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THE EFFECT OF INFLUENT ORGANIC COMPOUNDS ON THE PERFORMANCE OF BIOLOGICAL NUTRIENT REMOVAL SYSTEMS

by

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(ABSTRACT)

The main objective of the research was to investigate the effect of influent organic compounds on the performance of biological nutrient removal system. To carry out the investigation, a pilot plant system was designed and constructed. The system was operated as a UCT process at an influent flow rate of 0.15 liters/minute and a sludge age of 13 days. The influent wastewater was domestic sewage. Excess biological phosphorus removal and steady-state conditions were established before making experimental measurements, or adding supplemental substrate. The effects of separate addition of formic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acid, plus glucose, addition on phosphorus release under anaerobic conditions, and phosphorus uptake under aerobic conditions, were studied. The effects of the organic acid additions on the removal of nitrogen and COD, and changes in SOUR, MLVSS, and metals such as iron, magnesium, calcium and potassium, were also studied. In all experiments, the specific substrate was added continuously to the first anaerobic reactor for three days at an influent concentratin of 100 mg COD/liter. Samples were collected from each reactor at the end of the addition period and analyzed for orthophosphate, nitrate, nitrite, sulfate, volatile fatty acids, COD, MLVSS, pH and metals. All added substrates, except formic acid and dextrose, caused significant increases in phosphorus release in the anaerobic

stage, and phosphorus uptake, in the aerobic stage, and consequently, an increase in phosphorus removal efficiency. The molar ratios of phosphorus release to volatile fatty acid added obtained for propionic acid, acetic acid, butyric acid, and valeric acid were 0.44, 0.77, 0.78, and 1.72 respectively. However, on a COD basis, the greatest ratios of mg phosphorus released to mg COD utilized was produced by the addition of acetic acid (0.37) and valeric acid (0.19). It was also found that the branched organic acids, isobutyric and isovaleric, caused more phosphorus release in the anaerobic stage and better phosphorus removal efficiencies as compared with the nonbranching forms of the same organic acids. The molar ratios of phosphorus release for these two acids were 0.8 and 2.3, respectively, and on a COD basis were 0.16 and 0.25. For engineering applications, it is suggested by this research that at least 20 mg COD equivalent of acetic acid is needed for the removal of 1 mg phosphorus. The results obtained by this investigation were consistent with the hypothesis proposed by Marais et al., 1983. The most recent biochemical models, proposed by Comeau et al., 1986 and Wentzel et al., 1986, were also tested using the data collected in the present investigation. Both models, in most cases, overestimated the ratios of phosphorus release to volatile fatty acid utilized. A speculative model for anaerobic metabolism by poly-p bacteria of volatile fatty acids which contain both odd and even numbers of carbon atoms was proposed.

All added substrates produced no effect on both COD and TKN removals. Metal release were found to correlate with the amount of phosphorus release.

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I. INTRODUCTION

Excessive fertilization (eutrophication) of natural waters is the most significant cause of both water quality and aquatic life deteriorations. Eutrophication problems are not restricted to lakes; they also occur in estuaries, impoundments, water supply reservoirs, rivers and coastal waters. Eutrophication is dependent on solar radiation, temperature, morphology and concentration of nutrients present in the water. Nutrients include carbon, nitrogen and phosphorus. Carbon is naturally abundant from organic compounds, carbonate, bicarbonate and carbon dioxide. Nitrogen can be fixed by blue-green algae and some other organisms which provide a continuous supply to the system and hence cannot be a limiting nutrient except for limited periods of time. Huchinson (1957) has reported that phosphorus is the most important nutrient for biological productivity and can be effectively used to limit the biological growth in any surface water.

The main sources of phosphorus to lakes are: surface runoff, fertilizer application, phosphate mining and industrial and municipal wastewater treatment plant discharges. Although the phosphorus discharges from nonpoint sources, such as urban runoff, is very significant in many cases, point sources such as industrial and municipal wastewater

treatment plants, are usually the dominant source except during high rainfall periods, and the easiest to control. Many researchers and organizations have recommended controls to reduce the phosphorus content in detergents (Maki, et al. 1984). As a result, laws limiting the phosphorus content of detergents were enacted in Minnesota, Michigan, Vermont, Wisconsin, Florida and others (Grakstatter et al., 1978). More recently, such laws have been enacted in Maryland, Washington, D.C., and Virginia. In contrast, some investigators have reported that external P source is less important than expected and small changes in external P loads have insignificant effects on water quality, that is, detergent P-bans produce small changes in P-loading and no significant water quality effect is achieved by such bans, if that is the only control measure implemented (Maki, et al., 1984). Thus, a reduction in the treatment plant discharges is needed to control eutrophication. From the foregoing discussion, the need for effective phosphorus removal from wastewater discharges is a must for better water quality and productive aquatic life.

Removal of phosphorus from wastewater has been achieved by physical, chemical and biological processes. Chemical removal of phosphorus is achieved by the addition of chemicals such as iron salts, aluminum salts and lime. Chemical processes require large quantity of chemicals and generate large quantities of sludge containing lots of phosphorus. Processing and handling of such sludge requires high labor and maintenance costs. The physical process is very costly and is usually accomplished by ultrafiltration, reverse osmosis, and ion exchange-systems. PhoStrip and contact stabilization adaptation are two biological-chemical processes used for phosphorus removal. Biological phosphorus removal, which has been developed more recently, is considered to potentially be the most economical method of removing phosphorus from wastewaters. Sludge handling and disposal problems are greatly reduced by the biological process of phosphorus removal because chemical addition is eliminated. All systems

are modifications of the activated sludge system in which alternating anaerobic and aerobic environments are employed. Systems used for biological phosphorus removal include: Modified Bardenpho process (phoredox); UCT Process (University of Cape Town process); A/O process (Anaerobic/oxic process) and sequencing batch reactors. In the anaerobic reactor, which is essential for enhanced biological P-removal, large quantities of organics are removed from solution, which in turn lessens the organic strength of the waste and results in some energy savings due to reductions in aeration requirements, (Randall et al., 1987).

Although there has been cumulative evidence that enhanced biological phosphorus removal is the most economical and effective method of nutrient removal from wastewater, the acceptance and application of this process has been very limited. This is primarily due to the wide variety of performance efficiencies observed and reported by different operators and investigators. In addition, at present, there is no widely accepted or proven mechanistic explanation for the phenomenon of enhanced biological P-removal. There are several models which have been proposed to describe biological P-removal, but most of the systems are designed based on empirical observations.

Numerous parameters and variables have been reported to affect the performance of biological phosphorus removal systems such as: influent wastewater characteristics; anaerobic retention time; sludge age; aerobic retention time; and nitrogen oxide and/or oxygen concentrations in anaerobic reactors. Influent wastewater characteristics include: BOD; COD; P/COD; BOD/MLSS; BOD/P; COD/TKN; ORP; Temp; pH and the organic nature of wastewater. Many researchers have stressed the importance of the organic nature of wastewater, mainly the concentration of short chain volatile fatty acids, on the performance of biological phosphorus removal systems. The beneficial effect of short chain volatile fatty acid addition to poly P microorganisms under anaerobic conditions is well recognized (Fukase et al., 1982; Potgieter and Evans, 1983; Lotter,

1984; Iwema and Meunier, 1985; Wentzel et al., 1986; Comeau, 1987). However, most of these studies were conducted using pure cultures grown in a batch system, and the cultures were exposed to media containing the substrate to be investigated. This technique may not sufficiently represent the actual conditions that occur in excess biological phosphorus removal plants. Bacterial interaction, heterogeneity of biomass, and the complex nature of municipal wastewaters are all important factors to be considered.

The effect of the organic composition of wastewater on biological phosphorus removal was the principal objective of this study. The investigation was developed and performed because of the variations of phosphorus removal efficiency observed in similarly designed and operated biological P-removal plants treating different wastewaters. It was the purpose of this study to gain better understanding of the phosphorus removal process and thus, contribute toward the improvement of present design procedures for biological phosphorus removal. Specifically, the main objectives of the investigation were to:

1. investigate the effect of the influent organic composition of wastewater on the performance of excess biological phosphorus removal systems in particular, and on biological nutrient removal in general;
2. determine how biological phosphorus removal system design can be adjusted to account for variations in the nature of influent wastewater;
3. investigate the fate of fermentation products (short chain volatile fatty acids) in excess biological phosphorus removal systems; and
4. gain further insight into the biochemistry involved in excess biological phosphorus removal.

II. LITERATURE REVIEW

Phosphorus is one of the essential nutrients for the growth of microorganisms. Based on the formula suggested by McCarty, 1970, to quantify the relative nutrient composition of a typical microbial biomass C_{60} H_{87} O_{23} N_{12} P phosphorus requirement is 2.3 percent of cell biomass. Using the formula proposed by Metcalf and Eddy, 1979, the phosphorus content is also 2.3 percent. A lower value, 2.0, has been reported by Bundgaard et al., 1983. However, potentially higher percentages of phosphorus in bacterial biomass have been observed for sludges obtained from wastewater treatment systems operating under specific operating conditions. That is, cases have been reported in which microorganisms were capable of accumulating phosphorus in excess of the normal growth requirements (2 to 3 percent). Rensink et. al., 1981, have reported values in the range of 6 to 7 percent. Oldham and Kock, 1982, have reported a value of 6.5 percent. Values up to 8 percent have been reported by Fukase et al., 1982.

The terms Excess Biological Phosphorus Removal and Enhanced Biological Phosphorus Removal are used to describe the phenomenon in which

microorganisms accumulate phosphorus in excess of their normal growth requirements without chemicals addition.

II.1 Observations Supporting Biological Phosphorous Removal

According to Levin and Shapiro (1965), excess biological phosphorus was first observed by Srinath, Sastry and Dilla (1959) in India and by Alavcon (1961) in America. Levin and Shapiro (1965) extensively investigated the release and uptake of phosphorus. They observed polyphosphate granules inside isolated phosphorus accumulating cells. They hypothesized that those granules were the storage of excess phosphorus uptake. Addition of 2,4-dinitrophenol (DNP) caused a decrease in phosphorus uptake during the aerobic stage. Since 2,4-dinitrophenol inhibits the breakdown of high energy compounds and thus the transfer of energy from one form to another, the phenomenon of phosphorus uptake was believed to be truly biological, mediated mainly by the Kreb cycle. Similar results were obtained by Rensink et al. (1981) on the effects of DNP on phosphorus uptake as shown in Figure 1. Yall et al. (1970) investigated the mechanism of phosphorus removal using radioactive P and Ca. As a result of their study, they concluded that biological phosphorus removal was the main removal mechanism and calcium phosphorus precipitation played a minor role in the removal process. Rensink et al. (1981) conducted a series of experiments investigating the association between Ca and P in plants obtaining excess P re-

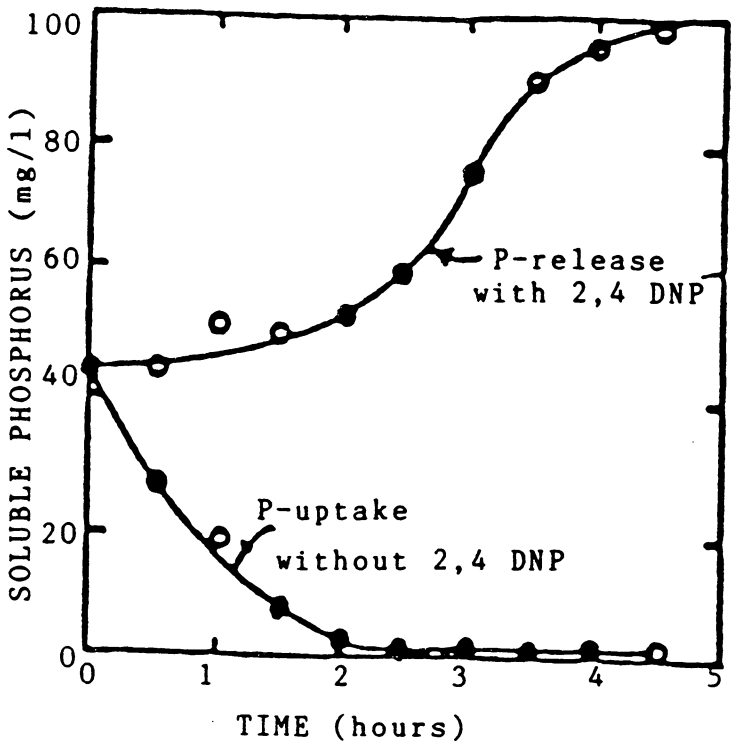


Figure 1. Effect of 2,4-Dinitrophenol addition on phosphate uptake under aerobic condition (after Rensink et al., 1982).

moval. They found no association taking place between P and Ca which suggested that phosphorus removal could be attributed to biological mechanisms. Barnard (1976) conducted experiments to study the effect of pH on phosphorus removal in a Bardenpho process. He found no significant increase in excess P removal which indicates that the physical-chemical mechanism was not the mechanism by which P was removed from the system. In conclusion, there is cumulative evidence that the phenomenon of excess phosphorus removal in wastewater treatment systems is biological.

II.2 Microorganisms Capable of Biological Phosphorus Uptake

Several investigators frequently have found bacteria of the genus *Acinetobacter* in systems where excess phosphorus uptake was observed (Fuhs and Chen, 1975; Buchan, 1983). Fuhs and Chen (1975) isolated the *Acinetobacter* genus and reported some of its characteristics. They found that *Acinetobacter* were obligate aerobes and have the ability to accumulate polyphosphate and poly-β-Hydroxybutyrate (PHB). These bacteria can grow on acetate and ethanol, but cannot grow on glucose, lactate and propionic acid. Brodisch and Joyner (1983) have studied the composition of microflora in the anaerobic, anoxic and aerated units. They concluded that microorganisms in addition to *Acinetobacter* can be responsible for biological phosphorus removal in activated sludge systems. *Aeromonas* and *pseudomonas* species were the predominant

groups of bacteria existing in the processes they investigated. Osborn and Nicholls (1978) reported many other bacteria capable of excess phosphorus uptake as shown in Table 1. Filamentous organisms of the species *Mierathrix* and *Nocardia* were also observed and believed to be capable of accumulating polyphosphate in their cell's granules. However, *Acinetobacter* is usually the predominant organism found in the activated sludge systems as shown in Table 2 (Buchan, 1983). According to Juni, (1978), *Acinetobacter* species are aerobic microorganisms, i.e., use only oxygen as the terminal electron acceptor and thus, cannot withstand extended anaerobic conditions. They are short plump rods 1 to 1.5 mm diameter by 1.5 - 2.5 mm in length, gram-negative, non sporeforming bacteria found in soil, water and sewage. Quantitative phosphorus analysis in *Acinetobacter* indicated that the intracellular polyphosphate granules contained in excess of 25% phosphorus and a Ca:P ratio between 0.15 - 0.36 (Buchan, 1983). *Acinetobacter* has the ability to accumulate lipids as a carbon reserve material, such as Poly-β-Hydroxybutyrate (PHB) and release polyphosphates in the phosphate form to solution under anaerobic conditions (Arvin, 1985).

In conclusion, various groups of bacteria are present in biological phosphorus removal systems, which are capable of storing polyhydroxybutanoic acid and can accumulate polyphosphate granules in their cells.

Table 1. List of organisms capable of excess phosphorus uptake (after Osborn and Nicholls, 1978).

Acetobacter suboxydans	M. smagmatis
Aerobacter aerogenes	M. thamnaphaeos
Azotobacter agilis	M. tuberculosis
A. vinelandii	Rhodopseudomonas palustris
Bacillus subtillis	Rhodospirillium rubrium
Bacterium aerogenes	Serratia marcascens
B. cloacae	Thiobacillus thioxydans
B. friedlanderi	
Caulobacter vibroides	Nitrobacter
Chlorobium thiosulphatophilum	Micrococcus denitrifians
Chromatium	Staphylococcus aureau
Clostridium spec.	Chlamydomoda
Corynebacterium diptheria	Mucor racemosus
C. xerose	Claviceps purpurea
Escherichia coli	
Hydrogenomonas spec.	Acinetobacter
Mycobactyerium avium	Zoogloea ramigera
M. chalonei	Nitrosococcus
M. phlei	Beggiatoa

Table 2. Relative population ratios of hetrotrophic, Gram-Negative bacteria in four nutrient removing activated sludges (after Buchan, 1983)

	Goudkoppies[1]	Northern 1[2]	Cape Town[2]	Brits
Acinetobacter/Morazella	53.6%	48.0%	66.2%	62.8%
Pasteurella spp	1.2	-	1.4	-
Alcaligenes	11.9	6.0	-	24.5
Aeromonas hydrophila	10.7	6.0	2.8	2.1
Pseudomonas spp	9.5	7.0	19.8	4.3
Flavobacterium odoratum	*5.9*	*31.0*	-	-
E. coli	*1.2*	-	-	-
Other genera	*4.8*	*2.0*	*8.4*	*.2*
Unidentified	*1.2*	-	*1.4*	*2.1*

Note: 1 Johannesburg multi-zone plants
2 University pilot plant
3 Single basin works (limited aeration)

II.3 Factors Affecting Biological Phosphorus Removal

There are numerous parameters which have been observed to have a significant effect on the performance of biological phosphorus removal systems. Some are process and operational parameter, others, are related to wastewater characteristics and plant configuration. Important parameters and their effect on the performance of biological phosphorus removal systems will be briefly discussed in this section. Summary of reported factors and their effect on phosphorus release and subsequent uptake is presented in Table 3.

II.3.1 Anaerobic Retention Time

According to Deinema et al. (1985), the purpose of the anaerobic stage is to provide fermentation products, such as acetate, ethanol and lactate, which will be utilized by poly-phosphate (poly-P) bacteria as carbon sources. Nicholls and Osborne (1979) considered the anaerobic stage as a stress factor necessary to stimulate the phosphate uptake in the aerobic stage. The anaerobic retention time enables two microbial processes. First: the organic material is stabilized and converted to readily biodegradable volatile acids such as acetic acid, propionic acid and lactic acid by fermentation. The second function is the uptake of the fermentation products by the poly-P organism and the release of phosphate to solution. Therefore, sufficient anaerobic time should be provided

Table 3. Reported observations on factors affecting phosphorus release and subsequent uptake in excess biological phosphorus removal

Reference	Factor	Change	P-release	P-uptake
Florentz et al., 1985	Temp.	Decrease	None	None
Jones et al. 1985	Temp.	Increase	Slight increase	Slight increase
Vassos and Oldham, 1985	Temp.	Increase above 17°C	Significant increase	Significant increase
Jones, et al. 1987	Temp.	Increase from 24 to 29°C	75% increase	33% increase
Shapiro et al. 1967	ORP	Decrease	Increase	Increase
Randall et al. (1970)	ORP	Decrease	None	None
Koch and Oldham 1984	ORP	Decrease to -125 to -150	Indirect increase	-
Levin and Shapiro 1965	pH	Decrease below 4	Increase	-
Fuhs and Chen 1975	pH	Decrease below	Increase	-
Potgieter and Evens, 1983	pH	Decrease below 4	Increase	-
"	pH	At pH = 8	-	Increase
Fuhs and Chen 1975	VFA	Added acetate	Increase	-
Fukase et al. 1982	VFA	Added acetate	Increase by 0.38 mg PO_4-P/mg acetate	-
Siebritz et al. 1983	VFA	Added acetate	Increase by .25 mg P/mg acetate	
Potgieter and Evens, 1983	VFA	Added acetate and propionate	Significant increase	-
	VFA	Formate	Increase	-
	VFA	Butyrate and hydroxybutyrate	Little increase	-
Deinema et al. 1983	VFA	Added acetate	High increase	-
Wentzel et al. 1986	VFA	Added acetate	Significant increase (1 mole PO_4-P/mole acetate)	-
Lotter, 1985	VFA	Added acetate	High increase (1 mole PO_4-P/mole acetate)	-
Marais and Ekama	VFA	Added acetate	High increase	-
Jones, et al. 1987	VFA	Added butyrate Added acetate	High increase Little increase	- -
Comeau, et al. 1987	VFA	Added acetate and propionate combination	Significant increase	-

for the above two functions to occur. The desired anaerobic retention time is determined by the characteristics of the incoming wastewater. Septic wastewaters will require shorter retention time than fresh wastewaters to achieve the same phosphorus removal (Gerber and Winter, 1984).

Gerber and Winter (1984) conducted a laboratory scale experiment to determine the effect of the anaerobic rentetion time on phosphorus removal efficiency. They observed an increase in phosphate removal when the anaerobic retention time was increased. They concluded, in part, that as the strength of the influent wastewater increases, the anaerobic retention time should be increased to achieve a predetermined effluent phosphorus concentration. A longer anaerobic retention time than the customary 0.5 to 3 h was recommended by their study to achieve high phosphorus removal efficiency.

It is worth mentioning that high anaerobic retention time may lead to sludges having poor settling characteristics and poor dewaterability. In addition, prolonged anaerobic retention time may result in high reduction of organic carbon and, consequently, carbon might become the limiting nutrient for denitrifying bacteria which are responsible for the removal of oxidized nitrogen, and thus affecting the efficiency of biological phosphorus removal.

II.3.2 Organic Loadings to the System

Influent COD and BOD effects on biological treatment systems have been studied by many investigators. Fukase et al. (1985) have published results of their investigations on factors affecting biological removal of phosphorus. Their

results suggested that BOD loading rate and BOD to MLSS ratio have a significant effect on phosphorus release in the anaerobic unit and must be maintained below 2.9 kg-BOD/kg-MLSS and 0.1 kg-BOD/kg-MLSS, respectively, to achieve good P-removal. It is well known, that enhanced biological phosphorus removal requires enough readily biodegradable concentration in the anaerobic reactor as a prerequisite for phosphorus release. Readily biodegradable substrate (fermentation products such as acetic acid, propionic acid and butyric acid) is indicated by measuring soluble BOD or soluble COD. Siebritz et al. (1982) suggested that the minimum readily biodegradable COD concentration in the anaerobic reactor should be more than 25 mg/l.

Manning and Irvine (1985) studied the effect of COD on phosphorus release from sludge under anaerobic condition. A sludge sample was divided into two 1 l beakers. Sludge sample in each beaker was slowly agitated to keep sludge in suspension. To one beaker, COD was added whereas no COD was added to the other. Figure 2 shows the results obtained from their experiment. The sludge to which 320 mg/l COD was added showed rapid phosphorus release during the initial 2 hours to reach its maximum value of 55 mg/l.

II.3.3 Nature and Characteristics of Influent Organic Substrate

Levin and Shapiro (1965) investigated the effect of carbonaceous energy addition on the performance of phosphorus uptake. They first obtained two samples from the Columbia Sewage Treatment Plant. Then they added carbon sources such as succinate, glucose and fresh wastewater to one sample, but

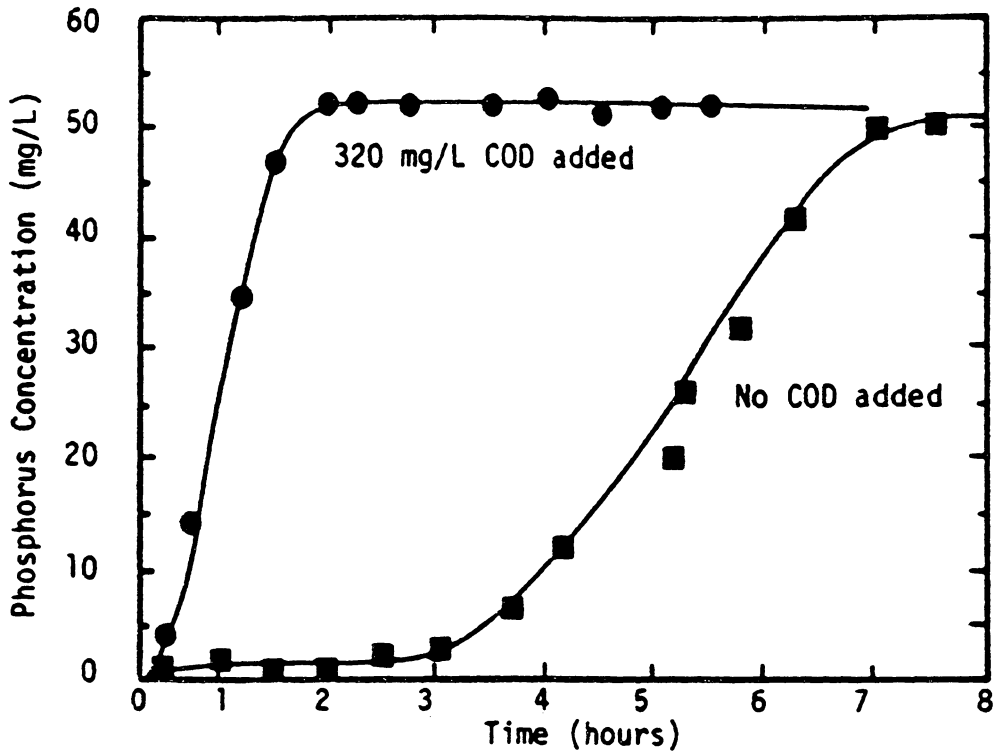


Figure 2. Phosphate release from sludge (after Manning and Irvine, 1985).

nothing to the other. The sample to which the carbon source was added showed a higher phosphorus uptake than the second sample. When the bacterial growth entered the endogenous respiration phase, phosphorus release to the solution was observed. This experiment indicated that the uptake was due to the active fraction of the sludge. Levin and Shapiro explained that P uptake in the aerobic state occurred due to P uptake via the formation of ATP from ADP, and P by substrate level phosphorylation and oxidative phosphorylation, by ETC. However, during the anaerobic state, the Embden-Meyerhof pathway, some ATPs are formed by substrate level phosphorylation but no P uptake was observed.

Fuhs and Chen (1975) studied the effect of specific substrate addition on phosphate release under anaerobic conditions. As shown in Figure 3, addition of CO_2 or acetic acid stimulated release whereas noncarbon substrate addition, such as N_2 and H_2, showed no effect on P-release. Fukase et al. (1982) studied the effect of different acetic acid levels on P-release. As the level of acetic acid present in the anaerobic unit increased, an increase in phosphate release was observed, 0.38 mg PO_4-P/l per mg acetic acid added, as shown in Figure 4. Similar results were reported by Siebritz et al. (1983), and the ratio of acetate added to phosphate released can be obtained from Figure 5 as 0.25 mg PO_4-P/mg acetic acid.

Potgieter and Evans (1983) studied, in part, the influence of several substrates on phosphate release under anaerobic conditions. Various types of substrate were added on a COD equivalent basis to anaerobic units containing sludge samples. It was found that acetic acid and propionic acid had an enhancing effect on phosphate release as shown in Table 4. Formate also enhanced the release but to a lesser extent. Butyrate, hydroxybutyrate and glucose showed little enhancement affect to release, and ribose, glycerol and GDTA had no effect

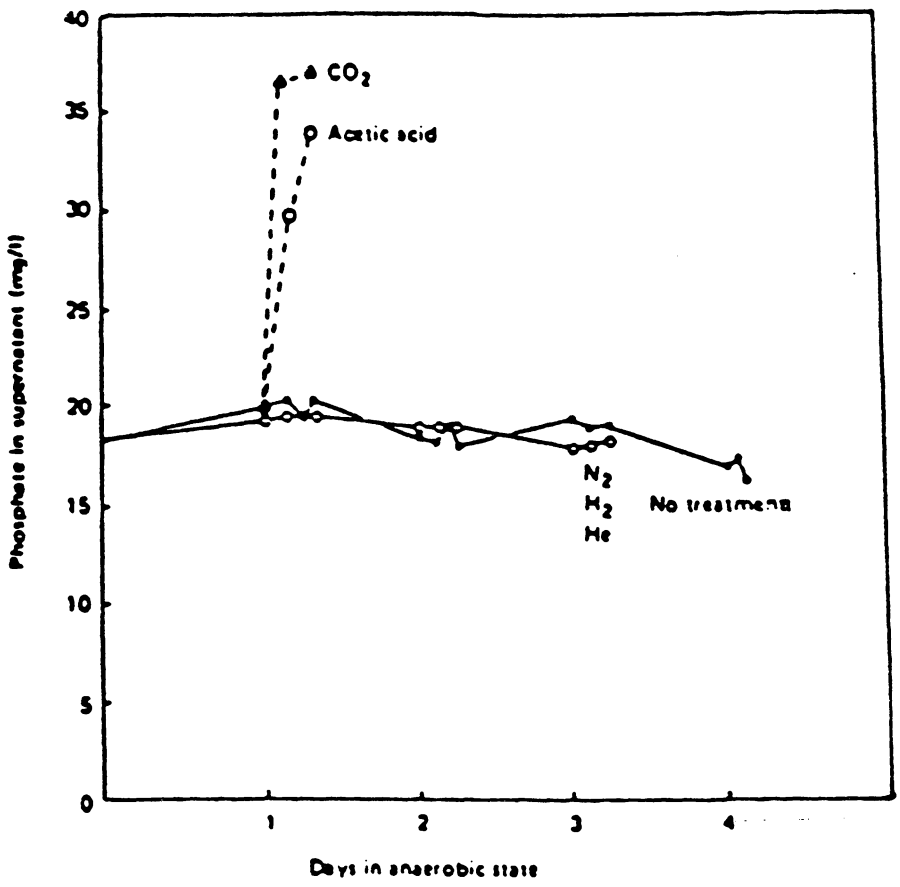


Figure 3. Effect of various substrate additions on phosphate release (after Fuhs and Chen, 1975).

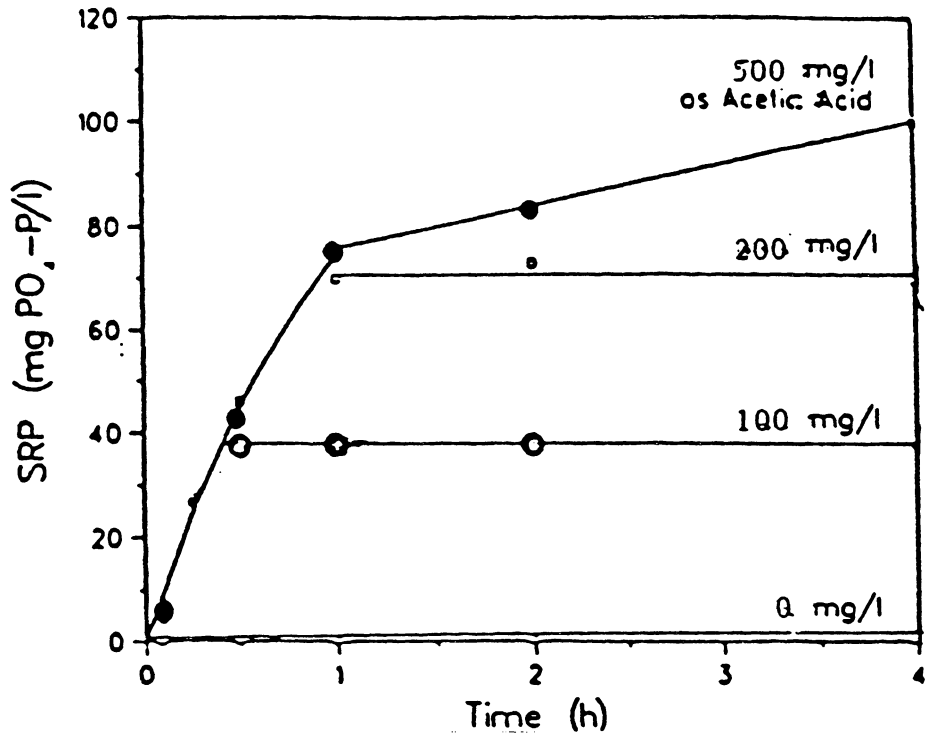


Figure 4. Effect of acetic acid concentration on phosphate release (after Fukase et al., 1983).

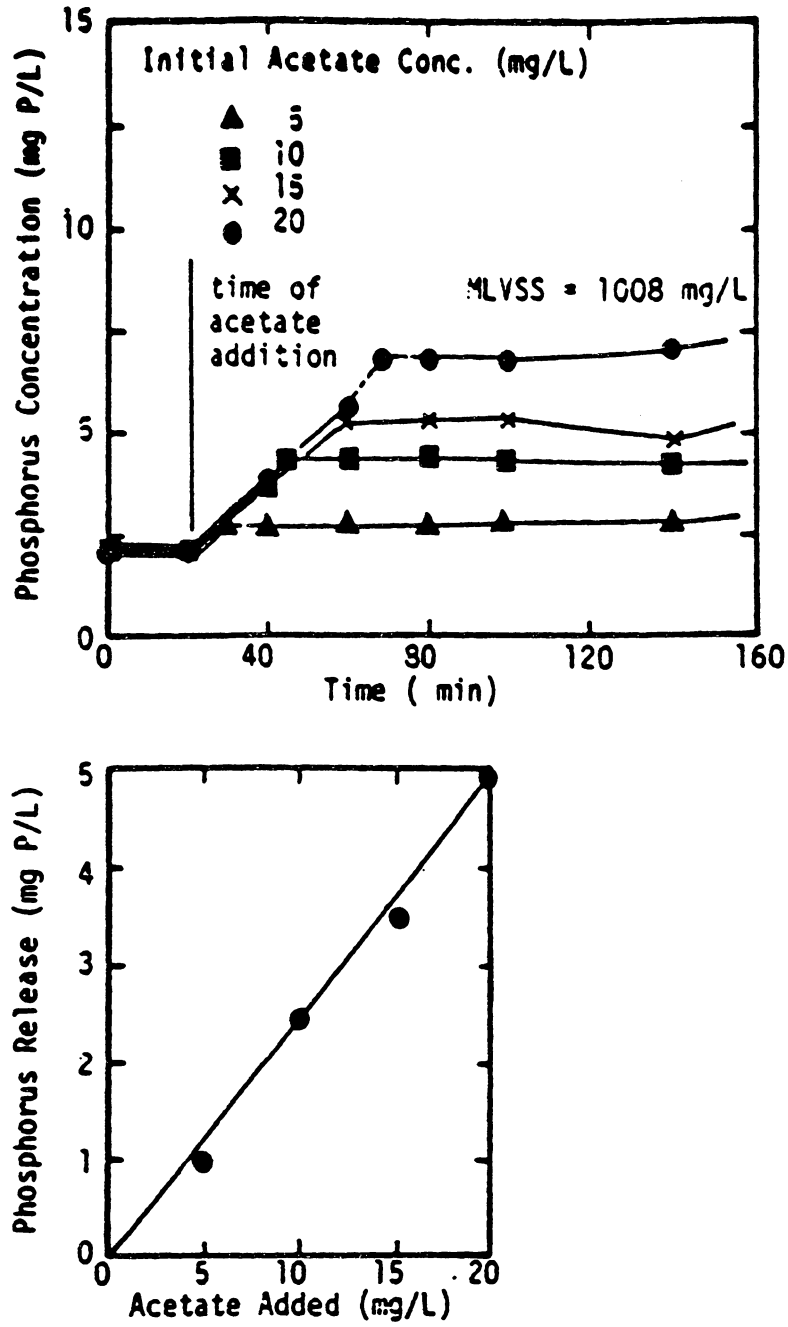


Figure 5. Phosphate release in a batch anaerobic reactor with different acetate concentrations (after Siebritz et al., 1983).

Table 4. Effects of different substrates on phosphate release (after Potgieter and Evans, 1983)

Substrate COD conc. 110 $mg\ell^{-1}$	Phosphate Release $mg\ell^{-1}$
Formate	23.4
Acetate	58.5
Propionate	54.5
Butyrate	8.2
Hydroxybutyrate	5.9
Glucose	5.0
Ribose	0.0
Glycerol	0.0
EDTA	0.0

on phosphorus release under anaerobic conditions. A similar study was conducted by Deinema et al. (1983). They observed high phosphate release following acetate and ethanol addition as shown by the data in Table 5.

Arvin and Kristensen (1985) conducted a series of batch experiments using 2-l closed beakers containing sludge samples. The sludge samples were obtained from the aerobic reactors at two plants operated for biological phosphorus removal, one at Lyngby and the other one at Frederikssund. The sludge was agitated at a low rate to keep it in suspension. Sludge was sampled at different time intervals and analyzed for specific volatile organic acid and the corresponding, if any, phosphate release. Figures 6, 7, 8, illustrate phosphorus released and the uptake of various organic acids such as: glucose, ethanol, lactate, propionic acid, acetate, and butyric acid by sludges taken from 84-Lyngby, 83-Frederikssund and 84-Frederikssund respectively. From these figures, substrate uptakes for lactate, acetate, propionic acid and butyric acid are closely correlated with phosphate release and required more than 4 hours for the exchange with phosphate to occur.

For glucose and ethanol addition, the two sludges showed different substrate uptake and phosphate release patterns. The sludge from the 83-Frederikssund plant, fed with wastewater from a juice factory, showed a quick pickup of glucose and released the phosphate at a moderate rate (see Figure 9). Although ethanol uptake rate by sludge taken from the 1983 Frederikssund was very low, phosphate release by the same sludge was relatively high. Sludge obtained from the pilot plant at Lyngby showed very low ethanol uptake rate and very low phosphate release as shown in Figure 10. The author provided no explanation for such behavior.

Table 5. Phosphate release in continuous culture after 24 Hours of Anaerobiosis at 30°C (after Deinema et al., 1983)

Added reduced carbon source	Rate of phosphate release mg P/ℓ.h	Phosphate release coefficient
Acetate	0.14	1
Acetate + ethanol	1.10	8
Acetate + butane-1.2di	1.23	9
Acetate + isocitrate	1.56	11
Acetate + citrate	1.43	10
Acetate + lactate	1.10	8

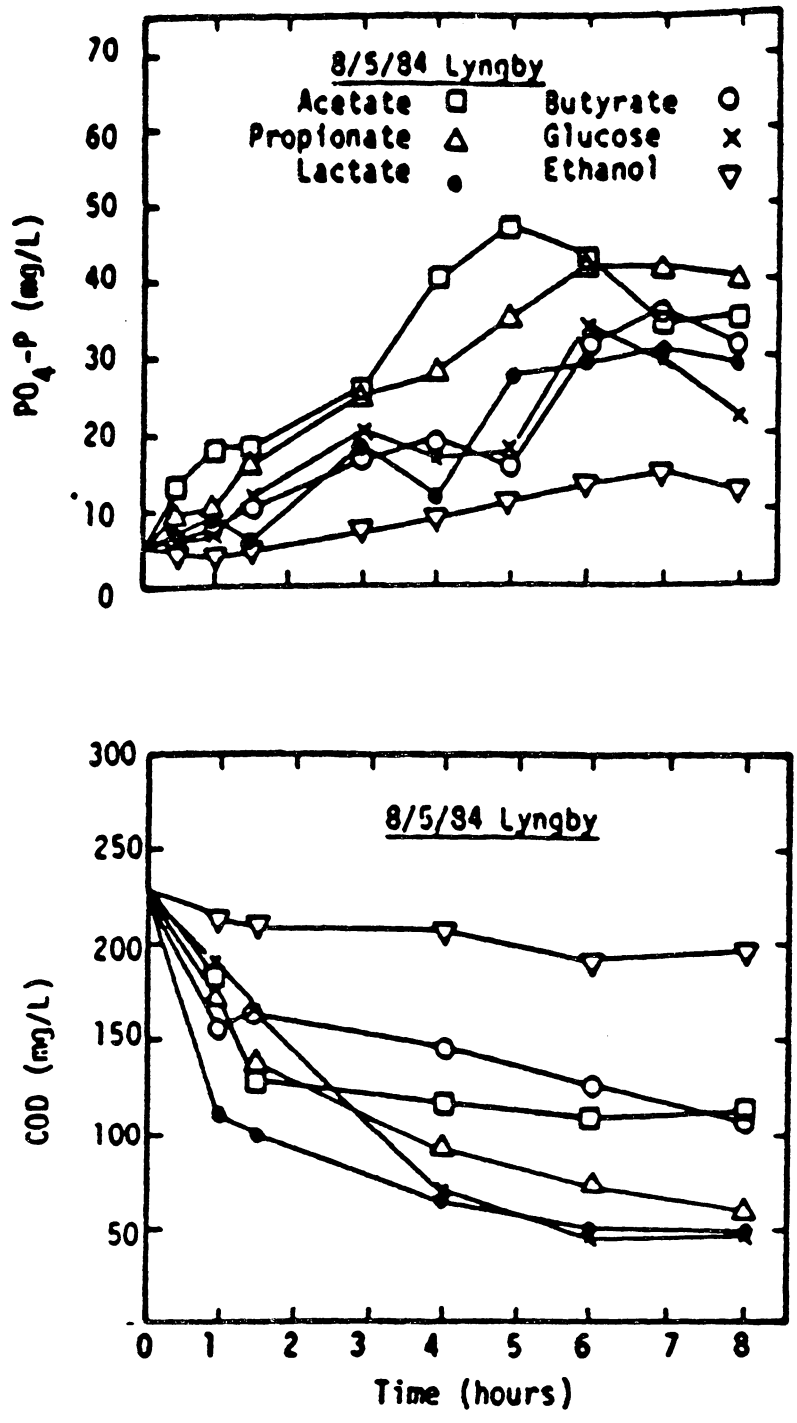


Figure 6. Anaerobic absorption of different substrates and phosphate release. (after Arvin and Kristensen, 1985).

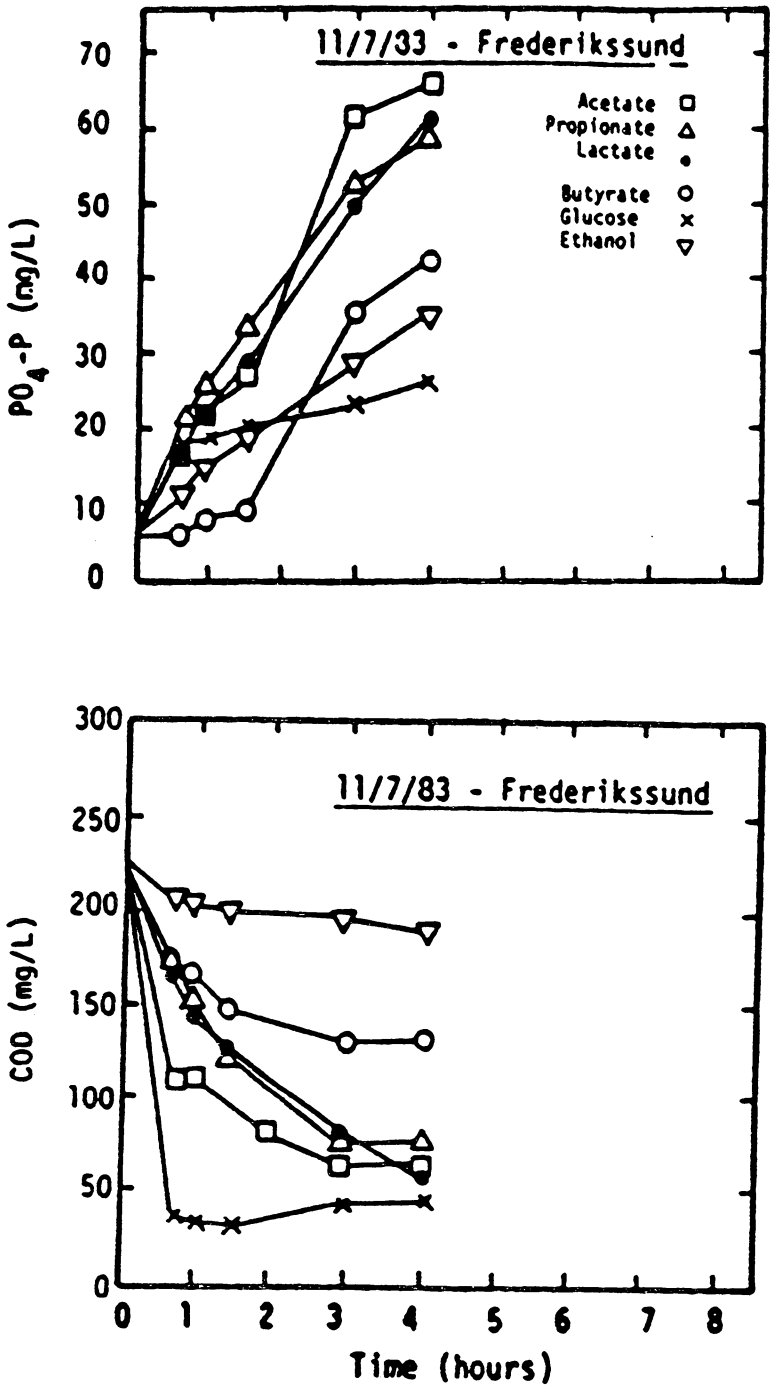


Figure 7. Anaerobic absorption of different substrates and phosphate release. (after Arvin and Kristensen, 1985).

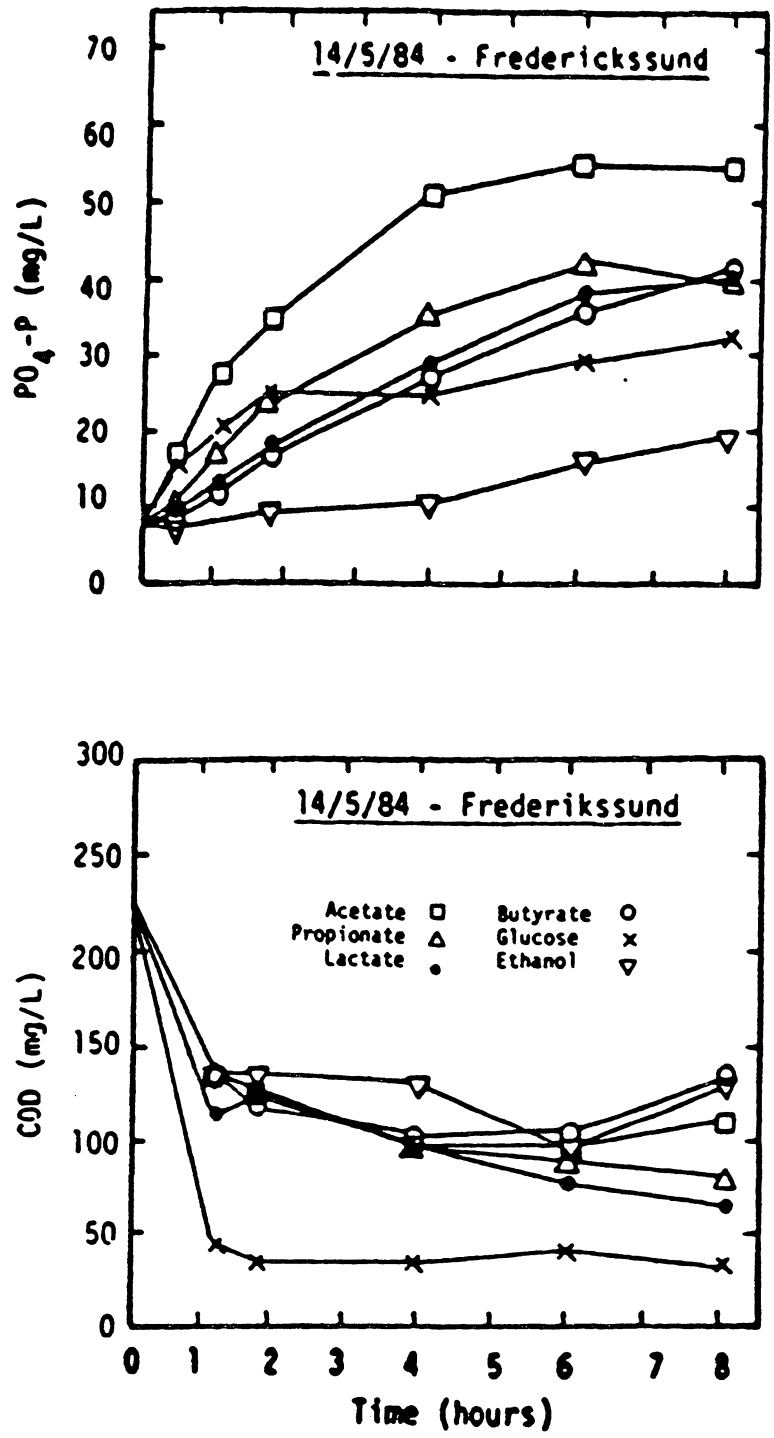


Figure 8. Anaerobic absorption of different substrates and phosphate release. (after Arvin and Kristensen, 1985).

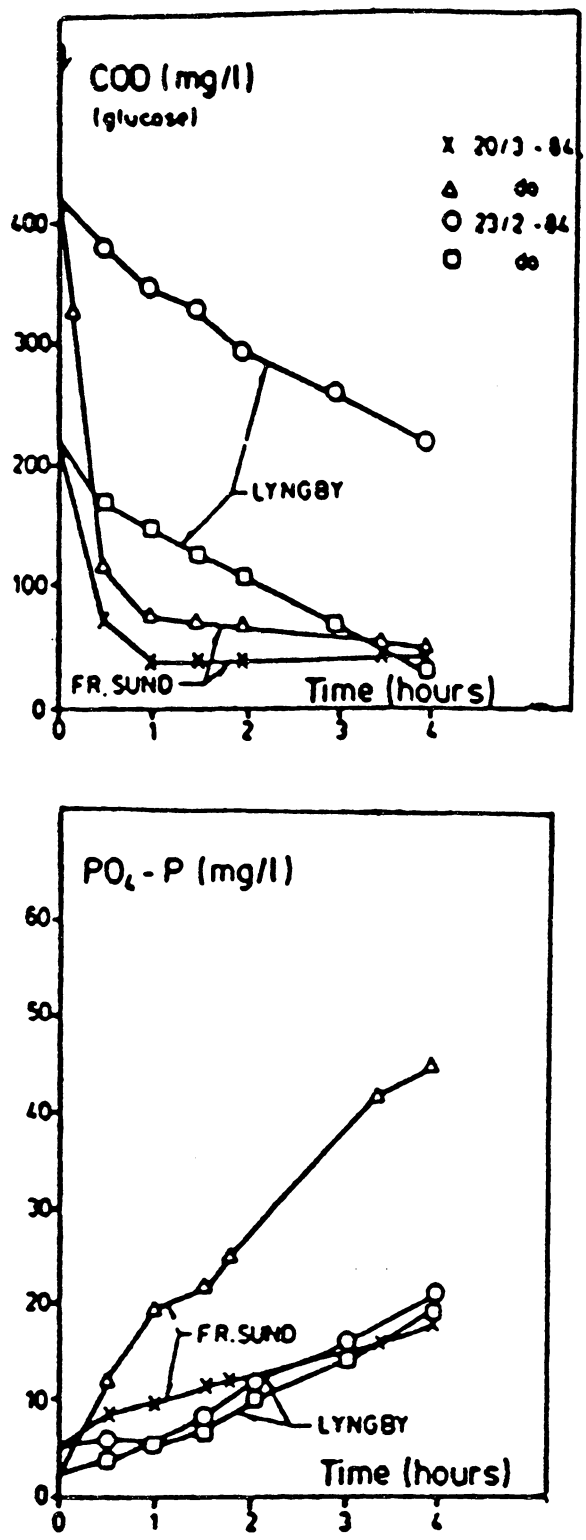


Figure 9. Glucose uptake patterns and corresponding phosphorus release for glucose-adapted and non-adapted sludges (after Arvin and Kristensen, 1985).

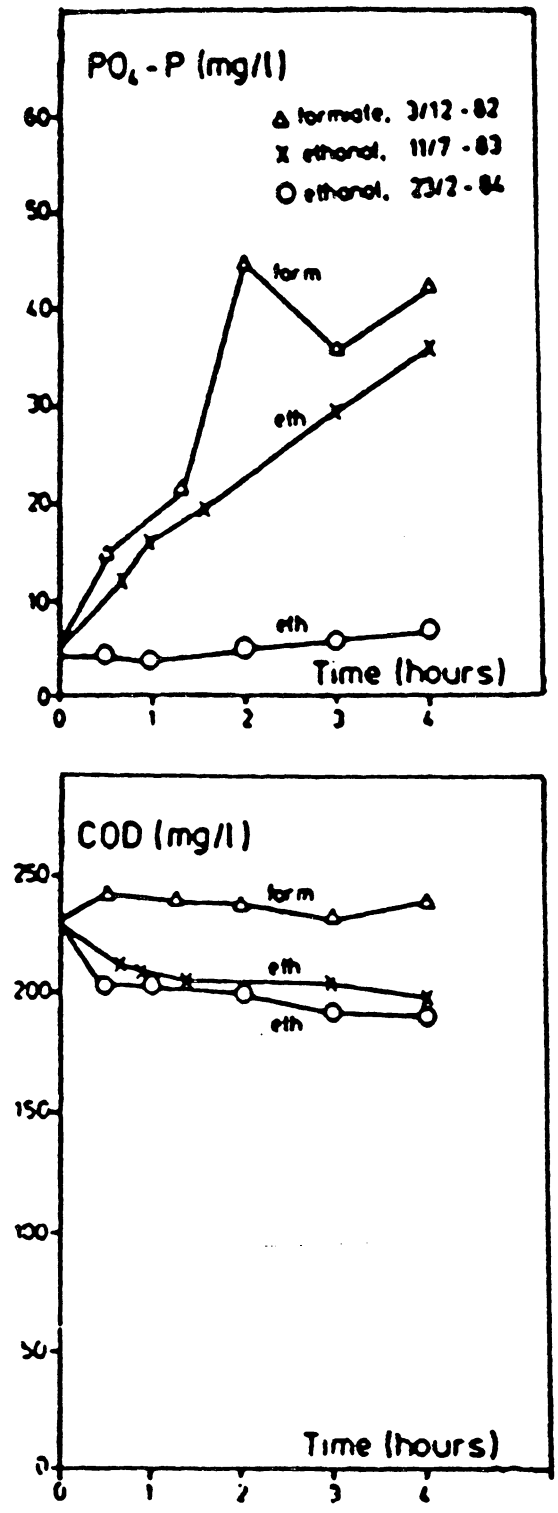


Figure 10. Examples of significant P-release with very small or no COD absorption of ethanol and formate (after Arvin and Kristensen, 1985).

The P/COD exchange ratios for acetate and propionate under steady state conditions were found to be:

Acetate: P/COD = 0.68 mg P/mg COD

Propionate: P/COD = 0.41 mg P/mg COD

Both substrates showed a molor ratio of about 1.5 as compared to a value of 1.0 reported by Fukase et al. (1982).

Lotter (1985) conducted series of batch experiments to study the effect of various substrate additions on phosphorus release from sludge under anaerobic condition. Sludge was taken from the aerobic zone of a 5-stage Bardenpho plant and placed in a beaker. Acetate and succinate were added to separate sludge samples to give a concentration of 100 mg/l. Samples were taken at 20 minute intervals and analyzed for orthophosphate. The sludge sample containing acetate showed a very rapid increase in orthophosphate concentration, about 55 mg/l, whereas the sample receiving succinate showed only a slight increase in phosphorus release, about 15 mg/l, as shown in Figure 11. These results agreed with results reported by Marais and Ekama (1982) on the stimulation of phosphate release by acetate.

Jones et al., (1987) have studied the effect of substrate addition on the release and subsequent uptake of phosphorus in biological phosphorus removal systems. A laboratory scale system was used which consisted of two anaerobic and four aerobic reactors, 1.5 l each, connected in series and oeprated with a sludge recycle ratio of 0.75. The total detention time of the system was about one hour. Different substrates, namely sodium acetate, acetic acid, butyric acid, ethanol and methanol were added to the first anaerobic reactor at different concentrations. Samples were analyzed for ortho and total phosphorus. The authors observed increases in phosphorus release in the anaerobic zone as the

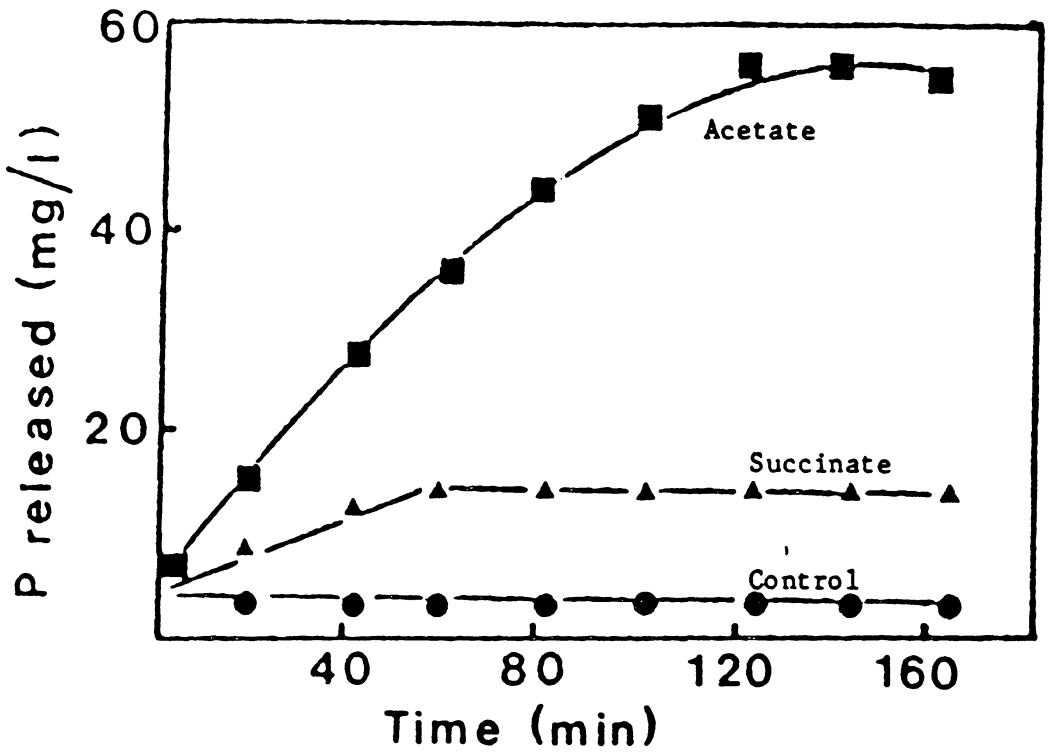


Figure 11. Phosphate release on addition of different substrates (after Lotter, 1985).

sodium acetate dose was increased (as shown in Figure 12). They also reported an optimum dose of sodium acetate which caused the most significant release of phosphorus. According to their observations, the release of phosphorus was substrate specific. Butyric acid caused the greatest release, whereas, acetic acid caused the least (as shown in Figure 13).

Comeau et al., (1987) conducted a series of batch experiments using 2.5 l Erlenmeyer flasks stirred with magnetic bars, to study the effects of short chain fatty acid, mainly acetate and propionate, additions on phosphorus release and uptake. In their study ten reactors were used and, to each reactor, about 30 mg of COD of the following substrates were added: acetate, acetate and propionate; propionate, formate, lactate, butyrate, valerate, fermented primary sludge and a control reactor. As shown in Figure 14, all substrate additions stimulated phosphorus release in the anaerobic period and phosphorus uptake in the aerobic period. The effect of substrate addition was greatest when a combination of acetate and propionate solution was added (as shown in Figure 15). They have also observed that the uptake of acetate and propionate, when added separately, occurred in 33 min whereas the uptake required only 22 min when the two substrates were added together. The author also observed the storage of PHB (poly-β-hydroxybutyrate) and PHV (poly-B-hydroxyvalerate) in the presence of various simple carbon substrates.

II.3.3.1 Factors Affecting VFA Production in BPR Systems

As mentioned earlier, it has been observed that the uptake of phosphorus in the anoxic and aerobic reactors is a function of the amount of phosphorus released in the anaerobic reactor, which is proportional to the concentration of volatile fatty acid (VFA) within the anaerobic reactor (Fukase et al., 1983; Arvin,

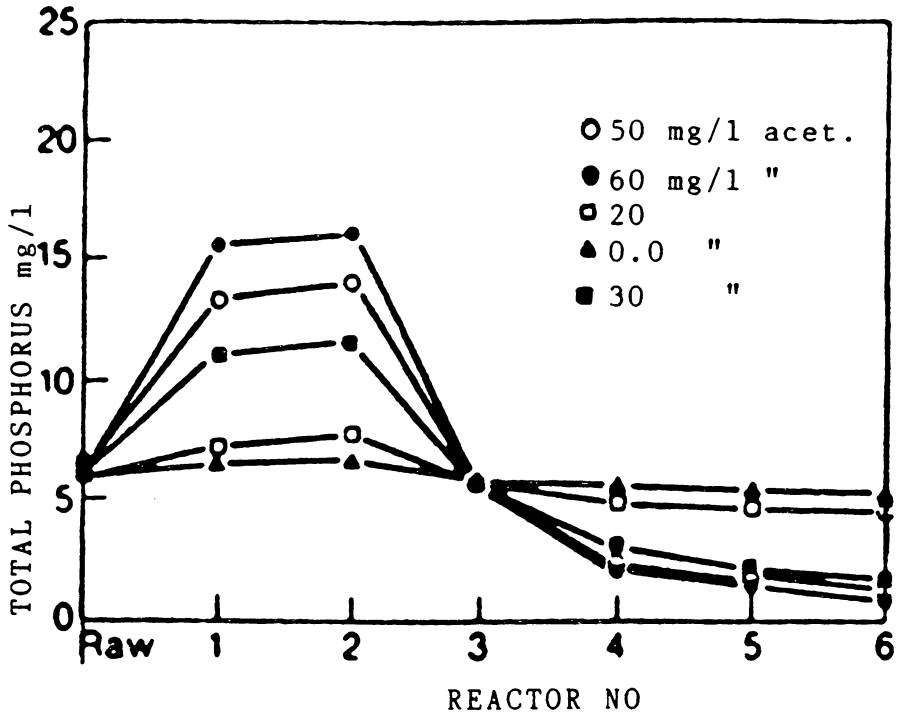


Figure 12. Phosphorus release and uptake at different doses of sodium acetate (after Jones et al., 1987)

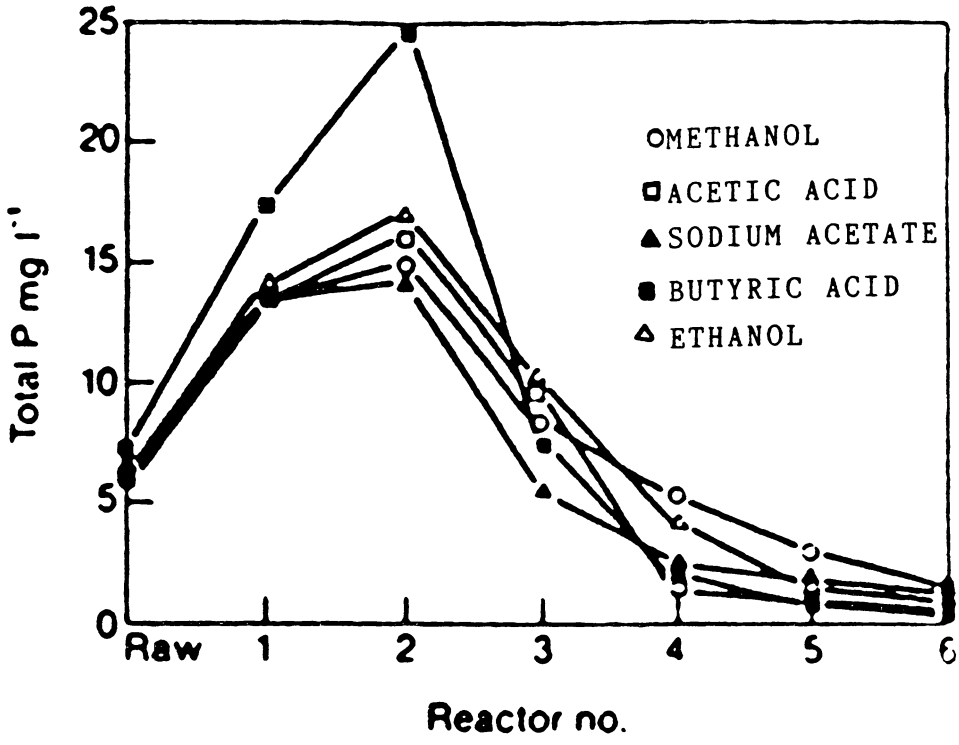


Figure 13. Phosphorus release and uptake by adding different substrates at a concentration of 50 mg/l as COD (after Jones et al., 1987)

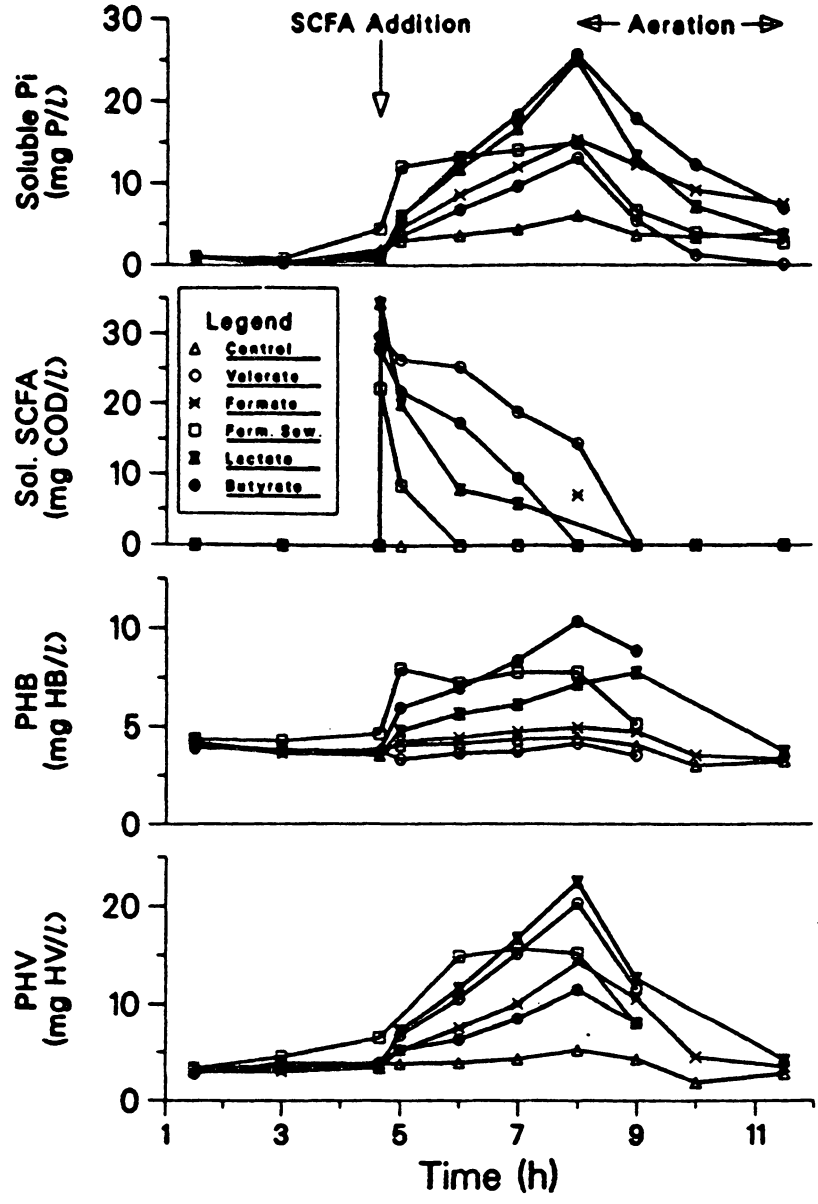


Figure 14. Effects of various volatile fatty acids addition on phosphate and PHB. (after Comeau et al., 1987)

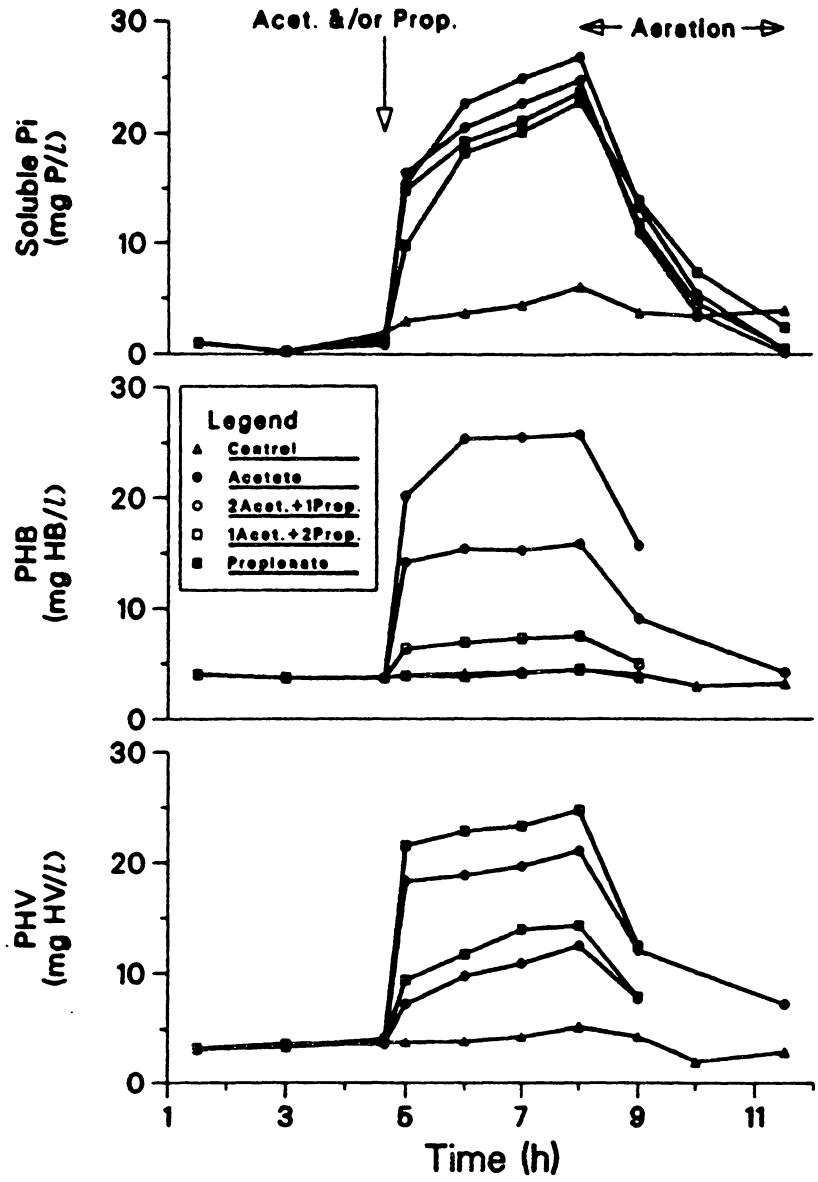


Figure 15. Effects of combinations of acetate and propionate addition (30 mg COD/l) on phosphate and PHA (after Comeau et al., 1987)

1985; Wentzel et al., 1984; Jones et al., 1987). Hence, many investigators studied different methods which can cause an increase in the production of VFA and potentially improve the efficiency of biological phosphorus removal plants.

Barnard, (1984) has proposed the activated primary sedimentation tank to enhance the production of VFA and, consequently, increase the primary phosphorus release, due to acetate uptake, which ultimately improves the phosphate removal ability of the plant. The proposed modification consists of recycling the thickened underflow to the primary sedimentation tank, thus providing a separate process for the production of VFAs which can be utilized in the anaerobic zone of the plant.

Oldham (1984), and Rabinowitz and Oldham (1985), have suggested the use of a fermentation unit ahead of the treatment plant to ferment primary sludge, thus forming acid-rich liquor which can be added to the anaerobic zone to improve the efficiency of the plant.

II.3.4 Effect of pH

Levin and Shapiro (1965) observed that the addition of hydrochloric acid caused phosphate stripping from sludge to solution. A similar effect was reported by Fuhs and Chen (1975) when carbon dioxide was added to a sludge sample under anaerobic condition. More recently, Potgieter and Evans (1983) studied the effect of pH on phosphate release from sludges obtained from an aerobic reactor. They observed maximum phosphate release at pH 4 and maximum phosphorus uptake at pH 8 as shown in Table 6.

Table 6. Effect of different pH levels on phosphate release (after Potgieter and Evans, 1983)

pH	Phosphate Release* $mg\ell^{-1}$
2	6.0
3	18.3
4	20.6
5	11.0
6	9.8
7 (Control)	5.9
8	-1.6
9	-5.0

*Negative value indicates phosphate uptake.

II.3.5 Effects of Oxidation-Reduction Potential (ORP)

Shapiro et al. (1967) proposed that phosphate release can be triggered by low oxidation reduction potential and that the anoxic release of phosphorus occurred because of the decrease in the redox potential and not due to decrease in dissolved oxygen concentration. Using laboratory-scale, batch-type, activated sludge units, Randall et al. (1970) studied different factors that affected phosphorus release. They observed that significant changes in oxidation reduction potential did not cause any soluble phosphorus release and phosphorus release usually preceded the change in ORP. On the basis of their observations, they concluded that anoxic phosphate release was not a function of the change in oxidation-reduction potential, but was more closely related to the availability of dissolved oxygen.

Koch and Oldham (1985) conducted a series of batch experiments, designed to simulate biological nutrient removal processes, to evaluate the usefulness of using (ORP) measurements as a control parameter. Specifically, they studied the relationship between measured ORP and phosphorus release in the anaerobic zone. As a result of their investigation, they observed, at times, that there was a good correlation between ORP and phosphorus release, as can be depicted from Figure 16. However, the observed relationship was not completely consistent and the authors proposed that ORP was more correlated to the disappearance of readily biodegradable organics than to phosphorus release.

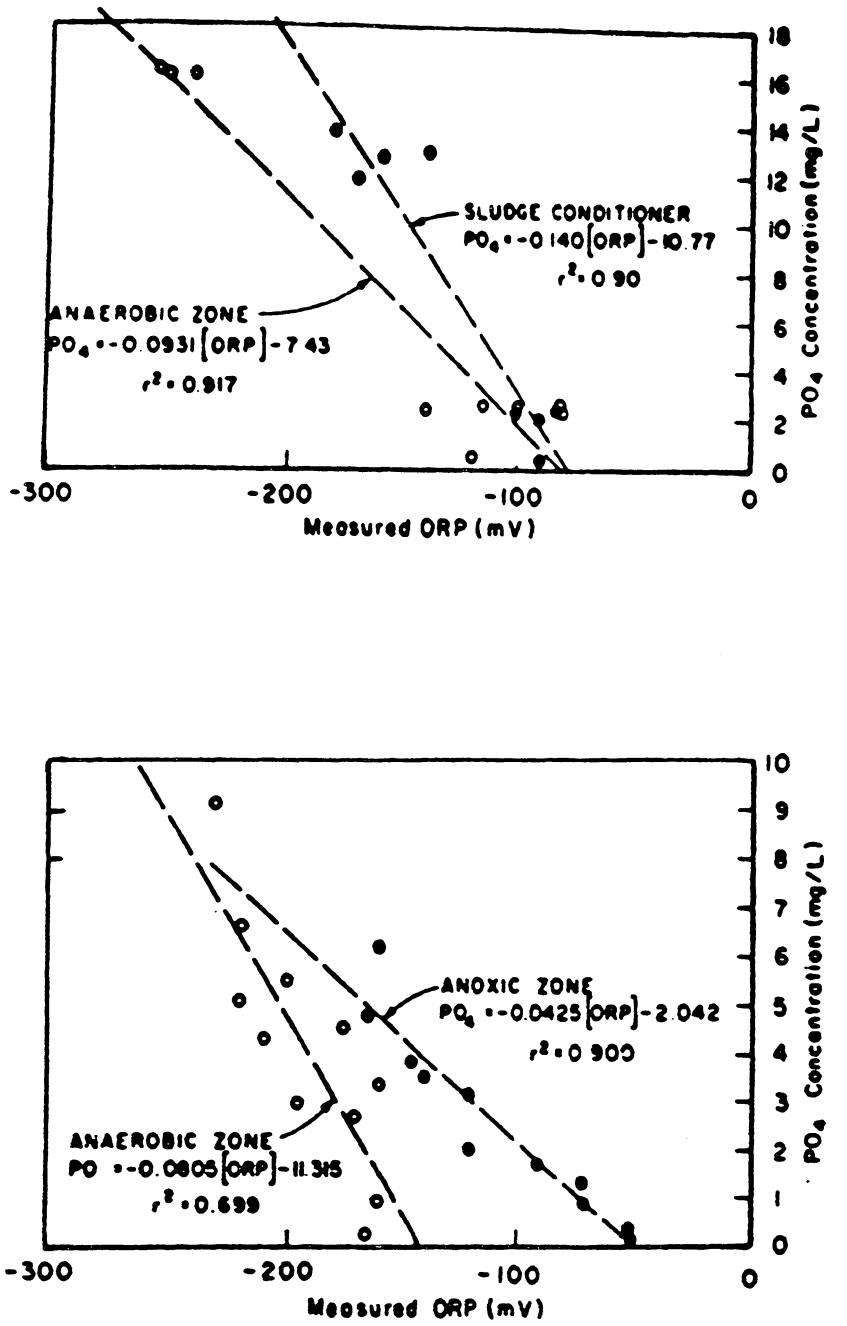


Figure 16. Linear regression of ORP and phosphate data - transient response testing (after Koch and Oldham, 1985)

II.3.6 Effect of Nitrate

The effect of nitrate on phosphorus release in biological phosphorus removal systems has been subjected to many investigations. It has been reported that the presence of nitrate in the anaerobic stage of excess biological phosphorus removal systems potentially reduces phosphorus release and ultimately reduces phosphorus uptake under aerobic conditions (Barnard, 1975; Kock and Oldham, 1984). Osborn and Nicholls (1978) reported that the effluent nitrate concentration should be kept less than 2 mg/l in order to achieve phosphorus release in the anaerobic stage. Venter et al., (1978) reported that low phosphorus release was observed when the effluent nitrate concentration was greater than 5 mg/l. Using series of batch experiments, Manning and Irvine (1985) studied the effect of nitrate on phosphorus release using continuous nitrate addition. Results of their investigation are shown in figure 17. As shown in the figure, although COD was added continuously and anaerobic condition was maintained, no phosphorus was released.

Nitrate elimination in the effluent could be achieved by complete denitrification or by suppressing nitrification in the aerobic stage of the treatment (Barnard, 1975). Pitman et. al., (1983) has reported that the addition of primary sludge of a BPR plant increased the amount of denitrification in the anoxic stage. Ekama et. al., 1983 have have reported different considerations in selecting treatment plant schemes based on the TKN/COD ratio of the wastewater, mainly to insure complete denitrification and accordingly good phosphorus release in the anaerobic zone of the treatment. Anoxic uptake of phosphorus ,that is nitrate is used as an electron acceptor, has been observed by several investigators

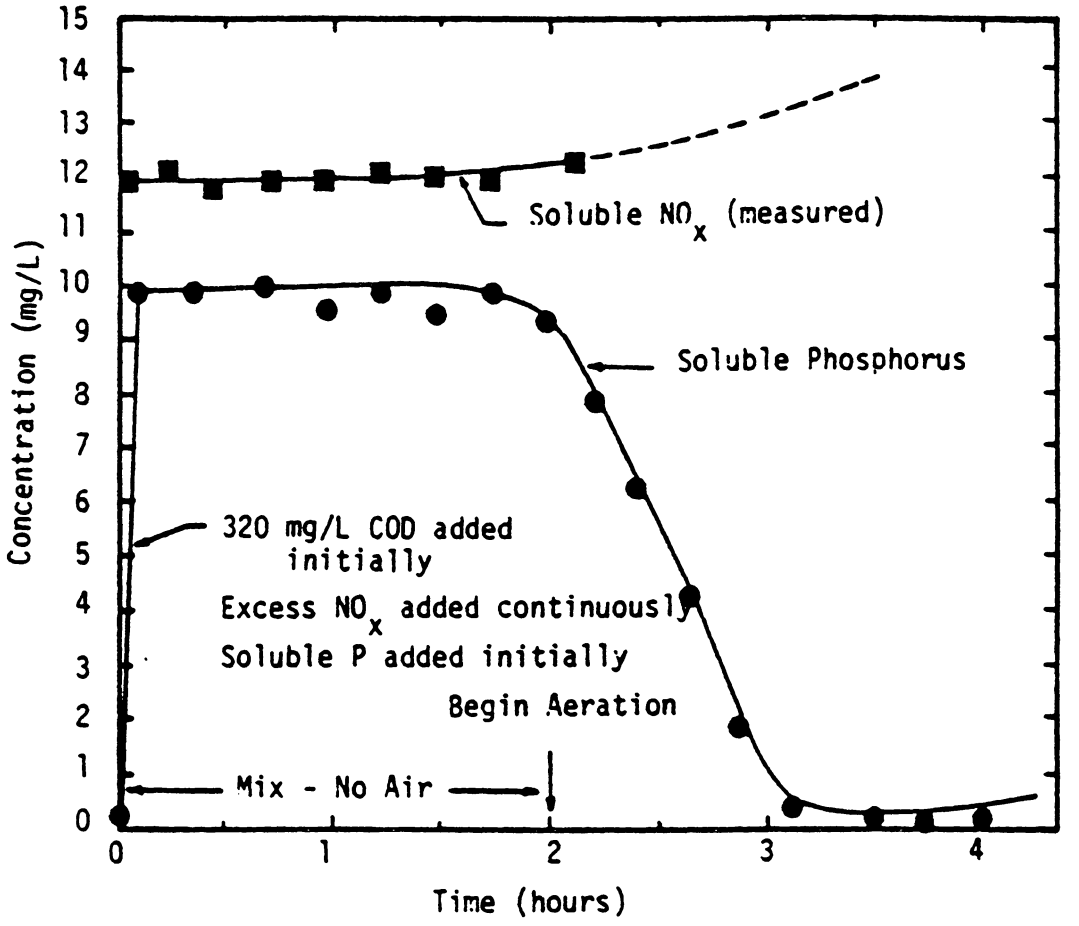


Figure 17. Effect of nitrate on phosphorus release/uptake (after Manning and Irvine (1985).

(Hascoet et al., 1984; Gerber and Winter, 1984). However, the anoxic uptake rates of phosphorus were found slower than uptake rates in the aerobic stage (Osborn and Nicholls, 1978; Gerber and Winter, 1984).

II.4 Mechanisms Proposed to Explain Biological Phosphorus Removal

Harold (1966) proposed two mechanisms to explain the accumulation of polyphosphate in volutin granules of some organisms, namely, "luxury uptake" and "overplus uptake."

The "luxury uptake" occurs when an essential nutrient other than phosphate was limited. This caused a nutrient imbalance, and nucleic acid synthesis was arrested. Since sufficient energy is available, polyphosphate will be transported across the membrane and accumulated inside the cell. When the limiting nutrient is made available, stored polyphosphate will be degraded and used for nucleic acid biosynthesis. Figure 18 shows the effect of sulfate starvation on polyphosphate accumulation by the "luxury uptake" mechanism. When certain microorganisms were first deprived of phosphate and then placed in an environment containing phosphate, rapid accumulation of polyphosphate occurred. This phenomenon was referred to as "polyphosphate overplus". Harold (1966) studied major enzymes evolved in the biosynthesis and degradation of polyphosphate. Polyphosphate kinase was found to be the major enzyme responsible for the synthesis of polyphosphate as illustrated in Figure 19. The

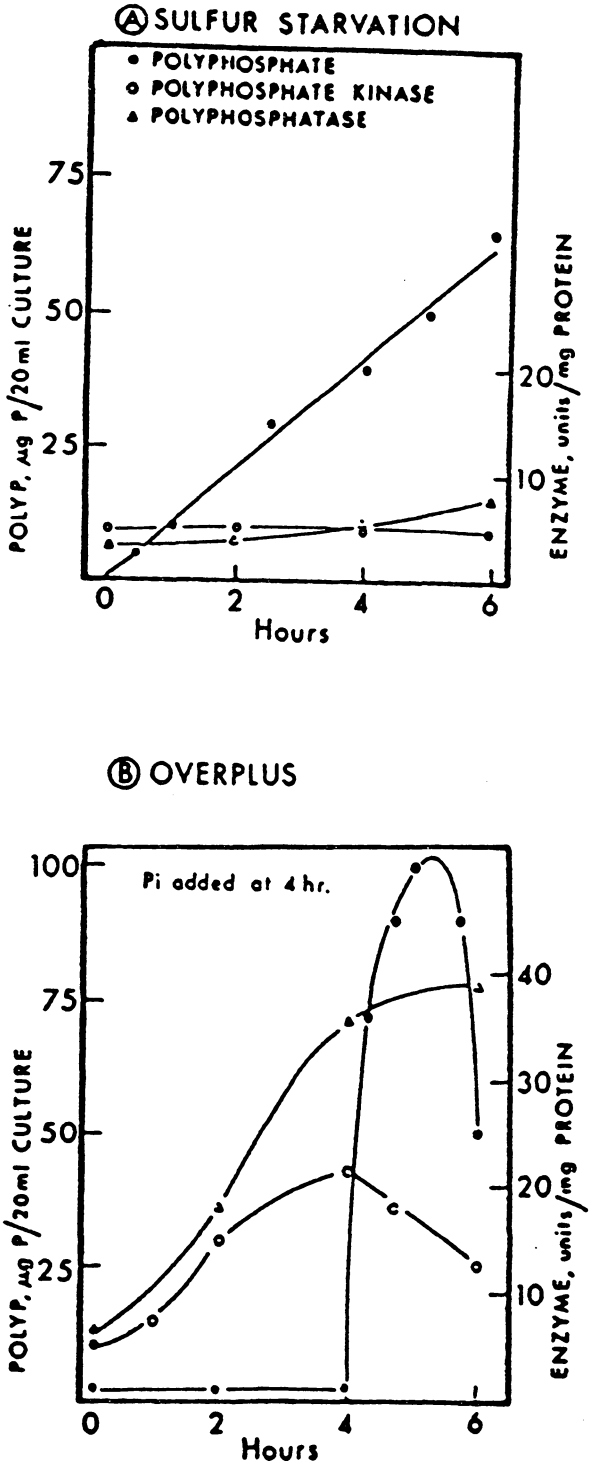


Figure 18. Effect of sulfate starvation on polyphosphate uptake (after Harold, 1966).

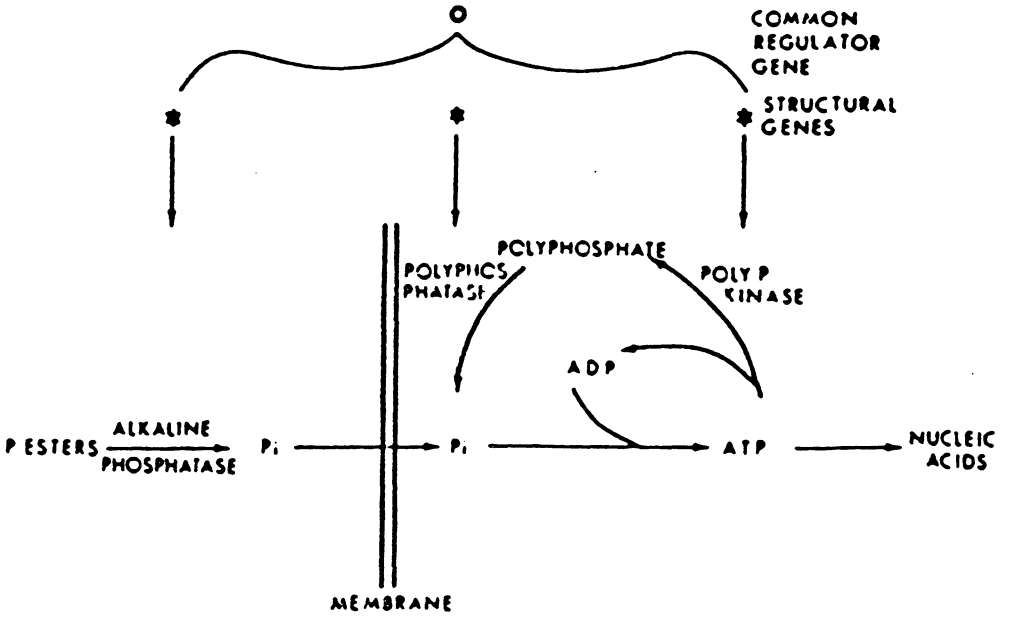


Figure 19. Polyphosphate cycle in aerobacter aerogenes and its genetic control (Harold, 1966).

major enzyme involved in the degradation of polyphosphate was the enzyme polyphosphatase. Other enzymes were also found to participate in the degradation process.

1. Polyphosphate kinase - catalyzing the high energy ATP formation from ADP.

2. Polyphosphate - AMP-phosphotransferase: transfer one phosphate group from polyphosphate to AMP and form ADP.

3. Polyphosphate glucokinase: catalyzing the phosphorylation of glucose in the sixth position

$$(P^1)_n + \text{glucose} \rightarrow \text{glucose 6-phosphate} + (P_1)_{n-1}$$

4. Polyphosphate fructokinase - a regulatory enzyme in the glycolysis, catalyzing the phosphorylation of fructose 6-phosphate in the first carbon position

$$(P_1)_n + \text{Fructose-6-phosphate} \rightarrow (P_1)_{n-1} + \text{Fructose 1,6-Diphosphate}$$

5. Polyphosphatase - catalyzing the hydrolysis of polyphosphate to phosphate units.

According to Harold the physiological functions of polyphosphate are an energy source, phosphate reserve, and metabolic regulator. Phosphate storage was considered to be the main function of polyphosphate in the cell, but polyphosphate is also used for the biosynthesis of nucleic acids, phosphalipids and other cell structures.

Fuhs and Chen (1975) found that some microorganisms accumulate polyphosphate under aerobic condition following an anaerobic stage during which phosphate is released due to polyphosphate degradation. They also stressed the importance of the anaerobic stage and its role in the establishment of organisms to carry out the fermentation process.

Nicholls and Osborn (1979) proposed the Poly-β-Hydroxybutyrate hypothesis in which Poly-p served as an energy reservoir to sustain the organism during the anaerobic stress and to build a storage of poly-β-Hydroxybutyrate (PHB). They proposed that PHB serves as a sink for hydrogen ions and electrons and, thus, the regeneration of NAD^+ required for the continuation of the fermentation process (conversion of glucose to pyruvate). They also indicated that a temporary stress producing situation must be introduced in order to enhance the storage mechanism. In order to achieve temporary stress, they suggested cutting the supply of oxygen and nitrates (producing anaerobic conditions) at certain locations in the treatment process. As a result, the aerobic bacteria will be forced to utilize the energy which has been already stored inside the cell in order to store available organics as PHB.

Marais et al. (1983) proposed the following hypothesis: poly-P accumulation does serve as an energy reservoir to sustain the organism during the anaerobic stressed state, but more importantly, it gives the poly-P organism a positive advantage over non-P accumulating organisms. They partition readily biodegradable COD (in the lower fatty acid form) in the anaerobic state for their exclusive use subsequently in the aerobic state. According to Marais et al. (1983), the ATPs formed under the aerobic condition would be used to transport and store the available readily biodegradable substrate inside the cell. When acetate was present in the anaerobic stage, only poly-P organisms could utilize it and converted it into acetyl-CoA by the use of two ATPs, thus, two phosphate molecules would diffuse to the surrounding solution according to the following reaction:

$$\text{acetate} + 2\,\text{ATP} + \text{CoA} \rightarrow \text{acetyl-CoA} + 2\,\text{ADP} + 2\,P_i$$

For continuity of the above reaction, the enzyme CoA would be regenerated by the following reaction:

$$2 \text{ acetyl-CoA} + 2 \text{ ATP} + 2 P_1 \rightarrow \text{acetoacetate} + 2 \text{ ATP} + 2 \text{ CoA}.$$

When glucose was available, two possible situations would arise: glucose cannot be utilized by poly-P organism or glucose can be utilized by poly-P organisms. In the former case, glucose would be fermented by facultative organisms into two acetate molecules, generating 2 ATPs for each glucose molecule fermented. This is done through the Embden-Meyerhof pathway.

$$\text{glucose} + 2 \text{ ADP} + 2 P_1 \rightarrow 2 \text{ acetate} + 2 \text{ ATP}$$

The acetate would then be utilized by the poly-P organisms as mentioned earlier.

In the second case, glucose can be utilized by poly-P organisms. Then, glucose would be utilized by both fermenters and poly-P organisms present in the anaerobic stage. However, Marais et al. (1983) proposed additional reactions by the poly-P organisms resulting in the formulation of PHB according to the following overall reaction:

$$\text{glucose} + 3 NAD^+ + 2 \text{ ADP} + 2 P_1 \rightarrow \beta\text{-Hydroxybutyrate} + 2 CO_2 + 2 \text{ ATP} + 2 \text{ NADH} + 2 H^+$$

The regeneration of NAD+ from NADH was suggested as being accomplished by other bacteria fermenting glucose to acetate according to the following reaction:

$$\text{acetoacetate} + \text{NADH} + H^+ \rightarrow \beta\text{-hydroxybutyrate} + NAD^+$$

Comeau et al. (1985) have proposed an improved biochemical model, compared to Marais; to describe the mechanism of excess biological P-removal. According to the model, see Figure 20, under anaerobic conditions substrate such as acetate will be transported across the membrane, by facilitated diffusion, in its ionic form, thus neutralizing H^+, which would cause a decrease in the pH gradi-

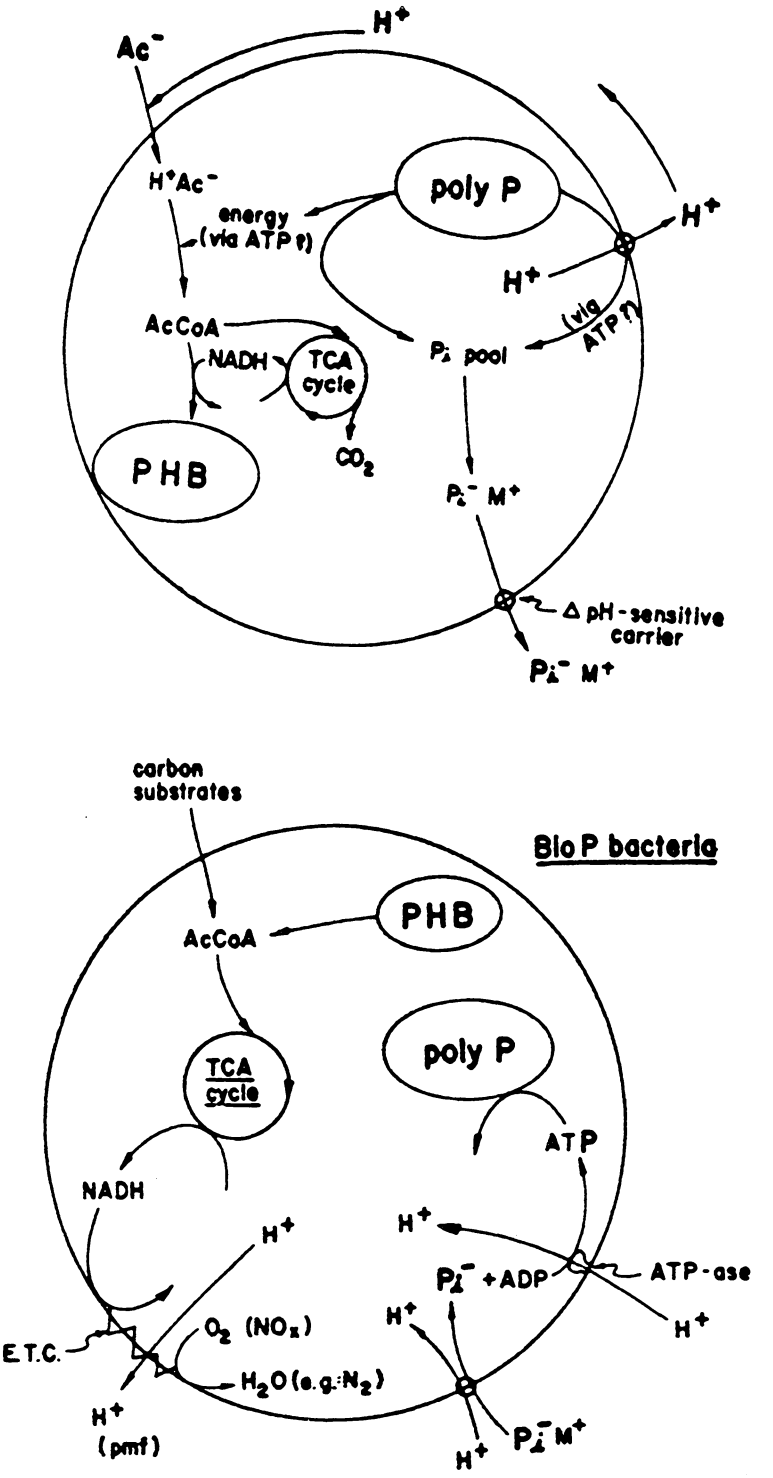


Figure 20. Postulated biochemical model for a) anaerobic and b) aerobic metabolism of bio-P bacteria (after Comeau et al., 1985)

ent and, thus, in the proton motive force. The proton motive force will be reestablished by phosphate expulsion from polyphosphate reserves inside the cell. The transported acetate will be metabolized and stored inside the cell as PHB. Under aerobic conditions, poly-P bacteria convert PHB into acetyl-CoA which is further metabolized via the TCA cycle with the generation of ATPs, CO_2 and reducing power (NADH, $FADH_2$). Regeneration of NAD^+ and FAD occur via the electron transport system in which electrons are transported to the terminal electron acceptor (oxygen or NO_x), and proton gradient is developed across the intracellular membrane. Thus establishing the H^+ gradient used for ATP generation and consequently, for polyphosphate synthesis and storage inside the cell.

In summary, there is no universally acceptable or conclusively proven mechanistic explanation for the phenomenon of excess biological phosphorus removal. Therefore, all systems have been designed based on empirical observations reported by different researchers and plant operators. The following are general behavior characteristics for biological phosphorus removal systems observed by different investigators.

1. Anaerobic-aerobic sequencing is essential in order to accomplish excess biological phosphorus removal.

2. Phosphorus is released only under strict anaerobic conditions and when readily biodegradable organics (such as acetate) are present.

3. Excess phosphorus uptake occurs in both aerobic and/or anoxic conditions if P release has been obtained in the anaerobic stage, (Comeau, et al., 1985).

4. Under anaerobic condition, neither oxygen nor nitrate is present. The bacteria release phosphate into the solution and accumulate PHB or other readily biodegradable organics. Under aerobic or anoxic conditions, poly-P accumulates

in the cells by the overplus mechanism and stored PHB is utilized as an energy source.

5. Influent wastewater characteristics should be known with great surety before selecting and designing specific biological phosphorus removal systems.

6. For TKN/COD ratios less than 0.08 mg N/mg COD, the phoredox process can be selected. For TKN/COD ratio greater than 0.08 the UCT should be selected. The modified UCT should be selected when the influent wastewater flow and/or characteristics is highly variable with time.

7. Sufficient quantity of readily biodegradable substrate (fermentation products, mainly acetate) is a prerequisite for excess biological phosphorus removal.

8. The larger the mass of sludge recycled through the anaerobic reactor, the higher the phosphorus removal (Siebritz et. al., 1982)

9. Higher phosphorus removal efficiencies occur at high COD:P ratios and lower sludge ages (Daigger et. al., 1987)

10. Polyphosphate and PHB metabolism seems to play an important role in biological phosphorus removal.

II.5 Treatment Systems Used for Biological Phosphorus Removal

All systems used for biological phosphorus removal are modifications of the activated sludge system. Such modifications were made to stimulate the best

environmental conditions for the release and uptake of phosphorus. These systems include: PhoStrip; modified Bardenpho (Phoredox); A/O (anaerobic-oxic); UCT (Modified Phoredox); the modified UCT; and Rotanox processes. Process configurations and system analysis will be discussed in this section.

II.5.1 PhoStrip Process

The PhoStrip system is an activated sludge system in which phosphorus is removed from effluent wastewaters by biological-chemical processes. The phosphorus is concentrated in a sidestream and precipitated by chemical additions. As depicted in Figure 21, part of the settled sludge is subjected to phosphorus release using an anaerobic unit. The supernatant from the anaerobic unit, which contains concentrated orthophosphate, is then chemically treated using lime or iron salts for phosphorus precipitation. PhoStrip systems have operated successfully with MLSS concentrations ranging from 600 to 5000 mg/l. Tetreault et al. (1986) have reported that an effluent phosphorus concentration of 1 mg/l can be achieved by PhoStrip process under nitrifying condition without tertiary treatment. The PhoStrip system is a patented process, with patent rights owned by Biospherics, Inc., of Beltsville, Maryland.

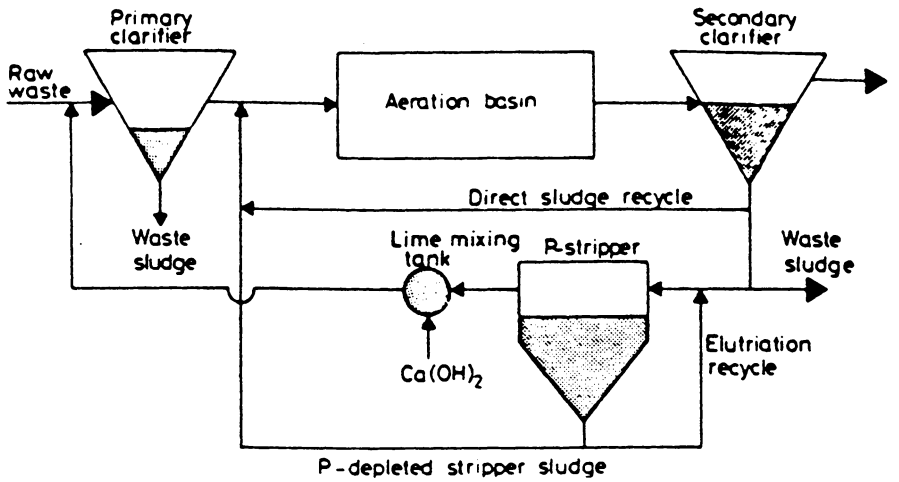


Figure 21. Schematic diagram of PhoStrip process

II.5.2 A/O Process

The A/O (anaerobic/oxic) process is an activated sludge process for biological phosphorus removal which consists of an anaerobic stage followed by an aerobic stage. The stages may be divided into sections as shown in Figure 22. Underflow from the secondary clarifier is returned to the anaerobic reactor. Phosphorus is released in the anaerobic unit as orthophosphate and organics are stored. Phosphorus uptake and organics metabolism occurs in the oxic units (aerobic stage). This system is not feasible when operated at a high sludge age because of the inhibitory effect of nitrogen oxides, i.e., nitrification products, on the fermentation process in the anaerobic zone and consequently on the performance of biological phosphorus removal. The recommended mixed liquor suspended solids in the oxic unit is around 2000 mg/l, with design organic loadings between 0.15 and 0.7 kg BODs/kg MLSS-d (WPCF Manual of Practice, 1983). This process is identical to the three-stage Phoredox (modified Bardenpho) process. The A/O system is a patented process with patent rights owned by Air Products and Chemicals, Inc., Allentown, Pennsylvania.

II.5.3 Phoredox Process

The three-stage Phoredox process is a modification of the Bardenpho process designed to accomplish biological removal of both nitrogen and phosphorus. The sechamatic diagram for the Bardenpho process is shown in figure 22 and that for the phoredox process is shown in figure 23. As shown in Figure 24, the

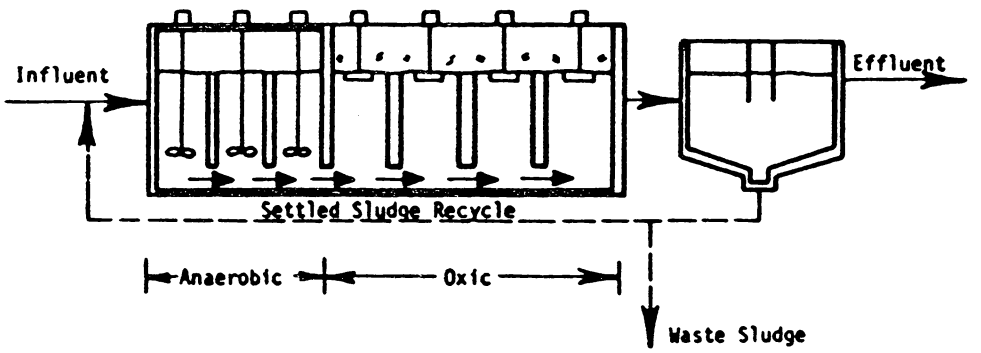


Figure 22. A/O process configuration

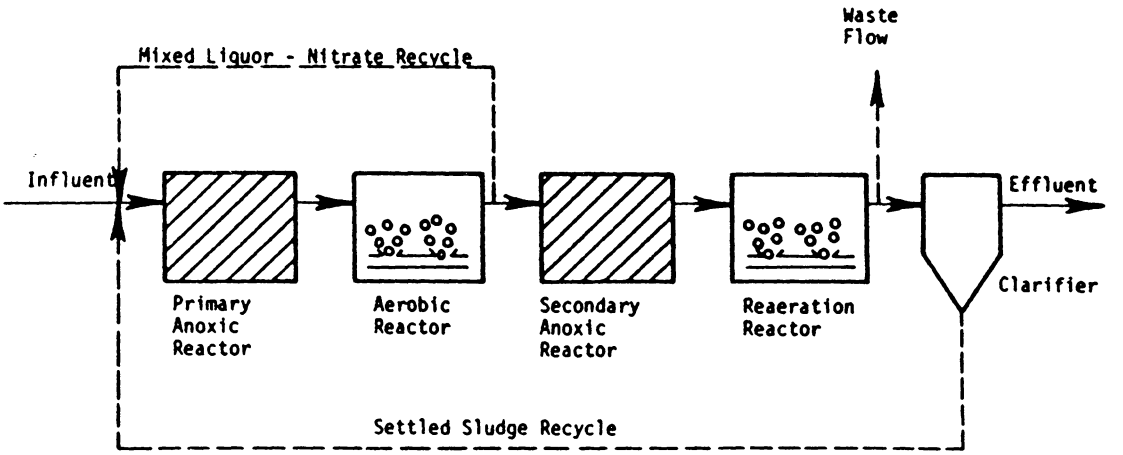
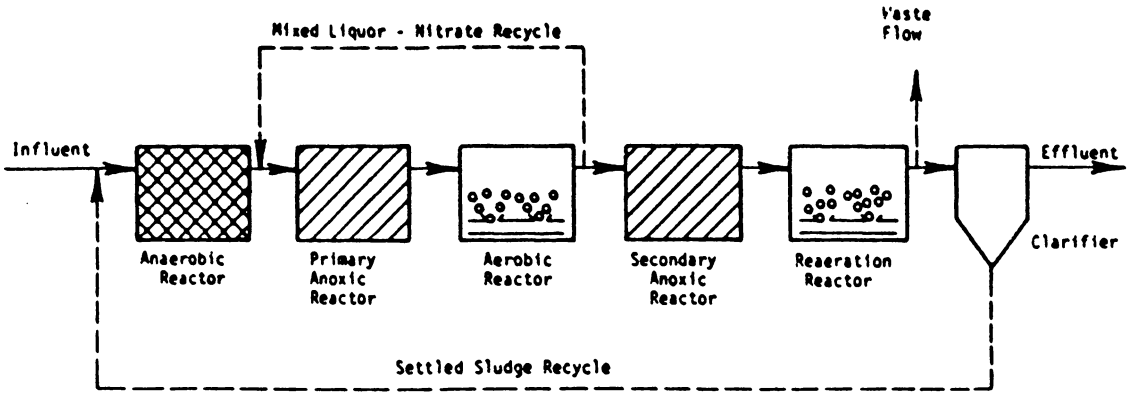


Figure 23. Bardenpho process configuration



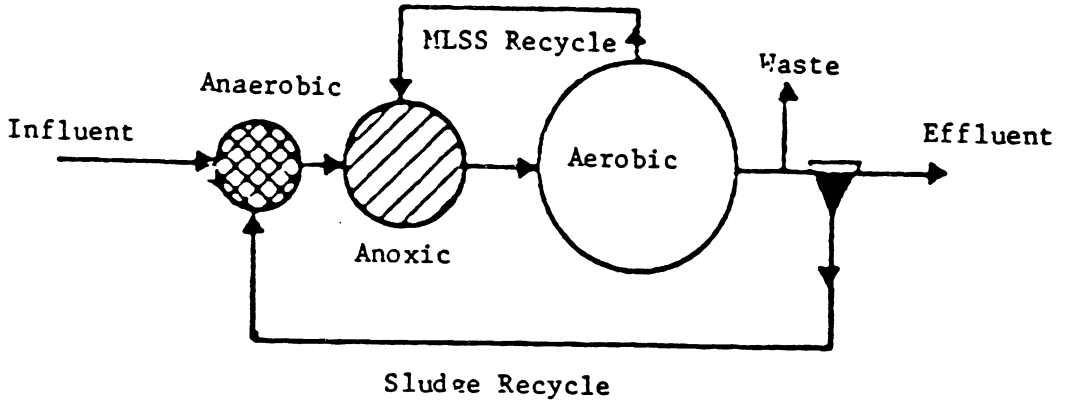


Figure 24. Phoredox process configuration: a) 5-Stages, b) 3-Stages

three-stage phoredox process consists of one anaerobic, one anoxic and one aerobic reactor. Underflow from the clarifier is returned to the first anaerobic basin and the mixed liquor from the aeration basin effluent is recycled to the first anoxic basin. In the anaerobic unit, fermentation and phosphorus release are achieved. In the aerobic unit, nitrification and phosphorus uptake are achieved, along with stabilization of stored organics. Denitrification and some phosphorus uptake occur in the anoxic units. Phosphorus is removed from the system in the waste sludge.

In this process, less anoxic mass is available for denitrification purposes because fraction of the total mass should be established in the anaerobic unit as prerequisite for excess phosphorus removal. To accomplish complete denitrification in the anoxic section, a lower influent TKN/COD ratio should be allowed for in the Phoredox process as compared with the Bardenpho process, which has a larger anoxic sludge fraction. Siebritz et al. (1983) proposed an upper limit of the TKN/COD ratio of 0.07 to 0.08 mg N/mg COD for successful operation of the Phoredox process. They defined successful operation as a treated effluent with less than 1.0 mg/l total phosphorus. Since the raw influent TKN/COD ratio of municipal wastewaters usually ranges between 0.07 to 0.09 mg N/mg COD, and is about 0.10 mg N/mg COD for settled sludge, this process will frequently not achieve an effluent concentration of less than 1.0 mg/l from municipal wastewaters (Siebritz, 1983).

II.5.4 UCT Process

The UCT process (Figure 25) is a modification of the Phoredox process. In this process, the settled sludge in the secondary clarifier is returned to the anoxic unit rather than the anaerobic unit. The mixed liquor from the effluent of the anoxic unit is then recycled to the anaerobic unit, therefore, the anaerobic reactor is protected from the nitrate concentration in the effluent. This configuration will cause a greater reduction of the nitrate concentration in the underflow recycle to the anaerobic unit. Therefore, higher phosphorus removal is expected as compared with the Phoredox process.

Siebritz et al. (1983) have reported an upper limit to the TKN/COD ratio of about 0.14 mg N/mg COD (at 14°C and 25 days sludge age) for adequate operation of the UCT process, assuming maximum recycle of nitrate (4 Q) to the anoxic unit. When the TKN/COD ratio of the effluent wastewater is above 0.14 mg N/mg COD, nitrate will be returned with the settled sludge to the anaerobic reactor leading to a decline in P removal efficiency. Since the upper limit of the TKN/COD ratio of the UCT process is 0.14 mg N/mg COD, the UCT process can successfully be used for both nitrogen and phosphorus removal from municipal wastewaters (TKN/COD ratio is about 0.08 mg N/mg COD). When the TKN/COD ratio increases above the upper limit (0.14 mg N/mg COD) the concentration of nitrates in the recycle line to the anaerobic reactor will increase causing a decline in the phosphorus removal. To avoid excess discharge of nitrate to the anaerobic unit, the recycle ratio should be reduced. However, the decrease in the recycle ratio will cause an increase in the anoxic retention time

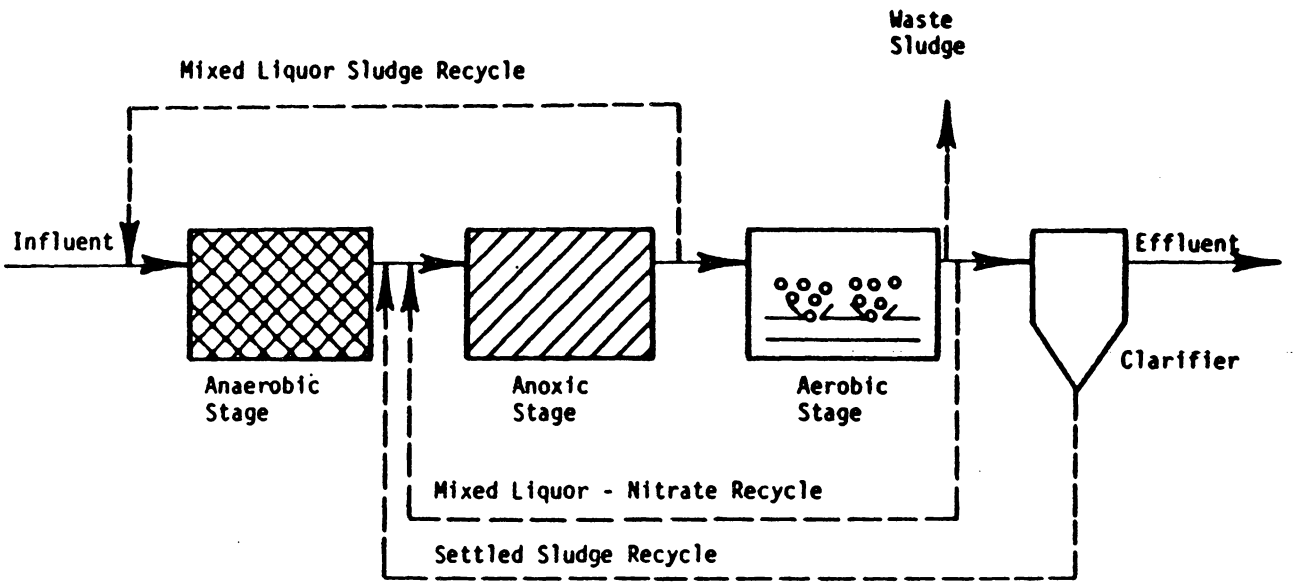


Figure 25. The UCT process for biological nitrogen and phosphorus removal

leading to poor sludge settling characteristics. To overcome this problem a modified UCT was developed.

II.5.5 Modified UCT Process

The modification of the UCT process was developed to overcome the poor settling characteristics of the sludge generated by the UCT process (Ekama et al., 1983). This process is made by using two anoxic units following the anaerobic reactor. The first anoxic unit receives the recycled sludge from the secondary clarifier and discharges the mixed liquor to the anaerobic reactor by another recycle (Figure 26). Low anoxic detention time could be maintained, improving sludge settleability, by increasing the mix liquor nitrate recycle flow without causing excess nitrate level in the anaerobic unit. Therefore the modified UCT process is believed, by its developers, to be the most consistent phosphorus removal process that generates settleable sludges. The modified UCT process can be employed successfully for excess biological phosphorus removal for wastewaters having influent TKN/COD ratios below 0.11 mg N/mg COD (Ekama et al., 1983).

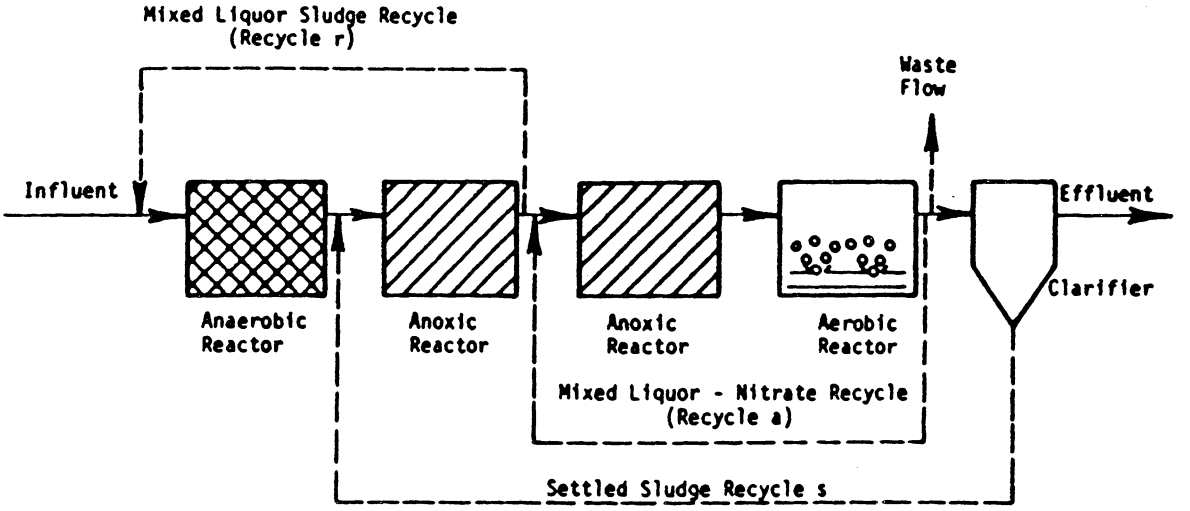


Figure 26. The modified UCT process for biological nitrogen and phosphorus removal

III EXPERIMENTAL METHODS

To enable this investigation, a pilot plant system was designed and constructed. The pilot plant system will be described in detail in this chapter. Start up, mode of system operation, monitoring and maintenance of the pilot plant will also be discussed. Further, the nature and characteristics of the wastewater influent to the pilot plant system, sample collection, preservation and analytical methods used in this study will be presented. Finally, the experimental approach used to carry out the present investigation will be described.

III.1 Description of the Pilot Plant System

To carry out the research investigation, a pilot plant system was constructed and housed in a small room, 8 feet wide, 10 feet long and 7 feet high, located near a sewage manhole 100 feet west of Oak Road, Virginia Tech, Blacksburg. The temperature inside the room was controlled during cold weather using a portable

thermostatted convection electric heater. thermostat control knob. A ventilation fan was also installed.

The pilot plant system consisted of six identical reactors and a clarifier. Each reactor, constructed of 9.5mm plexiglass sheets, and each reactor was 20 inches high, 8 inches wide and 8 inches long. Openings for wastewater flow, sampling ports, and recycle lines were 1 inch diameter. A free board of 4 inches was provided to avoid possible overflow during mixing and aeration. Therefore, the liquid volume contained by each reactor was about 19 liters. Removable covers were made for three reactors in which centered holes were drilled for paddle rods. Although the calculated head loss for the desired gravitational flow rate between two reactors was 1 inch, two inches of head was provided between each two units to insure sufficient head. A large wooden table was designed and constructed to support the reactors and to provide the necessary head between the units. Mixing was provided using variable speed mixers (1-100 rpm) from which stainless steel paddle rods extended. To each paddle rod, three plexiglass blades of 6 inches long and 2.5 inches high were equally positioned along the rod.

The clarifier was constructed by joining a 10-inch diameter plastic funnel to a 10-inch diameter industrial sewage pipe. The clarifier inlets and outlets were constructed using 1-inch diameter PVC pipe. The clarifier was designed as a vertical feed type. To scrape the solids attached to the bottom of the clarifier, central V-shaped steel blades were provided. The scraper was rotated at 3 rpm using an electrical motor mounted above the clarifier. The clarifier was housed in a wooden structure. The outlet from the clarifier was connected to the manhole using 1 inch diameter PVC pipe.

Five pumps were used to operate the system. The wastewater from the manhole to the system feeding tanks. Recycles were operated using a Cole-

Palmer (Chicago, IL) 7553-50 drive with 7017-20 Master-flex pump heads. Similar pumps were used to pump the raw wastewater and the tested chemical, to the first anaerobic reactor. To avoid possible clogging, the sludge recycle line was operated using a Cole-Palmer (Chicago, IL) 7553-60 drive with 7018-20 Master-flex pumphead which uses larger size of tubing. For anoxic and MLSS recycles, 4.5mm diameter Tygon tubing was used where, 6.0mm diameter tubing was used for sludge recycle. To aerate the system, two air compressors were used. A Gast (Benton Harbor, Michigan) Model DOA-P106-AA air compressor was used to aerate the first two aerobic reactors and a Thomas Industrial Inc. (Sheboygan, Wisc.) Model 107CA18 air compressor was used to aerate the third aerobic reactor. Compressed air was connected to two porus diffuser stones placed in each aerobic reactor. The aeration rate was adjusted using a control valve, to provide the desired dissolved oxygen concentration in each reactor of the aerobic zone of the treatment.

A 26 gallon plastic tank was connected, with PVC pipe, to a 32 gallon tank and used as an equalization basin for the feed wastewater. A nitrogen gas cylinder was installed beside the feeding tanks and connections were made, using 6mm dia. plastic tubing, to bubble nitrogen gas in the feeding tanks. Two large stone diffusers, 8 inches long, were used to strip the dissolved oxygen from the raw wastewater.

III.2 Mode of Operation

The pilot plant system was operated as a UCT process. All reactors were operated in series. The first two reactors were operated anaerobically, the third reactor was operated as an anoxic reactor and the last three reactors were operated aerobically. Configuration of the pilot plant system is shown in Figure 27. There were three recycle lines within the pilot plant, sludge recycle from the underflow of the clarifier to the anoxic unit, mixed liquor and nitrate recycle from the last aerobic reactor to the anoxic reactor and the anoxic recycle from the anoxic unit to the first anaerobic unit. The designed flow rate of the pilot plant system was 0.15 liter/minute. The hydraulic detention times in the anaerobic, anoxic and aerobic zones of the treatment were 4.1, 2.1, and 6.1 hours, respectively. The actual hydraulic retention times in the anaerobic, anoxic, and aerobic zones were 2.1, 0.7, and 3.2 hours, respectively. The design parameters of the pilot plant system are shown in Table 7.

III.3 Nature and Characteristics of the Influent Wastewater

Raw domestic wastewater was used as the feed to the pilot plant system. The wastewater was pumped from a 10 foot deep manhole at the rate of about 15 gallons per minute and stored in a 58 gallon volume feeding tank. Pumping

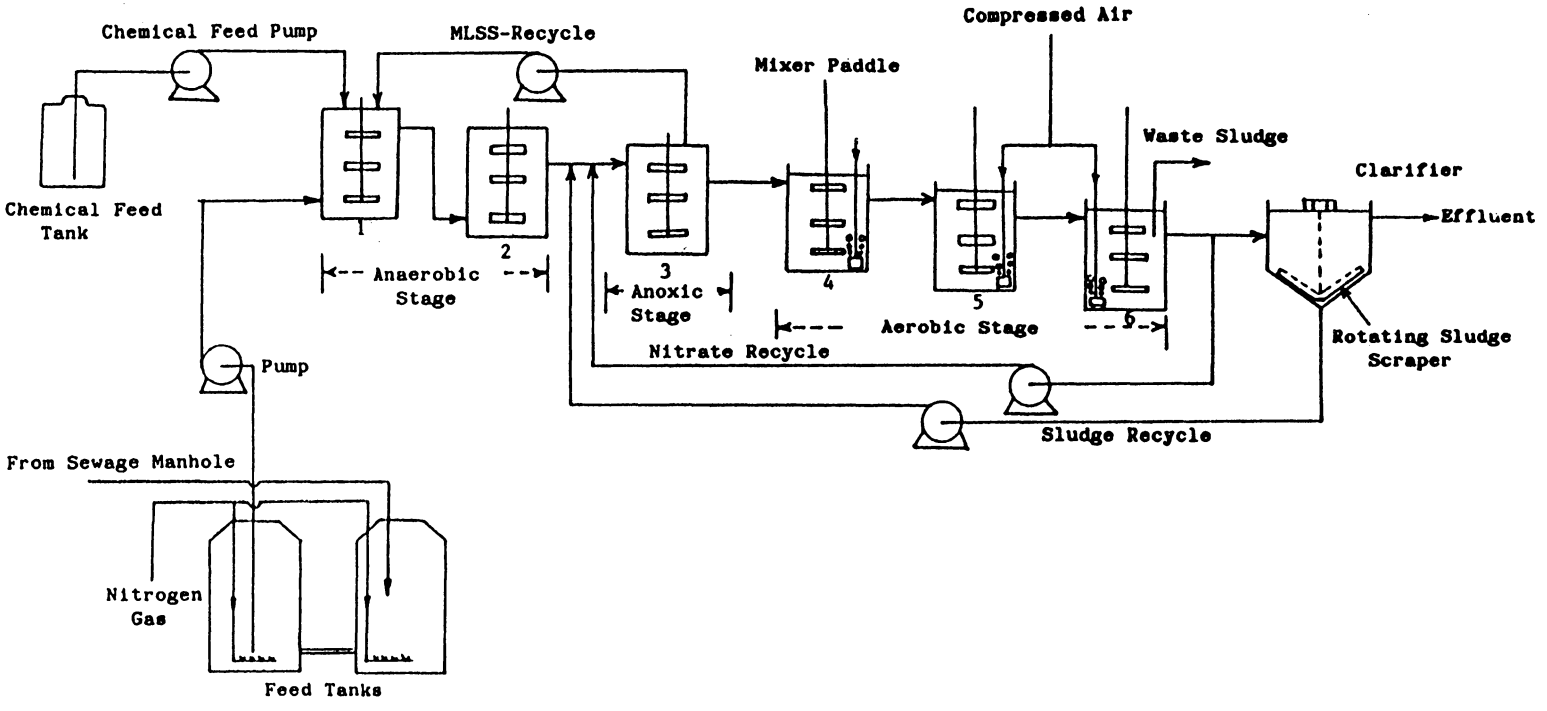


Figure 27. Schematic diagram of the pilot plant system

Table 7. Design parameters for the pilot plant system

Stage	Reactor	Depth (inches)	Volume (liters)	Nominal Detention time (hrs)	Total Nominal HRT of stage (hrs)	Actual HRT (hrs)
Anaerobic	1	17.3	18.1	2.0	4.1	1.0
	2	18.0	18.9	2.1		
Anoxic	1	18.0	18.9	2.1	2.1	0.7
Aerobic	1	18.0	18.9	2.1	6.1	1.1
	2	18.0	18.9	2.1		1.1
	3	16.0	16.8	1.9		1.0
Total Biological Reactor Nominal HRT					12.3	
Clarifier		24	25	2.8	2.8	
Total System Nominal HRT					15.1	

Note: System was operated at an influent wastewater flow rate of 0.15 liter/min.

the wastewater from the sewage manhole was one of the major problems faced during the present investigation. Initially, a centrifugal pump was used to pump the wastewater from the manhole, and it did not perform satisfactorily because of the high velocity and shallow depth of the wastewater flowing through the sewer. That is, when pumping was started a vortex was formed allowing the air to enter the free end of the suction pipe. To overcome the problem, a submersible effluent pump (Model 55, Zoeller Co., Louisville, KY) was installed inside the manhole. When the pump was not in operation, it was kept above the surface of the flowing wastewater to avoid possible clogging of the pump's inlet or the pump being carried away by the flow. During pumping, the pump's inlet was totally submersed in the wastewater.

The composition of the influent wastewater to the pilot plant system is shown in Table 8. As can be learned from the table, the wastewater was a typical medium strength municipal wastewater. All essential nutrients required for biological growth were present. The average TKN/COD ratio was 0.083 and the average P/COD ratio was 0.02. No measurable concentrations of any volatile fatty acid were found in the wastewater, indicating that the wastewater was not undergoing any fermentation while traveling in the sewer system. The dissolved oxygen concentration in the wastewater averaged 2.5 mg/l. Because maintaining anaerobic conditions in the anaerobic zone of excess biological phosphorus removal systems has been stressed by many investigators as an important factor for the success of these systems, efforts were directed towards reducing the dissolved oxygen concentration in the wastewater before treatment. Initially, 8 mg/l sodium sulfite, as a reducing agent, and 2 mg/l cobalt chloride, as a catalyst, were added to the wastewater. As a result, the dissolved oxygen concentration in the wastewater decreased to about 1.5 mg/l. To further decrease the level of dis-

Table 8. Composition of influent wastewater to the pilot plant system

Constituent	Mean Value, mg/l
Solids, Total	590 ±80
Dissolved, Total	340 ±70
Fixed	180 ±40
Volatile	160 ±30
Suspended, Total	250 ± 60
Fixed	25 ±15
Volatile	225 ±50
Organics	130 ±45
DOC	84 ±15
COD	330 ±40
BOD_5	200 ±35
Phosphorus, Total	6 ±2.5
Organic	2.1 ±0.7
Ortho	4.0 ±1.5
Nitrogen, Total	27 ±2.3
TKN	27 ±2.3
Ammonia	17 ±1.8
Organic	8 ±0.5
Nitrate	0.0
Nitrite	0.0
SO_4	20 ±4.9
DO	2.5 ±0.5
Fatty Acids	0.0
Propionic	0.0
Acetic	0.0

Note: Values are based on eight observations taken in June, July, and August. Both means and standard diviations are given.

solved oxygen, the sodium sulfite dose was increased to about 24 mg/l, and the cobalt chloride dose was also increased to 6 mg/l. Then, the dissolved oxygen concentration decreased to about 1.0 mg/l which was still too high for establishing phosphorus release during the anaerobic stage of treatment. Next, nitrogen gas was used, as a second alternative, to strip out the dissolved oxygen and decrease it to the required level. The nitrogen gas was bubbled through the wastewater, for about 20 minutes, until the dissolved oxygen level was less than 0.20 mg/l. To insure that phosphorus would not be the limiting nutrient for bacterial growth, an additional 5.0 mg/l orthophosphate was added to the wastewater before pumping it to the pilot plant.

III.4 Start Up and Operation

To examine the hydraulics of the system, the system was first operated by pumping tap water through the pilot plant system. The provided hydraulic heads between the reactors were found sufficient for the design flow rate. No leaks were observed in any of the reactors or the clarifier. The system was then drained and the raw wastewater was introduced. The first three reactors were seeded using 20 liters of activated sludge obtained from the aerobic zone of the York River treatment plant, owned and operated by Hampton Roads Sanitation District, and located at Seaford, Virginia. When all reactors and the clarifier were filled, all recycle pumps were turned on. Influent flow rate, anoxic recycle and nitrate recycle all were operated at 0.15 liter/minute. The sludge recycle was operated at 0.125 liter/minute. Other operating parameters are shown in Table

9. No sludge was wasted in the first two weeks. The system was then operated at the same sludge age throughout the investigation.

The sludge age, or biological solids retention time, can be derived for the completely mixed activated sludge system with solids recycle by making a mass balance around the whole system. The following expression will be obtained:

$$BSRT = \frac{V_a X}{(Q - Q_w) X_e + Q_w X}$$

where

X = biomass concentration in the aeration basin

V_a = volume of aeration tank

Q = influent flow rate

Q_w = sludge wasting rate

X_e = biomass concentration in the effluent

For good sludge settling characteristics, the biomass concentration in the effluent will be very small, thus the BSRT can be approximated by:

$$BSRT = \frac{V_a}{Q_w}$$

That is, the amount of sludge to be wasted daily is equal to (1/BSRT) times the reactor volume. The sludge age can also be calculated using the following expression:

$$BSRT = \frac{\text{Mass of Total Active Biomass in the System}}{\text{Total Quantity of Active Biomass Withdrawn from the System Daily}}$$

Table 9. Pilot-plant operating parameters

Influent Flow Rate, lit/min	0.15
Anoxic Recycle, lit/min	0.15
NO_x Recycle	0.15
Sludge Recycle	0.125
Total Biological Reactor Nominal HRT	12.30
Sludge Age, days	13.0
F/M ratio	0.30 $\pm$0.04

Throughout the current research, 7.3 liters of active biomass was wasted daily from reactor No. 6. When operated at a 13 day sludge age, the average biomass concentration in the anoxic and aerobic stages of treatment was 2400 mg/l, and the average biomass concentration in the anaerobic stage of treatment was 1300 mg/l. Using the former expression, the system was operating at 13 days actual sludge age.

Wastewater was obtained and the system was maintained and adjusted two times a day. For each maintenance period, the influent pump was turned off, the inside walls of the feeding tanks were cleaned and the remaining wastewater was siphoned back to the manhole. The submersible pump, installed inside the manhole, was lowered until its inlet screen was totally submersed under the surface of the wastewater flow, and pumping was started. When the two feeding tanks were filled with sewage, the pump was turned off and pulled up out of the sewer wastewater flow. Next, the dissolved oxygen was stripped out of the stored sewage using nitrogen gas. About 20 minutes of nitrogen bubbling through the wastewater was required to reduce the dissolved oxygen concentration to a value less than 0.2 mg/l. About one gram of orthophosphate was then added to the influent wastewater to provide an additional 5 mg/l of orthophosphate to the influent wastewater. The influent pump was then started.

The inside of the tubing connecting the reactors and the inside walls of all reactors and the clarifier were cleaned to minimize attached growth. During every night's maintenance period, the Tygon tubing of one pump head was replaced and 7.3 liter of sludge was wasted from the last aerobic reactor. The system was operated for about one month (2 sludge ages) in order to achieve steady state conditions and to insure consistent system performance.

During the investigation, maintaining true steady state conditions for extended periods of time was difficult to achieve because of fluctuations in the influent wastewater characteristics. However, after one month of operation, most of the parameters measured to evaluate the system performance remained approximately the same. At that time, one month after the operation was started, the system was not performing as an excess biological phosphorus removal system, that is, there was no phosphorus release in the anaerobic stage, and no high phosphorus uptake, in the aerobic stage, were observed. Since, it was essential to establish excess biological phosphorus removal operation to accomplish the objectives of the research, efforts were directed toward achieving the desired system performance. Two months later, phosphorus concentration patterns typical of excess biological phosphorus removal systems had been established in the various stages of the treatment plant. The main factor that had made it difficult to establish excess biological phosphorus removal was the high oxygen loading to the anaerobic stage of the plant. Although the dissolved oxygen concentrations measured in the anaerobic reactors were almost zero, no phosphorus release was observed indicating that real anaerobic conditions had not been reached. When the oxygen loading to the anaerobic reactors were reduced, by reducing the dissolved oxygen concentration in the influent (from 1.0 mg/l to 0.2 mg/l), and reducing the dissolved oxygen concentration in the aerobic stage (from 4.5 mg/l to 2.0 mg/l), phosphorus release was observed within less than 24 hours after the previously mentioned changes were made. The system was then operated for one month to re-establish the steady state conditions.

III.5 Organic Chemical Additions

Seven different short chain volatile fatty acids, namely: formic acid; acetic acid; propionic acid; butyric acid; isobutyric acid; valeric acid and isovaleric acid; plus glucose, were separately added to the influent wastewater and their effects on the performance of the biological nutrient system was observed. Chemical forms and concentrations of substrates used in the present investigation are tabulated in table 10. For each chemical, 22 liters of solution was prepared using tap water which had been previously bubbled with nitrogen gas to reduce the dissolved oxygen to a value less than 1 mg/l. The concentration of each chemical was calculated so that when the chemical was pumped, at a rate of 5 ml/min, to the first anaerobic reactor, 100 mg/l COD equivalent would be added to the COD of the influent wastewater. Each chemical was added continuously for about three days. One day was allowed, without chemical addition, between every two *consecutive* chemical additions. All tested chemicals were added at 5:00 p.m. of the first day of addition and stopped at 9:00 a.m. of the fourth day of addition.

III.6 Regular Maintenance Tasks Performed on the Pilot Plant System

Following are the maintenance tasks regularly performed during operation of the pilot plant.

Table 10. List of chemicals and concentrations used in the present investigation

Chemical	Stock Conc. (%)	Quantity Used Per 22 liter	Final Feed Conc. gm/l	Chemical Conc. mg/l In Reactor 1
Formic Acid	90	211 g	9.59	287
Acetic Acid	100	64 g	2.91	94
Propionic Acid	99	45 g	2.05	66
n-Butyric Acid	99	38 g	1.73	55
Isobutyric Acid	99	38 g	1.73	55
n-Valeric Acid	99	24 g	1.09	35
Isovaleric Acid	99	24 g	1.09	35
Dextrose	99	64 g	2.91	94

Note. All added chemicals were fed at a flow rate of 5 ml per minute to the first anaerobic reactor.

1. Lines connecting the units and the line connecting the last reactor and the clarifier were cleaned, using a small brush, two times a day to minimize attached growth.
2. The inside walls of each reactor and the clarifier were cleaned two times a day.
3. The inside walls of the feeding tanks were cleaned and the remaining wastewater was siphoned back to the manhole once a day.
4. The Tygon tubing of each pump head was replaced once every four days.
5. Both air compressors, used to aerate the last three reactors, were drained weekly.
6. All influent lines and recycle lines were disinfected using concentrated chlorine bleach solution and then cleaned with tap water once in every two weeks.
7. The submersible pump was pulled out from the manhole and cleaned whenever a decrease in the pumping rate was observed indicating that the pump's screen was clogged.

III.7 Samples Collection and Preservation

All samples were collected during the early morning of the forth day of continuous organic chemical addition, that is, at the end of the night operation. Before starting the regular procedures followed for day operation (as explained in section four), parameters such as dissolved oxygen concentration, oxygen uptake rate and pH were measured in situ (the procedures described in Standard

Methods ,1980, were followed). One hundred ml grab samples were collected from each reactor, the influent tank and the clarifier, in labeled plastic containers. On sampling days only 6.8 liters of the mixed liquor was wasted from the last aerobic reactor to compensate for the additional volume withdrawn from the system for analytical purposes. Following sampling and monitoring, the system was prepared for day operation and chemical addition was stopped.

The samples were taken to the laboratory (within 60 minutes after sampling) to continue the measurements of the parameters listed in Table 11. In the laboratory, a 25 ml, well-mixed portion of each sample (except the influent) was filtered through 0.45 μm glass fiber filters for mixed liquor suspended solids and volatile suspended solids determinations. An additional 15 ml sample was also filtered, that is, a total of about 40 ml of filtrate of each sample was made available for analysis. From each sample, a 5 ml portion of the filtrate was transferred to a 15 ml test tube for anion determinations (orthophosphate, nitrate, nitrite and sulfate). Another 5 ml portion of filtrate of each sample was also transferred to 15 ml test tubes, and 0.25 ml of isopropanol was added to each sample for the analysis of volatile fatty acids (VFAs). A third 10 ml portion was transferred to bottles for determination of the chemical oxygen demand (COD) in each reactor. A fourth 5 ml filtrate portion of each sample was transferred to 15 ml test tubes and preserved by adding 3 drops of concentrated nitric acid (the pH of the adjusted samples were less than 2 units). Samples for total phosphorus determination, in the influent and in the last aerobic (reactor number 6) were preserved by freezing in plastic bottles for later measurements. All standard solutions, for anions and volatile fatty acids, were stored at 4°C in glass bottles with teflon inserts inside the plastic caps.

Table 11. Parameters monitored during the pilot plant operation

		Reactor						
Parameter	Influent	1	2	3	4	5	6	Effluent
COD	X	X	X	X	X	X	X	X
TKN	X							X
Orthophosphate	X	X	X	X	X	X	X	X
Total Phosphorus	X						X	
Phosphorus in Sludge							X	
Nitrite	X	X	X	X	X	X	X	X
Nitrate	X	X	X	X	X	X	X	X
Sulfate	X	X	X	X	X	X	X	X
Oxygen Uptake Rate						X	X	
pH	X	X	X	X	X	X	X	X
MLSS		X	X	X	X	X	X	X
MLVSS		X	X	X	X	X	X	X
Volatile Fatty Acids*	X	X	X	X	X	X	X	X
Iron	X	X	X	X	X	X	X	X
Magnesium	X	X	X	X	X	X	X	X
Calcium	X	X	X	X	X	X	X	X
Potassium	X	X	X	X	X	X	X	X
Dissolved Oxygen		X	X	X	X	X	X	X

*This included: Acetic acid, formic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid and valeric acid.

Parameters including pH, OUR, COD and anions were measured within a few hours of sample collection. Measurements of all parameters listed in Table 11 except the metals and total phosphorus were completed within 48 hours of sample collection.

III.8 Analytical Methods

III.8.1 Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS)

The mixed liquor suspended solids was determined as material remaining after filtering a known amount of sample and evaporating the filtrate at 104°C to a constant weight (Section 209D, Standard Methods, 1980). The mixed liquor volatile suspended solids was determined as the material lost after heating at 550°C for two hours (Section 209E, Standard Methods, 1980). Both MLSS and MLVSS were measured in all reactors and the effluent. Measurements were done by filtering 25 ml sample through 0.45 μm Whatman (Whatman, Limited, Maidstone, England) 934-AH glass fiber filters.

III.8.2 Chemical Oxygen Demand (COD)

The chemical oxygen demand of unfiltered influent wastewater, filtered reactor and clarifier samples was determined using the dichromate reflux method in accordance to Section 508 outlined in Standard Methods, 1980. Samples were diluted before the analysis so that the COD of the diluted sample would be less than 100. Two well-mixed filtered portions of each sample were analyzed. The average of the two values was reported. The test was done by adding 2 milliliter 0.0167 N potassium dichromate digestion solution and 7 milliliter sulfuric acid reagent to 5 milliliter sample. Heating for digestion was accomplished using a cast aluminum heating plate operated at 150°C.

III.8.3 Total Organic Carbon (TOC)

Total organic carbon and dissolved organic carbon concentrations in samples were determined using a CD-80 series, Dohrmann Division, Xertex Corporation (Santa Clara, CA) carbon analyzer system. Samples were filtered, for DOC measurement, and acidified to convert inorganic carbon to CO_2, which was then purged out of the sample before the analysis.

III.8.3 Total Phosphorus

Samples were digested using the persulfate digestion method as outlined in Section 424(C)(III). Digested samples were then analyzed for orthophosphate

using the ascorbic acid method outlined in Section 424(F), Standard Methods (1980). Unfiltered influent sample and unfiltered sample obtained from the last aerobic reactor were analyzed for total phosphorus. Samples were diluted before analysis so that the measured value would be less than one mg/l phosphorus. A blank and four phosphorus standards were prepared during each analysis for the construction of a standard curve used for quantitative purposes. All samples, including standards, were analyzed spectrophotometrically using a Beckman (Irvine, California) DU-6 UV-visible spectrophotometer.

III.8.5 Total Kjeldahl Nitrogen (TKN)

TKN was measured using the Semi-Micro-Kjeldahl Method described in Section 420(B), Standard Methods (1980). Unfiltered influent wastewater samples and filtered effluent samples were digested using a semi-micro-Kjeldahl digestion apparatus. For both ammonia and TKN determinations, samples were distilled using distillation apparatus following pH adjustment, and ammonia was absorbed in plain boric acid solution. The organic nitrogen was determined by subtracting the ammonia nitrogen from the TKN values.

III.8.6 Volatile Fatty Acids (VFA)

Acetic, propionic, butyric, isobutyric valeric and isovaleric acids were analyzed, on filtered samples, using a Dionex 2000i Ion Chromatograph (Dionex Corporation, Palo Alto, CA), an ion exclusion chromatography. The instrument

was equipped with a suppressor device to reduce the background conductivity of the effluent, and a conductivity detector. Fatty acids were separated using a HPICE-AS1 (Dionex Corp.) column and the conductivity of the element was reduced using a resin exchange column. The suppressor column was regenerated using 10 mM tetrabutyl ammonium hydoxide (*TBAoH*) flowing, under pressure, at a rate of 3.0 ml/min.

The eluent, carrier of fluid and ions closer to the separator column, used for the analysis, was 1 mM perfluoroheptanoic acid in 5% isopropanol solution. The eluent was pumped using a Dionex 2000 i analytical pump, which is a constant pressure/constant flow pump, at a rate of 0.80 ml/min. For analysis, 3 ml of filtered sample was gently introduced into the injector valve using a plastic syringe and, hence, a 50 microliter loop was filled at atmospheric pressure. The injection mode was then selected to introduce the sample into the flowing element stream and onto the column. The conductivity was then measured and recorded with a Spectra-Physics 4270 integrator. Ionized distilled water was injected before each use of the instrument to check the base line representing the zero conductivity. A representative sample was then tested in order to select the appropriate output range and standard concentration which will be used for the analysis of test results. The output range was set at 10 μs full scale, and the standard concentration was made up by mixing 20 ml of 10 mg/l acetic, propionic, butyric, isobutyric, valeric and isovaleric acids. The identity of each peak produced during sample analysis was decided according to the retention time in the column. Sample spiking, with the suspected acid, was then made to confirm the identity of that peak.

III.8.7 Anions: Orthophosphate Phosphorus (PO_4-P), Nitrate (NO_3-N), Nitrite (NO_2-N), and Sulfate (SO_4)

Anions such as PO_4, NO_3-N, NO_2-N, SO_4 were measured in filtered samples using the predescribed ion chromatograph. However, different columns, eluents, retention times and pumping flow rates were used for analysis of the anions. The anions were separated using a HPIC-AS4A (Dionex Corp.). The eluent was 1.8 mM sodium carbonate (Na_2CO_3)/1.7 mM sodium bicarbonate ($NaHCO_3$) pumped at a flow rate of 2.0 ml/min. The suppressor anion micromembrane was continuously regenerated using 0.025 N $NaSO_4$ flowing at a rate of 3.0 ml/min. The output range was set at 30 μs full scale. The standard was 10 mg/l as phosphorus, 10 mg/l as nitrate nitrogen, 10 mg/l as nitrite nitrogen and 20 mg/l as sulfate. The standard was stored in the refrigerator at 4°C.

III.8.8 pH

The pH of the influent wastewater, reactor and effluent samples were measured using a Model 610A Fisher (Springfield, NJ) pH meter. Before each measurement the pH meter was standardized using commercially prepared buffer solution. Procedures outlined in Section 423, Standard Methods (1980), were followed for pH measurements.

III.8.9 Dissolved Oxygen

A YSI Model 54 A (Yellow Springs Instrument Company, Yellow Springs, OH) oxygen meter equipped with a YSI Model 5739 submersible probe was used for the measurement of dissolved oxygen concentrations in influent wastewater and different reactors. The membrane of the probe was replaced with a new membrane every two weeks. The instrument was calibrated before taking dissolved oxygen measurements in accordance with the instruction provided by the manufacturer. The probe was always kept in a humid environment when it was not in use.

III.8.9 Specific Oxygen Uptake Rate

The oxygen uptake rate (OUR) was measured in reactors 5 and 6 (last two aerobic reactors) using the procedure outlined in Section 213A, Oxygen-Consumption Rate, Standard Methods, 1980. Well-mixed mix liquor from each reactor was analyzed using a BOD bottle and direct measurement of dissolved oxygen concentration as a function of time. No oxygenation was made prior to taking readings. The tested sample was poured back in the reactor and the test was repeated for another sample obtained from the same reactor. The data was then analyzed using regression analysis and the slope of the regression line was calculated which represented the value of the oxygen uptake rate. The oxygen uptake rate was divided by the mixed liquor volatile suspended solids (MLVSS) concentration, in the same reactor, to determine the specific oxygen uptake rate (SOUR).

III.8.11 Iron, Magnesium, Calcium and Potassium

Preserved, 5 ml portions of filtered reactor and clarifier samples were analyzed for iron, magnesium, calcium, and potassium using a Model 703, Perkin-Elmer (Norwalk, CN) atomic absorption spectrophotometer. The procedural methods used for the analysis of iron, magnesium, calcium and potassium were in accordance with EPA Method 236.2; EPA Method 242.1; EPA Method 215.2 and EPA Method 258.1 respectively.

IV. RESULTS

As mentioned earlier, 100 mg/l COD equivalent of short chain volatile fatty acids, namely: formic acid; propionic acid; acetic acid; butyric acid; isobutyric acid; valeric acid and isovaleric acid; plus glucose, were separately added continuously to the first anaerobic reactor for three days. Thus, the resulting average influent COD to the pilot plant was 430 mg/l (330 mg/l from the wastewater and 100 mg/l from the added substrate). All samples were taken and analyzed at the end of the night operation of the forth day of addition. In this chapter, the results obtained throughout the investigation will be presented. The chapter is divided into eight sections. In the first two sections the establishment of excess biological phosphorus removal (BPR) and the treatment performance of the pilot plant system will be presented. Each of the last six sections will present the effects of various fatty acids on a specific parameter. These parameters included: phosphorus release, uptake and removal, TKN removal, nitrification, COD removal, MLVSS and SOUR, metals (iron, calcium, magnesium, potassium), and sulfate.

IV.1 Establishment of Excess Biological Phosphorus Removal

The following observations were reported in the literature for systems accomplishing excess biological phosphorus removal:

1. high phosphorus release during the anaerobic stage of treatment, and high phosphorus uptake during the aerobic stage of treatment;
2. significant removal of readily biodegradable substrate during the anaerobic stage of treatment; and
3. high percentage of phosphorus in the sludge of the aerobic stage of the treatment process.

In this investigation, the system established excess biological phosphorus removal three months after the start of the operation. The reason for the late establishment of excess biological phosphorus removal is explain in the previous chapter. Figure 28 shows the profiles of orthophosphate concentration when the system was operating as an activated sludge system and when it was operating as an excess biological phosphorus removal system. For both operations, no additional substrate was added. When the system was operating as a conventional activated sludge system, no phosphorus release or uptake were observed. In addition, the phosphorus content of the sludge was 2.6 percent, which is a typical percentage of phosphorus content, 2-3 percent, for sludge in a conventional, completely aerobic activated sludge system. However, when the system was accomplishing excess biological phosphorus removal, high release of orthophosphate occurred in the anaerobic stage, and high uptake of orthophosphate occurred in the aerobic stage of the treatment. The phosphorus content of the sludge

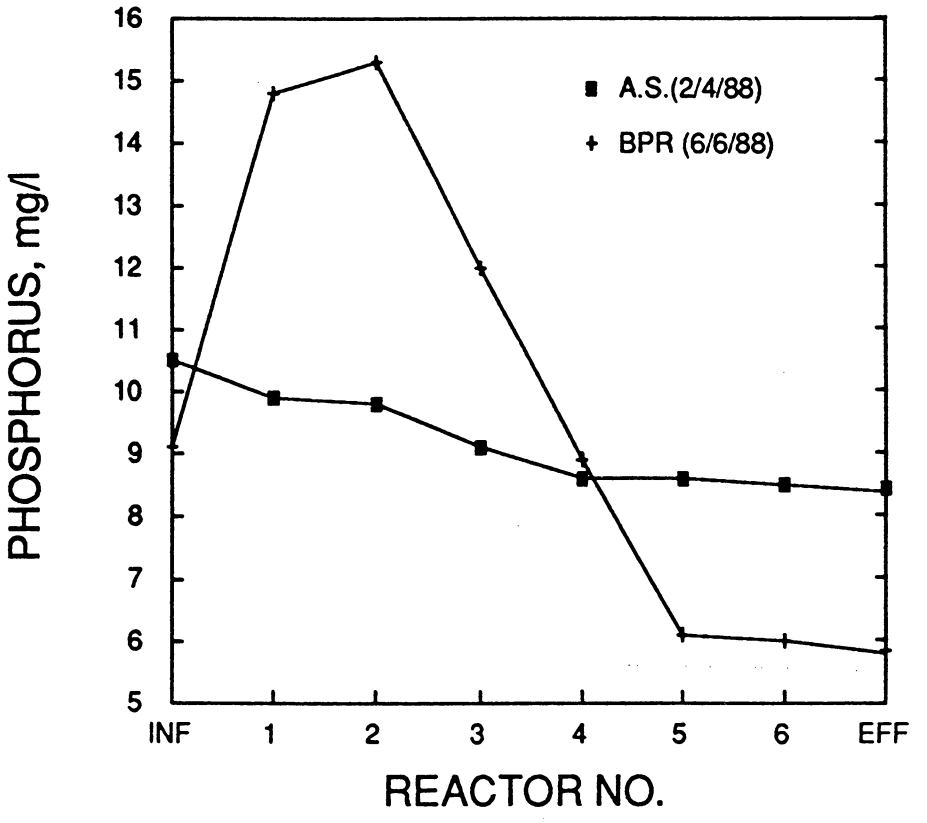


Figure 28. Comparison of Phosphorus Profile in Treatement System Before and After Excess Biological Phosphorus Removal (BRP)

was 4.4 percent, much higher than the value (2.6 percent) obtained before excess biological phosphorus removal had been established.

It is worth mentioning that all measurements of phosphorus release and uptake were expressed in terms of orthophosphate phosphorus because orthophosphate is the primary form of phosphorus involved in phosphorus release and uptake mechanisms.

IV.2 Performance of the Pilot Plant System

Following establishment of excess biological phosphorus removal (BPR), the system was operated for one month to reach steady-state conditions. After one month of operation, at a sludge age of 13 days, most of the monitored parameters remained approximately the same, indicating very stable operation. During that period, no additional substrate was added to the pilot plant, and no volatile fatty acid was present in the influent wastewater. The performance of the pilot plant system after one month of operation, with no additional volatile fatty acid added, is shown in Table 12. A typical COD concentration profile through the system is shown in Figure 29. The average influent COD to the pilot plant was 330 mg/l, and the average effluent COD was 22 mg/l; thus, the average COD removal efficiency of the plant was 93 percent. About 5 mg/l orthophosphate was added to the plant influent. Thus, the average influent phosphorus to the pilot plant was 11.75 mg/l, and the average effluent phosphorus concentration was 7.0 mg/l. The average content of phosphorus in the sludge was 4.5 percent. The average influent total kjeldahl nitrogen (TKN) was 27 mg/l, and the average effluent TKN was 3.8 mg/l. No nitrate or nitrite concentrations were detected in the anoxic reactor. The average effluent nitrate was 4.7 mg/l. The produced sludge showed very good

Table 12. Average performance of the pilot plant system when no additional substrates was added

Parameter	Influent	Effluent	Reactor
COD, mg/l	330 ±40	22 ±10	-
Phosphorus, mg/l	11.75 ±2.1	7.0 ±1.2	-
TKN, mg/l	27 ±2.3	3.8 ±1.0	-
Nitrate, mg/l	0.0	4.7 ±1	-
TSS, mg/l	-	20 ±10	-
VSS, mg/l	-	8 ±3	-
*MLSS, mg/l	-	-	3000 ±200
*MLVSS, mg/l	-	-	2400 ±175
SVI, ml/g	-	-	<100
*SOUR	-	-	0.28
* % P in MLVSS	-	-	4.4

Note. Operating Sludge Age (MCRT) of 13 days
* Reactor 6

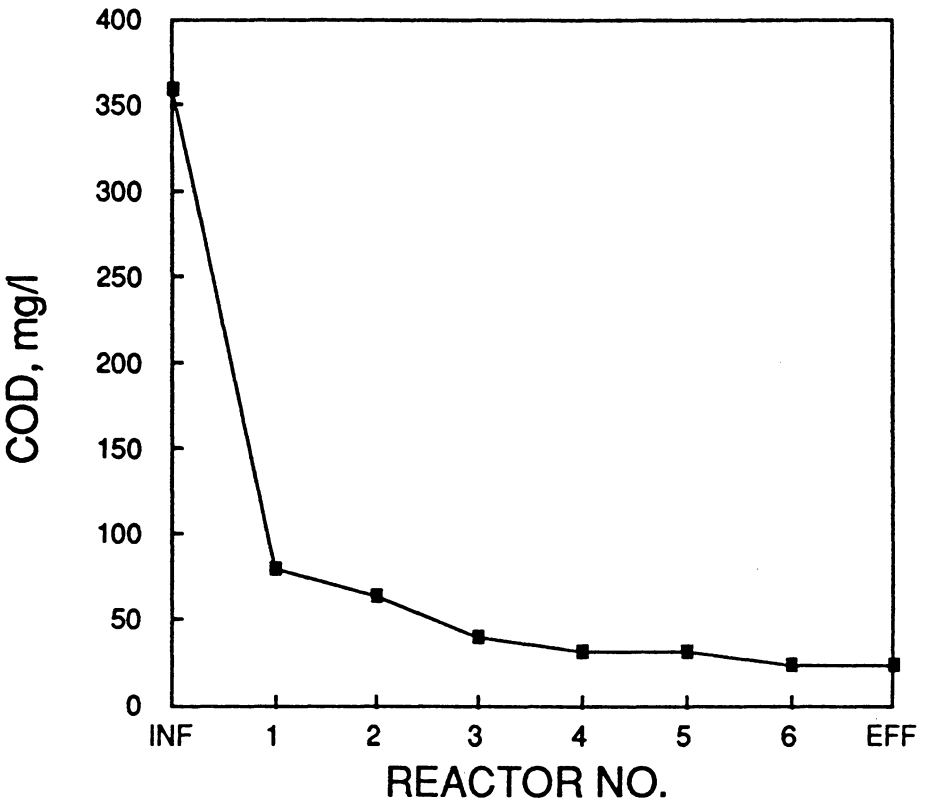


Figure 29. Typical COD concentration profile through the system

settling characteristics, the sludge volume index was always less than 100 and the average solids concentration in the effluent was 20 mg/l. The average mixed liquor volatile suspended solids concentration in the effluent was 8 mg/l.

IV.3 Influence of Substrate Addition on the Performance of Biological Phosphorus Removal

All tested organic compounds were added continuously to the first anaerobic reactor at a concentration of 100 mg COD/l. The corresponding chemical concentrations were: 287, 94, 66, 55, 55, 35 and 35 mg/l for formic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids, respectively. Volatile fatty acid profiles in the pilot plant system during substrate spiking experiments are shown in Figure 30. Figure 31 shows the mass balance volatile fatty acid changes in the system. As shown in Figure 31, all added organic acids (with the exception of formic acid) were completely removed (consumed) from solution in the first anaerobic reactor. In contrast, only 7 percent of the added formic acid was removed in the first anaerobic reactor. Most of the added formic acid, 67 percent, was consumed in the first aerobic reactor of the aerobic stage of treatment.

The reproducibility of the results obtained during the investigation was assessed by repeating some of the experiments and comparing the profiles of orthophosphate concentration. The reproducibility of the results was found to be very good, particularly when the time interval between the experiments was short (less than four weeks). In this section the effect of various volatile fatty acid additions on phosphorus release, phosphorus uptake, phosphorus removal, and phosphorus content of the aerobic sludge

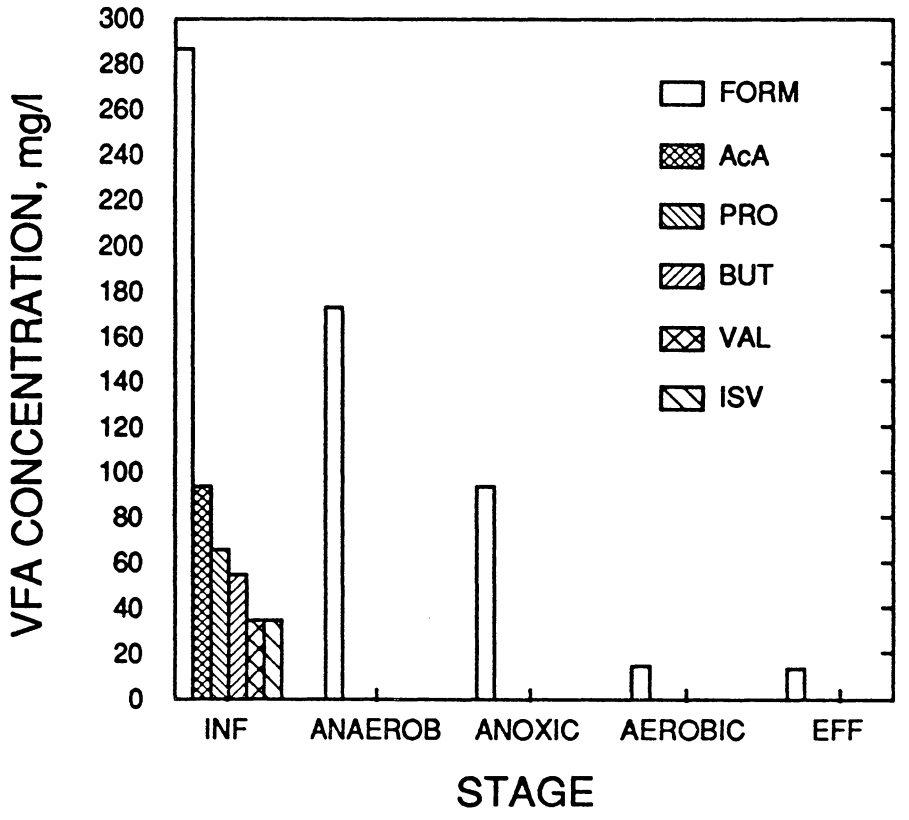


Figure 30. Volatile fatty acid profiles in the pilot plant system during substrate spiking experiments (see appendix A for abbrevation)

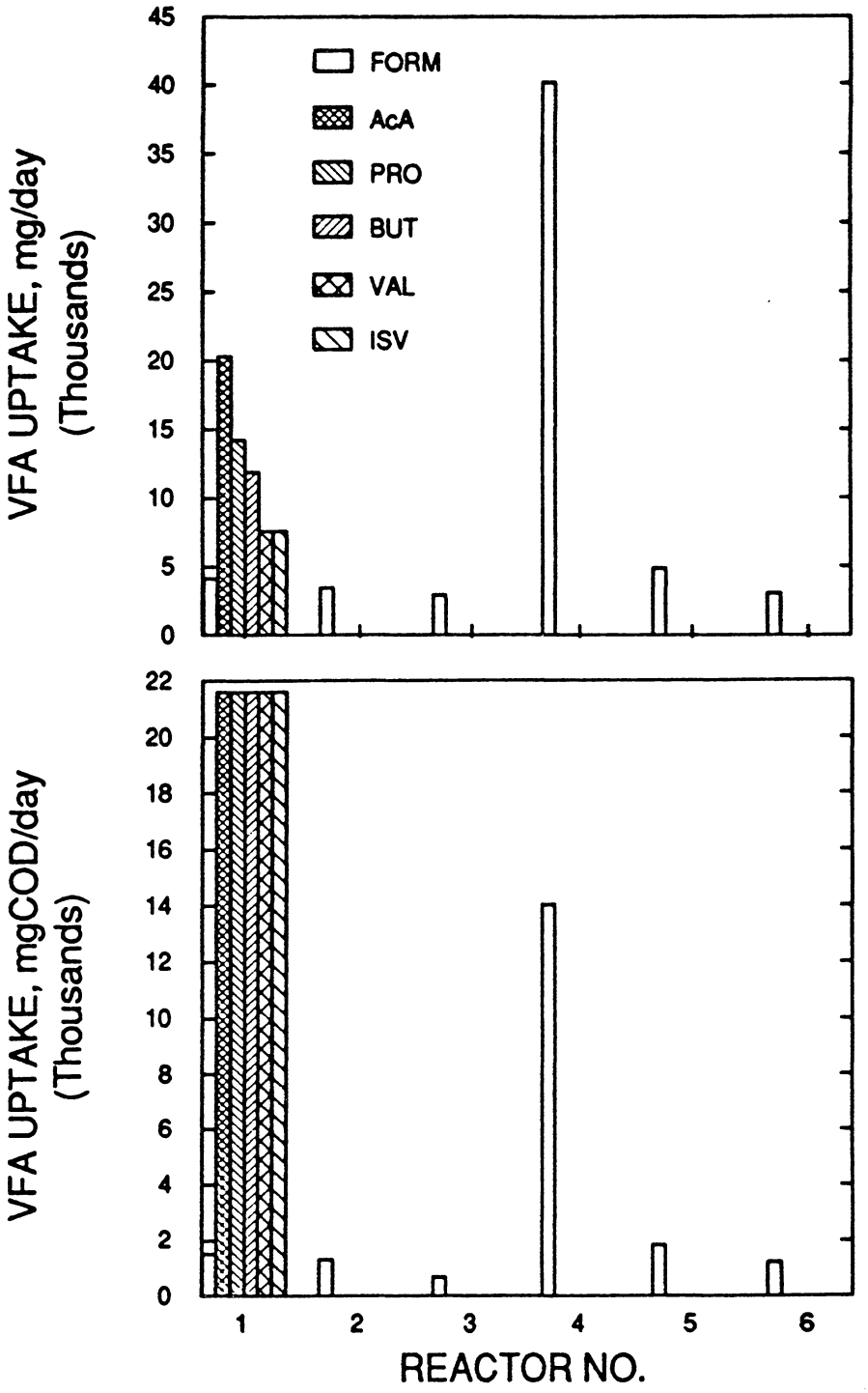


Figure 31. Mass balance volatile fatty acid changes in the system a) Acid concentration basis, b) COD basis

will be presented. In the last subsection, the effect of formic acid and dextrose additions on biological phosphorus removal will be presented.

IV.3.1 Influence of Substrate Addition on Phosphorus Release

Profiles of orthophosphate concentration for the pilot plant system obtained during control operation (no additional substrate was added) and with various substrate additions are shown in Figure 32. Different substrates caused variable degrees of phosphorus release in the anaerobic stage (reactors 1 and 2). All added substrates caused significant phosphorus release as compared with the control. However, orthophosphate profiles do not provide adequate information regarding the actual releases which occurred in the different reactors, because of the dilution effects caused by the different recycles. Mass balance calculations were performed around each reactor to show the actual phosphorus changes in each reactor, and the results are shown in Figure 33. The figure shows that most of the phosphorus was released in the first anaerobic reactor. The second anaerobic reactor showed low phosphorus release except for acetic acid which showed relatively high release in that reactor as well. With the exception of butyric acid, all added substrates showed phosphorus release in the anoxic stage of the treatment. The butyric acid addition caused relatively high phosphorus uptake during the aerobic stage of the treatment.

Figure 34 shows the total phosphorus release and phosphorus uptake obtained for different volatile fatty acid additions. Acetic acid caused the greatest phosphorus release, and propionic acid caused the least phosphorus release. The Ratios of phosphorus release to short chain volatile fatty acid addition during the investigation were calculated and are summarized in Table 13. Isovaleric acid showed the highest molar ratio, 2.31,

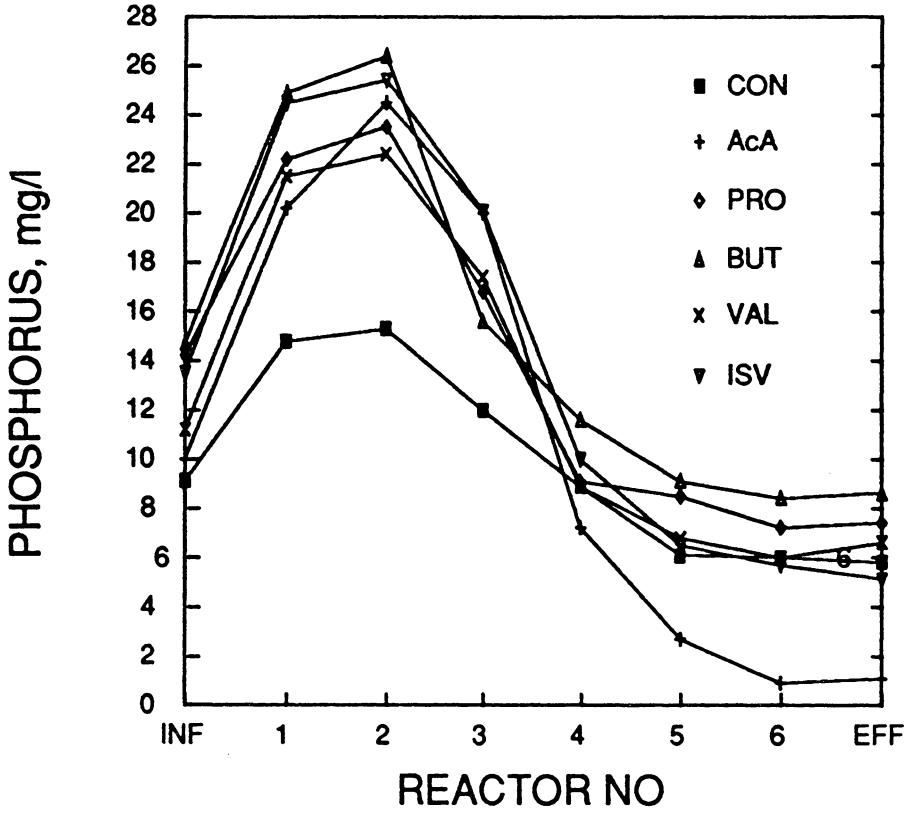


Figure 32. Profiles of observed orthophosphate concentrations in the pilot plant at various organic acids additions

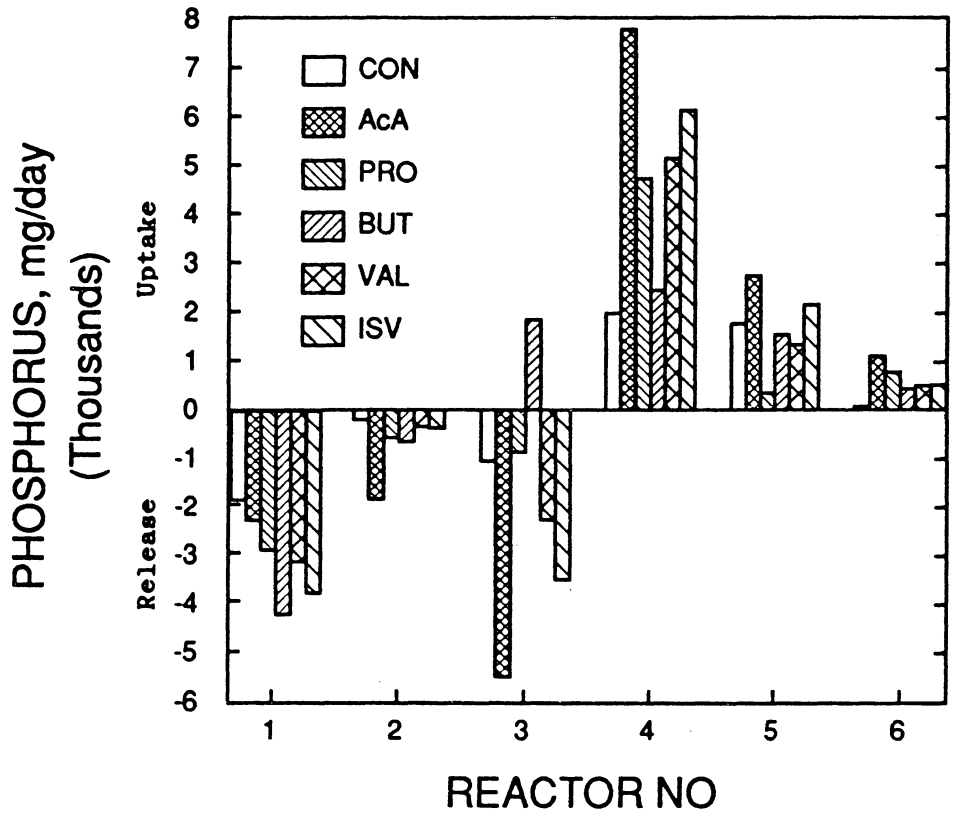


Figure 33. Mass balance phosphorus changes in reactors of the pilot plant for various organic acid additions

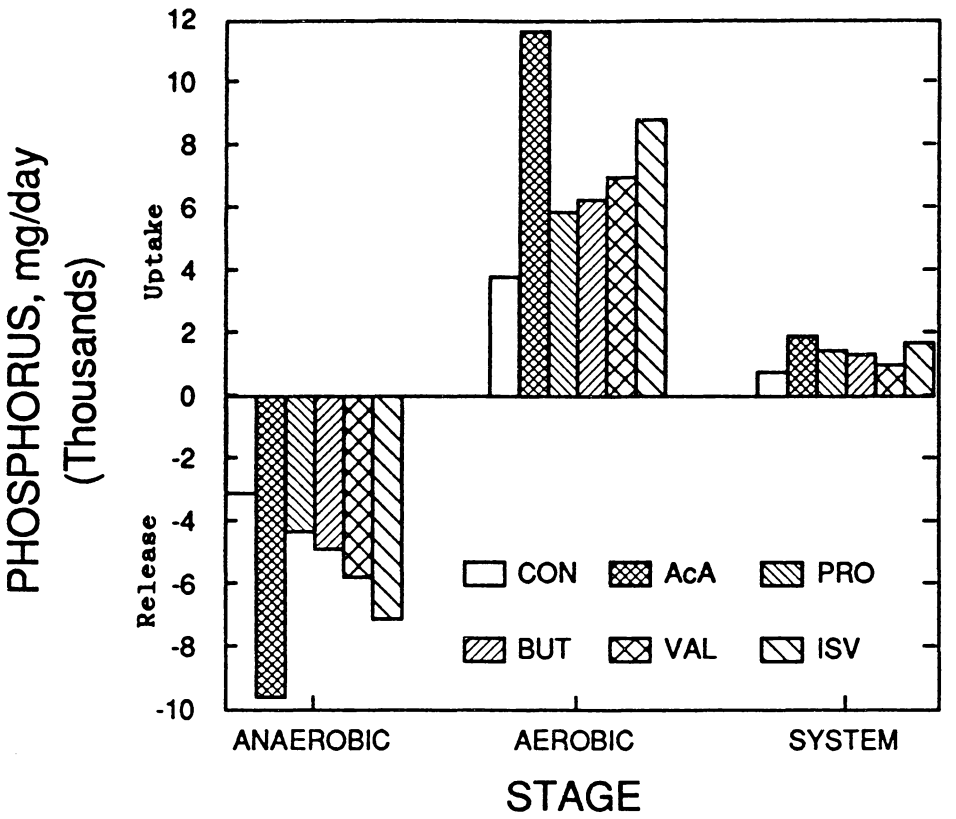


Figure 34. Mass balance phosphorus changes in different stages of the system for various organic acid additions

and propionic acid showed the lowest value, 0.44. The branched volatile fatty acids added, isobutyric acid and isovaleric acid, showed better molar ratios of phosphorus release to volatile fatty acid utilized, as compared with the nonbranched forms of these acids. The difference between the branched and nonbranched forms was found to be particularly significant for the five carbon organic acid, valeric acid.

IV.3.2 Influence of Substrate Addition on Phosphorus Uptake

As shown in Figures 33 and 34, added volatile fatty acids increased phosphorus uptake during the aerobic stage of treatment as compared with the control. Most of the uptake occurred in the first aerobic reactor. Anoxic uptake of phosphorus occurred only during the butyric acid experiment. Acetic acid produced the greatest phosphorus uptake, and propionic acid the least. The ratios of phosphorus uptake to volatile fatty acid added are shown in Table 14. Isovaleric acid showed the largest molar ratio, and propionic acid the smallest value. The iso forms (branched) of the tested organic acids stimulated higher molar ratios of phosphorus removal and better performance, as compared with the non-branched forms. As can be seen in Figure 35, regardless of the substrate added, there was a very good direct relationship correlation between phosphorus release in the anaerobic stage of the system and phosphorus uptake in the aerobic stage of the system. The correlation coefficient was 0.99, that is, 99 percent of the generated data could be well correlated to the regression line, which had a slope of 1.2. The molar ratio of phosphorus uptake to phosphorus released obtained during the current work was 1.20. This implies that the excess uptake was wasted from the system with the waste sludge.

Table 13. Ratios of phosphorus released and fatty acid utilized under anaerobic conditions

Volatile Fatty Acid	Moles Phosphorus Released / Moles VFA Utilized	mg Phosphorus Released / mg COD Utilized
Propionic Acid	0.44	0.12
Acetic Acid	0.77	0.37
Butyric Acid	0.78	0.15
Isobutyric Acid	0.80	0.16
Valeric Acid	1.72	0.19
Isovaleric Acid	2.31	0.25

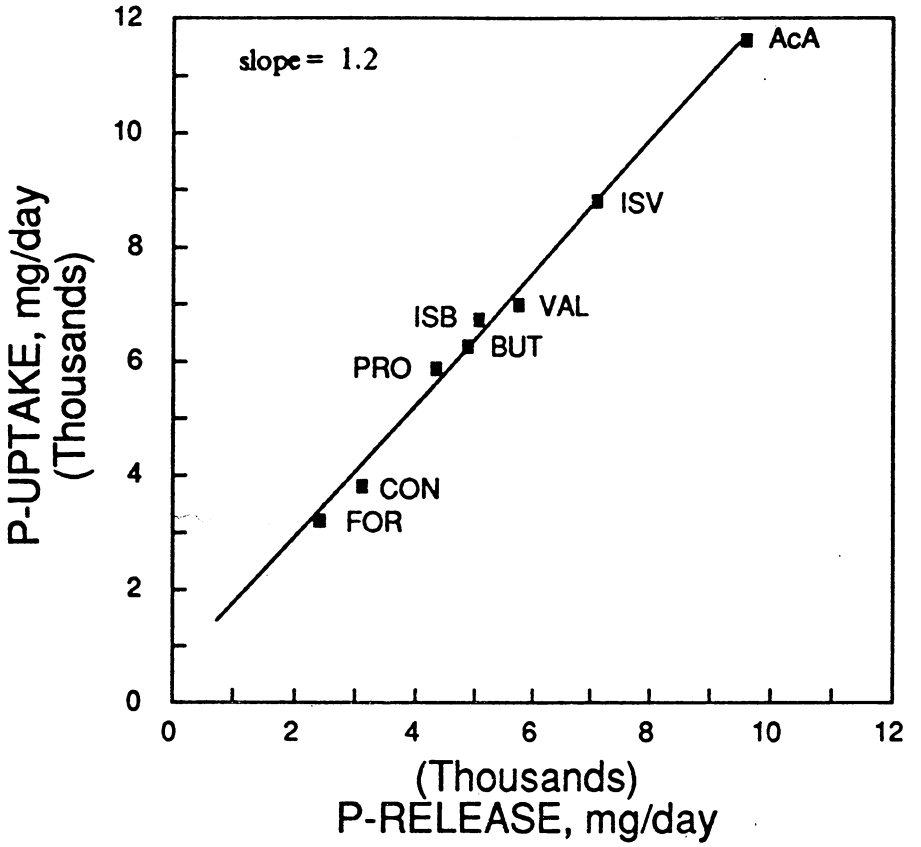


Figure 35. Relationship between phosphorus release and phosphorus uptake

Table 14. Ratios of phosphorus uptake and fatty acid utilized under aerobic conditions

Volatile Fatty Acid	Moles Phosphorus Uptake / Moles VFA Utilized	mg/l Phosphorus Uptake / mg/l COD Utilized
Propionic Acid	0.36	0.10
Acetic Acid	0.74	0.37
Butyric Acid	0.61	0.12
Isobutyric Acid	0.72	0.14
Valeric Acid	1.44	0.15
Isovaleric Acid	2.23	0.24

IV.3.3 Influence of Substrate Addition on Phosphorus Removed

With the exception of formic acid, all added short chain volatile fatty acids significantly enhanced biological phosphorus removal (the effect of formic acid addition will be presented in the last section of this chapter). Acetic acid caused the greatest removal of phosphorus, and valeric acid the least, as shown in Figure 36. Isovaleric acid addition caused much greater phosphorus removal than valeric acid addition. Similarly, isobutyric acid addition caused more phosphorus removal than butyric acid addition, although the difference was not very much. The ratios of grams COD utilized to moles of phosphorus removed are listed in Table 15.

In summary, on a COD basis, the effect of the short chain volatile fatty acids on phosphorus removal in the pilot plant system had the following decreasing order of effect with the first named organic acid being accompanied by the greatest removal:

acetic acid > isovaleric acid > propionic acid > isobutyric acid > butyric acid > valeric acid > formic acid.

IV.3.4 Phosphorus Content of Sludge

The phosphorus content of the sludge was estimated by mass balance calculations and indirect measurement. The phosphorus content of the sludge was measured by subtracting the total soluble orthophosphate from the total phosphorus measured using unfiltered mixed liquor examples from Reactor 6. Both measured and calculated percentages of phosphorus in the aerobic sludges were obtained at the end periods of continuous additions of the various volatile fatty acids. All measured and calculated values were much higher than the 2-3 percent content typically observed for conventional ac-

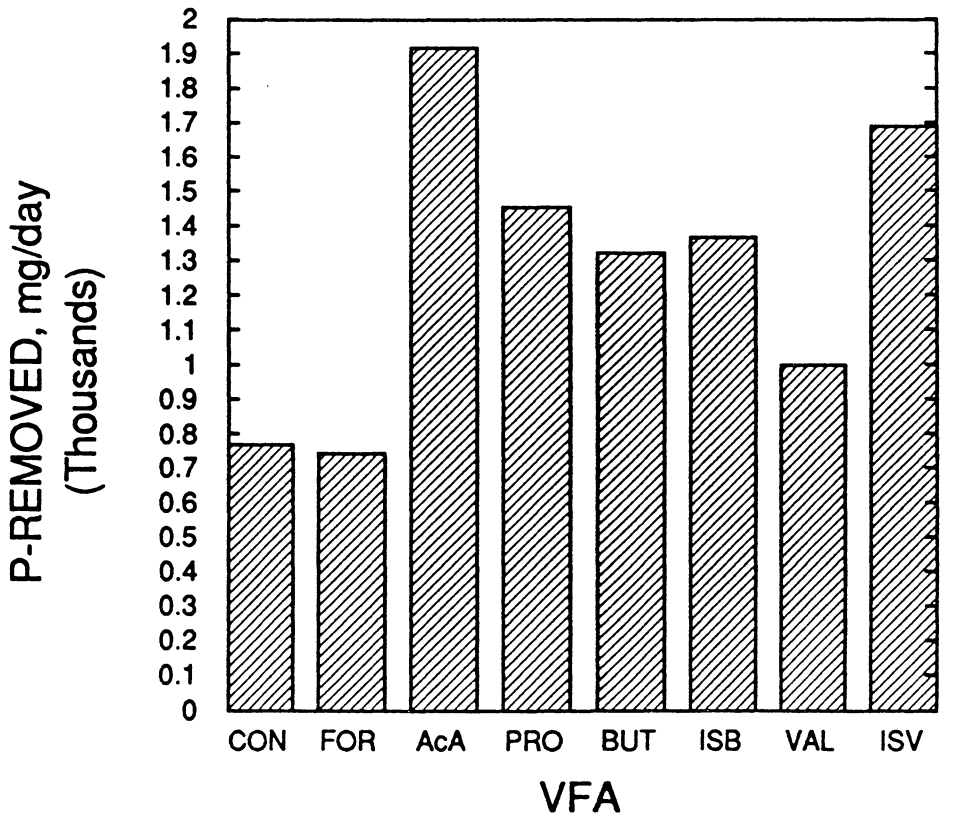


Figure 36. Effect of various volatile fatty acid additions on phosphorus removed

Table 15. Ratios of COD utilized per mole of phosphorus removed

Volatile Fatty Acid	g of COD Utilized / Moles of Phosphorus Removed	mg COD Utilized / mg Phosphorus Removed
Valeric Acid	2.90	94.0
Butyric Acid	1.21	39.0
Isobutyric Acid	1.11	36.1
Propionic Acid	0.97	31.5
Isovaleric Acid	0.73	23.5
Acetic Acid	0.58	18.8

tivated sludge, as shown in Figure 37. The average percent of phosphorus in the sludge, expressed as a percentage of MLVSS, obtained for acetic acid, isovaleric acid, propionic acid, butyric acid, isobutyric acid, and valeric acids, were 9.8, 8.2, 7.4, 7.0, 6.9, and 6.1, respectively. The control percentage was 4.4. All added organic acids except formic caused a significant increase in the percentage of phosphorus in the sludge. Acetic acid showed the greatest value whereas valeric acid showed the least. The value obtained for isovaleric acid was much higher than the value obtained for valeric acid and was second only to acetic acid. Although the isobutyric acid showed a value of 7.0, which was larger than that obtained for butyric addition experiment, 6.9, the difference was not significant. The average error of the MLVSS percent phosphorus measurements was 0.11, with a standard deviation of 0.07, which was larger than the difference observed between the two experiments.

IV.3.5 Influence of Formic Acid and Dextrose Additions on Biological Phosphorus Removal

The effect of continuous addition of formic acid on phosphorus release and phosphorus uptake was investigated. 100 mg/l COD equivalent of formic acid (284 mg/l) was added continuously to the first anaerobic reactor on day 3, and the addition was stopped on day 5. Formic acid neutralized to pH 7 using sodium hydroxide was added on day 7 and was stopped on day 9. The addition of unneutralized formic acid caused a significant decrease in anaerobic stage phosphorus release as compared with the amount released when no additional substrate was added. The addition of formic acid also caused deterioration in phosphorus uptake during the aerobic stage, accompanied with a significant drop in the pH of the first anaerobic reactor. Neutralized formic acid

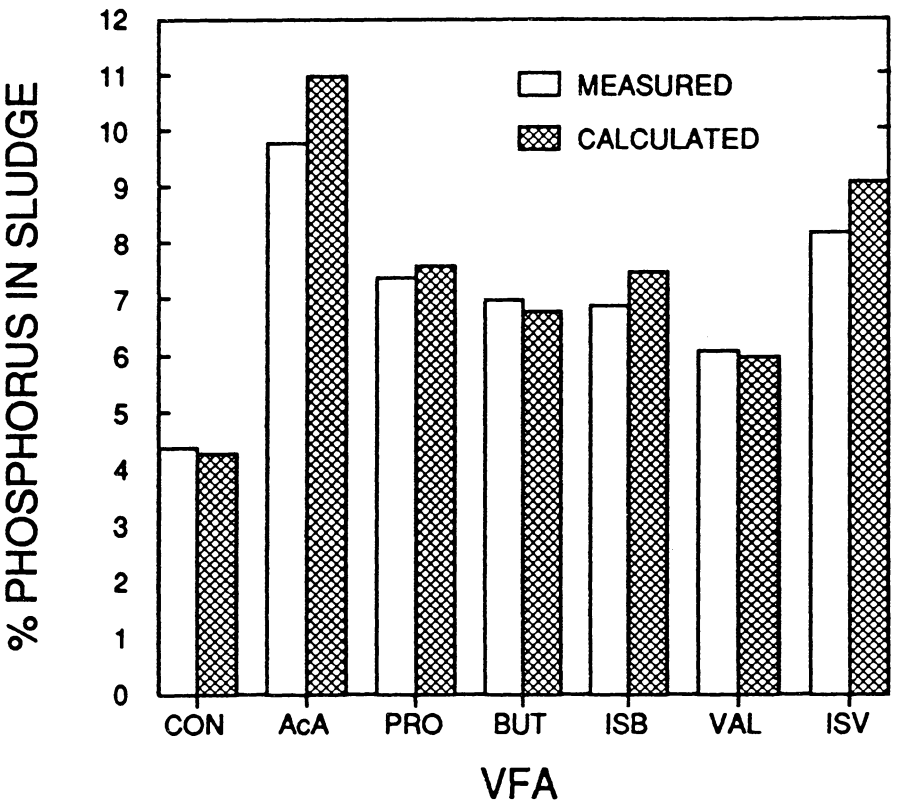


Figure 37. Phosphorus content of sludge at various fatty acids additions

in Figure 38. Profiles of pH in the pilot plant system for various fatty acid additions are shown in Figure 39.

The influence of dextrose addition on phosphorus release and phosphorus uptake is shown in Figure 40. 100 mg/l COD equivalent of dextrose was added continuously to the first anaerobic reactor. The chemical addition was started on day 3 and stopped on day 5. There was no apparent effect of dextrose addition on phosphorus release and uptake. It is worth mentioning that this experiment was carried out during the month of September, and significant changes had been observed in the wastewater characteristics compared to those of the VFA experiments. The COD of the wastewater in September was approximately double what it was during the previous experiments, and the wastewater contained high levels of acetic and propionic acids (fermented wastewater), whereas before none was observed. Concentrations of acetic and propionic acids were also detected in the first anaerobic reactor.

IV.4 Influence of Various Substrate Additions on Nitrogen Removal.

For all organic acid addition experiments, the total kjeldahl nitrogen in both the influent and effluent was measured. As shown in Figure 41, there was no apparent effect of volatile fatty acid additions on the kjeldahl nitrogen removal efficiency. The average influent TKN concentration was 27 mg/l, and the average effluent concentration was 3.8 mg/l. Thus, the average kjeldahl nitrogen removal efficiency of the pilot plant was 86%. The nitrate concentrations in the last aerobic reactor ranged from 3-6 mg/l as nitrogen.

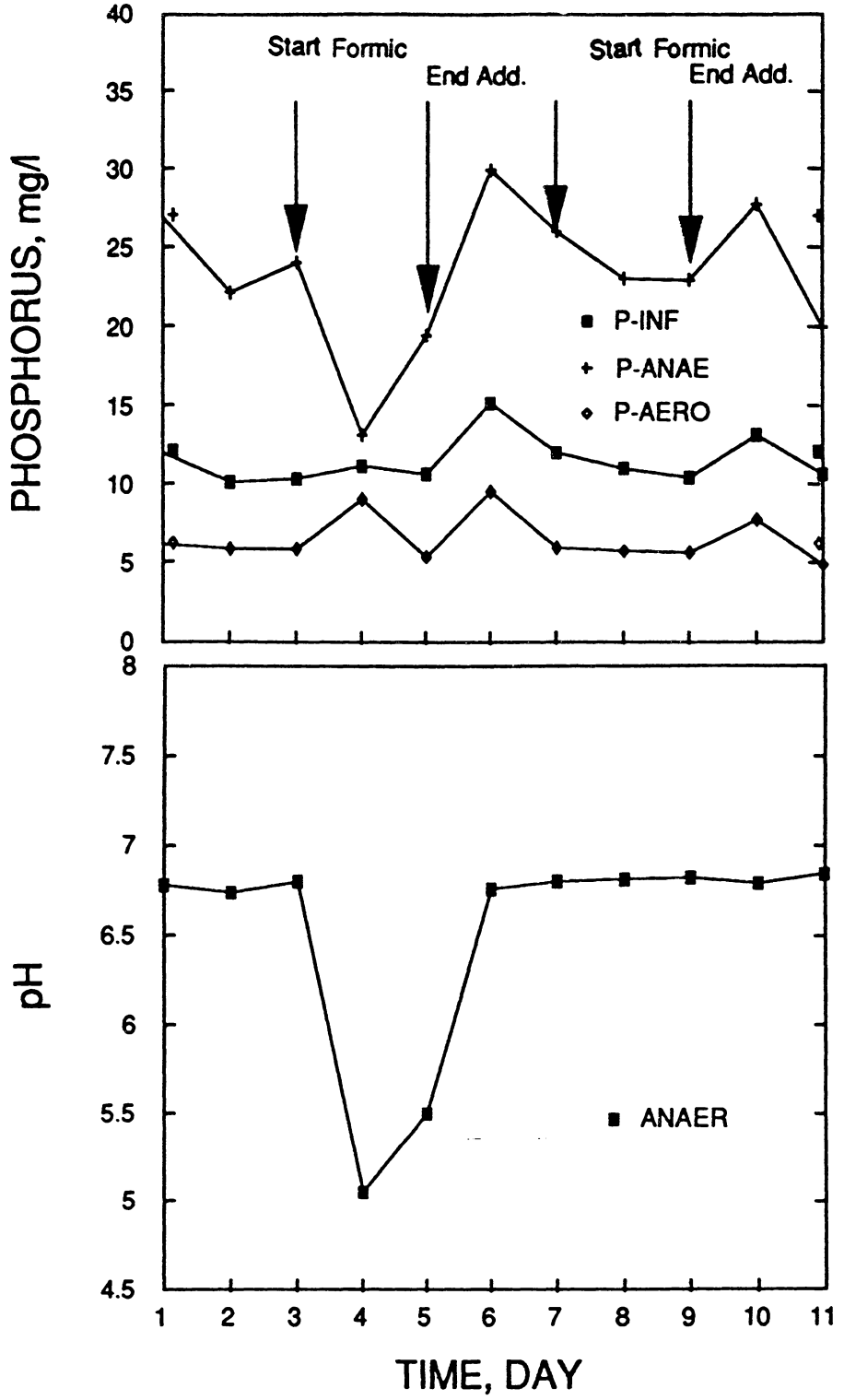


Figure 38. Effect of formic acid addition on phosphorus release / uptake and the corresponding pH profile in the first anaerobic reacto

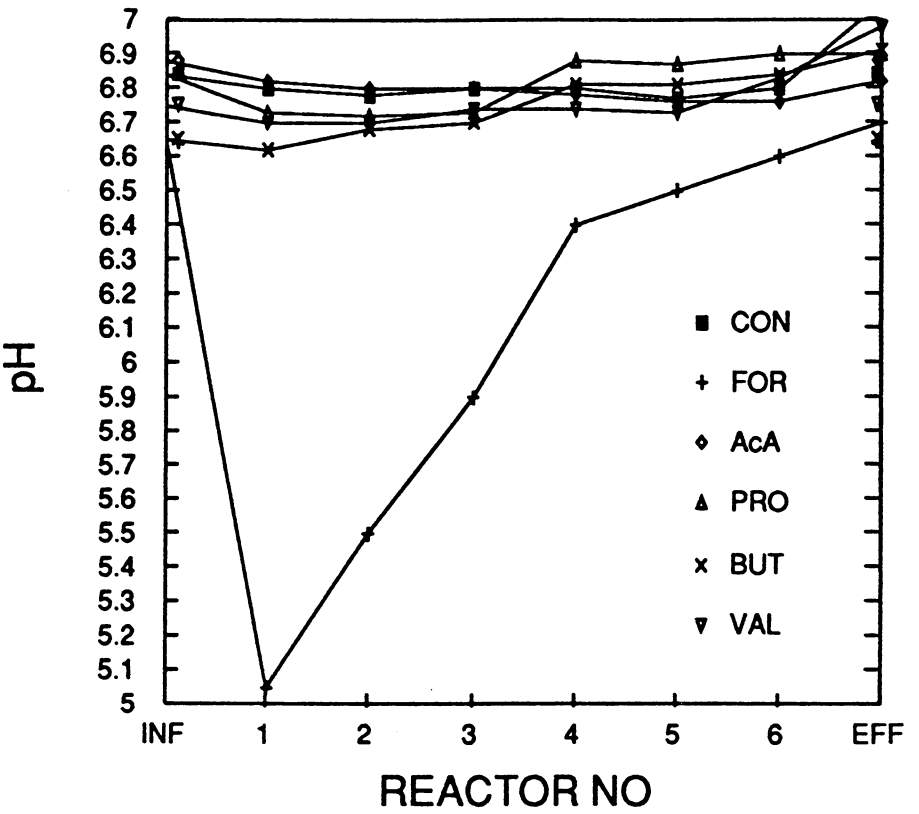


Figure 39. pH profiles in the pilot plant at various fatty acids additions.

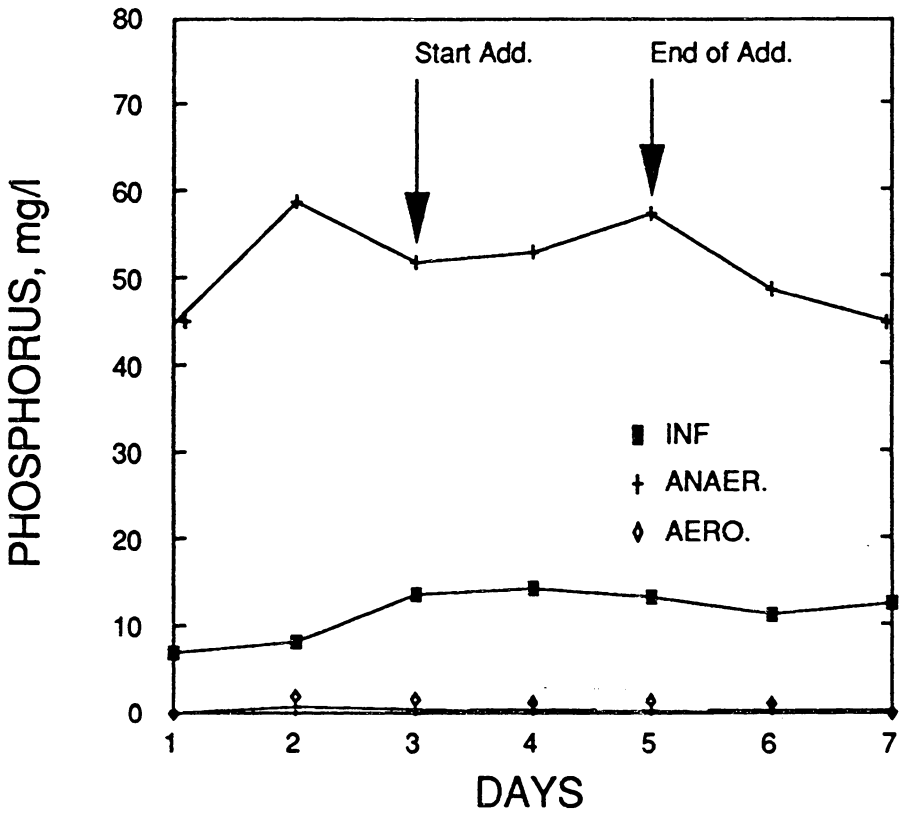


Figure 40. Effect of dextrose addition on phosphorus release and uptake.

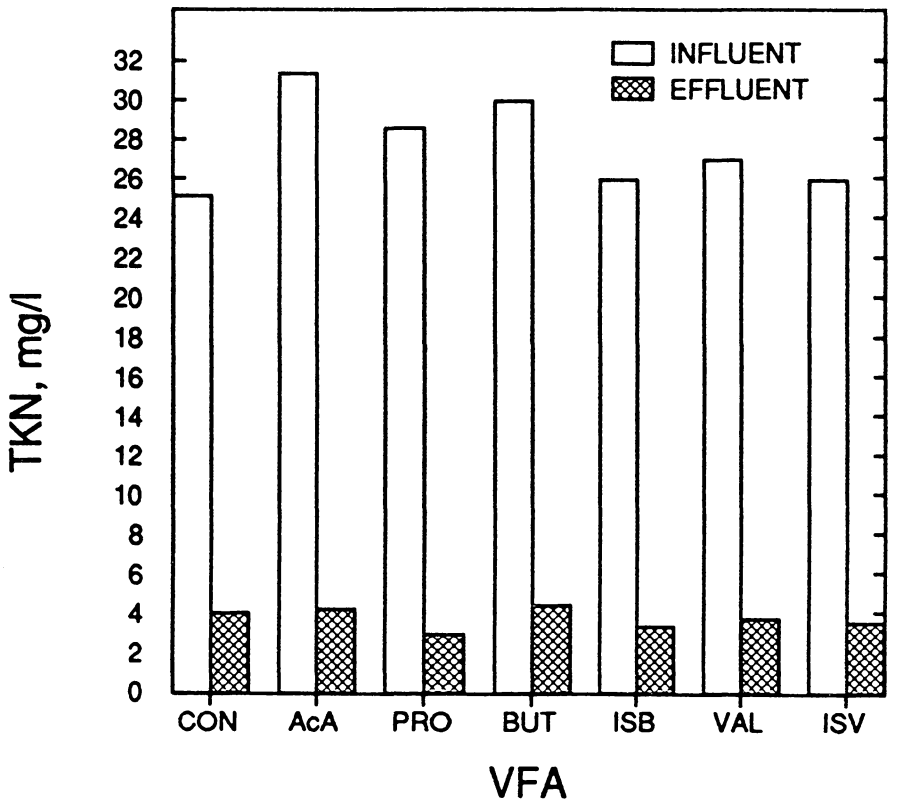


Figure 41. Effect of various fatty acids additions on TKN removal.

All nitrate formed during the aerobic stage of the treatment and recycled with the nitrate recycle or return sludge was totally removed and reduced during the anoxic stage, that is, no level of nitrate was detected in the anaerobic and anoxic reactors throughout the investigation.

IV.5 Effect of Various Substrate Additions on COD Removed

Figure 42 shows the total COD loading to the pilot plant including the COD of the added chemical. The average COD loading for the volatile fatty acids addition experiments was 90.3 g/day, having a standard deviation of 7.2 g/day. The greatest COD loading was observed for the isobutyric acid addition experiment (102 g/day) whereas the isovaleric acid addition experiment showed the least value (79.9 g/day). The COD loading for the dextrose addition experiment was potentially high (159.8 g/day) as compaired with other experiments. The dextrose addition experiment was conducted in early September ,begining of the Fall semester, whereas all other data were obtained during the Summer. Figure 43 shows the mass balance COD removed in different stages of the treatment for various fatty acids addition experiments. Figure 44 shows the percent of COD removed in each of the three stages of the treatment system and how it varied with addition of the different short chain volatile fatty acids. These percentages were calculated based on the total COD fed to the pilot plant, that is, the COD of the wastewater plus the COD of the added substrate (100 mg/l). As can be seen from the figure, the most dramatic reduction in COD occurred in the anaerobic stage of the treatment.

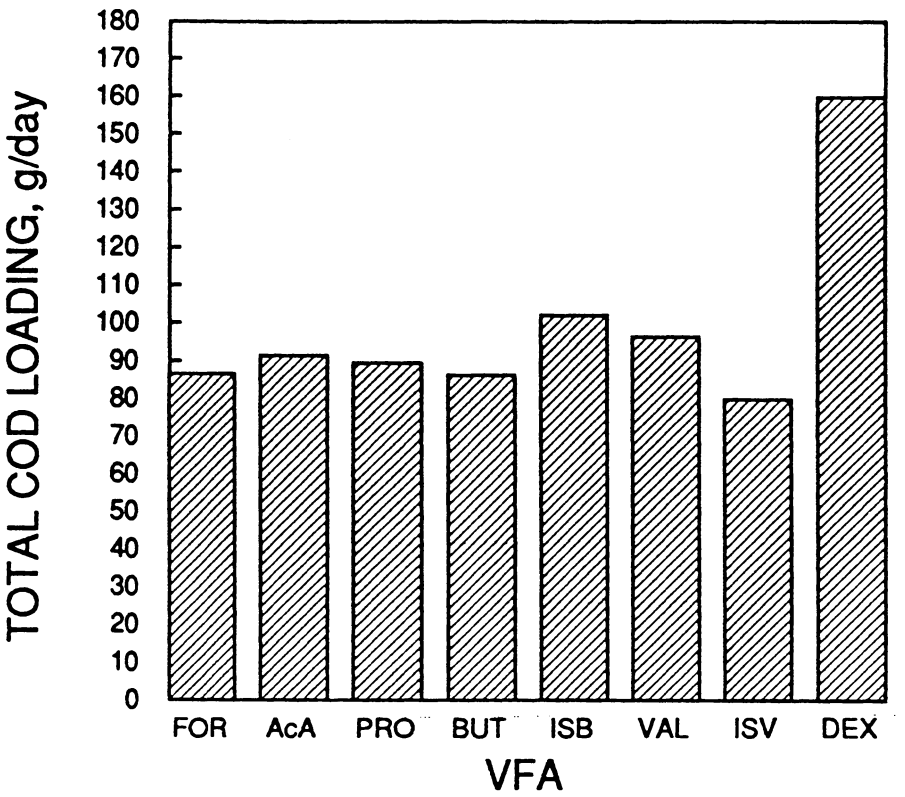


Figure 42. **Total COD loadings to the pilot plant system for various fatty acid addition experiments.**

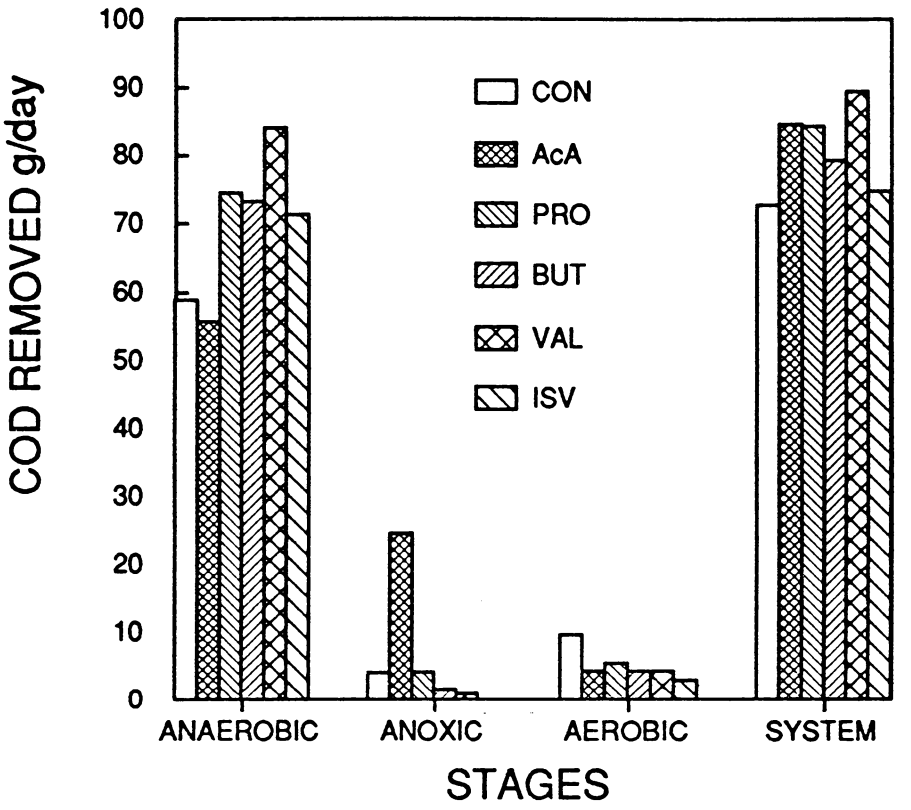


Figure 43. Mass balance COD removed in different stages of the pilot plant for various fatty acid addition experiments.

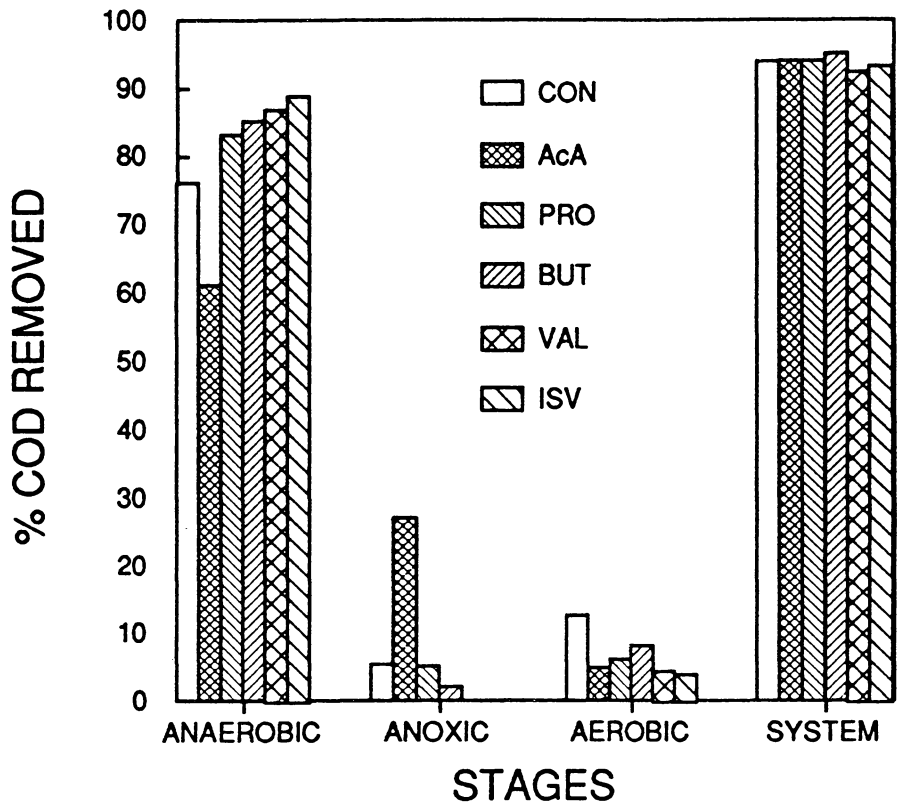


Figure 44. Percent of COD removed in different stages of the pilot plant at various fatty acids additions

Values ranging from 72-89% removal were observed in that stage. All added organic acids showed higher COD removed in the anaerobic stage as compared with the value obtained from the control (76%), to which no additional substrate was added, except the acetic addition experiment which showed relatively low percent COD removed (61%) during the anaerobic stage of treatment. Isovaleric acid addition showed the highest COD removed (89%) during the anaerobic stage. With the exception of acetic acid, all added acids showed low COD removal during the anoxic stage of treatment. Values of COD removed in the anoxic stage ranged from 1-5% for the other acids, whereas a value of about 27% was observed in the anoxic stage for the acetic acid addition experiment. The COD removed during the aerobic stage of treatment ranged from 4-8% for all substrate addition experiments except the formic acid addition experiment which showed a value of 20%. However, the overall COD removal by the pilot plant system remained approximately the same (ranged from 93-97%) throughout the current investigation.

IV.6 Effect of VFA Substrate Addition on MLVSS and SOUR

The mixed liquor volatile suspended solids (MLVSS) concentrations in each reactor obtained during different volatile fatty acid addition experiments are shown in Figure 45. For all control experiments where no fatty acid addition was made, the MLVSS concentrations remained almost the same for all reactors throughout the investigation. The average MLVSS in the first two anaerobic reactors was 1300 mg/l, and the average in the last four reactors was 2400 mg/l. As shown in Figure 45, the addition of different

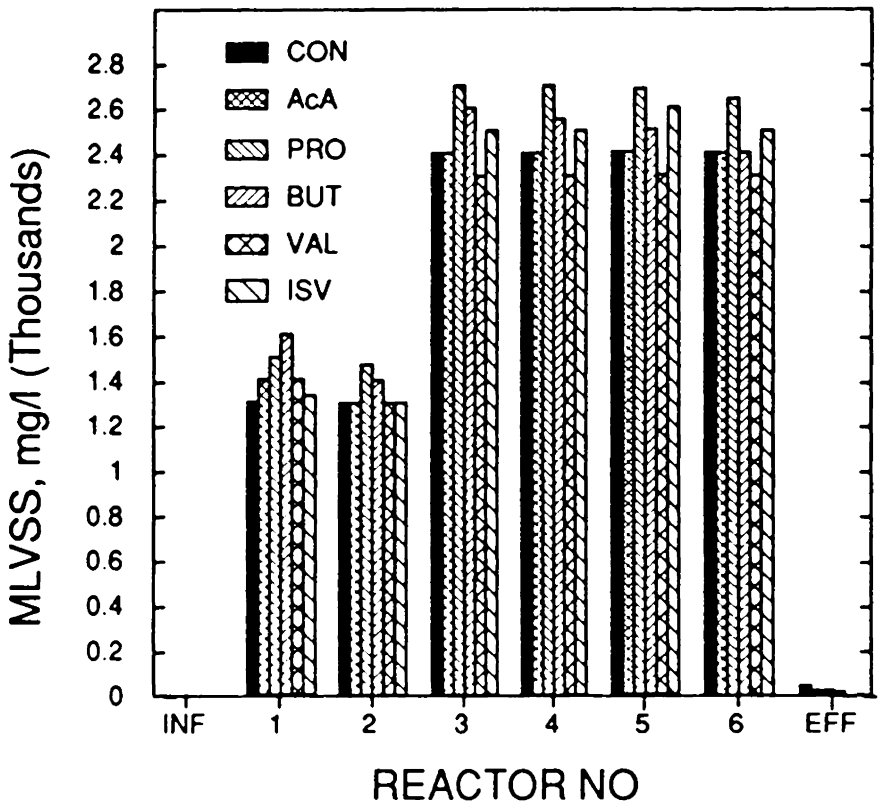


Figure 45. MLVSS concentrations in different reactors of the pilot plant at various fatty acids additions

short chain volatile fatty acids caused a slight increase in the MLVSS concentration in the first anaerobic reactor as compared with the value obtained for the control. Of course, the system did not have time to reach equilibrium with the increased substrate, so the changes observed are short-term responses that reflect the amount of energy that became available to the activated sludge for growth purposes when the VFAs were added. The magnitudes of the increase varied from 100 to 300 mg/l for all acids except isovaleric, which was only 30 mg/l. Volatile suspended solids remained at 80% of the total suspended solids. Both propionic acid and butyric acid additions showed relatively higher MLVSS concentrations, in all reactors, than all other acid additions. Except for the isovaleric acid, the increases in MLVSS were larger than the average MLVSS measurement error, which was 70 mg/l with a standard deviation of 28 mg/l, which implies that less of the substrate was available for phosphorus removal but more was available for growth. The MLVSS data are tabulated in Table B-13 in the Appendix.

Oxygen uptake rates for the last two aerobic reactors (Reactors 5 and 6) were measured for all experiments. The results for the organic acid addition experiments are shown in Figure 46. As shown in the figure, values obtained for Reactor 5 were the same as that for Reactor 6 except during the butyric acid addition experiment, in which the specific oxygen uptake rate for Reactor 5 was considerably higher than that for Reactor 6. It may be also noted that the addition of each organic acid caused an increase in specific oxygen uptake rates, as expected considering that each addition increased the organic loading rate.

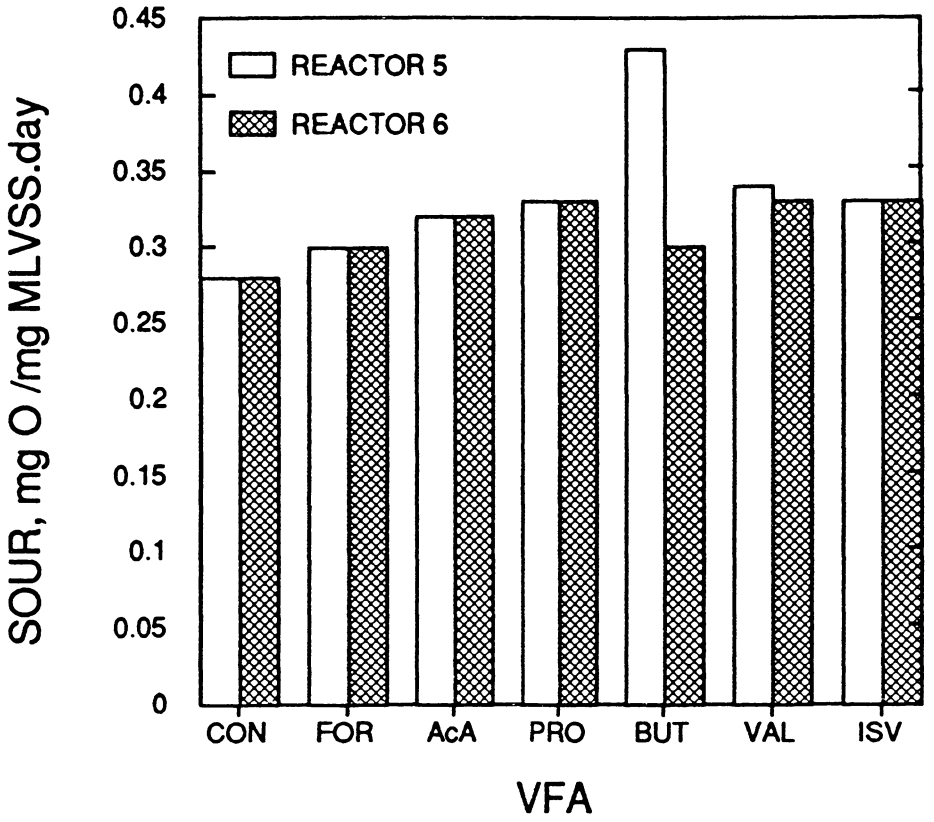


Figure 46. Specific oxygen uptake rates in reactors 5 and 6 during the volatile fatty acid addition experiments

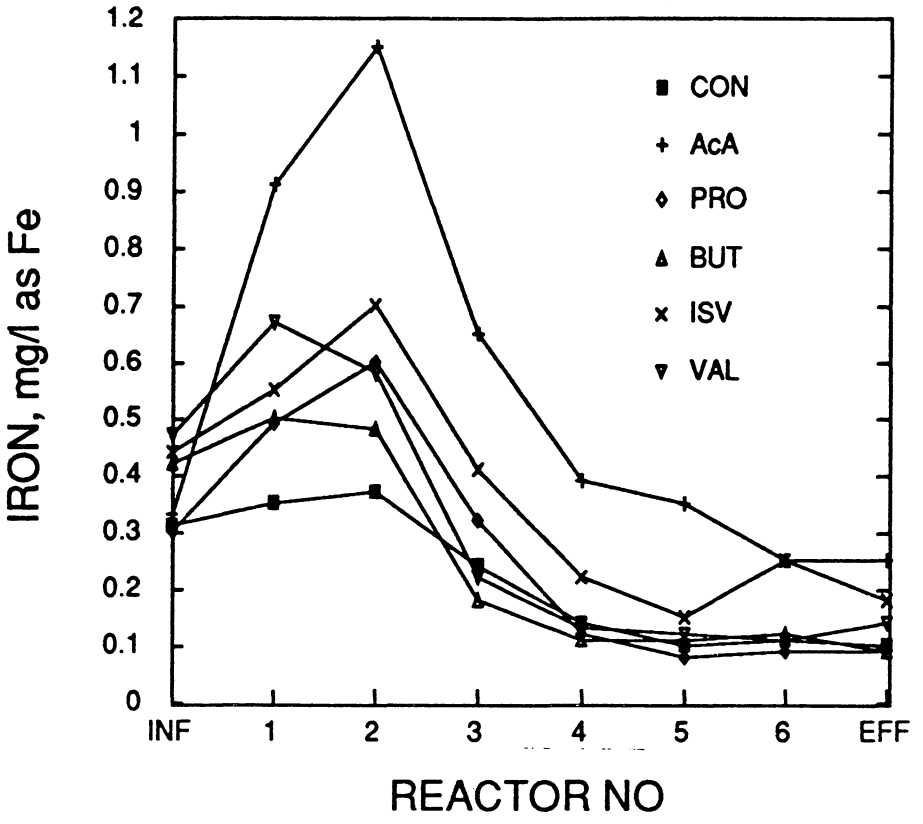


Figure 47. Profiles of iron concentration in the pilot plant system for various fatty acid additions

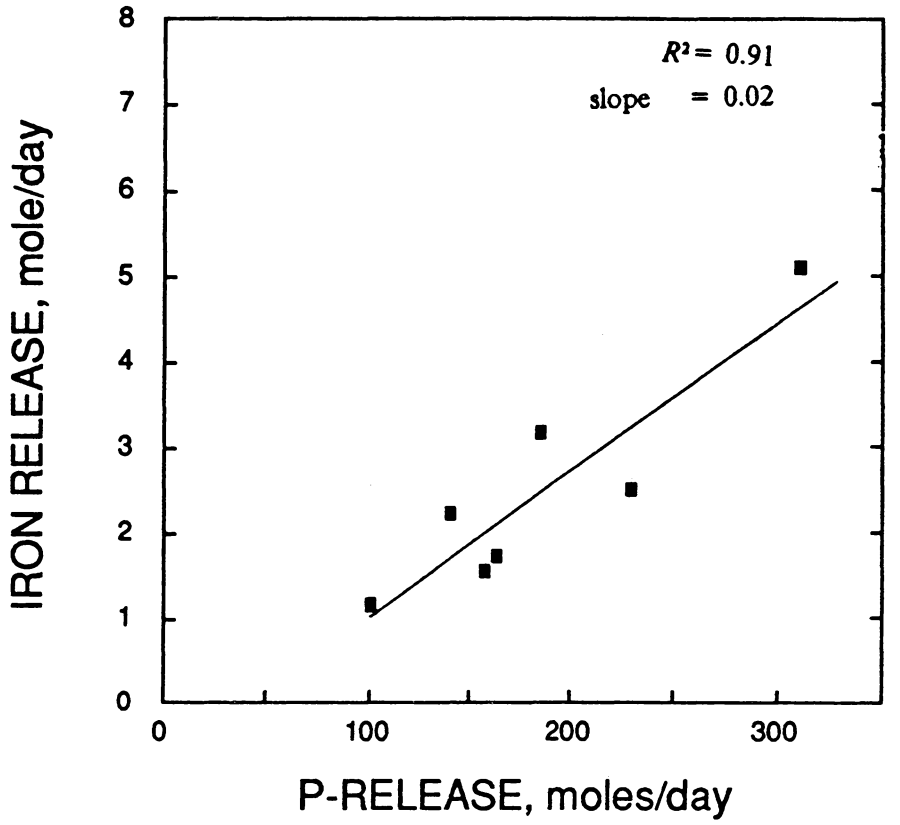


Figure 48. Relationship between iron release and phosphorus release in the anaerobic stage of the pilot plant

IV.7 Effect of Various Substrate Additions on Metals

IV.7.1 Iron

Profiles of iron concentration in the pilot plant system, obtained for the volatile organic acid additions, are shown in Figure 47. Release and uptake patterns of iron were very similar to those for orthophosphate (see Figure 32). Iron release took place each time VFAs were added to the anaerobic stage, and uptake always took place in the aerobic stage of treatment. Figure 48 shows the correlation between iron release and phosphorus release obtained during the various organic acid addition experiments. There was a very good correlation (the correlation coefficient was equal to 0.91) between the actual amount of phosphorus released and the actual amount of iron released. As can be observed in the figure, acetic acid addition resulted in the greatest iron release, and butyric acid addition caused the least. However, valeric acid addition caused more iron release than did isovaleric acid, which was opposite the phosphorus release results. However, the quantities of iron released were very small, and one erroneous sample could have distorted the results. The molar ratio of iron release to phosphorus release was 0.02.

IV.7.2 Calcium

Figure 49 shows profiles of calcium ion concentration for various organic acid addition experiments. The profiles did not show patterns similar to those observed for orthophosphate. That is, there was no indication of a calcium release/uptake mech-

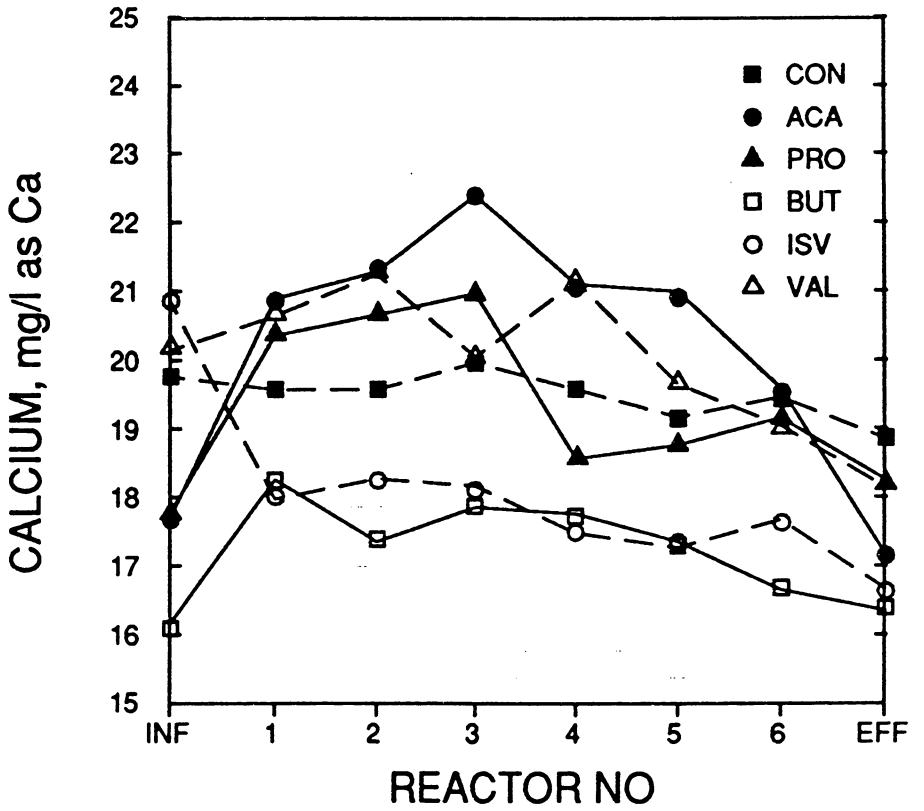


Figure 49. Profiles of calcium ion concentration in the pilot plant system at various fatty acids additions

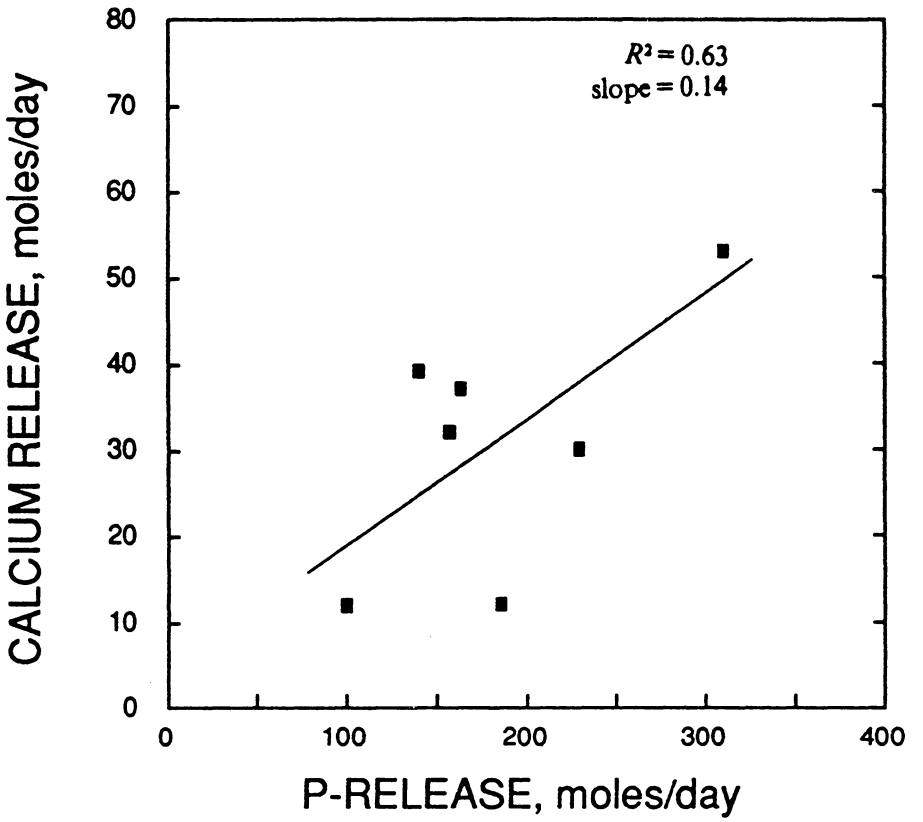


Figure 50. Relationship between calcium release and phosphorus release

anism related to phosphorus uptake and release. In addition, the correlation between calcium release and phosphorus release, on a mass basis, was very poor (the correlation coefficient was 0.63), as shown in Figure 50. Although there was no good correlation between calcium release and orthophosphate release, a large amount of calcium was released in the first three reactors of the pilot plant, as compared to the amount of iron released in the same reactors. The molar ratio of calcium release to phosphorus release was 0.14. Figure 45 clearly shows that the addition of COD resulted in more calcium release.

IV.7.3 Magnesium

The release and uptake of magnesium obtained during the different fatty acid addition experiments are shown in Figure 51. In all experiments, a significant amount of magnesium was released during the anaerobic stage of treatment, and significant uptake occurred during the aerobic stage. Figure 52 shows the relationship between the actual quantity of magnesium released and phosphorus released. There was a good straight-line relationship between magnesium release and phosphorus release (the correlation coefficient was 0.93). A comparison of Figure 51 with Figure 32 reveals that the effect of each organic acid on magnesium release was very similar to the effects on orthophosphate. Acetic acid resulted in the largest release of magnesium, and butyric acid showed the least. Both isovaleric acid and isobutyric acid showed larger magnesium release as compared to the nonbranched forms of these acids. The molar ratio of magnesium release to phosphorus release was 0.16.

IV.7.4 Potassium

Profiles of potassium ion concentration obtained during the different volatile fatty acid experiments are shown in Figure 53. Moderate potassium release in the anaerobic stage was observed during all organic acid addition experiments. The correlation between the actual amounts of potassium released and the corresponding amounts of phosphorus released during the organic acid addition experiments are shown in Figure 54. Acetic acid and isovaleric acid additions caused the greatest amount of potassium release while butyric acid addition resulted in the least release. Branched organic acid additions caused more potassium release as compared to nonbranched forms of the same acids. The molar ratio was 0.14.

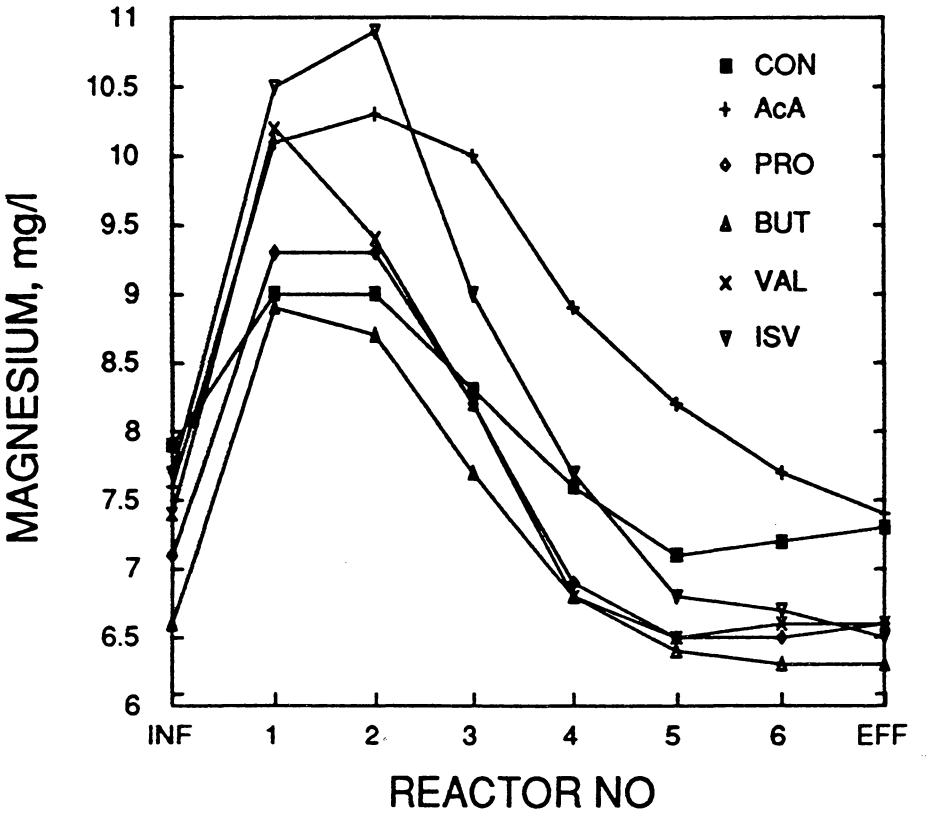


Figure 51. Profiles of magnesium ion concentration in the pilot plant at various fatty acids additions

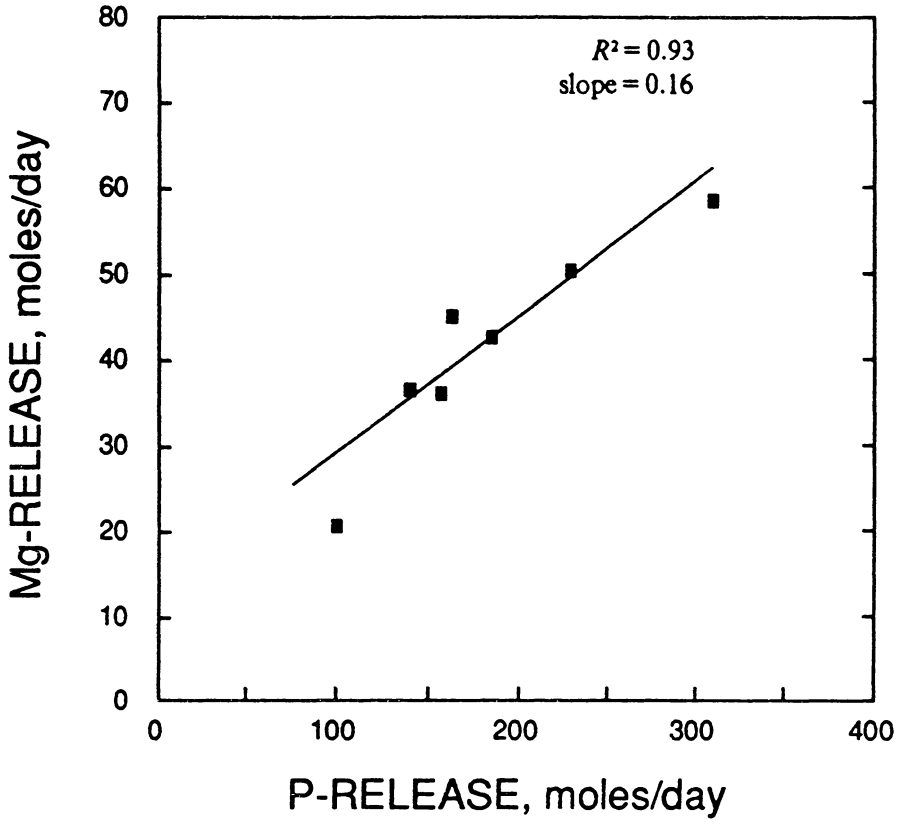


Figure 52. Relationship between magnesium release and phosphorus release

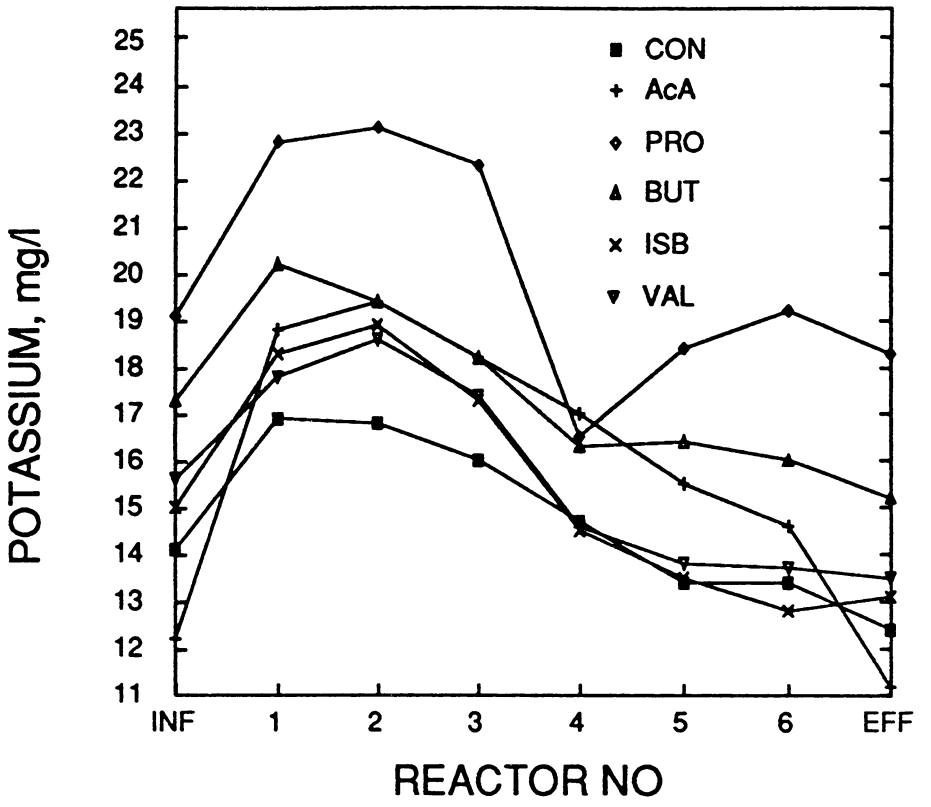


Figure 53. Profiles of potassiom ion concentration in the pilot plant at various fatty acids additions

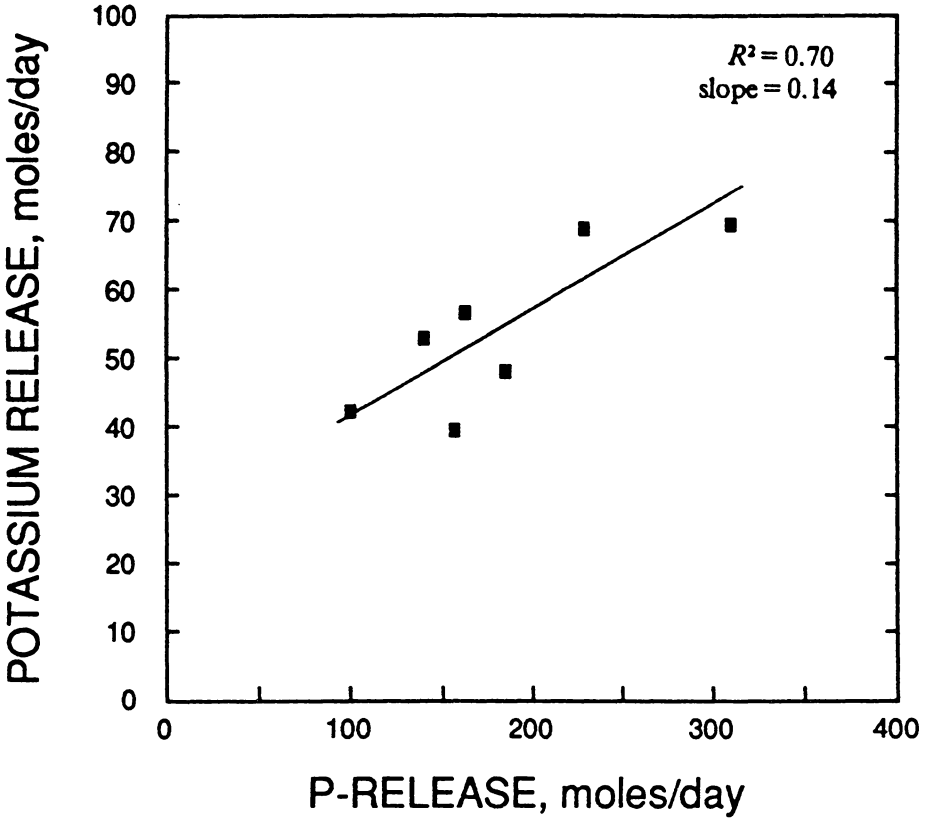


Figure 54. Relationship between ptasium release and phosphorus release

V. DISCUSSION

In this chapter, experimental results, obtained from the pilot plant presented in chapter IV, will be discussed. As mentioned earlier, all data were collected from experiments designed to accomplish the objectives of the current investigation. As identified in chapter I, the main objective of the research was to investigate the effect of various short chain volatile fatty acids (fermentation products) on excess biological phosphorus removal in particular, and biological nutrient removal in general. Other objectives included the fate of these organic acids in biological nutrient removal systems. An additional objective of the research was to gain further insight into the biochemistry involved in excess biological phosphorus removal.

The most accepted theory that explained the role of volatile fatty acids in excess biological phosphorus removal systems evolved to eventually suggest that under anaerobic conditions poly P bacteria absorb short chain organic acids and convert them into storable carbon reserves. The energy required for the conversion process is supplied by the hydrolysis of poly phosphate chains stored inside the poly P bacteria. The stored carbon reserves would then be metabolized under aerobic condition and used as exclusive carbon source for the biosynthesis of polyphosphate storage and for the growth of

poly P bacteria when they are growing under carbon limited aerobic zone (Fuhs and chen, 1975; Rensink, et. al., 1981; Marais, et. al., 1983; Comeau et. al., 1986; and Wentzel, et. al., 1986).

V.1 Establishment of Excess Biological Phosphorus Removal

Although the dissolved oxygen concentrations in the anaerobic reactors were approaching zero and the ORP (oxidation reduction potential) was about -140 mv during the first two months of operation, no phosphorus release was observed in the anaerobic reactors. During that period, the influent dissolved oxygen concentration was kept below 1.5 mg/l by the addition of a reducing agent. Since it was essential to establish excess biological phosphorus removal to accomplish the objectives of this investigation, effects were directed toward finding out the reason for unsuccessful operation.

It was suspected that the oxygen loading to the anaerobic stage of the treatment system was the cause of unsuccessful operation. To reduce the dissolved oxygen loading to the anaerobic stage, two steps were taken. First, the dissolved oxygen concentration in the influent wastewater was further decreased to a value less than 0.2 mg/l, using nitrogen gas bubbling through the wastewater. Secondly, the dissolved oxygen concentrations in the last three reactors were decreased to about 2.0 mg/l (the initial value was 4.0 mg/l) by reducing the air flow rate to these reactors. These changes substantially reduced the oxygen loading to the anaerobic reactor. The load was reduced from 1188 mg/day to 475 mg/day, a reduction of about 60 percent. Within 24 hours after these

changes were made, high phosphorus release was observed in the anaerobic stage, followed by high phosphorus uptake in the aerobic stage. This clearly confirmed that the oxygen loading during the first two months of operation to the anaerobic stage was too high for true anaerobic conditions.

It is accepted theory that phosphorus release from excess poly-P bacteria occurs, under anaerobic conditions, because of the breakdown by hydrolysis of polyphosphate bonds for energy, which is needed by the bacteria for the transport, activation and storage of readily biodegradable substrates (fermentation products). Under aerobic conditions, the accumulated carbon reserved would be used as both a carbon and energy source for growth of the poly-P bacteria plus storage of polyphosphate (Fuhs and Chen, 1975; Rensink et al., 1981; Marais et al., 1983; Comeau et al., 1986; Wentzel et al., 1986). If an electron acceptor such as oxygen or nitrate is present in the anaerobic zone, the readily biodegradable substrate would be fully metabolized by non poly-P bacteria, or the bio-p bacteria themselves, for energy production and a source of carbon. Consequently, no readily biodegradable substrate would be available for the poly-P bacteria to sequester and store, which is a prerequisite for phosphorus release. This was the case during the first two months of pilot plant operation. Dissolved oxygen entered the anaerobic zone and was used as an electron acceptor. In the anaerobic zone most, if not all, of the fermented organics were taken up immediately from solution and metabolized, accompanied by total utilization of all available dissolved oxygen. Thus, the measured dissolved oxygen concentrations in the anaerobic reactors were approaching zero and no phosphorus release was observed.

Successful excess biological phosphorus removal operation was evident by the following observations:

1. large phosphorus release in the anaerobic stage accompanied by high COD reduction;
2. large phosphorus uptake during the aerobic stage of treatment; and

3. high phosphorus content of the sludge (4 to 5 percent) in the aerobic stage as compared with conventional activated sludge values (2 to 3 percent), or values obtained when the pilot plant system was operating as an activated sludge system (2.6 percent).

During the course of the investigation, the phosphorus content of the sludge in the aerobic zone was further increased by short chain volatile fatty acid additions. Values more than 10 percent were measured, expressed as percent of MLVSS.

V.2 Effect of Added Substrates on Phosphorus Release and Uptake

It is generally agreed that anaerobic-aerobic sequencing is the essential prerequisite for enhanced biological phosphorus removal. Under anaerobic conditions, in which neither dissolved oxygen nor oxidized nitrogen (nitrate and nitrite) are present, phosphorus is released from the microorganisms to the bulk of the solution followed by high phosphorus uptake under the aerobic conditions. Various hypotheses have been proposed to explain the rate of phosphorus release in anaerobic conditions. It has been proposed that the anaerobic state causes a stress leading to some enzymatic reactions which eventually cause the synthesis and accumulation of polyphosphate granules. Fuhs and Chen (1975) have proposed that the anaerobic condition was necessary for the establishment of a single bacterial group, *Acinetobacter moraxella-mima.* Later studies have reported that *Acinetobacter* has the ability to store both carbon and phosphate (Deinema et al., 1980; Brodisch and Joyner, 1983; Lotter, 1985). The main function of

the anaerobic stage was also thought to be the continuous production of readily biodegradable substrate, i.e., volatile fatty acids, by the fermenters.

In this investigation, in all experiments, phosphorus was released mainly in the first anaerobic reactor (see Figure 33), and very little release was observed in the second anaerobic reactor, where conditions were even more reduced and the stress would have been greater. This observation indicates that the main role of the anaerobic stage is to produce readily storable and biodegradable substrates (fermentation products) rather than to cause stress conditions. The fatty acids produced would be absorbed by poly-P bacteria and stored in the form of organic polymer. The energy required for the transport and storage of these organics would be supplied by the hydrolysis of polyphosphate reserves. During the aerobic stage, the stored polymers would be metabolized and the liberated energy, which exceeds the amount needed for all growth and maintenance, would be used for the synthesis and storage of polyphosphate.

To date, the precise nature and function of the organic storage polymer in poly-P bacteria have not been identified. Poly-β-hydroxybutyrate (PHB) is the most commonly reported organic storage polymer in poly-P bacteria. Accumulation of this polymer by *Acinetobacter* species under anaerobic conditions, and its aerobic consumption, have been reported by different investigators.

Concentration of PHB in samples of activated sludge treating domestic sewage was found to be in the range from 0.0 to 0.2% on a dry weight basis (Deinema, 1972). Other organic storage polymers, such as glycogen, triglycerides and poly-β-Hydroxyalkanoate (PHA), have also been isolated from microorganisms. The polyhydroxyalkanoate was composed primarily of β-hydroxyvaleric acid and β-hydroxybutyric acid, along with lesser amounts of higher molecular weight compounds (Wallen and Rohwedder, 1974).

According to the literature (refer to Chapter II), PHB can only be produced using two acetyl CoA. Two acetyl CoA can be condensed to acetoacetyl CoA which can be

reduced to PHB. This implies that a readily biodegradable substrate in the presence of poly-p bacteria should be converted to acetyl CoA before it could be stored inside the microorganism. No pathway is proposed for the biosynthesis of BHV (β-hydroxyvaleric acid).

From the foregoing discussion, a large amount of phosphorus release under anaerobic conditions is an indication of a large amount of organic carbon storage as PHB or other organic reserve polymers, which consequently results in a high amount of polyphosphate accumulation under aerobic conditions. Organic storage polymers other than PHB, such as PHV or PHA, also might be involved in the biochemistry of enhanced biological phosphorus removal. High phosphorus release can also be an indication that a large amount of energy is being used for the biosynthesis of organic storage polymers from the building blocks, i.e., fermentation products such as VFAs.

The effect of each short chain volatile fatty acid addition, plus glucose, on biological phosphorus release and subsequent phosphorus uptake will be discussed, based on the above analysis. The fate of each added substrate in the pilot plant system will also be discussed.

V.2.1 Formic Acid

As shown in Figure 38, formic acid addition, when made after neutralization to pH 7 using sodium hydroxide, did not significantly affect either phosphorus release under anaerobic conditions, or phosphorus uptake under aerobic conditions. As mentioned before, acetyl CoA formation is an essential building block for the biosynthesis of poly-β-Hydroxybutyrate (organic storage polymer). Formation of acetyl CoA from formate can only be achieved by carboxylation reaction of formic acid. However, this

reaction is not energetically favorable. When 100 mg/l COD equivalent of formic acid was added, the measured pH in the first anaerobic reactor dropped to a value around 5 (as shown in Figure 38). The dissociation constant of formic is about 14 times more than all other organic acids. In addition, since formic acid is in a more highly oxidized state as compared with other organic acids, a much higher acid concentration is needed to obtain the equivalent of 100 mg/l COD. The drop in the pH of the first anaerobic reactor was accompanied by a decrease in both phosphorus release under anaerobic conditions and phosphorus uptake under aerobic conditions. This observation supported the biochemical model proposed by Comeau, et al., 1985. According to their model, under anaerobic conditions phosphate would be ejected from the interior of the cell during organic storage to reestablish the proton motive force. A decrease in the pH of the solution causes an increase in hydrogen concentration outside the microorganism and consequently an increase in the pH gradient across the membrane. Therefore, a smaller amount of phosphate would be expelled from poly-P bacteria to reestablish the pH gradient or the proton motive force.

V.2.2 Acetic Acid

Acetic acid addition caused the greatest phosphorus release and the greatest phosphorus uptake under aerobic conditions and, consequently, stimulated the highest phosphorus removal by the system (refer to Figures 33, 34 and Tables 13, 15). All added acetic acid was totally removed (consumed) in the first anaerobic reactor (refer to Figure 31). Synthesis of PHB from acetic acid has been reported by Dewes and Senior (1973) and Stanier et al. (1976). Since no detectable acetic acid was measured in the first anaerobic reactor (to which acetic acid addition was made), phosphorus release was

probably limited by the availability of readily biodegradable substrate. Similarly, phosphorus uptake under anaerobic conditions can be limited by the amount of PHB stored inside the microorganisms. The molar ratio of phosphorus released to acetic acid utilized was 0.8, as shown in Table 13. This value is close to the values reported by other investigators as shown in Table 16.

The theoretical molar ratio of phosphorus release to acetic acid storage can be calculated using the models proposed by Comeau et al., 1986 and Wentzel et al., 1986. For the biosynthesis of one β-hydroxybutyrate, two acetic acids and one electron need to be supplied. Electrons can be provided by the TCA cycle (tricarboxylic acid cycle) as proposed by Wentzel et al., 1986. The metabolism of one mole of acetic acid through the TCA cycle generates eight electrons; thus, only 0.125 moles of acetic acid is needed. Therefore, 2.125 moles of acetic acid are required for the synthesis of one mole of β-hydroxybutyrate. Acetate can only pass across the cytoplasmic membrane in the protonated form or by active transport system. That is, 0.5 ATP will be used for the transport of each mole of acetic acid, and 2.125 moles of ATP will be used for activation purposes. Thus, a total of 3.20 moles of ATP are needed for the biosynthesis of one mole of β-hydroxybutyrate. Since one mole of orthophosphate is needed for the biosynthesis of one mole of ATP, 3.2 moles of orthophosphate will be generated and released to solution. In summary, 3.2 moles of orthophosphate will be released for every 2.25 moles of acetic acid absorbed or every mole of β-hydroxybutyrate formed. Therefore, the calculated molar ratio of phosphorus released to acetic acid utilized is 1.5, and the molar ratio of PHB stored to acetic acid utilized is 0.4. The calculated molar ratios for acetate and other organic acids, based on biochemical models proposed by Comeau et al., 1986 and Wentzel et al., 1986 are presented in Table 17. Table 18 shows ratios of units COD utilized to mg phosphorus released calculated for the biosynthesis of one mole PHB.

Comparing the calculated molar ratio of phosphorus release to acetic acid utilized with values listed in Table 16, it can be seen that Wentzel's model (modification of model proposed by Comeau et al., 1986) overestimated the measured values reported by different investigators (including present research).

V.2.3 Propionic Acid

Propionic acid addition also caused an enhancing effect in phosphorus release and phosphorus uptake (refer to Figures 33 and 34). The molar ratio of phosphorus release to volatile fatty acid utilized was 0.44 as shown in Table 13. Theoretical molar ratio was calculated using the biochemical models proposed by Comeau et al., 1986 and chemical reactions reported by Hodgson and McGarry 1968, and Brock et al., 1983, as shown in Table 17. The molar ratios of phosphorus release to propionic acid utilized, and PHB formed to propionic acid utilized, were 1.5 and 0.5 respectively. The ratio of moles of PHB formed to moles of propionic acid utilized is very close, 0.5, to the value calculated for acetic acid, 0.4. Comparing the calculated value of moles of phosphorus released to moles of propionic acid utilized, using the value obtained by this investigation, it can be noted that the measured value is 30 percent of the calculated value. The precise reason for the high difference between the two values is not known. However, this difference may indicate that organic storage polymers other than PHB, such as PHV, are formed or that the biochemical models do not provide the actual mechanism of phosphorus release. Another possibility is that some anaerobic microorganisms were present in the anaerobic reactors and were capable of utilizing the propionate. It is worth mentioning that the conversion of propionyl CoA to acetyl CoA is accompanied by the generation of electrons which can be used for the biosynthesis of PHB from

Table 16. Reported molar ratios of phosphorus released to acetate utilized

Reference	Mole Phosphorus Released / Moles Acetate Utilized
Fukase et al. (1982)	0.9
Potgieter and Evans (1983)	1.1
Lotter (1984)	1.0
Iwema and Meunier (1985)	1.32
Wentzel et al. (1986)	1.0
Comeau, Y. et al. (1987)	1.50
Current Research (1988)	0.8

Note: The above values except current research were obtained using series of batch experiments.

Table 17. Molar ratios calculated for the biosynthesis of one mole of β-Hydroxybutyrate.

	Moles Needed for		Required Energy, ATP			
Substrate	synthesis of PHB	electron supply	Transport	Activation	Moles P released / Moles VFA Used	Moles PHB formed / Moles VFA Used
Acetic Acid	2	0.13	1.06	2.13	1.5	0.47
Propionic Acid[1]	2	0.00	1.00	2.00	1.5	0.50
Butyric Acid[2]	1	0.00	0.50	2.00	2.5	1.00
Valeric Acid[3]	1	0.00	0.50	2.00	2.5	1.00

Calculations are based on models proposed by Comeau et al (1986) and Wentzel et al (1986).

[1]Propionate + HSCoA + NAD^+ + ATP ---> acetyl CoA + ADP + Pi + NADH + H^+ + CO_2
(Hodgson and McGarry 1968, Koch, et al. 1983)

[2]Butyric Acid + ATP + 2HSCoA + FAD + NAD^+ + $2H_2O$ ----> 2 acetyl CoA + $FADH_2$ + NADH + H^+ + AMP + PPi

[3]Valeric Acid + ATP + 2HSCoA + FAD + $2NAD^+$ + $2H_2O$ ----> 2 acetyl CoA + Co_2 + $FADH_2$ + NADH + H^+ + AMP + PPi

Table 18. Ratios of units COD utilized to mg Phosphorus released for the biosynthesis of one mole β-Hydroxybutyrate.

	Moles Needed for		Required Energy, ATP			
Substrate	synthesis of PHB	electron supply	Transport	Activation	Unit COD Used / mg P Released	Unit COD Used / mg PHB Formed
Acetic Acid	2	0.25	1.06	2.13	1.4	1.5
Propionic Acid[1]	2	0.00	1.00	2.00	2.4	2.3
Butyric Acid[2]	1	0.00	0.50	2.00	2.0	1.5
Valeric Acid[3]	1	0.00	0.50	2.00	3.8	2.8

Calculations are based on models proposed by Comeau et al (1986) and Wentzel et al (1986).

[1] Propionate + HSCoA + NAD^+ + ATP ---> acetyl CoA + ADP + Pi + NADH + H^+ + CO_2
(Hodgson and McGarry 1968, Kock, et. al. 1983)

[2] Butyric Acid + ATP + 2HSCoA + FAD + NAD^+ + $2H_2O$ ----> 2 acetyl CoA + $FADH_2$ + NADH + H^+ + AMP + PPi

[3] Valeric Acid + ATP + 2HSCoA + FAD + $2NAD^+$ + $2H_2O$ ----> 2 acetyl CoA + Co_2 + $FADH_2$ + NADH + H^+ + AMP + PPi

acetoacetyl CoA. Thus, no additional substrate metabolism, through the TCA cycle, is needed for electron generation under anaerobic conditions.

V.2.4 Butyric Acid

Although, the nitrate concentration in the anoxic reactor was zero and the COD loading was an average value for the butyric acid addition experiment, anoxic uptake of phosphorus was observed as shown in figure 33. This implies that the anoxic uptake of phosphorus is substrate specific. The calculated ratio of moles of phosphorus released to moles of butyric acid utilized (2.5) is also higher than the measured value (0.78) shown in Table 13. This significant difference might have been observed because not all of the added butyric acid was utilized by the poly-p microorganisms or because of deficiencies in the proposed model. As shown in Table 17, butyric acid storage is 100 percent as PHB.

V.2.5 Valeric Acid

Based on the measured molar ratios of phosphorus released to volatile fatty acid utilized, valeric acid showed the greatest ratio, and better phosphorus removal, as compared with all tested organic acids except the branched form of the same acid as shown in Table 13. However,valeric acid produced the worst phosphorus removal per unit COD utilized as shown in Table 15 and the least molar ratio of phosphorus removed (or released) per unit of COD utilized, as shown in the same Table. This is because fewer moles are required to make 100 mg/l COD equivalent of valeric acid as compared to

other organic acids. However, the molar ratio of phosphorus release to valeric acid utilized obtained by the present research was 1.72, less than that obtained using the biochemical model proposed by Comeau et al., 1986, as shown in Table 17. The overall chemical reaction for the conversion of valeric acid to acetyl CoA (β-Oxidation of fatty acid) is used for the calculation of parameters presented in the table.

V.2.6 Branched Organic Acids

As mentioned before (refer to section IV.1), both isovaleric and isobutyric acids caused more phosphorus release than the normal (non-branched) forms of these acids. In terms of molar ratios of phosphorus released to acid utilized, or phosphorus removed to unit COD removed, isovaleric acid showed the second highest degree of effect on phosphorus removal (acetic acid being the first). The effect of these acids on biological phosphorus removal has never been investigated. Furthermore, there is no pathway reported in the literature for the metabolism of branched volatile fatty acid by any microorganism. Metabolism of isobutyric acid might be similar to the biochemical reactions of valine metabolism by *Pseudomonas aeroginosa* in which isobutyryl-CoA leads to the formation of propionyl-CoA. As mentioned before, propionyl-CoA can be stored inside the poly-p microorganism in PHB form. The metabolism of isovaleric acid might be similar to Leucine catabolism in *Pseudomonas putida* in which isovaleryl-CoA is converted to acetyl CoA and acetoacetate which could be ultimately converted to the organic storage polymer PHB. Pathways for aerobic metabolism of valine and Leucine catabolism in *Pseudomonas putida* are shown in the Appendix. It is also possible that organic storage polymers other than PHB or PHA may be involved in the storage of branched organic acids. Understanding the mechanisms involved in the metabolism of

these organic acids should enable determination of the precise mechanisms of biological phosphorus removal.

V.2.7 Dextrose

As shown in Figure 40, dextrose addition did not show any measurable effect on either phosphorus release under anaerobic conditions, or phosphorus uptake under aerobic conditions. In chapter II, the biochemical equations proposed by Marais et al., 1983 for the utilization of glucose in the anaerobic conditions by poly-p bacteria were discussed. Referring to the proposed equations, if glucose cannot be utilized by poly-p bacteria, it would be utilized by fermenters and lead to the production of three acetate molecules. The produced acetate ions would be utilized by poly-p organisms and would result in the release of three moles of phosphate. However, the COD loading in the dextrose addition experiment was potentially high as compaired with other experiments (refer to figure 42) The high COD loading results in more reduced conditions in the anaerobic stage and also results in an increase in the fermentation activities, that is more fermentation products would be made available for poly P bacteria. In this experiment concentrations of both acetic acid (24 mg/l) and propionic acid (16 mg/l) were measured in the influent wastewater. Low concentrations of both acetic acid (14 mg/l) and propionic acid (8 mg/l) were measured in the first anaerobic reactor of the pilot plant system. As shown in figure 40, although the influent phosphorus was increased to 14 mg/l, the effluent phosphorus concentration was almost zero which clearly indicated that phosphorus, rather than fermentation products limited the biosynthesis of organic storage polymer by poly P bacteria. This is supported by the concentrations of some

fermentation products (acetic and propionic acids) which were measured in the anaerobic stage of the treatment.

V.3 Proposed Model for Excess Biological Phosphorus Removal

Different hypotheses have been proposed to provide a satisfactory explanation for the mechanism of biological phosphorus removal by microorganisms. Nicholls and Osborn (1978) proposed that polyphosphate accumulation assists the microorganism to survive under anaerobic stress. According to them, the main role of organic polymer storage as PHB is to accumulate hydrogen ions and electrons so that more substrate can be metabolized under anaerobic conditions. This model suggested that the main role of the anaerobic stage is to develop stress conditions. However, this viewpoint was not supported by the results obtained during this research (refer to chapter III) and those of many other investigators (Rensink et al., 1981, Marais et al., 1983, and Comeau et al., 1985). Rensink et al., 1981 proposed that fatty acids generated in the anaerobic zone are stored as PHB using energy liberated from the hydrolysis of stored polyphosphate in the form of ATPs. Marais et al., 1983 stated that poly-p accumulation provides the microorganisms performing it with a positive advantage over non-poly p accumulating organisms because they absorb and store the readily biodegradable substrates under anaerobic conditions for energy production under subsequent aerobic conditions.

The most recent model proposed to explain biological phosphorus removal was that suggested by Comeau et al., 1985 and further extended by Wentzel, 1986. This

model was reviewed in chapter II. The model suggests that the transport of any volatile fatty acid will decrease the pH gradient by one H^+. This is logical because substrates cannot transport across the membrane in the ionic form. In addition, at pH about 7 most of the volatile fatty acids (except formic acid) will be present in the ionic form. Thus, the transport of fatty acids would lead to a decrease in the hydrogen gradient and, consequently, a decrease in the proton motive force since microorganisms have to maintain a constant level of proton motive force at all times (Bakker and Mangerich, 1981, Schuldiner and Padan, 1982). Reestablishment of the proton motive force can be achieved by the expulsion of H^+ to the outside of the membrane. According to Harold (1977), this could be done by the breakdown of ATP by the membrane-bound ATP-ase enzyme. Comeau et al., 1985 suggested that phosphate expulsion from the cell is a way for cells to expel H^+ and reestablish the pH gradient. However, phosphate can only be expelled across the membrane to the inside or to the outside in neutral form because, as mentioned earlier, the plasma membrane is impermeable to positive or negative ions. Therefore, one would not expect a change in the H^+ gradient because the number of H^+ transported with the phosphate when entering the plasma membrane would be the same as that when phosphate is expelled to the outside. As mentioned in the previous section, however, the molar ratio of phosphorus released to acetic acid utilized calculated using the Comeau model was much higher than the values obtained by most studies (including this research).

The Comeau model was extended by Wentzel. He proposed that under anaerobic conditions, the electrons required for the reduction of acetoacetyl CoA to PHB could be supplied by the TCA cycle. However, this is likely to be true only when acetic acid is the substrate under anaerobic conditions. Conversion of substrates such as butyric acid and valeric acid to acetyl CoA would be accompanied by the generation of electrons which could be used as the source of reducing power.

Most ,if not all, proposed models have strssed the importance of organic compounds stored in the form of PHB in the mechanism of excess biological phosphorus removal. It was shown that PHB storage could be increased by the addition of short chain volatile fatty acids (Potgieter and Evans, 1982; Lotter, 1984; Iwema and Meunier, 1985; Jones et. al., 1987). According to the literature, PHB can only be synthesized from acetyl CoA (Stanier et. al., 1976 ; Dawes and Senior, 1973), which indicates that various fatty acids must be converted to acetyl CoA before PHB can be produced. However, under anaerobic conditions, the Beta oxidation pathway for the conversion of fatty acids to acetyl CoA is thermodynamically unfavorable (Gaudy, A. and Gaudy, E., 1980). Therefore, a new model is proposed, and it is illustrated by Figures 55 and 56.

The proposed model suggests, mainly, that acetyl CoA is not the only building block for the synthesis of organic storage polymer under anaerobic conditions. Secondly, that the nature and quantity of the carbon storage polymer is dependent on the chemical structure of the organic acid present under anaerobic conditions. Chemical structure includes the number of carbon atoms and the degree of branching. Organic storage polymers ,such as poly-β-Hydroxyvalyrate (PHV) and poly-β-Hydroxyalkanoate (PHA), in addition to poly-β-Hydroxybutyrate (PHB), are involved in the phosphorus release and uptake mechanisms. These polymers have been found to accumulate in microorganisms isolated from wastewaters (Wallen, L. and Davis, E., 1972). The proposed model also suggests that more than one metabolic pathway can be functioning simultaneously in the anaerobic metabolism of a specific substrate. The model further suggests that during anaerobic conditions, fatty acids undergo activation, oxidation, hydrolysis, and condensation, consecutively. Other pathways are given to make the continuity of specific reactions possible. The reoxidation of NADH to NAD can be achieved by the reduction of acetoacetyl CoA to PHB as suggested by Stanier, et. al., 1976. Accumulated hydrogen atoms can also be converted to hydrogen gas by many

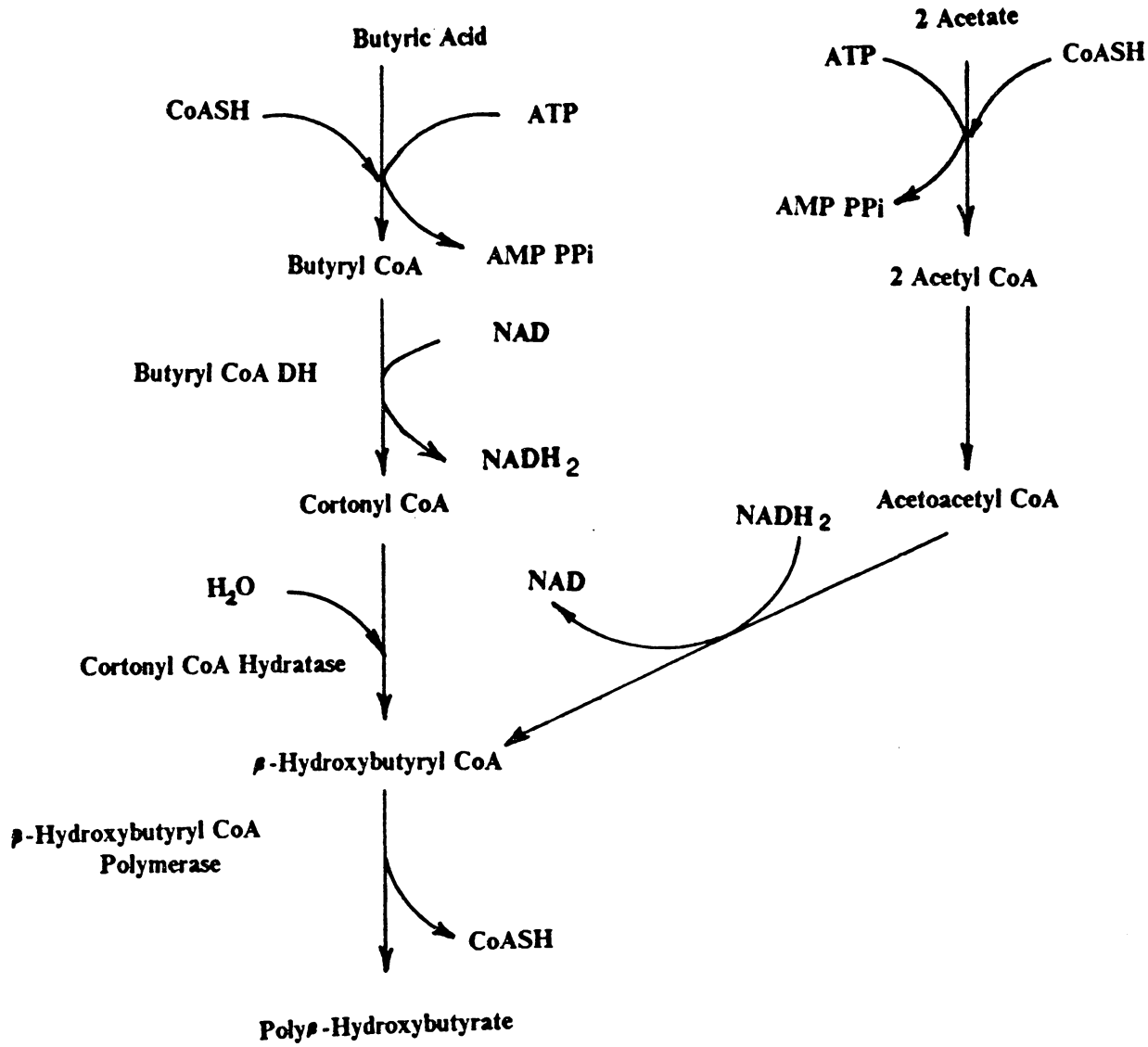


Figure 55. Proposed biochemical model for anaerobic metabolism of VFA containing an even number of carbon atoms

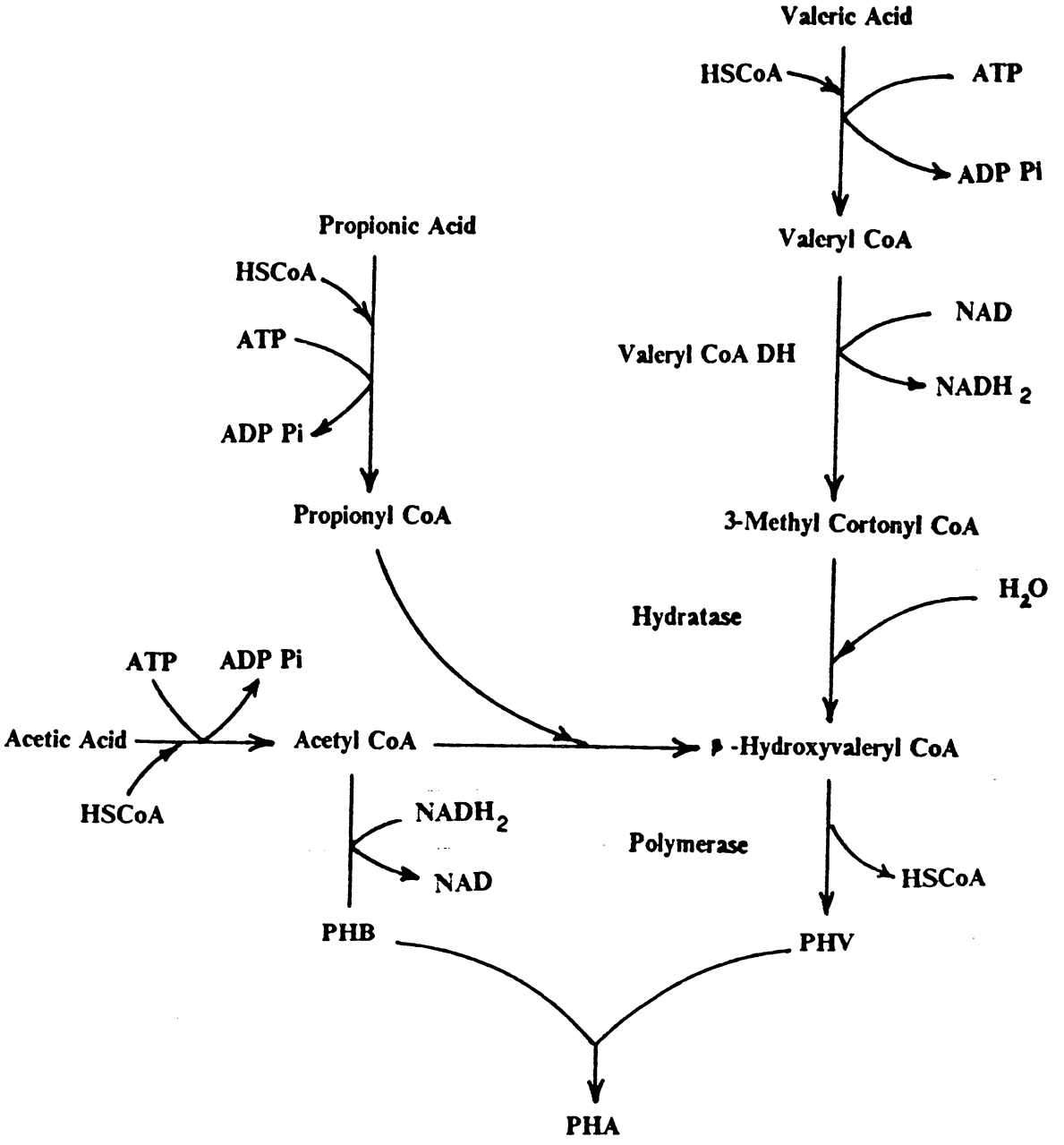


Figure 56. Proposed biochemical model for anaerobic metabolism of VFA containing an odd number of carbon atoms

to hydrogen gas by many anaerobic bacteria using the enzyme, hydrogenase (Gaudy, A. and Gaudy, E., 1980). The proposed model can be applied to both volatile fatty acids containing an even number of carbon atoms such as acetic and butyric acids (as shown in Figure 55), and those containing an odd number of carbon atoms, such as propionic and valeric acids (as shown in Figure 56). However, the proposed model is a speculative model and needs to be verified and tested by conducting series of biochemical experiments.

V.4 The Role of Metals in Phosphorus Release and Uptake

It is well known that both polyphosphate degradation (hydrolysis) and biosynthesis play an important role in the biochemistry of enhanced biological phosphorus removal, and they are coupled with the hydrolysis and formation of ATP (phosphate donor). The participation of monovalent and divalent cations in the stabilization of the structure of polyphosphates, pyrophosphates, and ATP is well recognized. Magnesium, one of the divalent cations, is found to have an important role in the biochemistry of these compounds, basically on the free energy of hydrolysis of ATP. The high affinity of these compounds for magnesium results in a high concentration of magnesium ions in intracellular fluid (Lehninger, 1978). Therefore, some cations, such as magnesium, iron, potassium, and calcium, would be expected to be released or co-transported with phosphate in the anaerobic stage of excess biological phosphorus removal systems.

The correlation between phosphorus release and metal release has been studied by different investigators (refer to chapter II). In most cases, a linear relationship was found to correlate between phosphorus release and metal release under anaerobic con-

ditions. The reported molar ratios of magnesium release to phosphorus release ranged between 0.26 and 0.33. The molar ratios of potassium release to phosphorus release ranged between 0.20 and 0.28 as shown in Table 19. The moles of positive charges associated with each mole of transported phosphate were in the range of 0.80 to 1.04. According to the biochemical model proposed by Comeau et al, 1985, metallic cations such as potassium and magnesium can be used to neutralize the charges on the phosphate molecules, and the ratio of the metal ion charges to moles of phosphate released should be equal to unity.

In this investigation, the relationship of metal release, such as iron, calcium, magnesium, and potassium, to orthophosphate release was studied. Profiles of soluble metal concentrations in the pilot plant system, under anaerobic conditions, are shown in Figures 47, 49, 51, and 53 (in the previous chapter). Correlations between the amount of iron metal release and orthophosphate release are shown in Figures 48, 50, 52 and 54 (chapter IV). Magnesium and iron release showed very good linear relationships with phosphorus release. However, the amount of iron release was very small as compared with the amount of magnesium released. This is logical because, as mentioned earlier, magnesium is the most abundant intracellular divalent cation. Potassium release also showed good correlation with orthophosphate release under anaerobic conditions and was released in amounts comparable to magnesium. Although calcium release showed poor correlation with phosphorus release, a large amount of calcium was released, as compared with iron released.

In general, there was a definite linear relationship between metal release and phosphorus release under anaerobic conditions. The involvement of metals, especially magnesium, in the mechanism of phosphorus release, is evident. Calcium seems to have a less important role in the mechanism of phosphorus release, and its release and uptake is probably controlled by other factors. The molar ratio of metal release to phosphorus

Table 19. Reported and measured molar ratios of metal release to phosphorus release

Reference	Fe^{++}:P	Mg^{++}:P	K^{+}:P	Ca^{++}:P	Sum of charges:P
Arvin and Kristensen (1984)	--	0.32	0.23	---	0.87
Fukase et al (1984)	--	0.33	0.28	0.05	1.04
Miyamoto-Mills et al (1983)	--	0.26	0.27	0	0.79
Comeau et al (1985)	--	0.27	0.23	0.12	1.01
Current Research*	0.02	0.16	0.14	0.14	0.78
Current Research**	0.01	0.23	0.29	0.14	1.05

* Values obtained by regression analysis

** Values obtained assuming no metal would be released when no phosphorus is released, i.e., line of best fit passes through the origin.

release was calculated using regression analysis (slope of the regression line), that is, assuming that metal release can occur even when no phosphorus is released. The molar ratios were also estimated by passing a straight line from the origin to fit most of the data points, that is, assuming that no metal would be released when no phosphorus was released. The calculated molar ratios were compared with values reported in the literature as presented in Table 19. Values obtained by regression analysis were very low as compared with reported values, whereas values obtained by assuming that no metals would be released when no phosphorus was released showed good agreement with the reported values. The molar charge ratio of released cations and released phosphorus was approximately one which supported the hypothesis proposed by Comeau et al., 1985 that metallic cations are co-transported with orthophosphate in a molar charge ratio of approximately one.

VI. CONCLUSIONS

This research was an investigation of the effects of additions of various readily biodegradable substrates to sewage being treated by a biological nutrient removal (BNR) activated sludge system on the performance of the treatment system. The substrates investigated were formic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids, plus dextrose. The following conclusions were developed based on the results obtained from this research:

1. Addition of short chain fatty acids to fresh sewage enhances phosphorus release in the anaerobic stage and enhances subsequent phosphorus uptake in the aerobic stage of biological nutrient removal (BNR) activated sludge systems.
2. The amount of enhanced phosphorus uptake in the aerobic stage of a BNR system will be proportional to release in the anaerobic stage, regardless of the organic composition of the influent substrate. Therefore, the amount of phosphorus released in the anaerobic stage could be used to predict phosphorus uptake in the aerobic stage.
3. Different types of volatile fatty acids (VFA's) cause different amounts of phosphorus release and subsequent phosphorus uptake depending on the number of carbon atoms and degree of branching of the fatty acid.
4. Formic acid addition showed no effect on phosphorus release in the anaerobic stage and, thus, no effect on phosphorus removal efficiency.
5. For normal forms of VFA's of two carbons and above, the amount of phosphorus removed per unit COD added decreased as the number of carbons increased.

6. Apparently the branched forms of VFA's enhanced phosphorus removed compared to VFA's with the same number of carbons. The isovalric form greatly enhanced phosphorus removal compared to the valeric form.

7. The pH in the anaerobic stage can significantly affect the amount of phosphorus released. In this investigation, a pH below 5.5 significantly decreased phosphorus release and subsequent uptake.

8. Both magnesium and iron release were strongly correlated to the amount of phosphorus release, whereas calcium and potassium releases, though positive, were less strongly correlated. The amount of iron involved was very small compared to the other three metals.

9. Volatile fatty acids addition produced no effect on either COD or TKN removal efficiencies when added to the anaerobic stage of a BNR system.

10. The change of phosphorus in the anoxic reactor may be substrate specific. All added substrates except butyric acid caused anoxic phosphorus release whereas butyric acid caused anoxic phosphorus uptake.

11. The main role of the anaerobic stage of a BNR system is both the production of storable substrates, and their storage inside microbial cells, rather than the development of stress conditions for the microorganisms.

A speculative comprehensive model for the anaerobic storage of VFA's by poly-p storing bacteria has been proposed.

VII. ENGINEERING APPLICATIONS

This investigation aimed to improve the efficiency of bio-p removal processes and explain the variations of phosphorus removal efficiency observed in similarly designed and operated biological phosphorus removal plants treating different wastewaters. The investigation confirmed that excess biological phosphorus removal is directly proportional to the amount of phosphorus released in the anaerobic stage of BNR treatment (see Figure 35). It was also concluded, in accordance with other investigators, that the role of the anaerobic conditions is to synthesize and accumulate PHB or PHA storage which will then be used under aerobic condition to remove phosphate from the bulk of the solution. Thus, in actual treatment plants, maximizing the organic storage under anaerobic conditions is an essential factor to establish successful enhanced biological phosphorus removal.

The stored organic polymer can be maximized by maximizing the amount of readily biodegradable substrates (fermentation products) such as short chain volatile fatty acids, particularly acetic and isovaleric acids (see Figure 34). In addition, to establish high organic storage, both oxygen and nitrate should not be allowed to enter the anaerobic zone of the treatment even if both electron acceptors are not detectable in the anaerobic

reactors. This recommendation is underscored by the unsuccessful operation which faced this investigation during the first two months of operation (refer to section V.1). As mentioned earlier, the available amount of fermentation products in the anaerobic zone highly enhances the amount of stored organic polymer, which results in high phosphorus release and, ultimately, high phosphorus removed from the system. Maximizing the amount of volatile fatty acid in the anaerobic zone of treatment plants can be achieved by different methods. Supernatant of fermented primary sludge in anaerobic digesters can be applied to the anaerobic zone (Nicholls et al., 1984). The primary sludge can be added directly to the anaerobic stage (Pitman et al., 1984). Primary sludge thickener supernatant can be applied to the anaerobic reactors (Oldham, 1984). Fermentation basins can be constructed ahead of the plant and used for the generation of short chain volatile fatty acids, mainly acetic acid. Municipal wastewaters can be combined with industrial wastewaters that are rich in volatile fatty acids such as acetic acid and isovaleric acid. Finally, short chain volatile fatty acids can be obtained from the market and added directly to the anaerobic stage of excess biological phosphorus removal plants. The above measures are very important when the treated wastewater is fresh (unfermented), that is, when no volatile fatty acid is present. This can be the case for many treatment plants due to the low hydraulic detention time in the sewer system or low organic content of the influent wastewater. In this research, it was estimated that about 20 mg COD/liter acetic acid concentration in the influent wastewater was needed to remove 1 mg/l phosphorus from the wastewater.

Oxygen and nitrate loadings to the anaerobic stage are very important parameters to consider. Both oxygen and nitrogen loading can be used as electron acceptors in the anaerobic zone and, thus, fermentation products would be preferentially metabolized for energy production and growth instead of being used by the poly-p bacteria and stored as organic polymers for energy production and subsequent phosphorus uptake under

aerobic conditions. Oxygen loading to the anaerobic zone could be reduced by reducing the dissolved oxygen concentration in the aerobic stage of treatment to the lowest possible value (a value of 2.0 mg/l was found sufficient in this investigation). Equipment that may introduce air to the upstream of treatment plants should be avoided. For example, aerated grit chambers, screw pumps, and other air entraining devices should not be used. Vigorous mixing of the anaerobic reactors should also be avoided. All turbulence, such as hydraulic jumps that may cause air entrainment should be prevented to establish good system performance.

VIII. RECOMMENDATIONS FOR FUTURE STUDY

Based on the results obtained during this investigation, several research topics are suggested for future investigation. These recommendations are discussed below.

1. Pure cultures of poly-p bacteria or activated biomass obtained from excess biological phosphorus removal systems, should be grown in batch systems to study the effects of various types of short chain volatile fatty acid additions under anaerobic conditions on the identity and quantity of organic storage polymers (such as poly-β-hydroxyalkanoate). Such a study is essential to verify the actual biochemical mechanisms involved in enhanced biological phosphorus removal systems.

2. Organic storage polymers should be identified and quantified when branched organic substrates such as isobutyric acid, isovaleric acid and isocitric acid are added to pure cultures or active biomass in batch systems under anaerobic conditions.

3. The effect of volatile fatty acid concentration on the rates of phosphorus release and phosphorus uptake, organic reserves and intercellular polyphosphate concentration should be investigated using both batch and continuous systems. such a study is important to understand the kinetics of biological nutrient removal.

4. In this investigation, phosphorus change (release and uptake) in the anoxic reactor was found to be substrate specific. This finding needs to be confirmed using active biomasses of poly-p bacteria in batch systems, under anoxic conditions, and exposed to various volatile fatty acids.

5. In this investigation, the pH in the anaerobic zone had a significant effect on both phosphorus release and phosphorus uptake. However, the observation was made for only one pH value (5.0) in the anaerobic zone, which was not sufficient to generalize the pH effect on biological phosphorus removal. Research should be conducted in which the effect of various pH values in the anaerobic reactor of continuous systems, rather than batch systems, could be investigated.

6. The proposed mechanism, suggested in the present investigation, for the synthesis of organic storage polymers, under anaerobic conditions, from various volatile fatty acids can be verified by measuring the various intermediates and enzyme activities proposed in the model.

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Appendix A. Nomenclature

Ac.A	Acetic Acid
ADP	Adenosine Diphosphate
A. S.	Activated Sludge System
ATP	Adenosine Triphosphate
BNR	Biological Nutrient Removal
BOD	Biochemical Oxygen Demand
BPR	Biological Phosphorus Removal
BSRT	Biological solids Retention Time
BUT.	Butyric Acid

DEX. Dextrose

DOC Dissolved Organic Carbon

COD Chemical Oxygen Demand

CON. Control

EFF. Effluent

FOR. Formic Acid

INF. Influent

ISB. Isobutyric Acid

ISV. Isovaleric Acid

MCRT Mean Cell Residence Time

MLSS Mixed Liquor Suspended Solids

MLVSS Mixed Liquor Volatile Suspended Solids

NAD Nicotinamide Adenine Dinucleotide

NEU. Neutralized

ORP Oxidation Reduction Potential

OUR	Oxygen Uptake Rate
PHA	Poly-β-Hydroxyalkanoate
PHB	Poly-β-Hydroxybutyrate
PHV	Poly-β-Hydroxyvalerate
Poly P	Polyphosphate
PRO.	Propionic Acid
SOUR	Specific Oxygen Uptake Rate
SVI	Sludge Volume Index
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
VAL.	Valeric Acid
VFA	Volatile Fatty Acid

Appendix B. Experimental Data

Table B-1. Orthophosphate Data in Different Reactors for Various Fatty Acids Addition Experiments

VFA	Orthophosphate in Reactors (mg/L)							
	INF	1	2	3	4	5	6	EF
Con.	9.1	14.8	15.3	12.0	8.9	6.1	6.0	5.8
Ac.A	10.0	20.2	24.5	20.0	7.2	2.7	0.9	1.1
Pro.	14.2	22.2	23.5	16.8	9.1	8.5	7.2	7.4
But.	14.7	24.9	26.4	15.6	11.6	9.1	8.4	8.6
Isb.	11.7	22.2	23.5	15.7	7.9	5.4	4.7	5.4
Val.	11.2	21.5	22.4	17.4	8.9	6.8	6.0	6.6
Isv.	13.5	24.5	25.4	20.1	10.0	6.5	5.7	5.1

Table B-2. Orthophosphate Data When the System was Operating as an Activated Sludge System and as a PNR System

Reactor No.	Phosphorus, mg/l	
	A.S (2/4/88)	BPR (6/6/88)
INF	10.5	9.1
#1	9.9	14.8
#2	9.8	15.3
#3	9.1	12.0
#4	8.6	8.9
#5	8.6	6.1
#6	8.5	6.0
EFF	8.4	5.8

Table B-3. Orthophosphate and pH Data for Formic Acid Addition

Time, days	Orthophosphate, mg/L			pH
	Influent	Anaerobic	Aerobic	Anaerobic
1	12.1	27.0	6.2	6.78
2	10.2	22.2	5.9	6.74
3	10.4	24.1	5.9	6.80
4	13.2	13.2	9.1	5.05
5	10.7	19.5	5.4	5.50
6	15.2	30.0	9.6	6.76
7	12.1	26.1	6.0	6.80
8	11.1	23.1	5.8	6.81
9	10.5	23.0	5.7	6.82
10	13.2	27.8	7.8	6.79
11	10.7	20.0	4.9	6.84

Note. Formic acid addition was started on day 3 and was stopped on day 5. The neutralized formic acid was started on day 7 and was stopped on day 9.

Table B-4. Orthophosphate Data for Dextrose Addition Experiment

Day	Orthophosphate, mg/L		
	Influent	Anaerobic	Reactor 6
1	7.1	45.0	0.0
2	8.3	59.0	0.8
3	13.8	52.0	0.5
4	14.5	53.2	0.1
5	13.5	57.6	0.3
6	11.5	48.9	0.0
7	12.8	50.0	0.1

Note. Dextrose addition was started on the third day and was stopped on the fifth day.

Table B-5. Percent Phosphorus in Sludge (MLVSS)

VFA	Percent Phosphorus in MLVSS		
	Reactor 6	Reactor 6	Average
Con.	4.30	4.41	4.4
Ac.A	9.77	9.80	9.8
Pro.	7.33	7.40	7.4
But.	6.88	6.93	6.9
Isb.	6.98	7.10	7.0
Val.	6.05	6.22	6.1
Isv.	8.08	8.30	8.2

Table B-6. COD Concentration Data for Various Fatty Acid Addition Experiments.

VFA	COD Concentration Data in Reactors, mg/l							
	INF	1	2	3	4	5	6	EFF
Con.	362	40	80	64	40	32	32	24
For.	401	95	85	58	34	32	29	29
Ac.A	423	106	102	39	35	32	32	32
Pro.	414	77	51	33	30	27	24	24
But.	399	72	50	40	39	37	33	33
Isb.	472	64	43	26	12	12	12	10
Val.	446	62	47	37	36	31	30	27
Isv.	370	59	34	28	27	23	23	19

Table B-7. Mass balance COD removed for various fatty acid addition experiments.

VFA	COD Removed in Stages, g/day			
	Anaerobic	Anoxic	Aerobic	System
Con.	58.8	4.1	9.7	72.6
For.	62.4	0.27	17.7	80.4
Ac.A	55.7	24.5	4.3	84.5
Pro.	74.5	4.2	5.5	84.2
But.	73.2	1.6	4.3	79.1
Isb.	89.0	1.5	8.5	99.0
Val.	84.0	1.0	4.3	89.3
Isv.	71.3	0.3	3.0	74.6

Table B-8. Percent COD Removal Data for Various Fatty Acids Addition Experiments.

VFA	Percent COD Removed			
	Anaerobic	Anoxic	Aerobic	System
Con.	76	5.3	12.5	93.8
For.	72	0.3	20.4	93
Ac.A	61	27	4.7	93
Pro.	83	5.0	6.0	94
But.	85	2.0	8.0	95
Isb.	87	2.0	8.0	97
Val.	87	1.0	4.5	93
Isv.	89	0.4	4.0	93

Table B-9. TKN Data for Various Fatty Acids Addition Experiments

VFA	TKN (mg/L)	
	INF	EFF
Con.	25.2	4.10
Ac.A	31.4	4.30
Pro.	28.6	3.00
But.	30.0	4.50
Isb.	26.0	3.40
Val.	27.4	3.80
Isv.	26.0	3.60

Table B-10. Nitrate Data for Various Fatty Acids Addition Experiments

VFA	Nitrate Concentration Data in Reactors, mg/l							
	INF	1	2	3	4	5	6	EF
Con.	0.0	0.0	0.0	0.0	0.5	2.7	4.5	5.1
For.	0.0	0.0	0.0	0.0	0.1	1.5	3.7	3.9
Ac.A	0.0	0.0	0.0	0.0	0.4	0.9	4.3	4.4
Pro.	0.0	0.0	0.0	0.0	0.2	1.2	5.1	5.0
But.	0.0	0.0	0.0	0.0	1.4	3.3	4.2	6.0
Isb.	0.0	0.0	0.0	0.0	0.3	0.9	3.0	3.5
Val.	0.0	0.0	0.0	0.0	0.9	4.8	4.9	4.9
Isv.	0.0	0.0	0.0	0.0	0.6	2.4	4.1	5.1

Table B-11. Specific Oxygen Uptake Rates for Various Fatty Acids Addition Experiments.

VFA	Specific Oxygen Uptake Rate mg O_2/mg MLVSS.d	
	Reactor 5	Reactor 6
Con.	0.26	0.26
For.	0.30	0.30
Ac.A	0.32	0.32
Pro.	0.33	0.33
But.	0.43	0.30
Val.	0.34	0.33
Isv.	0.33	0.33

Table B-12. pH Data for Various Fatty Acids Addition Experiments.

VFA	pH Values in Reactors							
	INF	1	2	3	4	5	6	EF
Con.	6.84	6.80	6.78	6.80	6.80	6.77	6.80	7.05
For.	6.64	5.05	5.50	5.90	6.4	6.5	6.60	6.7
Ac.A	6.88	6.82	6.80	6.80	6.78	6.76	6.76	6.82
Pro.	6.84	6.73	6.72	6.73	6.88	6.87	6.90	6.90
But.	6.65	6.62	6.68	6.70	6.81	6.81	6.84	6.91
Val.	6.75	6.70	6.70	6.74	6.74	6.73	6.83	6.98
Isv	6.75	6.65	6.68	6.72	6.75	6.75	6.85	6.97

Table B-13. MLVSS Data for Various Fatty Acids Addition Experiments

VFA	Reactor Number						
	1	2	3	4	5	6	EF
Con.	1300	1300	2400	2400	2400	2400	35
Ac.A	1400	1300	2400	2400	2400	2400	15
Pro.	1500	1470	2700	2700	2680	2640	16
But.	1600	1400	2600	2550	2500	2400	10
Isb.	1400	1400	2500	2500	2500	2500	28
Val.	1400	1300	2300	2300	2300	2300	0
Isv.	1330	1300	2500	2500	2600	2500	0

Table B-14. MLSS Data for Various Fatty Acid Addition Experiments

VFA	MLSS in Reactors, mg/l						
	1	2	3	4	5	6	EFF
Con.	1500	1500	2960	2900	2900	2900	44
For.	1500	1500	3000	3000	3000	3000	--
Ac.A	1700	1600	2900	2900	3000	2900	44
Pro.	1860	1780	3300	3300	3280	3300	40
But.	2060	1700	3350	3240	3230	3100	8
Isb.	1700	1700	3200	3200	3200	3200	4
Val.	1700	1600	3000	3000	3000	2950	0
Isv.	1700	1600	3300	3300	3400	3300	8

Table B-15. Sulfate Concentration Data for Various Fatty Acids Addition Experiments

VFA	Sulfate Concentration in Reactors (mg/L)							
	INF	1	2	3	4	5	6	EF
Con.	27	27	28	34	39	41	39	38
For.	22	22	23	24	24	23	28	25
Ac.A	20	21	21	28	32	35	35	35
Pro.	31	27	31	38	33	37	40	36
But.	23	23	23	25	33	34	34	34
Isb.	29	27	24	30	36	36	40	32
Vale	20	17	20	26	30	31	32	30
Isv.	24	24	24	33	39	39	40	35

Table B-16. Iron Concentration Data for Various Fatty Acids Addition Experiments

VFA	Iron Concentration in Reactors (mg/L)							
	INF	1	2	3	4	5	6	EF
Con.	0.31	0.35	0.37	0.24	0.14	0.10	0.11	0.10
For.	0.89	1.56	1.86	0.82	0.32	0.16	0.13	0.16
Ac.A	0.33	0.91	1.15	0.65	0.39	0.35	0.25	0.25
Pro.	0.30	0.49	0.60	0.32	0.12	0.08	0.09	0.09
But.	0.42	0.50	0.48	0.18	0.11	0.11	0.12	0.09
Isb.	0.35	0.38	0.45	0.10	0.08	0.07	0.14	0.27
Val.	0.47	0.67	0.58	0.22	0.13	0.12	0.11	0.14
Isv.	0.44	0.55	0.70	0.41	0.22	0.15	0.25	0.18

Table B-17. Magnesium Concentration Data for Various Fatty Acids Addition Experiments

VFA	Magnesium Concentration in Reactors (mg/L)							
	INF	1	2	3	4	5	6	EF
Con.	7.9	9.0	9.0	8.3	7.6	7.1	7.2	7.3
For.	7.9	9.0	9.5	9.8	9.3	8.0	8.6	8.9
Ac.A	7.6	10.1	10.3	10.0	8.9	8.2	7.7	7.3
Pro.	7.1	9.3	9.3	8.2	6.9	6.5	6.5	6.6
But.	6.6	8.9	8.7	7.7	6.8	6.4	6.3	6.3
Isb.	7.8	9.8	10.0	8.9	7.3	6.9	6.7	7.1
Val.	7.4	10.2	9.4	8.2	6.8	6.5	6.6	6.6
Isv.	7.7	10.5	10.9	9.0	7.7	6.8	6.7	6.5

Table B-18. Calcium Concentration Data for Various Fatty Acids Addition Experiments

VFA	Calcium Concentration in Reactors (mg/L)							
	INF	1	2	3	4	5	6	EFF
Con.	19.8	19.6	19.6	20.0	19.6	19.2	19.5	18.9
For.	20.6	28.6	29.6	30.8	26.8	22.9	24.9	23.8
Ac.A	17.7	20.9	21.3	22.4	21.1	21.0	19.6	17.1
Pro.	17.8	20.4	20.7	21.0	16.6	18.8	19.2	18.3
But.	16.2	18.3	17.4	17.9	17.8	17.4	16.7	16.4
Isb.	18.6	19.0	19.2	20.4	18.8	18.5	18.2	18.2
Val.	20.9	18.0	18.3	18.2	17.5	17.3	17.7	16.7
Isv.	20.2	20.7	21.3	20.1	21.2	19.7	19.1	18.2

Table B-19. Potassium Concentration Data for Various Fatty Acids Addition Experiments

VFA	INF	Potassium Concentration in Reactors (mg/L)						
		1	2	3	4	5	6	EFF
Con.	14.1	16.9	16.8	16.0	14.7	13.4	13.4	12.4
For.	12.8	16.7	18.4	18.1	15.4	12.9	10.1	14.6
Ac.A	12.2	18.8	19.4	18.2	17.0	15.5	14.6	11.2
Pro.	19.1	22.8	23.1	22.3	16.5	18.4	19.2	18.3
But.	17.3	20.2	19.4	18.2	16.3	16.4	16.0	15.2
Isb.	15.0	18.3	18.9	17.3	14.5	13.5	12.8	13.1
Val.	15.6	17.8	18.6	17.4	14.6	13.8	13.7	13.5
Isv.	18.4	21.0	22.8	20.0	16.4	14.6	14.4	13.6

Table B-20. Mass Balance Metals Released in the System for Various fatty Acid Addition Experiments.

VFA	Moles of metals released/day			
	Iron	Potassium	Magnesium	Calcium
Con.	1.15	42.4	20.6	11.9
Ac.A	5.09	69.5	58.5	52.9
Pro.	2.22	53.0	36.5	38.9
But.	1.54	39.6	36.0	32.0
Isb.	1.72	56.7	45.0	36.7
Val.	3.17	48.1	42.6	11.5
Isv.	2.50	68.9	50.3	30.0

Appendix C. Time schedule for experimentation

DATE	RESEARCH ACTIVITY
8/12/1987	Start System Construction
12/4/1987	Start System Operation
5/2/1988	Establishing BRP
5/30/1988	Begin Experimentation
9/28/1988	End of Experimentation

Appendix D. Glycolysis and TCA cycle pathways

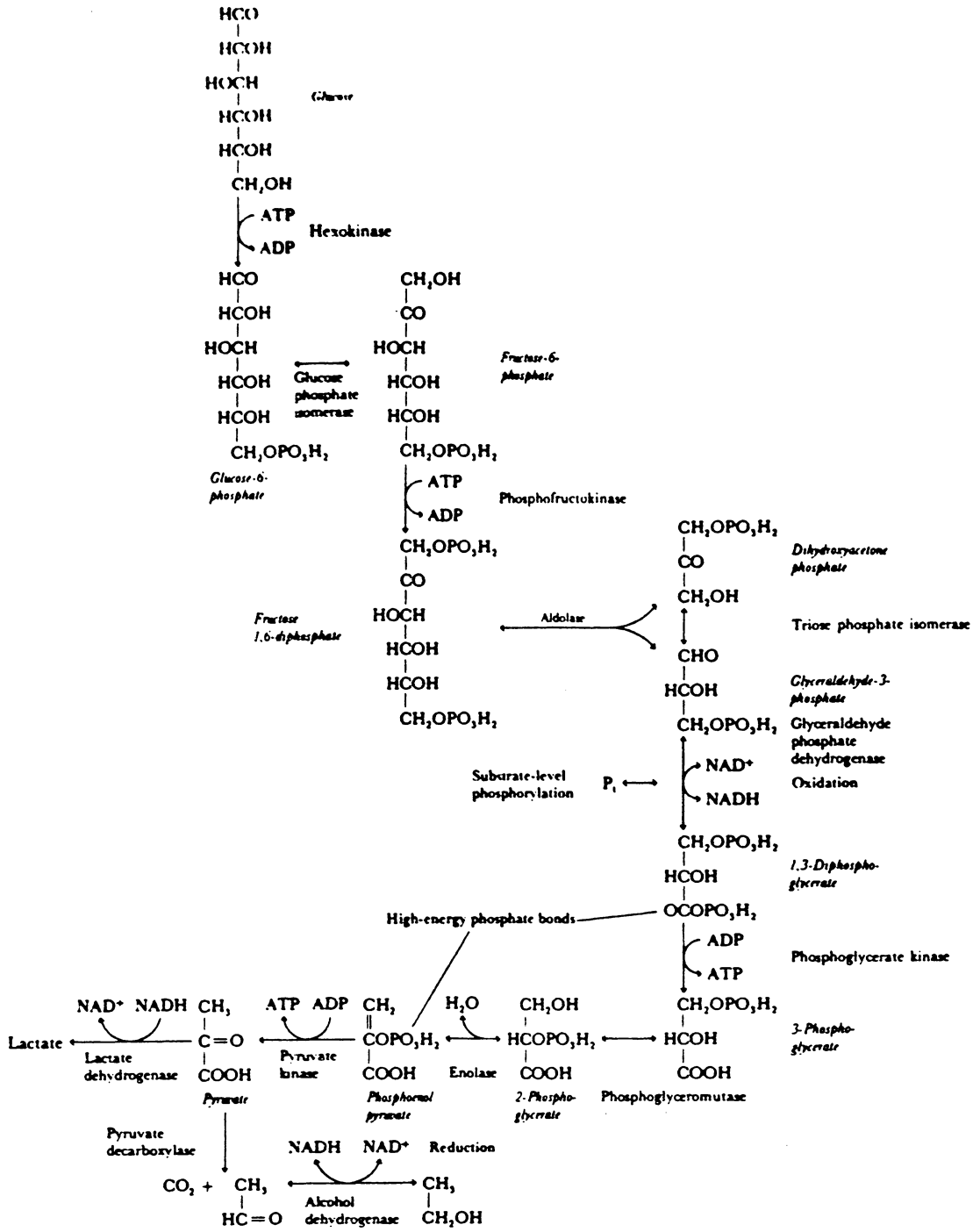

Figure D-1. Glycolysis, the sequence of enzymatic reactions in anaerobic metabolism of glucose (after Brock, et. al., 198

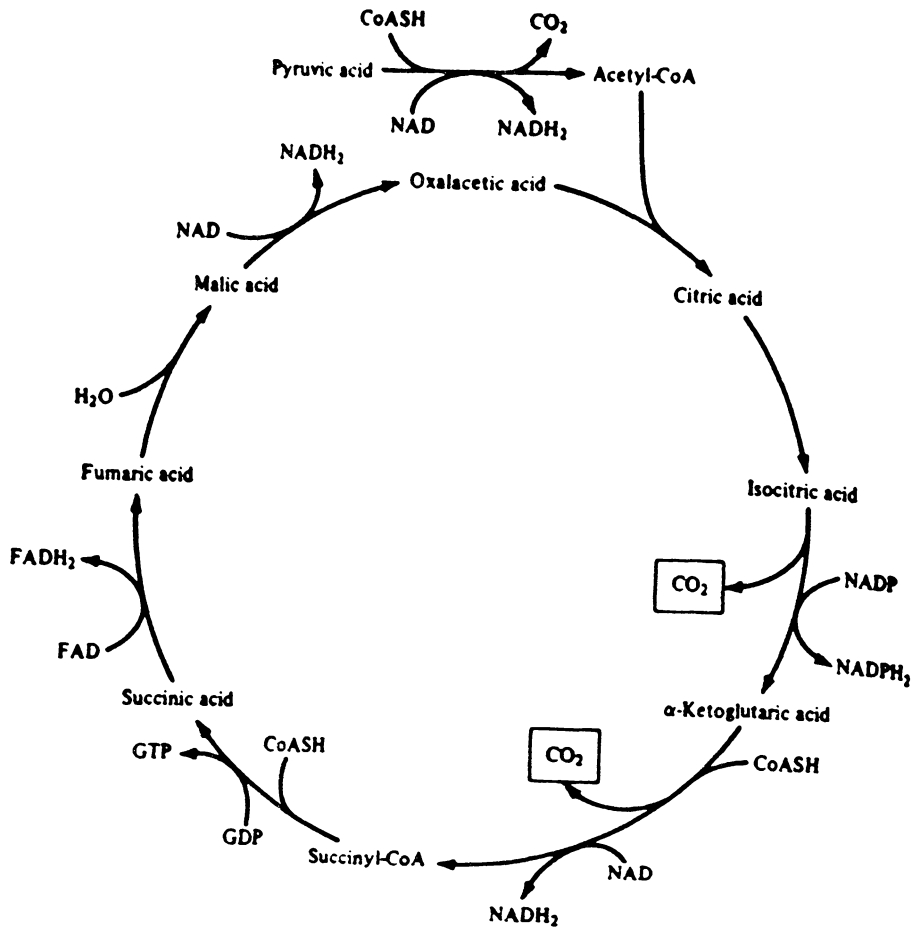

Figure D-2. The tricarboxylic acid (TCA) cycle (after Gaudy, A., and Gaudy, E., 1980)

Appendix E. Aerobic metabolism of valine

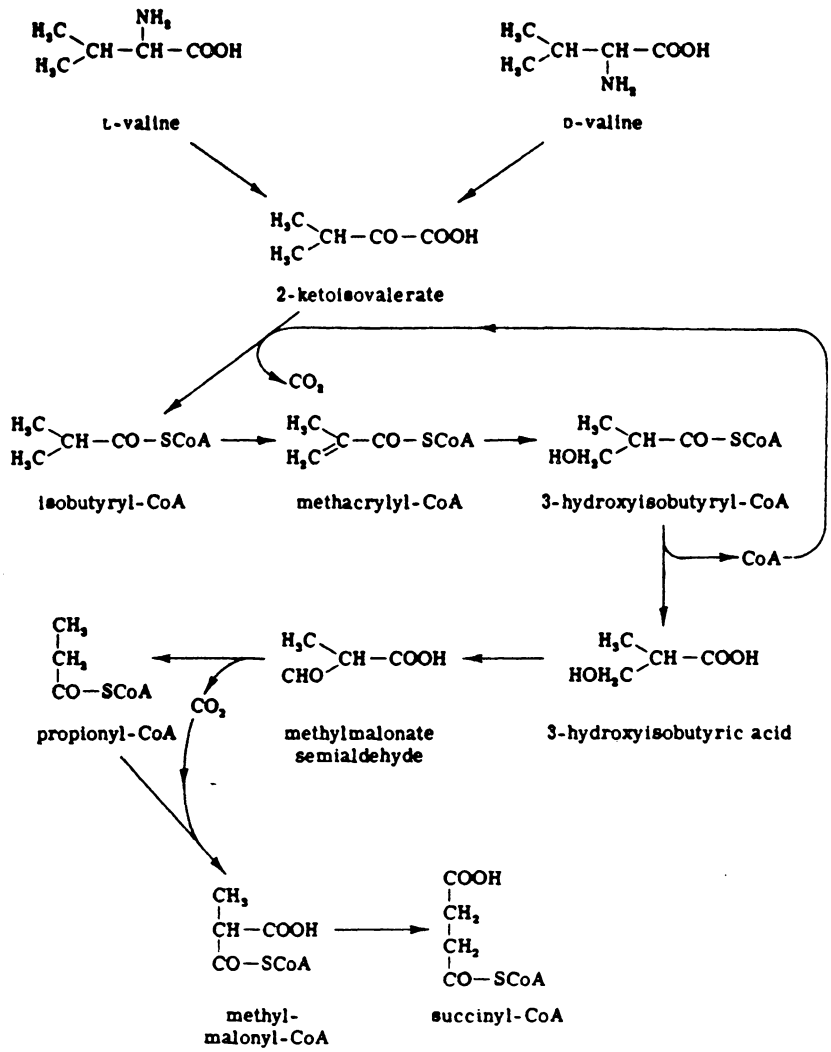

Figure E-1. Aerobic metabolism of valine (after Doelle, H., 1975)

Appendix F. Leucine catabolism

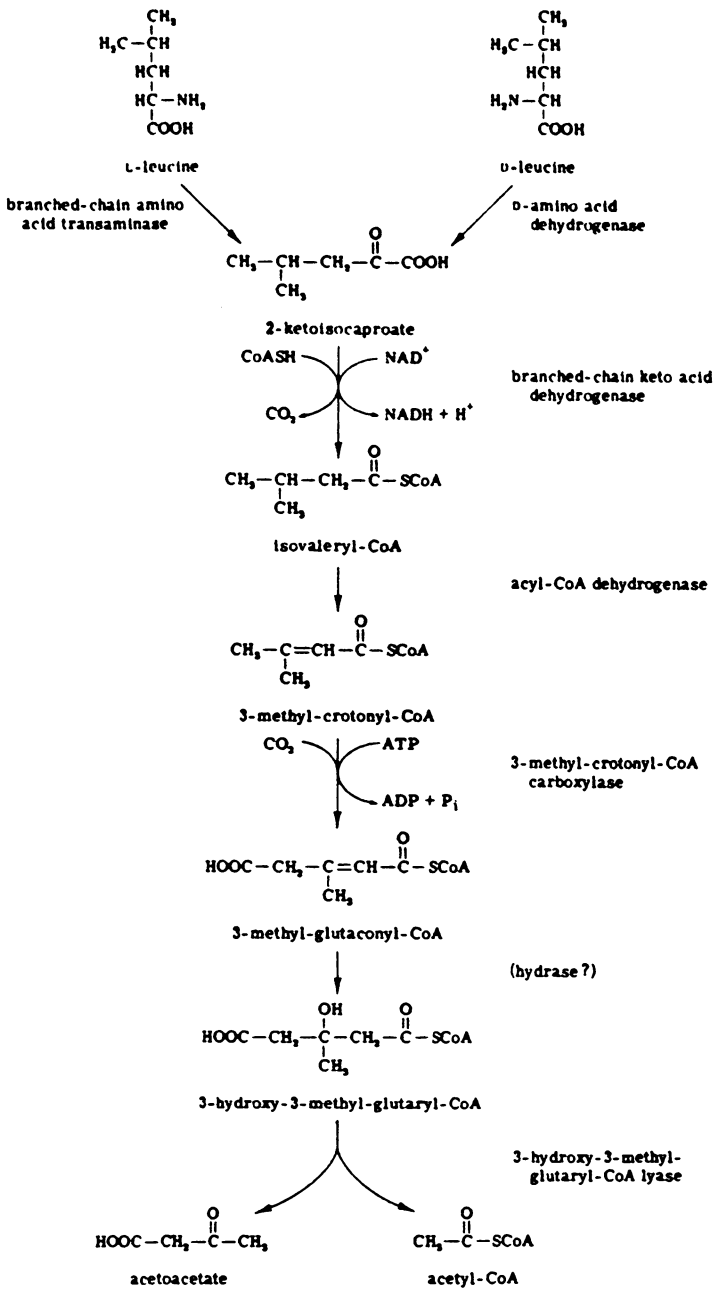

Figure F-1. D- and L-Leucine catabolism in Pseudomonas putida (after Doelle, H., 1975)

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