Phosphorus uptake in an activated sludge pilot plant as a function of cell residence time
by Owen Kenneth Boe

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree Of MASTER OF SCIENCE in Civil Engineering
Montana State University

Abstract:
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The purpose of this research was to study phosphorus removal as a function of cell residence time in a continuous flow stirred tank reactor. Several data in the literature excessive uptake of phosphorus in activated sludge at either high growth rates or low food to microorganism ratios.

A pilot plant which modeled a continuous flow stirred tank reactor was used for the research. An artificial sewage was used which was designed to be substrate-limiting. Sucrose was used as the substrate. Phosphorus at 7.9 mg/l-P was introduced as orthophosphate and was traced through the treatment process. The experimental variable was the cell residence time and was controlled by the waste rate.

Cell residence times calculated ranged from 2.0 to 13 days. Phosphorus in activated sludge was found to average 1.5 percent for the range of the cell residence times. Phosphorus removal due to iron phosphate and calcium phosphate precipitates were negligible in these experiments. Growth rates and food to microorganism ratios were found to have no significant effect on phosphorus uptake.

The decrease in cell residence time produced by and increase in sludge wastage, was found to be the only significant factor affecting phosphorus removal. The maximum phosphorus removed by the biomass was 35.5 percent.

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Date May 12 1972

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Approved:

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VITA

Owen Kenneth Boe was born in Missoula, Montana on December ninth, 1946, and is the son of Mr. and Mrs. K.N. Boe who reside in Arcata, California. He graduated from Arcata High School in June 1965 and then attended University of California at Davis California where he received his B.S. degree in Chemical Engineering in March 1970. He entered the California National Guard and upon completion of basic training in August 1970 he enrolled in Montana State University for graduate study.

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The nutrient enrichment or eutrophication of water supplies has been a subject of considerable concern for many years. One means that uuuu has been proposed to control eutrophication is to limit the critical nutrient from entering the water supplies. Phosphorus and nitrogen are the two major nutrients required by all aquatic life. Phosphorus in many cases has been chosen as the nutrient to control. Phosphorus is introduced to the aquatic environment through erosion and run-off and through domestic and industrial wastes. While nitrogen may be introduced by the same means as phosphorus, some algae and bacteria can also make use of free nitrogen in the atmosphere.

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The decrease in cell residence time produced by and increase in sludge wastage, was found to be the only significant factor affecting phosphorus removal. The maximum phosphorus removed by the biomass was 35.5 percent.

CHAPTER I

INTRODUCTION

The process of eutrophication and the role of phosphorus in eutrophication has been studied for many years. The concern, however, of both the general public and scientific community (5,27,38) has increased in recent years.

McGauhey (22) describes the process of eutrophication as the maturation of a lake from a nutrient - poor to a nutrient - rich body of water. Since nutrient enrichment is one factor in the process of eutrophication, a conclusion drawn by many scientists is control the nutrients entering the aquatic environment.

The two nutrients of greatest concern are nitrogen and phosphorus. These two nutrients have been shown to be the major mineral nutrients required by all algae (32). In addition to major nutrients, various minor ones are required by algae. The importance of minor nutrients as limiting growth factors is overshadowed, however, due to the larger requirements for the major nutrients.

Sawyer (32) reviewed the need for nutrient control and discussed several aspects relating to the control of nitrogen and phosphorus. He found that phosphorus can be introduced to the aquatic environment only through erosion and runoff and through domestic and industrial wastes. While nitrogen, may be introduced by the same means as phosphorus, some algae and bacteria can also make use of free nitrogen in the atmosphere, provided other nutrients are present in adaquate

supply.

The removal of the major nutritional elements from sewage should be one goal of sewage treatment, but this goal may not be economical with today's technology. As a consequence, present strategy is to remove the critical nutrient to the extent that growth of algae becomes rate limiting in the aquatic environment. Nesbitt (26) and Asano (2) concluded the critical nutrient in many cases was phosphorus. Asano summarized the rational supporting phosphorus removal as:

1. Phosphorus is present in small quantities in oligotrophic lakes.
2. Tributary streams running into these lakes contain little phosphorus but may contain relatively larger quantities of inorganic nitrogen.
3. Phosphorus removal decreases the phosphorus - to - nitrogen ratio and allows phosphorus to become a limiting factor in the growth of algae. Also, phosphorus removal will reduce the stimulating effect causing the growth of nitrogen - fixing blue-green algae.
4. In the presence of all nutrients but nitrogen, bacteria and blue-green algae are able to utilize atmospheric nitrogen for organic synthesis.
5. Phosphorus can be more readily removed from sewage effluents than nitrogen compounds.

If the removal of a critical nutrient limits the growth of algae then for the above reasons phosphorus should be removed from sewage effluents. There has been much literature published on chemical removal of phosphorus and some studies have been published on biological removal. On the basis of a literature review, Randall (29) concludes, a properly controlled activated sludge process can remove large quantities of phosphorus.

Most studies of activated sludge plants have been concerned with plug flow type aerators. Nowadays, most sewage treatment plants are designed for completely mixed aeration. There is a need to have a better understanding of the parameters in a completely mixed system as to their effects on nutrient removals. Such parameters as loading rates, residence times, and mixed liquor suspended solids, affect growth rates and yield coefficients; therefore, these same parameters would be expected to affect nutrient removals.

OBJECTIVES

General Purpose

The purpose of this research project was to study phosphorus uptake in activated sludge. An activated sludge pilot plant was designed and built that would operate on a continuous flow stirred tank reactor (CFSTR) basis. Cell residence time (CRT) was chosen as the experimental variable for use in this study of the uptake of phosphorus in the activated sludge process.

The cell residence time in the activated sludge process is a measure of the mean time that sludge or the mixed liquor suspended solids remains in the system. There are essentually two controls on the cell residence time. The first control is through clarification. An increase in the amount of suspended solids in the effluent will reduce the cell residence time. The second and primary method of control is the amount of cell material wasted each day. An increase in the mass of cell material wasted will result in a decrease in cell residence time.

A completely mixed culture of microorganisms is considered to be at steady state when the cell mass in the reactor does not change with respect to time. At steady state operation, therefore, the rate of cell mass leaving the system equals the cell mass grown.

Specific Objectives

There are four premises for the removal of phosphorus in an activated sludge process.

1. A decrease in CRT increases sludge grown, (increasing sludge wastage), thereby removing more phosphorus which is incorporated in the cell biomass.
2. Phosphorus uptake is enhanced by increased growth rates which results from decreased CRT's.
3. Phosphorus uptake is promoted by low food to microorganism ratios, which result from an increase in

CRT.

4. Chemicals in the raw sewage, notably calcium and iron aid in phosphorus removal by precipitation with phosphorus.

SCOPE

This research was limited to the study of the four premises above. The analysis was made by studying a CFSTR at steady state conditions. Both ortho and total phosphates were traced throughout the system. These measurements, in conjunction with growth rates, substrate removal rates, and yield coefficients were used to determine the mechanics or nature of phosphorus uptake in the completely mixed activated sludge process.

CHAPTER II

LITERATURE REVIEW

PHOSPHATES IN SEWAGE

In nature, phosphorus is predominately found in the form of inorganic phosphates. Phosphates can be divided into the following groups according to Van Wazer (41):

a) orthophosphates
b) polyphosphates (chain phosphates)
c) metaphosphates (ring phosphates)
d) ultraphosphates (branched ring structures)

The last three groups are commonly referred to as condensed phosphates. The ultraphosphates are relatively unimportant in water analysis because of their extreme unstability.

Cellular material contains phosphorus in the form of inorganic phosphates and organic phosphates. Organic phosphates are commonly found as:

a) phospholipids (cell membranes)
b) nucleic acids (genetic material such as DNA and RNA)
c) nucleotides (organic phosphate carriers composed of an organic base, a sugar molecule and one to three phosphate groups, ie ATP)

The three chemical states of phosphorus of particular interest in wastewater treatment are then; orthophosphates, condensed phosphates,

and organic phosphates. According to Hurwitz (16), the major form and scources of the three groups of phosphates are:

a) Orthophosphates. These occur mainly as trisodium phosphate. The sources are dishwashing compounds, hydrolytic products of polyphosphates, urine, and fertilizers.

b) Condensed phosphates. These occur mainly as polyphosphates and tetrasodium pyrophosphate. They are mainly derived from builders in synthetic detergents, phosphate glasses used in corrosion control, and complex phosphates from biological growths.

c) Organic phosphates. The sources are animal feces, urine and biological growth.

The amounts of phosphorus in sewage vary diurnally and from day to day. The amount of phosphorus excreted from body wastes, feces, and urine depends on the intake of phosphorus. Rudolfs (34) in reviewing literature on phosphates in sewage in 1947, found the range of excretion for phosphorus to be 0.7 to 1.5 g P /person - day. He estimated that humans excreted 50 to 65 percent of the total phosphorus in the urine and 35 to 50 percent was excreted in the feces.

Heinke (15) in 1966, reported values for phosphorus in wastewater. His figures were based on production figures for condensed phosphates. Per capita values were determined by using population figures from

1966 census and assuming a wastewater generation of 80 gpcd. The contribution of phosphorus from detergents and human waste is shown in Table 1.

TABLE 1

PHOSPHORUS IN WASTEWATER

Heinke (15)

	Amount, g P / person - day		
	U.S.	Canada	W. Germany
Human Waste	1.6	1.6	1.6
Detergents	3.4	1.7	1.6
Total	5.0	3.3	3.2
Conc. mg/l-P based on 80 gpcd	16.5	10.5	10.6

Heinke's derived value of 1.6 g P/ person - day shows only a slight increase from the range of 0.7 - 1.5 g P/ person - day given by Rudolfs (31) in 1947. One would not expect much difference in the amount of phosphorus excreted by the body. Heinke's figures, however, show the effects of the increase use of phosphate - based detergents in recent years. On a per capita basis Heinke determined a value of 3.4

g P/ person - day added to the wastewater in the United States. This gives a total of 5.0 g P/ person - day in the wastewater of the United States or an equivalent of 16.5 mg/l-P concentration in sewage based on 80 gpcd. These values indicate that the incoming phosphate in sewage was 67 percent condensed phosphates from detergents. Finstein (11) has estimated detergents contribute 42 percent of the phosphates in sewage.

Table 2 gives values of phosphate removals in various sewage treatment plants. Heinke's derived value of 16.5 mg/l-P is within the range of the various values of phosphorus reported in raw sewage, but higher than most values. This could be explained because 80 gpcd is probably low for the United States due to increased water use and combined storm and sewer lines. Phosphorus removal according to Table 2 ranged from a low value of 14 percent in a trickling filter plant to a maximum of 95 percent for an activated sludge plant.

Phosphorus contents in sludge has been often reported. Reid et al (30) reported 1-3 percent P in dried protoplasm. The APHA Committee on Sewage Disposal (1) gave comparable values of 1 to 4 percent P in dried activated sludge. Vacker et al (40), however, have reported values of 6.5 to 7.2 percent P in activated sludge.

TABLE 2

PHOSPHORUS CONTENT IN SEWAGE

Plant	Date	Raw Sewage mg/l-P	Secondary Effluent mg/l-P	Treatment	Percent Reduction %	Source
Ave of 12 Plants Unknown Location	1947	2.3	0.2	T F	91	31
Hyperion L.A., Calif.	1968	7.5	3.8	A S	50	4
	1969	10.0	0.5	A S	95	4
Ave of 2 Plants Unknown Location	1966	15.5	12.8	A S	17	11
Rilling San Antonio, Texas	1964	10.5	1.3	A S	87	40
	1964	12.4	1.5	A S	88	40
East Plant San Antonio, Texas	1964	10.5	5.4	A S	49	40
	1964	12.4	5.4	A S	33	40
West Plant San Antonio, Texas	1964	10.5	5.9	A S	48	40
	1964	12.4	5.9	A S	53	40
Austin Plant Austin, Texas	1965	13.4	6.9	A S	49	40
Village Creek Fort Worth, Texas	1965	12.4	8.2	A S	33	40
River Side No. Fort Worth, Texas	1964	13.0	10.8	T F	17	40
Dallas Plant Dallas, Texas	1964	11.8	10.1	T F	14	40
White Rock Dallas, Texas	1964	14.0	11.8	T F	16	40

A S - Activated Sludge

T F - Trickling Filter

PHOSPHATE METABOLISM

Lehninger (20) has described metabolism as a highly integrated activity in which many sets of multienzyme systems participate for the purpose of exchanging matter and energy between the cell and its environment. One of the most important exchanges of matter and energy occures in the TCA cycle, also called Krebs cycle. Thimann (39) in discussing the importance of Krebs cycle as the main pathway of oxidation in bacteria, concludes it is now clear that virtually all aerobes oxidize pyruvate and acetate in the Krebs cycle. The importance of the Krebs cycle in aerobic organisms is the large amounts of energy conserved in high energy phosphate bonds from the oxidation pathway. Thimann shows that complete oxidation of pyruvate yields seventeen high energy phosphate bonds from the reactions in the Krebs cycle.

ATP (adenosine triphosphate) is the universal functional group which carries the high energy phosphate bond in living matter (21). The two terminal phosphate bonds in ATP exhibit high energy characteristics, but only the last phosphate bond is generally used in energy transformations. The chemical structure of ATP as given by Conn and Stumpf (6) is shown in Figure 1.

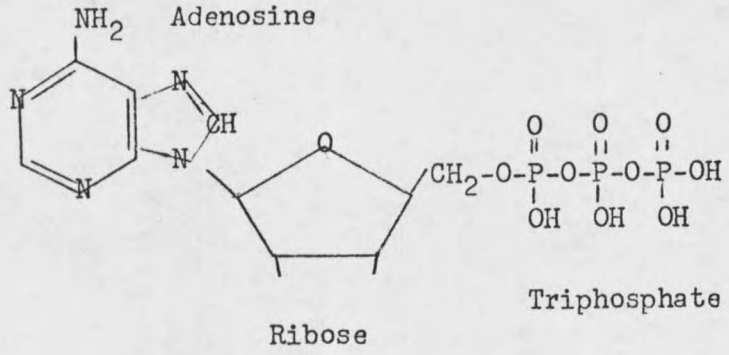

Fig. 1 ADENOSINE TRIPHOSPHATE

According to Lehninger (20) metabolism is divided into catabolism and anabolism. Anabolism is the synthesis of relatively large molecular components of cells (polysaccarrides, nucleic acids, proteins and lipids). Anabolism requires the input of free energy, which is furnished by the phosphate-bond of ATP.

Catabolism is the enzymatic degredation, largely oxidative reactions, of relatively large nutrient molecules (carbohydrates, lipids, and proteins). Catabolism is accompanied by release of free energy which is generally conserved in the form of phosphate bond energy

of ATP. In some cases energy is stored as polyphosphates. Polyphosphates accumulate in volatin granules. This accumulation is herein defined as luxury uptake (q.v. LUXURY UPTAKE). The high energy bond of polyphosphates is probably transferred to ATP before the energy is utilized by the cell. ADP (adenosine diphosphate) reacts with free phosphate within the cell, absorbing the energy from catabolism and forming ATP. The absorbed energy in ATP is available to the organism in the form of high energy reactions for energy requiring functions such as: locomotion, protoplasm synthesis, cell division and respiration. The ATP - ADP cycle is shown in Figure 2.

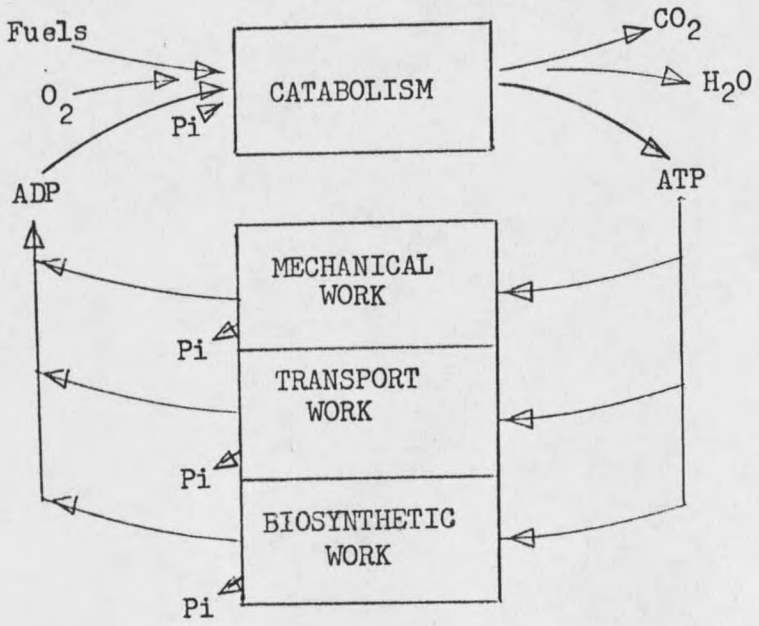

Fig. 2. ATP - ADP CYCLE from Lehninger, (20)

MECHANISMS OF PHOSPHORUS REMOVAL

There is considerable disagreement to the mechanism of phosphorus removal in activated sludge. One common assumption is phosphorus removal is strictly dependent on BOD removal. Helmers et al (14) have reported values of 0.006, 0.007, 0.005 lb. P per lb. BOD_5 removed at 10, 20, and 30°C respectively. A temperature dependency of phosphorus removal would indicate, however, that phosphorus removal is a function of biological activity. Other investigators have also indicated a relation between food and microorganisms as being the important factor in phosphorus removal. Connell and Vacker (7) have reported a high concentration of operation solids (and therefore a resultant low BOD to solids loading ratio) promote phosphorus uptake.

Hall (13) reported that soluble phosphate uptake, in the activated sludge units increased with increasing initial soluble substrate concentration. He also found the length of aeration time influenced the uptake of soluble phosphorus. Several investigators (5), (21), (40), have reported results indicating activated sludge microorganisms are capable of removing and storing far more phosphorus than is required for growth.

Phosphorus uptake has been related to different physical parameters such as BOD and MLSS, but there have been few attempts to discern what is responsible for the phosphorus uptake. Yally et al (46) reported that much of the phosphate taken up by the sludge is associated

with a mechanism involving the synthesis of ATP. Borchardt (5) reported phosphorus uptake reactions are energy dependent and would be expected to increase with increasing temperatures.

Varma and Reed (42), using labeled phosphorus have shown phosphorus is assimulated at a rate exceeding the rate of absorption as growth increases. Absorption is a physical uptake of phosphorus and assimulation is a metabolic intake of phosphorus.

In studying substrate utilization Krishman (19) demonstrated substrate is initially channeled into carbohydrate synthesis and after this, protein is synthesized from the carbohydrate store. Protein synthesis is a high energy demanding process. In discussing protein synthesis Lehninger (20) concluded at least three high - energy bonds is ultimately required for the synthesis of each peptide bond of the complete protein. Each high-energy bond represents one molecule of ATP. A peptide bond joins together two amino acids, and there may be hundreds of amino acids in one protein molecule.

Some discussion in the literature suggests the removal of phosphorus in activated sludge is predominately a physical property. Menar and Jenkins (23) have postulated high removal of phosphorus occurs because of chemical precipitates and entrapment of precipitants in biological floc. Increased aeration and low growth rates promote conditions for reduced concentrations of CO_2 and, therefore, increased pH values. Increased pH values promote precipitates of phosphorus as

complexes with calcium and iron.

Bargman (4) reported the Hyperion treatment plant was removing 50 percent of the phosphorus; but the amount removed could be accounted for by precipitation of calcium, aluminum, zinc and iron phosphate.

LUXURY UPTAKE

Luxury uptake has been a term used to describe any phosphorus uptake in excess of "normal" cell requirements. Borchardt (5) stated that with carbohydrate-rich wastes the phosphorus requirement is about one percent of the BOD value. Phosphate requirements in terms of cell growth were given by Levin and Shapiro (21) and Vacker et al (40) at 1 to 2 mg-P for 100 mg of cell solids formed. The "normal" uptake of phosphorus can then be defined as either 1 mg/l-P for 100 mg/l BOD or as 1 to 2 mg/l-P for 100 mg/l cell material produced.

Many researchers have found phosphorus stored in microorganisms in excess of the "normal" cell requirement. Vacker et al (40) have reported activated sludge is capable of storing phosphorus up to seven percent of the dry sludge weight.

Several other researchers (37),(43), (35) have reported bacteria, algae, and fungi store phosphorus in volutin granules. This can be demonstrated by the metachromatic effect, (appearing red when stained with blue dye) which is caused by the presence of large amounts of inorganic polyphosphates. The polyphosphates composed of orthophosphates are polymers of varying chain lengths.

Borchardt (5) summarized conditions for the excessive accumulation of phosphorus as: 1) when cells are aged or exposed to certain chemical inhibitors, 2) when cells are exposed to phosphate after prolonged phosphate starvation, 3) or to any condition unfavorable to growth and synthesizing activities which normally would consume ATP. Sulfate starvation has been found to be particularly effective in leading to rapid and massive accumulation of polyphosphate (37). Tracer studies of these accumulations with P^{32}, shows that when phosphorus reserves are used the phosphate is incorporated into nucleic acids.

The results of the various investigations indicate that volutin granules appear to function primarily as an intracellular phosphate reserve, formed under a variety of conditions when nucleic acid synthesis is impeded.

Chapter III

THEORETICAL DEVELOPMENT

The activated sludge process utilizes a continuous culture of microorganisms. In most plants the process goes beyond the simple chemostat principle and incorporates some form of cell recycle. The operation of cell recycle, or the recirculation of cells, enables the cell residence time to be greater than the hydraulic residence time.

The activated sludge process is a microbial system which incorporates the complexities of a diverse food chain. The life cycles of bacteria, protozoa, and even mites play a role in the activated sludge process. Also, within each organism there is a complex network of enzymatically controlled metabolic pathways. In a symbiotic relationship the various pathways and various organisms degrade the wastewater by removing the organic and inorganic nutrients. The efficiency and operation characteristics must be described in rational terms meaningful both from a process or operational standpoint, and from a microbiological standpoint (17).

The complexity of the ecology of activated sludge leads to some limitations of evaluation from a microbial standpoint. Growth kinetics, which have been developed from enzymatic reactions and from studies of pure cultures of microorganisms may not be directly applicable to the mixed cultures of activated sludge. However, similar terminology and theoretical development will produce relationships which can be utilized in the analysis and design of waste treatment systems.

Symbols used in the following are shown in Table 3.

TABLE 3

SUMMARY OF SYMBOLS

Symbol	Units	Dinemsion	Terminology
x	mg/l	ML^{-3}	Concentration of Microorganisms
s	mg/l	ML^{-3}	Concentration of Substrate
μ	day^{-1}	T^{-1}	Specific Growth Rate
q	day^{-1}	T^{-1}	Substrate Removal Rate
t	day	T	Time
V	l	L^3	Volume of the System
F	l/day	L^3T^{-1}	Flow Rate
θ	day	T	Hydraulic Residence Time
θ_c	day	T	Cell Residence Time
Y	--	--	Yield Coefficient
α	--	--	Recycle Ratio
		SUBSCRIPT DESIGNATION	
0		Inflow to the System	
1		Within the Reactor	
w		Waste from the System	
r		Recycle	
e		Effluent from the System	

GROWTH RATE RELATIONSHIP

An analysis of the growth of microorganisms in a batch system with no limiting factors, yields the relationship:

$$dx/dt = \mu x \qquad (III - 1)$$

Equation (III-1) defines the specific growth rate as a proportionally constant to the amount of cellular material, X. Integration of equation (III-1) yields equation (III-2), assuming μ to be independent of time.

$$x = x_o e^{\mu t} \qquad (III - 2)$$

As long as there is no hindrance to growth, μ remains constant and the amount of cellular material increases logarathmically. Eventually carbon or some nutrient may become limiting or a toxic material excreted by the organisms may become inhibiting. When any one of or any combination of these events occur, the net growth of cells diminishes until a zero net growth is established. Finally conditions become too severe and the culture goes into a negative growth rate called the death phase. These relationships are shown in a generalized growth curve for bacteria in Figure 3.

Several attempts have been made to describe a kinetic relationship between the specific growth rate and a limiting substrate for activated sludge (17), (28). One major kinetic evaluation is based on work by Monod (25) which incorporates a substrate enzyme reaction mechanism first proposed by Michaelis and Menten (24), for growth of bacteria.

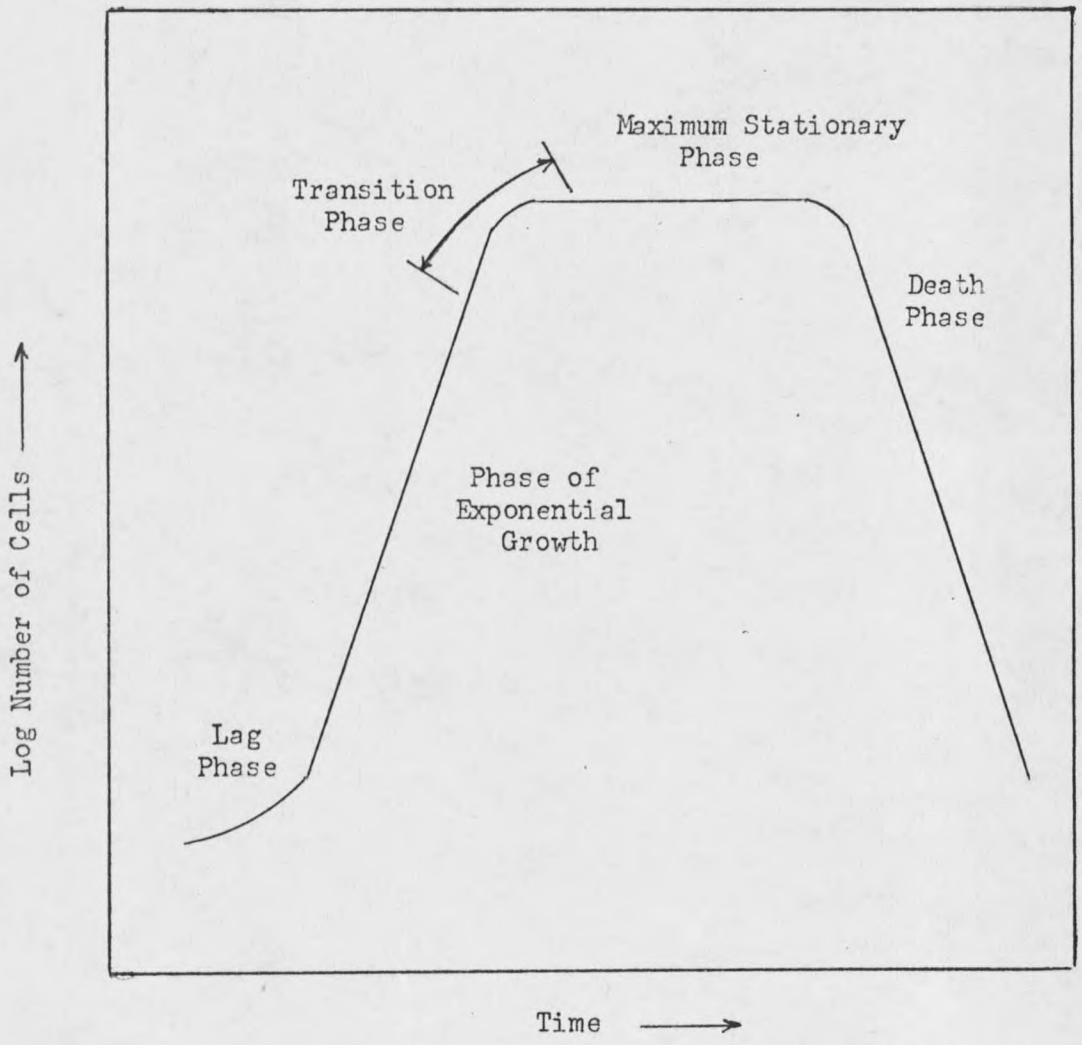

Fig. 3 GENERALIZED GROWTH CURVE OF A BACTERIAL CULTURE

These kinetic equation were developed for pure cultures of microorganism. Activated sludge, however, incorporates a diverse culture of microorganisms, which leads to ambiguity. This study, therefore, will leave the definition of specific growth rate in terms of process parameters, such as MLSS.

Plants treating domestic sewage have generally been considered to operate on a substrate - limiting basis. The transition phase (between late exponential and early stationary phases of Figure 3) represents the range of operation of a sewage treatment plant where substrate is begining to limit and or limiting. When substrate becomes limiting, or if a toxic material becomes inhibiting, the specific growth rate no longer remains a constant. The net growth rate decreases until it reaches zero. At this point the growth of new cells is just balanced by the death of old cells.

The yield coefficient or cell yield is a term used to describe some correlation between the cell growth and substrate uptake. It is common to assume the yield coefficient is constant. This assumption implies a direct and linear correlation between cell growth and substrate removal, or:

$$dx/dt = -Yds/dt \qquad (III - 3)$$

Where Y is the correlation constant known as the yield coefficient. From equation (III - 3) and equation (III - 1) we find:

$$\mu x = -Yds/dt \qquad (III - 4)$$

and:

$$\mu = (-Yds/dt)/x \qquad (III - 5)$$

Now if we define a substrate removal rate as:

$$q = \frac{\text{g substrate removed per day}}{\text{g cellular material}}$$

$$q = (ds/dt)/x \qquad (III - 6)$$

then:

$$\mu = -Yq \qquad (III - 7)$$

PROCESS MATERIAL BALANCE

The following equations for material balances are derived mainly from the work of Schroeder, Jenkins and Friedman (34), (17), (11), and applied to the system used in this research. If the system is substrate limiting, two material balances are necessary: one for substrate and one for microorganisms. The schematic of the system is shown in Figure 4.

Microorganism Balance

A material balance for microorganisms around the entire system in Figure 4 leads to the equation:

Input + Growth = Output + Accumulation

$$FoXo + VX1\mu = (Fo-Fw)\ Xe + F'wX1 + Vdx/dt \qquad (III - 8)$$

Figure 4 shows a schematic diagram of the system.