD reduction shown in Figure 23 was sufficiently large enough to justify application of the contact stabilization process if the presence of a lag is accepted as a suitable indicator. However, the lag in Figure 24 would not support a similar conclusion.

In Figures 27 and 28 the lag was found to be quite substantial, however, the oxygen uptake fell quite rapidly from its maximum value to a much lower level of approximately the same point that maximum value to a much lower level at approximately the same point that maximum COD reduction had occurred. The oxygen uptake was then observed to approach the endogenous rate very slowly. In both cases, the use of a stabilization tank would probably not be warranted because of the much reduced oxygen uptake rate after complete COD reduction. It appears that utilization of oxygen uptake data to justify contact stabilization treatment would require more analysis than simply the time of return to endogenous level compared to time of substrate transfer.

The energy and endogenous oxygen were determined using the Bhatla et al. (36) approach as discussed in the literature. The

endogenous oxygen is expressed in terms of oxygen uptake per unit of MLVSS. Energy oxygen is expressed in terms of oxygen uptake per unit of chemical oxygen demand utilized. The results of these determinations for the three wastewaters at varying MLVSS concentrations are summarized in Table V. The endogenous oxygen is also expressed in terms of oxygen uptake per unit of cellular protein. The data, presented in Table V, shows that the observed energy oxygen and endogenous oxygen values were relatively constant for the paper mill and food processing wastewaters. For the domestic waste, however, there was a substantial increase in the energy oxygen with increasing MLVSS concentrations, and a substantial decrease in the endogenous oxygen with an increasing MLVSS concentration.

The energy oxygen concentration, as presented in Table V and expressed as mg/l, was observed to increase with an increasing MLVSS concentration. The variation of energy oxygen with MLVSS concentrations is depicted in Figure 35. A linear relationship was observed for all three wastewaters. The observed relationships were not unexpected because energy oxygen should correlate with the amount of available substrate present. Since relatively constant F/M ratios were used, the available substrate increased linearly with MLVSS increase. The linear increase simply illustrates that approximately the same fraction of available substrate was used for cell energy regardless of the MLVSS concentration because essentially constant F/M ratios were used.

TABLE V. Summary of oxygen uptake data for the domestic, paper mill, and food processing wastewaters.

Wastewater	Initial Loading mg/l COD / mg/l MLVSS	Initial MLVSS mg/l	Energy Oxygen for Filtered COD		Endogenous Oxygen		
			mg/l	mg/l / mg/l COD	mg/l	mg/l day / mg/l MLVSS	mg/l day / mg/l protein
Domestic	0.358	1660	78.24	1.07	25.80	0.09	0.15
	0.358	990	36.00	0.76	28.35	0.14	0.19
	0.358	570	4.13	0.28	16.51	0.35	0.40
Paper Mill	0.462	2825	189.60	0.22	88.80	0.15	0.15
	0.454	1800	110.10	0.23	63.60	0.19	0.18
	0.455	740	31.38	0.22	8.25	0.15	0.15
Food Processing	0.512	2560	101.50	0.11	22.50	0.17	0.26
	0.487	1685	72.50	0.14	17.00	0.19	-
	0.528	640	35.70	0.22	9.38	0.28	0.25

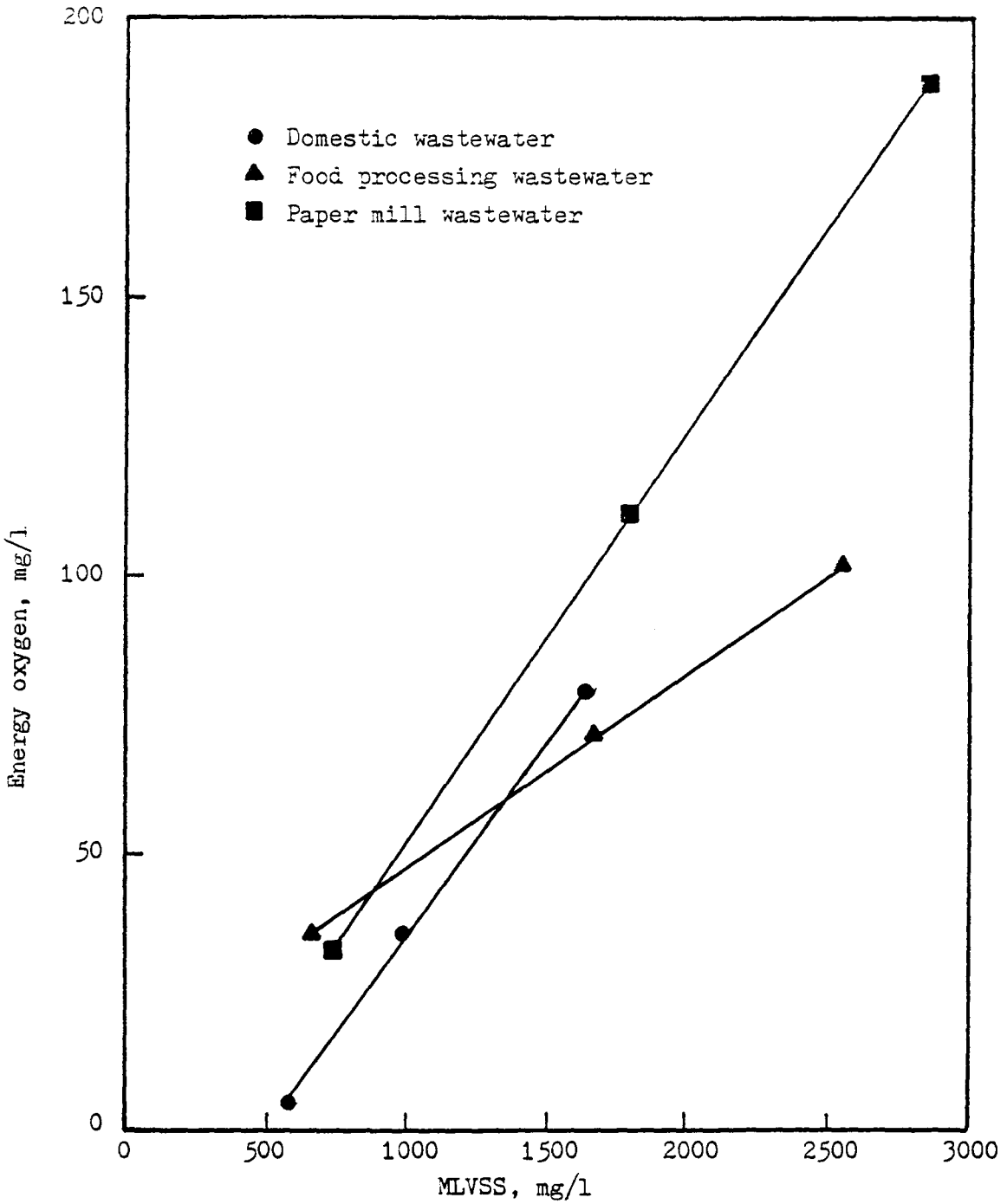

Figure 35. Variation of energy oxygen with mixed liquor volatile suspended solids (MLVSS) concentration for the domestic, paper mill, and food processing wastewaters.

MICROBIAL STORAGE

The variations of cellular carbohydrate with time for the three wastewaters investigated, are shown in Figures 26, 30 and 34. In Figure 26, for the domestic wastewater, there was an apparent storage phenomenon occurring at the 1660 and 990 mg/l MLVSS concentrations, as evidenced by the relative rate of increase of the carbohydrate concentration with respect to the protein concentration. No storage was observed for the 570 mg/l MLVSS concentration. The difference in the initial carbohydrate content values for the three curves in Figure 26 can be attributed to inorganic particulate matter held in the sludge which, in later sludge suspensions, may have resulted in higher absorbance readings when conducting the colorimetric analyses for determining the protein and carbohydrate concentrations of the sludge. At higher MLVSS concentrations more of the organic matter would be retained in the sludge and, therefore, the initial values will be slightly higher than those at the lower concentrations. However, this interference would have been uniform within the experimental series and should not have caused the relative increase of cellular carbohydrate with time, with respect to the initial cellular carbohydrate fraction. Figure 26 suggests that, for treatment purposes, aeration of the microorganisms at the highest and intermediate MLVSS concentrations, should continue without further addition of substrate for a time sufficient to insure that the stored cellular carbohydrate has been depleted and the sludge is in a suitable condition to rapidly sorb and utilize additional substrate.

Similar results were obtained for the paper mill waste, as shown in Figure 30, and demonstrate that the storage phenomenon at the intermediate MLVSS concentration was not nearly as pronounced as was that for the highest concentration of MLVSS. Again, no storage phenomenon was observed for the lowest MLVSS concentration.

The food processing waste results, illustrated by Figure 34, demonstrated an immediate and rapid storage of carbohydrate, followed by a gradual reduction in cellular carbohydrate for the 2560 MLVSS concentration. No noticeable storage phenomenon was observed for the 640 MLVSS concentration. An explanation for the higher initial value of cellular carbohydrate, at the 640 MLVSS concentration, has already been discussed in chapter four. The rapid increase and then decrease in the storage of carbohydrate, at the high MLVSS concentration, suggest that an extended period of aeration would not be necessary to insure that the stored carbohydrate has been depleted.

For each of the three experimental series of batch runs utilizing a different wastewater, there was a pronounced increase in carbohydrate storage associated with the higher MLVSS concentrations, and no observable storage phenomenon for the lowest MLVSS concentrations. This, as was discussed in an earlier section, is probably related to the higher rates of organic substrate removal observed for the higher MLVSS concentrations. As a result of higher rates of removal of organic substrate at higher MLVSS concentrations, it

is probable that a larger percentage of the substrate removed was stored rather than utilized for growth and maintenance.

The ratios of carbohydrate per unit MLVSS, and of protein per MLVSS were determined and are presented in Tables VI, VII, and VIII for the domestic, paper mill, and food processing wastewater respectively. For the highest MLVSS concentrations of each experimental series, there was an increase in carbohydrate per MLVSS at approximately the same time intervals as those observed in Figures 26, 30, and 34, which is suggestive of a storage phenomenon. However, it is considered undesirable to express the variation in storage products by the change in the concentration of the solids, since the solids weight is not an accurate measure of the number of cells (45). For the lower two MLVSS concentrations of each experimental series, there was no observable increase in the carbohydrate per MLVSS ratio. The protein/MLVSS ratio was observed to decrease with increasing MLVSS concentration for the experiments conducted using the domestic waste. This suggests that at higher MLVSS concentrations a larger percentage of the substrate removed was stored rather than utilized for growth and maintenance. The protein/MLVSS ratios were not observed to vary to any degree with the MLVSS concentration for the paper mill or the food processing wastewater.

As previously mentioned, inorganic particulate matter held in the sludge may have resulted in higher absorbance readings and therefore higher protein and carbohydrate concentrations. However,

TABLE VI. Summary of carbohydrate/unit MLVSS and protein/unit MLVSS ratios for the experimental runs, conducted at three different initial MLVSS concentrations, using the domestic wastewater.

Time Minutes	Initial MLVSS concentration of 1660 mg/l		Initial MLVSS concentration of 990 mg/l		Initial MLVSS concentration of 570 mg/l	
	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS
0	0.23	0.57	0.27	0.72	0.26	0.86
30	-	-	0.25	0.69	0.25	0.85
75	0.25	0.58	0.26	0.72	0.25	0.86
105	-	-	0.25	0.67	0.24	0.86
150	0.28	0.59	0.27	0.70	0.23	0.83
210	-	-	0.23	0.59	0.25	0.93
240	0.26	0.58	0.27	0.74	0.24	0.85
300	0.22	0.55	0.27	0.78	0.22	0.80
360	0.20	0.53	0.25	0.76	0.23	0.83
420	-	-	0.23	0.79	0.24	0.82

TABLE VII. Summary of carbohydrate/unit MLVSS and protein/unit MLVSS ratios for the experimental runs, conducted at three different initial MLVSS concentrations, using the paper mill wastewater.

Time Minutes	Initial MLVSS concentration of 2825 mg/l		Initial MLVSS concentration of 1800 mg/l		Initial MLVSS concentration of 740 mg/l	
	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS
0	0.22	1.03	0.21	1.06	0.14	0.93
30	0.21	0.95	0.20	0.96	0.12	0.89
60	0.21	0.92	-	-	-	-
75	-	-	0.19	0.80	0.12	0.86
105	0.22	0.89	0.18	0.77	0.12	0.80
150	0.27	0.91	0.19	0.82	0.13	0.90
210	0.25	0.74	0.19	0.85	0.11	0.82
240	-	-	0.19	0.90	0.10	0.73
300	0.26	0.82	0.20	0.91	0.11	0.72
360	0.22	0.92	0.19	0.90	-	-
420	0.22	0.98	0.19	0.93	-	-
480	0.20	0.98	-	-	-	-

TABLE VIII. Summary of carbohydrate/unit MLVSS and protein/unit MLVSS ratios for the experimental runs, conducted at three different initial MLVSS concentrations, using the food processing wastewater.

Time Minutes	Initial MLVSS concentration of 2560 mg/l		Initial MLVSS concentration of 640 mg/l	
	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS	mg/l carbohydrate / mg/l MLVSS	mg/l protein / mg/l MLVSS
0	0.18	0.64	0.28	1.14
15	0.25	0.61	-	-
30	0.21	0.61	0.11	0.59
75	0.18	0.57	0.12	0.58
105	0.17	0.56	0.12	0.58
150	0.15	0.53	0.14	0.62
180	0.16	0.57	-	-
210	-	-	0.13	0.56
240	0.17	0.59	-	-
300	0.17	0.59	-	-
360	0.18	0.65	-	-

this would have been uniform within the experimental series and would not have effected any relative increase of cellular carbohydrate with time. This explains why the protein/MLVSS and carbohydrate/MLVSS fractions together exceed unity in some cases.

VI. CONCLUSIONS

Evaluation of the data from the series of activated sludge batch experiments utilizing domestic, paper mill and food processing wastewaters has led to the following conclusions:

1. There was no significant uptake and subsequent release of organic substrate observed for any of the wastewaters studied. All the wastewaters, regardless of the colloidal fraction, demonstrated organic removal patterns suggestive of soluble substrates, which conflicts with current and popular concepts of colloidal removal in biological systems. It was concluded that resolubilization of colloidal substrate is not an important mechanism in activated sludge treatment.

2. For the same F/M ratio, the rate of removal of organic substrate and the degree to which it is removed in an activated sludge system is a direct function of the mixed liquor volatile suspended solids (MLVSS) concentration.

3. A substantial lag in the time required for the oxygen uptake rate to return to the endogenous level was observed only for the experimental run utilizing (1) domestic wastewater as a source of organic substrate, and (2) the highest mixed liquor volatile suspended solids concentration of that experimental series (1660 mg/l). It was concluded that stored substrate will not necessarily cause a continuation of high oxygen utilization activity after substrate removal is complete.

4. A decrease in MLVSS concentration generally resulted in an increase in the endogenous oxygen utilization rate per unit MLVSS. The variation of energy oxygen utilization with MLVSS concentration was more complex, however. For the domestic wastewater there was a substantial increase in the energy oxygen with increasing MLVSS concentration, but for the food processing wastewater energy oxygen utilization increased with decreasing MLVSS concentration. Utilization was constant for the paper mill waste.

5. At a constant food-to-microorganism ratio, the energy oxygen concentration, expressed as mg/l of oxygen utilized for energy reactions, increases linearly with increases in the MLVSS concentration of an activated sludge system.

6. Cellular carbohydrate in activated sludge operated at a constant F/M ratio increases as the MLVSS concentration increases. Greater substrate storage during substrate removal, as measured by an increase in cellular carbohydrate, occurred when higher MLVSS concentrations were used for each of the experimental series of batch tube runs, utilizing a different wastewater, even though the food-to-microorganism ratio was nearly constant. No significant storage of carbohydrates was observed at the lowest MLVSS concentrations for any of the three experimental series.

7. In a batch activated sludge system, the absolute value of the carbohydrate fraction in activated sludge apparently increases as the MLVSS concentration increases. The relative initial value between sludges varies with the nature of the substrate utilized.

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APPENDIX

BATCH EXPERIMENTAL DATA

Domestic Wastewater (initial MLVSS of 1660 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.054	105.0	187.7	1660	7.10	-	-
5	0.618	96.0	207.5	-	-	951	384
10	0.657	-	-	-	-	-	-
15	0.574	71.1	110.7	-	-	951	410
20	0.584	-	-	-	7.65	-	-
25	0.639	56.1	131.2	-	-	912	362
30	0.635	-	-	-	7.70	-	-
40	0.599	49.4	77.1	-	-	984	414
50	0.596	-	-	-	7.80	-	-
60	0.461	45.4	71.1	-	-	955	416
75	0.439	35.6	-	1650	7.80	-	-
90	0.413	34.8	65.2	-	-	925	424
105	0.400	33.6	71.1	-	-	-	-
120	0.458	33.6	65.2	-	7.78	967	424
150	0.422	29.6	61.3	1635	-	-	-
180	0.435	31.6	57.3	-	7.70	903	476
240	0.119	32.8	61.3	1625	7.74	945	394
300	0.109	41.5	57.3	1645	7.79	905	286
360	0.122	33.6	53.4	1580	7.89	840	348
420	0.152	29.6	53.4	1530	7.90	-	-

Domestic Wastewater (initial MLVSS of 990 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.094	80.3	156.7	990	7.30	-	-
5	0.612	80.7	136.5	-	7.45	696	224
10	0.624	-	-	-	-	-	-
15	0.594	64.7	108.6	-	-	696	292
20	0.558	-	-	-	7.68	-	-
25	0.438	58.8	88.7	-	-	695	230
30	0.396	-	-	1020	7.70	-	-
40	0.354	45.8	89.8	-	-	721	286
50	0.396	45.2	94.6	-	7.78	-	-
60	0.294	46.6	88.7	-	-	668	224
75	0.276	44.0	83.1	960	7.80	-	-
90	0.318	42.6	79.9	-	-	648	-
105	0.240	43.4	72.7	1000	7.76	-	-
120	0.240	41.3	70.3	-	7.81	681	256
150	0.156	35.9	64.7	890	7.81	-	-
180	0.126	36.5	56.8	-	-	566	226
210	0.120	-	-	1020	-	-	-
240	0.114	35.7	58.8	860	7.78	670	214
300	0.096	34.4	47.6	820	7.91	630	230
360	0.096	31.0	56.8	850	7.90	623	218
420	0.096	33.2	48.0	880	7.81	699	202

Domestic Wastewater (initial MLVSS of 570 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.110	33.3	72.2	570	-	-	-
5	0.322	34.6	-	-	7.20	496	128
10	0.324	-	-	-	-	-	-
15	0.228	36.7	24.2	-	-	420	154
20	0.204	-	-	-	7.54	-	-
25	0.186	34.6	63.8	-	-	525	134
30	0.186	-	-	590	7.63	-	-
40	0.156	30.8	57.5	-	-	545	144
50	0.162	27.2	-	-	7.67	-	-
60	0.156	27.2	36.7	-	-	545	151
75	0.150	25.2	51.3	595	7.70	-	-
90	0.144	25.2	36.7	-	-	507	137
105	0.150	24.2	36.7	605	7.66	-	-
120	0.132	25.2	35.8	-	7.70	485	138
150	0.156	-	34.6	635	7.62	-	-
180	0.162	24.0	29.2	-	7.50	575	158
210	0.132	-	-	580	-	-	-
240	0.138	19.2	30.4	613	7.73	533	146
300	0.144	20.6	28.3	665	7.40	525	146
360	0.132	14.8	28.3	625	7.38	505	147
420	0.072	18.3	-	608	7.42	495	154

Paper mill Wastewater (initial MLVSS of 2825 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.152	1049.8	1052.4	2825	-	2900	616
5	2.748	717.7	781.9	-	7.10	-	-
10	2.865	-	-	-	-	2750	648
15	3.120	605.2	633.3	-	-	-	-
20	2.870	-	-	-	7.15	-	-
25	3.073	483.9	528.9	-	-	3050	554
30	3.060	-	-	3100	7.20	-	-
40	3.000	370.7	365.1	-	-	2520	672
50	2.443	291.9	316.1	-	7.27	-	-
60	1.563	203.6	226.1	3220	-	3050	690
75	0.712	195.6	203.6	-	7.31	-	-
90	0.607	-	202.8	-	-	-	-
105	0.546	170.7	201.2	3215	7.39	-	-
120	0.512	163.5	187.6	-	7.38	3010	708
150	0.470	153.8	193.9	2865	7.30	-	-
180	0.447	171.5	203.6	-	7.40	2380	806
210	0.414	179.5	187.6	3175	7.45	-	-
240	0.428	165.9	187.6	-	7.40	-	-
300	0.332	227.7	227.7	2865	7.48	2290	780
360	0.278	223.7	195.6	2770	7.37	2550	550
420	0.265	147.4	194.0	2690	7.35	-	-
480	0.247	169.9	231.7	2835	7.39	2780	614

Paper mill Wastewater (initial MLVSS of 1800 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.185	583.7	615.7	1800	-	-	-
5	2.063	481.7	521.6	-	7.01	-	-
10	1.991	-	-	-	-	-	-
15	1.950	403.2	440.9	-	-	1644	419
20	1.809	-	-	-	7.00	-	-
25	1.828	324.4	352.2	-	-	1821	365
30	1.826	-	-	1815	7.00	-	-
40	1.600	252.6	284.1	-	-	1909	368
50	1.471	209.6	236.7	-	7.02	-	-
60	1.395	192.8	188.8	-	-	1721	334
75	0.480	131.5	141.8	1940	7.10	-	-
90	0.403	126.7	145.0	-	-	-	-
105	0.368	121.1	145.0	1965	7.08	-	-
120	0.339	125.1	151.4	-	7.09	1447	348
150	0.320	108.4	118.3	1900	7.09	-	-
180	0.287	105.2	119.5	-	7.15	1726	356
210	0.271	145.0	117.1	1950	7.11	-	-
240	0.243	97.2	113.2	1890	7.07	-	-
300	0.217	105.2	117.1	1885	7.10	1715	360
360	0.219	94.0	117.1	1920	7.10	1730	315
420	0.217	101.2	135.1	1895	7.14	1754	404

Paper mill Wastewater (initial MLVSS of 740 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.240	191.0	211.0	740	-	-	-
5	0.700	184.8	224.8	-	7.23	-	-
10	0.660	-	-	-	-	-	-
15	0.617	163.6	177.2	-	-	728	103
20	0.579	-	-	-	7.21	-	-
25	0.567	136.4	145.1	-	-	770	93
30	0.558	-	-	755	7.10	-	-
40	0.524	107.6	104.0	-	-	566	73
50	0.464	89.6	97.6	-	7.10	-	-
60	0.411	73.6	84.0	-	-	663	98
75	0.133	63.6	74.4	755	7.05	-	-
90	0.120	63.6	-	-	-	-	-
105	0.097	60.8	68.8	775	7.09	-	-
120	0.084	51.6	61.6	-	7.06	750	95
150	0.075	40.8	61.6	690	7.05	-	-
180	0.061	60.8	53.6	-	7.04	600	101
210	0.063	44.4	56.8	735	7.03	-	-
240	0.059	53.6	56.8	805	7.07	-	-
300	0.052	43.6	56.8	755	7.00	521	74

Food processing Wastewater (initial MLVSS of 2560 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.09	976.4	1090.2	2560	-	1650	454
5	3.29	667.2	770.7	-	7.01	-	-
10	3.30	-	-	-	-	-	-
15	3.12	478.5	573.3	-	-	1380	598
20	3.11	-	-	-	7.27	-	-
25	2.86	345.1	368.0	-	-	1305	442
30	2.41	-	-	2170	7.27	-	-
40	2.37	138.9	232.1	-	-	1230	436
50	0.61	71.9	154.8	-	7.40	-	-
60	0.31	67.9	138.9	-	-	1289	340
75	0.28	67.9	135.0	2060	7.40	-	-
90	0.25	60.0	119.2	-	-	-	-
105	0.24	64.0	131.1	2090	7.40	-	-
120	0.26	67.9	143.0	-	7.36	1188	390
150	0.25	67.9	123.2	2230	7.31	-	-
180	0.23	67.1	121.6	2100	7.35	1190	336
210	0.22	67.9	115.3	-	7.38	-	-
240	0.21	67.1	135.0	2025	7.36	-	-
300	0.20	60.0	103.4	2035	7.25	1205	374
360	0.19	75.8	114.4	1880	7.38	1218	-

Food processing Wastewater (initial MLVSS of 1685 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.09	572.2	646.7	1685	-	-	-
5	2.43	432.5	497.2	-	7.01	-	-
10	2.48	-	-	-	-	-	-
15	2.57	330.2	337.1	-	-	-	-
20	2.60	-	-	-	7.03	-	-
25	2.31	209.3	254.4	-	-	-	-
30	2.03	-	-	1795	7.04	-	-
40	1.92	86.2	135.5	-	-	-	-
50	0.34	55.4	120.1	-	7.04	-	-
60	0.29	63.1	116.2	-	-	-	-
75	0.28	47.7	97.0	1645	7.03	-	-
90	0.26	53.9	100.8	-	-	-	-
105	0.26	51.6	100.1	1560	7.10	-	-
120	0.24	51.6	116.2	-	7.09	-	-
150	0.26	47.7	108.5	1665	7.20	-	-
180	0.23	55.4	98.9	1775	7.12	-	-
210	0.22	47.7	93.1	-	7.17	-	-
240	0.22	-	83.5	1455	7.10	-	-
300	0.19	47.7	95.1	1380	7.20	-	-

Food processing Wastewater (initial MLVSS of 640 mg/l)

SAMPLE TIME (min)	OXYGEN UPTAKE (mg/l)/(min)	COD (mg/l)		MLVSS (mg/l)	pH	CELLULAR PROTEIN (mg/l)	CELLULAR CARBOHYDRATE (mg/l)
		FILTERED	SETTLED				
0	0.11	200.8	230.9	640	-	730	180
5	0.78	195.6	244.8	-	6.92	-	-
10	0.89	-	-	-	-	-	-
15	0.90	155.6	173.6	-	-	379	76
20	0.91	-	-	-	6.96	-	-
25	0.90	107.6	133.6	-	-	400	86
30	0.77	-	-	735	6.98	-	-
40	0.76	61.6	101.6	-	-	400	93
50	0.66	39.6	63.6	-	7.02	-	-
60	0.15	35.6	53.6	-	-	519	76
75	0.13	35.6	53.6	695	6.97	-	-
90	0.13	33.6	51.6	-	-	-	-
105	0.12	27.6	45.6	650	7.00	-	-
120	0.12	29.6	57.6	-	7.05	404	66
150	0.10	27.6	51.6	585	7.09	-	-
180	0.11	-	49.6	-	7.12	386	80
210	0.11	33.6	49.6	620	7.10	335	71

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AN INVESTIGATION OF SUBSTRATE REMOVAL AND STORAGE IN THE ACTIVATED SLUDGE PROCESS

by

Steven Robert Hearne

(ABSTRACT)

The objective of this study was to investigate what effect the concentration of microorganisms would have on substrate removal, microbial substrate storage, and oxygen utilization at a constant food-to-microorganism ratio. Batch experiments were conducted, under aerated and completely mixed conditions, using a domestic wastewater, a paper mill wastewater, and a food processing wastewater. A series of three batch experiments were run for each of these wastes. The food-to-microorganism ratio for each series was kept constant while the mixed liquor suspended solids concentration was varied for each of the experiments within the series. The following analyses were conducted on samples that were withdrawn at specified time intervals: filtered and settled COD, oxygen uptake, mixed liquor volatile suspended solids (MLVSS), pH, protein concentration and carbohydrate concentration.

No significant uptake and subsequent release of organic substrate was observed for any of the wastewaters studied. For the same F/M ratio, the rate of removal of organic substrate and the degree to which it was removed in the activated sludge system was found to be

a direct function of the MLVSS concentration. The change in the cellular carbohydrate to cellular protein ratio in the activated sludge during substrate metabolism was a function of the MLVSS concentration. As the MLVSS concentration increased, the carbohydrate to protein ratio, which is an indicator of substrate storage, also increased, even though the F/M ratio was held constant.