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THE EFFECTS OF GLUCOSE AND FATTY ACIDS ON ENHANCED
BIOLOGICAL PHOSPHORUS REMOVAL USING A SEQUENCING BATCH
REACTOR

by

TAREK ZAKI KHOURI
B.S. University of Central Florida, 1994

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Civil and Environmental Engineering
in the College of Engineering
at the University of Central Florida
Orlando, Florida

Spring Term
1996

ABSTRACT

Two anaerobic/aerobic sequencing batch reactors (SBRs) were used to evaluate enhanced biological phosphorus removal (EBPR). The first SBR, designated the Glucose SBR, was run for a period of four months. It received a synthetic wastewater plus glucose as a supplemental carbon source. The second SBR, the Isovaleric SBR, was run for three months. During the first month, isovaleric acid was its supplemental carbon source while for the remaining time period, no supplemental carbon source was added to the feed.

Steady-state data from the SBR receiving isovalerate yielded the highest phosphorus (P) removals observed during the study, with a mixed liquor volatile suspended solid (MLVSS) P content of 7.2%.

The next highest removals were observed when prefermented glucose was received, which yielded a MLVSS P content of 6.4%. The lowest removals were observed when no supplemental carbon source was added to the SBR influent, with a 4.4% MLVSS P content.

Batch experiments were also conducted to quantify the effect on EBPR of glucose and the volatile fatty acids (VFAs) acetic acid, propionic acid, valeric acid, and isovaleric acid. Compounds giving the largest anaerobic P release ultimately yielded the lowest effluent P concentrations. At 0.80 mmol/l, isovaleric acid resulted in anaerobic P releases 9.5 mg/l

greater than an equal amount of glucose or propionic acid, but ultimately gave effluent P values roughly 4 mg/l lower than either. Ratios of aerobic P uptake/anaerobic P release were found to be roughly equal for all the VFAs when the VFAs were compared on a molar basis.

Propionic acid had aerobic P uptake/anaerobic P release ratios similar to the other VFAs. It also behaved the same as all the other VFAs with respect to the effect of concentrations added to the batch experiment; however, the magnitude of its removal was significantly lower than all the other substrates.

Glucose, on the other hand, behaved differently from all the VFAs. Glucose aerobic P uptake/ anaerobic P release ratios varied with concentration, which was not the case for the other substrates. Also, glucose P net removals decreased at concentrations higher than 0.60 mmol/l. Glucose also resulted in net P removals roughly 2 mg/l higher than propionic acid, but ultimately gave lower net P removal than isovaleric, valeric and acetic acids.

ACKNOWLEDGMENTS

I would like to express my utmost gratitude to the faculty members of the University of Central Florida who have contributed to my learning experience during the past three years. I would also like to extend my thanks to my graduate committee: Dr. Andrew A. Randall, Dr. Debra R. Reinhart, and Dr. John D. Dietz for their encouragement and support.

Special thanks, gratitude and admiration to my advisor Dr. Andrew A. Randall. I am very fortunate and honored to have had the privilege to work closely with him. Thank you very much for your support and your trust Dr. Randall .

I would also like to thank Dr. Reinhart for her continuous support and concern throughout my studies at UCF.

Most of all, I thank all my friends who have made me feel like I am home during my stay at UCF and in Orlando.

I must also acknowledge the prayers and the support of my family, in the states and back home in Lebanon. Without their love I could have never achieved what I am today.

To my brother Zaki: You are the greatest .

To my late grandfather, Father Kamel Zakka El-Khoury, I dedicate this work with love and tears. I wish you were around at this stage of my life.

TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS AND ACRONYMS	x
I. INTRODUCTION	1
II. LITERATURE REVIEW	4
Biochemical Models for EBPR	4
Substrates Effects	12
Bacterial Population	15
Electron Acceptors	17
Cation Effects	19
Reactor pH	19
Mean Cell Residence Time	21
Statement of Objectives	21
III. METHODS AND MATERIALS	24
Sequencing Batch Reactors	24
Operation of SBRs	26
Maintenance of SBRs	28
Temperature	29
Batch Experiments	30
Analytical Procedures	31
Turbidity	31
Suspended Solids	31
Ortho-Phosphorus	32
IV. RESULTS AND DISCUSSION	33
Influent Feed	33
Reactor pH	36
Mean Cell Residence Time	38
Mixed Liquors Percent Phosphorus	38

SBR Effluent Phosphorus	41
Sequencing Batch Reactors	41
Batch Experiments	45
V. CONCLUSIONS AND RECOMMENDATIONS	68
APPENDIXES	71
A. Routine Operational Data	71
B. SBR Data	76
C. Batch Experiments Data	81
D. Sample Calculations for Percentage P in the Mixed Liquors	104
LIST OF REFERENCES	107

LIST OF TABLES

Table		
3.1	Control program for influent feed and SBR cycles	25
3.2	Composition of the synthetic wastewater	27
4.1	Changes in the influent feed turbidity and pH due to fermentation	35
4.2	Percentage of P in the mixed liquors for both SBRs	39
4.3(a)	Net ortho-P removal and ThOD for all batch experiments relative to control	47
4.3(b)	Net ortho-P removal relative to control per mg/l of substrate added	47
4.4	Ortho-P uptake/release ratio relative to control	60

LIST OF FIGURES

Figure		
2.1	Biochemical models of EBPR for anaerobic and aerobic conditions	6
2.2	Division of acetate between TCA and PHB under aerobic conditions as proposed by Comeau/Wentzel model	7
2.3	Biochemical pathways of extended Mino model	9
2.4	Biochemical models for anaerobic phase of VFAs containing an even number (top) and an odd number (bottom) of carbon atoms	10
2.5	Glycolysis and the propionate pathway supplying energy for anaerobic substrate uptake without the need of energy from poly-P	11
2.6	Correlation of P release and uptake	14
2.7	Profiles of phosphorus, magnesium and potassium	20
2.8	Influence of MLSS on P release and uptake	22
4.1	Influent feed pH and turbidity	34
4.2	Phosphate release and uptake compared to pH behavior for both SBRs	37
4.3	Effluent ortho-P for both SBRs	42
4.4	Phosphorus release and uptake of both SBRs for cycle 1	44

4.5	Net P removal for all four fatty acids relative to control	46
4.6	Ortho-P release and uptake with 0.0 mmoles/l substrate for React 1 and React 2	49
4.7	Batch experiments for all substrates	50
4.8	P uptake versus P release for batch experiments using 0.20 and 0.40 mmoles/l substrates	52
4.9	P uptake versus P release for batch experiments using 0.60 and 0.80 mmoles/l substrates	53
4.10	Net P removal for glucose substrate relative to control	54
4.11	Net P removal for all substrates relative to control	56
4.12	P uptake versus P release for batch experiments using 0.20 mmoles/l substrate, showing glucose as an outlier	57
4.13	Ortho-P for the Glucose SBR and the batch experiment using 0.80 mmoles/l glucose	58
4.14	Net P removal for all concentrations of substrates relative to control	61
4.15	Ortho-P for the Isovaleric SBR and the batch experiment using 0.80 mmoles/l isovaleric acid	63
4.16	Ortho-P release and uptake for batch experiments with 0.40 mmoles/l substrates	65
4.17	P uptake/release ratio for all substrates including the controls	66

LIST OF ABBREVIATIONS AND ACRONYMS

ADP	Adenosine Dinucleotide Phosphate
ATP	Adenosine Trinucleotide Phosphate
BOD	Biological Oxygen Demand
C _n	n Carbon (ie. C ₁ is one carbon)
CoA	Coenzyme A
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
ED pathway	Entner-Doudoroff pathway
EMP pathway	Embden-Meyerhof-Panas pathway
EPA	Environmental Protection Agency
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
NAD	Nicotinamide Adenine Dinucleotide (oxidized form)
NADH	Nicotinamide Adenine Dinucleotide (reduced form)
NO _x	Nitrogen Oxides
NTU	Nepthalometric Turbidity Unit

P	Phosphate
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvalerate
SBR	Sequencing Batch Reactor
TCA cycle	Tricarboxylic Acid cycle
ThOD	Theoretical Oxygen Demand
VFA	Volatile Fatty Acid

I. INTRODUCTION

Any natural waterway has a limited capacity for purifying itself. When this capacity is overwhelmed, the waterway becomes polluted. One way pollution can occur is through a phenomena called "eutrophication". Eutrophication occurs when large concentrations of nutrients, like phosphorus, are present in the system. This leads to heavy alga growth which is detrimental to the natural water system. It has been reported that a minimum phosphorus concentration of 10 micrograms/liter is required before algal growth can occur (Horan, 1990).

Phosphorus, which is normally found in municipal wastewater in concentrations of 6-9 mg/l, is present as organic phosphorus, inorganic phosphate or orthophosphate, and polyphosphate forms. Polyphosphate can be stored as a bacterial storage product which is involved in enhanced biological phosphorus removal. Typically, bacterial cells contain approximately 2.3 % phosphorus. However, under the appropriate environmental conditions, the biomass will be capable of accumulating up to 6-8 % phosphorus or more, far in excess of its nutritional requirements. This phenomena is known as "luxury uptake" or enhanced biological phosphorus removal (EBPR) (Randall et al., 1992).

EBPR can be achieved by polyphosphate (poly-P) bacteria during anaerobic-aerobic sequencing. Under aerobic conditions, these bacteria have no growth advantage, and thus

they do not predominate. However, under intermittent anaerobic conditions, poly-P bacteria use the energy stored in polyphosphate bonds to sequester and polymerize organic compounds such as the short chain volatile fatty acids (VFAs) for intracellular storage as polyhydroxyalkanoates (PHAs). When the poly-P bacteria exit the anaerobic phase, and are exposed to aerobic conditions, they metabolize the stored substrate for growth and energy. Excess energy is generated beyond the need for growth, and they remove phosphorus from the solution and store the energy in phosphate bonds. As a result, the poly-P bacteria will contain large amounts of polyphosphate granules when they leave the aerobic phase (Dold, 1990).

A proposed biological phosphorus removal mechanism was also presented in the Environmental Protection agency (EPA) design manual (U.S. EPA, 1987). It assumes that acetate and other fermentation products, ie. VFAs, are produced from fermentation reactions catalyzed by facultative organisms in the anaerobic phase. These fermentation products are assimilated by the microorganisms present, and are stored as the intracellular storage products PHAs, of which polyhydroxybutyrate (PHB) is the most common. In order to force the storage of PHBs in a normally operating wastewater treatment plant, a temporary stress-producing situation must be created. This situation is achieved by the introduction of the anaerobic phase to the system, thus restricting the availability of oxygen and nitrates. Under such conditions, aerobic bacteria will be forced to utilize internally stored energy sources, such as polyphosphate. On the other hand, facultative bacteria can still continue their metabolic activity using a fermentative metabolic pathway (Nicholls and Osborn, 1979).

Under aerobic conditions, hydrogen and electrons generated by substrate utilization would pass through the Krebs cycle and the electron transport system and form water. However, oxygen is unavailable under anaerobic conditions, and the disposal of hydrogen and electrons will not occur unless other pathways are made available. It was found that poly-P bacteria are capable of converting these hydrogen atoms to PHBs, an alternative "hydrogen sink" to the Krebs cycle (Dawes and Senior, 1973). Lafferty et al.(1988), on the other hand, offered a more updated mechanistic model. Under aerobic conditions, the substrate goes to the tricarboxylic acid (TCA) cycle. Under the anaerobic conditions and due to the absence of oxygen, the Nicotinamide Adenine Dinucleotide (NADH) builds up and causes several TCA cycle enzymes to be inhibited, which causes substrate carbon to be shunted towards PHA biosynthesis instead of the TCA cycle. This assimilation and storage is aided by the energy made available from hydrolysis and release of polyphosphates previously stored in the cell, thus causing an increase in the soluble phosphorus concentration.

The anaerobic phase is followed by the aerobic one where PHBs stored inside the cells are metabolized, generating energy that is stored as polyphosphate. Soluble phosphorus from the surrounding bulk liquid is taken up for this purpose. The phosphate utilizing bacterial population increases during this time due to substrate utilization and growth (U.S. EPA, 1987).

II. LITERATURE REVIEW

Biochemical Models for EBPR

Many mechanistic models have been proposed to explain the EBPR process. One model was introduced by Marais et al. (1983) which presented a biochemical pathway for the synthesis of PHBs under anaerobic conditions. However, with acetate as the substrate, it was not possible to identify pathways for PHB synthesis since no source of protons and electrons were present to reduce acetoacetate to PHB. To explain this matter, Comeau et al. (1986) suggested that the need for protons and electrons can be supplied from the TCA cycle operating in reverse, while Mino et al. (1987) proposed that the reducing power could be supplied from the degradation of glycogen.

Comeau et al. (1986) came up with a model, often referred to as the Comeau model, based on their data and biochemical literature, in which they assumed the importance of the *Acinetobacter* genus, isolated in South Africa by Marais et al. (1983), to the EBPR mechanism. Under anaerobic conditions, the energy for PHB synthesis was observed to be provided by poly-P which was modelled as being co-transported with protons across the membrane. Furthermore, Acetyl-CoA would be metabolized by the TCA cycle to provide the reduced form of Nicotinamide Adenine Dinucleotide (NADH) needed for the reduction of Acetyl-CoA

in PHB synthesis. Under aerobic conditions, the energy is generated by using the PHB carbon reserves, and thus, the energy obtained is stored as poly-P (Figure 2.1).

The Comeau model was later modified by Wentzel et al. (1986), and is referred to as the Comeau/Wentzel model. The new model considered carbon and phosphorus biochemical metabolism as though specific to the *Acinetobacter* spp., being the genus typifying poly-P/bio-P organisms. As such, two parameters were identified to control polyphosphate and PHB synthesis and degradation, the Adenosine Trinucleotide Phosphate/ Adenosine Dinucleotide Phosphate (ATP/ADP) and NADH/NAD ratios, NAD being the oxidized form of NADH. Due to the lack of a terminal electron acceptor under anaerobic conditions, the NADH/NAD ratio rises, while the repression of oxidative phosphorylation leads to a decrease in ATP/ADP ratios. Such phenomena controls the TCA cycle and PHB synthesis and ensures the proper sharing of Acetyl-CoA between the two pathways (Figure 2.2).

Under aerobic conditions, the NADH/NAD ratio decreases due to the absence of exogenous carbon compounds and the prevalence of electrons transport, thus, leading to PHB degradation and activation of the TCA and glyoxylic acid cycles. As PHB degrades, it provides the cell with energy which will increase the ATP/ADP ratio and enhance polyphosphate synthesis (Randall et al., 1992).

Another mechanistic model to explain EBPR was contributed by Mino et al. (1987). The model suggests that the reduction of acetate to PHB is driven by some reducing equivalents supplied by glycolysis. Both the Entner-Doudoroff (ED) and the Embden-Meyerhof-Panas (EMP) pathways have been proposed as pathways for this glycolysis. Arun et al. (1988) proposed a model in which *Acinetobacter* spp. can utilize glucose via the EMP or the ED pathways.

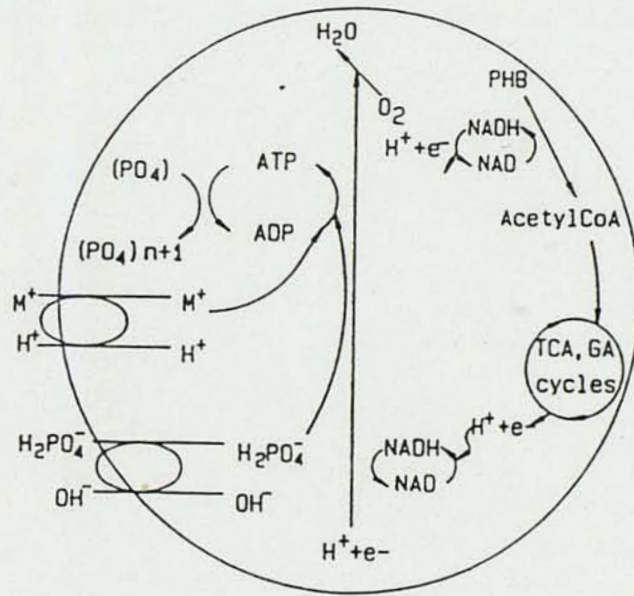
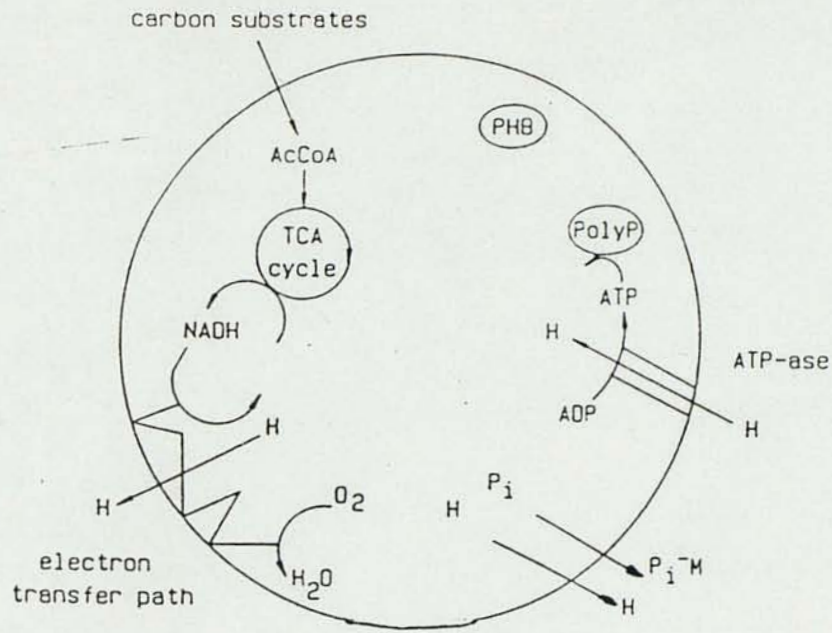


Figure 2.1- Biochemical model of EBPR for anaerobic (top) and aerobic (bottom) conditions (after Comeau et al., 1986).

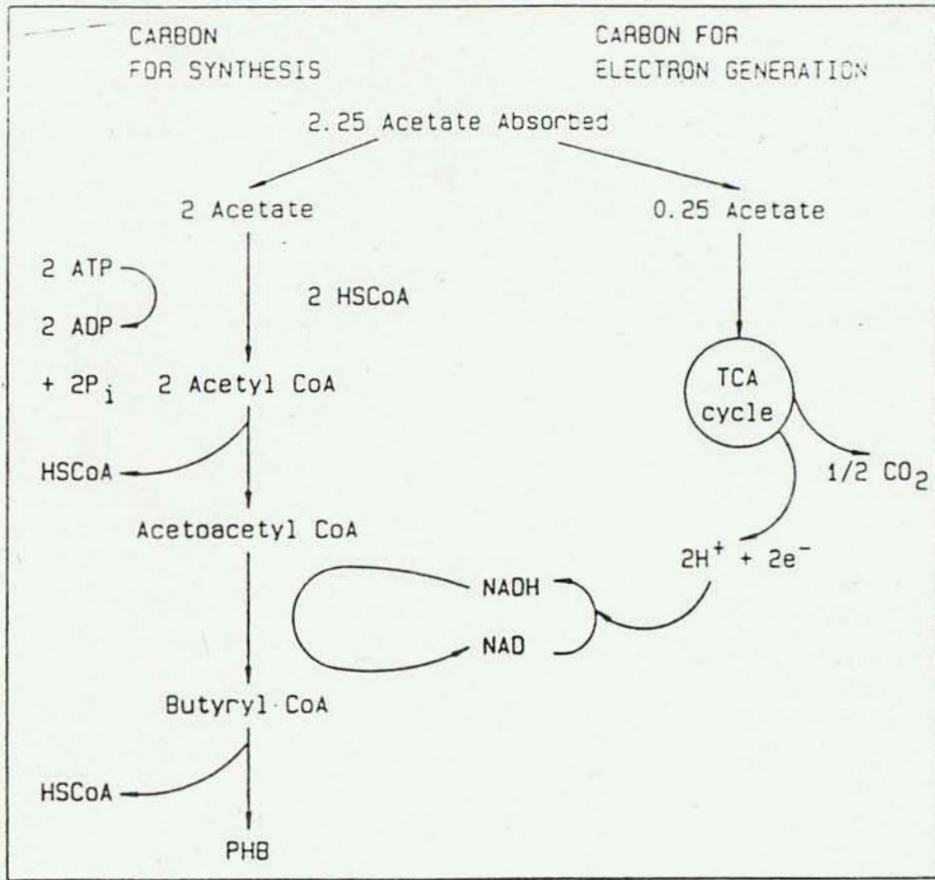


Figure 2.2- Division of acetate between TCA and PHB under anaerobic conditions as proposed by Comeau/Wentzel model (after Randall et al., 1992).

Since TCA cycle needs a way to oxidize reduced electron carries, they suggested that NADH might be supplied from carbohydrates via the EMP pathway. The EMP pathway produces more energy per glucose unit but since *Acinetobacter* spp. conduct glycolysis solely via the ED pathway, the ED pathway has been proposed. This was supported by Bergey (1984) who pointed out that *Acinetobacter* spp. does not possess the EMP pathway, but does have the ED pathway. Furthermore, Wentzel et al. (1985) suggested that the short-chain fatty acids that *Acinetobacter* takes up are generated by heterotrophs which convert glucose to the acids in the anaerobic phase. As such, more energy production via poly-P breakdown is needed to convert acetate to Acetyl-CoA using the ED pathway instead of the EMP (Wentzel et al., 1991). In both cases, the power for PHA biosynthesis comes from glycogen, not the TCA cycle operating in reverse as Comeau et al. (1986) proposed. Figure 2.3 shows the generic glycolysis pathway of the extended Mino model (Arun et al., 1989).

Extensions of the EBPR models were later on proposed by Abu-ghararah and Randall (1989). In addition to acetate, a C₂ compound, Abu-ghararah and Randall (1989) incorporated the C₃-C₅ VFAs as additional substrates that resulted in PHA formation. Figure 2.4 shows the proposed biochemical models for PHBs and PHAs formation using the C₂ and C₃-C₅ substrates.

Other extensions to the EBPR mechanistic models were proposed by Matsuo et al. (1992) and Satoh et al. (1992). They both emphasized the use of glycogen as an energy and a reducing power source for PHA synthesis. In the pathway shown in Figure 2.5, polyphosphate is no longer needed to supply energy. The energy is now supplied using both glycolysis and the propionate fermentation pathways in the anaerobic phase, and thus subsequent aerobic

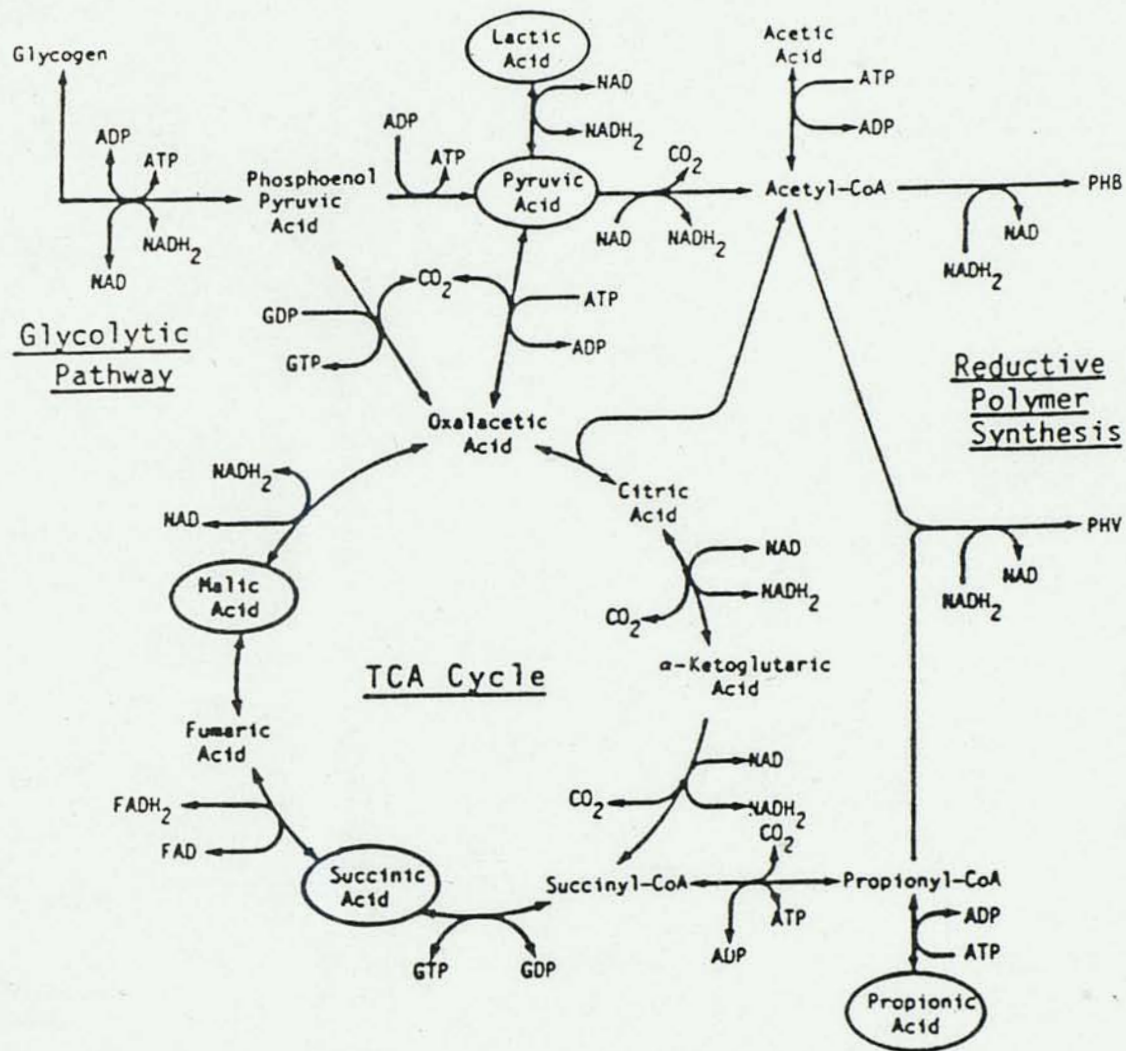


Figure 2.3- Biochemical pathways of extended Mino model (after Arun et al.,1989).

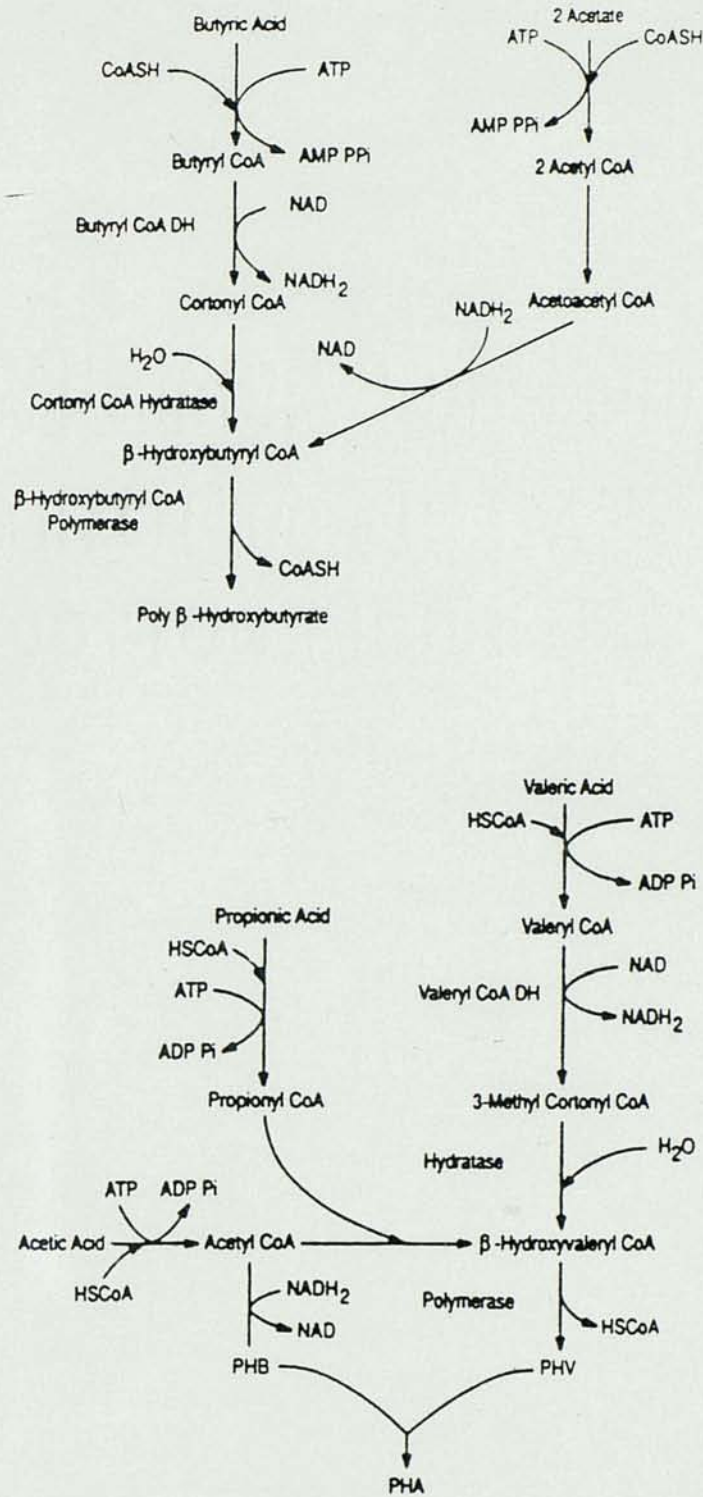


Figure 2.4- Biochemical models for anaerobic phase of VFAs containing an even number (top) and an odd number (bottom) of carbon atoms (after Abu-ghararah and Randall, 1989).

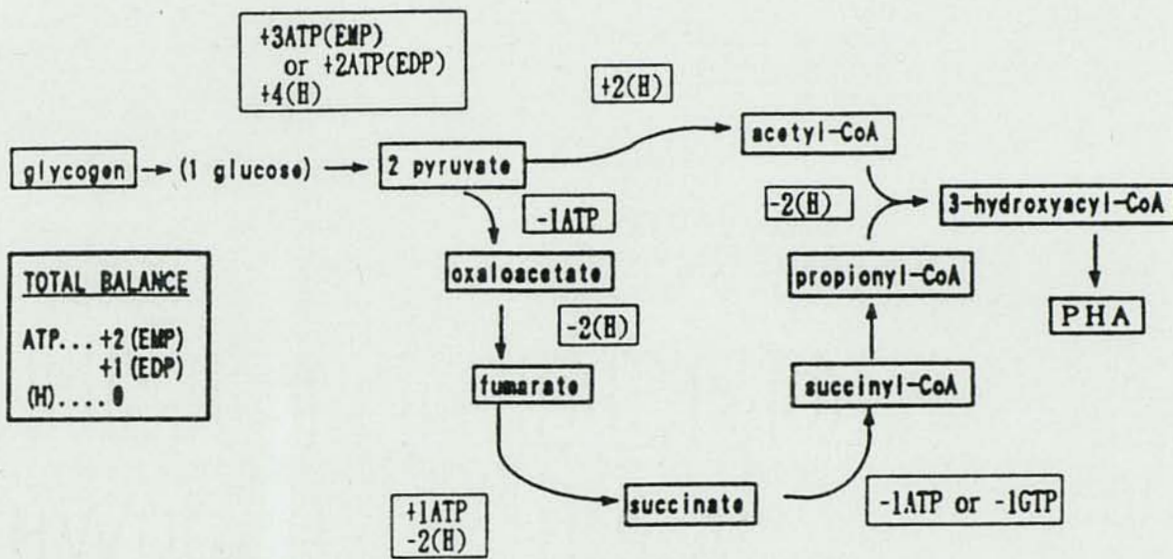


Figure 2.5- Glycolysis and the propionate pathways supplying energy for anaerobic substrate uptake without the need of energy from poly-p (after Satoh et al., 1992).

EBPR will no longer be required. Furthermore, this metabolism may be utilized by non-P EBPR organisms. It may also use non-VFA substrates, such as glucose and lactate for the storage of PHAs without the involvement of polyphosphate (Matsuo et al., 1992; Satoh et al., 1992).

Substrates Effects

The biochemical models presented were limited to situations where acetate was the primary carbon source in the anaerobic phase. However, natural wastewaters may contain other substrates which will affect the operation of the EBPR process.

Another study performed by Shin and Jun (1992) showed that a mixture of glucose/acetate as carbon sources provided better phosphorus removal than using glucose alone. In both cases, the substrate was completely removed during the anaerobic phase. For the mixture of glucose/acetate, they found that the released phosphorus in a three-hour anaerobic phase was five times higher than that of the influent containing glucose alone.

Tam et al. (1992) found that, during the anaerobic phase, high phosphorus release was recorded in reactors treated with acetate. Also, they observed that the higher the dose of acetate introduced, the larger the phosphorus release in the reactor in the anaerobic phase and the greater the phosphorus uptake in the subsequent aerobic period. Furthermore, when the same amount of glucose and acetate was added to separate sequencing batch reactors (SBRs), acetate showed more phosphorus release. Acetate is known to be an easily biodegradable substrate which enters the TCA cycle or PHA synthesis directly without any processing. On the other hand, glucose is a complex organic molecule and must be fermented to simpler forms

before being sequestered by poly-P bacteria and shunted to PHA biosynthesis. It can also be sequestered by competing bacteria and shunted to glycogen synthesis instead (Cech and Hartman, 1990; Satoh et al., 1992). Another study done by Marais et al. (1983) showed that the amount of phosphorus uptake during aerobiosis is proportional to phosphorus released in the anaerobic period. Accordingly, phosphorus removal by P-removing bacteria would be proportional to the amount of phosphorus released under anaerobic conditions.

Abu-ghararah and Randall (1991) also studied the effects of organic compounds on EBPR. All the VFAs used supported a proportional relationship between the amount of P uptake in the aerobic phase and the P released in the anaerobic one (Figure 2.6). The addition of VFAs ranging from acetic to valeric enhanced the BPR process. However, different types of VFAs caused different amounts of net P removals. Abu-ghararah and Randall (1989) also observed that for VFAs of two carbons and more, except for propionic acid, net P removal decreased as the number of carbons increased. On the other hand, branched isomers of the same VFAs exhibited better net P removal than their linear counterparts.

Randall et al. (1995) also observed the superiority of branched VFA isomers compared to linear ones. Furthermore, they observed that the C₂-C₅ VFAs, except propionic acid, induced the EBPR process. In addition, unfermented glucose substrate was found to be consistently detrimental to the P removal mechanism, while prefermented glucose substrate caused a dramatic improvement in the performance of their SBRs. Randall et al. (1995) also reported that the carboxyl functional group was more important to EBPR than the length of the carbon chain (i.e., acids vs. alcohols) which reflected the superiority of such compounds for PHA biosynthesis.

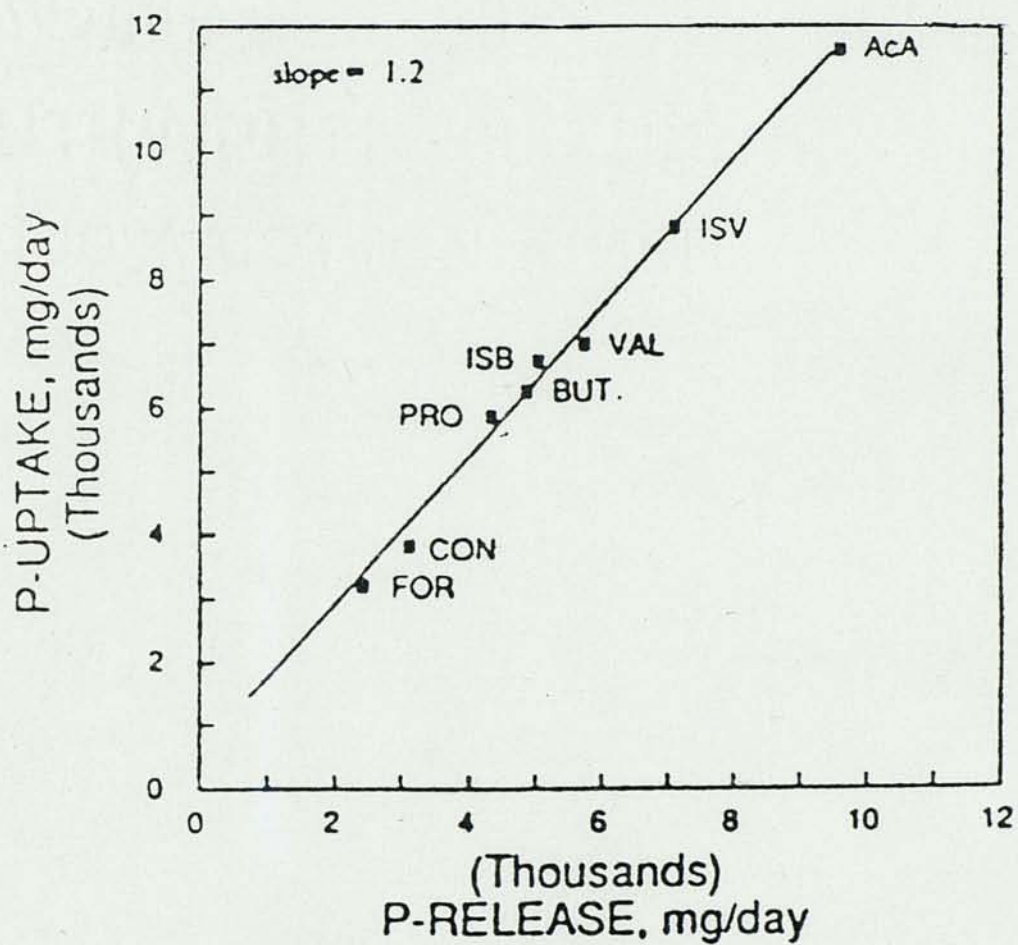


Figure 2.6- Correlation of P release and uptake (after Abu-ghararah and Randall, 1991).

Bacterial Population

From the biochemical models that were proposed to explain EBPR, three classifications for the bacteria responsible for such a process arose. The bacteria were classified based on energy, carbon and reducing equivalents sources (Liu et al., 1996).

The first category of bacteria was reported by Wentzel et al. (1991). These bacteria use poly-P as the energy source for the uptake of the substrate. They also store simple forms of fatty acids as PHBs and use acetate assimilation through the TCA cycle to supply the NADH required for the PHB synthesis.

The next category differs from the first only with respect to the reducing equivalents sources. The required NADH for PHB synthesis from acetate are supplied from degradation of cellular glycogen through glycolysis (Arun et al., 1989; Cech and Hartman, 1993; Satoh et al., 1992).

The final category refers to the group that is believed to use poly-P to supply ATP for the uptake and storage of glucose into poly-glucose under anaerobic conditions. Under aerobic conditions, this final category uses the stored poly-glucose for P uptake and poly-P accumulation as well as for VFA uptake. Therefore, they will circumvent poly-P so they don't participate in EBPR but rather compete with it (Liu et al., 1996).

Brodich (1984) noted that the phosphorus removal in biological systems containing *Acinetobacter* spp. can only be significantly efficient after the development of an *Aeromonas* population. The *Aeromonas* bacteria is important to the EBPR process in that it produces fermentation products in the anaerobic phase for the *Acinetobacter* population.

Randall et al. (1996) observed that an SBR with prefermented influent glucose achieved higher removal levels than an SBR with non-prefermented starch. The prefermented influent SBR was dominated by *Pseudomonas* population while the other SBR was dominated by an *Aeromonas* one. In spite of the presence of *Aeromonas*, which presumably hydrolyzed starch to glucose, there was marginal EBPR. This was probably because glucose was sequestered, by *Aeromonas*, too rapidly to ferment.

Cech and Hartman (1993) observed in their first SBR, where the substrate was a mixture of acetic acid and glucose, that "G" bacteria, which accumulate polysaccharide instead of polyphosphate, competed with poly-P accumulating bacteria, and were able to dominate the SBR system even though acetic acid was present in the mixture. For their second SBR, where no glucose was present and only acetic acid was the supplemental carbon source, poly-P bacteria dominated the system. They also observed that when only "G" bacteria and no poly-P bacteria were present in the system, the anaerobic phosphate release and the phosphate uptake/acetate supplied ratio was equal to zero. When the poly-P bacteria started increasing, anaerobic P release was observed and the phosphate uptake/acetate supplied ratio was observed to increase as well. It was also shown that the "G" bacteria were able to completely overgrow poly-P bacteria in the EBPR systems if the influent contained glucose. Since the phosphate concentration in "G" bacteria cells is very low, their presence in large numbers will negatively influence the efficiency of EBPR.

Knight et al. (1995) isolated a large number of strains of *Acinetobacter* from a biological nutrient removal plant to determine their carbon substrate utilization profiles. They reported the percentage of strains of each *Acinetobacter* genospecies positive for utilization of each

carbon source. When the carbon source was acetic acid, 99% of the strains were positive while it dropped to 87% when propionic acid was the carbon source. Such results emphasized the carbon utilization pattern of the *Acinetobacter* genospecies and their nutritional diversity.

Kavanaugh (1991) observed that many genera of bacteria are involved in the biological nutrient removal process. *Aeromonas/Vibrio*, coliform, *Pseudomonas* spp. and *Acinetobacter* spp. were identified as the main organisms capable of achieving BPR as well as biological nitrogen removal. *Acinetobacter* spp. only accounted for 5% of the total bacterial population while *Aeromonas/Vibrio* and *Pseudomonas* spp. were highly predominant. All the genera listed above showed EBPR in batch experiments, thus accumulating significantly in excess of 2% to 3% P by weight. They all also exhibited the presence of poly-P granules in their cells.

Okada et al. (1992) studied the slow recovery of EBPR from a short-term disturbance test which was run using SBRs. *Acinetobacter* and *Pseudomonas* were the predominant organisms, and they exhibited slow growth rates which in turn was detrimental to their predominance, thus slowing the recovery of the EBPR.

Electron Acceptors

Schon et al. (1993) studied the effect of dissolved oxygen (DO) on the performance of EBPR. In their activated sludge system, phosphorus release in the anaerobic phase did start until the DO content was reduced to 0.5 mg/l oxygen. However, the maximum release rate was reached only when there was no more measurable DO in the reactor. In the aerobic phase, phosphorus uptake started when the DO measured 0.1 mg/l oxygen, and it reached

a maximum at 0.2 mg/l oxygen. They concluded in their results that the actual oxygen concentrations, in both anaerobic and aerobic phases, exhibited a gradient within the sludge flocs. Investigations of pure cultures of *Acinetobacter* showed that as long as there is a functional respiratory metabolism, the cells will not release any phosphate in the medium.

Comeau et al. (1987) observed in their study that EBPR was achieved with nitrate present in an anoxic phase. Nitrate was used as an electron acceptor by denitrifying bacteria in the absence of oxygen. The denitrifying bacteria also needed a source of carbon substrate which, in their case, was PHB, and denitrification and P uptake was observed to occur simultaneously.

On the other hand, the stream containing nitrate should not be recycled to the anaerobic reactor because the available carbon source from the influent feed would be consumed rather than stored. Denitrification in this case will remove some of the easily biodegradable organic matter which was supposed to be stored in the P accumulating bacteria. The result is that P removal is reduced as the amount of easily biodegradable matter available is reduced (Henze et al., 1995).

Moreover, the effect of nitrate on EBPR and its possible utilization as an electron acceptor was studied by Kuba et al. (1993). Their anaerobic-anoxic SBR showed stable P removal and accumulation of phosphorus removing bacteria that use nitrate as an electron acceptor. The released P during the anaerobic phase was completely removed during the anoxic one, but P leakage occurred at the end of the anoxic phase leading to a decrease in the overall P removal efficiency. This behavior was easily corrected by controlled addition of nitrate.

Cation Effects

Pattarkine (1991) reported that the cations magnesium and potassium are essential for the proper function of the EBPR system. Both cations must be available in certain quantities for the poly-P bacteria for P release and uptake (Figure 2.7). Randall et al. (1992) reported the minimum amounts of magnesium and potassium that should be available for the removal of phosphorus. For each mole of P taken up, 0.25 moles of magnesium and 0.23 moles of potassium are needed to achieve acceptable level of EBPR, whereas at lower quantities limited P removal has been demonstrated (Randall et al., 1992). Some limitations of P removal have been observed when potassium and magnesium are severely limited, but the threshold or quantification of inhibition for this effect has never been assessed (Pattarkine, 1991). It should also be noted that if potassium and magnesium do act in a limiting fashion with respect to EBPR, they will be limiting to the net P removal.

Reactor pH

Smolders et al. (1994) presented a model that describes how the energy required for acetate uptake (i.e. transport) is strongly dependant on pH. They observed that at a pH ranging from 6 to 8, a higher fraction of poly-P organisms will exist in the system since less energy and thus less degradation of poly-P would be needed for the uptake of the acetate. This should directly influence the efficiency and possibly the economics of the EBPR process. Acetate transport is more energy efficient at low pH since only protonated acetate crosses the cell membrane.

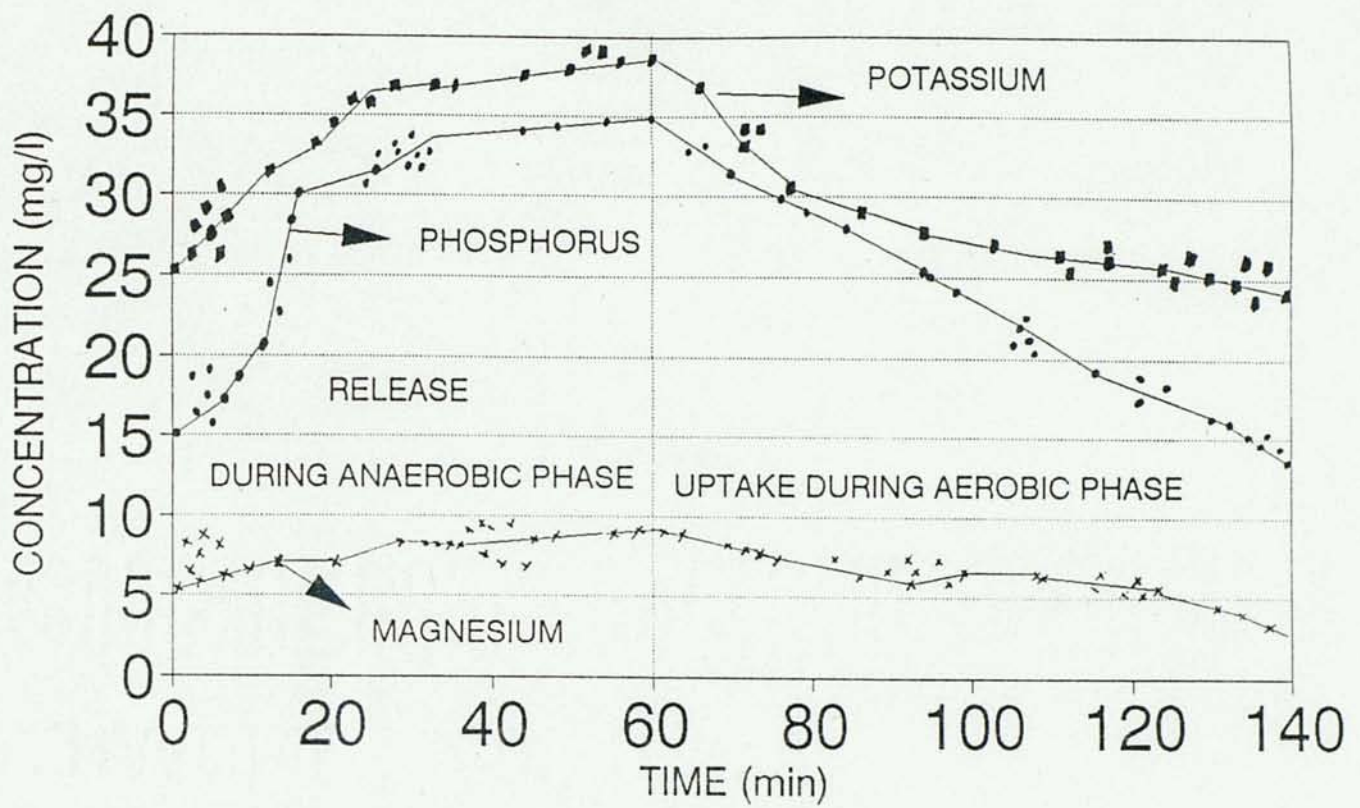


Figure 2.7- Profiles of phosphorus, magnesium, and potassium (after Pattarkin, 1991).

The pH profiles also relate to the biochemical reactions that take place within an SBR. When fermentation occurs in the influent feed, VFAs are produced, resulting in a decrease in the reactor pH. The VFAs are then transported into the cell from energy provided by breaking down the poly-P and ATP bonds, increasing the pH of the outside medium solution, since phosphate is co-transported with a proton. When the aerobic phase is introduced, the PHBs will be oxidized to carbon dioxide, water and energy, thus causing the pH of the solution to rise slightly.

Mean Cell Residence Time

Determination of a limiting mean cell residence time (MCRT) is very crucial to the efficiency of the EBPR process. Data developed by Reddy (1989) showed that below a minimum MLSS concentration, phosphorus release in the anaerobic zone would be reduced. Figure 2.8 indicates that the phosphorus removal efficiency increases with increasing MLSS until phosphorus release reaches a maximum value.

It is thought that at longer MCRTs, a larger part of the active mass will be represented by *Acinetobacter* spp., which will result in higher P removal and higher % P content within the cell (Randall et al., 1992).

Statement of Objectives

This study was performed to achieve the following objectives:

1. Due to fermentation reactions in domestic sewage, many VFAs are produced. Typically, the VFAs concentration averages 50 mg/l and the more septic the sewage, the greater

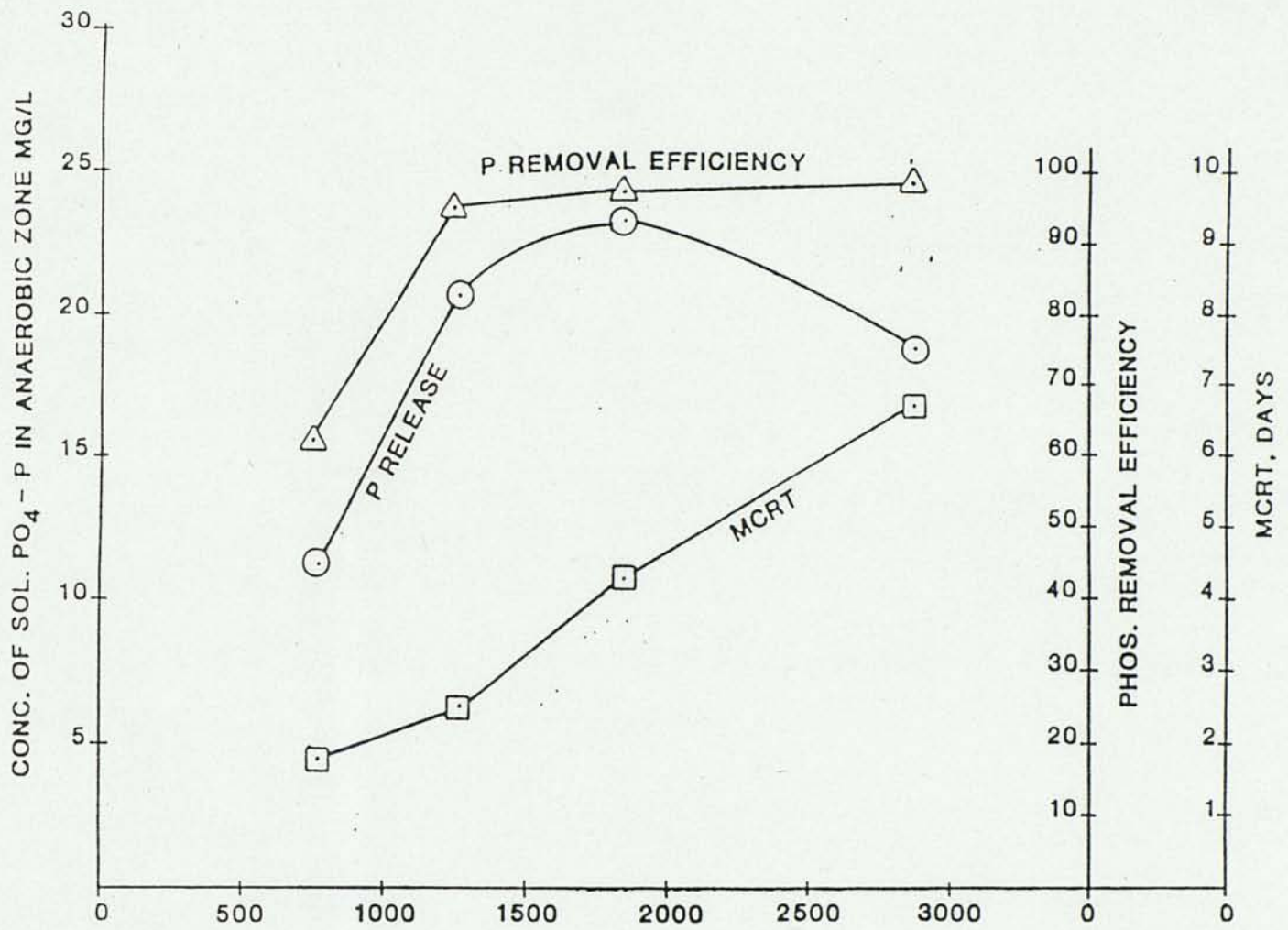


Figure 2.8- Influence of MLSS on P release and uptake (after Reddy, 1989).

the concentration. Acetic acid averages 45 % of the total VFAs produced, propionic acid averages 30 %, butyric and isobutyric acids average 15 %, while valeric and isovaleric acids constitute the remaining 10 %. As such, it was important to study the effects of these substrates on the performance of EBPR, because their presence in wastewater greatly affect the efficiency of wastewater treatment plants.

2. In the chapters to come, most of the data presented will be compared to prior studies done by Abu-ghararah and Randall (1991), Randall (1993), and Randall et al. (1995). However, this research went beyond these previous studies. This research also filled the gap for many other studies performed to evaluate EBPR. Also, very limited data were presented to show the effects of glucose and VFAs on EBPR; as such, additional studies on these substrates needed to be performed.

3. Abu-ghararah and Randall (1991) did not look at glucose effect on EBPR. However, they studied the effect of VFAs on P release/P uptake ratios, but only at one concentration.

4. Randall (1993) and Randall et al. (1995) studied glucose effects on EBPR, but they never looked at its anaerobic P release. Also, the effects of VFAs at different concentrations were not addressed in their research or any other research concerning EBPR.

5. This study was the first to look at the effects of isovaleric acid at steady state situation, on EBPR. It was also the only extensive research to study the time and concentration profiles for glucose, acetic acid, isovaleric acid, valeric acid, and propionic acid.

III. METHODS AND MATERIALS

Sequencing Batch Reactors

Two sequencing batch reactors (SBRs) were used in this research in order to evaluate enhanced biological phosphorus removal (EBPR). The first SBR, designated the Glucose SBR, was run for a period of four months, and it received synthetic wastewater plus glucose as a supplemental carbon source in its feed.

The second SBR, the Isovaleric SBR, was identical to the Glucose SBR except for the supplemental carbon source and was run for a period of three months. During the first month, isovaleric acid at an influent concentration of 0.80 mmol/l (roughly 80 mg/l) was the supplemental carbon source. For the remaining time period, no supplemental carbon source was added to the feed.

Both SBRs were plexiglass cubical reactors with a 3.0 liters volume. They both run non-stop, 24 hours a day and seven days a week for the entire research period. Both SBRs operated on eight hours-cycles with three cycles a day. Every cycle included a two-hour anaerobic reaction period followed by a four-hour aerobic period. This was followed by one hour of settling, then half of an hour for effluent draining, and another half of an hour idle period (Table 3.1).

Table 3.1 - Control programs for influent feed (top) and SBR cycles (bottom).

PROGRAM	FUNCTION	ON TIME	OFF TIME
1	FEED #1	12:10 am	12:40 am
2	FEED #2	8:10 am	8:40 am
3	FEED #3	4:10 pm	4:40 pm

PROGRAM	FUNCTION	CYCLE	ON TIME	OFF TIME
1	AIR	1	6:15 pm	10:15 pm
2	AIR	2	2:15 am	6:15 am
3	AIR	3	10:15 am	2:15 pm
4	WAS	1	10:10 pm	10:11 pm
5	WAS	2	6:10 am	6:11 am
6	WAS	3	2:10 pm	2:11 pm
7	EFFLUENT	1	3:30 pm	3:59 pm
8	EFFLUENT	2	11:30 pm	11:59 pm
9	EFFLUENT	3	7:30 am	7:59 am
10	MIXER	1	4:15 pm	10:15 pm
11	MIXER	2	12:15 am	6:15 am
12	MIXER	3	8:15 am	2:15 pm

Air was supplied through an aeration stone placed at the bottom of a side wall of each reactor. Sufficient quantities of air were introduced to assure well mixed conditions and to maintain a high (above 7.0 mg/l) dissolved oxygen (DO) concentration in the SBR during the aerobic phase. Similar reactors have been used in previous studies by Jig et al. (1992), Randall (1993), and Randall and Chapin (1994).

The original inoculates used for the SBRs were taken from the Iron Bridge Wastewater Treatment Plant in Orlando, Florida from the anoxic stage of a Bardenpho process. The SBRs were allowed to reach a steady state before any batch experiments were conducted; they were run for 3 MCRTs with every MCRT averaging 6 days. This sludge exhibited good EBPR ability at the full-scale plant as well as after reaching steady state in the laboratory reactors.

Operation of SBRs

The influent feed for the SBRs was a synthetic wastewater composed of yeast extract, nutrient broth, and some inorganic salts (Table 3.2). Phosphorus was added to the water in the form of KH_2PO_4 in order to ensure an influent concentration of 20 mg/l total phosphorus.

The feed was initially prepared as 10 liters of concentrate feed, as described in Table 3.2. To make the daily influent feed, 300 ml of the concentrate was diluted to 7.5 l using tap water. For the Glucose SBR, 1.05 g of glucose was added to the 7.5 l solution as a supplemental carbon source. For the Isovaleric SBR, pure liquid isovaleric acid was added to the system as the supplemental carbon source for a final concentration of approximately 80 mg/l. This concentration was chosen to be roughly equal to the mmoles of glucose substrate used as the supplemental carbon source for the Glucose SBR. The 7.5 liters influent feed was equally

Table 3.2 - Composition of the synthetic wastewater.

PARAMETER	CONCENTRATE FEED g/10 liters	FINAL CONCENTRATION mg/l
NUTRIENT BROTH	75.0	300
YEAST EXTRACT	8.00	32.0
MgSO ₄ .7H ₂ O	5.00	20.0
MnSO ₄ .H ₂ O	3.20	12.8
(NH ₄) ₂ SO ₄	8.60	34.4
NH ₄ CL	16.9	67.6
KH ₂ PO ₄	22.0	88.0
KOH	5.80	23.2

divided into three feed jars. Each cycle began by introducing 2.5 liters of the influent feed from the feed jars into the SBR, which already contained 0.4 l of settled biomass from the previous cycle.

Waste activated sludge (WAS) was pumped out of the reactor during the final minutes of the aerobic phase. Enough WAS was removed to ensure an MCRT of six days. The operational characteristics, such as SBR's cycles and influent feed composition, intentionally duplicated those used in prior studies (Randall et al., 1995).

Maintenance of SBRs

The SBR maintenance procedure, described below, had to be done on a daily basis anytime after the last feed reservoir was empty (8:20 am) and before the first feed reservoir release valve was opened (4:15 pm). First, the feed remaining was decanted from the feed jars from each system to graduated cylinders so the untreated feed volume could be recorded. The feed reservoir was then washed with tap water and a brush. After that, the wasting tubing of each system was backwashed with tap water by running the pump in reverse. Next, the reactor's interior walls were cleaned by a brush. This step was performed during the aeration period in order not to disturb the reactor anaerobic and settling phases. Afterward, the new influent feed was poured into the three feed jars for each system (1 per cycle). At this time, the back wash of the wasting tubing was stopped and the pump set to the forward mode. The volumes of feed residue and wasted mixed liquor were recorded for each system and discarded after that. Also, the temperature and the DO of the reactors were measured using a biological oxygen demand (BOD) meter. Finally, the operating conditions of the lab and

the reactors, such as the compressed air pressure, temperature, and backup batteries, were checked.

Temperature

It has been reported that EBPR processes are relatively insensitive to temperature changes compared to other biological processes. However, they are sensitive to the types of poly-P bacteria present. Some poly-P bacteria can be psychrophiles, mesophiles, or thermophiles. It was observed that psychrophiles, which grow better at low temperatures, are the poly-P bacteria capable of giving the best EBPR (Randall et al., 1992). However, the group of poly-P that would dominate the system depends on the initial culture and the operating conditions (Randall, 1992). Regardless of the bacteria present, the potential to recycle and entrain the electron acceptors, DO and nitrogen oxides (NO_x) into the anaerobic mixed liquor is much greater at low temperatures than high ones. Reducing the amount of substrate stored in the MLVSS in the anaerobic phase in the reactor and, consequently, reducing phosphorus removal by the system (Barnard, 1984).

In this research, care was taken to maintain a steady-state temperature environment. The reactor and the room temperatures were both maintained around 25 °C at all times. However, due to heat stripping in the aeration phase, the reactor temperature sometimes got as much as 5 °C lower than that of the room temperature.

Batch Experiments

Glucose SBR P removal was largely dependant on fermentation products of influent glucose. As a result, batch experiments using the Glucose SBR's biomass were performed for four high purity liquid carboxylic acids: acetic acid, propionic acid, valeric acid, and isovaleric acid, and for glucose. All batch experiments were conducted in six two-liter glass reactors with a liquid volume of approximately 500 ml per reactor. Two of 6 reactors (React 1 and React 2) served as experimental controls and received 0.0 mmoles/l substrate, while reactors 3 to 6 received 0.20, 0.40, 0.60, and 0.80 mmoles/l respectively of each substrate. Mixing was supplied by a magnetic mixer that delivered minimum agitation, but enough to keep the activated sludge in suspension. This same technique was used in earlier studies by Arun et al. (1988) and Randall et al. (1995).

For each batch experiment, all the biomass from the Glucose SBR was taken out at the end of the last idle phase of cycle 3 and before the start of cycle one. The volume of the biomass was around 210 ml. The biomass was then mixed in a separate flask with 2.5 liters of the synthetic wastewater feed without any supplemental glucose, and the total volume of 2.71 liters was divided equally between the 6 batch reactors. The acid substrates were then introduced as pure liquid form (99% assay) at time zero of the anaerobic phase by means of a pipet, while the glucose substrate was introduced in a powder form. All batch experiments were run on eight-hours cycles identical to the SBRs' treatment cycle. Each cycle included a two-hour anaerobic period followed by a four hour aerobic one. Samples from each reactor were taken at approximately hourly intervals and analyzed for ortho-P, to follow the time course of phosphorus as the reactors moved through the sequence of anaerobic and aerobic

phases. Once the analysis of each batch experiment was done, the mixed liquor was allowed to settle, and the settled biomass was returned to the Glucose SBR to resume the daily cycles.

Analytical Procedures

Ortho-phosphorus, temperature, pH, total suspended solids (TSS) and turbidity were measured throughout the course of the research following the guidelines described in Standard Methods for the Examination of Water and Wastewater (APHA, 1985). Further details about each procedure follows.

Turbidity

The turbidity of the influent feed as well as of the reactors was consistently monitored for both SBRs. Samples were taken at different time intervals within the anaerobic, aerobic, and settling phases. A Hatch Ratio Turbidimeter (Model# 910505913) was used.

Suspended Solids

Suspended solids were measured for the effluent, the mixed liquors and the waste activated sludge. Such measurements were needed to establish a mass balance around the SBR systems and to calculate the MCRT as well as to determine the biomass P content. 934-AH glass fiber filters (Whatman) were used to measure the suspended solids.

Ortho-Phosphorus

To measure soluble ortho-P, the sample was centrifuged for a period of 2 minutes, and then filtered through a 45 micron membrane filter. The ortho-P was then measured using the Vanadate - Molybdate technique, method # 424-D of the Standard Methods (APHA, 1985). Ortho-P was analyzed for the influent feed, the reactors, the effluent, and the WAS at different time intervals for both SBRs to conduct mass balances for phosphorus. In addition, total phosphorus measurements were performed by directly measuring the P content of the MLSS, using the total P persulfate digestion method (APHA, 1985).

IV. RESULTS AND DISCUSSION

Influent Feed

The Glucose SBR influent feed was monitored for pH and turbidity. During the first feed cycle, the pH decreased from 7.6 to 5.4 within a time period of 8 hours while the turbidity increased from 3.8 to 81 NTUs (Figure 4.1). Such results were an indication that glucose decreased in the influent feed due to fermentation, and were identical to SBR behavior, using the same feed, in prior studies (Randall, 1994).

These observations were consistent with prefermentation (acidogenesis) of glucose in the influent feed so the Glucose SBR routinely received VFAs in its influent. As fermentation proceeded, the turbidity and acidity increased in the feed due to bacterial growth and VFA production. Table 4.1 compares the influent feed values of this study to those observed by Randall (1993). The results showed greater change in the turbidity of the influent feed during the fermentation period than those observed by Randall (1993), which might be related to the difference in temperatures at which the SBRs were run during the two studies. The average temperature during Randall's study (1993) was approximately 5°C less than this study. Since 10°C increase is known to double the reaction rate, it was clear that this difference in temperature should have resulted in slower growth rate, explaining the lower turbidity change in Randall

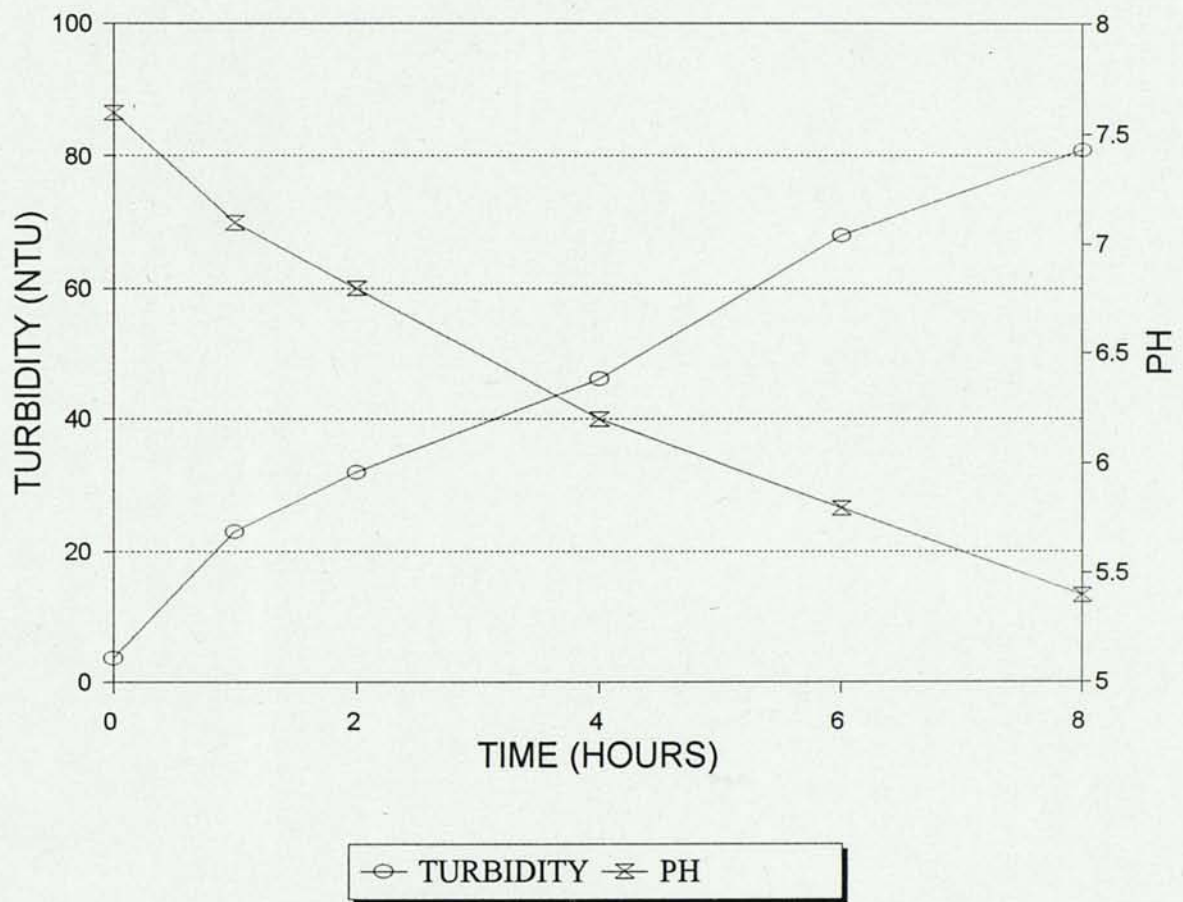


Figure 4.1 - Influent feed pH and turbidity (using average values).

Table 4.1 - Changes in the influent feed turbidity and pH due to fermentation.

SEQUENCING BATCH REACTORS	TURBIDITY INCREASE (NTU)	pH DECREASE	TIME FRAME (hours)
EBPR (This study)	77	2.2	8.0
RANDALL (1993)	12	2.6	10

(1993). The pH change was insignificant, and both studies clearly showed pH depression due to acid production.

Reactor pH

Data were taken throughout the research to examine how the reactor pH of both the Glucose and the Isovaleric SBRs would behave during the treatment cycle phases in the EBPR process. As pH increased in the anaerobic period, phosphorus release increased as well (Figure 4.2). This increase in pH corresponded to the sequestration of VFAs within the reactor during anaerobiosis. As VFAs were transported into the cells, phosphate was released into the outside medium.

During the aerobic phase, phosphorus uptake accompanied by a decrease in the pH was observed for both SBRs. In Randall's study (1993), the pH increased during aerobiosis. One hypothesis that could explain this difference in behavior is that nitrification was taking place in the aerobic phase during our study, while it was not significant in Randall's (1993). The SBRs in this study were operated at a temperature about 5°C higher (around 25°C) probably leading to much more complete nitrification, which would in turn destroy the alkalinity present and cause the observed decrease in the reactors' pH. Nitrification was not quantified since the ortho-P profiles were consistent with EBPR phenomena observed by Comeau et al. (1986) and Randall et al. (1993).

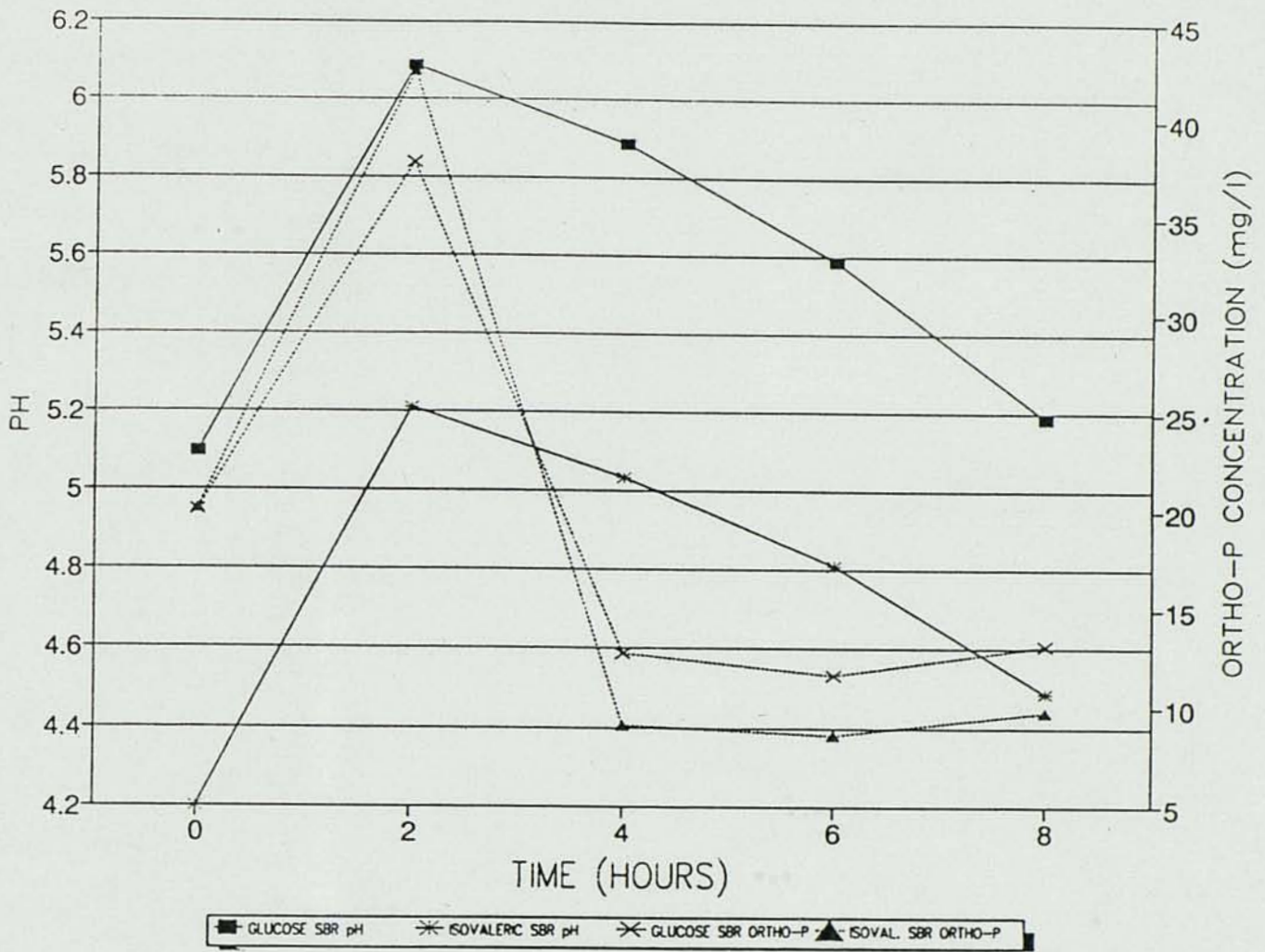


Figure 4.2- Phosphate release and uptake compared to pH behavior for both SBRs (using average values).

Mean Cell Residence Time

A mass balance for MLSS was performed in order to quantify the MCRT of the system. For both SBRs, the MCRT ranged roughly between 5.7 and 5.9 days. An MCRT of 6 days was desired to duplicate prior studies done by Randall (1993). However, the actual MCRT value was less than the design value because the solids in the effluent were considered negligible when determining the wasting rate. Randall et al. (1992) reported that EBPR can take place at MCRTs of less than 3 days, but the process is not stable and the effluent is not clear. They reported that at an MCRT of 6 days, the EBPR is most stable.

Mixed Liquors Percent Phosphorus

A mass balance for P was performed around each system using data from the influent and effluent streams as well as the WAS stream. For the Glucose SBR, the percentage P calculated in the MLVSS was 6.4%, assuming that fixed suspended solids P content was negligible (refer to Appendix D for sample calculations). For the Isovaleric SBR, it was found to be 7.2% when isovaleric acid was the supplemental carbon source, and 4.4% with no supplemental carbon source. To confirm our mass balance analysis, direct measurement of MLSS % P was performed using a total phosphorus persulfate digestion measurements, and it was found to be 5.9% for the Glucose SBR and 6.8% for the Isovaleric SBR when isovaleric acid was received (Table 4.2). To make a rough estimate of the % P in the MLVSS from direct measurements, the MLVSS/MLSS ratio calculated in this study (Appendix A) was used and it was assumed that the fixed suspended solids P content was insignificant. A ratio of MLVSS/MLSS of 0.74 was used as the conversion factor for the Glucose SBR. This ratio

Table 4.2 - Percentage of P in the mixed liquors for both SBRs.

SEQUENCING BATCH REACTOR	% P OF MLVSS FROM MASS BALANCE	% P OF MLSS FROM DIRECT MEASUREMENTS	% P OF MLVSS CALCULATED FROM DIRECT MEASUREMENTS
GLUCOSE SBR	6.4	5.9	8.0
ISOVALERIC SBR (WITH SUPPLEMENT)	7.2	6.8	8.6
ISOVALERIC SBR (WITHOUT SUPPLEMENT)	4.4	4.0	5.0

represents the average ratio observed during this study. For the Isovaleric SBR, the MLVSS/MLSS ratio was found to be 0.78, while it was 0.80 for the same SBR when it was run without any supplemental carbon source. An average MLVSS/MLSS ratio of 0.79 was used as a conversion factor for the Isovaleric SBR, since the slight difference between the 2 ratios for this SBR was insignificant with respect to the error inherent in the testing procedure. The MLVSS % P content from direct measurements was significantly higher than the values obtained from mass balance. This observation was expected since the mass balance calculations assume that all the effluent total suspended solids were biomass.

Randall (1993) reported average P values for MLVSS of 5.0 % and 5.3 % calculated from direct measurements of the MLSS, while Arun et al. (1988) reported 5.4% P in the effluent sludge. Our data reported even greater values for % P in both the MLVSS and MLSS, certainly in excess of the stoichiometric value of 2.3% (Randall et al., 1992), clearly establishing that EBPR was taking place. The reason this study had better P removal efficiency than that of Randall (1993) was due to the temperature difference between the two studies. Two bacterial populations would compete for glucose fermentation and sequestration: The fermenters and the "G" bacteria. The 5°C temperature increase in this study would increase the reaction rate of the fermenters; thus, they will sequester glucose and ferment it to VFAs. On the other hand, if the "G" bacteria dominated the system, the glucose substrate would not be as efficient in P removal, such as the case in the study of Randall (1993).

SBR Effluent Phosphorus

For both SBRs, the only difference in the influent feed was the supplemental carbon source, while all other operating and environmental conditions remained constant like pH, temperature, MCRT, and feed composition. The effluent ortho-P values shown in Figure 4.3 begin on the first day of the SBR's operation and steady-state conditions were assumed after 3 MCRTs at day 18 for the Glucose SBR and day 50 for the Isovaleric one. Also, the Isovaleric SBR was run for around 5 MCRTs after it reached steady state- a process that has never been done before. The Isovaleric SBR showed lower phosphorus effluent values when receiving isovaleric acid than when isovaleric addition was halted (Figure 4.3). The glucose system received a mixture of VFAs, and had significantly higher effluent values, while the Isovaleric SBR showed the poorest performance observed when it received no VFAs. The effluent ortho-P was observed to be 12.4 mg/l for the Glucose SBR and 9.2 mg/l for the Isovaleric SBR, but it was 17.1 mg/l when the Isovaleric SBR was run without any supplemental carbon source. A possible explanation for Glucose SBR showing less P removal than its counterpart, the Isovaleric SBR, was that part of the supplemental carbon source was still in the form of glucose which could have been transported and stored as glycogen by non-fermenting bacteria, such as the "G" bacteria before any fermenting bacteria could metabolize it (Randall, 1993).

Sequencing Batch Reactors

Two kinds of carbon supplements were used to study the effect of substrates on P Removal using SBRs: A glucose supplement and an isovaleric acid supplement. Both SBRs

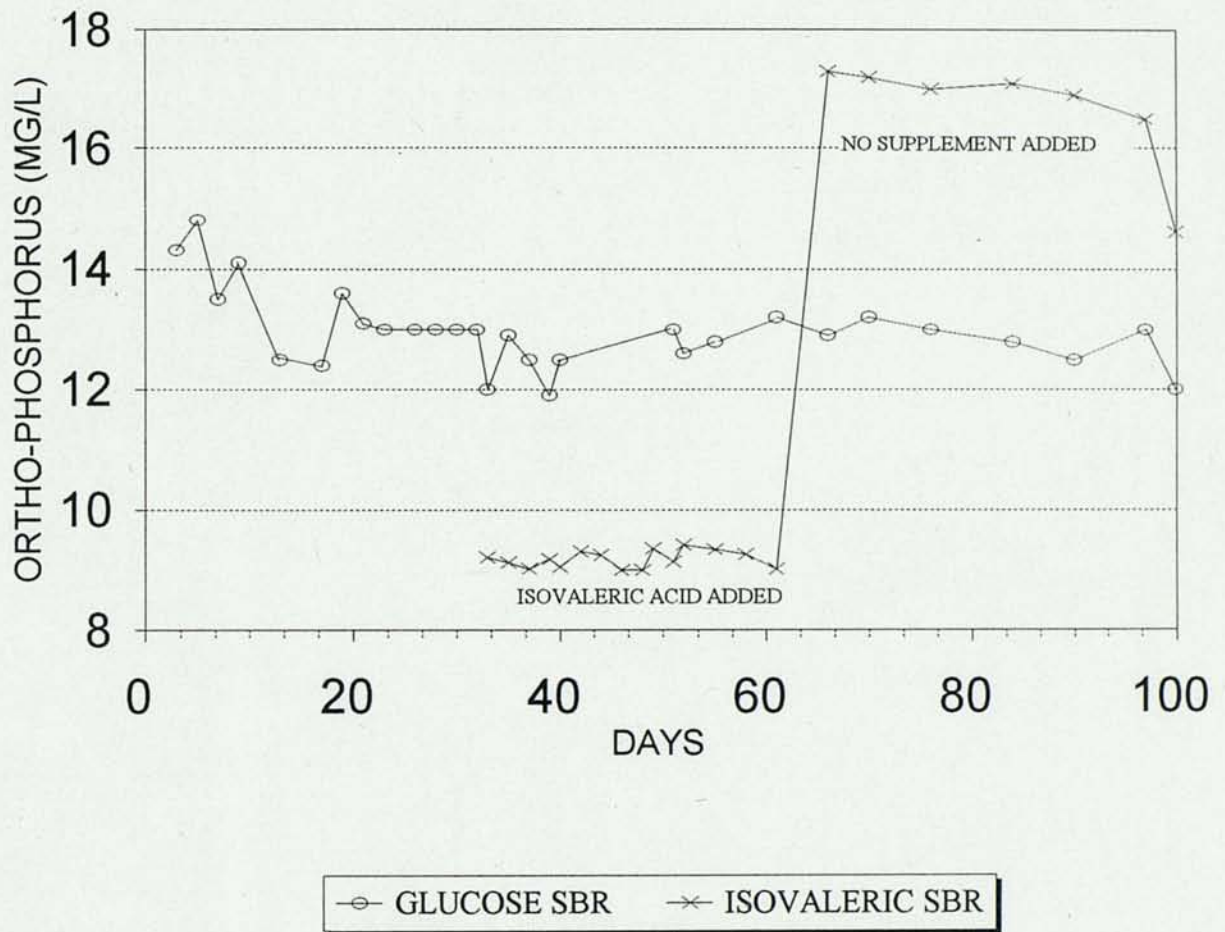


Figure 4.3 - Effluent ortho-P for both SBRs.

had sludge ages of around 6 days. The MCRT or sludge age was maintained around 6 days by withdrawing out the needed volume of mixed liquor from the reactors, once every cycle. Figure 4.4 clearly shows the phosphorus release and uptake trends associated with this system. This figure represents the average P values of cycle 1 for both the Glucose and the Isovaleric SBRs and compares them to a no-supplement option. The Glucose SBR achieved good P removal, with effluent P of 12.4 mg/l, similar to the P effluent values observed by Randall (1993).

The SBR data also showed that P increased in the anaerobic phase and decreased in the aerobic one. This behavior indicates that P was released from the cells of microorganisms in activated sludge during the first phase, and also that it was taken up by the cells in the next phase. In addition, phosphate concentrations in the effluent were observed to be slightly higher than those at the end of the aerobic period. The reason was that the biomass was exposed for at least an hour to an anaerobic state without an exogenous carbon source during the idle period. This exposure could result in secondary release of P without a corresponding uptake of readily available chemical oxygen demand (COD), such as VFAs (Barnard, 1992). The reason could also be related to the death and lysis of some microbial cells or the depletion of the carbon source which limits the metabolic activities of the cells (Tam et al., 1992). The phosphorus release and uptake trends and the effect of readily available substrates on the release/uptake observed, were typical of EBPR systems studied earlier (Comeau et al., 1987; Randall, 1993).

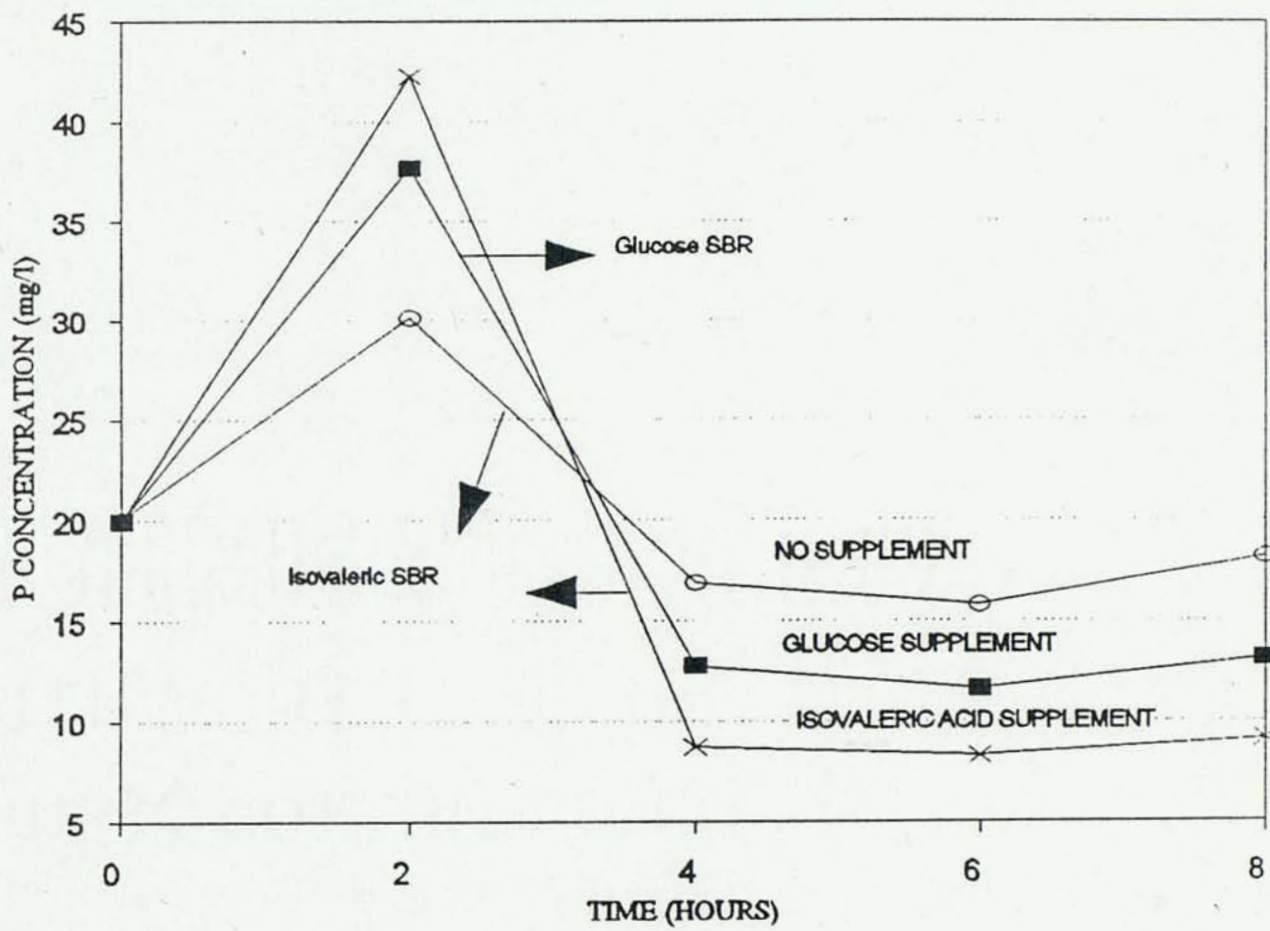


Figure 4.4- Phosphorus release and uptake of both SBRs for cycle 1 (using average values).

Batch Experiments

Batch experiments were performed using the entire Glucose SBR biomass which was removed before the beginning of cycle 1 each day of an experiment. The biomass was only used for the batch experiments after the system exhibited very good phosphorus removal, up to 6.4 % P in the MLVSS, and 3 MCRTs.

Each substrate was studied over a period of 2 month, using the same SBR biomass and under the same operating conditions for each batch experiment. Six batch experiments were performed using each of the VFAs studied and five experiments using the glucose substrate. For the purpose of this study, average values, for the two month period's data, of all batch experiments are used in the result and discussion analysis, unless specified otherwise. Net removals were taken after 6 hours, at the end of the two hour anaerobic/ four hour aerobic treatment cycle.

The reproducibility of the results for all the VFAs' batch experiments was found to be good with a correlation coefficient ranging between 0.8 and 0.9. When the concentration of any of the four VFAs was increased, the phosphorus removal was stimulated over that accomplished with no substrate addition. The phosphorus removal increased in direct proportion to the increase in VFA concentration (Figure 4.5); however, some substrates were more beneficial than others to the EBPR system. The best P removal was obtained by isovaleric acid substrate while the least effective one was obtained when propionic acid was added.

The results of the batch experiments for the addition of the five substrates and their respective theoretical oxygen demands (ThOD) are shown in Table 4.3(a). Comparing isovaleric acid and acetic acid on a net P removal/ThOD basis, acetic acid showed better P removal

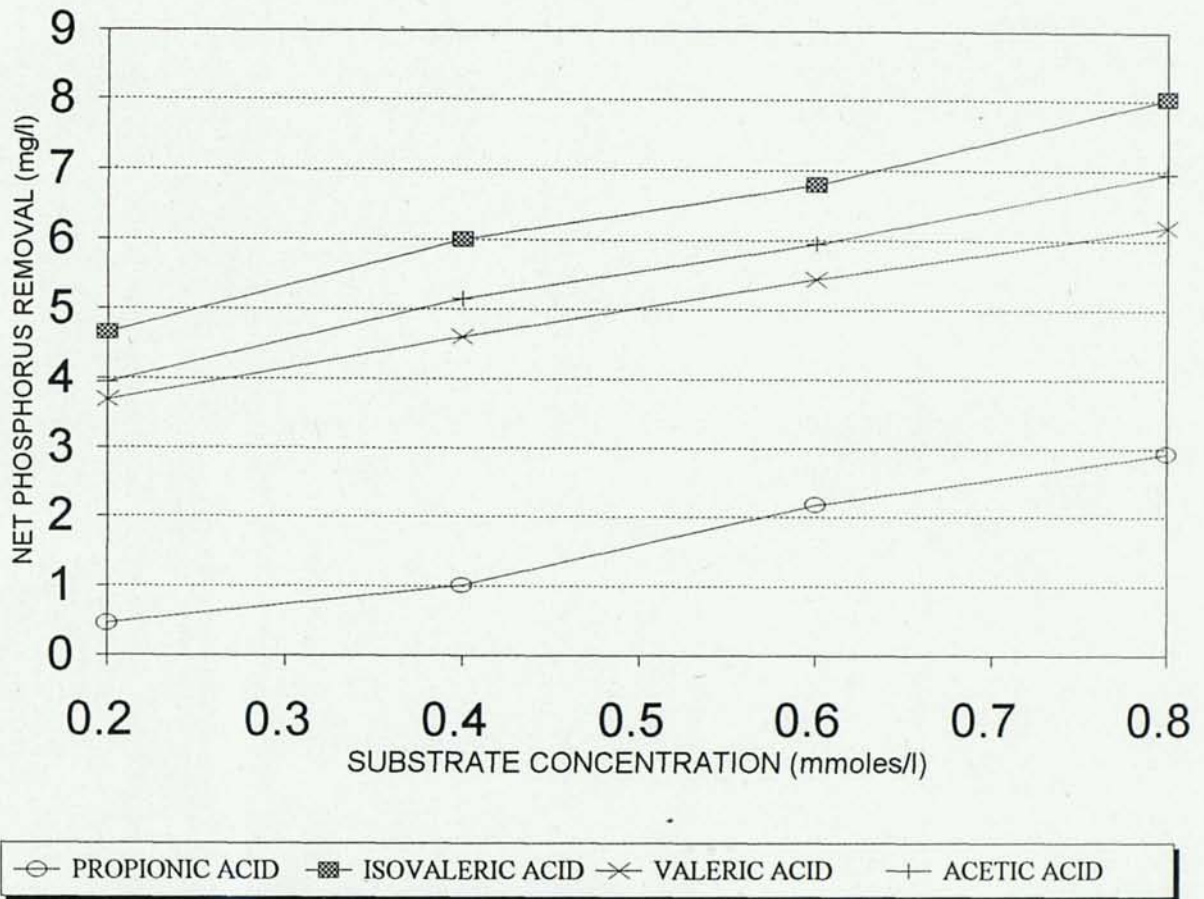


Figure 4.5 - Net P removal for all four fatty acids relative to control (using average values).

Table 4.3 (a)- Net ortho-P removal and ThOD for all batch experiments relative to control (Using average values).

SUBSTRATE mmoles/L	PROPIONIC ACID		ISOVALERIC ACID		GLUCOSE		VALERIC ACID		ACETIC ACID	
	ThOD mg/l	P mg/l	ThOD mg/l	P mg/l	ThOD mg/l	P mg/l	ThOD mg/l	P mg/l	ThOD mg/l	P mg/l
0.20	22.4	0.50	41.6	4.7	38.4	2.6	41.6	3.7	12.8	3.9
0.40	44.8	1.0	83.2	6.0	76.8	3.7	83.2	4.6	25.6	5.2
0.60	67.2	2.2	125	6.8	115	4.5	125	5.4	38.4	6.0
0.80	89.6	2.9	166	8.0	154	4.3	166	6.2	51.2	7.0

Table 4.3 (b)- Net ortho-P removal relative to control and mg/l of substrate added.

SUBSTRATE mmoles/L	PROPIONIC ACID		ISOVALERIC ACID		GLUCOSE		VALERIC ACID		ACETIC ACID	
	mg/l added	mg/l P rem.	mg/l added	mg/l P rem.	mg/l added	mg/l P rem.	mg/l added	mg/l P rem.	mg/l added	mg/l P rem.
0.20	14.07	0.50	20.4	4.7	35.9	2.6	20.4	3.7	12.0	3.9
0.40	29.5	1.0	40.9	6.0	71.8	3.7	40.9	4.6	23.9	5.2
0.60	44.2	2.2	61.2	6.8	107	4.5	61.2	5.4	35.9	6.0
0.80	59.0	2.9	81.6	8.0	144	4.3	81.6	6.2	47.8	7.0

than isovaleric acid as well as all the other substrates. Furthermore, Table 4.3(b) clearly shows that more isovaleric acid on a mass basis was needed to remove the same amount of P. Similar behavior was also observed by Abu-ghararah and Randall (1991), (refer to Figure 2.6). On a molar basis, however, isovaleric acid was more effective than acetic acid. Isovaleric acid is a five carbon molecule while acetic acid is a two carbon one. In terms of P removal per mole of carbon added, acetic acid was the most efficient substrate studied. This study differed from an identical study by Randall (1993) where isovaleric acid was observed to be the most efficient VFA over a similar concentration range. It may be that the 5 degree Celsius temperature difference affected the metabolism or population distribution of the poly-P bacteria, resulting in somewhat different performance characteristics between the two studies (Randall et al., 1992).

Batch reactors 1 and 2 (React 1 and React 2) were run, without the addition of any supplemental substrates to the influent feed, as experimental controls and biological phosphorus removal was evident in these control reactors (Figure 4.6). React 1 and 2 exhibited average P release of 4.5 mg/l in the anaerobic phase followed by an average P uptake of 8.3 mg/l in the aerobic phase, a net P removal of 3.8 mg/l. This behavior, with no VFA addition, suggests that some fermentation activity was taking place in the reactors, or that a small amount of other suitable substrates were available in the synthetic wastewater.

For all batch experiments including the controls, each of the VFAs caused significantly more P release and subsequent net P removal than the control reactors (Figure 4.6 and Figure 4.7). Furthermore, there was greater P release during the anaerobic phase with increasing VFA concentration. These observations are in agreement with the biochemical model of Comeau

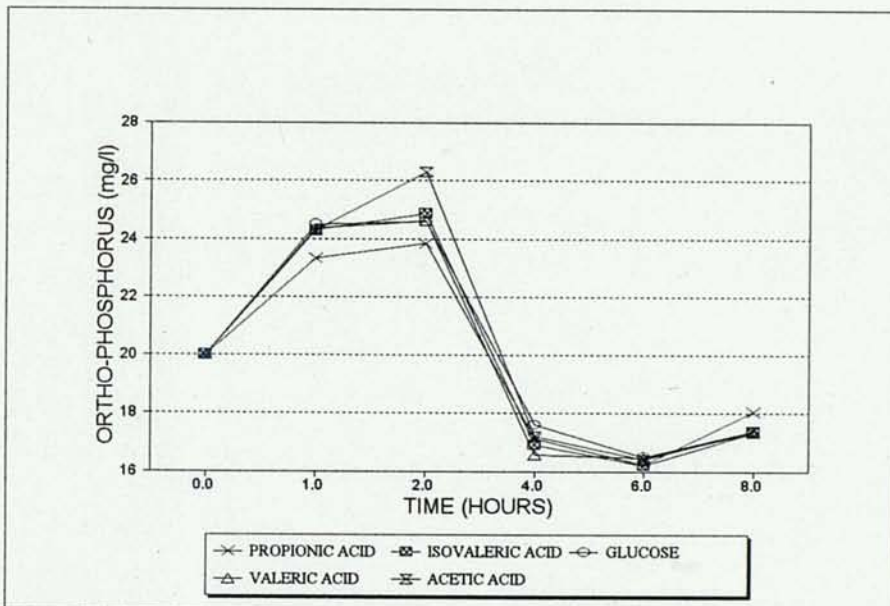
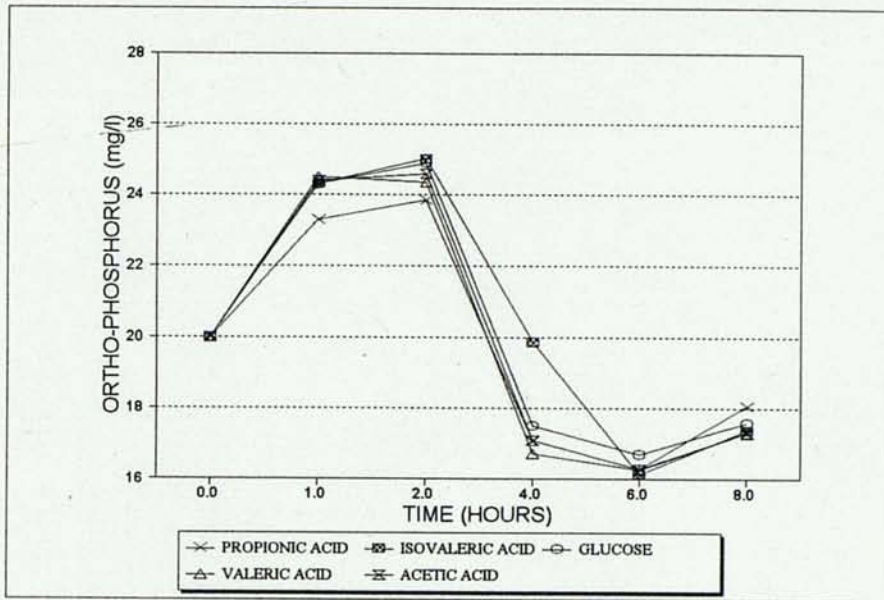


Figure 4.6 - Ortho-P release and uptake with 0.0 mmoles/l substrate for React 1 (top) and React 2 (bottom) (using average values).

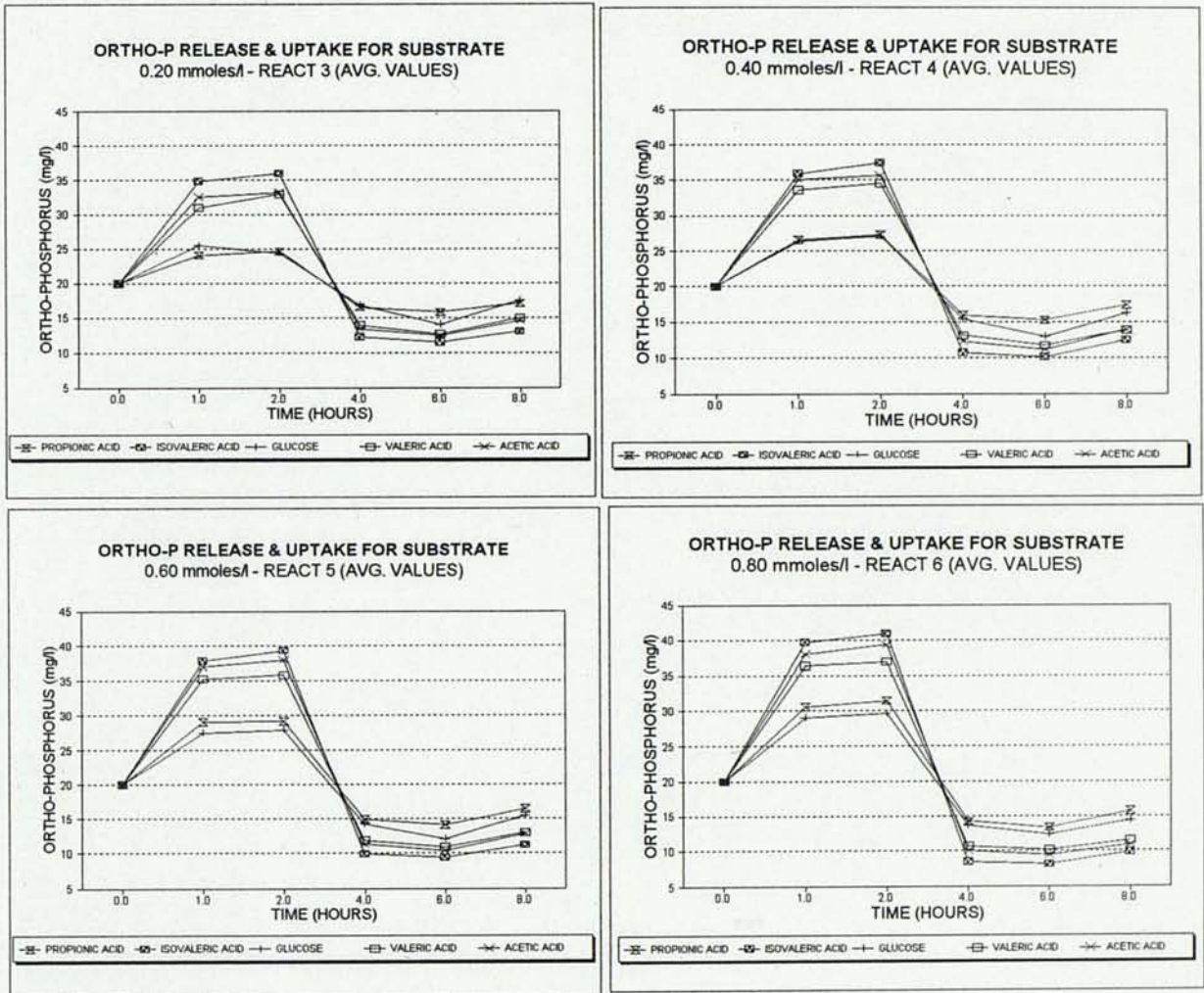


Figure 4.7 - Batch experiments for all substrates.

et al. (1986) which proposed that VFAs are stored as PHBs and that the P release reflects poly-P breakdown providing the energy needed for these anaerobic reactions.

Figures 4.8 and 4.9 show that there is a very good direct correlation between P release in the anaerobic phase and subsequent P uptake in the aerobic one regardless of the substrate used. These values were reported relative to controls (ie. React 1 and React 2). Also the glucose values were not included in the linear regression analysis, since glucose will later on be shown to behave differently than all the other VFAs, and its presence negatively affected the results. Without glucose in the linear regression, the coefficient of determination averaged 0.99 while the slope values ranged between 1.3 and 1.6 mg P uptake/ mg P release. In addition, at 0.80 mmoles/l substrate concentration, the slope was observed to decrease to 1.27 as compared to an average slope of 1.5 for the lower concentration levels. Abu-ghararah and Randall (1991) recorded a correlation factor of 0.99 with a slope of 1.2 for the same VFAs used in our studies. However, they presented their values based on an equivalent of 100 mg/l COD of each of the VFAs used while the VFAs in this study were compared on an equi-molar basis. In addition, their slope was not based on releases relative to a control, which may partially explain the differences on the slopes values. As a result, isovaleric acid gave superior results to acetic acid in our studies while the opposite was observed in their studies.

Increasing glucose addition improved P removal up to 0.60 mmoles/l. However, when the concentration of the influent glucose was increased above 0.60 mmoles/l, the EBPR system showed a decrease in the P removal efficiency (Figure 4.10). This behavior might be related to the presence of some non-poly P bacteria in the system (Cech and Hartman, 1990), or to some biochemical effects.

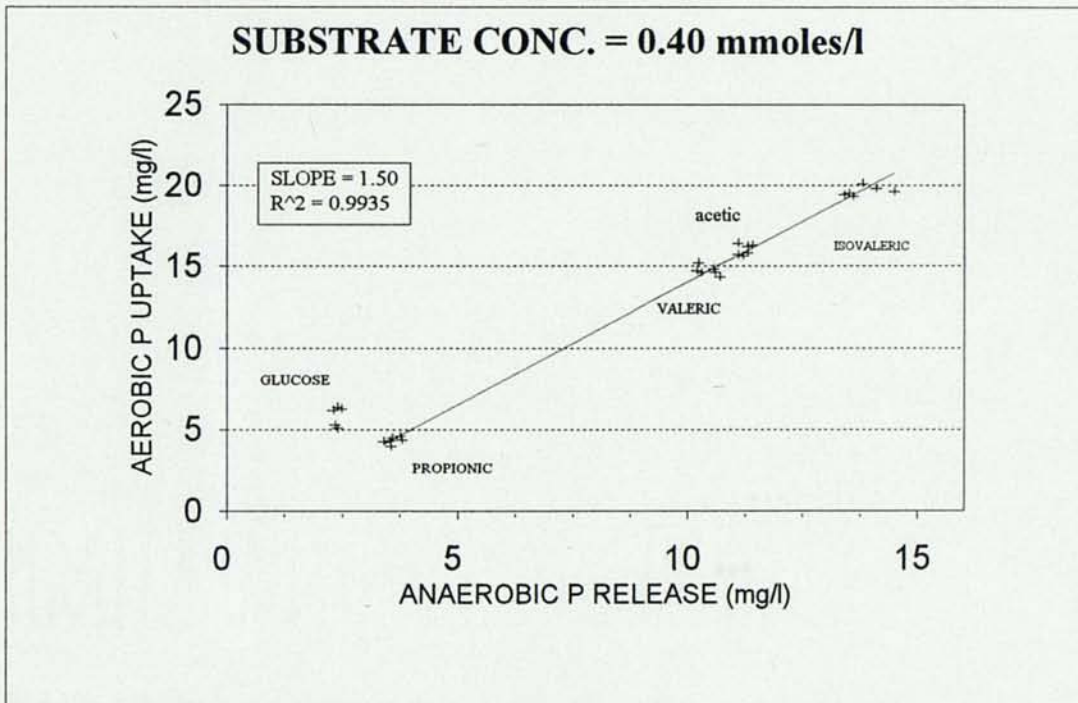
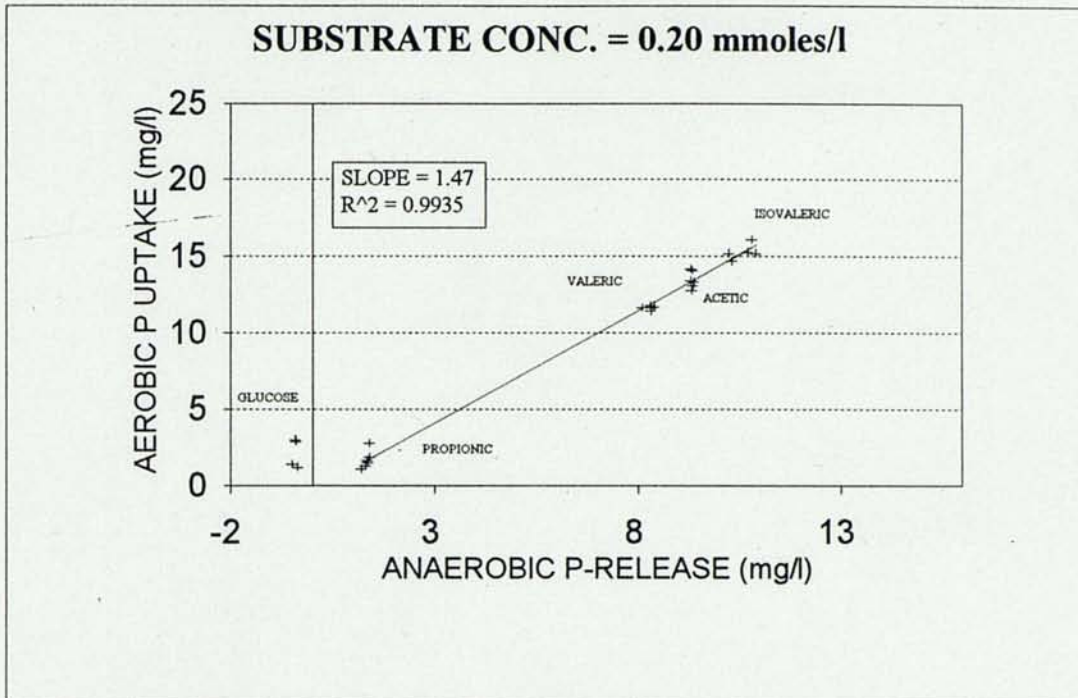


Figure 4.8 - P uptake versus P release for batch experiments using 0.20 and 0.40 mmoles/l substrates (relative to control).

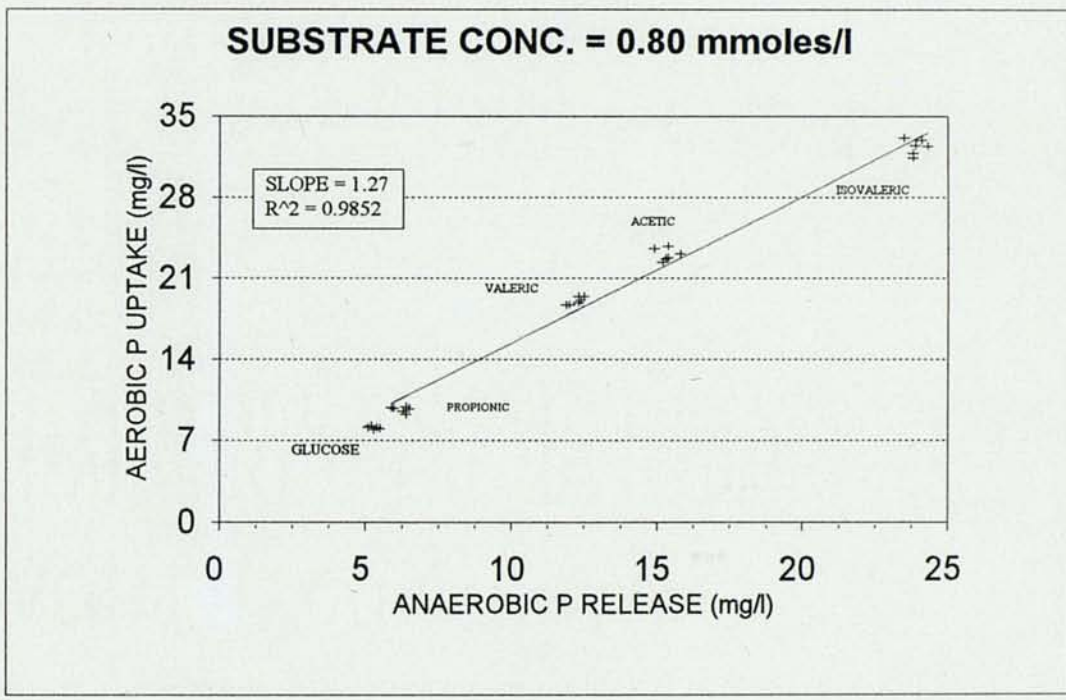
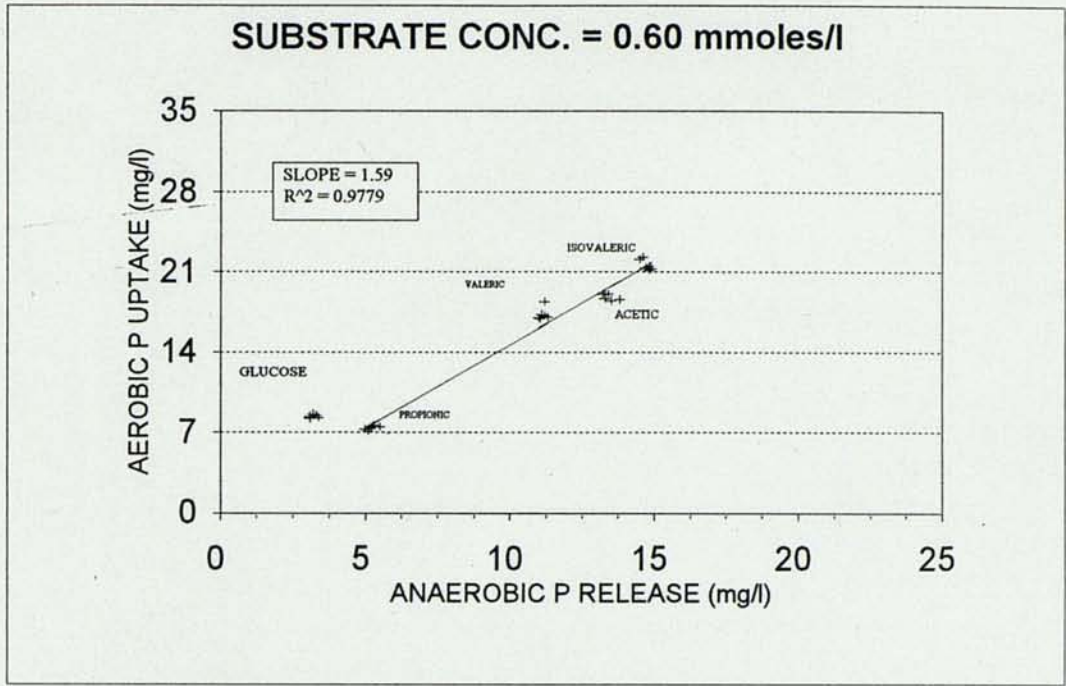


Figure 4.9 - P uptake versus P release for batch experiments using 0.60 and 0.80 mmoles/l substrates (relative to control).

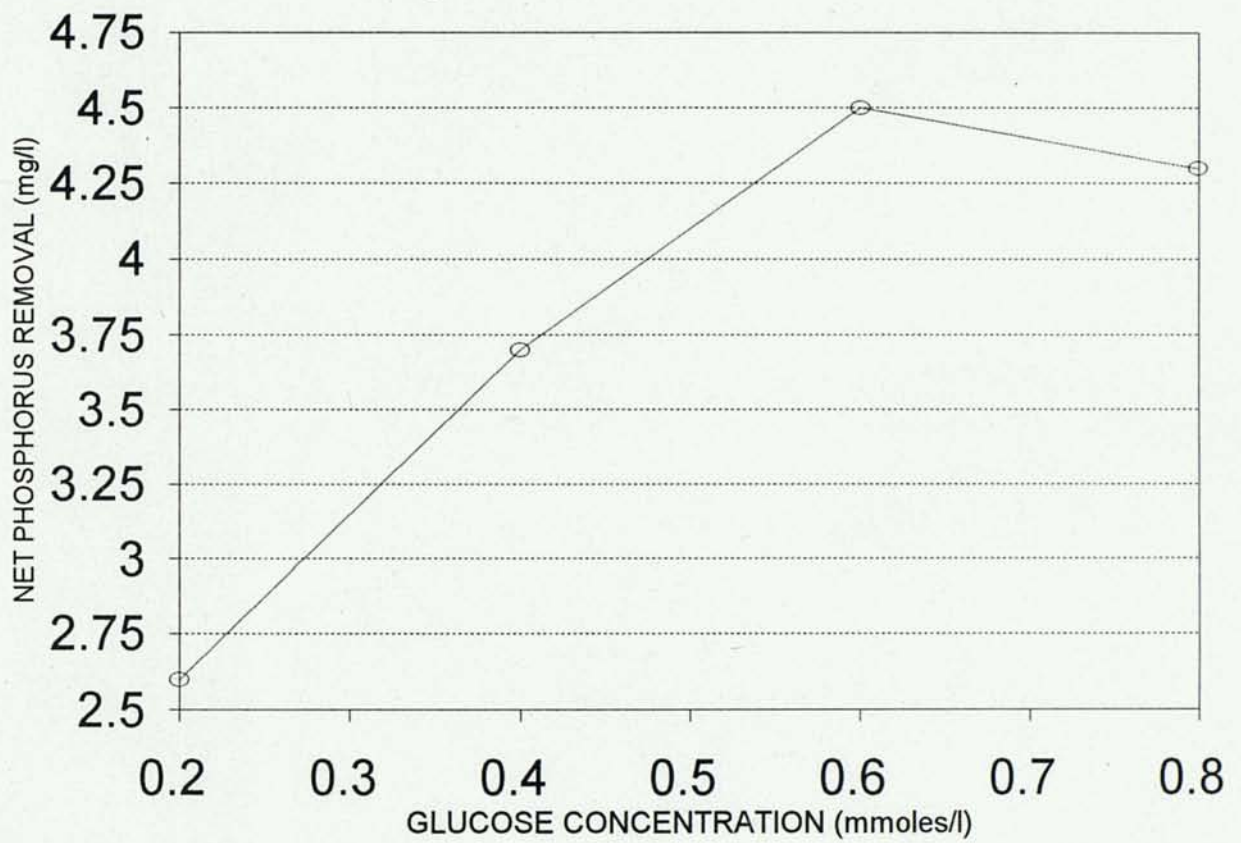


Figure 4.10 - Net P removal for glucose substrate relative to control (using average values).

It was also observed that the batch experiments using synthetic wastewater spiked with isovaleric acid performed better than the experiments spiked with the four other substrates analyzed. All four VFAs studied in batch experiments improved the phosphorus removal beyond the glucose experiment phosphorus removals, except for propionic acid (Figure 4.11). Isovaleric acid gave the highest removals followed by acetic acid and valeric acid. The difference in P removals observed for isovaleric and valeric acids may reflect energetic differences in processing of the two isomers during PHA biosynthesis.

From Figure 4.12, all the values for the glucose substrate at 0.20 mmol/l concentration showed P uptake in the anaerobic phase. The observed P uptake during the anaerobic period may have been the result of P requirements for glucose transport into the cell in group translocation, which requires phosphorylation of glucose (Moat and Foster, 1988).

Furthermore, it was observed that the Glucose SBR showed both greater P release and removal than batch experiments which received the same concentration of glucose (Figure 4.13). The reason was due to the prefermentation of the SBR influent so that a mixture of VFAs and glucose was actually received. The feed for the Glucose SBR did have sufficient time, around 8 hours, to undergo fermentation before it was introduced into the reactor. On the other hand, the glucose feed for the batch experiments had no time to preferment and was directly introduced into the reactor. This is because it has been shown that VFAs can be directly sequestered and polymerized to PHAs by poly-P bacteria (Sato et al., 1992). Glucose must first be fermented to VFAs but may be sequestered by bacteria competing with poly-P bacteria before this can happen. In addition, glucose may induce biochemical pathways

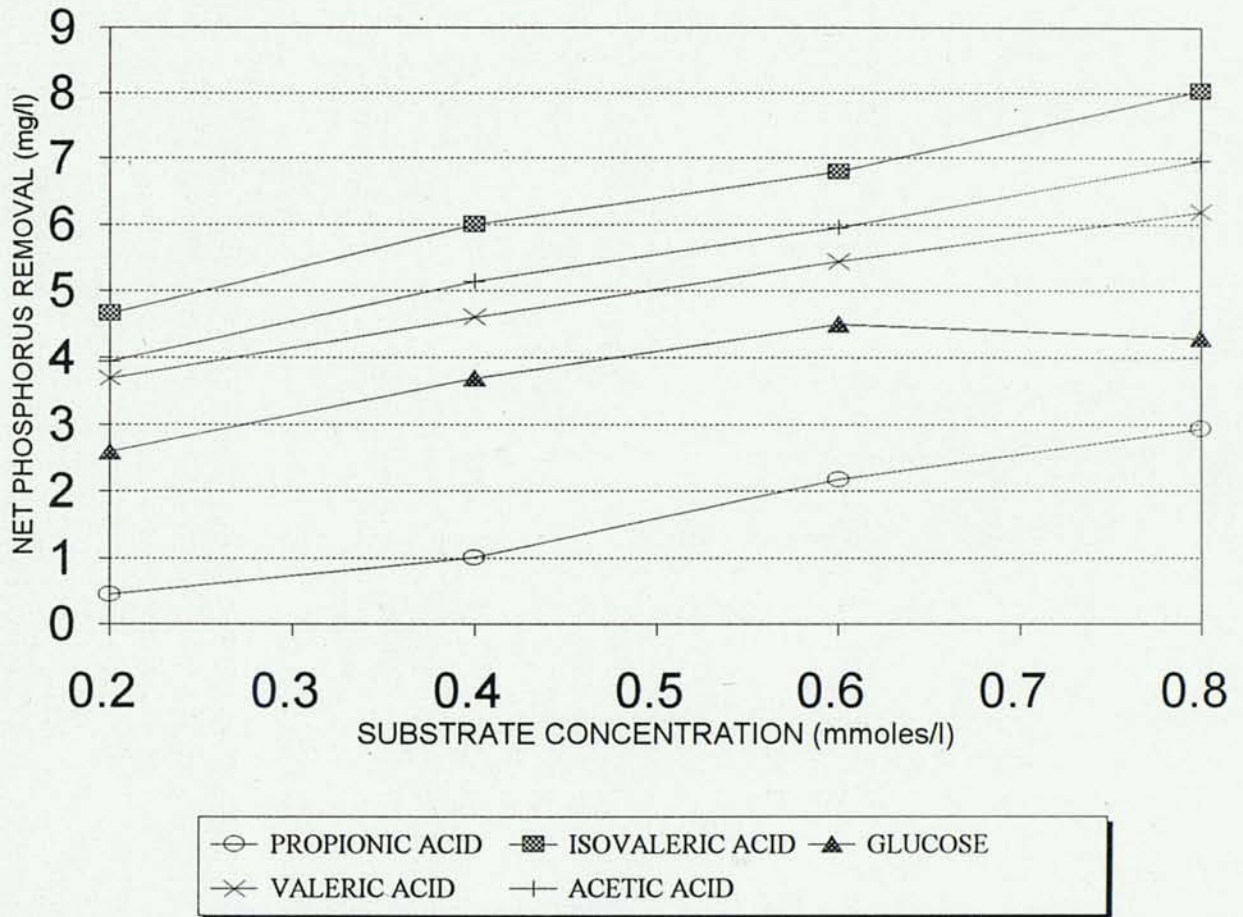


Figure 4.11 - Net P removal for substrates relative to control (using average values).

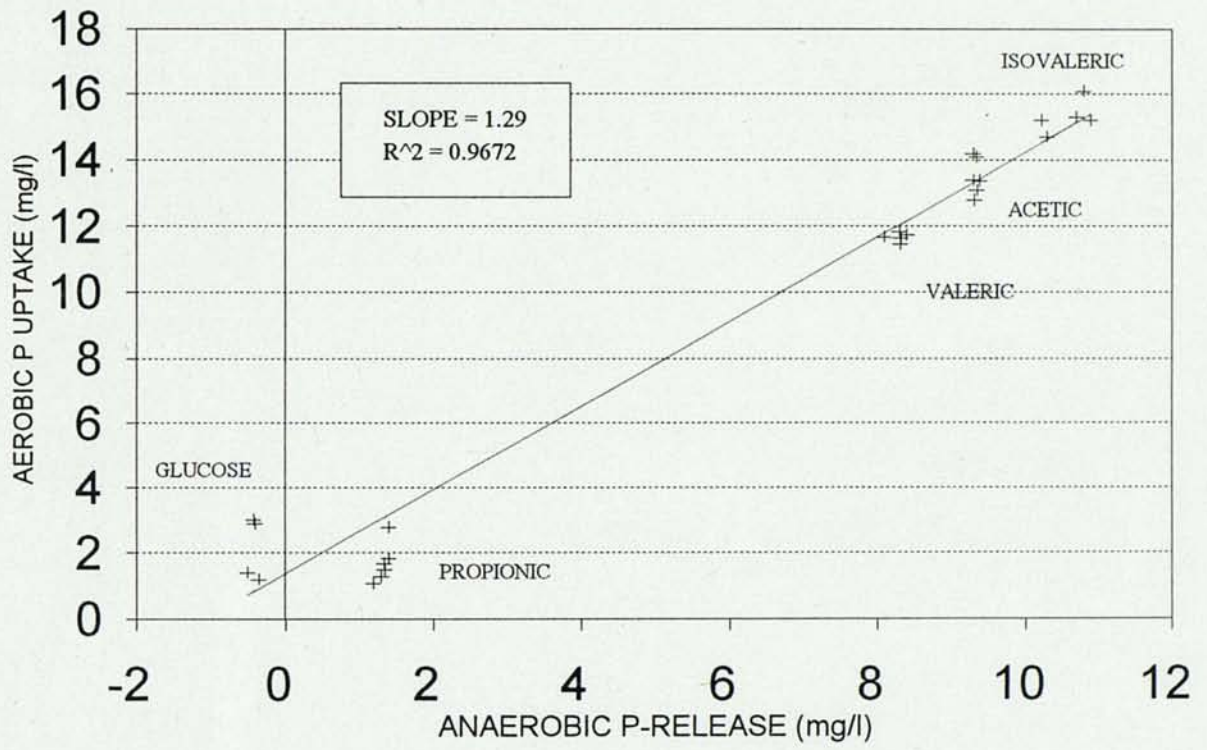


Figure 4.12 - P uptake versus P release for batch experiments using 0.20 mmol/l substrate, showing glucose as an outlier.

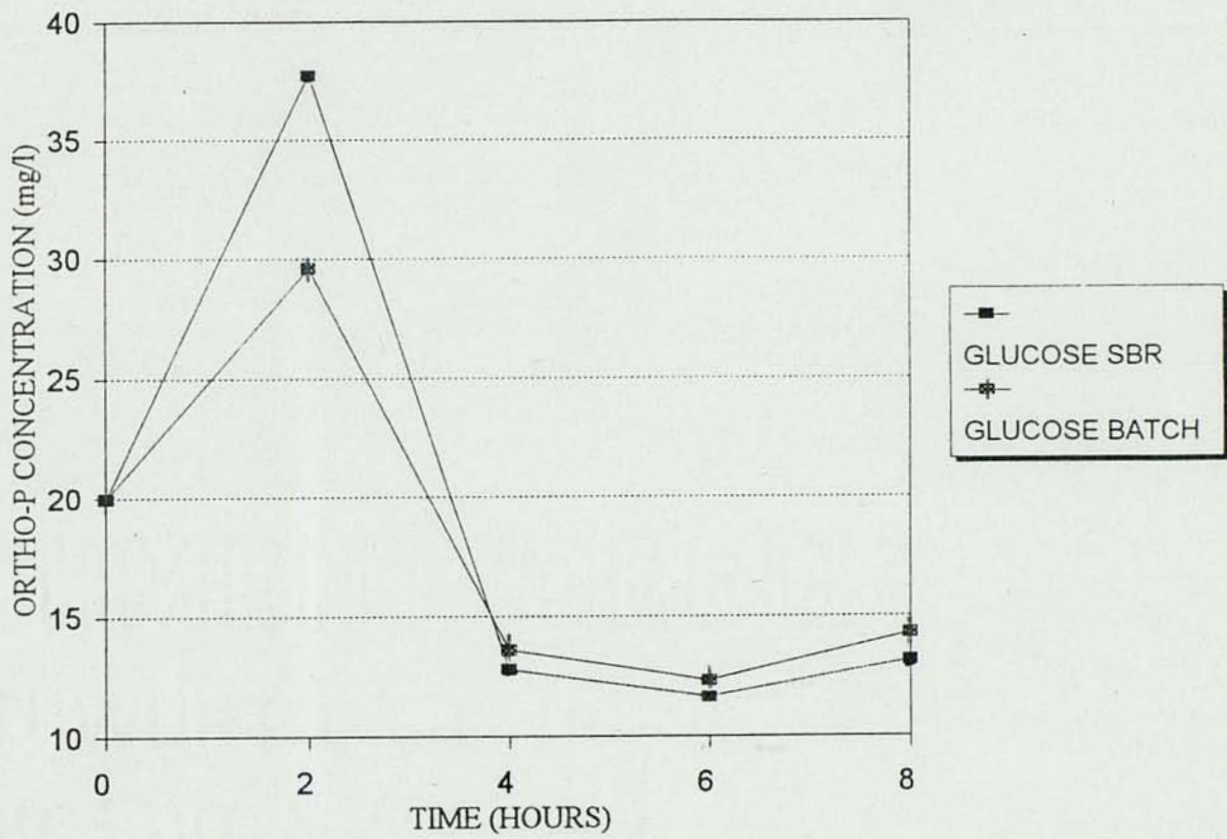


Figure 4.13- Ortho-P for the Glucose SBR and the batch experiment using 0.80 mmol/l glucose (using average values).

detrimental to EBPR in the poly-P bacterial population analogous to catabolic repression (Moat and Foster, 1988).

Table 4.4 shows that with respect to P uptake/release ratios, all four VFAs behaved in the same manner, while glucose behaved in a very different fashion. This behavioral difference probably because the P release and uptake for VFAs are dependent on the energy required for PHA biosynthesis and the energy derived from PHA biodegradation. Glucose, on the other hand, did not have ratios similar to any of the VFAs. In addition, its ratio varied greatly depending on the concentration of glucose administered in the experiments. The ratio difference may imply that glucose was sequestered and metabolized either by a different bacterial population than the poly-Ps (ie. "G" bacteria) and /or it induced in the poly-P bacteria other biochemical pathways that do not utilize VFAs. It could be that both mechanisms were operative and this resulted in the variability with dosage. Satoh et al. (1992) put forward a biochemical model of how glucose may induce non-EBPR metabolism in poly-P bacteria, or in "G" bacteria.

As stated earlier, for all the batch experiments, the addition of any of the VFAs and the glucose substrate increased the P removals beyond the levels achieved in the control reactors (Figure 4.14). The best substrate on a molar basis with respect to P removal was isovaleric acid. It achieved better P removal than all the substrates including its linear isomer valeric acid. Branched isomers have been observed to be superior in inducing P removal over their linear counterparts (Abu-ghararah and Randall, 1991; Randall, 1993). The metabolism of isovaleric acid was also related to Leucine catabolism by *Pseudomonas Putida* in which Isovaleryl-CoA is converted to Acetyl-CoA which might explain the superiority of isovaleric acid in EBPR (Abu-ghararah and Randall, 1991).

Table 4.4 - Ortho-P uptake/release ratio relative to control
(using average values).

SUBSTRATES mmoles/l	ORTHO-P UPTAKE/RELEASE RATIO				
	PRO.	IVAL.	GLU.	VAL.	ACE.
0.20	1.57	1.43	-6.43	1.43	1.51
0.40	1.29	1.48	2.68	1.46	1.50
0.60	1.41	1.47	2.43	1.48	1.48
0.80	1.39	1.50	1.89	1.50	1.50

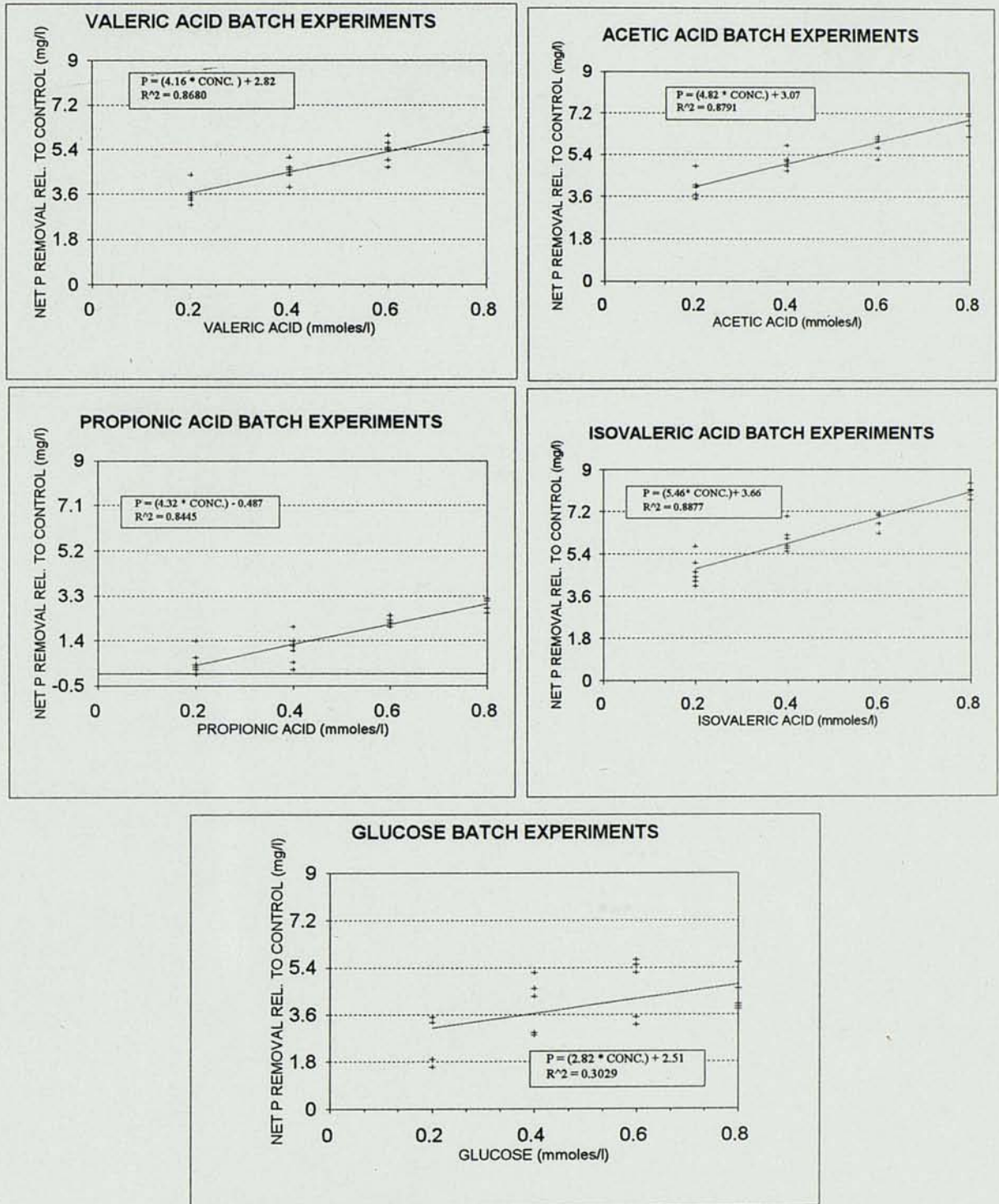


Figure 4.14 - Net P removal for all concentrations of substrates relative to control (using average values).

Furthermore, from Figure 4.14, the P removal of the glucose batch experiments are observed to be poorly correlated with a correlation coefficient of 0.303. This poor correlation might be because glucose was involved with metabolisms carried out by a diversity of bacterial groups within the system rather than being largely associated with a single type of metabolism as the VFAs are with the EBPR metabolism carried out by poly-P bacteria. It could also mean that glucose is fermenting during the anaerobic phase and this probably explains why we see improved P removals with glucose in this study, while it was detrimental in Randall's (1993). In addition, the scatter in the glucose data could also be related to the 5°C temperature difference. This higher temperature and the significant anaerobic phase fermentation probably resulted in lower observed yields than in the study by Randall (1993), and this explains the lower MLSS concentrations in this study.

When the VFAs were added, correlation coefficient values of 0.845 and higher were observed which is probably related to the exclusivity of VFA sequestration and metabolism by the poly-P population.

It was also observed that the Isovaleric SBR showed greater anaerobic P release and greater aerobic P uptake compared to the isovaleric batch experiments when using the roughly the same concentration, 0.80 mmol/l (Figure 4.15). This behavior was probably because the Isovaleric SBR received isovaleric acid as a supplemental carbon source on a regular basis, every 8 hours so the bacterial population was cultured in the presence of isovaleric acid. In batch experiments, the population was cultured by the presence of glucose and the variety of VFAs added, and the difference in resulting population distribution probably explains the relatively small differences observed between batch and steady state experiments.

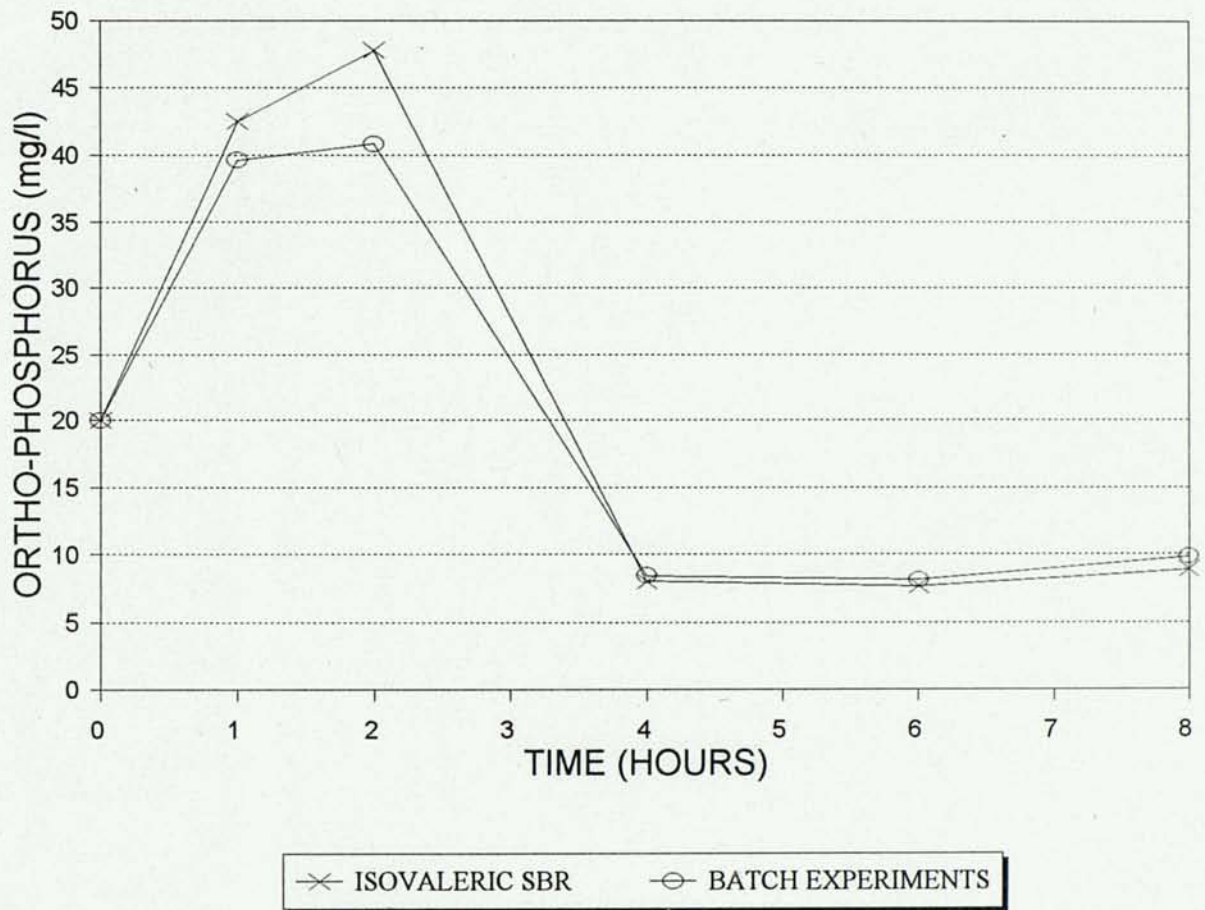


Figure 4.15 - Ortho-P for the Isovaleric SBR and the batch experiment using 0.80 mmoles/l isovaleric acid (using average values).

Even though EBPR did occur using propionic acid as a substrate, the magnitude of its removal was low compared to the other substrates. Propionic acid resulted in lower phosphorus release in the anaerobic period and lower phosphorus uptake in the aerobic phase relative to the other VFAs (Figure 4.16). This might be related to the availability of the strains of the *Acinetobacter* species capable of utilizing the propionic acid as the carbon source. Knight et al. (1995) reported 87% of the strains within the genospecies for utilization of propionic acid while the percentage was much higher for other substrates, such as acetic acid with 99% of the *Acinetobacter* strains.

Furthermore, propionic acid did not behave like the other VFAs with respect to ortho-P uptake/release ratio when compared in terms of absolute values (ie. relative to controls) (Figure 4.17). In addition, the magnitude of its P removal was much lower than all the other substrates. Also, all the other VFAs had very constant ortho-P uptake/release ratios compared to propionic acid. Such differences might be related to the fact that isovaleric acid, acetic acid, and valeric acid were directly stored into the cell as PHAs, whereas propionic acid had to be metabolized first, probably by other fermentative bacteria (Comeau et al., 1987). Comeau et al. (1987), however, obtained good P removal using propionic acid while its effect was detrimental in experiments performed by Randall et al. (1995). A possible explanation for the unique behavior of propionic acid was proposed by Satoh et al. (1992). They observed that propionic acid, a C₃ carbon VFA, resulted in a co-polymer of several PHAs instead of PHB which was observed for acetic acid. Thus, they proposed that propionic acid behaved differently from the other C₂-C₅ VFAs, affecting the overall energetics and end-product of PHA biosynthesis, by inducing the formation of polyhydroxyvalerates (PHVs) in addition to PHBs.

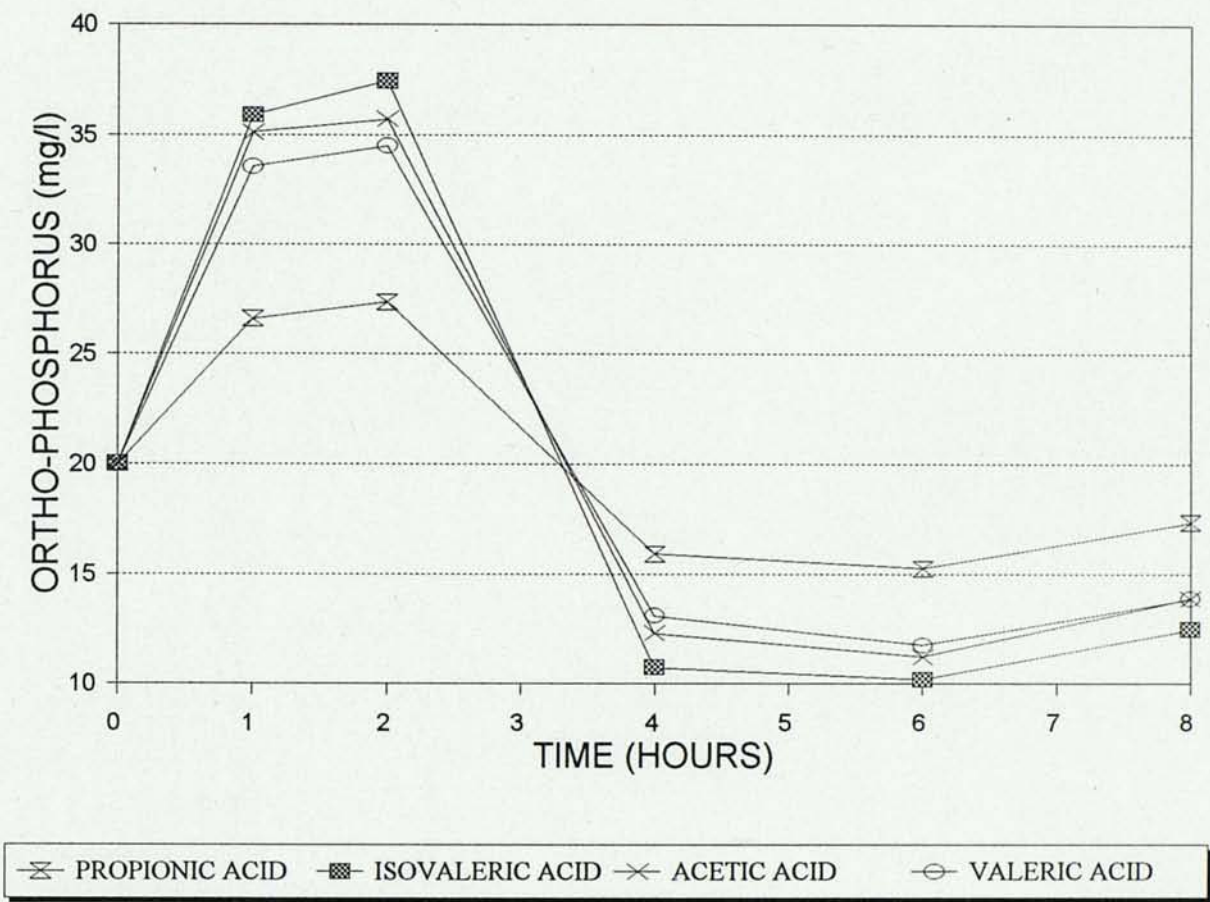


Figure 4.16 - Ortho-P release and uptake for batch experiments with 0.40 mmol/l substrates (using average values).

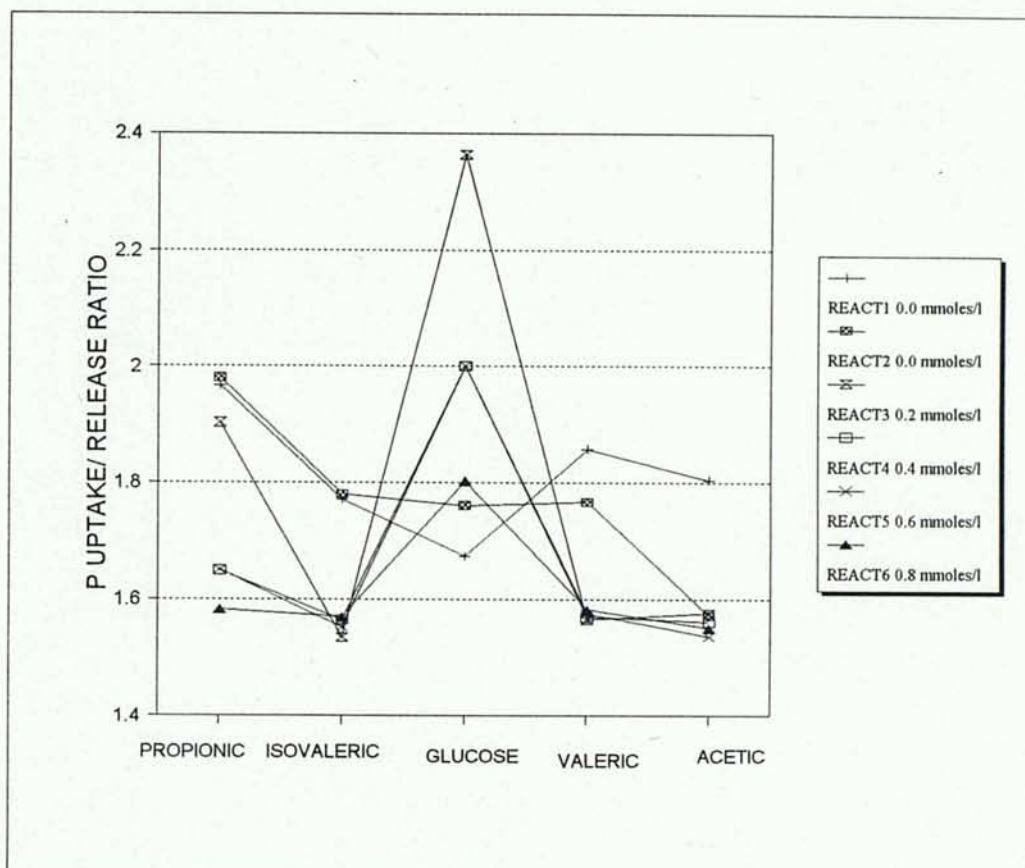


Figure 4.17 - P uptake/release ratio for all substrates including the controls (using average values).

There are two basic hypotheses of why propionic acid behaved differently. The first hypothesis is that the carbon chain length of propionic acid had biochemical and energetic consequences that resulted in different levels or types of PHA storage and this affected subsequent P uptake. The second hypothesis to explain the data would be that it depended on the predominant species selected in the system. Some poly-P bacteria may achieve very good P removal receiving propionic acid while others don't. Both these hypotheses may be true and could overlap.

The difference between propionic acid behavior in this study and that of Randall's (1993) may have been due to population effects (different predominant poly-P bacteria) resulting from the temperature difference. Also, Randall et al. (1996) suggest that chaotic dynamics may be operative in these systems, in which case, a small difference in initial conditions could result in significant population and metabolic differences between the systems.

V. CONCLUSIONS AND RECOMMENDATIONS

High P release in the anaerobic phase and subsequent P uptake in the aerobic phase was observed for the Glucose SBR when prefermented glucose was introduced as a supplemental carbon source. For the Isovaleric SBR, even higher EBPR efficiency was observed following the introduction of isovaleric acid as the supplemental carbon source.

The glucose SBR reported P percentages up to 6.4 % in the MLVSS while for the Isovaleric SBR, 8.7 % P was reported when isovaleric acid was received and 4.4 % P when no supplemental carbon source was added. Such results indicate that EBPR was established in all three cases. Prefermentation of glucose was also very important in inducing better P removal. The Glucose SBR, which received prefermented waste, gave net P removals roughly 3 mg/l greater than the same concentration of glucose used in batch experiments.

The data obtained from batch experiments showed that P removal increased as a result of substrate addition. All the substrates, the VFAs and the glucose, improved EBPR but the magnitude of their performance differed from one to another. Furthermore, the following can be concluded:

1. Isovaleric acid was shown to result in high levels of P removal at steady state. Thus, isovaleric acid not only induces P removal in a bacterial population where EBPR is active, as shown in batch experiments and prior studies (Abu-ghararah and Randall, 1991; Randall

et al., 1995) but it also selects for poly-P organisms and will result in higher levels of EBPR as a routine influent component.

2. Acetic acid and valeric acid also showed good P removal through out the batch experiments, and were therefore very beneficial to EBPR induction. Their P uptake/P release ratios were also similar to the other VFAs.

3. Propionic acid behaved like other VFAs with respect to the effect of concentration between 0.20 and 0.80 mmol/l, but the magnitude of its removal was lower than all the other substrates. When compared relative to control, the P uptake/P release ratio was the same for propionic acid as for the other VFAs.

4. Glucose showed significant differences in behavior from the VFAs. The P uptake/P release ratio was very different, and varied with concentration. In addition, P removals at concentrations higher than 0.60 mmol/l did not increase with concentration but rather decreased.

5. Improvements in removal with glucose as a substrate, in the SBR over the batch experiments, were probably the result of fermentation during the anaerobic phase. Contrast of this study and that of Randall's (1993) suggests that fermentable substrates may be detrimental at low temperatures, but result in EBPR when temperatures are higher.

For isovaleric, acetic, and valeric acids, the amount of P uptake in the aerobic phase was proportional to the P release in the anaerobic one. However, propionic acid exhibited low P release in the anaerobic phase and an unproportionally higher P uptake in the subsequent aerobic phase when compared at absolute values.

It is also important to note that with the addition of the appropriate substrate, EBPR could be achieved at great concentration levels. Our results also indicated that temperature has a major impact on fermentation in the anaerobic phase, and low temperatures may justify separate prefermentation of influent, especially with low influent readily available COD (ie. VFAs) to P ratios. A valuable experiment for future research would be to determine the differences in VFA type and quantity resulting from equal amount of isovaleric and valeric acids in an EBPR population, and link this information quantitatively to subsequent polyphosphate storage.

This study also confirmed the poor effect of propionic acid on EBPR. In addition, isovaleric acid was found to be very beneficial to EBPR processes. Since septic wastewater contains low concentrations of isovaleric acid, it is suggested to study the possibility of engineering fermenters that could produce high levels of isovaleric acid, thus improving EBPR to great extents.

Appendix A

Routine Operational Data

FILE TO STORE THE DATA COLLECTED THROUGH OUT THE RESEARCH FOR THE 2 SBRs												
(GLUCOSE SBR = sbr1, AND ISOVALERIC SBR = SBR2)												
SAMPLE DATE 1995	----- MLSS -----		----- MLVSS -----		-- % MLVSS/MLSS --		-- EFFLUENT TSS --		ORTHO-P AERATED		-- ORTHO-P EFFL --	
	SBR1	SBR2	SBR1	SBR2	SBR1	SBR2	SBR1	SBR2	SBR1	SBR2	SBR1	SBR2
JUNE												
	22	1200								15		
	24	1150								14		14.3
	26											
	28											
	30	1050								15.5		14.8
AVERAGE		1133								15		
STD.		76								16		13.5
JULY												
	2	1000								16		14.1
	4	900								14		
	6											
	8	1100		930		0.85		10		11		
	10											
	12	990		900		0.91		20		11.5		12.5
	14	1000		880		0.88		10		12		
	16	950		799		0.84		15		10		12.4
	18									11		13.6
	20	1100		815		0.74		11		10		13.1
	22	980		826		0.84		12		11		13
	24	1000		795		0.80		14		12		13
	26	1300		895		0.69		11				
	28	1250		925		0.74		10				13
	30	1100		928		0.84		11				13
AVERAGE		1032		855		0.8		13		12		13
STD		120		55		0.1		3		2		1
AUGUST												
	1	1250		940		0.75		11		12		13
	3		1100		841		0.76		12		8.51	9.21
	5	1354	1300	969	958	0.72	0.74	11	11	11.5	7.91	12.9
	7	1356	1655	988	1126	0.73	0.68	10	13	10.6	7.56	12.5
	9	1455	1799	985	1400	0.68	0.78	9	11	10.5	8.51	11.9
	11	1500	1815	1055	1528	0.70	0.84	11	12.1	11.1	7.98	12.5
	13		1768		1455		0.82		11.5		8.12	9.31
	15		1821		1498		0.82		12.7		8.11	9.24
	17		1959		1568		0.80		13.5		7.99	8.99
	19		1856		1458		0.79		11.1		8.13	8.99
	21		1799		1299		0.72		10.8		8.11	9.36
	23		1698		1378		0.81		10.9		7.99	9.14
	25		1924		1345		0.70		11.3		8.21	9.42
	27		1899		1465		0.77		11.5		8.16	9.34
	29	SBR2 IS BEING RUN WITHOUT ANY SUBSTRATE. ONLY THE USUAL DAILY FEED IS BEING INTRODUCED										9.26
	31		1400		1200		0.86		10.5		15.8	16.8
AVERAGE		1416	1723	999	1332	0.71	0.77	10.25	11.72	10.93	8.10	12.45
STDS		73	255	38	218	0.02	0.05	0.96	0.90	0.46	2.07	0.41
SEPTEMBER												
	2		1350		1150		0.85		11		15.9	17
	4	1480	1400	1100	1100	0.74	0.79	11	10	12.5	15.7	12.9
	6	1465	1385	1125	1055	0.77	0.76	10	10	11.5	16.1	13.2
	10	1525	1295	1095	1145	0.72	0.88	11	9.5	11.7	15.1	13
	14	MYRA JORDAN THREW AWAY MY SAMPLES.										
	20	1500	1325	1110	1095	0.74	0.83	12	10	11.8	15.4	12.8
	28	1500	1420	1111	1005	0.74	0.71	11	9.5	10.2	16.1	12.5
AVERAGE		1245	1363	1108	1092	1	1	11	10	12	16	13
STDS		610	48	12	55	0	0	1	1	1	0	0
OCTOBER												
	11	1600	1410	1100	1090	0.69	0.77	11	10	12	13	13
	13	1450	1280	1195	1050	0.82	0.82	11	9.8	13	14	12

SAMPLE FILE TO STORE THE DATA COLLECTED THROUGH OUT THE RESEARCH FOR THE 2 SBRs
 DATE (GLUCOSE SBR = SBR1, AND ISOVALERIC SBR = SBR2) (TEMPERATURES ARE IN DEGREE CELSIUS
 1995 -- REMAINING FEED -- -- WAS -- -- ROOM TEMP. -- -- REACTOR TEMP. - -- DISSOLVED OXYGEN --
 (mg/l) (mg/l) ANAEROBIC PHASE (mg/l)
 SBR1 SBR2 SBR1 SBR2 SBR1 SBR2 SBR1 SBR2

JUNE

	22	520		460		24	25		7
	24	560		450		25	24		8.5
	26	510		460		25	25		
	28	560		460		25	25		
	30	560		450		24	26		8.5
AVERAGE		542		456		25	25		9
STD.		25		5		1	1		9

JULY

	2	560		460		24	24		8.5	
	4	560		450		24	25		7.5	
	6	560		450		26	26			
	8	545		450		24	25		9.5	
	10	555		455		24	23			
	12	546		475		24	24		9.5	
	14	560		470		25	25			
	16	550		460		25	25		10	
	18	560		470		25	24		9.5	
	20	560		460		26	25			
	22	550		475		27	26			
	24	570		450		25	25			
	26	560		455		26	24		9.5	
	28	560	560	465	400	25	25	25	8.5	7
	30	560	550	460	420	25	24	25		12
AVERAGE		557	555	460	410	25	25	25	9	10
STD		7	7	9	14	1	1	0	1	4

AUGUST

	1	560	500	460	450	25	25	26	9.5	
	3	560	560	450	420	26	24	25		10.5
	5	550	550	455	425	25	25	24	9	10.3
	7	560	540	475	460	25	26	25	10	
	9	525	550	460	475	25	25	26	10	12
	11	530	560	450	465	24	25	25		12.5
	13	560	540	470	475	24	25	24		
	15	570	560	450	457	25	25	25		12
	17	575	560	460	456	24	24	26		12.5
	19	570	555	455	450	25	26	25		10
	21	560	550	460	450	25	25	25		11.5
	23	580	545	475	470	25	25	25		
	25	560	560	460	450	26	25	26	10	
	27	560	550	455	455	25	25	27	11	
	29	SBR2 IS BEING RUN WITHOUT ANY SUBSTRATE. ONLY THE USUAL DAILY FEED IS BEING INTRODUCED								
	31	560	560	425	425	24	25	25		13.5
AVERAGE		545	552	458	454	25	25	25	10	11
STD		17	8	10	18	1	1	1	1	1

SEPTEMBER

	2	560	560	460	450	24	25	26		13.5
	4	550	550	450	460	25	25	25	9.5	12.5
	6	560	540	450	470	25	25	25	10	13
	10	545	560	460	460	26	26	25	9.5	12.4
	14	MYRA JORDAN THREW AWAY MY SAMPLES.								
	20	560	560	460	460	24	25	26		
	28	550	550	450	450	25	26	24	9.5	
AVERAGE		475	553	455	458	25	25	25	10	13
STD		210	8	5	8	1	0	1	0	1

OCTOBER

	11	560	560	450	460	25	25	26	9.5	12.5
	13	550	550	460	460	25	25	25	8.5	12

TYPICAL OPERATIONAL PARAMETERS

	GLUCOSE SBR	ISOVALERIC SBR
MLVSS (mg/l)	990	1355
REACTOR VOL (ml)	2900	2900
X (mg)	2871	3930
AVE Qwas (ml/day)	460	460
AVE FEED WST (ml/day)	560	560
AVE FEED TREATED (ml/cycl)	1940	1940
AVE Qin _f (ml/day)	5820	5820
AVE Q _{eff} (ml/day)	5360	5360
AVE X _{was} (mg/day)	455	623
EFF VSS (mg/l)	8.13	8.19
X _{eff} (mg/day)	44	44
X _{was tot}	499	667
MCRT (days)	5.8	5.9

GLUCOSE SBR WAS ACTIVATED ON MAY 26th, 1995. ACTUAL DATA COLLECTION STARTED ON JUNE 15, 1995. GLUCOSE WAS USED AS A SUBSTRATE.

ISOVALERIC SBR WAS ACTIVATED ON AUGUST 3rd, 1995. ACTUAL DATA COLLECTION STARTED ON AUGUST 5, 1995. ISOVALERIC ACID WAS USED AS A SUBSTRATE.

ISOVALERIC SBR IS BEING FED ONLY THE DAILY FEED. NO SUBSTRATE IS BEING INTRODUCED AS OF AUGUST 29.

% P CALCULATED FROM P MASS BALANCE INF & EFF MEASUREMENTS

P _{inf} (mg/day)	116	116
P _{eff} (mg/day)	68.6	49.2
P _{was} (mg/day)	5.6	3.7
% P _{mlvss}	6.4	7.2

DIRECT MEASUREMENT OF MIXED LIQUOR % P

% P _{mlss}	5.9	6.8
---------------------	-----	-----

ADDITIONAL INFORMATION

TP _{inf} (mg/l)	20.0	20.0
AVE SP _{eff-acerated} (mg/l)	12.1	8.1
AVE SP _{eff} (mg/l)	12.8	9.18

ORTHO-PHOSPHORUS STANDARD CURVE

SAMPLE	CONCENTRATION mg/l	ABSORBANCE AT 400 nm
BLANK	0	0
S1	4	0.099
S2	5	0.122
S3	8	0.215
S4	10	0.262
S5	12	0.336
S6	15	0.404
S7	20	0.513

Regression Output:

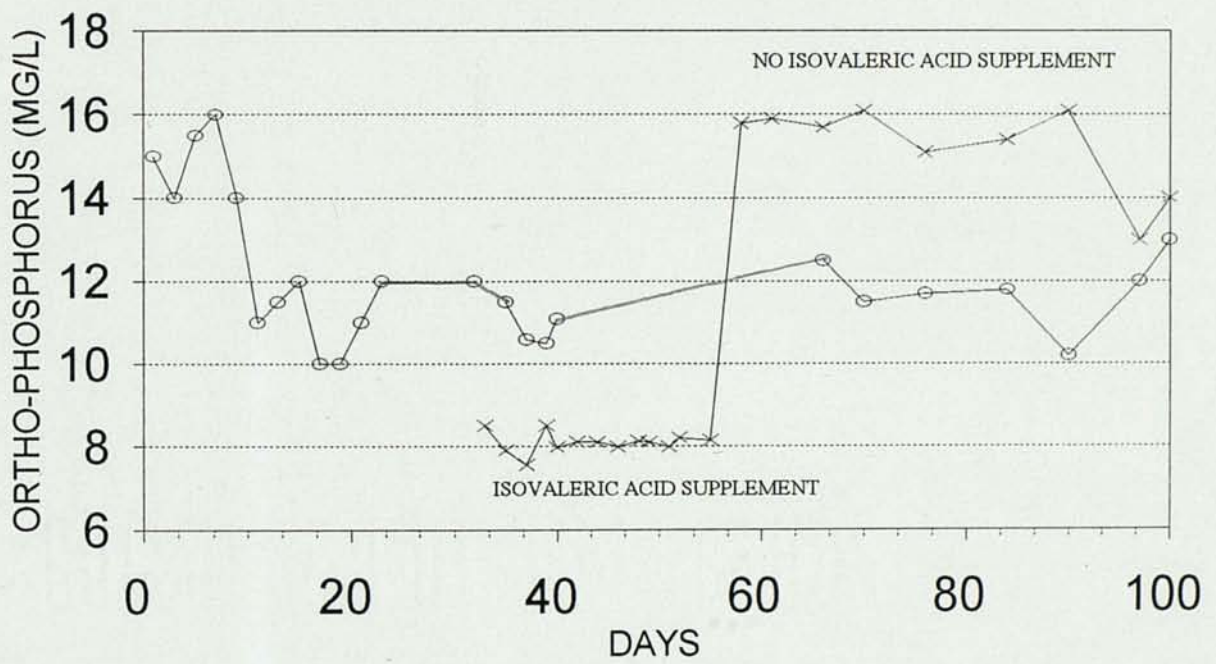
Constant	-0.000595
Std Err of Y Est	0.01168
R Squared	0.995968
No. of Observations	8
Degrees of Freedom	6

X Coefficient(s)	0.026429
Std Err of Coef.	0.000686

Appendix B

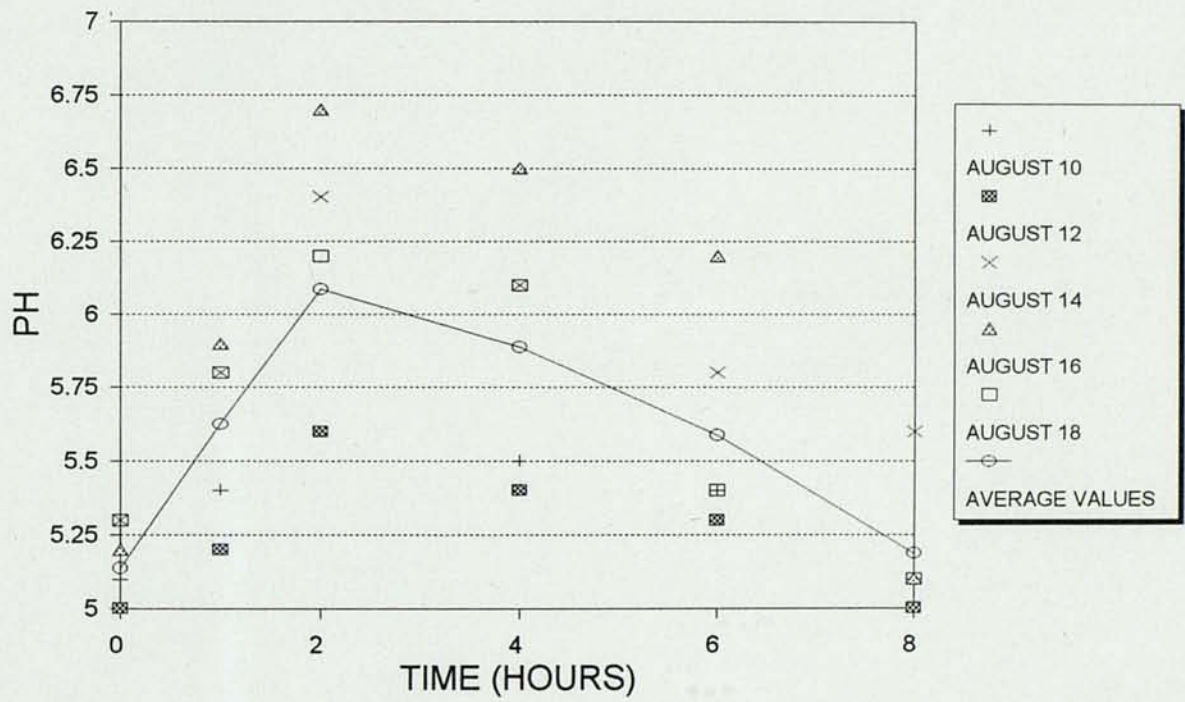
SBR Data

SBRs BEHAVIOR FOR ORTHO-PHOSPHORUS AERATED ORTHO-P (AT 6 hrs)



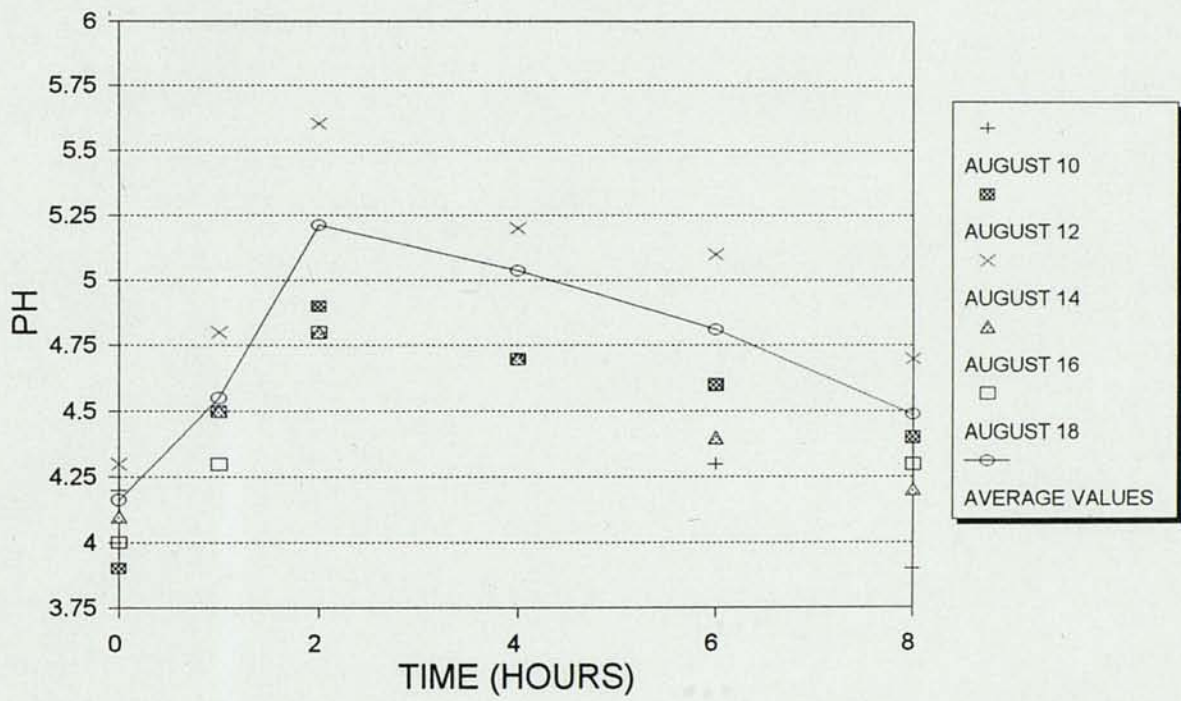
○ GLUCOSE SBR × ISOVALERIC SBR

GLUCOSE SBR PH VS TIME GLUCOSE SUPPLEMENT



ISOVALERIC SBR PH VS TIME

ISOVALERIC SUPPLEMENT



REACTOR ANALYSIS WITH RESPECT TO PH

THE FOLLOWING DATA ARE PERFORMED ON THE GLUCOSE SBR.

DATE	PH					
AUGUST	TIME (HOURS)					
	0	1	2	4	6	8
4	5.1	5.9	6.7	6.2	5.9	5.6
6	4.9	5.4	5.7	5.6	5.4	5.1
8	5.2	5.6	5.8	5.7	5.3	4.9
10	5.1	5.4	5.6	5.5	5.4	5.1
12	5	5.2	5.6	5.4	5.3	5
14	5.3	5.8	6.4	6.1	5.8	5.6
16	5.2	5.9	6.7	6.5	6.2	5.1
18	5.3	5.8	6.2	6.1	5.4	5.1
AVERAGE	5.1	5.6	6.1	5.9	5.6	5.2

REACTOR ANALYSIS WITH RESPECT TO PH

THE FOLLOWING DATA ARE PERFORMED ON THE ISOVALERIC SBR

DATE	PH					
AUGUST	TIME (HOURS)					
	0	1	2	4	6	8
4	4.1	4.3	5.6	5.4	5.1	4.7
6	4.2	4.6	5.5	5.3	5.1	4.6
8	4.5	4.9	5.7	5.6	5.3	5.1
10	4.2	4.5	4.8	4.7	4.3	3.9
12	3.9	4.5	4.9	4.7	4.6	4.4
14	4.3	4.8	5.6	5.2	5.1	4.7
16	4.1	4.5	4.8	4.7	4.4	4.2
18	4	4.3	4.8	4.7	4.6	4.3
AVERAGE	4.2	4.6	5.2	5.0	4.8	4.5

Appendix C

Batch Experiments Data

BATCH EXPERIMENTS USING DIFFERENT SUBSTRATES.

SUBSTRATE	MOLECULAR FORMULA	DATE
ACETIC ACID	C ₂ H ₄ O ₂	AUGUST 12 AUGUST 14 AUGUST 17 AUGUST 19 SEPTEMBER 9 SEPTEMBER 24
PROPIONIC ACID	C ₃ H ₆ O ₂	AUGUST 11 AUGUST 13 AUGUST 16 AUGUST 18 SEPTEMBER 2 SEPTEMBER 21
VALERIC ACID	C ₅ H ₁₀ O ₂	AUGUST 25 AUGUST 27 AUGUST 29 AUGUST 31 SEPTEMBER 19 SEPTEMBER 29
ISOVALERIC ACID	C ₅ H ₁₀ O ₂	AUGUST 15 AUGUST 20 AUGUST 21 AUGUST 23 SEPTEMBER 7 SEPTEMBER 23
GLUCOSE	C ₆ H ₁₂ O ₆	OCTOBER 2 OCTOBER 4 OCTOBER 9 OCTOBER 14 OCTOBER 16

ISOVALERIC ACID EXPERIMENTS WERE PERFORMED USING THE BIOMASS OF SBR 1. FOUR EXPERIMENTS WERE PERFORMED ON THIS SUBSTRATE OF MOLECULAR FORMULA = C₅H₁₀O₂ AND MOLECULAR WEIGHT OF 102 g/mole. THE VOLUME PER REACTOR WAS 485 ml.

THIS EXPERIMENT WAS PERFORMED IN AUGUST 15, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.6	24.6	17.1	16.6	17.9
0	2	20	24.5	24.5	17.3	16.6	17.9
0.2	3	20	34.6	35.3	12.3	11.9	13.4
0.4	4	20	35.8	36.4	11.1	10.4	12.5
0.6	5	20	37.6	38.7	10.1	9.87	11.1
0.8	6	20	38.9	39.7	8.95	8.45	9.23

THIS EXPERIMENT WAS PERFORMED IN AUGUST 21, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.6	24.6	17.1	16.6	17.9
0	2	20	24.5	24.5	17.3	16.6	17.9
0.2	3	20	34.5	35.4	12.4	11.8	13.2
0.4	4	20	35.2	36.8	11.4	10.6	13.1
0.6	5	20	36.9	37.9	9.89	9.54	10.9
0.8	6	20	38.6	40.1	8.26	8.11	9.84

THIS EXPERIMENT WAS PERFORMED IN AUGUST 20, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.1	24.2	16.9	15.9	16.9
0	2	20	24.1	24.3	16.9	15.9	16.8
0.2	3	20	34.9	35.6	12.1	12.1	13.1
0.4	4	20	35.8	37.1	10.6	10.1	12.8
0.6	5	20	37.9	39.3	9.98	9.45	11.3
0.8	6	20	39.1	41.1	8.54	8.23	9.67

THIS EXPERIMENT WAS PERFORMED IN AUGUST 23, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.3	24.5	16.8	16.2	17.8
0	2	20	24.3	24.6	16.9	16.3	17.9
0.2	3	20	35.1	35.8	12.1	11.6	12.9
0.4	4	20	36.9	37.4	11.1	10.7	12.4
0.6	5	20	38.1	39.5	10.1	9.16	11.6
0.8	6	20	39.9	41.1	8.24	8.15	9.87

THIS EXPERIMENT WAS PERFORMED IN SEPTEMBER 7, 1995.

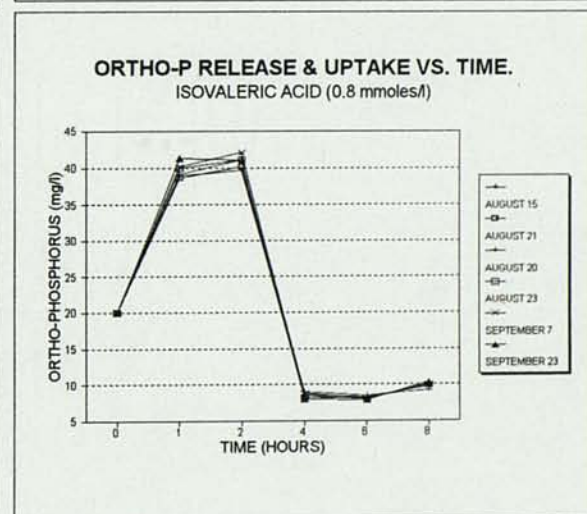
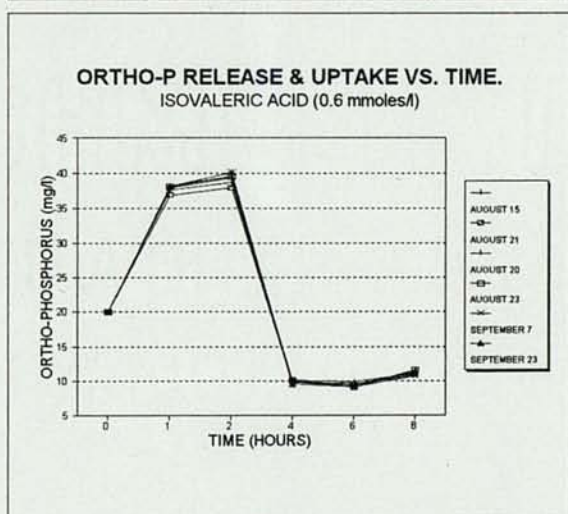
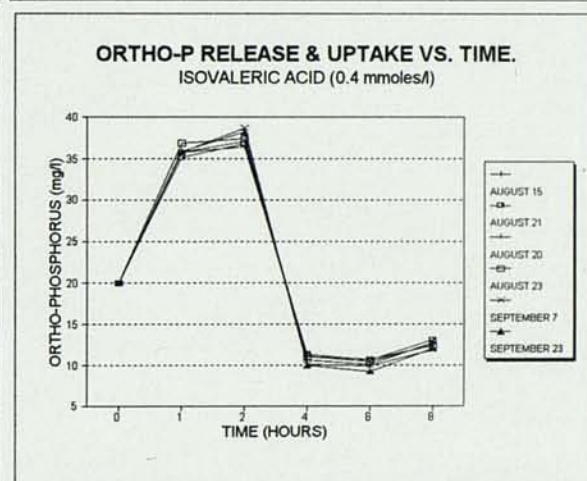
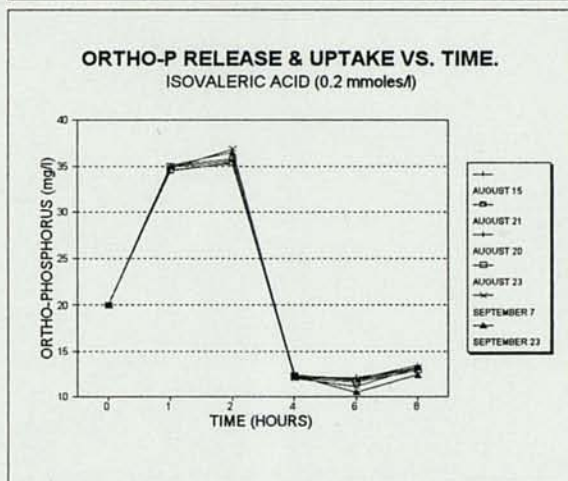
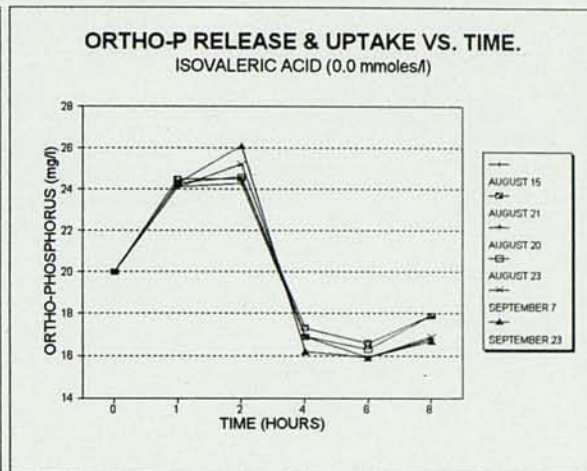
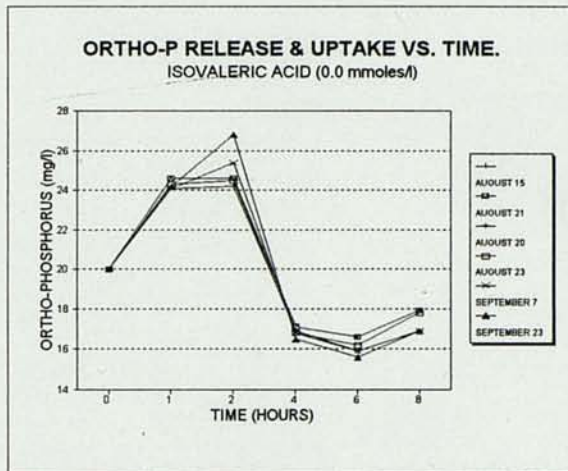
CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.1	25.4	16.8	15.9	16.9
0	2	20	24.1	25.2	16.9	15.9	16.9
0.2	3	20	34.8	36.9	12	11.1	13.2
0.4	4	20	35.8	38.7	10.1	9.94	11.9
0.6	5	20	37.9	40.2	9.88	9.11	10.7
0.8	6	20	40.1	42.1	8.78	8.01	9.98

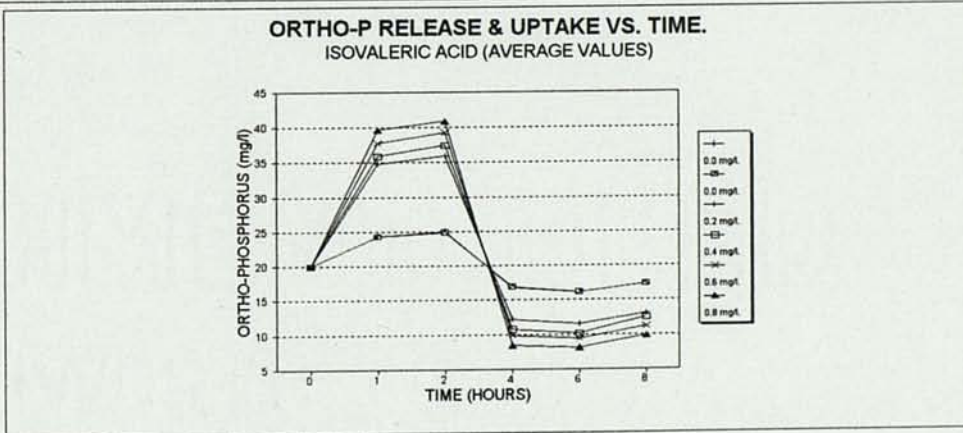
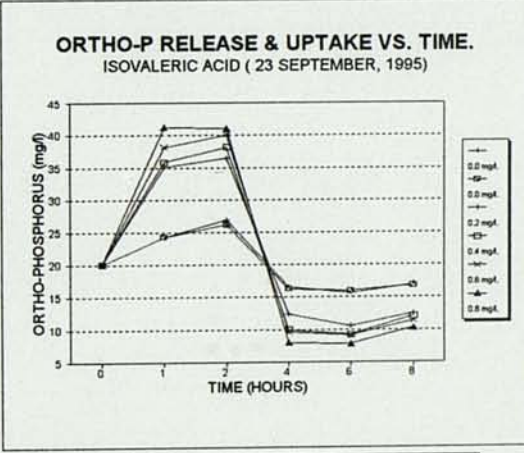
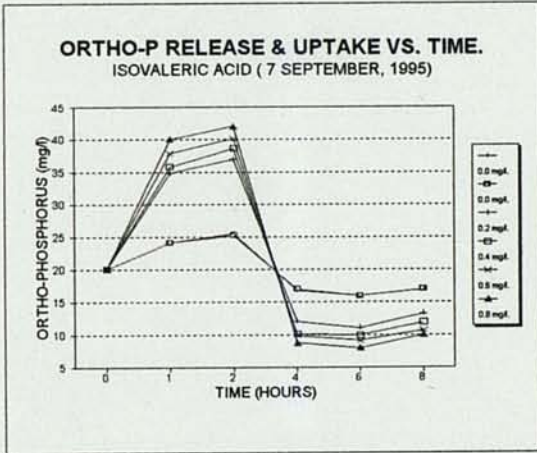
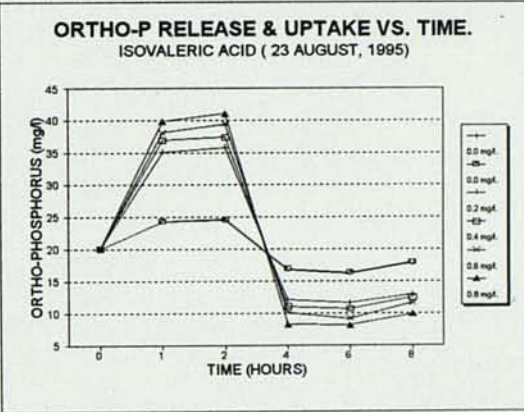
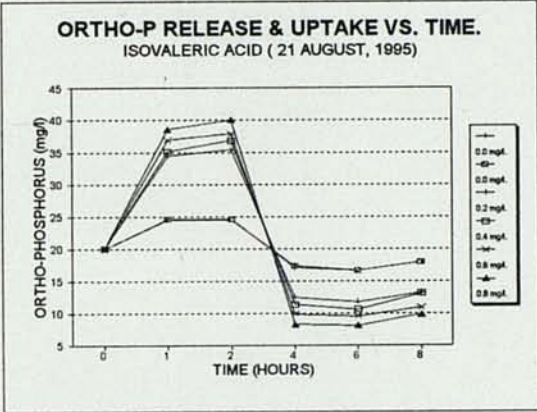
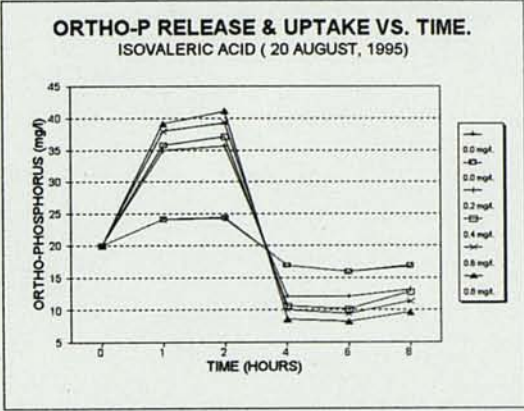
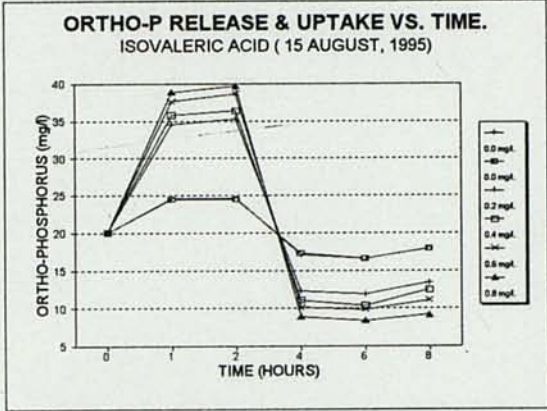
THIS EXPERIMENT WAS PERFORMED IN SEPTEMBER 23, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.2	26.8	16.5	15.6	16.9
0	2	20	24.3	26.1	16.2	15.9	16.7
0.2	3	20	35.1	36.5	12.3	10.5	12.4
0.4	4	20	35.9	38.2	10	9.23	12.1
0.6	5	20	38.2	40.1	9.55	9.1	11.4
0.8	6	20	41.3	41.1	7.99	7.8	10.3

THIS TABLE REPRESENTS THE AVERAGE VALUES FOR ALL THE BATCH EXPERIMENTS FOR ISOVALERIC ACID. THE FOLLOWING DATA WILL BE USED TO WRITE THE THESIS.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20.00	24.32	25.02	16.87	16.13	17.38
0	2	20.00	24.30	24.87	16.92	16.20	17.35
0.2	3	20.00	34.83	35.92	12.20	11.50	13.03
0.4	4	20.00	35.90	37.43	10.72	10.16	12.47
0.6	5	20.00	37.77	39.28	9.92	9.37	11.17
0.8	6	20.00	39.65	40.87	8.46	8.13	9.82





PROPIONIC ACID EXPERIMENTS WERE PERFORMED USING THE BIOMASS OF SBR 1. FOUR EXPERIMENTS WERE PERFORMED ON THIS SUBSTRATE OF MOLECULAR FORMULA = C₃H₆O₂ AND MOLECULAR WEIGHT OF 74 g/mole. THE VOLUME PER REACTOR WAS 485 ml.

THIS EXPERIMENT WAS PERFORMED IN AUGUST 11, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	22.9	23.1	17.2	16.8	17.9
0	2	20	22.8	23.1	17.3	16.8	17.9
0.2	3	20	23.2	24.1	17.1	16.3	17.3
0.4	4	20	25.9	26.5	16.8	16.1	17.1
0.6	5	20	28.1	29.1	15.1	14.3	16.5
0.8	6	20	29.9	30.4	14.9	13.7	16.1

THIS EXPERIMENT WAS PERFORMED IN AUGUST 13, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	23.3	23.6	17	15.9	18.4
0	2	20	23.3	23.5	17	15.9	18.3
0.2	3	20	24.2	25.1	16.8	16	18.1
0.4	4	20	26.4	27.3	15.7	15.1	17.6
0.6	5	20	29.5	28.9	14.8	14	16.5
0.8	6	20	31.3	32.9	13.9	13.2	15.8

THIS EXPERIMENT WAS PERFORMED IN SEPTEMBER 2nd, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	23.2	24.1	17.2	16.4	17.9
0	2	20	23.2	24.1	17.3	16.1	17.9
0.2	3	20	24.3	24.8	16.1	15.6	18.2
0.4	4	20	26.4	27.3	15.4	14.9	17.6
0.6	5	20	29.2	28.8	14.7	14.1	16.4
0.8	6	20	30.8	31.9	14.1	13.5	15.6

THIS TABLE REPRESENTS THE AVERAGE VALUES OF ALL THE BATCH EXPERIMENTS FOR PROPIONIC ACID. THIS DATA WILL BE USED TO WRITE THE THESIS.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20.00	23.30	23.85	17.08	16.28	18.07
0	2	20.00	23.32	23.85	17.13	16.23	18.05
0.2	3	20.00	24.08	24.65	16.53	15.80	17.50
0.4	4	20.00	26.58	27.32	15.92	15.25	17.35
0.6	5	20.00	29.07	29.13	14.93	14.07	16.42
0.8	6	20.00	30.58	31.47	14.18	13.32	15.65

THIS EXPERIMENT WAS PERFORMED IN AUGUST 16, 1995.

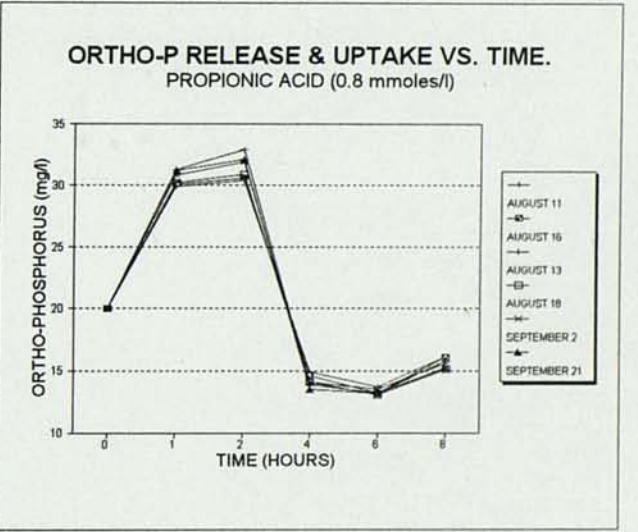
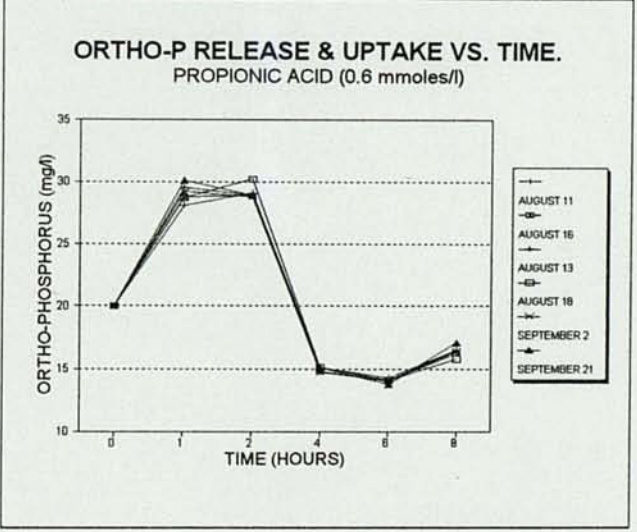
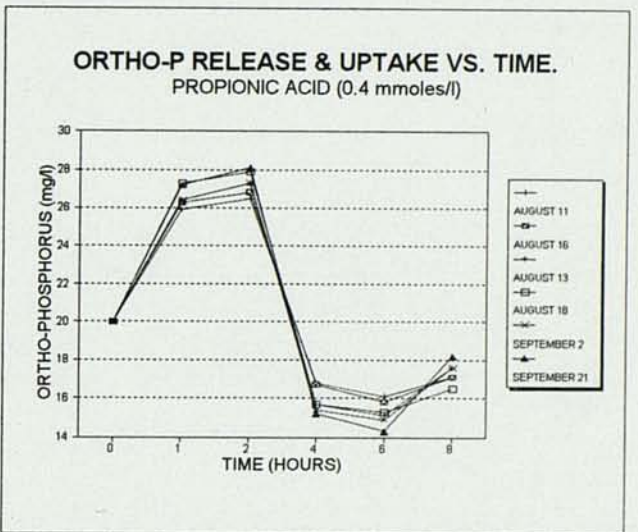
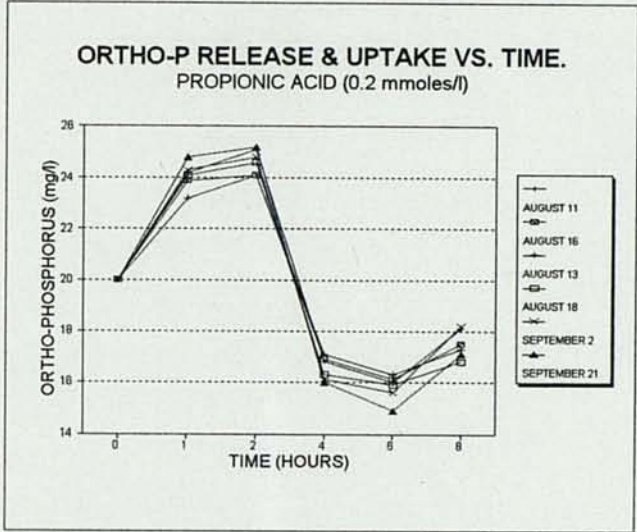
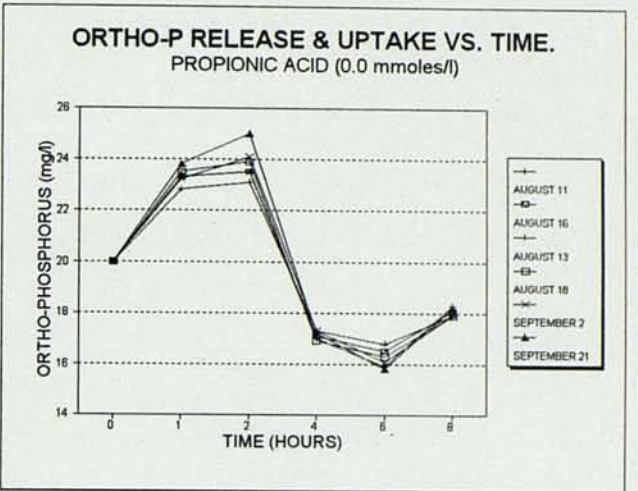
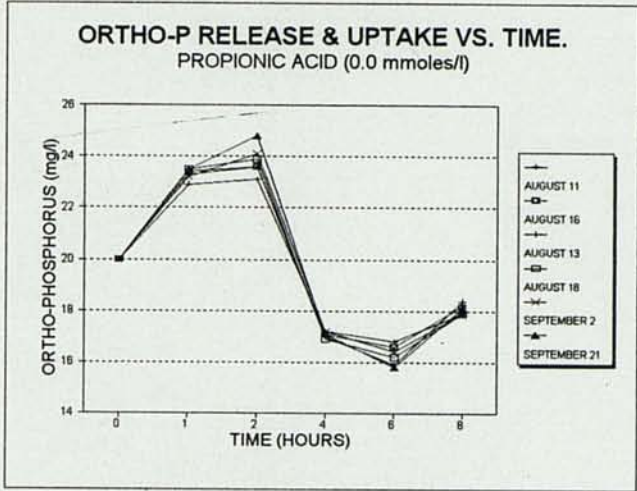
CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	23.4	23.6	17.1	16.6	18.2
0	2	20	23.3	23.5	17.1	16.5	18.1
0.2	3	20	23.9	24.1	16.9	16.1	17.5
0.4	4	20	26.3	26.8	16.7	15.8	17.1
0.6	5	20	28.8	28.9	14.8	14.1	16.2
0.8	6	20	30.1	30.6	14.6	13.2	16.1

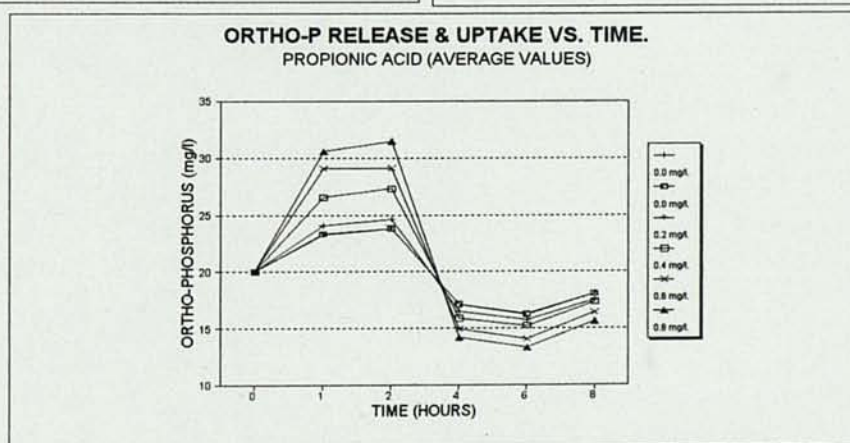
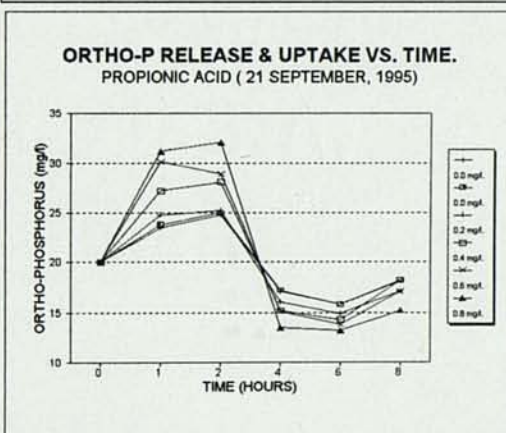
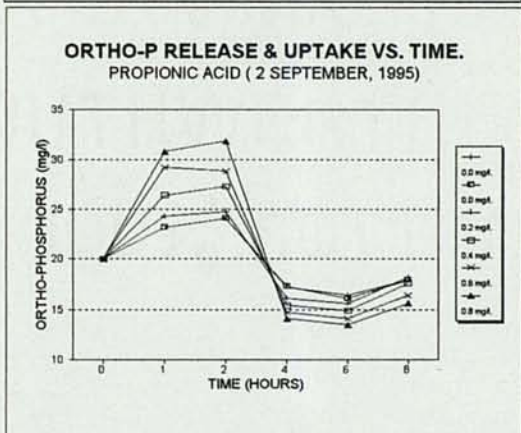
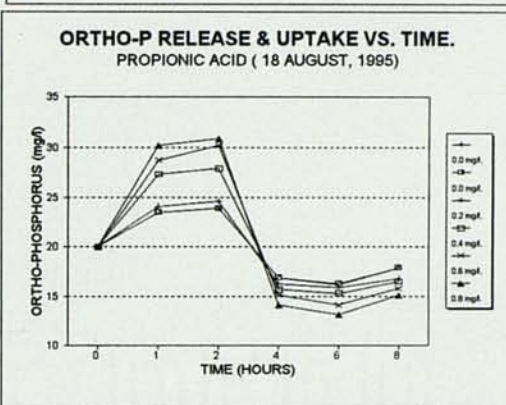
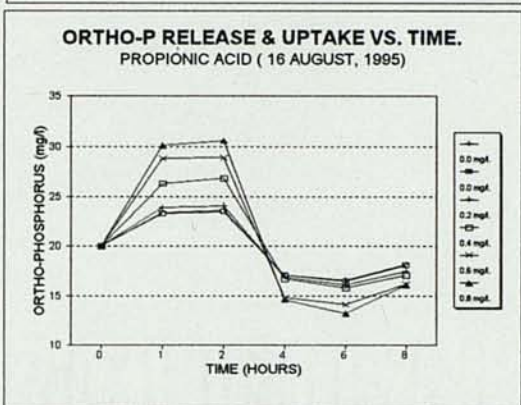
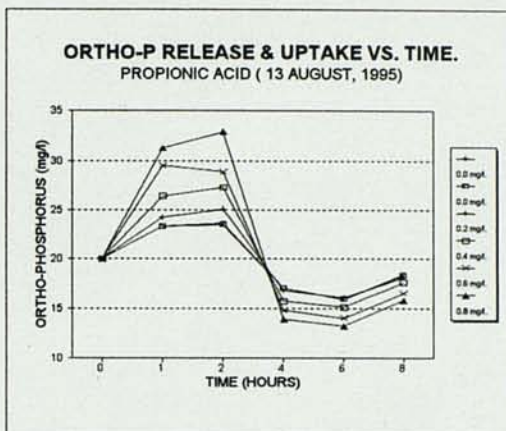
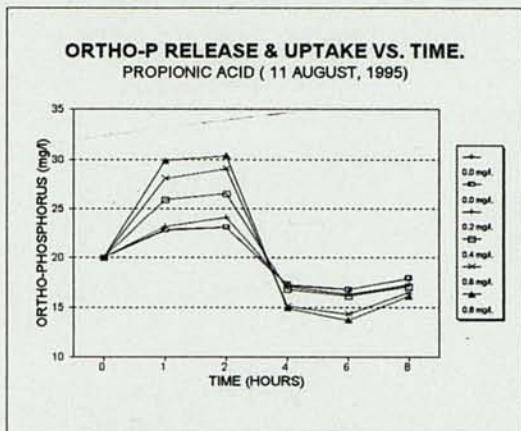
THIS EXPERIMENT WAS PERFORMED IN AUGUST 18, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	23.5	23.9	16.9	16.2	17.9
0	2	20	23.5	23.9	16.9	16.3	17.9
0.2	3	20	24.1	24.6	16.3	15.9	16.8
0.4	4	20	27.3	27.9	15.7	15.3	16.5
0.6	5	20	28.7	30.2	15.1	14.1	15.8
0.8	6	20	30.2	30.9	14.1	13.1	15.1

THIS EXPERIMENT WAS PERFORMED IN SEPTEMBER 21, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	23.5	24.8	17.1	15.8	18.1
0	2	20	23.8	25	17.2	15.8	18.2
0.2	3	20	24.8	25.2	16	14.9	17.1
0.4	4	20	27.2	28.1	15.2	14.3	18.2
0.6	5	20	30.1	28.9	15.1	13.8	17.1
0.8	6	20	31.2	32.1	13.5	13.2	15.2





VALERIC ACID EXPERIMENTS WERE PERFORMED USING THE BIOMASS OF SBR 1.
FOUR EXPERIMENTS WERE PERFORMED ON THIS SUBSTRATE OF MOLECULAR
FORMULA = C₅H₁₀O₂ AND MOLECULAR WEIGHT OF 102 g/mole.
THE VOLUME PER REACTOR WAS 485 ml.

THIS EXPERIMENT WAS PERFORMED IN AUGUST 25, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.5	24.5	16.9	16.6	17.6
0	2	20	24.5	24.5	16.9	16.6	17.6
0.2	3	20	32.7	33.1	13.9	12.7	14.5
0.4	4	20	33.7	34.3	12.7	11.6	13.7
0.6	5	20	35.1	36.5	11.8	10.9	12.9
0.8	6	20	36.4	36.9	10.3	9.97	10.6

THIS EXPERIMENT WAS PERFORMED IN AUGUST 27, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.3	24.2	16.4	15.9	16.9
0	2	20	24.2	24.3	16.4	15.9	16.8
0.2	3	20	31.9	33.2	13.8	12.6	14.8
0.4	4	20	33.4	34.1	12.7	11.7	13.6
0.6	5	20	35	35.9	11.4	10.3	12.7
0.8	6	20	36.1	36.7	10.4	9.97	10.9

THIS EXPERIMENT WAS PERFORMED IN SEPTEMBER 19, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.3	24.5	16.3	16.1	16.9
0	2	20	24.4	24.5	16.2	16.1	16.8
0.2	3	20	32.1	33.6	13.9	12.9	14.9
0.4	4	20	33.5	34.1	12.8	11.7	13.9
0.6	5	20	35.4	35.2	11.6	10.6	13.1
0.8	6	20	36.5	36.9	10.7	10.1	11.1

THIS TABLE REPRESENTS THE AVERAGE VALUES FOR THE BATCH EXPERIMENTS VALERIC ACID. THIS DATA WILL BE USED TO WRITE THE THESIS.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20.00	24.50	24.35	16.73	16.27	17.32
0	2	20.00	24.35	24.63	16.58	16.45	17.35
0.2	3	20.00	31.03	32.98	13.93	12.67	14.95
0.4	4	20.00	33.58	34.50	13.10	11.75	13.87
0.6	5	20.00	35.25	35.80	11.87	10.92	13.00
0.8	6	20.00	36.32	36.88	10.68	10.17	11.48

THIS EXPERIMENT WAS PERFORMED IN AUGUST 29, 1995.

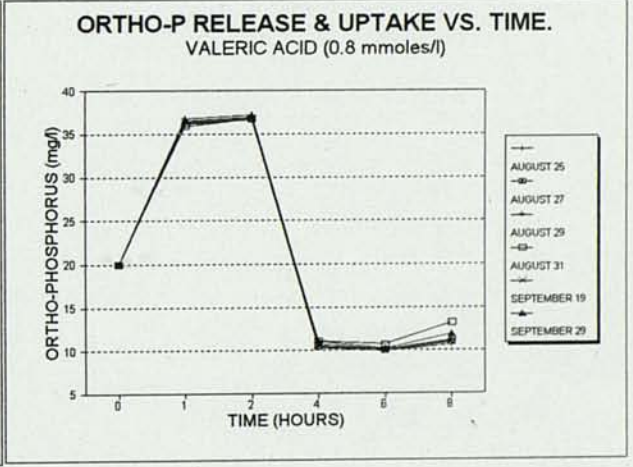
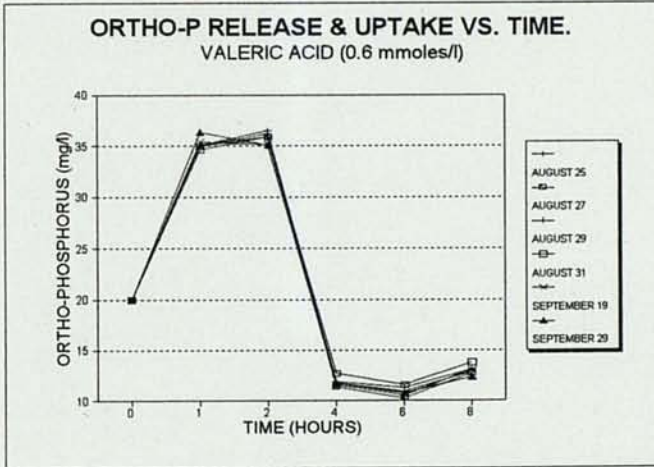
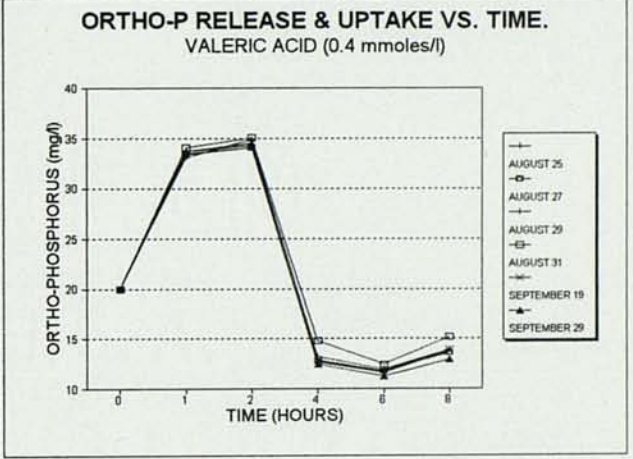
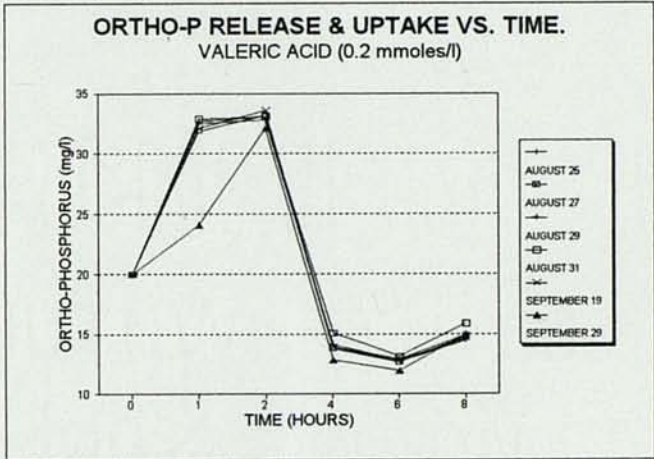
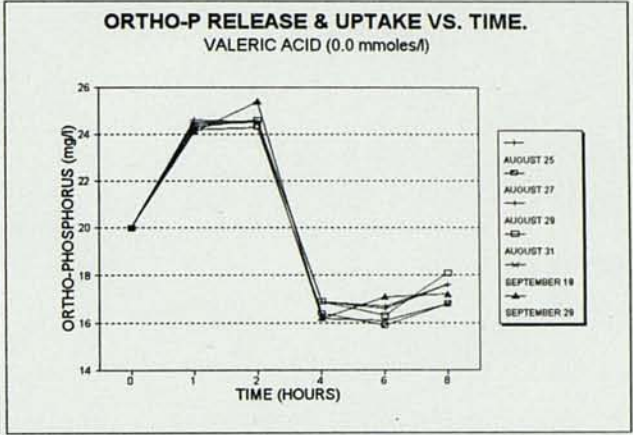
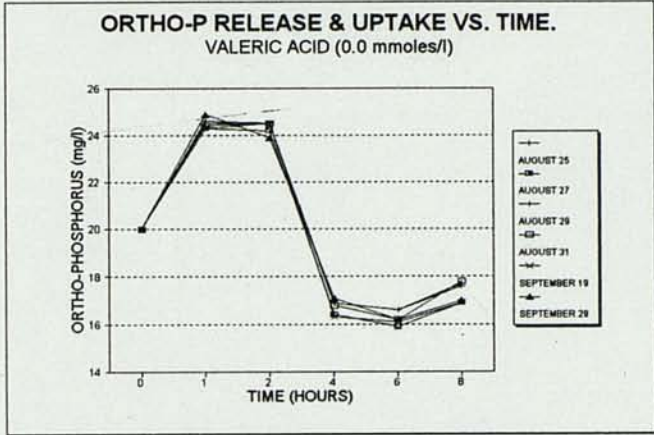
CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.6	24.5	16.9	16.6	17.7
0	2	20	24.6	24.5	16.9	16.7	17.6
0.2	3	20	32.5	32.8	14.1	12.8	14.6
0.4	4	20	33.1	34.9	13.2	11.9	13.9
0.6	5	20	35	36.2	11.9	11.3	13.1
0.8	6	20	36.2	36.8	10.5	10.1	11.2

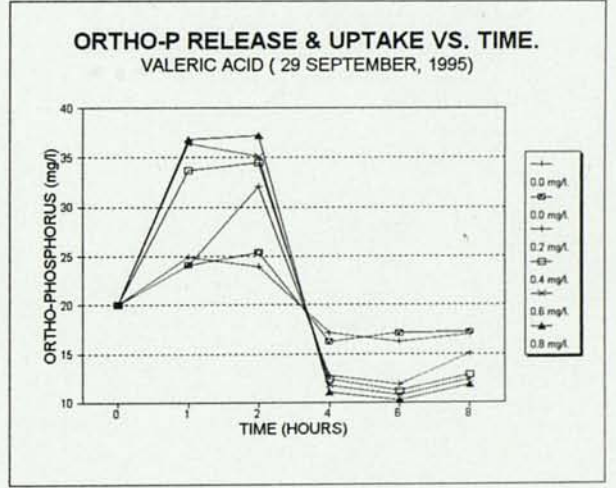
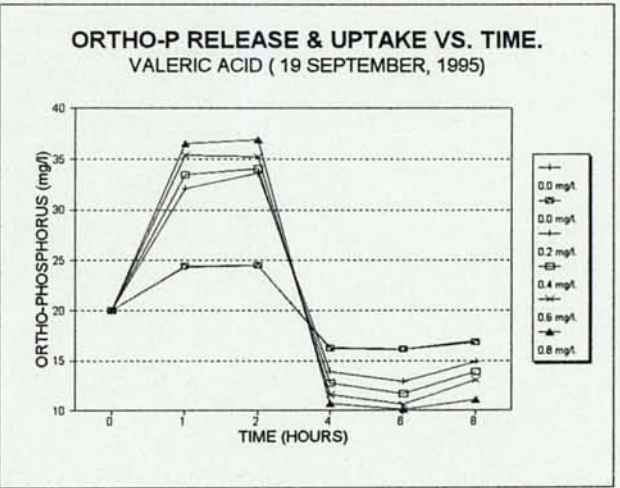
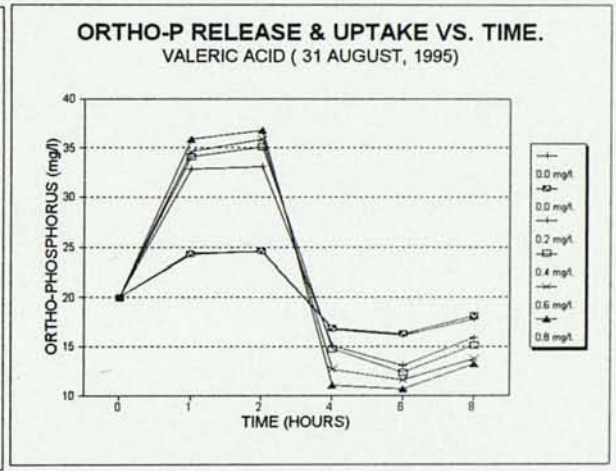
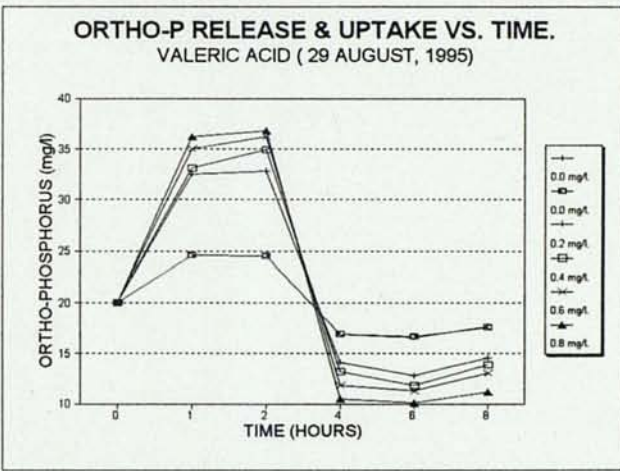
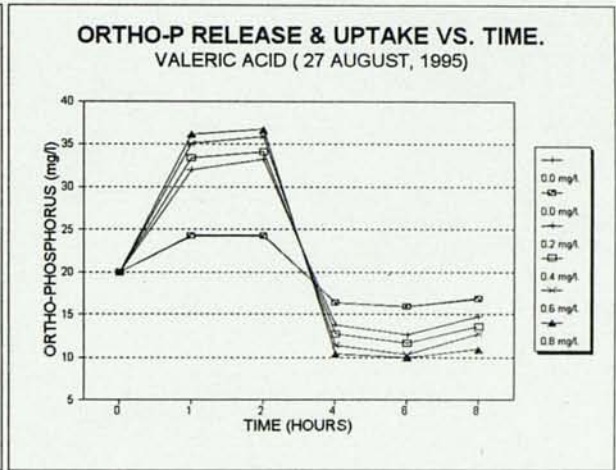
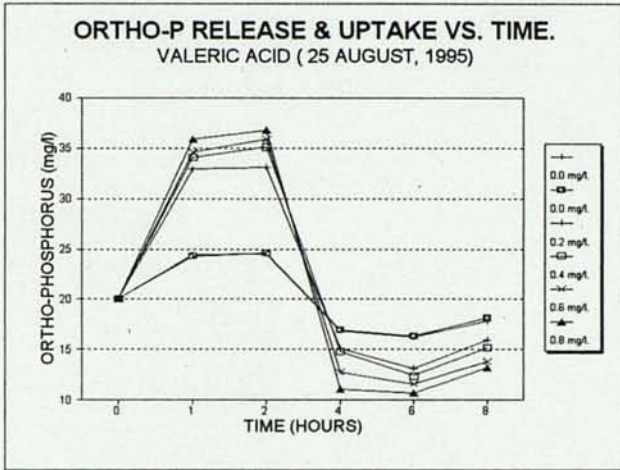
THIS EXPERIMENT WAS PERFORMED IN AUGUST 31, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.4	24.5	16.8	16.2	17.8
0	2	20	24.3	24.6	16.9	16.3	18.1
0.2	3	20	32.9	33.1	15.1	13.1	15.9
0.4	4	20	34.1	35.1	14.8	12.4	15.2
0.6	5	20	34.6	35.9	12.7	11.6	13.8
0.8	6	20	35.9	36.8	11.1	10.7	13.2

THIS EXPERIMENT WAS PERFORMED IN SEPTEMBER 29, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0	1	2	4	6	8
0	1	20	24.9	23.9	17.1	16.2	17
0	2	20	24.1	25.4	16.2	17.1	17.2
0.2	3	20	24.1	32.1	12.8	11.9	15
0.4	4	20	33.7	34.5	12.4	11.2	12.9
0.6	5	20	36.4	35.1	11.8	10.8	12.4
0.8	6	20	36.8	37.2	11.1	10.2	11.9





GLUCOSE EXPERIMENTS WERE PERFORMED USING THE BIOMASS OF SBR 1.
FOUR EXPERIMENTS WERE PERFORMED ON THIS SUBSTRATE OF MOLECULAR
FORMULA = C₆H₁₂O₆ AND MOLECULAR WEIGHT OF 180 g/mole.
THE VOLUME PER REACTOR WAS 490 ml.

THIS EXPERIMENT WAS PERFORMED ON OCTOBER 2, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/L	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.1	24.2	17.3	17.1	17.9
0.0	2	20.0	24.3	24.2	17.5	17.1	17.5
0.2	3	20.0	25.6	26.1	16.8	13.4	17.1
0.4	4	20.0	26.1	26.5	15.4	12.4	16.3
0.6	5	20.0	27.3	27.5	14.2	11.2	15.2
0.8	6	20.0	29.1	29.8	13.1	12.8	15.0

THIS EXPERIMENT WAS PERFORMED IN OCTOBER 4, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/L	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.2	25.8	17.8	16.4	17.9
0.0	2	20.0	25.1	25.1	17.5	16.8	17.8
0.2	3	20.0	25.2	27.0	17.1	13.2	17.5
0.4	4	20.0	26.3	27.1	15.6	12.1	16.1
0.6	5	20.0	27.1	27.6	14.1	11.0	15.4
0.8	6	20.0	29.8	29.9	13.5	12.1	13.8

THIS EXPERIMENT WAS PERFORMED IN OCTOBER 9, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/L	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.1	24.2	17.1	16.9	17.4
0.0	2	20.0	24.5	24.5	18.2	16.1	17.9
0.2	3	20.0	25.7	25.9	17.1	14.8	18.1
0.4	4	20.0	26.8	27.1	14.5	13.9	15.6
0.6	5	20.0	27.9	28.1	14.1	13.5	15.2
0.8	6	20.0	28.1	29.5	13.8	12.7	14.7

THIS EXPERIMENT WAS PERFORMED IN OCTOBER 14, 1995.

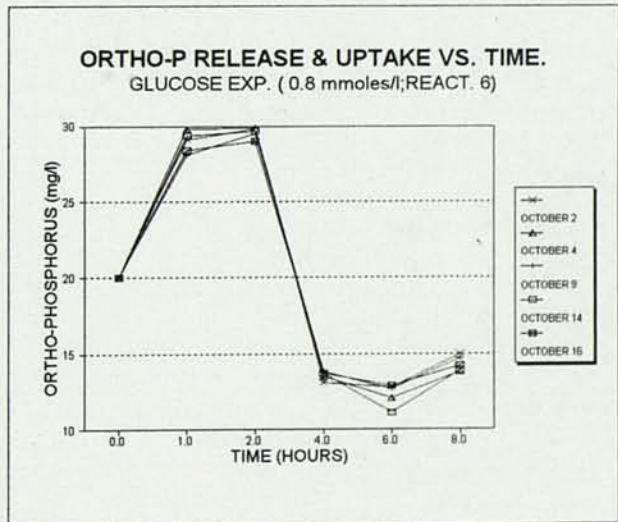
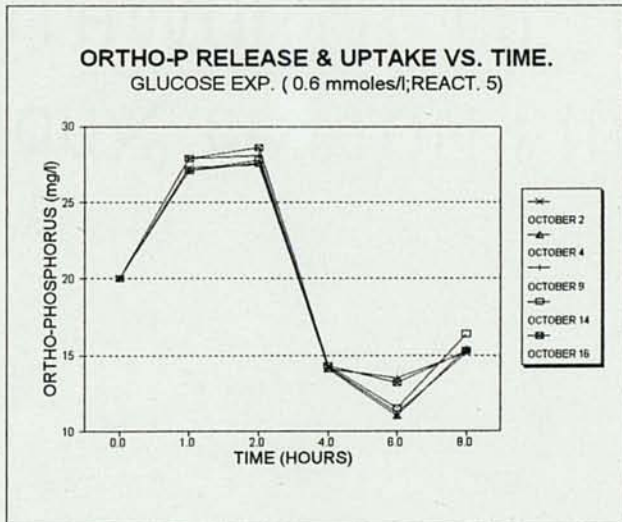
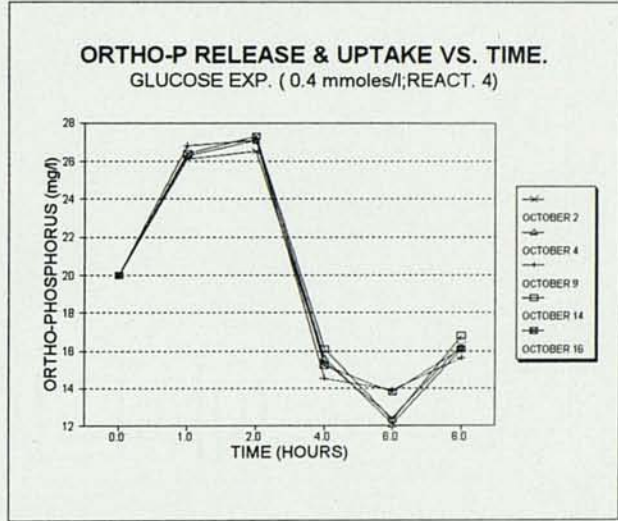
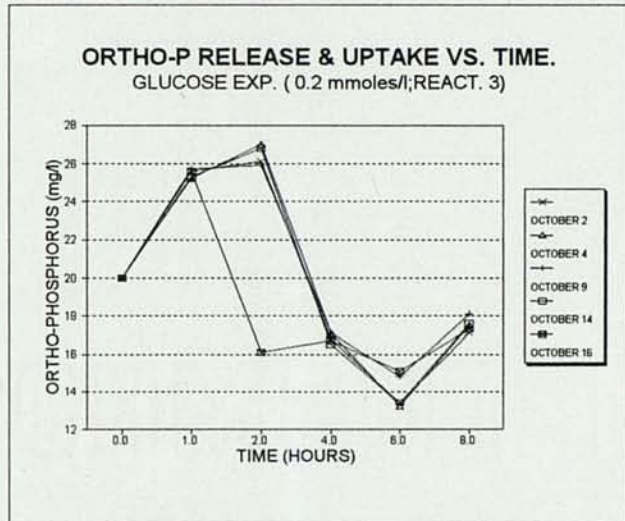
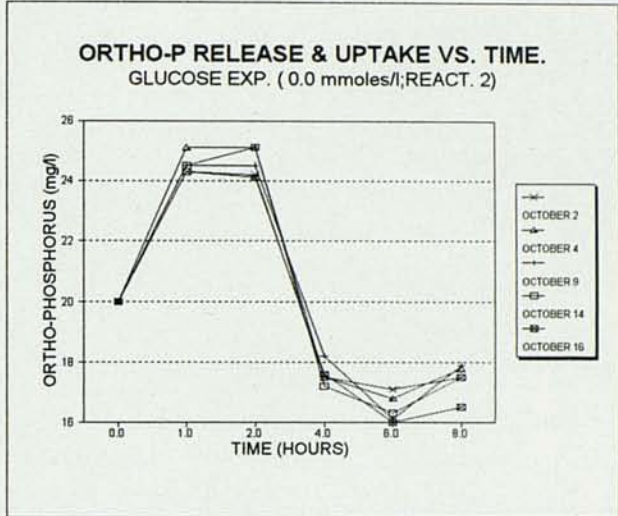
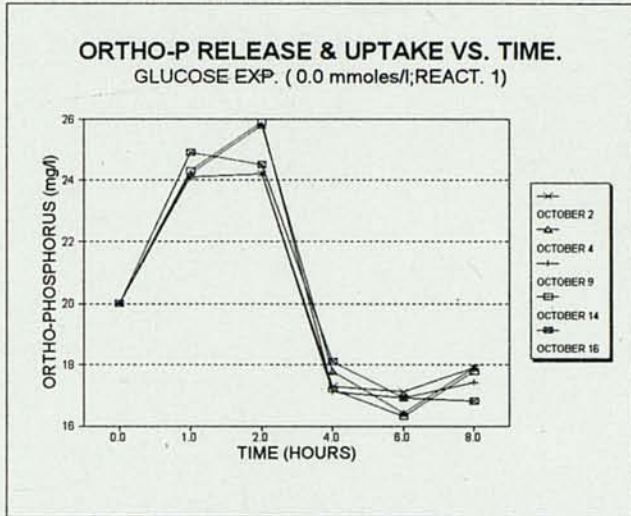
CONCENTRATION OF THE SUBSTRATE IN mmol/L	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.3	25.9	17.2	16.3	17.8
0.0	2	20.0	24.5	25.1	17.2	16.3	17.5
0.2	3	20.0	25.3	26.8	16.5	13.4	17.6
0.4	4	20.0	26.4	27.3	16.1	12.3	16.8
0.6	5	20.0	27.1	27.8	14.3	11.5	16.4
0.8	6	20.0	29.4	29.7	13.7	11.1	13.9

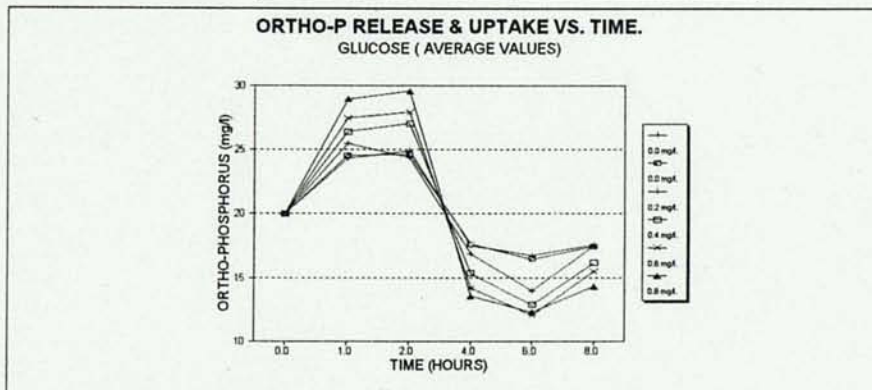
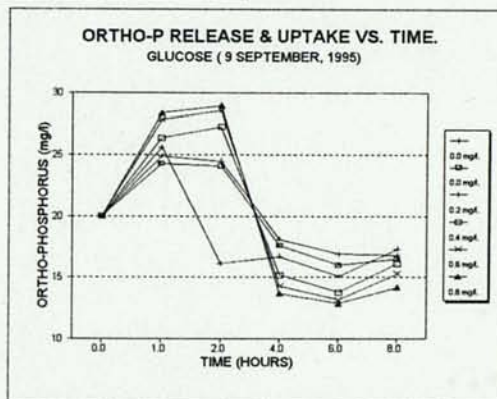
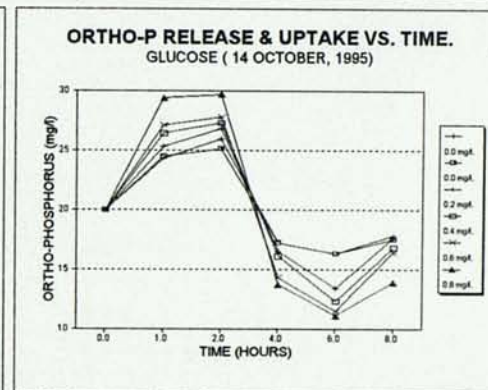
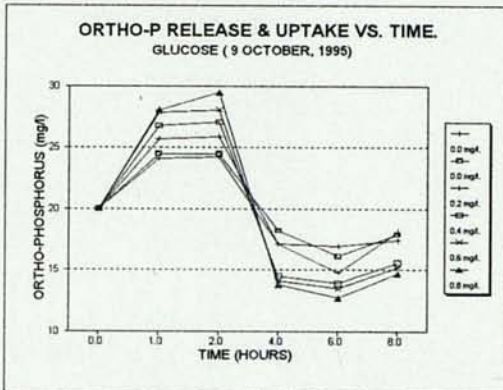
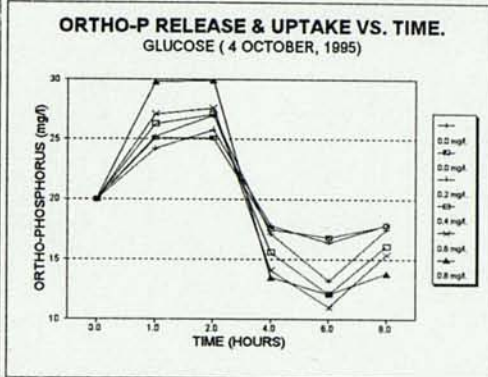
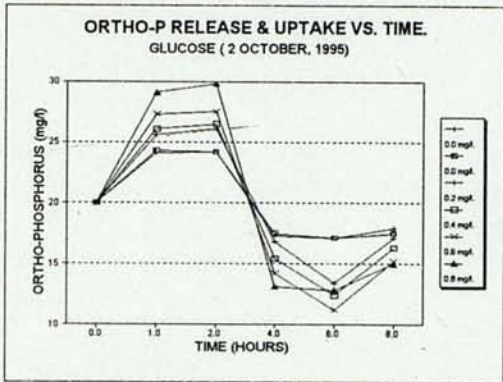
THIS EXPERIMENT WAS PERFORMED IN OCTOBER 16, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/L	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.9	24.5	18.1	16.9	16.8
0.0	2	20.0	24.3	24.1	17.6	16.0	16.5
0.2	3	20.0	25.6	16.1	16.7	15.1	17.3
0.4	4	20.0	26.4	27.3	15.2	13.8	16.1
0.6	5	20.0	27.9	28.6	14.3	13.2	15.3
0.8	6	20.0	28.4	29.0	13.7	12.9	14.2

THIS TABLE REPRESENTS THE AVERAGE VALUES FOR ALL THE BATCH EXPERIMENTS DONE ON THE SUBSTRATE GLUCOSE. THESE VALUES WILL BE THE DATA TO BE USED FOR WRITING THE THESIS.

CONCENTRATION OF THE SUBSTRATE IN mmol/L	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.3	24.9	17.5	16.7	17.6
0.0	2	20.0	24.5	24.6	17.6	16.5	17.4
0.2	3	20.0	25.5	24.4	16.8	14.0	17.5
0.4	4	20.0	26.4	27.1	15.4	12.9	16.2
0.6	5	20.0	27.5	27.9	14.2	12.1	15.5
0.8	6	20.0	29.0	29.6	13.6	12.3	14.3





ACETIC ACID EXPERIMENTS WERE PERFORMED USING THE BIOMASS OF SBR 1. FOUR EXPERIMENTS WERE PERFORMED ON THIS SUBSTRATE OF MOLECULAR FORMULA = C₂H₄O₂ AND MOLECULAR WEIGHT OF 60 g/mole. THE VOLUME PER REACTOR WAS 485 ml.

THIS EXPERIMENT WAS PERFORMED ON AUGUST 12, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.5	24.6	17.1	16.8	17.9
0.0	2	20.0	24.5	24.5	17.3	16.7	17.9
0.2	3	20.0	32.3	32.7	13.2	12.2	15.2
0.4	4	20.0	34.5	34.9	12.1	11.1	14.8
0.6	5	20.0	36.9	37.8	11.1	10.2	13.2
0.8	6	20.0	37.9	38.6	9.81	9.11	10.8

THIS EXPERIMENT WAS PERFORMED ON AUGUST 14, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.1	24.2	16.9	15.9	16.9
0.0	2	20.0	24.1	24.3	16.9	15.9	16.8
0.2	3	20.0	33.5	33.9	13.1	12.8	14.5
0.4	4	20.0	36.4	36.8	11.9	11.2	13.6
0.6	5	20.0	37.8	38.5	10.9	10.1	12.3
0.8	6	20.0	39.1	40.1	9.15	9.11	10.0

THIS EXPERIMENT WAS PERFORMED ON SEPTEMBER 9, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.2	24.2	17.1	16.0	16.8
0.0	2	20.0	24.2	24.3	17.1	16.0	16.8
0.2	3	20.0	33.2	34.1	13.5	12.6	14.5
0.4	4	20.0	36.2	36.9	12.2	11.1	13.9
0.6	5	20.0	37.1	38.8	11.3	10.3	12.8
0.8	6	20.0	38.9	40.2	10.1	9.21	10.5

THIS TABLE REPRESENTS THE AVERAGE VALUES FOR ALL THE BATCH EXPERIMENTS DONE ON THE SUBSTRATE ACETIC ACID. THESE VALUES WILL BE THE DATA USED IN WRITING THE THESIS.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.4	24.6	17.1	16.3	17.3
0.0	2	20.0	24.3	26.3	17.2	16.4	17.4
0.2	3	20.0	32.6	33.2	13.4	12.4	14.6
0.4	4	20.0	35.1	35.7	12.3	11.2	13.9
0.6	5	20.0	36.9	37.9	11.3	10.4	12.8
0.8	6	20.0	38.0	39.3	10.0	9.39	10.9

THIS EXPERIMENT WAS PERFORMED ON AUGUST 17, 1995.

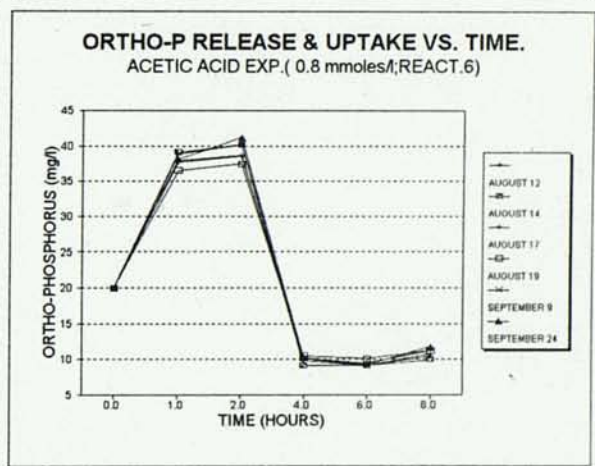
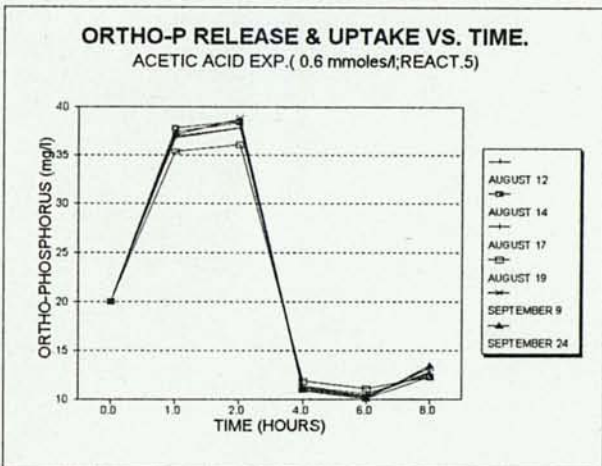
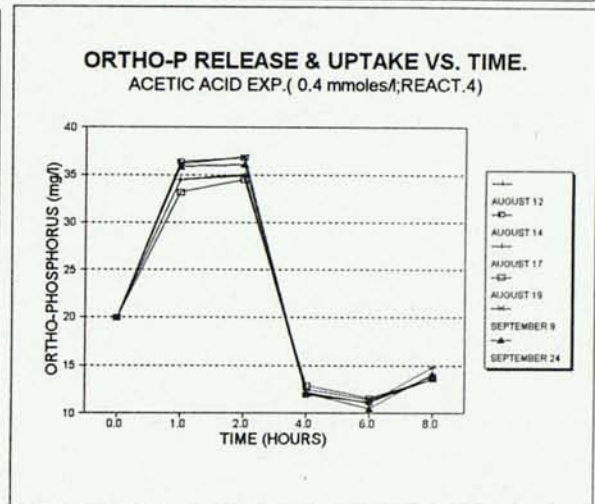
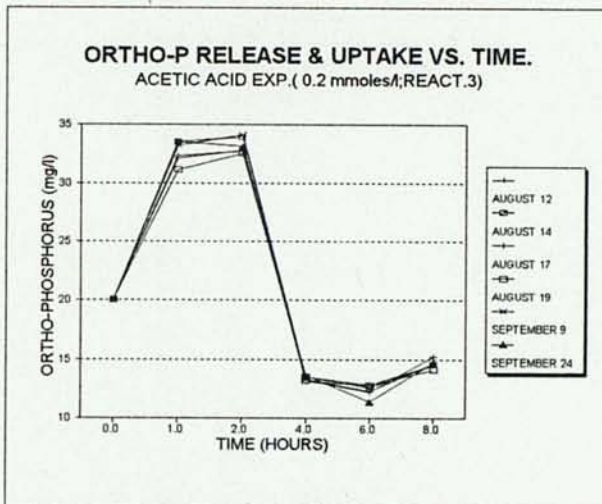
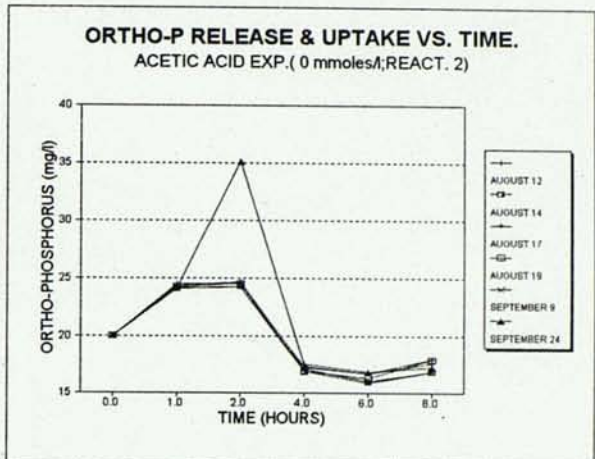
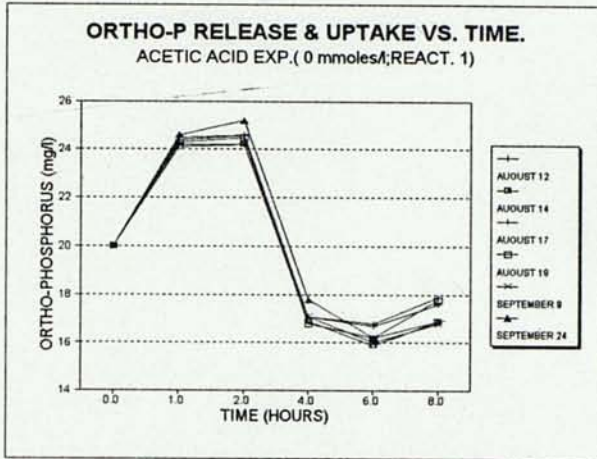
CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1.0	20.0	24.4	24.6	17.1	16.7	17.6
0.0	2.0	20.0	24.4	24.7	17.2	16.7	17.6
0.2	3.0	20.0	32.1	32.8	13.3	12.3	14.6
0.4	4.0	20.0	34.5	35.1	12.5	11.4	13.6
0.6	5.0	20.0	36.8	37.8	11.4	10.6	12.7
0.8	6.0	20.0	37.6	38.5	10.1	9.55	11.2

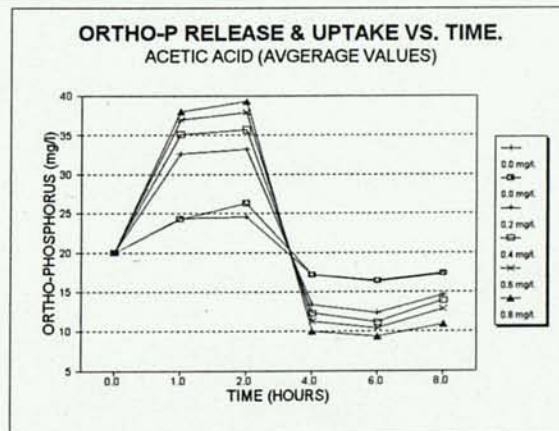
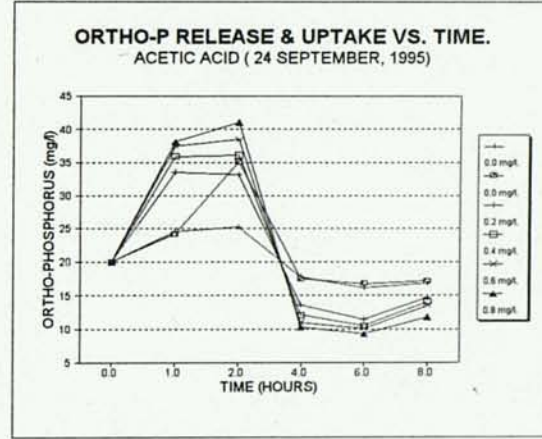
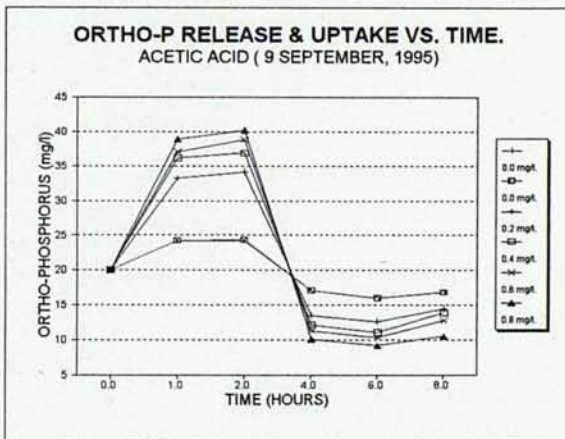
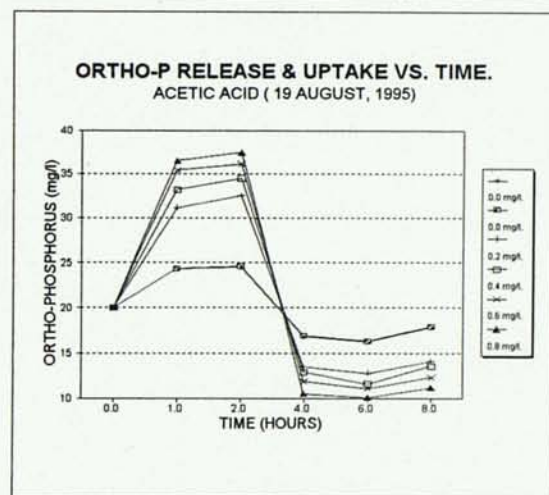
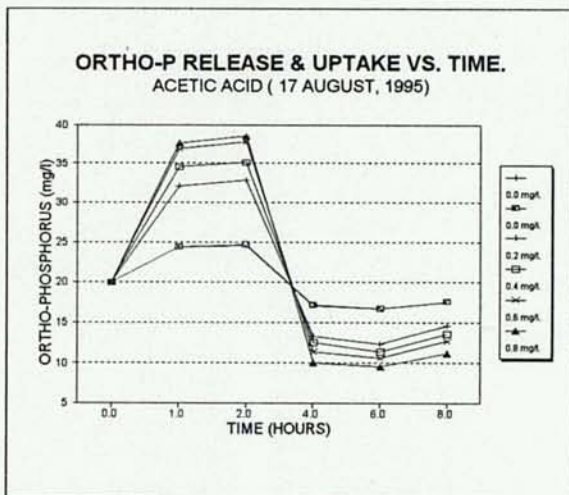
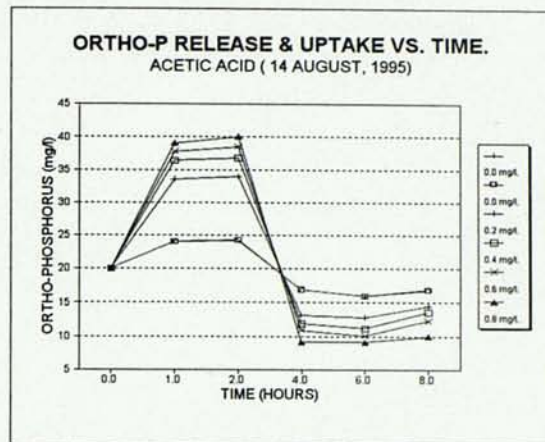
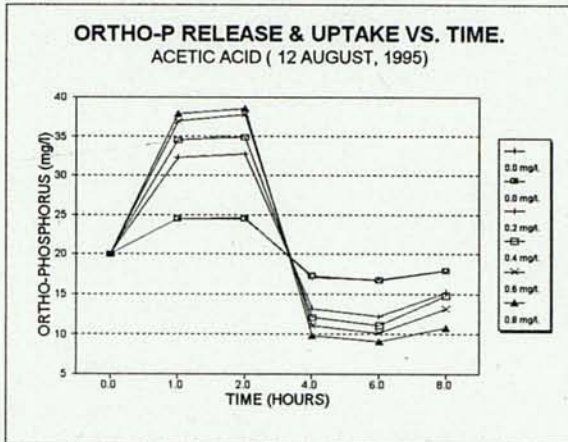
THIS EXPERIMENT WAS PERFORMED ON AUGUST 19, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1.0	20.0	24.3	24.5	16.8	16.2	17.8
0.0	2.0	20.0	24.3	24.6	16.9	16.3	17.9
0.2	3.0	20.0	31.1	32.5	13.5	12.8	14.1
0.4	4.0	20.0	33.2	34.5	12.9	11.6	13.6
0.6	5.0	20.0	35.4	36.1	11.9	11.1	12.3
0.8	6.0	20.0	36.5	37.4	10.5	10.1	11.2

THIS EXPERIMENT WAS PERFORMED ON SEPTEMBER 24, 1995.

CONCENTRATION OF THE SUBSTRATE IN mmol/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1.0	20.0	24.6	25.2	17.8	16.2	16.9
0.0	2.0	20.0	24.2	24.3	17.5	16.8	17.2
0.2	3.0	20.0	33.5	33.1	13.6	11.4	14.7
0.4	4.0	20.0	35.9	36.1	12.1	10.5	14.1
0.6	5.0	20.0	37.4	38.4	11.0	10.1	13.5
0.8	6.0	20.0	38.1	41.1	10.3	9.23	11.8





BATCH EXPERIMENTS COMPARAISON BETWEEN ALL THE SUBSTRATES
USING AVERAGE VALUES FOR ALL EXPERIMENTS PERFORMED.

PROPIONIC ACID AVERAGE BATCH VALUES

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	23.3	23.9	17.1	16.3	18.1
0.0	2	20.0	23.3	23.9	17.1	16.2	18.1
0.2	3	20.0	24.1	24.7	16.5	15.8	17.1
0.4	4	20.0	26.6	27.3	15.9	15.3	17.4
0.6	5	20.0	29.1	29.1	14.9	14.1	16.4
0.8	6	20.0	30.6	31.5	14.2	13.3	15.7

ISOVALERIC ACID AVERAGE BATCH VALUES

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1.0	20.0	24.3	25.0	19.9	16.1	17.4
0.0	2.0	20.0	24.3	24.9	16.9	16.2	17.4
0.2	3.0	20.0	34.8	35.9	12.2	11.5	13.0
0.4	4.0	20.0	35.9	37.4	10.7	10.2	12.5
0.6	5.0	20.0	37.8	39.3	9.92	9.37	11.2
0.8	6.0	20.0	39.7	40.9	8.46	8.13	9.82

GLUCOSE BATCH AVERAGE BATCH VALUES

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.3	24.9	17.5	16.7	17.6
0.0	2	20.0	24.5	24.6	17.6	16.5	17.4
0.2	3	20.0	25.5	24.4	16.8	14.0	17.5
0.4	4	20.0	26.4	27.1	15.4	12.9	16.2
0.6	5	20.0	27.5	27.9	14.2	12.1	15.5
0.8	6	20.0	29.0	29.6	13.6	12.3	14.3

VALERIC ACID AVERAGE BATCH VALUES

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1.0	20.0	24.5	24.4	16.7	16.3	17.3
0.0	2.0	20.0	24.4	24.6	16.6	16.5	17.4
0.2	3.0	20.0	31.0	33.0	13.9	12.7	15.0
0.4	4.0	20.0	33.6	34.5	13.1	11.8	13.9
0.6	5.0	20.0	35.3	35.8	11.9	10.9	13.0
0.8	6.0	20.0	36.3	36.9	10.7	10.2	11.5

ACETIC ACID AVERAGE BATCH VALUES

CONCENTRATION OF THE SUBSTRATE IN mmoles/l.	REACTOR	TIME (HOURS)					
		0.0	1.0	2.0	4.0	6.0	8.0
0.0	1	20.0	24.4	24.6	17.1	16.3	17.3
0.0	2	20.0	24.3	26.3	17.2	16.4	17.4
0.2	3	20.0	32.6	33.2	13.4	12.4	14.6
0.4	4	20.0	35.1	35.7	12.3	11.2	13.9
0.6	5	20.0	36.9	37.9	11.3	10.4	12.8
0.8	6	20.0	38.0	39.3	10.0	9.39	10.9

COMPARAISON OF RELEASE AND UPTAKE FOR ALL SUBSTRATES USING AVERAGE BATCH VALUES.
REACTOR 1 - 0.0 mmoles/l

SUBSTRATE	P-REL. 0-2 Hrs mg/l	P-UPT. 2-6 Hrs mg/l	NET UPT. mg/l	UPT/REL RATIO
PROPIONIC ACID	3.85	7.57	3.72	1.97
ISOVALERIC ACID	5.02	8.89	3.87	1.77
GLUCOSE	4.90	8.20	3.30	1.67
VALERIC ACID	4.35	8.08	3.73	1.86
ACETIC ACID	4.60	8.30	3.70	1.80

COMPARAISON OF RELEASE AND UPTAKE FOR ALL SUBSTRATES USING AVERAGE BATCH VALUES.
REACTOR 2 - 0.0 mmoles/l

SUBSTRATE	P-REL. 0-2 Hrs mg/l	P-UPT. 2-6 Hrs mg/l	NET UPT. mg/l	UPT/REL RATIO
PROPIONIC ACID	3.85	7.62	3.77	1.98
ISOVALERIC ACID	4.87	8.67	3.80	1.78
GLUCOSE	4.60	8.10	3.50	1.76
VALERIC ACID	4.63	8.18	3.55	1.77
ACETIC ACID	6.30	9.90	3.60	1.57

COMPARAISON OF RELEASE AND UPTAKE FOR ALL SUBSTRATES USING AVERAGE BATCH VALUES.
REACTOR 3 - 0.20 mmoles/l

SUBSTRATE	P-REL. 0-2 Hrs mg/l	P-UPT. 2-6 Hrs mg/l	NET UPT. mg/l	UPT/REL RATIO
PROPIONIC ACID	4.65	8.85	4.20	1.90
ISOVALERIC ACID	15.9	24.4	8.50	1.53
GLUCOSE	4.40	10.4	6.00	2.36
VALERIC ACID	13.0	20.3	7.33	1.56
ACETIC ACID	13.2	20.8	7.60	1.58

COMPARAISON OF RELEASE AND UPTAKE FOR ALL SUBSTRATES USING AVERAGE BATCH VALUES.
REACTOR 4 - 0.40 mmoles/l

SUBSTRATE	P-REL. 0-2 Hrs mg/l	P-UPT. 2-6 Hrs mg/l	NET UPT. mg/l	UPT/REL RATIO
PROPIONIC ACID	7.32	12.1	4.75	1.65
ISOVALERIC ACID	17.4	27.3	9.84	1.56
GLUCOSE	7.10	14.2	7.10	2.00
VALERIC ACID	14.5	22.8	8.25	1.57
ACETIC ACID	15.7	24.5	8.80	1.56

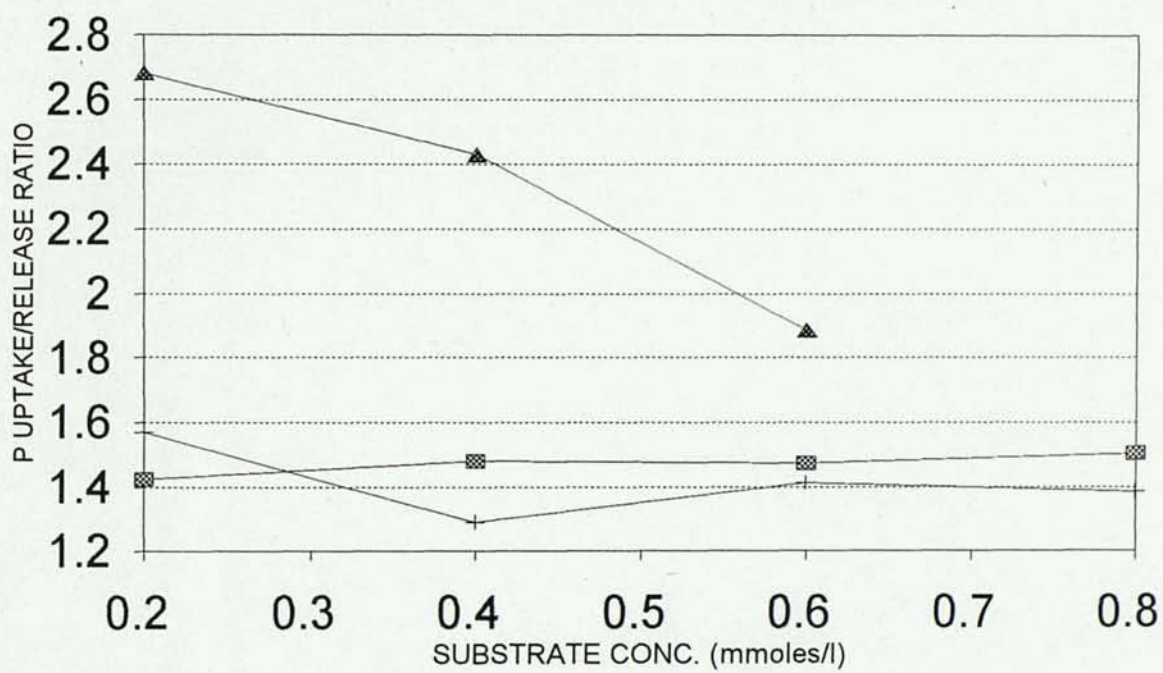
COMPARAISON OF RELEASE AND UPTAKE FOR ALL SUBSTRATES USING AVERAGE BATCH VALUES.
REACTOR 5 - 0.60 mmoles/l

SUBSTRATE	P-REL. 0-2 Hrs mg/l	P-UPT. 2-6 Hrs mg/l	NET UPT. mg/l	UPT/REL RATIO
PROPIONIC ACID	9.13	15.1	5.93	1.65
ISOVALERIC ACID	19.3	29.9	10.6	1.55
GLUCOSE	7.90	15.8	7.90	2.00
VALERIC ACID	15.8	24.9	9.08	1.57
ACETIC ACID	17.9	27.5	9.60	1.54

COMPARAISON OF RELEASE AND UPTAKE FOR ALL SUBSTRATES USING AVERAGE BATCH VALUES.
REACTOR 6 - 0.80 mmoles/l

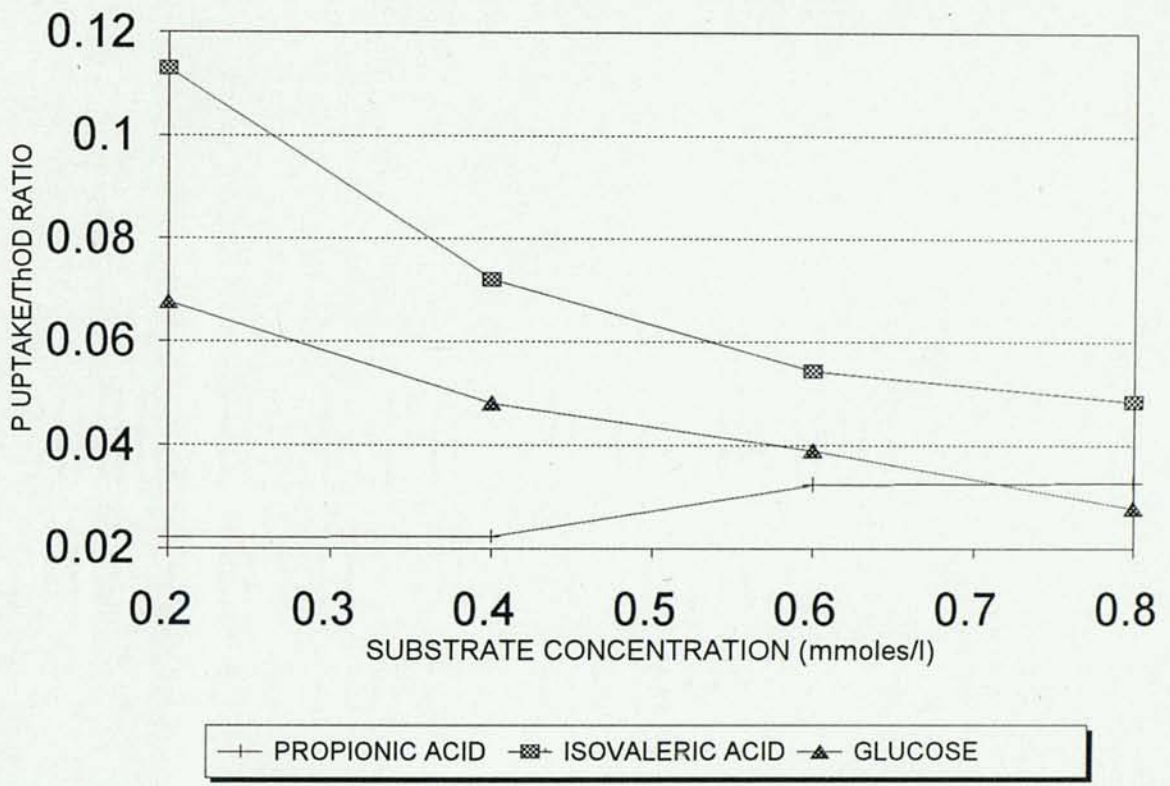
SUBSTRATE	P-REL. 0-2 Hrs mg/l	P-UPT. 2-6 Hrs mg/l	NET UPT. mg/l	UPT/REL RATIO
PROPIONIC ACID	11.5	18.2	6.68	1.58
ISOVALERIC ACID	20.9	32.7	11.9	1.57
GLUCOSE	9.60	17.3	7.70	1.80
VALERIC ACID	16.9	26.7	9.83	1.58
ACETIC ACID	19.3	29.9	10.6	1.55

P UPTAKE/RELEASE RATIO BATCH EXPERIMENTS

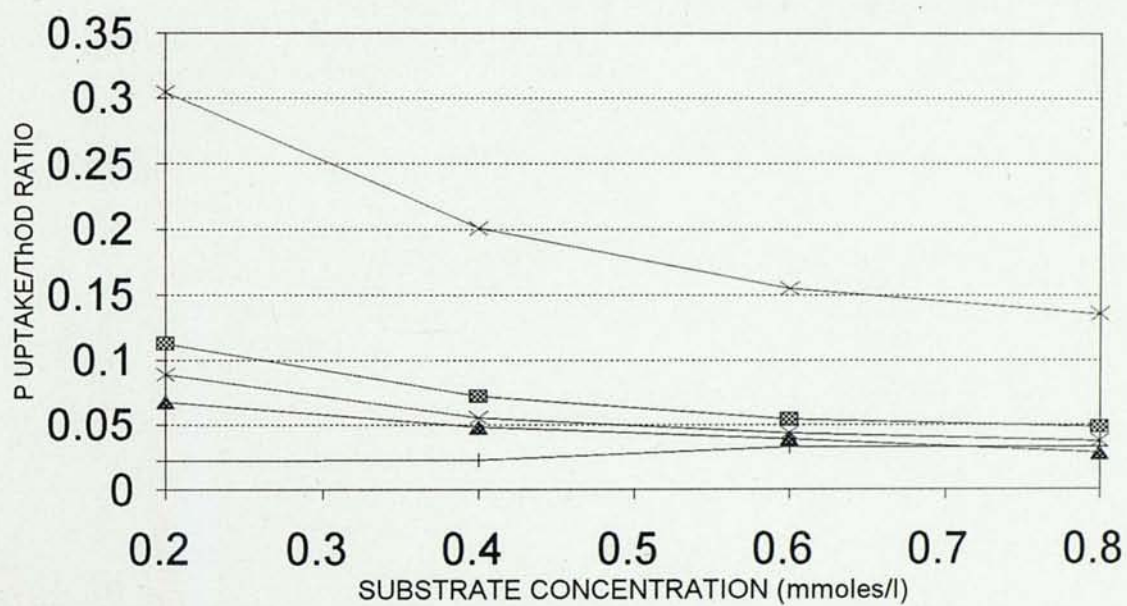


+ PROPIONIC x ISOVALERIC ▲ GLUCOSE

NET P UPTAKE/ThOD BATCH EXPERIMENTS

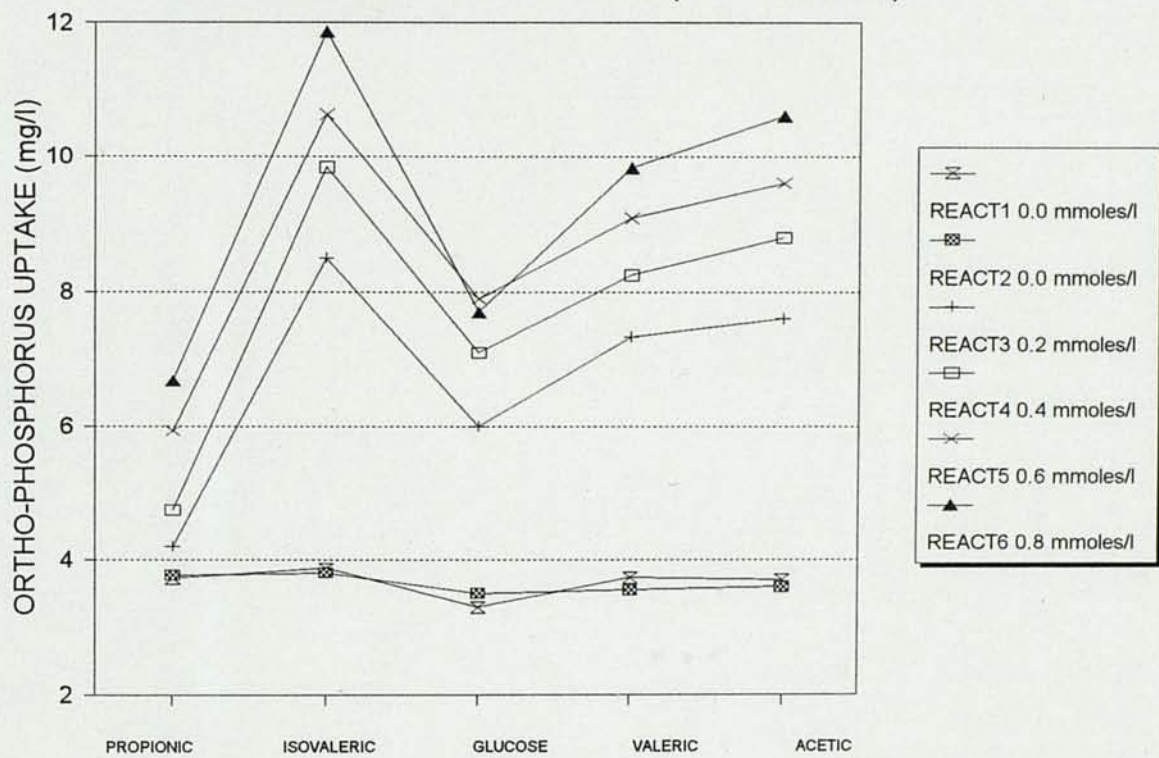


NET P UPTAKE/ThOD BATCH EXPERIMENTS



+ PROPIONIC ACID □ ISOVALERIC ACID ▲ GLUCOSE
x VALERIC ACID x ACETIC ACID

NET ORTHO-P UPTAKE FOR ALL SUBSTRATES GLUCOSE SBR BIOMASS (AVG VALUES)



Appendix D

Sample Calculations for Percentage of P in the Mixed Liquors

The following calculations are done for Glucose SBR, and average values are used to perform these calculations:

Operational Parameters:

OP = Ortho-P

SP = Soluble P

TP = Total P

MLVSS = 990 mg/l

Reactor volume = 2.9 liters

Total P in the influent = 20 mg/l

SP at end of aeration phase = 12.1 mg/l

SP in the effluent = 12.8 mg/l

Q of waste activated sludge = $Q_{was} = 0.46$ l/day

Feed wasted = 0.56 l/day

Effluent VSS = 8.13 mg/l

MLVSS/MLSS = 0.74

Calculations:

A. MCRT calculations:

$$X = (\text{MLVSS}) * (\text{reactor volume}) = 990 * 2.9 = 2871 \text{ mg}$$

$$\text{Feed treated per cycle} = 2.5 - 0.56 = 1.94 \text{ l/cycle}$$

$$Q_{\text{influent}} = (3 \text{ cycles/day}) * 1.94 \text{ l/cycle} = 5.82 \text{ l/day}$$

$$Q_{\text{effluent}} = Q_{\text{inf.}} - Q_{\text{was}} = 5.82 \text{ l/day} - 0.46 \text{ l/day} = 5.36 \text{ l/day}$$

$$X_{\text{was}} = Q_{\text{was}} * \text{MLVSS} = 0.46 \text{ l/day} * 990 \text{ mg/l} = 455 \text{ mg/day}$$

$$X_{\text{effluent}} = \text{Effluent VSS} * Q_{\text{effluent}} = 8.13 \text{ mg/l} * 5.36 \text{ l/day} = 44 \text{ mg/day}$$

$$\text{Total } X_{\text{was}} = X_{\text{effluent}} + X_{\text{was}} = 455 + 44 = 499 \text{ mg/day}$$

$$\text{MCRT} = (X) / (\text{Total } X_{\text{was}}) = 2871 / 499 = 5.8 \text{ days}$$

B. Percentage P in mixed liquors:

Assuming a steady state situation, we can write the following mass balance:

$$TP_{influent} = TP_{was} + TP_{effluent}$$

a- $TP_{influent} = (Volume)_{in.} * (P)_{in.} = 20 \text{ mg/l} * 5.82 \text{ l/day} = 116 \text{ mg/day}$

b- $TP_{was} = (OP_{was} * Q_{was}) + (PP_{was} * Q_{was})$

and $PP_{was} = \%P * MLSS$

where $MLSS = MLVSS / 0.74 = (455 \text{ mg/day}) / 0.74 = 615 \text{ mg/day}$

So $TP_{was} = (12.1 \text{ mg/l} * 0.46 \text{ l/day}) + (\%P * 615 \text{ mg/day})$

$$= 5.6 \text{ mg/day} + [(615 \text{ mg/d}) * \%P]$$

c- $TP_{effluent} = (OP_{eff.} * Q_{eff.}) + (\%P * TS_{Seff.} * Q_{eff.})$

$$= (12.8 \text{ mg/l} * 5.36 \text{ l/day}) + (\%P * 44 \text{ mg/day})$$

Finally, $a = b + c$

$$116 \text{ mg/day} = 5.6 \text{ mg/day} + (615 \text{ mg/day} * \%P) + 68.6 \text{ mg/day} + (\%P * 44 \text{ mg/day})$$

Solving for % P, we find it to be = 6.3 %

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