THE UNIVERSITY OF MANITOBA

ATP
A MONITORING PARAMETER
OF
EXTENDED AERATION
SEWAGE TREATMENT SYSTEMS

BY
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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF CIVIL ENGINEERING
WINNIPEG, MANITOBA
FEBRUARY, 1981

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ABSTRACT

Traditional means of measuring cell viability or the active portion of biomass in activated sludge sewage treatment plants are known to have severe limitations. The adenosine triphosphate, ATP, content of biomass is a relatively new viability parameter to be considered and additional information to determine ATP levels and its applicability to traditional activated sludge kinetics is required.

A model extended aeration, activated sludge sewage treatment plant was operated in a "steady state" condition for 56 days at 4°C. Loading parameters were monitored as BOD_5, COD and TOC; viability parameters of the raw sewage, mixed liquor and effluent were monitored as volatile suspended solids, VSS and ATP.

The average ATP concentration in the raw sewage, mixed liquor and effluent were 0.558, 0.9535 and 1.120μg ATP/mg VSS, respectively. The average kinetic growth and substrate utilization rate constants determined using ATP and VSS as viability parameters were as follows:

	Y	kd	μmax	K_s
ATP	0.470 (μg ATP/mg BOD_5)	1.247×10^{-3}/hr	2.35×10^{-3}/hr	5.72 (mg/L)
VSS	0.580 (mg VSS/mg BOD_5)	1.207×10^{-3}/hr	2.005×10^{-3}/hr	4.068 (mg/L)

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LIST OF SYMBOLS

ATP - adenosine triphosphate

BOD_5 - 5 day biochemical oxygen demand

COD - chemical oxygen demand

SRT - solids retention time

ADP - adenosine disphosphate

AMP - adenosine monophosphate

FAD - flavin dinucleotide

μm - micrometer; 10^{-9} meter

l - litre; L

ml - millilitre; mL

mg - milligram

CPM - unitless photometer digital count

SS - total nonfilterable residue

VSS - total volatile residue

TSS - total fixed residue

μg - microgram

min - minute

^{o}C - degree Celsius

MLSS - mixed liquor SS

MLVSS - mixed liquor VSS

RSS - raw sewage SS

RVSS - raw sewage VSS

ESS - effluent VSS

hr - hour

[ ] - concentration

SVI - sludge volume index

N - nitrogen

μmhos/cm - micro mhos per centimetre, measure of conductivity

NTU - nepthelometric turbidity unit

% - percent

TOC - total organic carbon

F/M - substrate concentration/microorganism concentration measured as VSS

Eff - efficiency

R/Q - sludge recycle ratio

cm - centimetre

PCA - perchloric acid, extraction technique

ĀTP - A MONITORING PARAMETER OF EXTENDED AERATION SEWAGE TREATMENT SYSTEMS

1. INTRODUCTION

Developments in waste treatment technology and constantly refined methods of monitoring waste treatment techniques have cultured a need for more accurate parameters to measure biological viability. As demands for more efficient waste treatment systems increase and the desire to automate large systems grows a need for more accurate means to measure the biological condition of these systems gains importance.

1.1. REASON FOR THE STUDY

Continuous and intermittent problems with poor activated sludge treatment efficiency have been related to problems with process control testing procedure and process controllability, which are identified amongst the primary causes (1)*. An indication that poor treatment efficiency

* The numbers in parentheses in the text indicate references in the Bibliography.

is being obtained is through the frequent difficulties encountered in controlling the loss of volatile suspended solids in the effluent.

Interest in the use of adenosine triphosphate, ATP, to measure biological waste treatment systems viability has increased in recent times (2), (3), (4), (5). The widely used methods for measuring or estimating activated sludge active cell content or viability are mass measurements of total suspended solids, TSS, and volatile suspended solids, VSS (4). These methods are, however, nonspecific and include variable concentrations of available substrate and cell debris.

These traditional means of measuring cell viability have been used extensively in modelling activated sludge kinetics (6), (7). Accumulations of biologically inert solids in an extended aeration system, however, tend to create a nonconstant relationship between suspended solids and viable organisms which is not, generally, considered to be necessary to consider, or true, in short term model studies (7). Recent studies indicate that ATP measurements may provide a useful means of indicating viable cells (8) which may provide a more precise means of interpretating the kinetics of full scale waste treatment systems.

Models developed for activated sludge systems include many generalizing assumptions to simplify interpretation.

These include a constant ratio of viable to non viable mass and that viable organisms in the influent is insignificant as compared to that of the mixed liquor (7). Further investigation into the validity of using ATP as a nonconservative measure of viability, that is, its association only with living cells, may aid in the refinement of models and increase the efficienty of plant control and operation.

2. SCOPE OF THE INVESTIGATION

2.1. OBJECTIVES

The objectives of this investigation using a continuous flow extended aeration plant were;

1) to evaluate the ATP concentration variation in the mixed liquor with respect to the sludge age,

2) to investigate the relationship between mixed liquor ATP concentration versus substrate removal rates,

3) and to investigate the use of ATP in biokinetic interpretation of continuous feed, complete mix activated sludge, that is, the kinetic growth and substrate utilization rate constants.

2.2. EXTENT OF THE INVESTIGATION

The laboratory model extended aeration plant and the operation were the same as that as described and used by Topnik (9). The plant was operated, after an initial start up period, for 56 days at $4 \pm .5^{o}C$ with the following daily testing;

1) ATP on raw, mixed liquor and effluent,

2) total carbon and organic carbon of raw sewage, mixed liquor and effluent,

3) BOD_5 on raw sewage and effluent,

4) volatile suspended solids and total suspended

solids on raw sewage, mixed liquor and effluent,

5) mixed liquor settleability and pH,

6) pH, turbidity and conductivity on raw sewage and effluent,

7) orthophosphate on raw sewage, mixed liquor and effluent,

8) temperature

9) and feed flow rate.

Other tests conducted intermittently throughout the investigation were;

1) total phosphate on raw sewage, mixed liquor and effluent,

2) organic nitrogen on mixed liquor,

3) COD total and COD soluble on raw sewage and effluent,

4) and oxygen uptake rates of the mixed liquor.

The plant was run for an additional 23 days wasting a predetermined volume of mixed liquor to maintain a desired solids retention time (SRT). The above mentioned tests were conducted twice weekly.

3. LITERATURE REVIEW

3.1. INTRODUCTION

Secondary wastewater treatment technology is based on the capability of a treatment process to biologically degrade animal and industrial wastes. The settleable solids in this type of treatment are active masses of microorganisms, or activated sludge. Wastes are mixed in an aeration tank with return activated sludge. The microorganisms aerobically degrade or stabilize the organic matter as the waste flows through the aeration tank. A final sedimentation chamber allows the activated solids to flocculate in quiescent conditions, separating the return activatved sludge and clear effluent.

The extended aeration activated sludge method of treatment, with a typical solids retention time of 20 to 30 days, is widely used in Canadian conditions being suited to the needs of current domestic waste treatment standards with minimal plant control. The current increasing demands for water quality control have resulted in an increasing need for treatment control technology. The application of any treatment control parameter, which may be applied to biological waste treatment systems, should reliably reflect the active or viable organisms in the treatment reactor which are responsible for degrading the organic wastes.

The use of adenosine triphosphate, ATP, as a monitoring parameter for activated sludge processes has recently been investigated by several researchers and the preliminary results merit further investigation. The purpose of this literature review, is to present the microbiological fundamentals of ATP and to examine the results of other investigators related to activated sludge operation.

3.2. BASIC CELL STRUCTURE

Microorganisms responsible for the aerobic degradation of organic material in activated sludge wastewater treatment processes are classified as heterotrophic protists. By definition, these organisms require an organic source of carbon for energy (10). The protists are subdivided into classes; higher, lower and viruses, refer to Figure 1. The higher protists or eucaryotic cells and the lower protists or procarytic cells are of interest in aerated biological waste treatment systems.

Procaryotes are simple cells with a single cell membrane, no membrane organelles such as a mitochondria and contain only one chromosome. The basic cell structure of the procaryote bacterium Escherichia Coli, a member of the coliform group of bacteria, is shown in Figure 2.

Eucaryotes are relatively complex cells containing nuclear material divided into several chromosomes and membrane organelles such as mitochondria and golgi bodies.

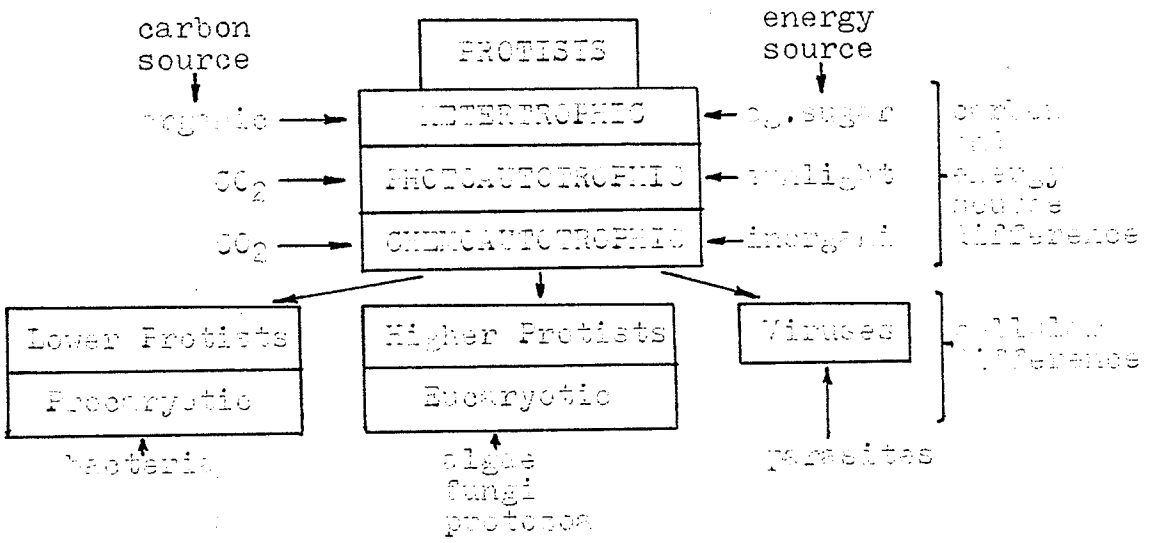


FIGURE 1. ORDERS OF THE PROTIST KINGDOM

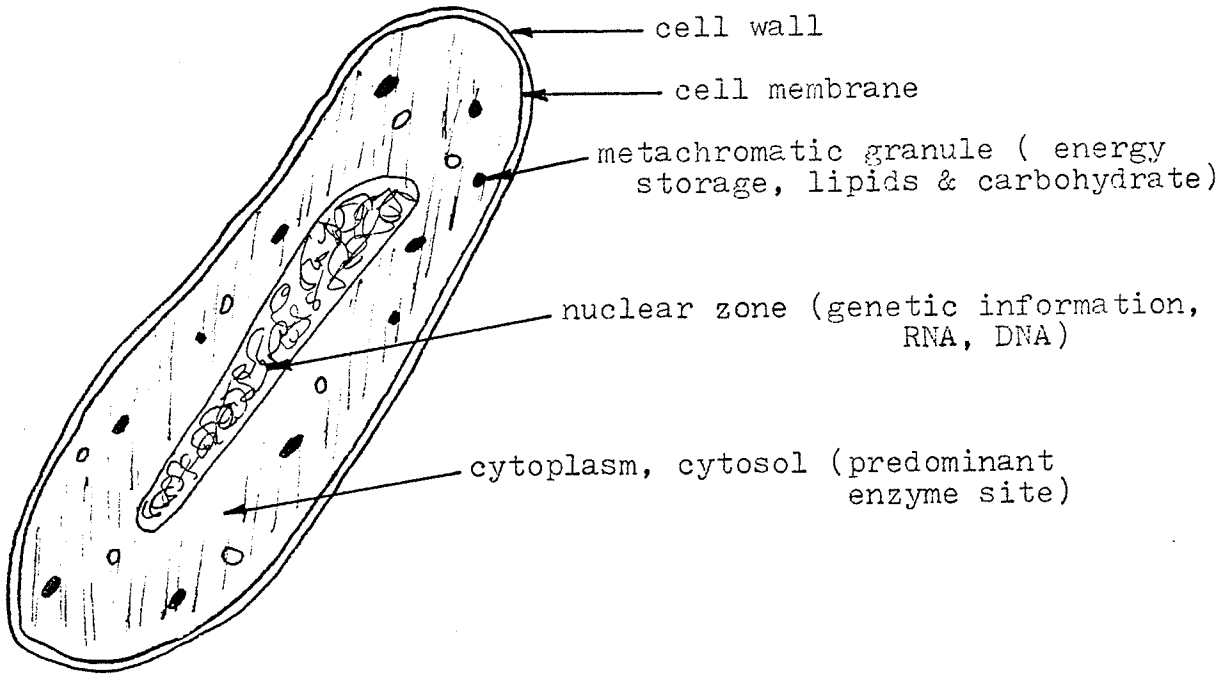


FIGURE 2. [illegible]

Many of the metabolic reactions in eucaryotes are segregated into structural compartments such as the formation of adenosine triphosphate, ATP, in the mitochondria. Within the mitochondria are the oxidation sites of carbohydrates, lipids and amino acids to CO_2 and H_2O by molecular oxygen (10). The enzymes of electron transport and energy conversion are located in the inner membrane of the mitochondria. Aerobic fungi and protozoa are eucaryotic and the basic cell structure of a eucaryote is shown in Figure 3.

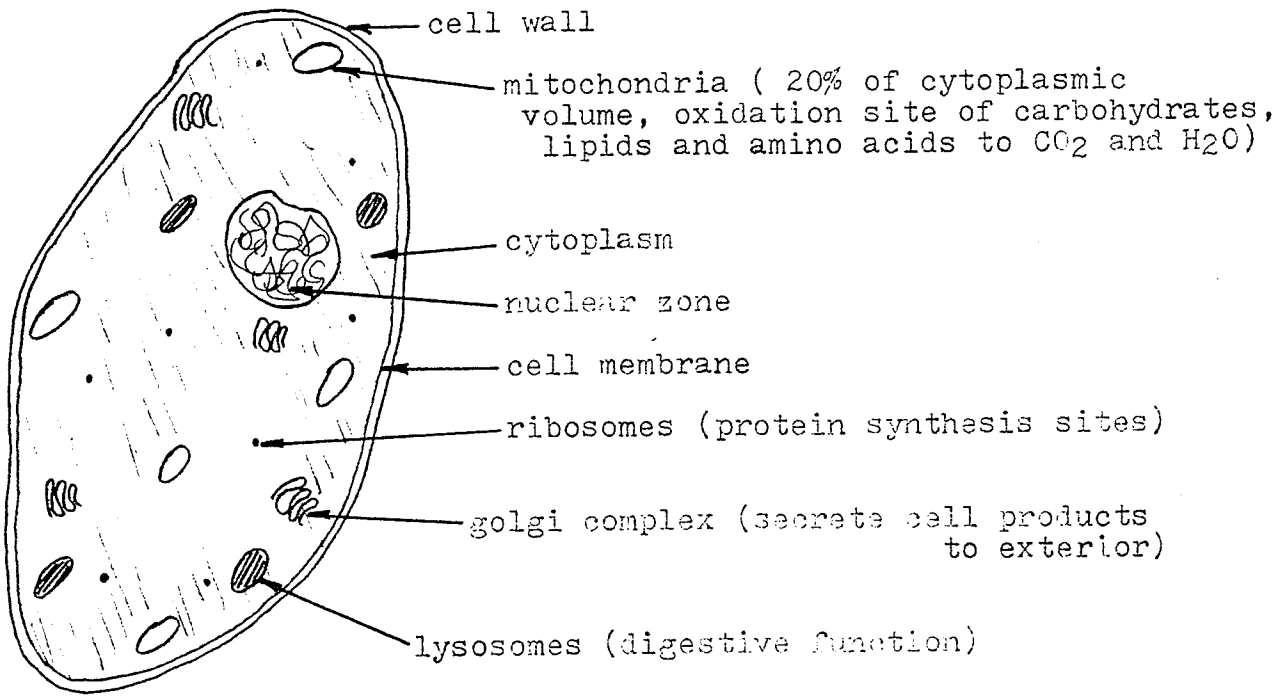


FIGURE 3. BASIC EUCARYOTIC CELL STRUCTURE

3.2.1. Metobolism

Microorganisms process, or metabolize, raw materials to the end product protoplasm. Most microorganisms are heterotrophic, utilizing organic matter for a source of carbon as a building block and for energy. The pathway of any metabolic reaction is dependent on the chemical structure of the compound being metabolized. The general metabolic reactions are summarized as follows;

1. addition or removal of hydrogen,
2. addition or removal of water,
3. carbon-carbon split or formation and
4. addition or removal of nitrogen (6)

Microorganisms obtain energy for the production of protoplasm, for motility and for cell maintenance from metabolism of organic and inorganic compounds.

3.2.2. Enzymes

Chemical reactions of metabolism induced by microorganisms are all aided by organic catalysts or enzymes. Enzymes are the largest and most highly specialized class of protein molecules (10), which catalyze thousands of chemical reactions. Some enzymes' function or activity depends only on their protein structure, while others require non protein structures, cofactors, for activity (10). A cofactor may be a metal ion, metallic activator, or a complex organic molecule coenzyme. Coenzymes usually function as intermediary carriers of electrons or specific atoms or functional groups

that are transferred in the overall enzymatic reaction (10). The ATP coenzyme, Figure 4, is responsible for energy transfer.

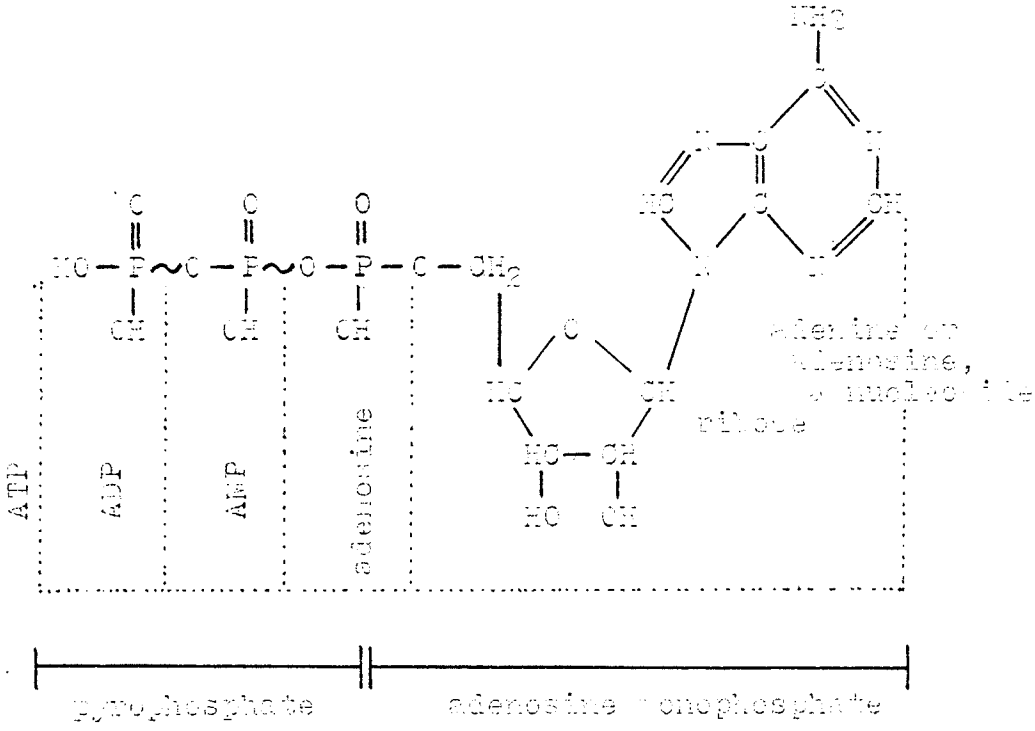


FIGURE 4. ATP STRUCTURE

3.2.3. Energy

The ability of microorganisms to obtain energy from the metabolism of organic and inorganic matter is essential for growth and survival. The oxidation of organic and inorganic matter releases heat energy which is partially conserved by microorganisms through coupled chemical reactions. Energy is transferred from one compound to another with relatively minor heat loss (6).

The phosphate enzyme system is now generally accepted as the basis of biological energy change. This energy is stored, and released as required by other chemical reactions, by the coenzymes adenosine diphosphate, ADP, and adenosine triphosphate, ATP. These coenzymes store energy in the high energy phosphate bondings, illustrated in Figure 4.

The transfer of energy during metabolism results in a net gain in energy of the viable cellular material and a net decrease in the energy level of the metabolized matter. The energy balance is completed with the loss of some heat energy. A schematic representation of energy change during metabolism is shown in Figure 5.

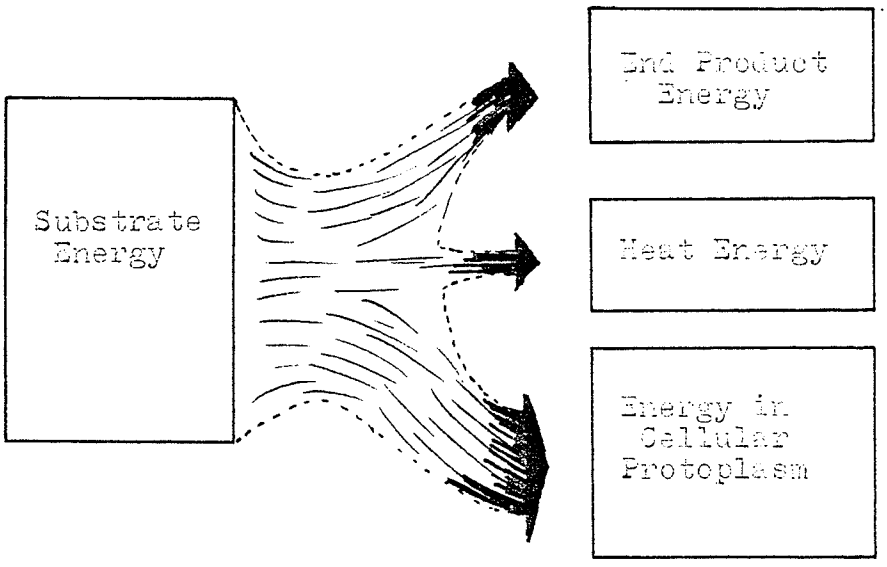


FIGURE 5. ENERGY CHANGES DURING METABOLISM

From [illegible] (6)

Energy is released in all oxidation reactions involved in the stabilization of waste water. The oxidation of organic matter by microorganisms is achieved indirectly by the process of hydrogen removal and addition of water. The removal of hydrogen is achieved by the coenzymes DPN or TPN, forming $DPNH_2$. The DPN is regenerated, in aerobic metabolism by flavin adenosine dinucleotide, FAD as follows:

$$DPNH_2 + FAD \longrightarrow DPN + FADH_2$$

The FAD is in turn regenerated by cytochrome pigments strictly by electron transfer as:

$$FADH_2 + 2Fe^{+3} \longrightarrow FAD + 2Fe^{+2} + 2H^{+}$$

The cytochromes are regenerated by the reduction of other pigments, with the final reaction between dissolved oxygen and hydrogen ions liberated from the organic matter. The complete simplified scheme of aerobic hydrogen transfer is shown in Figure 6 (6).

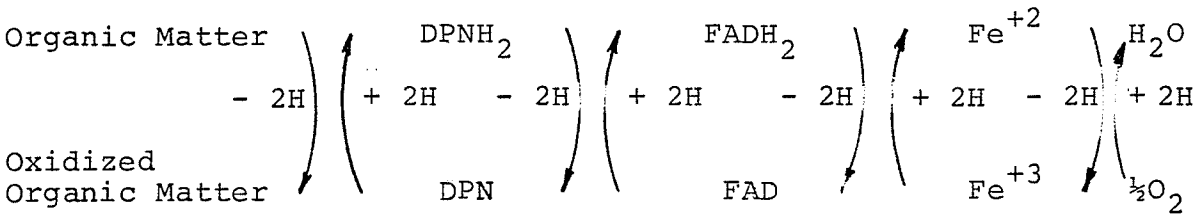


FIGURE 6. AEROBIC HYDROGEN TRANSFER

The chemical reaction removing hydrogen ions releases energy which is partially used in the formation of the high energy phosphate bond in ATP refer to Figure 7. Thus energy is stored in ATP during the entire cycle of hydrogen removal

in oxidation, that is during hydrogen removal from organic matter, and regeneration of cytochrome pigments. ATP is also formed as a result of the splitting of carbon-carbon bonds (6). The energy of a cell is used primarily for cell maintenance, however, excess ATP molecules which are formed through oxidation are used to drive other synthesis reactions within cells.

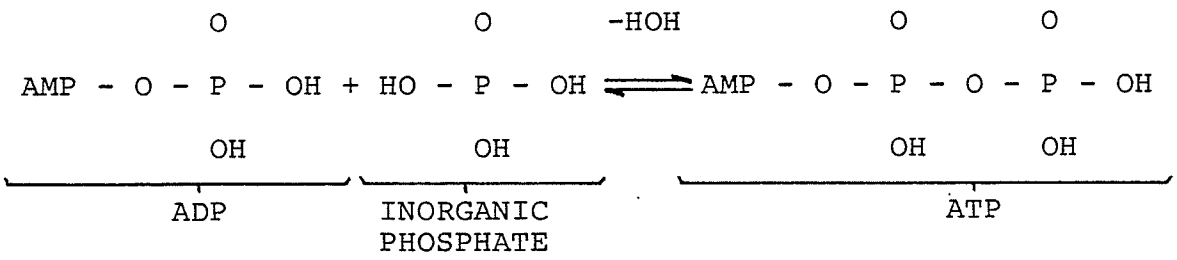


FIGURE 7. ADDITION OF INORGANIC PHOSPHATE TO ADP (6)

3.3. ADENOSINE TRIPHOSPHATE, ATP

3.3.1. ATP Biochemistry

Adenosine triphosphate, ATP, was first isolated in 1929 from acid extracts of muscle. Its structure was deduced by chemical synthesis in 1948 (10). ATP is a highly charged anion, an energy rich compound which serves as an energy carrier for use by organisms for the energy consuming function of cell material synthesis and numerous other cell functions (11).

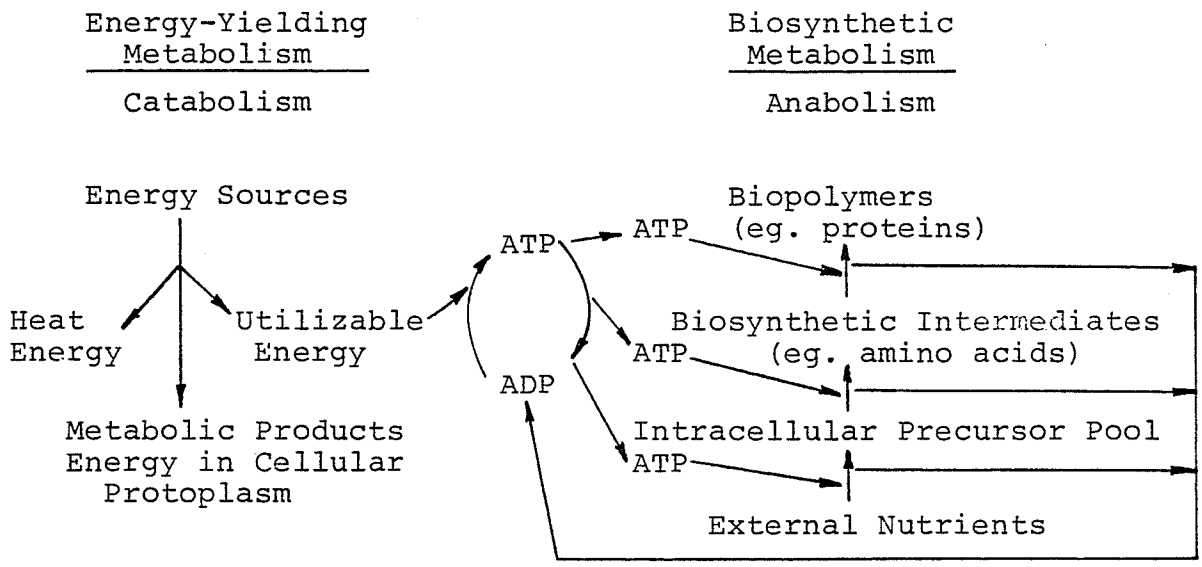
The formation of ATP in eucaryotic cells takes place in the mitochondria of cells where carbohydrates, proteins and fats, which are previously enzymatically broken down

outside the mitochondria, pass into the mitochondria (11). Enzymatic oxidative reactions continue in the mitochondria to form CO_2, water and chemical energy, ATP. Oxidative phosphorylation or phosphorylation of adenosine diphosphate, ADP, to ATP is one of the primary functions of the mitochondrial membrane and, coupled to respiration, represents a mechanism for aerobic energy recovery (10). Chemical energy is stored in ATP through a high energy phosphate bond. The energy is then transferred as required to metabolic chemical reactions as ATP is reduced back to ADP.

ATP has been shown to be universally distributed in plants, microorganisms and animals. The first and second phosphate bonds are energy rich, however, the third is not. ATP is, therefore, capable of supplying two energy rich units (12). This link between two phosphates is a pyrophosphate bond and is the primary group in the conservation of oxidation-reduction energy. The free energy of hydrolysis at pH 7.0 of the terminal phosphate of ATP is approximately 8000 calories (12). The ATP-ADP system operates at shifting levels of equilibrium and functions as a carrier of chemical energy since ADP can be phosphorylated to form a high energy bond during energy-yielding reactions of catabolism, a degraditive pathway. In turn, ATP can donate its terminal phosphate group, yielding energy through dephosphorylation during energy-requiring reactions of anabolism, a biosynthetic pathway (10). These two major pathways form the major cellular functions of microbial metabolism.

A cell will contain ATP, ADP and AMP in equilibrium. As the metabolic requirements of a cell vary the equilibrium shifts. The total concentration of ATP, ADP and AMP in intact cells is significant. The concentration of ATP is usually much higher than ADP or/and AMP combined (10). A simplified schematic of the role of ATP-ADP in cellular metabolism is shown in Figure 8. (8).

FIGURE 8. SCHEMATIC REPRESENTATION OF THE ROLE OF ATP IN CELLULAR METABOLISM (13)



3.3.2. Bioluminescence

Bioluminescence is a form of chemiluminescence which occurs when the energy released during biological oxidation forces an electron of a molecule to a higher energy level (12). The oxidation energy serves the same purpose as the incident proton does in photoluminescence.

There are numerous chemical types of bioluminescent reactions. An example, includes an oxidizable substance called luciferin. In the presence of oxygen and an enzyme, luciferase, luciferin is destroyed producing light. One type of bacteria emits a blue light, 495μm, a South American worm emits a red light, 640μm, and a type of fungi which grows on decaying wood emits light at 530μm. The firefly emits light due to the oxidation of a specific type of luciferin at 560 to 580μm.

Adenosine triphosphate, ATP, research has been conducted primarily by means of the enzyme system or bioluminescent reaction which occurs in the native firefly Photinus pyralis (14). With all reactants in excess, the amount of light produced in the reaction is directly proportional to the amount of ATP present. The reaction is specific for ATP when purified luciferin-luciferase is employed (15). The sequence of the firefly reaction is summerized from McElroy's proposed reaction sequence as follows:

$$LH_2 + E + ATP \xrightarrow{Mg^{++}} E.L.H_2AMP + PP \quad ...(1)$$

(luciferin) (luciferase) (luciferyl adenylate complex) (pyrophos-phate)

$$E.LH_2.AMP + O_2 \longrightarrow E + Product + CO_2 + AMP + h\nu \quad ...(2)$$

(light)

Luciferin, the light emitting substance in the firefly, combines, in the above reaction with the enzyme luciferase in the presence of ATP to form a complex which is oxidized. This reaction yields one photon of light emitted in the wavelength of 560 to 580μm for each molecule of ATP

expended (16), (15). The light emission is a product of the oxidation of the enzyme-luciferin-adenosine monophosphate, $E.LH_2.AMP$, to an excited oxyluciferyladenylate.

The reaction produces an initial burst of light which rapidly decays to a low uniform level of luminescence. Arsenate buffer in the reaction produces an intermediate level of light emission, decaying exponentially with time (8) and the presence of magnesium ions stimulate the reaction (17).

The ionic composition of the reaction medium affects light emission, reducing it linearly with increasing concentrations of several cations in the following order:

$Ca^{++} > K^{+} > Na^{+} > Rb^{+} > Li^{+} > choline^{+}$ (15)

The ionic strength of normal activated sludge in sample extraction dilutions is sufficiently low that inhibitory effects are considered to be negligible (5). Marine samples frequently require dilutions higher than 1:25 and sample filtration prior to ATP extraction due to high concentrations of inhibiting cations in the ATP detection reaction (5). The ionic strength of sewage in an area services with highly mineralized ground water may require special testing procedures or dilutions of at least 1:20 to reduce the salt concentration, similar to sea water, below inhibitory levels (18).

Light emission is also affected by pH and temperature. Rhodes and McElroy (19) found that light emission was signi-

ficantly altered by pH, rapidly increasing from pH 2.0 to 3.8, decreasing from pH 3.8 to 4.8 and increasing from pH 4.8 to 7.0. Patterson et al (5) found that maximum light emission in activated sludge samples occurred in the range of pH 7.5 to 8.0 and that light emission was reduced at pH 3.0 and 11.0. This reduction in ATP at extreme pH was indicated as either a reduction in viability or numbers, or increased energy demand to maintain homeostasis. McElroy and Strehler (20) stated that the optimum temperature for the light producing reaction was 25°C.

Transphosphorylase enzymes, contained in crude extracts of firefly may produce light in the presence of high energy phosphate molecules other that ATP, such as adenosine diphosphate, ADP, cytidine-5'-triphosphate, CTP, and inosine-5'-triphosphate, ITP (14). The relative effect of light emission with other mucleoside triphosphates in activated sludge studies are considered negligible with respect to ATP induced light emission due to the relative abundance of cellular ATP compared to other nucleoside triphosphates (14).

3.3.3. ATP as an Indicator of Viability

Adenosine triphosphate, ATP, as an energy mediating compound is considered to be a valid indicator to provide a more accurate index of living plankton and periphyton biomass as compared to empirical derivations from volatile suspended solids measurements and various counting techniques such as microscopic and plate counting (16). Visual counting

techniques are tedious and, due to the small size of the bacteria, are usually disregarded (16). Plate counting techniques are, in general terms, specific to certain bacterial populations and colonies of bacteria in particulate matter may show as only one colony on a plate colony. The reliability of volatile suspended solids to reflect viability is questionable and it is not able to reflect rapid changes in sludge viability (2).

Numerous parameters have been investigated in attempts to measure active cell mass; such as deoxyribonucleic acid or DNA, organic nitrogen, respiration, dissolved oxygen uptake rates, dehydrogenase activity and the measurement of other specific enzyme activity such as reported by Weddle and Jenkins (4). However, each of these parameters has its limitations. The use of DNA in anaerobic systems was reported to be superior to volatile suspended solids (4). DNA however, is considered to be conservative and is considered to be an unreliable indicator of viable biomass (2). A conservative indicator being, by definition, related to viable cells only. Alternately, Irgens (21) reports that extracellular DNA is less conservative than intracellular DNA and is rapidly degraded by sludge floc.

Organic nitrogen is conservative and therefore, is a poor indicator of environment stress on a biological system. The availability of nitrogen from the influent to an activated sludge mixed liquor system has been shown to affect nitrogen content in activated sludge (4).

The measurement of viable biomass concentrations in activated sludge by respirometric techniques can be inaccurate due to the formation of storage compounds and fast cell decay following shock loading which alters the rate of endogenous respiration (22). It is thought that respirometric techniques may only be of value in substrate limiting conditions.

Dehydrogenases are a collective group of enzymes which catalyze the transfer of electrons through the electron transport chain from substrate to molecular oxygen (dehydrogenation of a substrate). Kotze has concluded that quantitative values, obtained for the intermediary enzyme activities, allows an estimate of the overall efficiency of a culture through prediction of the end products (23). However, the use of DNA and other specific enzymes as an index for activated sludge viability are difficult to interpret. They may be too specific to be used as an indicator of the general level of sludge activity.

The validity of using ATP as an indicator of viable biomass; an indicator of general activated sludge activity and age; and as an indicator of specific plankton, periphyton and bacterial populations has recently been under increased investigation. Recently researchers have concluded that ATP is a specific measure of sludge activity and under constant environmental conditions and can be used to estimate viable biomass (24). ATP is non-conservative, has a relatively short survival time after death, and can provide a

sensitive and rapid indicator of toxic stress due to heavy metal in activated sludge (24). Recent applications of ATP measurement include monitoring the effectiveness of disenfection of wastewater streams to measure the residual microorganism viability (25).

Upadhyaya and Eckenfelder (26) compared the variation of ATP, with other activity parameters versus organic loading rates and sludge age in a bench scale conventional activated sludge plant. They concluded that the sludge activity per unit weight was higher in systems with lower concentrations of suspended solids in the sludge. This indicated that with increasing sludge age, or increased mean cell residence time, there may be an accumulation of non active mass. Nutt (8), investigating viability parameters in activated sludge systems, concluded that ATP showed good potential as a viability indicator and showed significant correlation to the organic carbon removal rate. ATP is a specific index of cellular viability in predominant culture studies.

3.3.4 Microbial Respiration and Growth

ATP is non conservative (27), (4) and must be associated only with living cells. Weddle and Jenkins (4) report that ATP also appears to be in an approximately constant amount in viable microbial cells of different species and growth rates. However, Leninger states that when the supply of respiratory substrate is high, the concentration of ATP is low and the rate of oxygen consumption is high with

corresponding high ADP and phosphate concetrations (10). The converse occurs when substrate is low. ADP is determined to be the most critical in setting the respiratory rate due to the high affinity of the mitochondria for ADP.

Investigators have determined that the ATP pool, or net concentration of ATP in a reactor, during growth is a balance between the rate of energy production and energy utilization. During exponential growth of Bdellovibric bacteriovorus, Gadkari and Stolp (28), found a parallel increase in ATP which indicated that the concentration of ATP per unit dry weight remained constant. During the initial stages of exponential growth, however, the ATP pool was low which reflected the energy consumption exceeding the energy production or a shift in equilibrium from ATP to ADP. Gadkari and Stolp (28) also found that the transition from exponential growth to endogenous respiration produced a peak in the ATP pool then a gradual decrease. The decrease in ATP was characterized by a series of ocillations or fluctuations in the equilibrium of ATP-ADP and a rapid fall in viability during the endogenous respiration was reflected by a drop in the ATP. The ocillations were regarded as production of ATP through degradation of the organisms own cellular material, which would tend to average out in hetrogenous cultures.

Forrest (29) found that the level of the ATP pool of Streptoccus faecalis rose through out the lag phase to a

maximum at the point where exponential growth began, then began declining as growth proceeded. He noted a corresponding lag in the growth of the ATP pool during the initial lag phase of growth and a rapid increase when growth began. As the rate of growth decreased the ATP pool increased more rapidly, to a peak, and decline preceeding the endogenous respiration phase. Forrest also determined that the rate of increase of the size of the ATP pool closely correlated to the first order rate constants for exponential organism growth.

Strange, Wade and Dark (30) found that starvation of A. aerogenes reduces the ATP concentration and the ability of bacteria to synthesize ATP when transferred from anaerobic to aerobic conditions or from solutions of high to low solute concentrations.

Knowles and Smith (31) found that the ATP levels in Azotobacter were about the same through a 10 fold range in respiratory rates and that the aerobic ATP levels were low in the early log phase of growth increasing to a maximum of 4.8 to 6.2 n moles/mg dry weight at the end of the log phase growth. They also concluded that oxidative phosphorylation does not appear to be tightly coupled to oxygen uptake.

Hamilton and Holm-Hanson (27) found that in the endogenous phase of marine cultures the concentration of ATP was, closely related to the number of viable cells during log growth and began to decline rapidly during the later log growth phase or early stationary phase. During the endogenous phase the

ATP/cell content was nearly constant at approximately 0.2×10^{-9} μg/cell. A summary of the ATP concentration in various organisms as determined by other researchers is included as Table 1.

The bacterial growth patterns, as described by McKinney, with respect to increasing time, are shown in Figure 9. The curves for relative numbers and mass of microorganisms show significant similarities despite the fact that the number relationship is frequently used in pure cultures and the mass relationship is usually applied to complex heterogenous cultures such as activated sludge. The numbers-mass curves versus time both exhibit log growth during a condition of excess food supply. In the declining growth phase, the food supply is limiting to growth and diminishing towards the death phase. This is considered to be the phase of growth where the system energy is declining and is characterized by better floc settleability and relatively lower sludge production.

The apparent wide range in results of other investigaters of ATP concentrations with respect to a decreasing food supply with increasing time are shown in Figure 9. There are discrepancies in these results as to the point at which the system energy, expressed as a concentration of ATP, reaches a peak. The system ATP generally increases through the log growth phase. The endogenous phase of respiration, which is of particular importance to this study since it is in this phase that extended aeration activated sludge systems

TABLE 1 ATP CONTENT OF BIOLOGICAL MATTER

Organism	ATP Content	Reference
Chlorella vulgaris	0.2% (dry wt.)	Syrett (1958)
Saccharomyces cerevisiae	0.04% (dry wt.)	Grylls (1961)
E. coli	0.3% (dry wt.)	Leninger (1956)
Streptococcus faecalis	0.2-1.2% (dry wt.)	Forrest (1965)
Z. ramingera	0.11-0.60% (dry wt.)	Biospherics (1972)
E. coli	0.092-0.13% (dry wt.)	Biospherics (1972)
Bacillus sp.	0.06-0.33% (dry wt.)	Biospherics (1972)
E. coli	0.1% (dry wt.)	Chappelle & Levin (1968)
S. cerevisiae	0.11% (dry wt.)	Chappelle & Levin (1968)
General Bacterial Cells	0.1-0.2% (dry wt.)	Holm-Hansen & Booth (1966)
General Algae Cells	0.003-0.16% (dry wt.)	Holm-Hansen & Booth (1966)
Marine Bacteria Isolates	0.018-0.72% (dry wt.)	Holm-Hansen & Booth (1966)
Activated Sludge	0.02-0.03% (dry wt.)	Patterson et at (1969)
Activated Sludge	0.03-0.15% (vol. wt.)	Weddle & Jenkins (1971)
Activated Sludge	0.06-0.16% (vol. wt.)	Chiu et at (1973)
E. Coli	$3.9\text{-}17.0 \times 10^{-10}$ µg/cell	Biospherics (1972)
Z. ramigera	$3.1\text{-}30.8 \times 10^{-10}$ µg/cell	Biospherics (1972)

TABLE (cont.)

Organism	ATP Content	Reference
Bacillus sp.	0.053-9.7 x 10^{-7} µg/cell	Biospherics (1972)
Marine Bacteria Isolates	0.5-6.5 x 10^{-9} µg/cell	Holm-Hansen & Booth (1966)
Endogenous Bacteria (13 species)	4.7 x 10^{-10} µg/cell	D'Eustachio & Johnson (1968)
Aspergillus niger fungi	.164% (dry wt.)	Qureshi and Patel (1976)
Trichoderma viride	.256% (dry wt.)	Qureshi and Patel (1976)
Penicillium sp. fungi	.237% (dry wt.)	Qureshi and Patel (1976)
Powdered skim milk (activated sludge)	0.73-0.80 µg/mg MLVSS .0073-.0080% (dry wt.)	Upadhyaya and Eckenfelder (1975)
Amphidinium carteri	.015% (dry wt.)	Holm-Hansen & Booth
Cyclotella nana	.084% (dry wt.)	Holm-Hansen & Booth
Ditylum brightwellii	.12% (dry wt.)	Holm-Hansen & Booth
Dunaliella tertiolectra	.15% (dry wt.)	Holm-Hansen & Booth
Monochrysis lutheri	.028% (dry wt.)	Holm-Hansen & Booth
Rhizosolenia sp.	.16% (dry wt.)	Holm-Hansen & Booth
Selenastrum capricornutum	.14-.34% (dry wt.)	Lee et al
Skeletonema costatum	.013% (dry wt.)	Holm-Hansen & Booth
Syracosphaera elongata	.003% (dry wt.)	Holm-Hansen & Booth

TABLE (cont.)

Organism	ATP Content	Reference
Chromobacterium marinum	.32% (dry wt.)	Hamilton & Holm-Hansen
Esckerichia coli	.30-.90% (dry wt.)	Cole et al
Micrococcus sp.	.12% (dry wt.)	Hamilton & Holm-Hansen
Pseudomonas sp. (C-6)	.44% (dry wt.)	Hamilton & Holm-Hansen
Pseudomonas sp. (GL-7)	.24% (dry wt.)	Hamilton & Holm-Hansen
Pseudomonas sp. (GU-1)	.24% (dry wt.)	Hamilton & Holm-Hansen
Serratia sp.	.20% (dry wt.)	Hamilton & Holm-Hansen
Vibrio sp.	.24% (dry wt.)	Hamilton & Holm-Hansen
Phaseolus vulgaris	.10-.70% (dry wt.)	Jones

(from Nutt and Glass) (8), (16)

*indicates mixed culture
** indicates pure culture

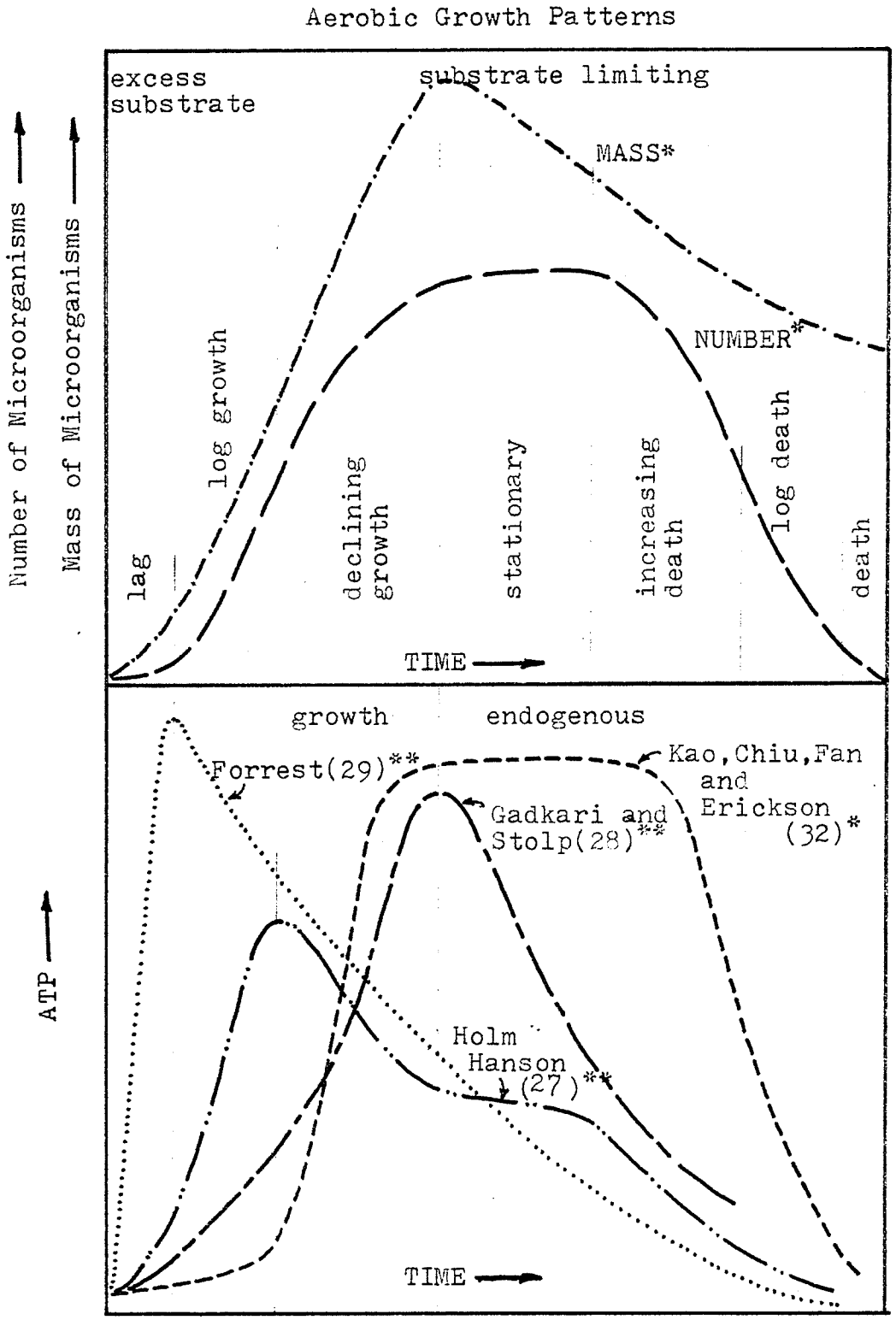


Figure 9 . MICROORGANISM GROWTH PATTERNS
after McKinney(6)

operate, does exhibit some common characteristics in terms of system energy. The results of other investigators show that the ATP concentration or system energy gradually decreases with decreasing food supply in pure and mixed cultures. (29), (27), (28), (32), (3). This system energy decrease is similar to the decrease in the number of microorganisms versus time curve. The ATP content of a mixed liquor operating in the endogenous phase therefore, may be a useful parameter to estimate the system viability.

3.4. KINETIC MODELS

Kinetic model development to describe or predict the behaviour of biological waste treatment systems is based primarily on the Monod model for continuous pure cultures and on the Michaelis-Menten equation based on enzyme behaviour. Mathematical model development is based on material balance equations of cells and of substrate, modified to a particular system being modelled. The analysis of heterogenous treatment systems using a form of the Monod equation or Michaelis-Menten equation is based on pure culture kinetics. In addition, these equations include the assumption that microbial growth is limited by the deficiency of only essential nutrient or substrate.

3.4.1. Kinetic Model for Extended Aeration

A mathematical model for an extended aeration plant has been developed by Middlebrooks and Garland (7), based on

the material balance equations. The complete mix with cellular recycle model was used to define the extended aeration process. A flow schematic after that used by Middlebrooks and Garland is included as Figure 10.

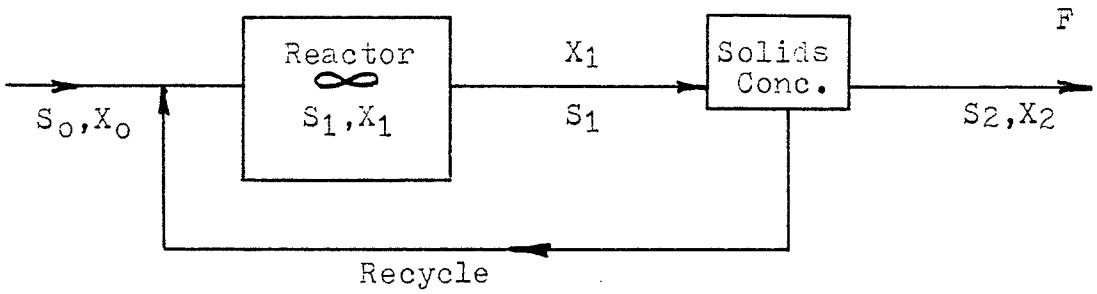


Figure 10. CONTINUOUS FLOW WITH CELLULAR RECYCLE

The material balance equation for the organisms in this system are written as (from Middlebrooks and Garland, 1968):

Organism change in reactor	=	Organisms in influent	+	Growth of organisms	-	Loss of organisms in effluent	-	Loss due to decay

OR

$$V(dx_1)net = X_oFdt + (dX_1)gV - X_2Fdt - k_dX_1Vdt \qquad ...3$$

Where:

V = reactor volume

$(dX_1)net$ = net change in reactor organism concentration

X_o = organism concentration in influent

F = flow rate through reactor

$(dX_1)g = \mu X_1 dt$, where μ = growth rate constant

k_d = decay rate constant

X_1 = organism concentration in the effluent

t = time

Θ = V/F = hydraulic retention time

The use of a constant k_d makes the assumption that the specific endogenous respiration rate does not vary significantly with the type of organism, the substrate, the substrate concentration or time, that is, the system must be in an ideal steady state. Further simplifying assumptions are made in equation 3:

1) Monods equation can be used to express the relationship between growth rate and essential nutrient concentration,

2) a constant proportion of the organisms is viable and,

3) the yield of the organisms is constant.

By further assuming that the influent organism concentration is insignificant as compared to the organism concentration in the reactor, at steady state, $\frac{dX}{dt}1$ net = 0, equation 3 reduces to:

$$\mu = kd + \frac{1}{b'} \qquad \ldots 4$$

where $b' = X_1/X_2$ and is always greater than or equal to 1.0.

The material balance equation for substrate in this system are written as (from Middlebrooks and Garland, (7)):

Substate change in reactor = Substrate in influent - Consumption by organisms - Loss of substate in effluent

OR

$$V(dS_1)net = S_oFdt - V(dS_1)g - S_2Fdt \qquad ...5$$

Where:

$(dS_1)net$ = net change in reactor substrate concentration

S_o = substrate concentration in the influent

S_1 = substrate concentration in the reactor

S_2 = substrate concentration in the effluent

$(dS_1)g = dX/Y$ = the change in substrate concentration due to growth

By assuming that;

1) most of the effluent substrate is soluble,

2) influent substrate is solubilized at a constant rate and,

3) substrate concentration in the effluent equals the substrate concentration in the reactor, at steady state,

$\frac{dS_1}{dt}$ = net = 0,

equation 5 reduces to:

$$X_1 = \frac{b'Y(S_o - S_1)}{(1 \pm b'\Theta kd)} \qquad ...6$$

Further substitution and rearranging yields:

$$S_1 = \frac{Ks(1 + b'\Theta kd)}{b'\Theta\mu_{max} - (1 + b'\Theta kd)} \qquad ...7$$

Where:

Ks = substrate concentration at ½ maximum growth rate, mass/volume

μmax = maximum growth rate, time $^{-1}$

Y = yield constant, $\frac{\text{weight of organisms formed}}{\text{weight of essential nutrient utilized}}$

Substituting equation 4 into equation, a reduced form of equation 5 yields:

$$\frac{\mu \max S_1}{K_s + S_1} = \frac{1 + b'\theta kd}{b'\theta} \qquad \ldots 8$$

The growth kinetics may be obtained from equations 6 and 8 by rewriting into a straight line form:

$$\frac{k_d\, b'\theta}{Y} + 1/Y = \frac{b'(S_o - S_1)}{X_1} \qquad \ldots 9$$

Where: slope = kd/Y and intercept = 1/Y

and ; $\frac{b'\theta}{1 + b'\theta kd} = \frac{Ks}{\mu \max} \frac{1}{S_1} + \frac{1}{\mu \max}$

Where: slope = Ks/μ max and intercept = 1/μ max ...10

3.4.2 Growth and Substrate Utilization Rate Constants

The kinetic growth and substrate utilization rate constants are widely used to describe and predict the performance of biological treatment processes. These constants are influenced by various environmental conditions such as temperature, substrate concentration, mixing and turbulence, as described by Middlebrooks and Garland.

The growth parameters are Y, the yield coefficient, and k_d, the endogenous decay rate. The substrate utilization parameters are K_s, the half velocity coefficient and μ max, the maximum growth rate.

Topnik (9) found that the growth and substrate utilization rate constants, calculated from basic Monod kinetics did not show any apparent variation due to an operating temperature range of 20 to 0°C. The use of VSS was considered to be responsible for lack of temperature effects. This and other research into kinetic constants utilize BOD_5 or COD typically for measuring substrate and traditionally use volatile suspended solids as a measure of the viable or active biomass concentration.

Benefield et al (33) have investigated the inherent errors associated with using volatile suspended solids, VSS, in the determination of kinetic constants and what correction is required to account for the active fraction of VSS only. They used oxygen uptake rates to determine viability through a series of biomass extractions and insertions into an environment containing excess substrate to maximize the substrate utilization and the growth rate. Benefield et al (33) stated that a large inactive fraction of the VSS tends to dampen out biokinetic data variability resulting from continual shifting in predominant species in a heterogenous culture. A summary of kinetic constants complied by Topnik (9) is included as Table 2.

Substrate	k_d	Y	μ max.	K_s (mg/l)	Culture	Reference
BOD_5	0.97×10^{-3} hr	0.33(VSS)	1.27×10^{-3} hr	6	extended aeration 20°C-0°C	Topnik
COD	0.98×10^{-3} hr	1.46(VSS)	1.11×10^{-3} hr	10		
domestic waste	0.04-0.05	0.5-0.6				Walker
COD	0.04	0.03-0.9(VSS)			θ_c =5-15 days	Jenkins
COD	0.2-0.26	0.4(VSS)			20°C	Eckenfelder
BOD_5	0.2-0.26	0.55			20°C	Eckenfelder
domestic waste		0.53 degradable 0.12 non degradable				McCarty & Broderson
skim milk solids		.58 ultimate O_2 demand			total oxidation activated sludge	Kountz & Forney
COD	0.16/day	0.445(SS) 0.39(VSS)	0.80/hr 1.59×10^{-3} hr	345 12.4		Reynolds & Yang, Middlebrooks & Garland
glucose	.116/hr	.841	.728/hr	1.7		Gosh et el

TABLE 2 SUMMARY OF GROWTH AND SUBSTRATE UTILIZATION CONSTANTS

Substrate	k_d	Y	max.	K_s (mg/l)	Culture	Reference
galactose	.027/hr	.586	.387/hr	3.9		Gosh et el
various organic substrates		.37 mg				Servizzi & Bogan
BOD_5	.05/day .01/day	.56			activated sludge load ranged low loading	Stewart & Ludwig

Θ_c = mean cell residence time

(compiled from Topnik, 1976) (9)

TABLE 2 SUMMARY OF GROWTH AND SUBSTRATE UTILIZATION PARAMETERS (cont).

3.4.3. Energy Change and Cell Yield Correlation

The principles of thermodynamics which apply to closed systems include the first law that all energy is conserved and the second that all energy transformations in a system take place in the direction of the increased entrophy between the system and its surroundings, towards equilibrium. Free energy is the difference between the energy of the initial and final state of a particular system (10). The problem of applying the laws of thermodynamics to a continuous flow process, such as activated sludge, is that is in an open system. In an open system, living matter exchanges matter, energy and heat with its surroundings, outside of the defined system.

Non equilibrium thermodynamics is applied to open biological systems. Two basic attributes of open systems are of significance. That is, an open system in a steady state is capable of doing work because it is away from the condition of equilibrium and only an open system away from equilibrium can be subjected to control and regulation (10).

ATP, as the mediator of most energy transfer within a cell, undergoes rapid turnover. The actual turnover rate of the terminal phosphate group of intercellular ATP is so rapid that is has not yet been accurately measured, although in rapidly expiring E. coli is calculated to be less that one second (10).

Growth is related to the free energy change or the free energy of oxidation of a particular substrate (34).

However, the energy of oxidation should be considered in two parts, that utilized for synthesis and that used for cell maintenance (34).

Schroeder and Busch (34), examined the validity of using free energy or biological energy for the prediction of growth in terms of net cell yield in a system which minimized endogenous respiration. They conclude that the validity of direct correlation between cell mass production and oxidative phosphorylation coupled to electron transport and substrate level phosphorylation free energy in an open system is questionable. They also question the validity of the assumption that growth is a function only of the amount of energy potentially available from the substrate. One reason being that there are alternate pathways of substrate oxidation existing in micro-organisms which do not produce equivalent quantities of ATP. Another is that where a compound serves the micro-organism as both a source of energy and carbon, such as glucose, a portion of the energy transfer to the cell is from substrate molecules not oxidized to carbon dioxide (34). Work by Bauchop and Elsden (34) conversely, have indicated that bacterial growth is proportional to the production of ATP.

Recent work by Stouthamer and Bettenhaussen has indicated culture growth rates influence the ATP yield to a large extent. They also found that by varying the type of limiting substrate different ATP yields were obtained. This is, a larger quantity of ATP was required for different substrates to produce one unit weight of new cell material. The dif-

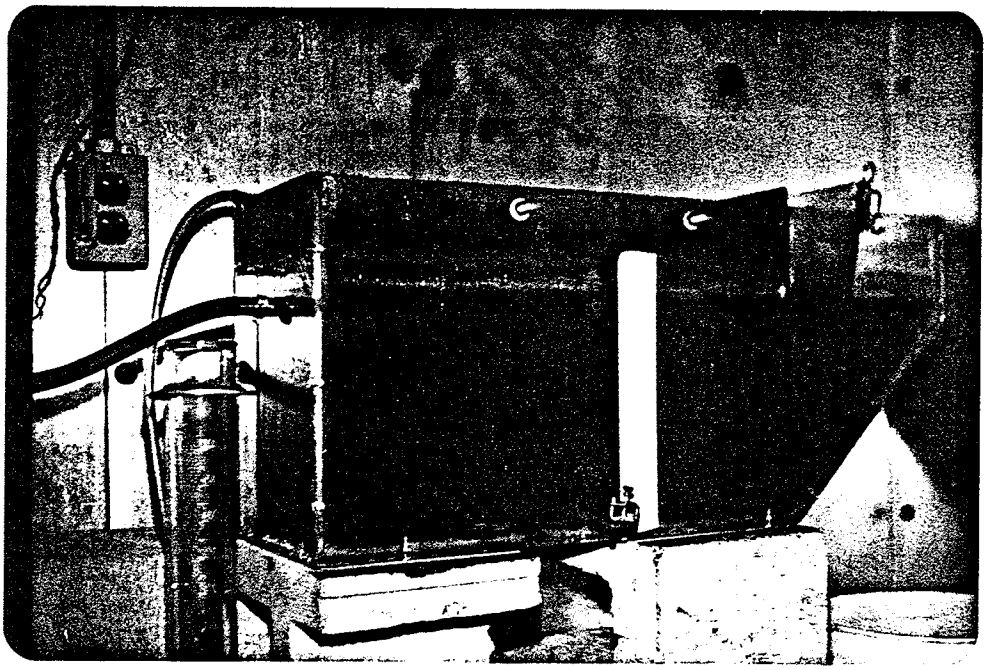
ference between the theoretical calculation for the amount of ATP required for cell formation and the actual, or measured ATP required for cell formation, is termed the maintenance coefficient, m_e (35). This "maintenance energy", or energy used independent of growth, varies for difference substrates. The reasons for this occurrence are not known at this time.

4. EXPERIMENTAL PROCEDURE

4.1. LABORATORY EXTENDED AERATION TREATMENT PLANT AND OPERATION

The laboratory model extended aeration treatment plant was of the same basic design and construction as that used in a previous investigation of low temperature biokinetics, conducted by the University of Manitoba, Sanitary Engineering Department (9). The model was constructed with an aeration basin volume of 19.8 litres and with a stilling chamber and clarifier volume of approximately 5.0 litres. The model was designed in accordance with Ten State Standards (36) to treat typical raw domestic sewage. The operating parameters of the laboratory model plant are included in Appendix A. The baffles were designed to dissipate the energy in the mixed liquor overflow and to ensure complete recycle of the sludge blanket. The laboratory model extended aeration treatment plant and feed equipment are shown in Photographs 1 and 2.

Raw, degritted domestic sewage from the City of Winnipeg South End Pollution Control Centre were used as feed for the laboratory model. Raw sewage samples were obtained weekly at approximately the same time and were stored in the laboratory at $4^{o}C$. Continuous feed was approximately simulated by a timer solenoid which, intermittently, admitted air into a sealed feed container. The volume of sewage feed



PHOTOGRAPH 1.
Model Laboratory
Extended Aeration
Sewage Treatment
Plant

PHOTOGRAPH 2.
Extended Aeration Plant
and Automatic Feed
Apparatus

was regulated by adjusting a clamp to pinch the air feed line to act as a capillary. The rate of flow was regulated in the same manner as the previous study (9). The feed cycle lasted approximately 3 seconds every 5 minutes.

The daily operation of the laboratory model consisted of refilling the plastic feed container and emptying the effluent collected in a plastic container. Samples of the raw feed, mixed liquor and effluent were taken for analysis.

The model plant start-up was achieved by filling the plant with at 50:50 mixture of return sludge from the City of Winnipeg South End Pollution Control Centre, SEPCC, final clarifier:degritted raw sewage from the SEPCC. The plant was run, as designed, for 30 days. Due to auxiliary equipment malfunction, the plant was restarted using a 10:50:40 mixture of mixed liquor from the model plant:return sludge from the SEPCC:degritted raw sewage from the SEPCC. This ratio was used to maintain some influence from the original culture. The plant was run, as designed, for 27 days prior to initiation of the daily testing program.

The temperature of the laboratory model plant was maintained at 4.0±0.5°C, an approximation of the minimum operating temperature of activated sludge treatment plants in western Canada (9). The temperature was continuously recorded on a weekly chart and verified by a laboratory thermometer in the environmental chamber.

Sludge wasting was not exercised during the first 56 days of monitored operation. Following this period of intensive monitoring, sludge was wasted daily by removing 600 ml of mixed liquor from the aeration basin, using tap water, previously cooled to 4.0±0.5 °C, to make up the reactor volume. Intentional sludge wasting was continued for 23 days. At this time, the daily feed was discontinued, the reactor was continuously aerated and tap water cooled to 4.0±0.5 °C was occasionally added to compensate for liquid volume lost due to evaporation.

4.2. MONITORING PROGRAM

The plant was tested daily for mixed liquor settleability. The raw sewage, mixed liquor and effluent were tested daily for pH, suspended solids and volatile suspended solids. The raw sewage and effluent were tested daily for turbidity, conductivity and biochemical oxygen demand. Samples of raw sewage, mixed liquor and effluent were daily collected and stored, and routinely tested for carbon (total, inorganic and organic), adenosine triphosphate (ATP) and phosphate (total and ortho).

Periodically, the raw sewage and effluent were tested for total chemical oxygen demand (COD) and soluble COD. Similarly, oxygen uptake rates were recorded in the mixed liquor. Occasional tests were conducted for mixed liquor organic nitrogen.

4.3. TESTS AND ANALYTICAL PROCEDURES

4.3.1. Adenosine Triphosphate;ATP

The adenosine triphosphate (ATP) content of raw sewage mixed liquor and effluent microorganisms was determined using a Model 3000 integrating photometer manufactured by S.A.I. Technology Company (18). The instrument was designed to measure low concentrations of ATP in aquatic environments through a bioluminescent, light emitting reaction, described in Chapter 3.3.2. The mechanics of the instrument are elaborated in Appendix B.

4.3.1.1. Reagent Preparation

Reagents were prepared in accordance with the manufacturer's recommendations. All glassware used in the ATP analyses was acid washed with concentrated HCl and rinsed four times with low response water. The low response water was distilled water, prepared daily. Glassware was rinsed with tris buffer prior to use.

4.3.1.1.1. Tris Buffer

The 0.02M tris buffer, tris (hydroxymethyl) aminomethane, was prepared by dissolving 2.5 grams of the buffer crystals in one litre of distilled (deionized) water. The pH was adjusted to 7.75 using a few drops of concentrated HCl. This is consistant with reported results of maximum ATP detection by the firefly enzyme in a pH range of 7.5 to 8.0 (5). The tris buffer was then dispensed into cotton stoppered flasks, sterilized by autoclaving for 20 minutes at 120°C, 15 pounds pres-

sure and stored at 4°C until use.

4.3.1.1.2. Firefly Lantern Extract

The firefly lantern extract, luciferase enzyme, supplied by the Sigma Chemical Company, was obtained in premeasured vials containing the soluble extract form of 50 mg dried lanterns. The extract, stored in a dessicator at 0°C was reconstituted with 15 ml tris buffer in the extract vial. The solution was mixed by hand for approximately 1 minute. The enzyme solution was then stored for 24 hours at 4°C prior to use to dissipate background light. Undissolved particulate matter was removed from the enzyme by sedimentation during the 24 hour storage period.

4.3.1.1.3. Standard ATP Solution

Standard ATP solution was prepared by dissolving a premeasured vial of ATP in 100 ml of tris buffer. One vial, supplied by Calbiochem, contained one milligram adenosine triphosphate and 40 milligrams magnesium sulfate. The standard ATP solution was dispensed into HCl acid washed vials with polyethylene sealed lids and frozen until use. One mg ATP standard solution contained 10^{-2} mg ATP.

The ATP standards were prepared by dilution in 100 ml volumetric flasks. All dilutions were prepared using tris buffer and stored during testing at 4°C.

4.3.1.2. Sample Preparation

The sample extraction apparatus consisted of three, 25 millilitre volumetric flasks suspended in a boiling water bath. Twenty millilitres of tris buffer in each flask was

brought to 95°C to 98°C in the tightly cotton stoppered flasks. The tris buffer temperature was verified by a fourth temperature control volumetric flask containing 20 ml of water and a laboratory thermometer. Samples of the raw sewage, mixed liquor and effluent were taken within 15 minutes of the extraction procedure. Sample sizes of 1.0, 0.5 and 1.0 ml of the effluent, mixed liquor and raw sewage, respectively, were added and the tight cotton stopper immediately replaced. The solutions were mixed occasionally and allowed to boil for 15 minutes. ATP extraction by heat in a buffered solution, has been shown to conserve 98% of standard ATP samples and eliminates cumbersome procedures of PCA or n-butanol extraction (37). The effluent sample, with the highest dilution was added in two steps to reduce the initial temperature drop in the extraction step. The samples were immediately dispensed into vials and frozen until testing, since extracellular ATP has been shown to be reduced by 50% after 5 minutes (38), disappearing within 2 hours after cell death (5). Extraction samples were routinely checked for volume loss and made up to original volume if required (very frequent) by adding tris buffer. During the latter stages of testing the volume of the extract was simply raised to 25 ml with tris buffer in the volumetric flask to simplify the operation. Samples extracted on the ATP testing day were stored at 4°C until testing, to preserve the extracted ATP, then immediately frozen until retested to measure possible ATP loss during sample storage.

4.3.1.3. Testing Procedure

The integrating photometer was energized for 30 minutes to warm up. The high voltage setting of 4.0 was selected for use to exclude "dark current" and because the three types of samples tested fell within the instrument range at this setting. The photometer was zeroed with the shutter closed. Then a radioactive calibration light source was inserted and recorded before and after all sample testing to monitor instrument drift.

A digital count, CPM, which is a direct function of the total light emitted in the reaction, integrated over a precisely timed interval, is displayed for each test sample. Sample testing was done by first pipetting 0.5 ml of enzyme into a scintilation vial and recording the 0 to 6 second background CPM.

A 0.5 ml sample was then pipetted into the vial and swirled for 5 seconds. The delayed assay footswitch was depressed the instant the sample was pipetted. A uniform 15 second delay (time 0 to 15 seconds) occurred from this point and each sample CPM was recorded over an integration time period of 45 seconds (time 15 to 60 seconds).

A series of ATP standards were run before, during and after sample testing to control the results. A mean ATP standards curve was prepared as CPM versus ATP for each series of tests which eliminated the enzyme background CPM from the results.

The effects of the ATP extraction temperature versus the ATP recovery was evaluated by a series of extractions of mixed

liquor ATP at declining tris buffer plus sample temperatures. The effect of the elapsed time of the mixed liquor sample from aeration basin versus the ATP concentration was evaluated by a series of extractions at a composite tris buffer plus mixed liquor sample temperature of approximately 96°C.

4.3.2. Standard Testing Methods

Standard Monitoring tests were conducted in accordance with procedures outlined in Standard Methods for the Examination of Water and Wastewater (39).

1) Biochemical Oxygen Demand, BOD, tests were performed in accordance with section 507 and 422 B.

2) Chemical Oxygen Demand, COD, tests were performed in accordance with section 508.

3) Total non-filterable residue, SS, were obtained in accordance with section 208 D.

4) Total volatile residue and total fixed residue, VSS and TSS, respectively, were obtained in accordance with section 208 E.

5) Total phosphorus tests were performed in accordance with section 425 CIII and 425 E.

6) Ortho phosphorus tests were performed in accordance with section 425 E.

7) Organic nitrogen tests were performed in accordance section 421.

4.3.3. Non Standard Testing Methods

Additional tests included:

1) Total carbon, inorganic carbon and organic carbon tests using a Beckman Model 915 A carbon analyzer.

2) Turbidity using a nepthelometric turbidimeter.

3) pH using a Radiometer pH meter.

4) Conductivity using a Radiometer conductivity probe, Type CDM 2e

5) Oxygen uptake rates using a YSI oxygen meter, and observing oxygen depletion versus time of a mixed liquor sample in a BOD bottle immersed in ice water. The ice water bath maintained the sample temperature at 4°C to 8°C.

6) ATP using an integrating photometer as outlined previously in section 4.3.1.3.

5. EXPERIMENTAL RESULTS

5.1. ANALYTICAL RESULTS

5.1.1. ATP Calibration Curve

The ATP calibration curve, selected in a sensitivity range which would span all sample concentrations tested, was in the lower half of the instrument sensitivity scale to reduce instrument drift. A typical ATP calibration curve obtained in this investigation was as shown in Figure 11.

The results of ATP extraction versus temperature of extraction are shown in Figure 12.

The net ATP extracted from a mixed liquor sample versus elapsed time, that is time from withdrawal from aeration basin to sample addition to the boiling tris buffer, is included as Figure 13.

5.1.2. ATP in Mixed Liquor

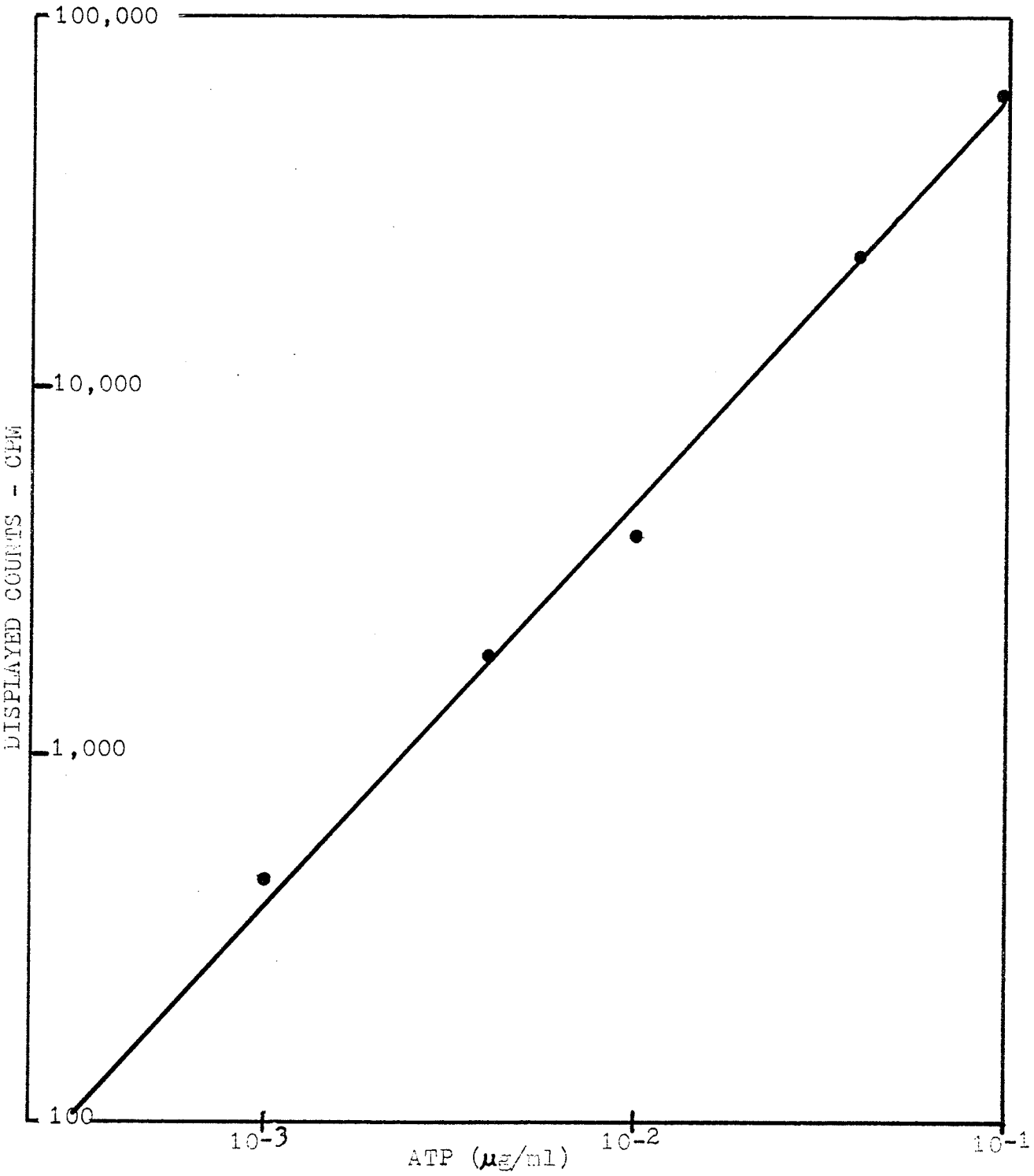
The concentrations of ATP versus MLVSS and ATP versus MLSS are shown in Figure 14 and are included in Table 3.

5.1.3. ATP in Raw Domestic Sewage

The concentrations of ATP versus RVSS and versus RSS are shown in Figure 15 and included in Table 4.

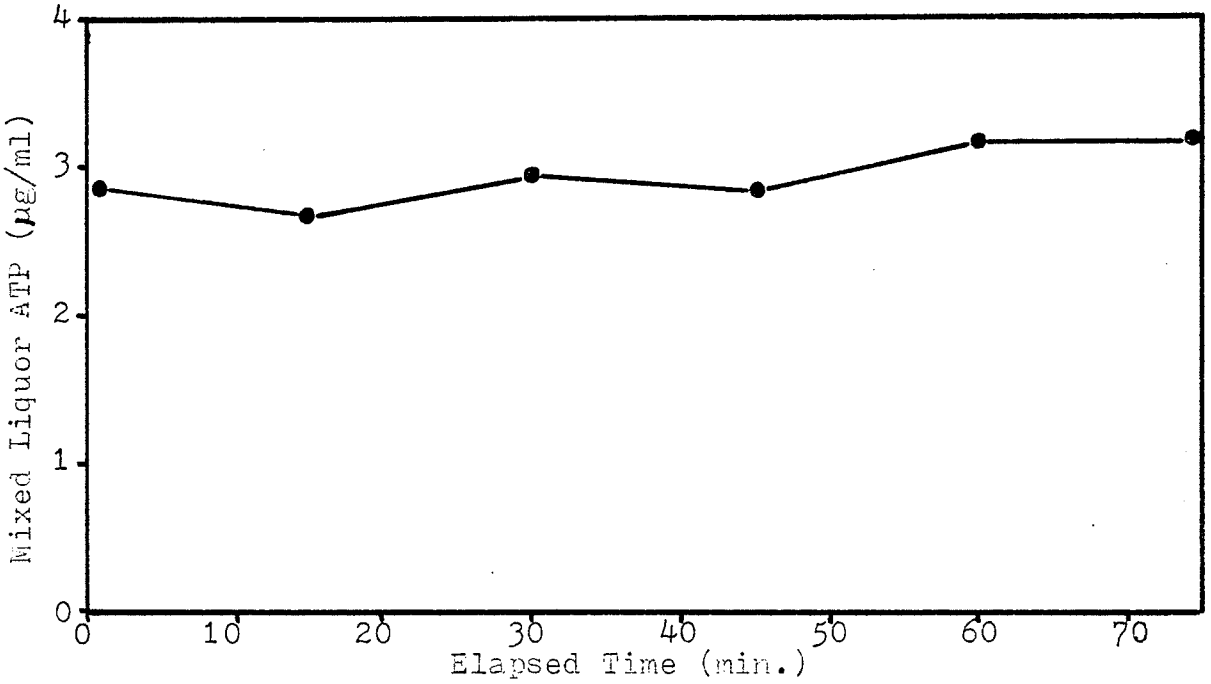
5.1.4 ATP in Effluent

The concentrations of ATP versus EVSS and ESS are shown in Figure 16 and are included in Table 5.



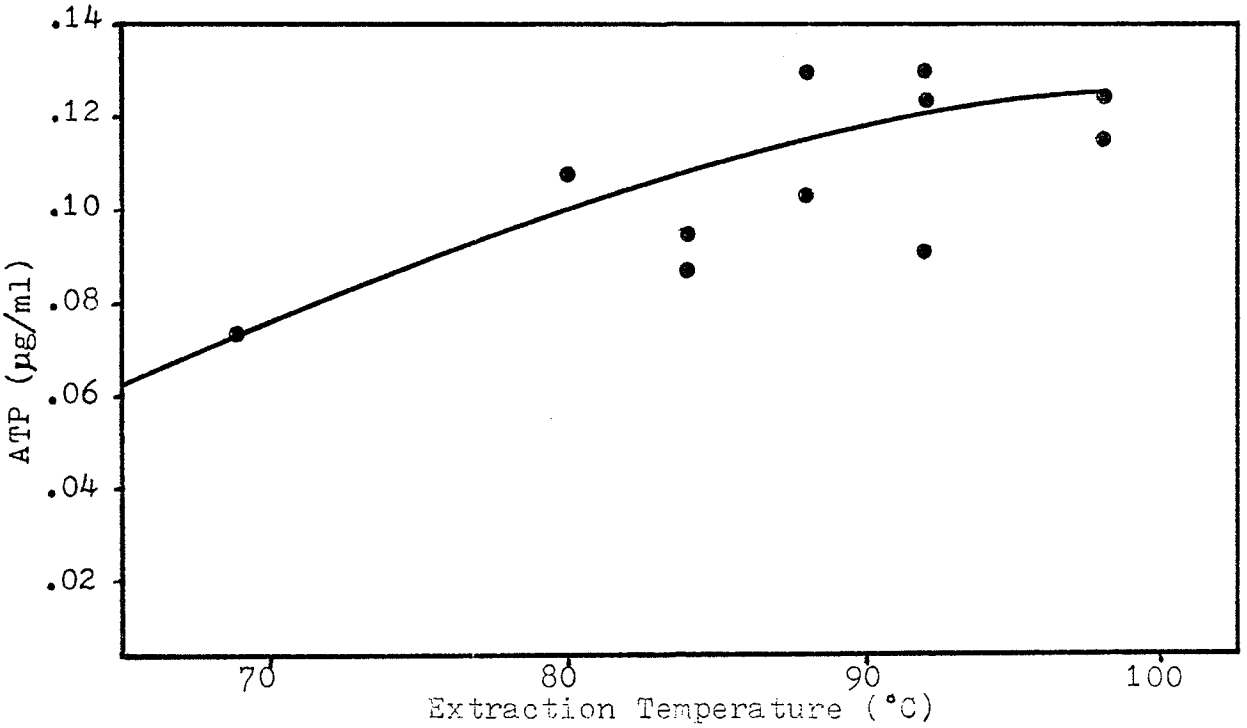
ATP STANDARD CALIBRATION CURVE

Figure 11.



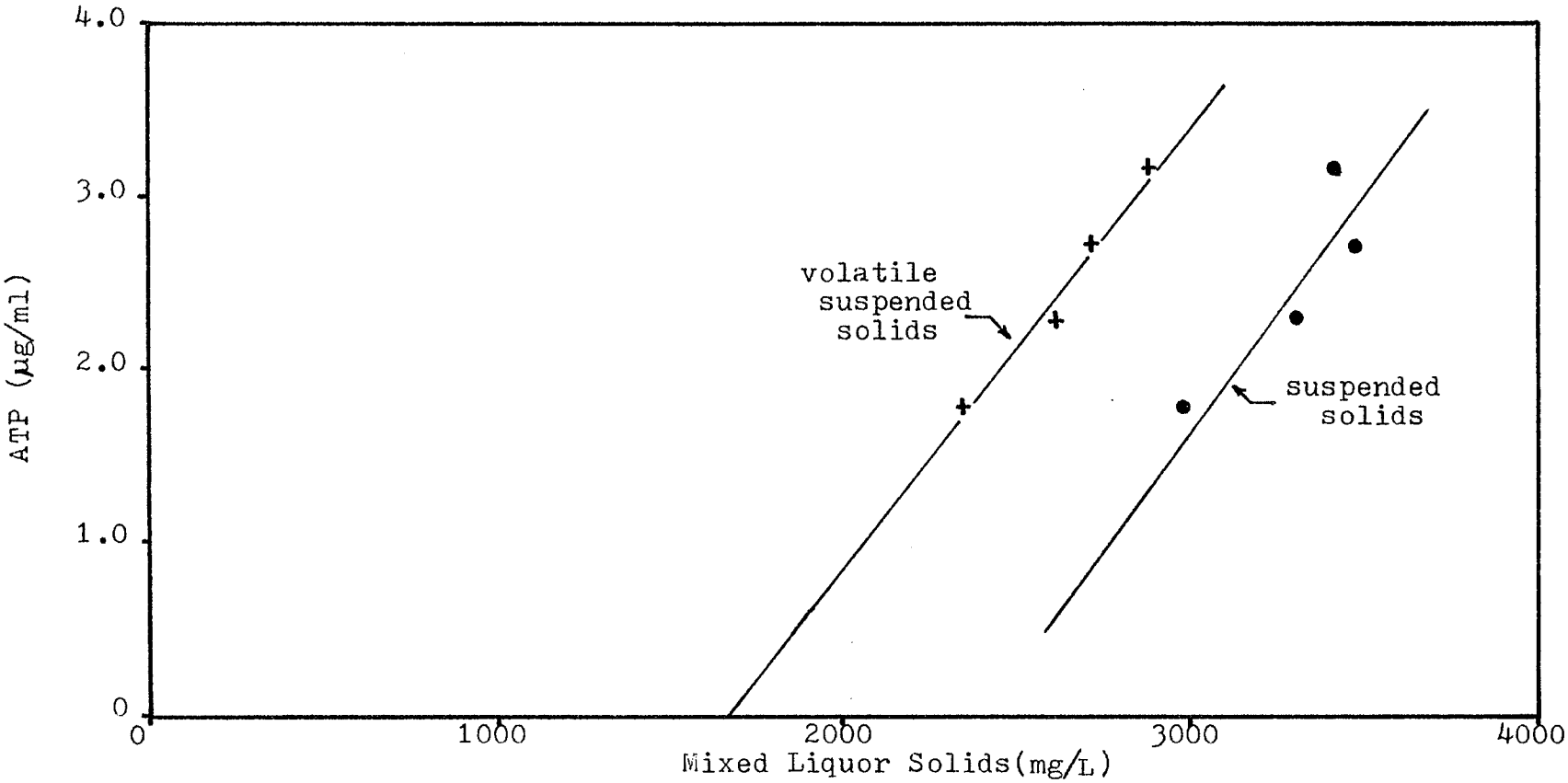
RELATIVE ATP RECOVERY VERSUS ELAPSED TIME , UNAERATED

Figure 12.



EFFECT OF TEMPERATURE OF EXTRACTION ON ATP RECOVERY

Figure 13.



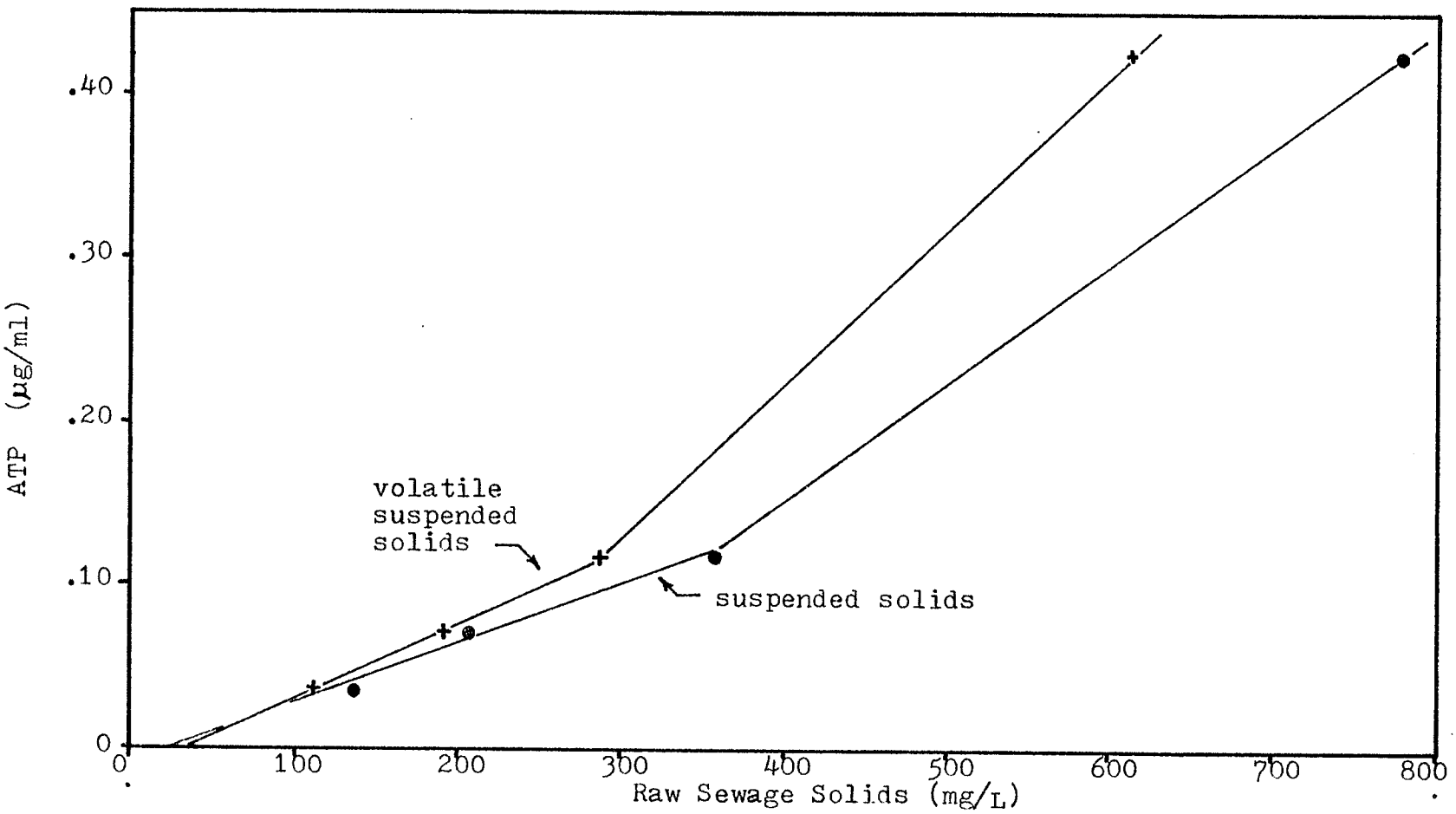
RELATIONSHIP BETWEEN ATP AND MIXED LIQUOR SOLIDS

Figure 14.

TABLE 3

MIXED LIQUOR ATP, SUSPENDED SOLIDS
AND VOLATILE SUSPENDED SOLIDS

Day	ATP µg/ml	SS mg/L	VSS mg/L	Day	ATP µg/ml	SS mg/L	VSS mg/L
1	2.31	2925	2725	30	2.82	3650	2710
2	3.15	3125	2875	31	2.04	2980	2240
3	3.78	2375	2200	32	1.70	2250	1690
4	2.94	5000	3950	33	2.52	3120	2480
5	3.30	3100	2650	34	2.59	3770	3000
6	2.52	3500	2850	35	2.65	3760	2940
7	2.94			36	2.77	4390	2980
8	4.06	2797	2266	37	2.42	3660	2810
9	2.42	2800	2333	38	2.30	3690	2890
10	1.71	4533	3600	39	3.08	4040	3100
11	1.93	2400	2100	40	2.40	3850	2930
12	2.79	2566	2100	41	2.65	3720	2820
13	2.96	2600	2140	42	2.67	3540	2720
14	2.79			43	2.56	3550	2730
15	3.98			44	1.90	2890	2250
16	2.46			45		2410	1850
17	2.62	3260	2600	46	1.73	2930	2270
18	2.79	3180	2600	47	1.71	2970	2270
19	2.83	3260	2640	48	1.72	2580	2350
20	2.46	3480	2840	49	1.91	3250	2450
21	2.79	3660	2860	50	1.90	3290	2530
22	2.01	3170	2500	51	1.87	3190	2430
23	2.67	3100	2440	52	1.98	3180	2420
24	2.71	3530	2740	53	2.22	3330	2510
25	2.23	2980	2350	54	2.37	3480	2640
26	2.71	3370	2620	55	1.68	2280	1730
27	2.58	3440	2680	56	2.01	3400	2670
28	2.88	3270	2540	57	1.78	3110	2340
29	1.66	2950	2240				



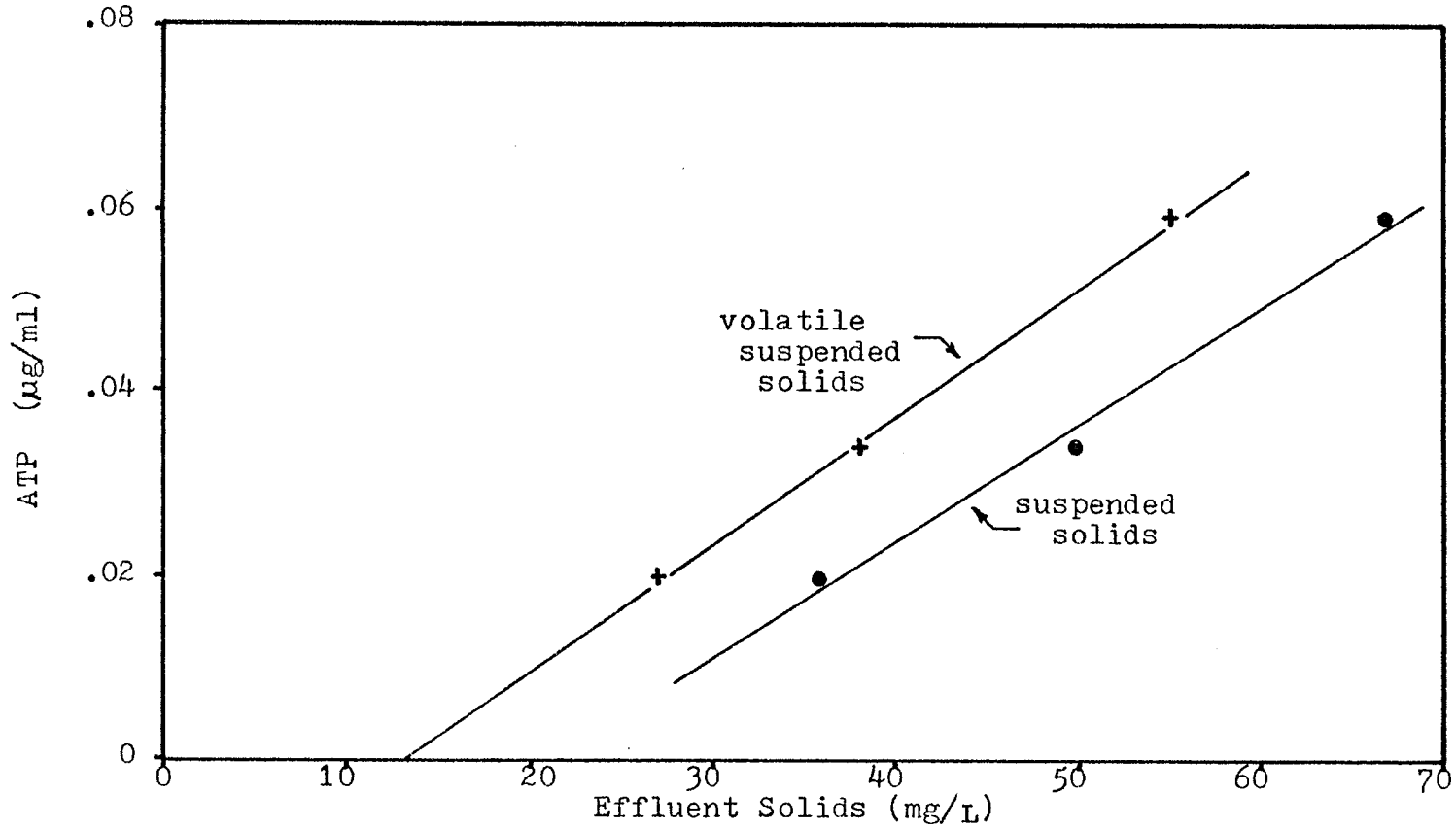
RELATIONSHIP BETWEEN ATP AND RAW SEWAGE SOLIDS

Figure 15.

TABLE 4

RAW SEWAGE ATP, SUSPENDED SOLIDS AND VOLATILE SUSPENDED SOLIDS

Day	ATP µg/ml	SS mg/L	VSS mg/L	Day	ATP µg/ml	SS mg/L	VSS mg/L
1	.139			30	.106	224	166
2	.420			31	.064	150	106
3	.840	1700	1350	32	.067	131	93
4	.097	170	133	33	.084	366	302
5	.076	145	128	34	.056	141	113
6	.080	163	143	35	.102	506	388
7	.040			36	.069	188	146
8	.218	265	223	37	.046	128	100
9	.168	357	102	38	.033	148	138
10	.120	356	288	39	.022	100	72
11	.134	430	366	40	.033	163	115
12	.151	425	345	41	.036	186	138
13	.105	312	248	42	.029	193	149
14	.126			43	.027	124	89
15	.034			44	.032	207	93
16	.076			45		314	210
17	.076	250	158	46	.043	129	89
18	.059	214	140	47	.065	218	150
19	.061	240	156	48	.048	202	138
20	.076	275	210	49	.064	228	152
21	.044	151	119	50	.056	226	152
22		136	102	51	.075	304	198
23	.068	129	97	52	.214	364	262
24	.062	134	96	53	.308	533	408
25	.094	138	101	54	.353	664	516
26	.116	182	128	55	.351	535	400
27	.056	113	86	56	.273	480	385
28		150	114	57	.134	500	435
29	.061	110	82				



RELATIONSHIP BETWEEN ATP AND EFFLUENT SOLIDS

Figure 16.

TABLE 5

EFFLUENT ATP, SUSPENDED SOLIDS AND VOLATILE SUSPENDED SOLIDS

Day	ATP µg/ml	SS mg/L	VSS mg/L	Day	ATP µg/ml	SS mg/L	VSS mg/L
1	.050			30	.061	70	47
2	.084			31	.055	72	52
3	.105	12.5	12.5	32	.034	36	27
4	.029	30	26	33	.051	75	73
5	.021	10	10	34	.053	74	63
6	.025	20	20	35	.043	76	61
7	.065			36	.058	124	96
8	.054	40	34	37	.033	65	51
9	.040	28	24	38	.047	146	100
10		23	21	39	.045	110	76
11	.069	30	28	40	.014	59	41
12	.043	42	37	41	.034	98	66
13	.029	23	22	42	.037	75	53
14	.030			43	.036	91	60
15	.286			44	.021	50	36
16	.040			45		52	40
17	.044	34	29	46	.019	23	19
18	.035	22	20	47	.027	61	46
19	.044	27	20	48	.030	43	31
20	.035	34	32	49	.030	52	36
21	.033	23	21	50	.030	45	32
22	.024	37	30	51	.027	50	36
23	.027	30	25	52	.127	52	36
24	.029	31	23	53	.032	50	38
25	.029	34	29	54	.030	48	35
26	.034	42	33	55	.040	44	34
27	.032	38	33	56	.074	50	50
28		45	38	57	.035	40	40
29	.018	34	24				

5.1.5. Mixed Liquor ATP versus Organic Constituent Removal Rates

The ATP concentration, expressed as the weight of ATP per unit volume and the weight of ATP per unit dry weight of MLVSS versus BOD_5, COD and TOC removal rates are shown in Figures 17 to 22, inclusive.

5.1.6. Phosphorous Tests

The results of the mixed liquor ortho phosphorous tests versus the ATP concentration in the mixed liquor is shown in Figure 23.

5.1.7. Oxygen Uptake Rate Tests

The oxygen uptake rates of the mixed liquor at an operating temperature of $4 \pm .5^{o}C$ with respect to the ATP levels in the mixed liquor are shown in Figures 24 and 25.

5.1.8. Sludge Volume Index and Sludge Age

The sludge volume index, SVI, of the mixed liquor is shown in Figure 26.

The ATP concentrations in the mixed liquor per unit dry weight of suspended solids, MLSS, and per unit dry weight of volatile suspended solids, MLVSS, versus elapsed time are shown in Figures 27 and 28, respectively.

The mixed liquor ATP concentrations per unit volume versus elapsed time are shown in Figure 29.

5.1.9. Miscellaneous Tests

The pH of the raw sewage typically ranged between 7.0 and 7.5. The pH of the mixed liquor and effluent was typically in the range of 8.0 to 8.4.

The turbidity of the raw sewage varied through a wide range from 58 to 295 NTU, typically in the range of 100 to 200 NTU. The turbidity of the effluent typically ranged between 20 to 80 NTU.

The conductivity of the raw sewage and the effluent ranged between 475 and 500 μmhos/cm. The conductivity of the effluent consistantly reflected any variation in the raw sewage conductivity.

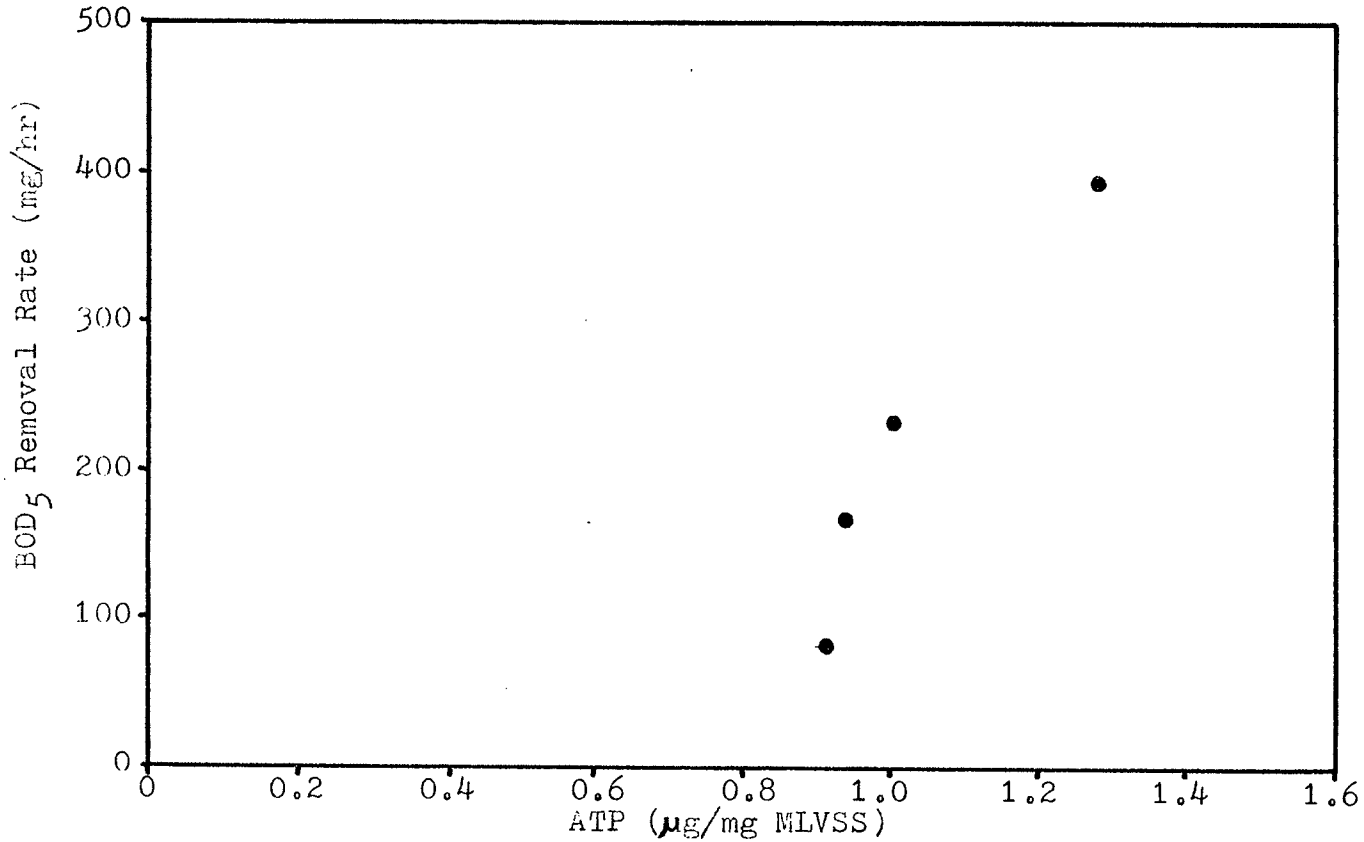
The average ATP content with respect to organic nitrogen content in the mixed liquor was 12.25 μg ATP/mg organic N.

5.2. Biokinetic Interpretation

5.2.1. Kinetic Growth and Substrate Utilization Rate Constants

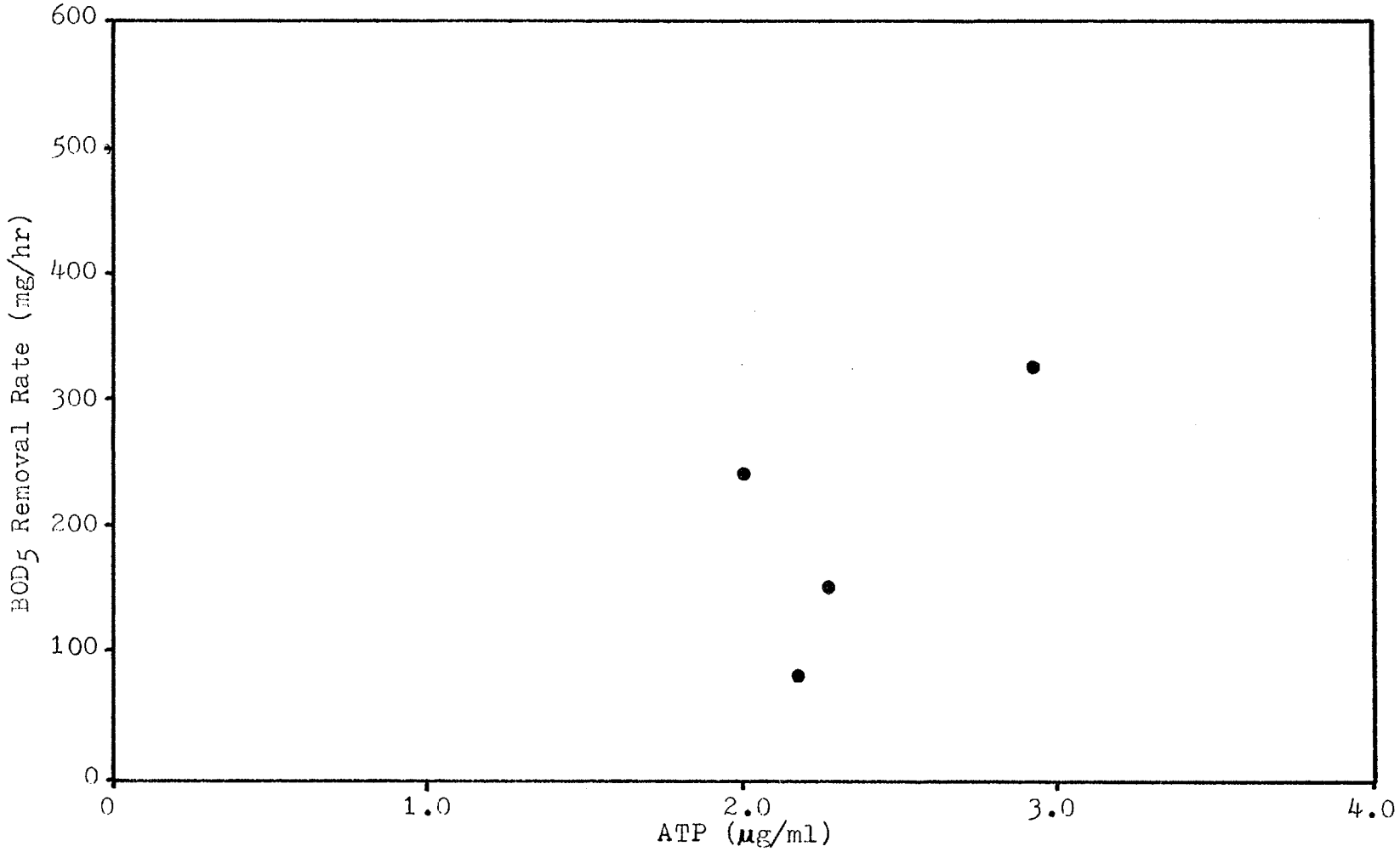
The kinetic growth and substrate utilization rate constants were calculated according to the kinetic theory summarized in section 3.3.1. The constants were calculated using adensoine triphosphate as an indicator of mixed liquor and effluent microorganism content and also, using the traditional volatile suspended solids, VSS, for comparison. The results are summarized in Table 6 and 7.

An example calculation for an average BOD_5 feed of 200 - 300 mg/L is included in Appendix C. The calculated data in this example is, also, included in Appendix C as Figures 33 and 34.



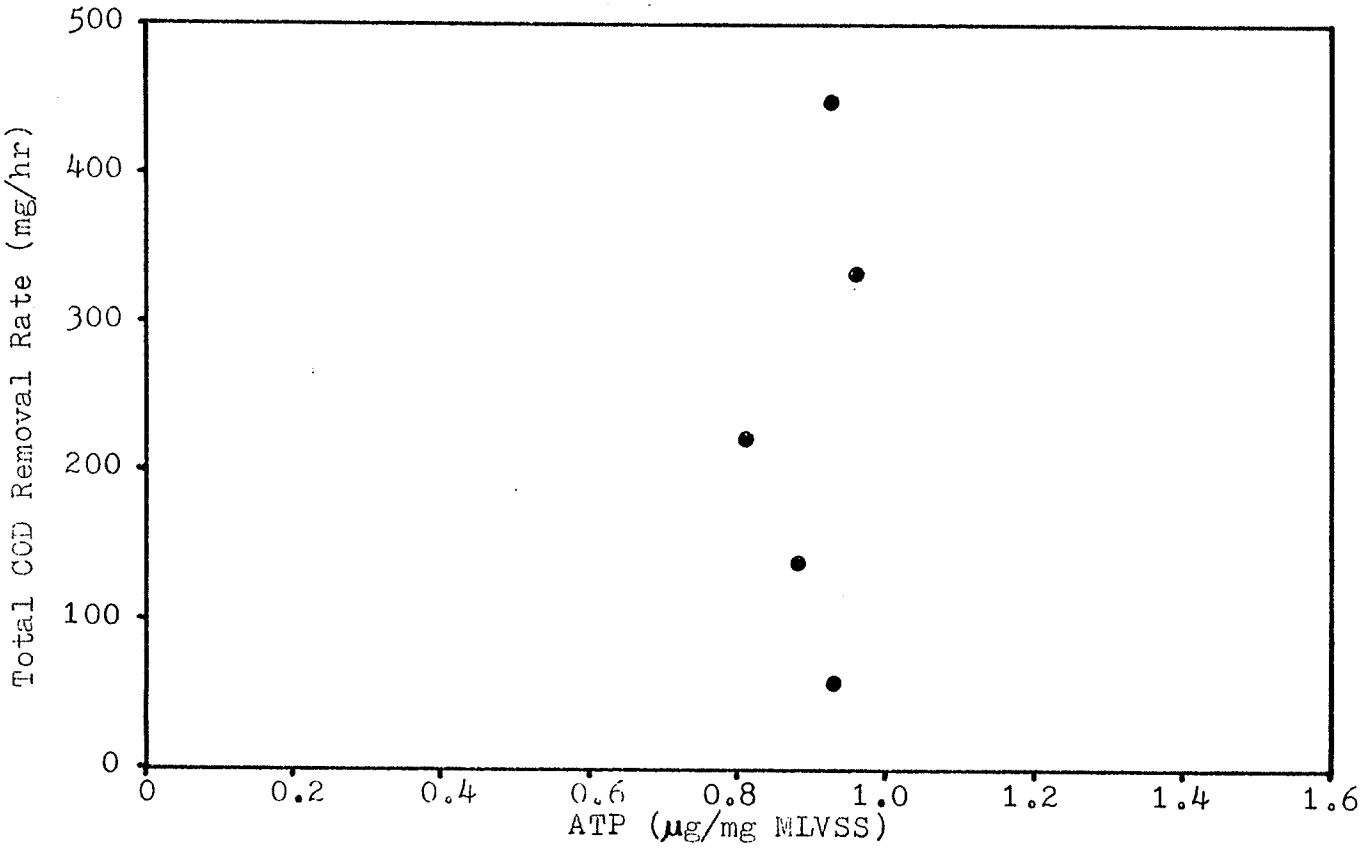
ATP CONCENTRATION
VERSUS
BIOCHEMICAL OXYGEN DEMAND REMOVAL RATE

Figure 17.



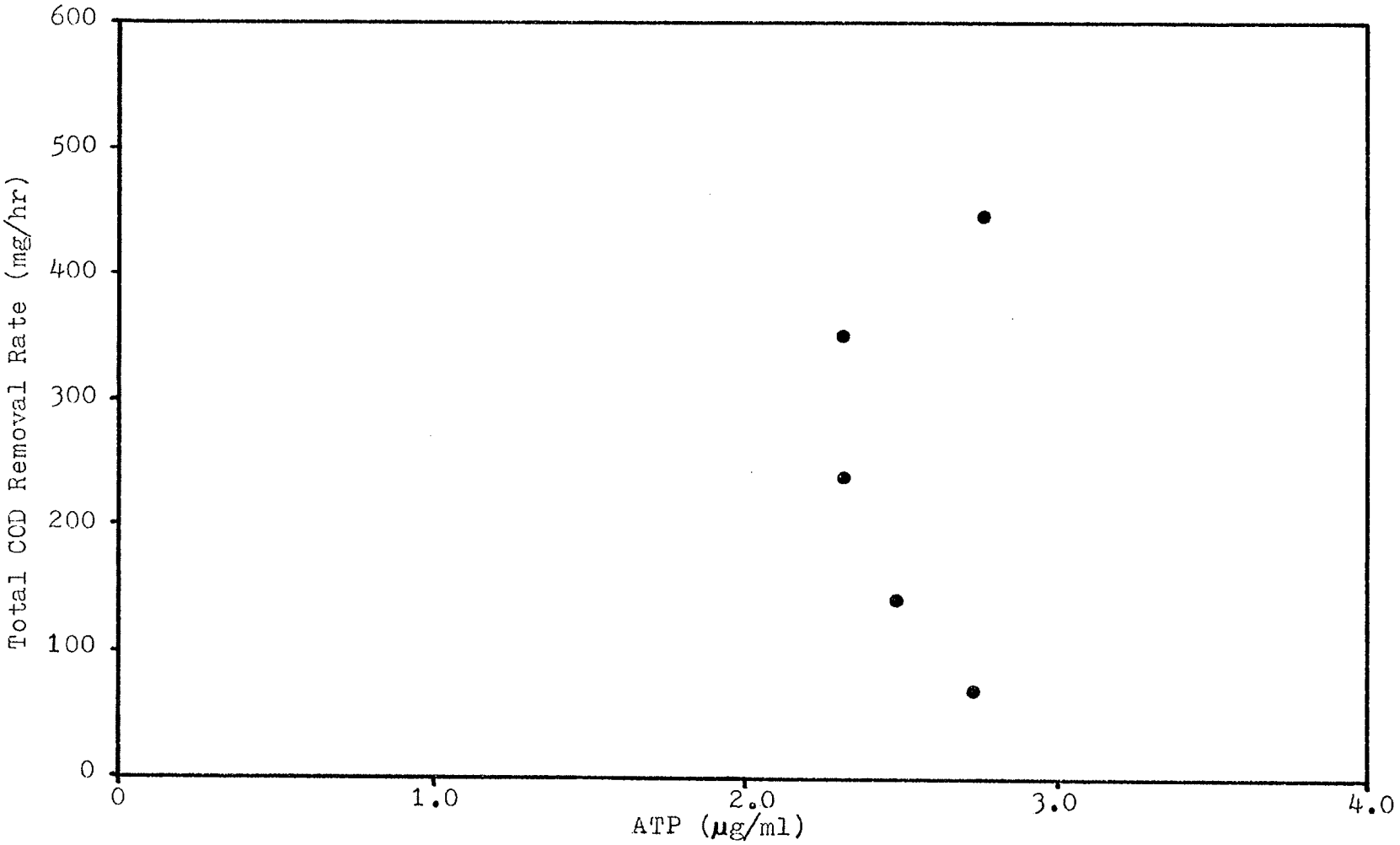
ATP CONTENT VERSUS BIOCHEMICAL OXYGEN DEMAND REMOVAL RATE

Figure 18.



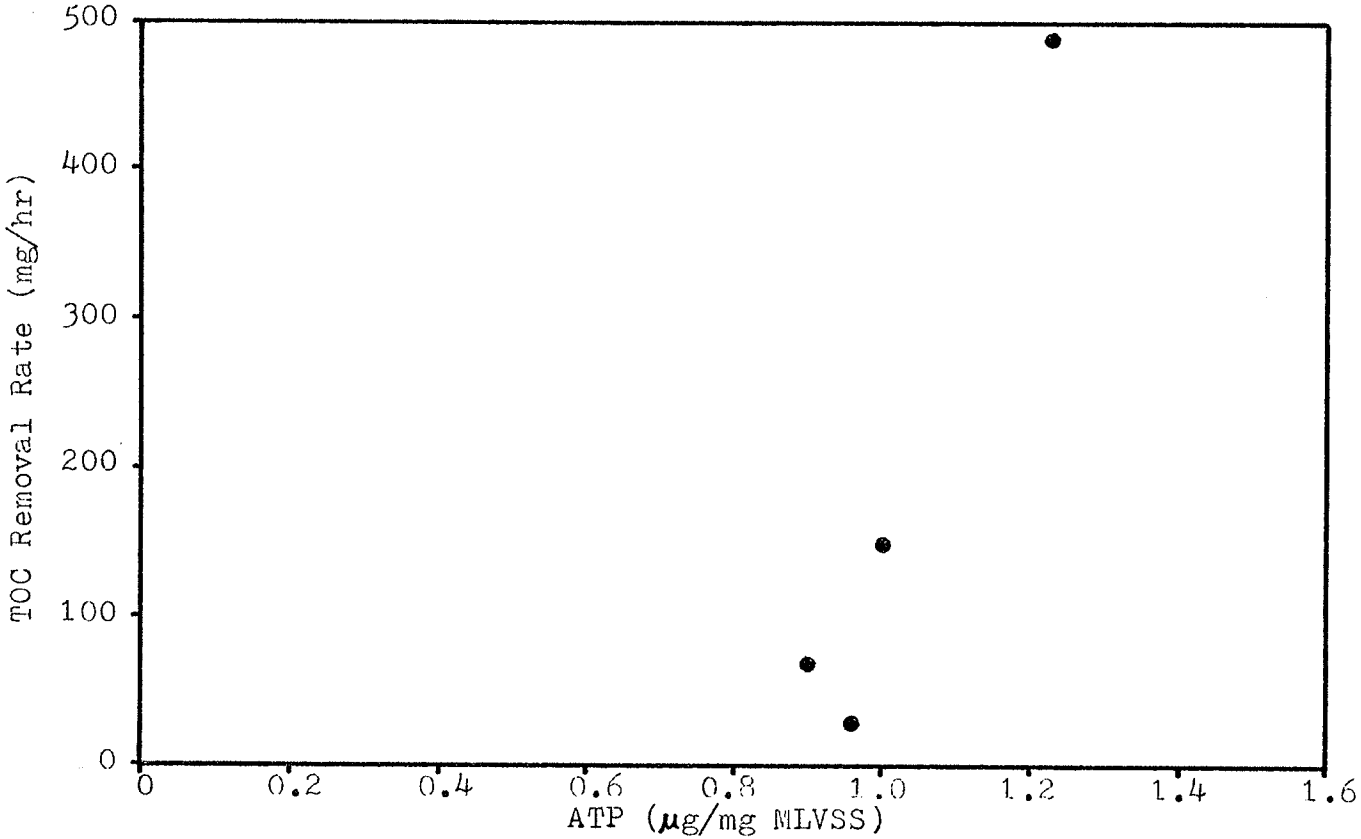
ATP CONCENTRATION
VERSUS
TOTAL CHEMICAL OXYGEN DEMAND REMOVAL RATE

Figure 19.



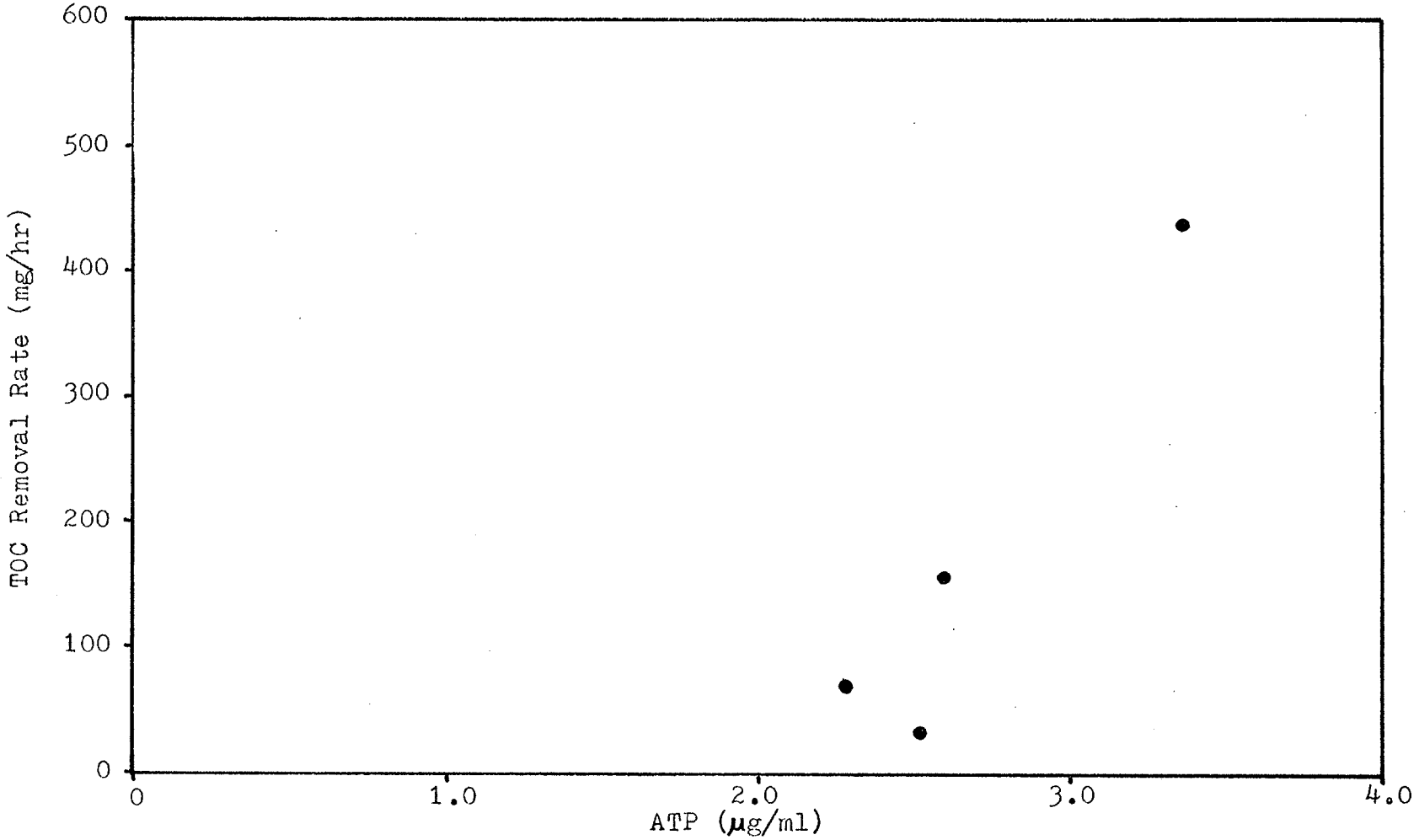
ATP CONTENT VERSUS TOTAL CHEMICAL OXYGEN DEMAND REMOVAL RATE

Figure 20.



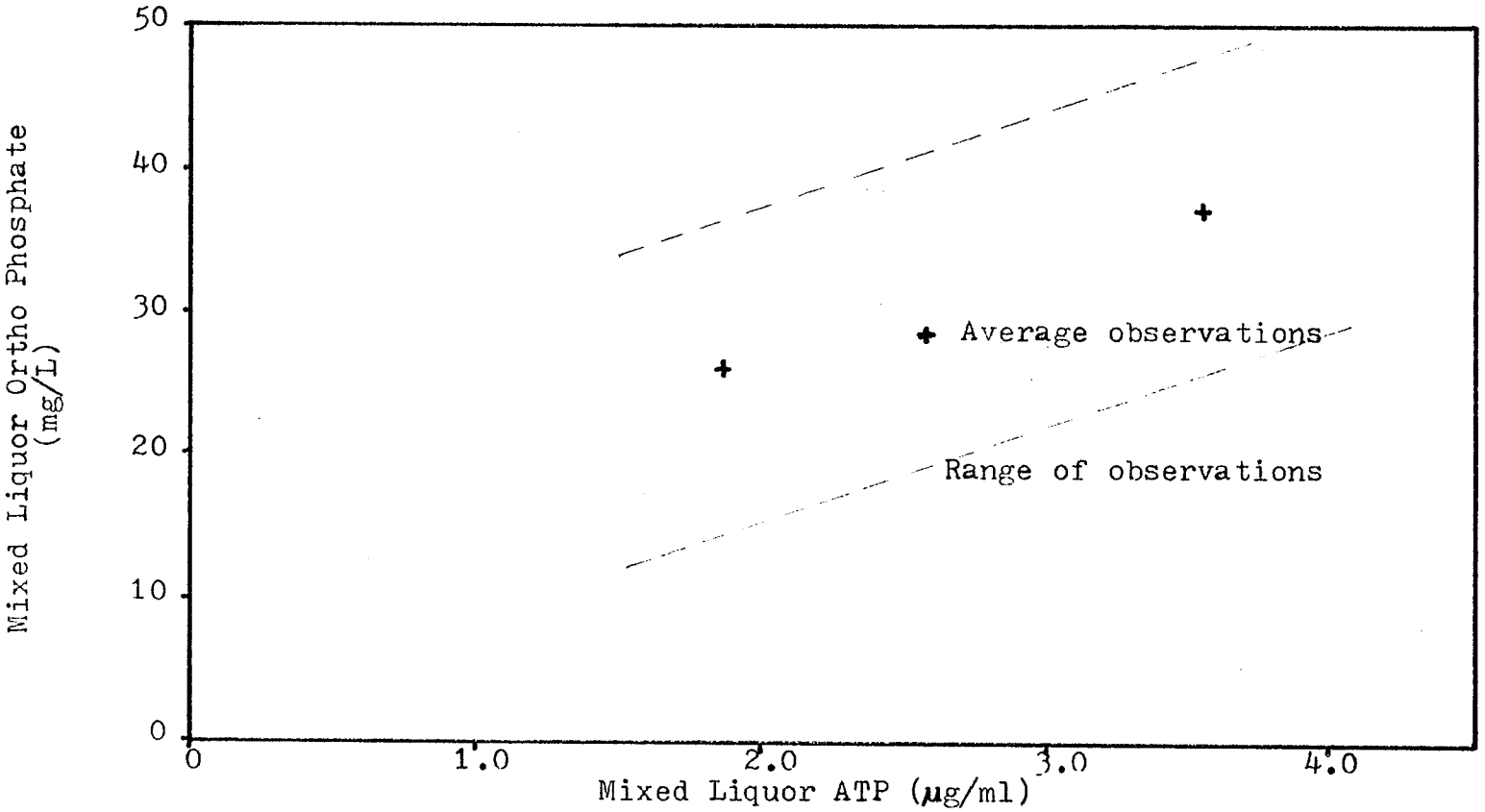
ATP CONCENTRATION
VERSUS
TOTAL ORGANIC CARBON REMOVAL RATE

Figure 21.



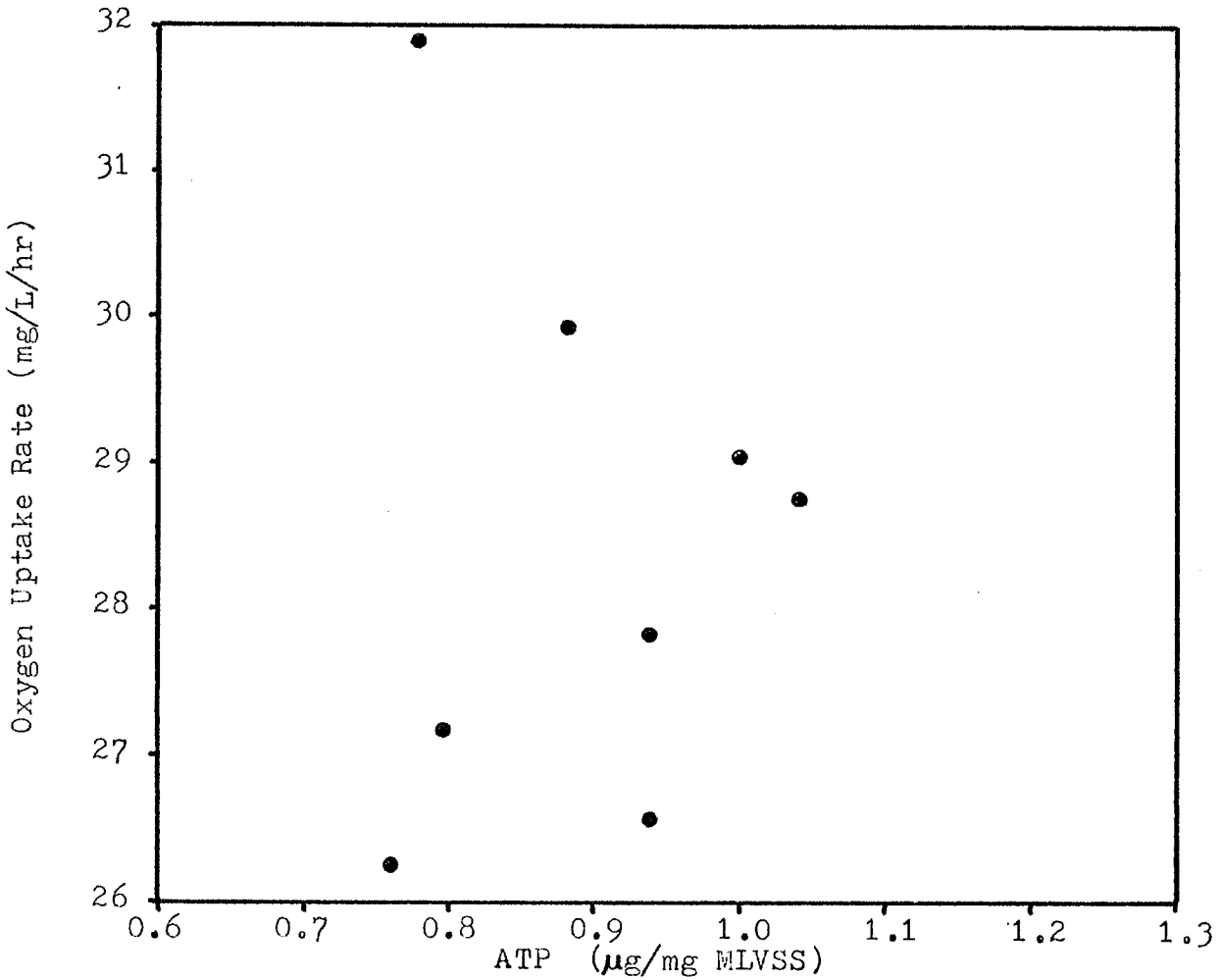
ATP CONTENT VERSUS TOTAL ORGANIC CARBON REMOVAL RATE

Figure 22.



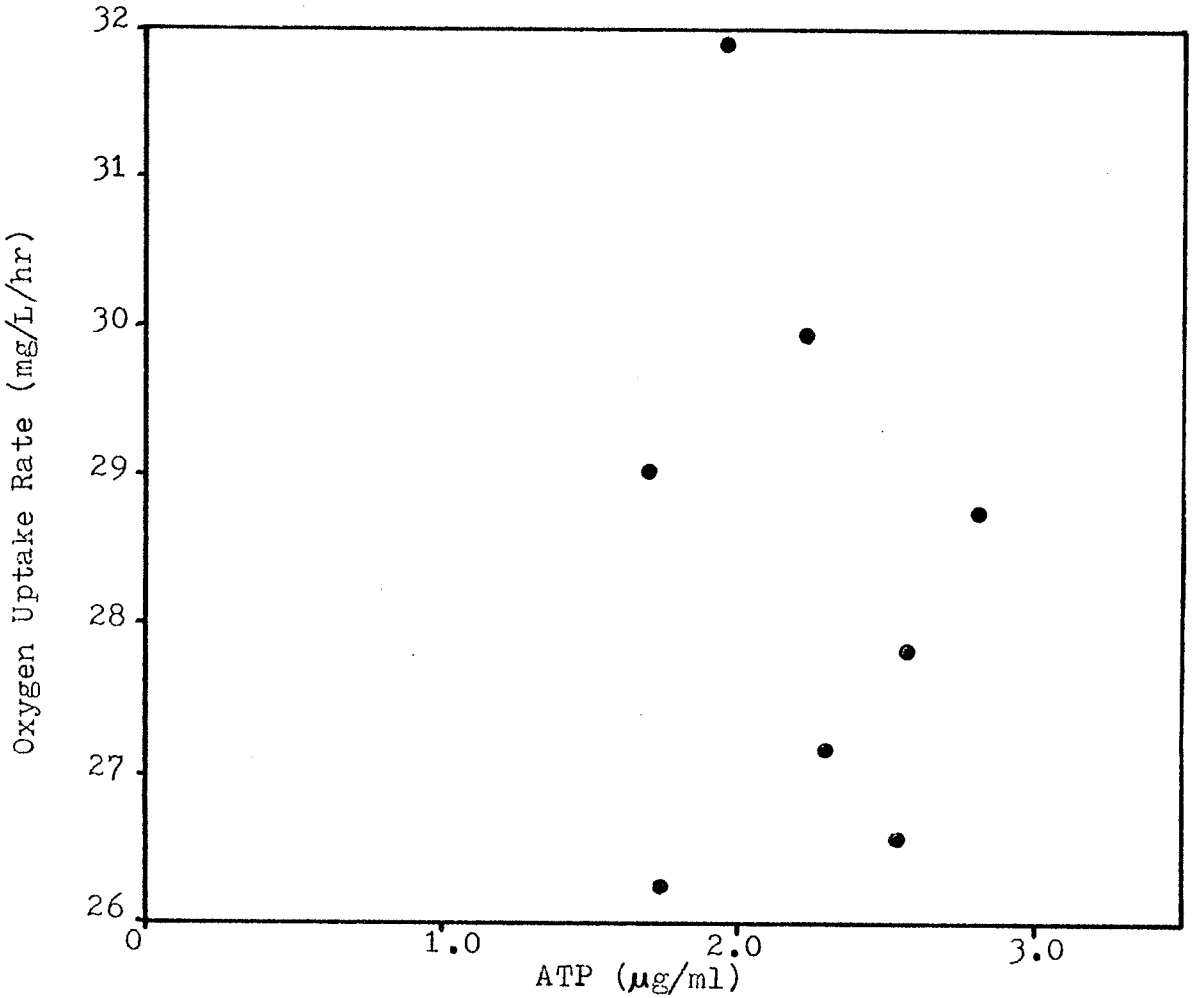
RELATIONSHIP BETWEEN MIXED LIQUOR ATP CONTENT AND MIXED LIQUOR ORTHO PHOSPHATE CONTENT

Figure 23.



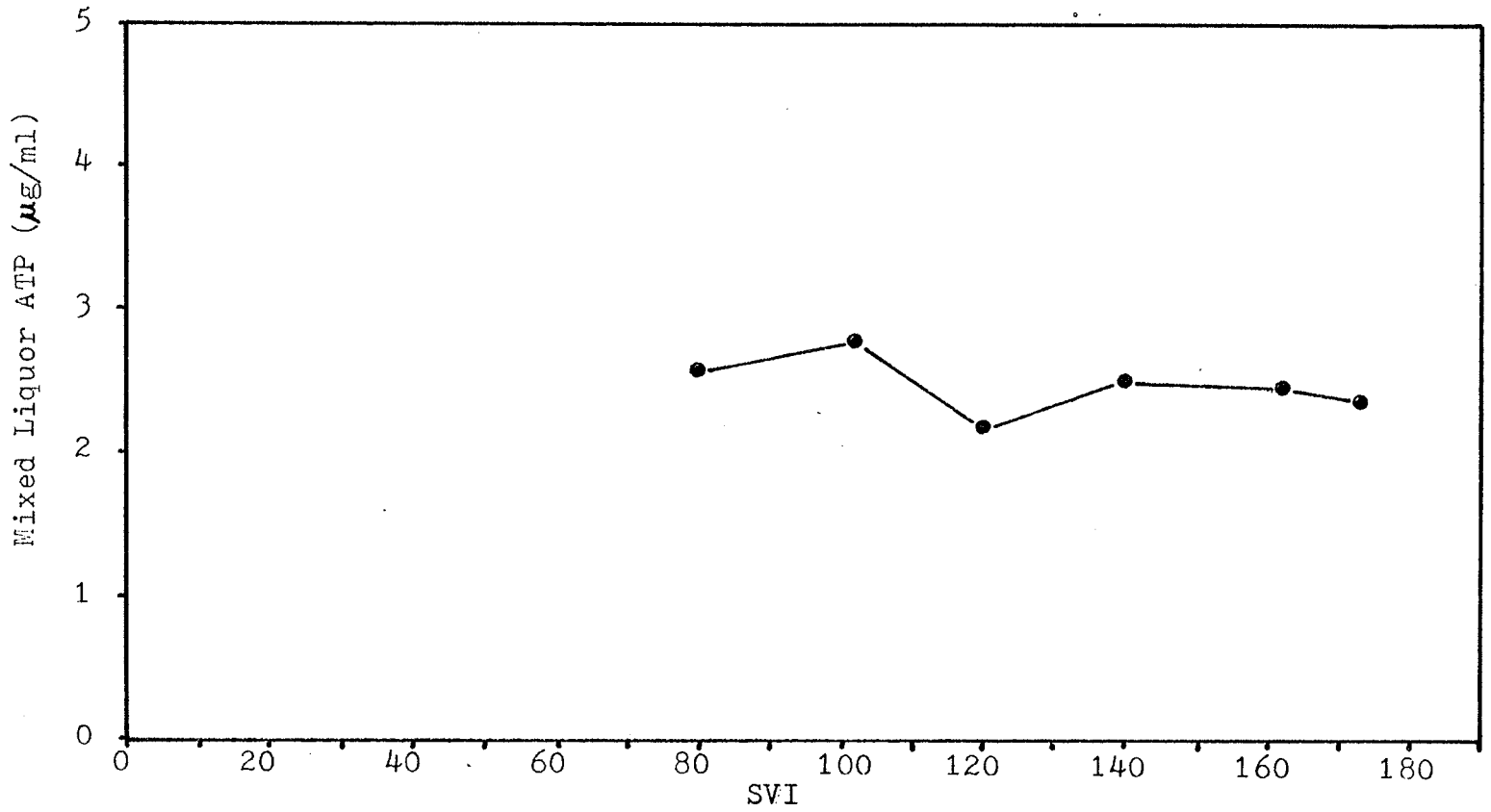
RELATIONSHIP BETWEEN ATP CONCENTRATION
AND MIXED LIQUOR OXYGEN UPTAKE RATE

Figure 24.



RELATIONSHIP BETWEEN ATP CONTENT
AND MIXED LIQUOR OXYGEN UPTAKE RATE

Figure 25.



SLUDGE VOLUME INDEX AND CORRESPONDING MIXED LIQUOR ATP CONTENT

Figure 26.

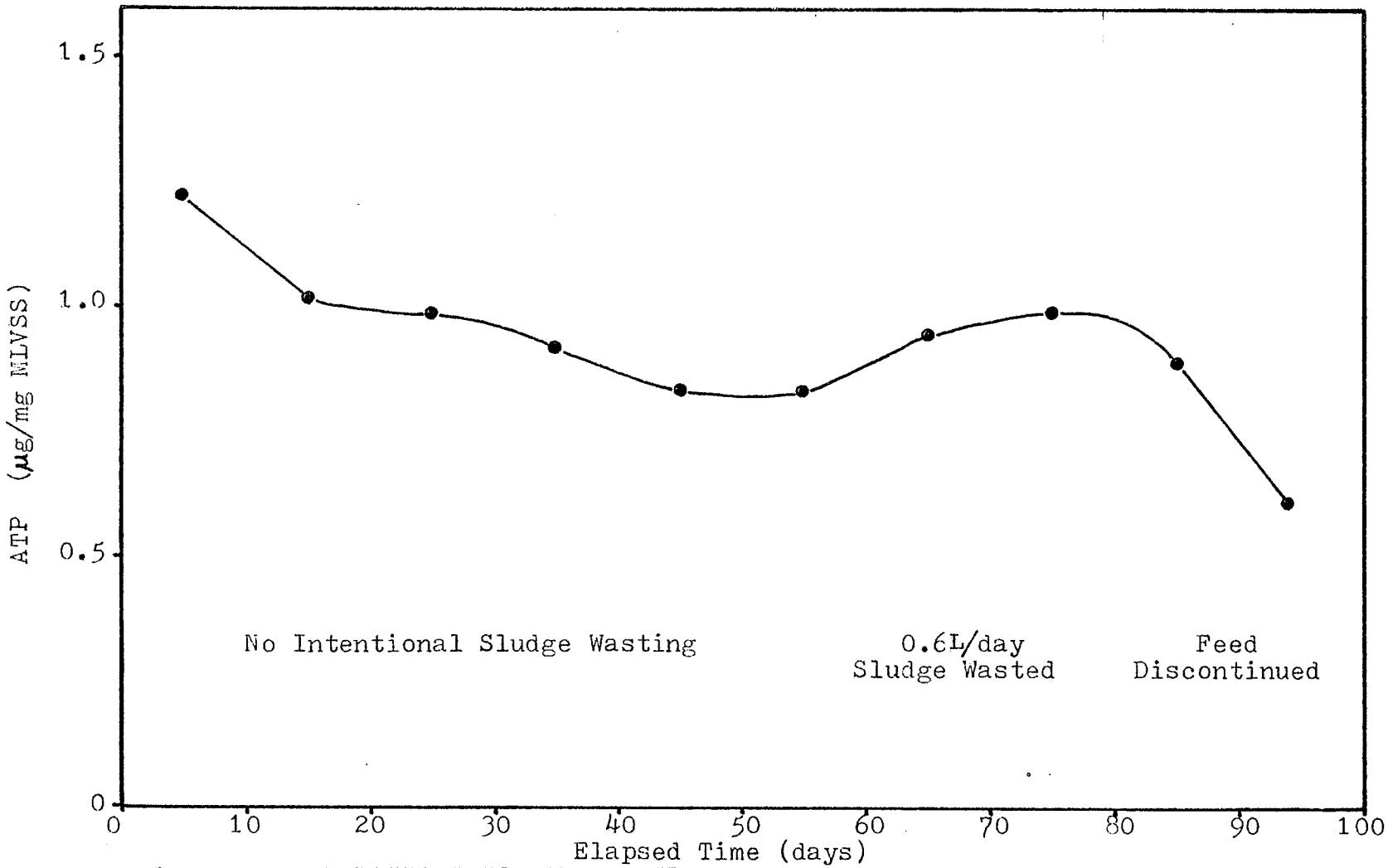


Figure 27. RELATIONSHIP BETWEEN SLUDGE CONDITION AND ATP CONCENTRATION WITH RESPECT TO MIXED LIQUOR VOLATILE SUSPENDED SOLIDS

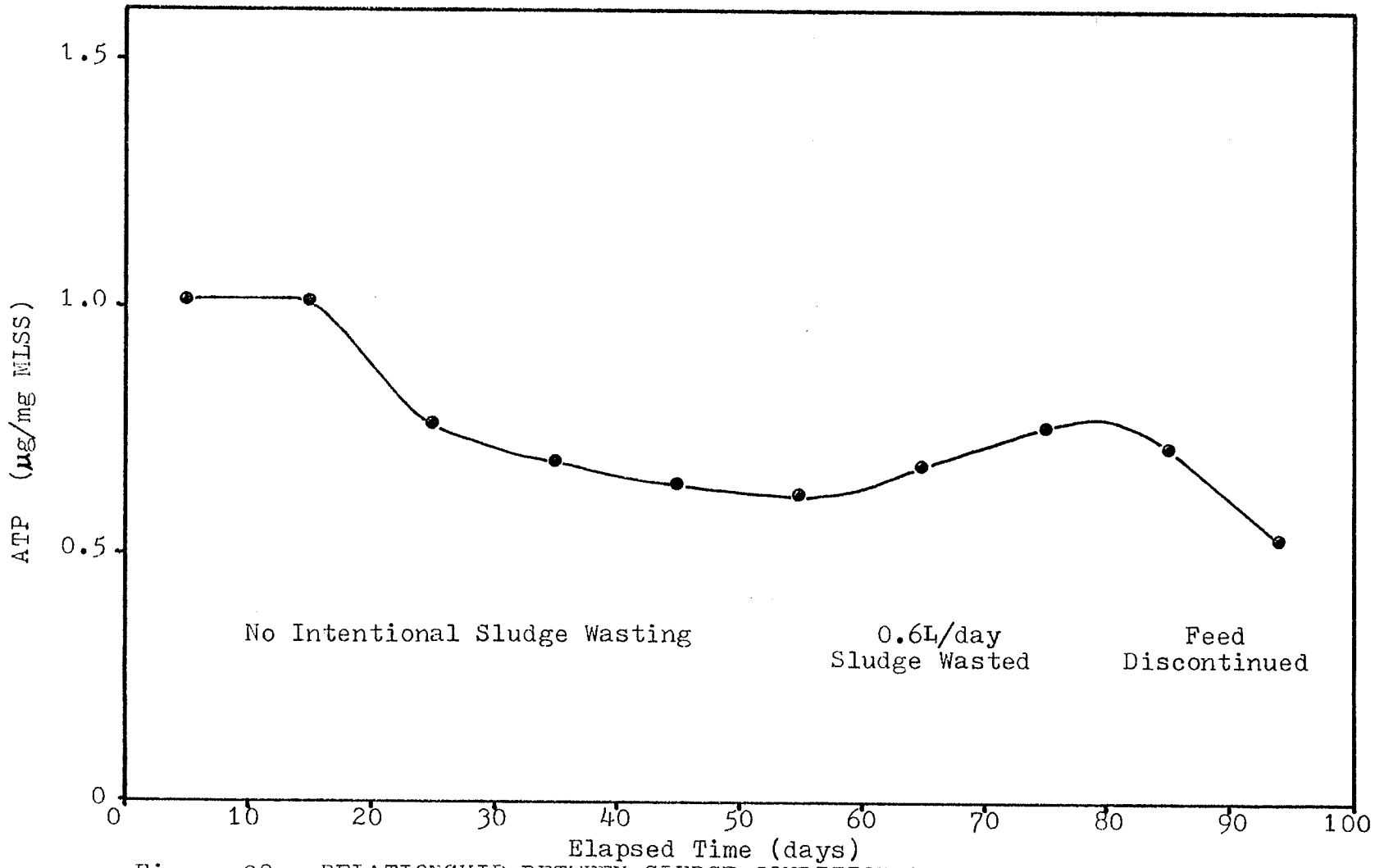


Figure 28. RELATIONSHIP BETWEEN SLUDGE CONDITION AND ATP CONCENTRATION WITH RESPECT TO MIXED LIQUOR SUSPENDED SOLIDS

Figure 29. RELATIONSHIP BETWEEN SLUDGE CONDITION AND ATP CONTENT

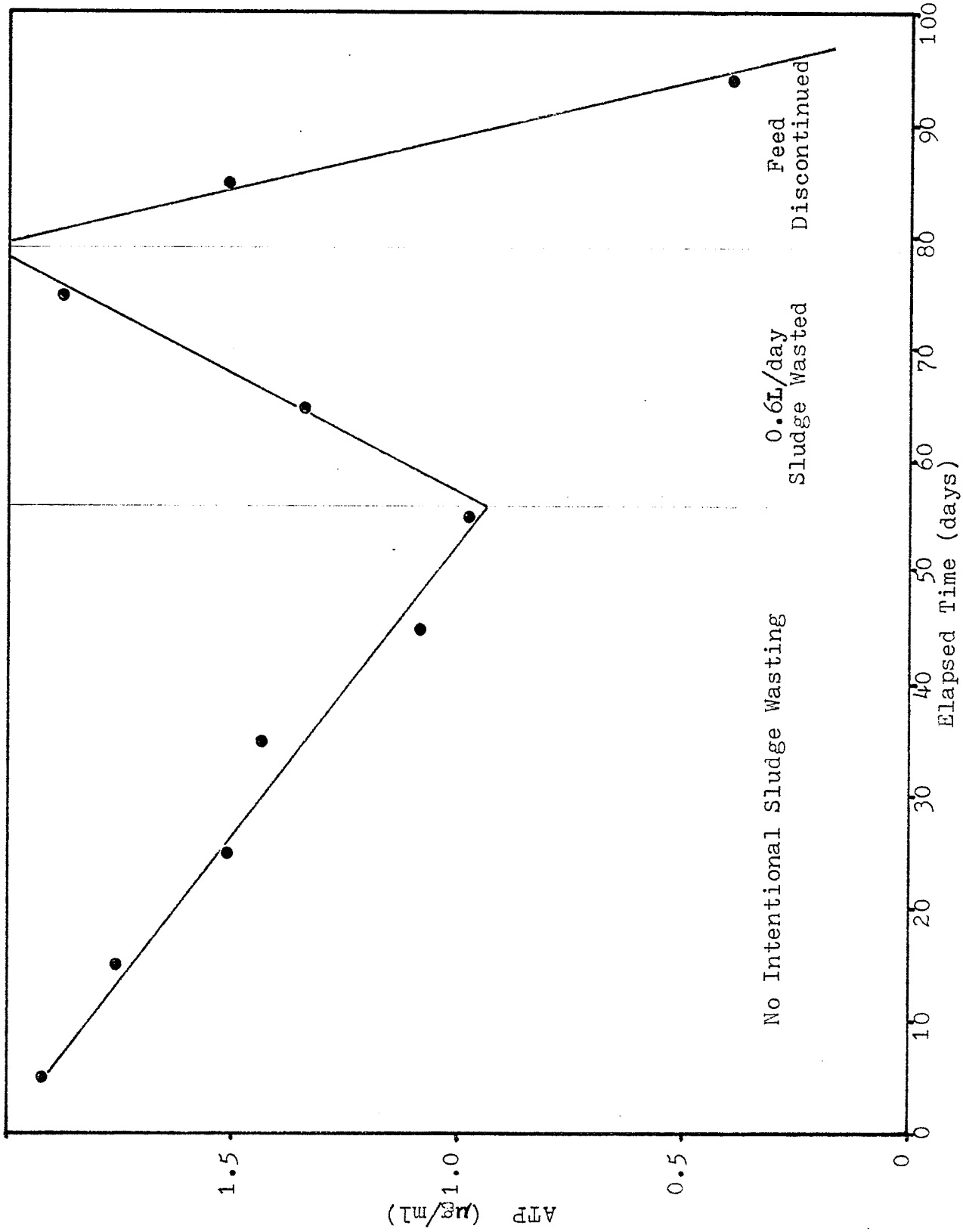


TABLE 6

No.	Avg. BOD_5 mg/L	Y $\frac{\mu g\ ATP}{mg\ BOD_5}$	k_d x_{10^3}/hr	r	t
15	165	0.782	1.411	0.727	5.35
21	260	0.336	0.665	0.565	3.53
7	366	0.483	1.596	0.772	3.83
6	458	0.277	1.315	0.843	5.81
		$\frac{mg\ VSS}{mg\ BOD_5}$			
15	165	0.533	0.175	0.293	1.15
21	260	1.000	2.429	0.856	13.99
7	366	0.302	0.469	0.812	4.78
6	458	0.486	1.755	0.874	3.604

Yield coefficient, Y, and Endogeneous Decay Rate, k_d, Based on BOD_5 Parameters at $4 \pm .5$°C.

TABLE 7

No.	Avg. BOD_5 mg/L	K_s mg/L	μmax x_{10^3}/hr	r	t
15	165	.047	2.00	.406	1.539
21	260	5.20	1.61	-.294	-1.305
7	366	17.59	3.69	.672	1.812
6	458	0.040	2.10	.359	0.768

Using ATP

TABLE 7 (cont)

No.	Avg. BOD_5 mg/L	K_s mg/L	μ max x_{10^3}/hr	r	t
15	165	11.67	1.30	.538	2.30
21	260	0.25	3.26	-.788	-5.58
7	366	1.42	0.99	.003	.007
6	458	2.89	2.47	.511	1.03

Using VSS

Maximum Growth Rate, μ max, and the Half Velocity Coefficient, K_s, based on BOD_5 Parameters at $4^{\pm}$ .5°C .

The average ATP yield per dry unit weight of volatile suspended solids preduced versus a range of average BOD_5 feed rates are shown in Table 8.

TABLE 8

ENERGY YIELD

Avg. BOD_5	μg ATP yield/mg VSS cells produced	
165	.782/.533	= 1.467
260	.336/1.000	= 0.336
366	.483/.302	= 1.599
458	.277/.486	= 0.570

6. DISCUSSION OF RESULTS

6.1. ANALYTICAL TEST RESULTS

6.1.1. ATP Response

The ratio of enzyme to prepared ATP sample volume used throughout this investigation was 1:1. This ratio ensured that the enzyme was in excess so that the optimum light intensity of a particular sample would result, slightly in excess for the optimal enzyme to sample ratio of 1:2 given by Nutt (8).

The optimal extraction temperature appears to be in the range of 90 to 100°C. Below 90°C the effective extraction of ATP dropped off at an increasing rate.

The effect of elapsed time of mixed liquor sample removal from aeration basis to ATP extraction appeared to have a negligible effect on the net ATP extracted from samples, up to certain limiting times. This effect was likely due to the high initial dissolved oxygen concentration (typically 10 mg/L) of the mixed liquor as a result of high air flows required to completely mix the reactor solids. The elapsed time would likely be more critical for a full scale plant operating at approximately 2 mg/L dissolved oxygen. The stress of oxygen depletion to anaerobic conditions, has been shown to result in a depletion of the ATP sample pool in a culture of aerobic and facultative microorganisms.

6.1.2. Mixed Liquor ATP

The average ATP concentration in this investigation was 0.9535 μg ATP/mg dry cell material. Chapelle and Levin (17)

found a mean ATP pool in 19 bacterial species of 2.09μg ATP/mg dry weight of cell material in the stationary phase. Patterson et al (5) have summarized the results of several investigators and concluded that a mean endogenous ATP pool of 2 μg ATP/mg cell material may be applied intergenerically to bacterial species in pure culture as a means to estimate viable biomass.

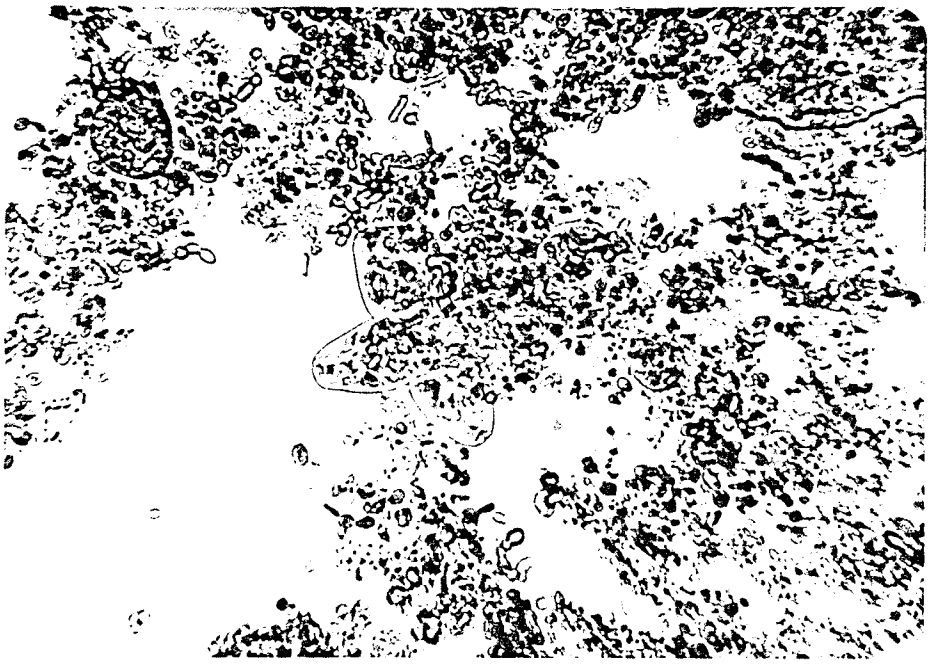
The application of this figure to extended aeration activated sludge (endogenous respiration) assumes that the predominant microfauna in this type of heterogenous culture is of a bacterial nature. This would represent a mean viability in the mixed liquor in this investigation of 47.7%, based on a mean ATP concentration of 0.9535 μg ATP/mg MLVSS. McKinney (33) suggested that the active biomass of the mixed liquor suspended solids, MLSS is 30 to 50% when treating domestic wastewater by the conventional activated sludge process. Similarly the active biomass is suggested to be only 10% of the MLSS in the extended aeration process, operating in the endogenous phase.

Weddle and Jenkins (4) indicated that the active heterotrophic content of activated sludge was 10 to 20% of the MLVSS.

Benefield (33) has indicated that for an activated sludge plant operating in the extended aeration mode the active biomass is approximately 50% of the MLVSS.

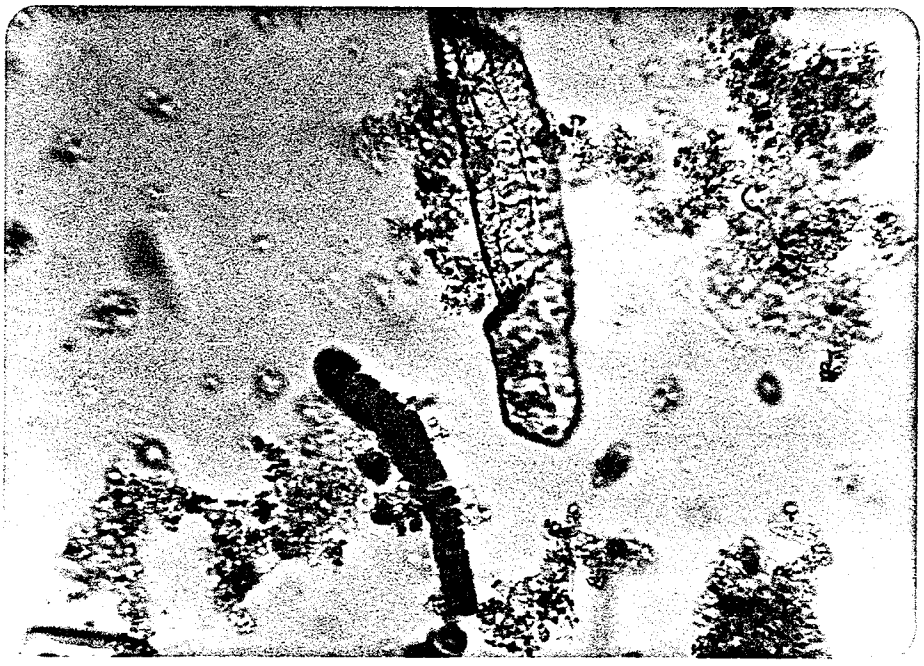
Microscopic examination of the mixed liquor consistantly showed a range in the types of microfauna living in the activated sludge. Photographs 3,4,5 and 6 show typical micrographic observations of the mixed liquor. Visual observations of stalked ciliata

PHOTOGRAPH 3. Mixed Liquor - Protozoa 25X



PHOTOGRAPH 4. Mixed Liquor - Ciliata 25X

PHOTOGRAPH 5. Mixed Liquor - Stalked Ciliata 25X



PHOTOGRAPH 6. Mixed Liquor - Spirogira 25X

showed roughly the same population within the accuracy of random visual examination throughout this investigation. The assumption made in any investigation, using one activity parameter, is that during steady state operation under reasonably "constant" environmental conditions of temperature, available substrate, pH and ionic strength, the overall balance of various species in the whole population remains "constant". This would result in a "constant" ATP concentration per unit dry weight of volatile solids within the reactor. Since ATP responds rapidly to population shifts and the phase of growth (3) and also to toxic effects in activated sludge, ATP would be considered a more meaningful parameter in identifying operational disturbances, with relative speed, as compared to conventional parameter such as VSS.

6.1.3 Raw Domestic Sewage ATP

The average concentration of ATP per dry weight raw sewage suspended solids, RSS, was 0.400 μg ATP/mg RSS. The average concentration of ATP per dry weight raw sewage volatile suspended solids, RVSS, was 0.558 μg ATP/mg RVSS.

The mean viability of the raw domestic sewage, based on the mean ATP concentration of 0.558 μg ATP/mg RVSS, would be estimated at 28%. However, to assume that the viable population in the raw sewage is predominantly bacteria in the endogeneous phase would be misleading since the population in the raw sewage is entirely different from the activated floc. Raw sewage contains aerobic, facultative and anaerobic bacteria, spores and viruses contained within a wide spectrum of discretely different genera.

PHOTOGRAPH 7. Raw Sewage 100X

Several investigators, including D'Eustachio and Levin (41), (5) report that the ATP pool is "constant" throughout all phases of growth. There is, however, sufficient evidence to suggest that the ATP concentration in pure cultures is higher during log growth and stationary growth as compared to the ATP concentration in the endogenous phase. Results of other investigators presented by Patterson et al suggest that growth prior to the endogenous phase may result in an ATP pool at least 6 times higher. The factor when applied to the above viability estimate results in a mean raw sewage viability of only 4.7%. Microscopic examination of the raw sewage feed, as shown in Photograph 7, confirms that a large proportion of the solids consist of non viable debris including viruses and spores and other viable and non viable matter which was beyond the resolution capability of the microscope.

6.1.4. Effluent ATP

The average concentration of ATP per dry weight effluent suspended solids, ESS, was 0.916 µg ATP/mg ESS. The average concentration of ATP, per dry weight effluent volatile suspended solids was 1.120 µg ATP/mg EVSS, was 56%. This is slightly higher than that in the mixed liquor which could be explained by a higher portion of non flocculant, viable organisms, being lost in the effluent.

6.1.5. ATP versus BOD_5, COD and TOC Removal Rates

The ATP concentration, expressed here in terms of µg per mg MLVSS, tended to reflect the removal rates of total organic carbon, (TOC), 5 day biochemical oxygen demand (BOD) and

chemical oxygen demand (COD).

The ATP concentration increased in all cases with an increase in the removal rates as follows:

$$0.1514 \ \frac{\mu g \text{ ATP}}{\text{mg MLVSS}} \text{ per } + 100 \ \frac{\text{mg}}{\text{hr}} \text{ TOC removed}$$

$$0.0691 \ \frac{\mu g \text{ ATP}}{\text{mg MLVSS}} \text{ per } + 100 \ \frac{\text{mg}}{\text{hr}} \text{ BOD removed}$$

$$0.0431 \ \frac{\mu g \text{ ATP}}{\text{mg MLVSS}} \text{ per } + 100 \ \frac{\text{mg}}{\text{hr}} \text{ COD removed}$$

By equating the increased concentrations of ATP for corresponding organic constituent removal rates, a change of 0.10 μg ATP/mg MLVSS would indicate a corresponding change in removal rates as follows:

66 mg TOC/hr

145 mg BOD/hr

232 mg COD/hr

The ATP concentration of the mixed liquor expressed as μg ATP/ml did not significantly reflect changes in the COD removal rate or the BOD_5 removal rate. The ATP in the mixed liquor did reflect, changes in the TOC removal rates to a higher degree than BOD_5 and COD.

The observed changes in ATP were as follows:

-8.93 μg ATP/ml per + 100 mg COD removed/hr

+4.53 μg ATP/ml per + 100 mg BOD_5 removed/hr

+1.82 μg ATP/ml per + 100 mg TOC removed/hr

The concentration of mixed liquor ATP expressed per unit mixed liquor volatile suspended solids showed a general trend of increasing ATP/MLVSS with increasing weight of BOD_5,

COD and TOC removed per hour, Kao et al (32) found that the ATP accumulation (mg/ml) was approximately linearly dependent on or increased proportionally with an increase in glucose consumption in both pure and mixed cultures. However, the ATP accumulation in mixed cultures was relatively less than that for pure cultures.

Nutt and Murphy (2) reported a significant dependency of oxidizable organic material removal rate, expressed as mg TOC removed/hr, with the ATP content, expressed as μg/ml, ATP increasing as the TOC removal rate increased. They also reported a corresponding lack of dependency between conventional parameters, oxygen uptake, MLVSS and dehydrogenase, and the removal rates of organic carbon.

ATP is therefore, considered to have good potential as a sludge activity parameter, reflecting the condition of the sludge. ATP concentrations appear to reflect mixed culture population dynamics, substrate concentration changes and toxic substances.

The results of this investigation reflect the same dependency of the ATP content of the mixed liquor with the substrate concentration. The BOD_5 is a measurement of the dissolved free oxygen used by aerobic microorganisms in the biochemical oxidation of the organic matter, available to the micro-organisms for a specified time and temperature (42). The COD is an oxygen equivalent of the oxidizable organic matter measured by means of a strong chemical oxidizing agent at acid conditions and elevated temperature. The TOC is

measured as the total organic carbon through high temperature combustion. Since BOD_5 measures the actual microbial activity of the micro-organisms and ATP is the mediator of most energy transfer within a cell, one could expect some correlation between BOD_5 and ATP. Also, since BOD_5 primarily measures carbonaceous oxygen demand, one could expect some correlation between TOC and ATP.

Investigations by Chiu et al (3), however, reflect a decrease in ATP content at steady state per unit MLSS with a corresponding increase in COD removal rates per unit MLSS. The least squares fit of the data, from this investigation, expressing ATP content per unit volume versus COD removal rates per unit volume shows a decrease in ATP for a corresponding increase in COD removal rates. This agrees with the theory of Leninger that in conditions of high available substrate the measurable ATP will drop due to a higher ATP turnover and a shift in the ATP-ADP equilibrium towards ADP (10). The scatter of the data points is high and the ATP content per unit volume shows a general increase with respect to BOD_5 and TOC removal rates per unit volume.

There is an apparent contradiction in the same data, that is, increasing ATP concentration per unit weight MLVSS and decreasing ATP per unit volume for corresponding increases in COD removal rate. This could occur with a more significant decrease in dry weight of MLVSS during periods of high substrate removal or the introduction of relatively high quantities of non volatile or fixed suspended solids in the influent

and subsequent loss of VSS in the effluent over a period of time at higher loading rates.

6.1.6. Phosphorous Results

The hydrolysis of a terminal phosphate group from ATP, or ADP and AMP, yields one inorganic orthophosphate molecule. Orthophosphates are, in turn, available for biological metabolism without further breakdown (42).

The mixed liquor orthophosphate content (mg/L) showed a general trend of increase with corresponding increase in the mixed liquor ATP (μg/L). This would be expected with a higher concentration of ATP.

6.1.7. Oxygen Uptake Rate

Oxygen as the final electron acceptor in the aerobic metabolic chain may be expected to show some correlation to the mixed liquor ATP, that is, an increase in oxygen uptake rates with an increase in the ATP concentration. The results of this investigation show no apparent correlation between the oxygen uptake rate and the ATP content or the ATP concentration with respect to MLVSS. This aspect was not investigated in detail, however, and the results are inconclusive due to the paucity of data points.

6.1.8. Sludge Age Results

The sludge volume index, SVI, is an empirical measurement defined as the volume in milliliters occupied by one gram of activated sludge mixed liquor solids, dry weight, after

settling for 30 minutes in a one litre graduated cylinder, (42). The activated sludge in this investigation exhibited "good" settling characteristics operating at $4^{\pm}$ .5°C, with a measured SVI of typically 90 to 170. The ATP content of the mixed liquor did not appear to have any definite dependency on the SVI. The ATP content did appear to decrease with increasing SVI which would be expected as a corresponding reduction in viable matter with a decrease in the settleability of the floc.

The ATP:MLVSS ratio decreased at a mean rate of 0.022 μg/ATP/mg MLVSS/day of operation which represents an accumulation of non viable volatile solids of approximately 2.31%/day. The ATP:MLSS ratio decreased at a mean rate of 0.019μg ATP/mg MLSS/day of operation. This indicates that there is an apparent continuous buildup of inert volatile suspended solids in the aeration basin, possibly unassimible polysaccharide cell wall material and similar cell debris. Middlebrooks and Garland (7) have stated that the accumulation of biologically inert solids must be considered in growth kinetics when the death rate is significant, however, this factor is traditionally considered to be negligible in model studies.

The decreasing concentration of ATP with respect to MLSS must at some point reach equilibrium. It is possible that this is the ultimate state where activated sludge plants experience excessive solids loss in the effluent, a function which is largely unpredictable.

Continued operation of the plant and daily wasting of mixed liquor from the aeration basin resulted in a mean cell residence time of 22 days, as compared to an average of 65 days during the initial phase of this investigation. The results indicated a gradual increase in the ATP concentration with respect to MLVSS and MLSS.

The mean cell residence time is defined by Jenkins and Garrison (43) as the mean residence time of an activated sludge particle in the system in terms of MLVSS concentration and effluent VSS and waste sludge flow. They concede that the use of VSS has limitations due to the inclusion of viable and non viable matter which may introduce serious errors in kinetic predictions, particularly at high rates of operation. Goodman and Englande (33) proposed that the percentage of MLVSS that is active, or viable, decreases with increasing sludge age. The relationship of percentage active mass to sludge age is shown in Figure 30. ATP measurements may prove to be of value in controlling the activated sludge process by providing a correction factor or in fact a substitute viability parameter in kinetic constant determinations.

Final operation of the plant during this investigation with no feed shows a gradual decrease in the mixed liquor ATP concentration per unit dry weight MLVSS and MLSS during the death phase.

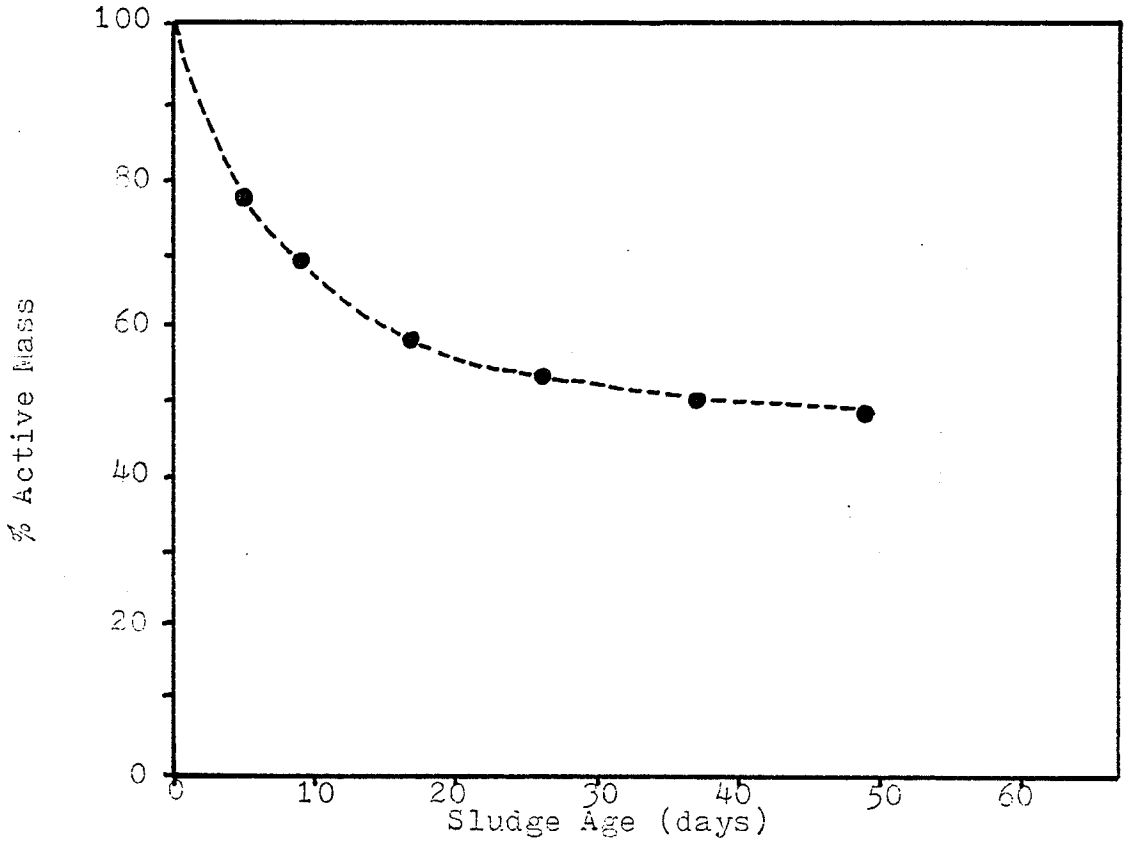


Figure 30. RELATIONSHIP BETWEEN PERCENT ACTIVE MASS AND SLUDGE AGE
(from Benefield et al. 33)

6.1.9. Miscellaneous Test Results

The pH or the negative logarithm of the hydrogen ion concentration of the raw sewage was fairly constant throughout the investigation. The pH of the mixed liquor (and effluent) was, also, fairly constant, however, somewhat higher than the influent. This was likely due to the agitation of the mixed liquor through air addition required for mixing and resultant air stripping of the metabolic by-product CO_2.

Turbidity is caused by a variety of suspended matter ranging in size from colloidal to coarse dispersions (44). The

turbidity of the effluent showed that good clarification was produced by the clarification chamber in the model plant. There was, however, a short period during the investigation where the solids overflow rose. This was rectified by reducing the air flow into the reactor while maintaining complete mixing conditions. The ATP concentration in the effluent during periods of high turbidity showed a slight increase with corresponding increases in SS and VSS.

The conductivity of a solution is a measure of its ability to carry an electrical current and it varies with the number and type of ions in solution (44). Since the ionic composition of a solution affects light emission in the firefly luciferin-luciferase reaction, conductivity measurements were recorded for control purposes. No significant variation in the influent conductivity was recorded so that any relative affects on the samples tested is considered to be consistent throughout this investigation. Patterson et al, also indicated that the ionic strength of "normal activated sludge" is sufficiently low so that inhibitory effects are unimportant at 1:25 dilutions used in their investigations.

The relative concentration of mixed liquor ATP with respect to mixed liquor organic nitrogen was not continuously monitored. Investigations by Symons and McKinney (1958), reported by Weddle and Jenkins (4), showed that the nitrogen content of activated sludge could vary between 1.5 and 9.7 per cent VSS depending on the availability of nitrogen. The ATP

measurements did reflect a linear dependence on the organic nitrogen content of the activated sludge, however, the number of data points produce inconclusive evidence.

6.2. Biokinetic Interpretation Results

6.2.1. Kinetic Growth and Substrate Utilization Results

The kinetic growth constants, that is the growth yield, Y, and the microbial decay coefficient, kd, were calculated using the system energy which was assumed to be directly proportional to the system viability. The system examined was determined to be operating in the endogenous phase of growth, by definition, and operating at steady state, indicated by consistant BOD_5 removal efficiency. The yield and decay coefficients determined using ATP did not demonstrate any specific trends with respect to the average influent BOD_5 with an average Y ATP of 0.470 μg ATP/mg BOD_5 removed. Similarly the ATP yield per unit dry weight of solids produced did not show any specific trend with respect to the average influent BOD_5 with an average of 0.993 μg ATP/mg VSS produced.

The decay coefficient expressed as weight ATP per unit time showed no specific trends with an average kd of $1.247/10^3$ hr. The significance of the data points on the growth constant curves using ATP and using MLVSS were both highly significant. The mean Y using MLVSS was 0.580 wt VSS/wt BOD_5 and the mean kd using MLVSS was $1.207/10^3$hr.

The substrate utilization constants were determined using both ATP and MLVSS. The ATP data showed that the average K_s, or substrate concentration when the rate of energy utilization is 1/2 the maximum, is 5.72 mg/L BOD_5. The average specific growth rate, μ max, from the ATP data is $2.35/10^3$hr. The average specific growth rate, μ max, from the MLVSS data is $2.005/10^3$hr and Ks using VSS was 4.068 mg/L.

The units of the growth constants using ATP versus VSS are not similar therefore, direct comparisons of the values obtained are not valid. However, if the figures estimating the mean viability of the mixed liquor and effluent, by means of ATP correlation to VSS, are inserted as correction factors into the growth constant equations some projections may be made.

Inserting .477 MLVSS and .560 Eff VSS into the equations, representing the fraction of viability the following results:

	Avg. Calculated Value (VSS)	Corrected for % viability
Y	.580	1.036
kd	1.207	0.322
μ max	2.005	1.391
K_s	4.068	8.445

Although the cell yield constant, Y, cannot exceed 1.0 the corrected constant does indicate that the average calculated value using VSS may be low by assuming all volatile solids are viable. This may also indicate the effects of

inherent errors produced by the assumption that the predominant microfauna are bacteria to estimate viability in terms of ATP content per unit weight of cell material (refer for 6.1.2.). This indicates that present designs may underestimate excess sludge production. An offsetting value which is, also, not included in this approach to kinetic constant evaluation is the viability of the raw sewage, however, this is not considered to be significant.

The decay rate and the specific growth rate constants corrected for viability were both less than the calculated values. This indicates that the method used may be over conservative. Similarly the calculated K_s constant is low when compared to the adjusted K_s constant.

These comparative growth constants indicate that the growth equations could be reevaluated to include a factor for viability of the mixed liquor and effluent. The resulting growth constants may provide a more accurate means of designing and operating full scale biological waste treatment systems. The basic assumption in this approach is that by determining growth and substrate utilization kinetics utilizing ATP provides a more accurate means of measuring the system dynamics by providing a more accurate means of measuring the system viability or system energy, as compared to traditional VSS measurements.

7. CONCLUSIONS

The following are the conclusions of this laboratory extended aeration study under controlled substrate feed and temperature, conducted at 4 ± .5° C.

1) The average ATP concentration of raw sewage, mixed liquor and effluent were 0.558, 0.9535, and 1.120 μg ATP/mg VSS, respectively.

2) Based on a mean endogenous ATP concentration in a mixed culture of 2 μg ATP/mg cell material, the average per cent viability of the mixed liquor and effluent were 47.7% and 56%, respectively.

3) The mixed liquor ATP concentration with respect to MLVSS and MLSS decreased with time at a mean rate of 0.022 μg ATP/mg MLVSS/day and 0.019 μg ATP.mg MLSS/day. A decrease in the sludge age resulted in a trend of increased ATP/unit dry weight VSS and SS.

4) The mean ATP pool variation and ATP concentration variation with respect to substrate removal rates are shown in Table 9.

ATP pool μg/ml	ATP concentration μg/mg MLVSS	per+100 mg removed/hr
-8.93	.0431	COD
4.53	.0691	BOD_5
1.82	.1514	TOC

TABLE 9 ATP VERSUS SUBSTRATE REMOVAL RATES

5) The average kinetic growth and substrate utilization rate constants determined using ATP and VSS are shown in Table 10.

	Y	kd	μmax	K_s
ATP	0.470 (μg ATP/mg BOD_5)	1.247 ($\times 10^{-3}$/hr)	2.35 ($\times 10^{-3}$/hr)	5.72 (mg/L)
VSS	0.580 (mg VSS/mg BOD_5)	1.207 ($\times 10^{-3}$/hr)	2.005 ($\times 10^{-3}$/hr)	4.068 (mg/L)

TABLE 10

KINETIC GROWTH AND SUBSTRATE UTILIZATION RATE CONSTANTS

6) ATP is a non conservative indicator of biomass viability. The ATP test is rapid and provides potential means to control biological treatment systems through direct measurement of the sludge condition.

8. FUTURE WORK

1) The effects of sludge age on the ATP pool, at controlled intervals, should be further investigated by running identical models simultaneously to better understand the characteristic ATP pool responses to varying sludge conditions.

2) The ATP in various biological treatment systems should be investigated through a range of controlled environmental conditions to examine the effects of, for example, temperature on the reactor ATP pool.

3) The ATP pool in different types of biological waste treatment systems and various substrates should be investigated to establish characteristic ATP levels.

4) Activated sludge mixed liquor wash-out due to high surcharging on existing systems has created problems such as a lag in treatment efficiency. Adenosine Triphosphate measurements should be examined in absolute amounts and in relative amounts, with respect to traditional viability indicator parameters, to determine the effects of wash-out on the mixed liquor ATP pool and mixed liquor recovery.

5) The ATP in raw sewage and mixed liquor should be monitored and evaluated in terms of activated sludge plant efficiency control by means of adjustment of the mixed liquor ATP. This approach should be guided at the ultimate goal of total automated plant control by the use of automatic ATP monitoring set up to operate as the control centre for all plant functions. For example, a sudden drop in the ATP pool of raw sewage may indicate the entry of toxic wastes into the

sewage.

6) Monitoring the ATP and the total adenosine nucleotide concentration in activated sludge to investigate the effects of organic feed rates on the equilibrium shift in the ATP-ADP-AMP concentrations.

The loss of solids in the effluent of continuously fed activated sludge plants is difficult to control so that the ideal steady state operation cannot be easily controlled. Research is currently in progress at the University of Manitoba in which the ATP levels are being monitored and investigated in a batch feed system.

9. ACKNOWLEDGEMENTS

I gratefully acknowledge the advise, guidance and support of my principal advisor, Dr. Brian Topnik of the Department of Sanitary Engineering. I, also, thank Dr. Arthur Sparling and Dr. Gregory Blank, members of my examining committee for their effort and interest.

Thanks as well to Judy who willingly helped haul the sewage for this study. I also acknowledge with thanks, my sister, Mary for reasonable typing rates, Teresa for typing the final draft and Cathy, for correcting the draft.

A special thanks to my wife Carol, for all the typing and listening she did for me. I've never seen anyone more interested in this subject.

10. APPENDICES

APPENDIX A

LABORATORY EXTENDED AERATION UNIT

Aeration Tank - 7.875" x 18.25" x 8.375"

or 20 cm x 46.4 xm x 21.3 cm

Volume - 19.77 L Clairifier Volume - 5 L

Extended Aeration Parameters	F/M	0.05	—	0.20
	V/Q	24	—	36 hrs.
	MLSS	3500	—	5000^{+}
	SRT	20	—	30 days
	Vol. loading	15	—	25 lbs BOD_5/1000 $ft.^3$
	%Eff BOD_5	75	—	85%
	R/Q sludge recycle	1.0	—	2.0

Extended Aeration Parameters (typical) model	FM	0.11
	V/Q	24 hrs.
	MLSS	3200
waste (.15L/day + eff SS)	SRT	64.6 days
	Vol. loading	22
	% Eff BOD_5	91.4%

R/Q = Sludge recirculation flow/raw flow into system

Total solids in reactor = (19.77L) (3200mg/L) = 63264 mg

SRT = (63264 mg) / (.15L) (3200) + (20L) (25 mg)

= 63264 / 480 + 500 = 64.6 days

for SRT - 30 days req. waste - 500 ml ML

Avg. BOD_5 loading = (350 mg/L) (20L) = 7000 mg/day

= (.007 lg) (2.2046 lbs/kg) = 0.154 lbs/day

Vol. = 19.77L or 19.77/28.31605 = .698ft^3 = .000698 x 1000 ft^3

Vol. loading = .0154/.000698 = 22.06 lbs BOD_5/1000 ft^3

% Eff BOD_5 = (350-30) / 350 = 91.4%

F/M = (350 mg/L) / (3200 mg/L) = .11

APPENDIX B

BIOLUMINESCENCE DETECTION - MODEL 3000 INTEGRATING PHOTOMETER

Manufactured by SAI Technology Co. (18)

The Integrating Photometer is a laboratory instrument designed to aid the user in studying aspects of many light emitting reactions. Specifically the instrument was designed for the measurements of extremely low concentrations of adenosine triphosphate (ATP) for microbial biomass determinations in aquatic environments. In addition, the Photometer can be used for many chemiluminescent and bioluminescent reactions and virtually any light emitting reaction system.

ATP

The quantitative determination of ATP by bioluminescence is dependent upon: (a) firefly luciferase enzyme being absolutely specific for ATP, (b) the rate of the reaction being directly proportional to the concentration of ATP, assuming that other reactants are in excess, and (c) the emission of one photon of light for each molecule of ATP which is hydrolyzed. The overall reaction is shown by:

$$\text{Luciferin (reduced)} + \text{ATP} + O_2 \xrightarrow[\text{LUCIFERASE}]{Mg^{++}} \text{luciferin (oxidized)} + PP_i + \text{AMP} + H_2O + h\gamma$$

When a sample containing ATP is injected into the enxyme preparation, there is an immediate burst of light in the range of 560 to 580 μm. The intensity of the light declines in a semi-exponential fashion as shown in Figure 31. The area under this curve is proportional to the amount of ATP in the

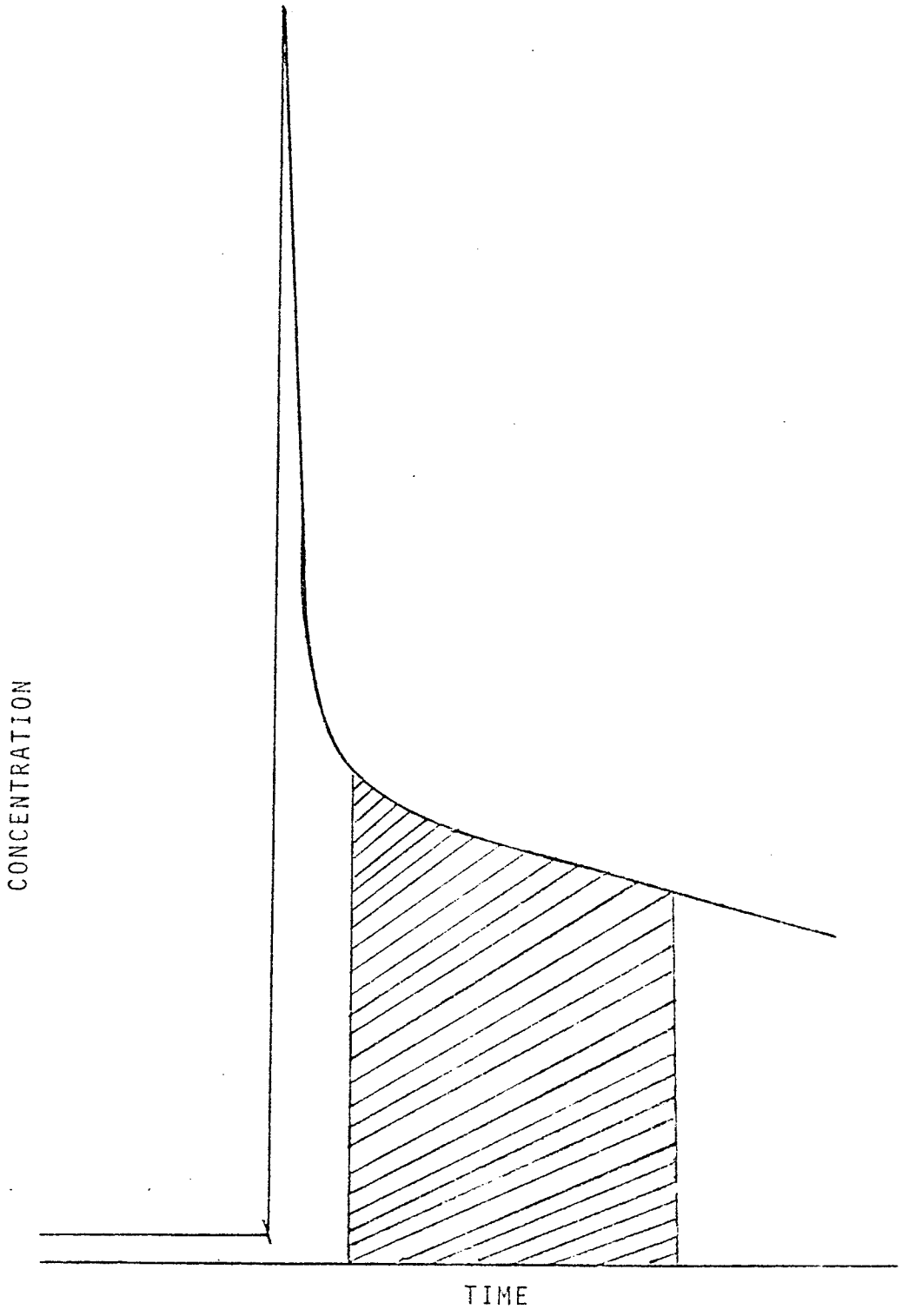


Figure 31. Light emission curve when a sample containing ATP is injected into the luciferin-luciferase enzyme preparation.

sample.

When the enzyme is placed in the sample chamber after introduction of ATP, light strikes the photocathode of the phototube, liberating electrons which cascade through the photomultiplier to produce a current proportional to the light intensity of the sample. This current is converted and amplified to produce a proportional voltage (see Figure 32). This voltage is fed into a voltage to frequency converter where a pulse train is produced with a frequency proportional to the unput voltage.

This pulse train is prescaled for increased accuracy and gated by a precision timer. This precision timer allows the pulse train to pass to a counter where it is accumulated for an adjustable or preset interval. At the end of the interval the count displayed represents the integral

$$C \int_{t_1}^{t_2} I_\nu \, dt = \text{displayed counts}$$

where C is a constant determined by a combination of the high voltage applied to the phototube (sensitivity control), phototube sensitivity, and optical geometry. I is the intensity of the light emitted from the sample and t_1 and t_2 represent the initial and final times respectively of the integration period.

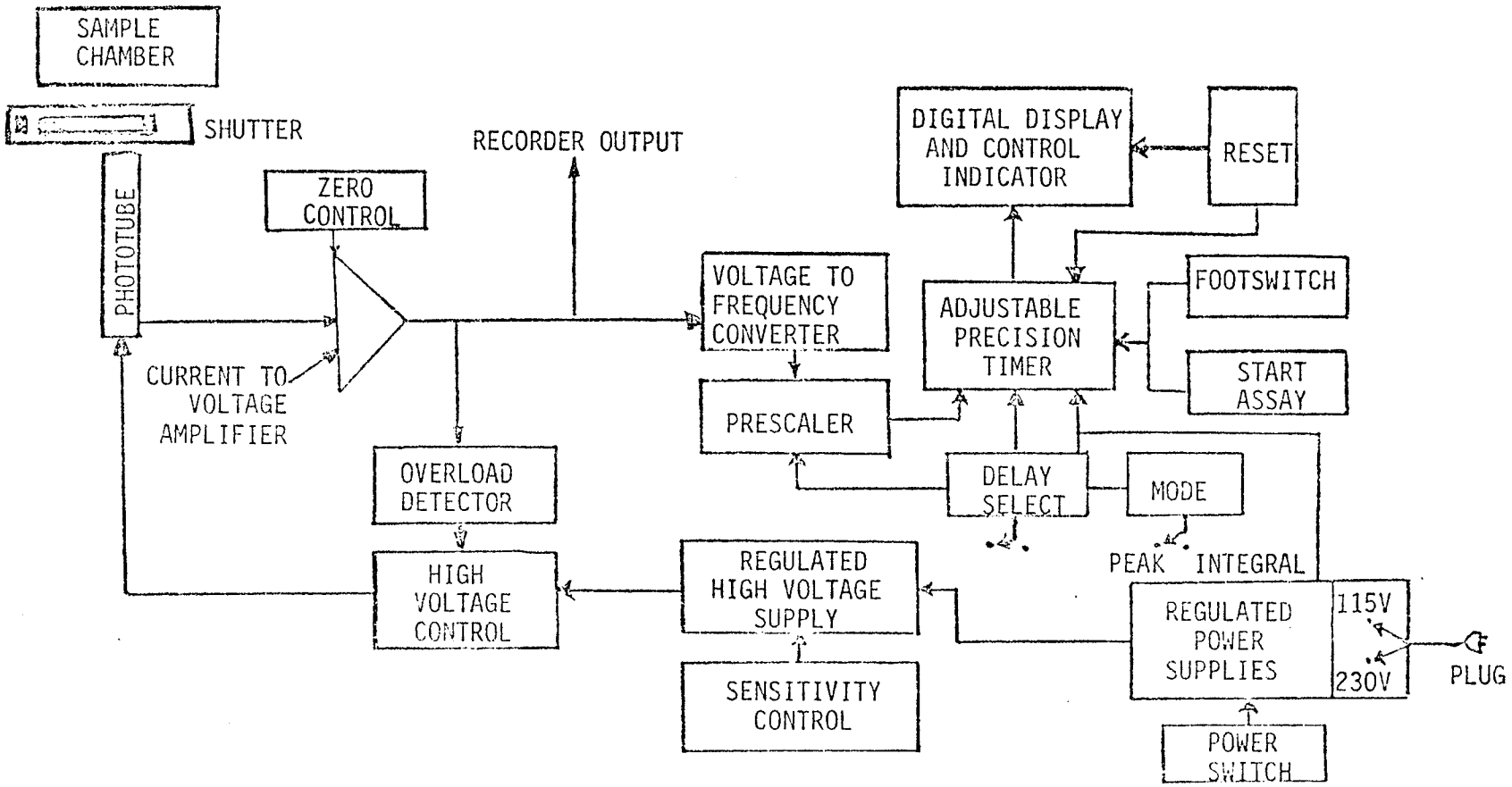


Figure 32. Block diagram of ATP Photometer.

APPENDIX C

EXAMPLE CALCULATIONS OF BIOKINETIC CONSTANTS

The biokinetic constants calculated were:

Y = growth yield

kd = endogenous decay rate constant

μmax = maximum substrate utilization rate

K_s = half velocity coefficient

r = correlation coefficient

t = student's t level of significance

Section 3.3.1. included the following straight line mass balance equations from Middlebrooks and Garland used to determine the growth and substrate utilization constants for the steady state extended aeration process:

$$\frac{(kd)}{(Y)}\, b^1\theta + \frac{1}{Y} = \frac{b^1\,(S_0-S_1)}{X_1}$$

where $\frac{kd}{Y}$ = slope

$\frac{1}{Y}$ = intercept

$$\frac{b^1\theta}{1+b^1\theta kd} = \frac{(K_s)}{(\mu max)}\,\frac{1}{S_1} + \frac{1}{\mu max}$$

where $\frac{K_s}{\mu max}$ = slope

and $\frac{1}{\mu max}$ = intercept

The kinetic growth and substrate utilization constants were determined using the data where the BOD_5 of the feed, S_o, was in the range of 100 - 200 mg/L, 200 - 300 mg/L,

300 - 400 mg/L, 300 - 400 mg/L and 400 - 500 mg/L.

Using the case where the BOD_5 range was 200 - 300 mg/L, the viability indicator expressed as concentrations of ATP, μg/ml, was substituted into the equation in the X terms. (In addition, for comparison, the viability indicator expressed as VSS was used in the equation. The calculation is the same and is not included here). The BOD_5 for the feed and effluent and the ATP for the mixed liquor and effluent are shown in Table 11.

TABLE 11

BOD_5 AND ATP DATA FOR
Kinetic Constants

Date	Feed BOD_5 (S_o) mg/L	Effluent BOD_5 (S_1) mg/L	ML ATP μg/ml (X_1)	Eff. ATP μg/ml (X_2)
Jan. 15	290	21	2.52	.025
22	270	23	2.96	.029
26	288	47	2.62	.044
28	295	24	2.83	.044
30	283	42	2.79	.033
Feb. 1	230	26	2.67	.027
2	258	23	2.71	.029
9	248	76	2.04	.055
10	233	36	1.70	.034
11	280	39	2.52	.051
12	238	34	2.59	.053
13	225	19	2.65	.043
17	253	55	3.08	.045

TABLE 11 (cont)

Date	Feed BOD_5 (S_o) mg/L	Effluent BOD_5 (S_1) mg/L	ML ATP μg/ml (X_1)	EFF. ATP μg/ml (X_2)
Feb. 19	230	33	2.65	.034
21	295	34	2.56	.036
23	255	25		
24	228	20	1.73	.019
25	243	29	1.71	.027
28	295	32	1.90	.030
Mar. 2	248	41	1.89	.127
7	290	26	1.78	.035

The parameters required for plotting the kinetic growth equation are shown in Table 12.

TABLE 12

KINETIC GROWTH EQUATION DATA

Date	$b^1(X_1/X_2)$	θ(hrs) V/F	$b^1\theta \times 10^3$ (hrs)	X_1 (μg/ml)	S_0-S_1 (mg/L)	$\frac{b^1(S_0S_1)}{X_1}$
Jan. 15	100.80	23.8	2.399	2.52	269	10760
22	102.07	25.3	2.582	2.96	247	8517
26	59.55	23.8	1.417	2.62	241	5478
28	63.32	23.3	1.970	2.83	271	6064
30	84.55	23.0	1.945	2.79	241	7303
Feb. 1	98.89	27.1	2.680	2.67	204	7556
2	93.45	23.5	2.196	2.71	234	8069
9	37.09	26.4	0.979	2.04	172	3127

TABLE 12 (cont)

Date	b^1 (X_1/X_2)	θ(hrs) V/F	$b^1\theta x10^3$ (hrs)	X_1 (μg/ml)	S_0-S (mg/L)	$\frac{b^1(S_0S_1)}{X_1}$
Feb. 10	50.00	25.7	1.285	1.70	197	5794
11	49.41	23.5	1.161	2.52	241	4725
12	48.87	31.9	1.559	2.59	204	3849
13	61.63	19.6	1.208	2.65	206	4791
17	68.44	50.7	3.470	3.08	198	4400
19	77.94	25.3	1.972	2.65	197	5794
21	71.11	27.8	1.977	2.56	261	7250
23		61.8			230	
24	91.05	26.7	2.431	1.73	208	10947
25	63.33	22.5	1.425	1.71	214	7926
28	63.33	31.4	1.989	1.90	263	8766
Mar. 2	14.88	23.0	0.342	1.89	207	1630
7	50.86	31.9	1.622	1.78	264	7543

The plotted growth equation is shown in Figure 33 using the least squares best fit. From the "best fit" straight line:

$$\text{intercept} = \frac{1}{Y} = 2.976$$

$$\text{slope} = \frac{kd}{Y} = 1.979$$

...Therefore:

$$Y = 0.336\ \mu g\ ATP/mg\ BOD_5$$

$$kd = 0.665 \times 10^{-3}/hr$$

The calculation of the substrate utilization curve are shown in Table 13 and illustrated in Figure 34.

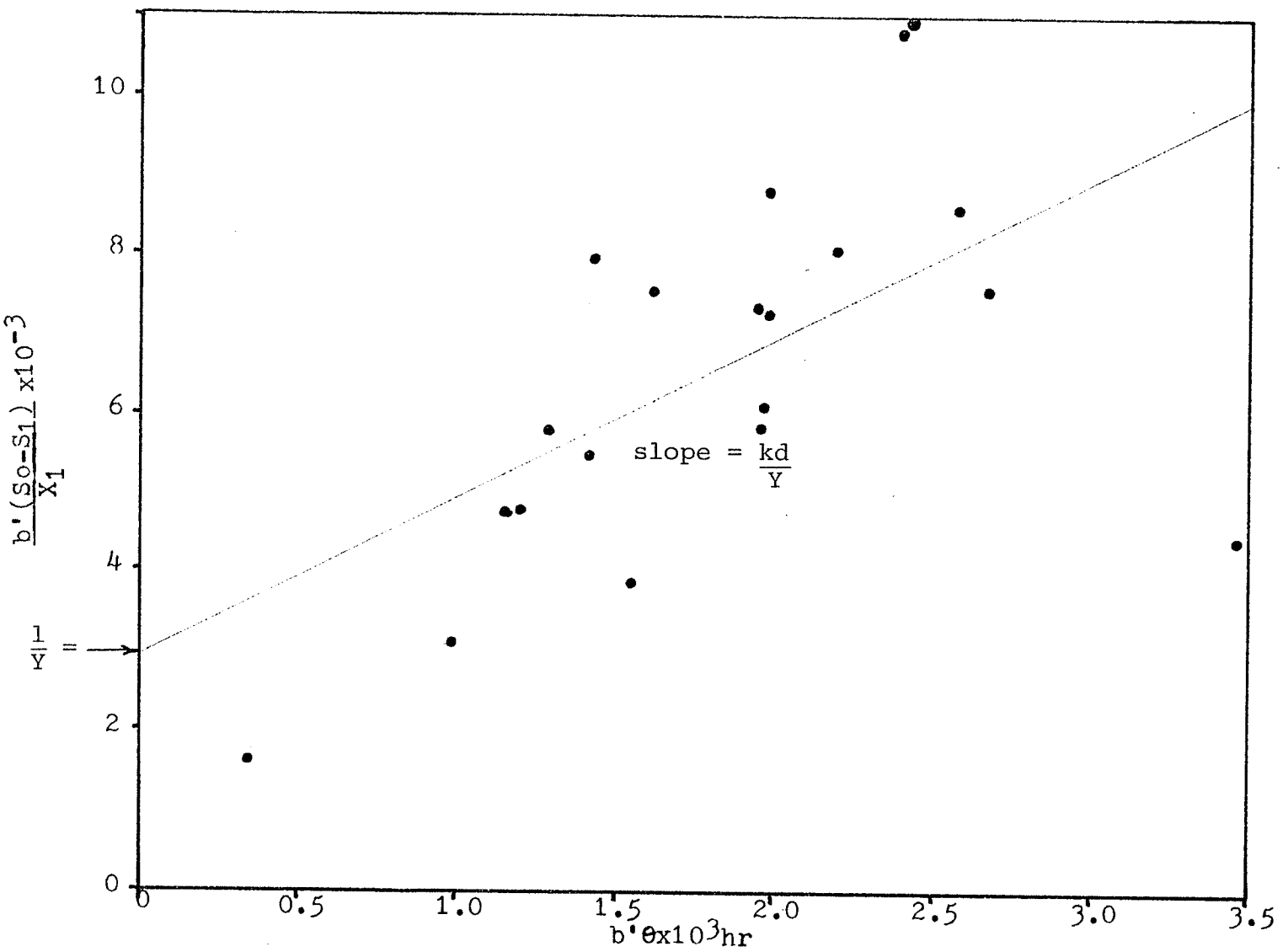


Figure 33. KINETIC GROWTH EQUATION CURVE

TABLE 13

SUBSTRATE UTILIZATION RATE EQUATION DATA

Date	$b^1\theta x10^3$ (hrs)	$kdx10^3/hr$	$b^1\theta kd$	$\frac{b^1\theta_1}{1+b\ \theta kdx10^3}$	S_1	$\frac{1}{S_1}$
Jan. 15	2.399	0.665	1.5953	0.924	21	0.0476
22	2.582	0.665	1.7170	0.950	23	0.0435
26	1.417	0.665	0.9423	0.730	47	0.0213
28	1.970	0.665	1.3101	0.853	24	0.0417
30	1.945	0.665	1.2934	0.848	42	0.0238
Feb. 1	2.680	0.665	1.7822	0.963	26	0.0385
2	2.196	0.665	1.4603	0.893	24	0.0417
9	0.979	0.665	0.6510	0.593	76	0.0132
10	1.285	0.665	0.8545	0.693	36	0.0278
11	1.161	0.665	0.7721	0.755	39	0.0256
12	1.559	0.665	1.0367	0.765	34	0.0294
13	1.208	0.665	0.8033	0.670	19	0.0526
17	3.470	0.665	2.3076	1.049	55	0.0182
19	1.972	0.665	1.3114	0.853	33	0.0303
21	1.977	0.665	1.3147	0.854	34	0.0294
23					25	0.0400
24	2.431	0.665	1.6166	0.929	20	0.0500
25	1.425	0.665	0.9476	0.732	29	0.0345
28	1.989	0.665	1.3227	0.853	32	0.0313
Mar. 2	0.342	0.665	0.2274	0.279	41	0.0244
7	1.622	0.665	1.0786	0.780	26	0.0385

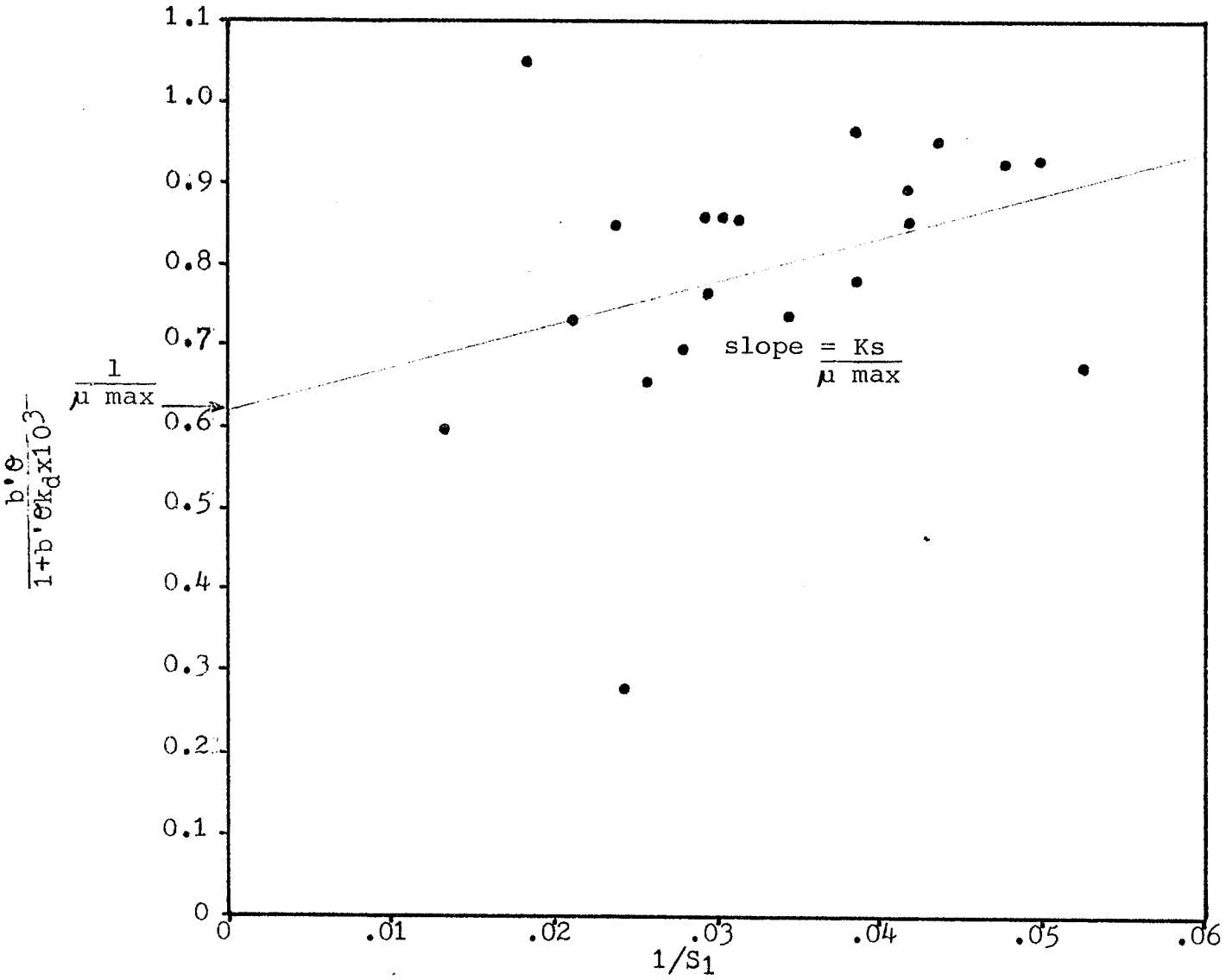


Figure 34. KINETIC SUBSTRATE UTILIZATION CURVE

From the "best fit" straight line:

$$\text{intercept} = \frac{1}{\mu max} = 0.621$$

$$\text{slope} = \frac{K_s}{\mu max} =$$

Therefore: $\mu max = 1.61$

$$K_s = 3.23$$

The calculation of the correlation coefficient and the student's t level of significance was calculated and evaluated according to the formulae given by Moroney, as used by Topnik (9), where:

$$\text{the correlation coefficient} = r = \frac{(\frac{1}{N} \Sigma xy) - \bar{x}\,\bar{y}}{S \times Sy}$$

$$\text{where } Sx = (\frac{1}{N})\ (\Sigma x^2) - \bar{x}^2$$

$$Sy = (\frac{1}{N})\ (\Sigma y^2) - \bar{y}^2$$

N = number of samples and

$$\text{the student's } t = \frac{r\ N - 2}{1 - r^2}$$

Substitution into the growth rate least squares straight line results in;

$r = 0.565$

$t = 3.53$ which indicates that the sample group is significant to a level of confidence of approximately 99.8%. Substitution into the growth yield least squares straight line results in:

$r = -.294$

$t = -1.305$ which indicates that the sample group is significant to a level of confidence of approximately 89.5%.

11. BIBLIOGRAPHY

1. Murphy, K.L., "Status of Biological Waste Treatment in Canada", Engineering Journal, Dec., P.6, (1979)

2. Nutt, S.G. and Murphy, K.L., "Parameters for Measuring the Activity and Viability of Activated Sludge", Proc. 9th Canadian Symp. Water Poll. Res. Canada, Vol. 9 p. 235, (1974).

3. Chiu, S.Y., Kao, O.C., Erickson, L.E., and Fan, L.T., "ATP pools in Activated Sludge", Jour. Water Poll. Control Fed., Vol. 45, No. 8, p. 1746, Aug., (1973).

4. Weddle, C.L. and Jenkins, D., " The Viability and Activity of Activated Sludge", Water Research, Vol. 5, p. 621, (1971).

5. Patterson, J.W., Brezonik, P.L. and Putnam, H.D., "Measurement and Significance of Adenosine Triphosphate in Activated Sludge", Envir. Science and Technology, Vol. 4, No. 7, P. 569, July, (1970).

6. McKinney, R.E., Microbiology for Sanitary Engineers, McGraw-Hill Book Co. Inc., New York, N.Y., (1962).

7. Middlebrooks, E.J. and Garland, C.F., "Kinetics of Model and Field Extended-Aeration Wastewater Treatment Units", Jour. Water Poll. Control Fed., Vol. 40, No. 4 P. 586, Apr., (1968).

8. Nutt, S.G. " The Activity and Viability of Activated Sludge- A Preliminary Investigation of Parameters", Master of Eng. Thesis, McMaster Univ.

9. Topnik, B.H., "Biokinetics of Low Temperature Waste Assimilation", Ph.D. Thesis, Univ. of Manitoba, Oct. (1976).

10. Lehninger, A.L., Biochemistry, Worth Publishers Inc., New York, N.Y. (1970).

11. Swanson, C.P., The Cell, 2nd Ed. Prentice-Hall, Inc. New Jersey, (1965).

12. McElroy, W.D., Cell Physiology and Biochemistry, Prentice Hall Inc. New Jersey, 2nd Ed. (1965).

13. Stanier, R.Y. Doudoroff, M. and Adelberg, E.A., The Microbial World, 3rd Ed. Prentice Hall N.J. 1970.

14. Qureshi, A.A. and Patel, J., "Adenosine Triphosphate (ATP) Levels in Microbial Cultures and a Review of the ATP Biomass Estimation Technique", Scientific Series No. 63, Envir. Canada, Inland Waters Directorate, (1976).

15. Dearborn Environmental Consulting Services, "The Use of the ATP Assay for Rapid Assessment of Water Quality", Tech. Bull. No. 109, Fisheries and Envir. Canada, Inland Water Directorate, (1979).

16. Glass, G.E., Bioassay Techniques and Environmentsl Chemistry, EPA, Ann Arbor Science Publishers Inc., (1973).

17. Chappelle, E.W. and Levin, G.V., "Use of the Firefly Bioluminescent Reaction for Rapid Detection and Counting of Bacteria", Biochem. Med., Vol. 2, p. 41 (1968)

18. SAI Technology Co., "Model 3000 Integrating Photometer Instruction Manual", March 1975.

19. Rodes, W.C. and McElroy, W.D., Jour. Biol. Chem. Vol. 223, P. 1528, (1958), from ref. (5).

20. McElroy, W.D. and Strehler, B.L. Arch. Biochem. Biophys, Vol. 22, p 420, (1949), from ref. (5).

21. Southwest Missouri State College, "DNA Concentration as an Estimate of Sludge Biomass", Proj. No. 17070 DHO, E.P.A., Wash., D.C. Feb., (1971).

22. Blok, J., "Measurements of the Viable Biomass Concentration in Activated sludge Respirometric Techniques," Water Research, Vol. 10, P. 919, (1976).

23. Kotze, J.P., "Methods for the Determination of Intermediary Enzymes in Mixed Cultures Used for the Purification of organic Polluted Waters", Water Research, Vol. 1, P. 351, (1967).

24. Brezonik, P.L. and Patterson, J.W., "Activated Sludge ATP: Jour. of the San. Eng. Div., ASCE, Vol. 97 No. SA6, P. 813 Dec., (1972)

25. Cairns, J.E., Nutt, S.G., Afghan, B.K. and Carlisle, D.B., "Application of the ATP Assay for Monitoring the Disenfection of Wastewater Streams", Scientific Series No. 108, Envir. Canada, Inland Waters Directorate, (1979).

26. Upadhyaya, A.K. and Eckenfelder Jr., W.W., "Biodegradable Fraction as an Activity Parameter of Activated Sludge", Water Research, Vol. 9, P. 691, (1975).

27. Hamilton, R.D. and Holm-Hansen, O., "Adenosine Triphosphate Content of Marine Bacteria", Limnol, Oceanog., Vol. 12, P. 319, (1967).

28. Gadkari, D. and Stolp, H., "Energy Metabolism of Bdellovibrio bacteriovorus, Arch. Microbial., Vol. 102, No. 3, P. 179, (1975).

29. Forrest, W.W., "Adenosine Triphosphate Pool During the Growth Cycle in Steptoccus faecalis", Jour. of Bacteriology, Vol. 90, No. 4, P. 1013, Oct. (1975).

30. Strange, R.E., Wade, H.E. and Dark, F.A., "Effect of Starvation on Adenosine Triphosphate Concentration in Aerobactor Arogenes", Nature, Vol. 199, No. 4888, P. 55, July, (1963).

31. Knowles, C.N. and Smith, L., "Measurements of ATP Levels of Intact Azotobacter Vinelandii Under Different Conditions", Biochem. Biophys, Acta. Vol. 197, P. 152, (1970).

32. Kao, O.C., Chiu, S.Y. Fan, L.T. and Erickson, L.E., "ATP Pools in Pure and Mixed Cultures", Jour. Water Poll. Control Fed., Vol. 45, No. 5, P. 926, May (1973).

33. Benefield, L. Lawrence, D. and Randall, C., "The effect of sludge viability on biokinetic coefficient evaluation", Jour. Water Poll. Control Fed., Vol 51, No. 1, p. 187, Jan. (1979).

34. Schroeder, E.D. and Busch, A.W., "Validity of Energy Change as a Growth Parameter", Jour. of the San. Eng. Div., ASCE, Vol. 94, No. SA2, P. 193, Apr., (1968).

35. Stouthamer, A.H. and Bettenhaussen, C.W., "Determination of the Efficiency of Oxidative Phosphorylation in Continuous Cultures of Aerobacter aerogenes:, Arch. Microbial., Vol. 102, No. 3 P. 187 (1975).

36. Great Lakes - Upper Mississippi River Board of State Sanitary Engineers, "Recommended Standards for Sewage Works, "Health Education Service, P.O. Box, 7283, Albany, N.Y. 12224, (1971).

37. Dhople, A.M. and Hanks, J.H., "Quantitative Extraction of Adenosine Triphosphate from Cultivable and Host-Grown Microbes: Calculation of Adenosine Triphosphate Pools", Applied Microbiology, Vo. 26, No. 3 p. 399, Sept. (1973).

38. Holm-Hansen, o. and Booth, C.R., "The measurement of Adenosine Triphosphate in the Ocean and Its Ecological Significance", Limnol, Oceanog., Vol. 11, P. 510, (1966).

39. APHA, Standard Methods for the Examination of Water and Wastewater, 14th Ed., (1975).

40. D'Eustachio, A.J. and Johnson, D.R., "Adenosine Triphosphate Content of Bacteria", Fed. Proc. Vol. 27, P. 761 (1968).

41. D'Eustachio, A.J. and Levin, E.I., "Levels of Adenosine Triphosphate During Bacterial Growth", Bacterial Proc., Vol. 67, No. 121, (1967).

42. Metcalf and Eddy Inc. Wastewater Engineering, McGraw-Hill Book Co., New York, N.Y., (1972).

43. Jenkins, D. and Garrison, W.E., "Control of Activated Sludge by Mean Cell Residence Time", Jour. Water Poll. Control Fed., Vol. 40, No. 11, Part 1, p. 1905, Nov., (1968).

44. Sawyer, C.N. and McCarty, P.L., Chemistry for Sanitary Engineers, 2nd Ed., McGraw-Hill Book Co., New York, N.Y. (1967).

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