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Biological Phosphorus Removal Using a Fixed Film Batch Reactor

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Abstract

Phosphorus, the limiting nutrient in eutrophication, usually has been removed from wastewater by precipitation with iron or aluminum salts. Interest has grown in biological phosphorus removal, a poorly understood behavior deemed "luxury uptake." Full scale systems now operate worldwide, employing modified versions of the activated sludge process. Periods of anaerobiosis preceeding aeration act as a selective stress for those organisms capable of storing phosphorus in greater than stoichiometric amounts.

Fixed film systems offer advantages over slurry systems at the expense of mass transfer limitations. Sequencing batch reactors have been used successfully in nutrient removal systems, but never with fixed films. This report explores the feasibility of a fixed film batch reactor in biological phosphorus removal.

This study was conducted at the University of Massachusetts during 1985 - 1986. A bench scale batch reactor filled with plastic attachment media was seeded and run on synthetic wastewater to promote biological phosphorus removal. Biological phosphorus removal was evident under certain conditions but the effects of mass transfer resistance pointed out the impracticality of fixed

films for this purpose.

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CHAPTER I

INTRODUCTION

Phosphorus has been identified as the limiting nutrient in eutrophication of most freshwater receiving waters.[1, 31] Historically, .point sources have been targeted to reduce phosphorus loading of these waters by imposition of local phosphate detergent bans, thus lowering input to treatment plants, and strict plant specific effluent limits.[17,31] Treatment plant operators have met these limits by coagulant addition and precipitation of phosphate rich sludges. As costs of chemical addition and sludge handling have increased, alternative phosphorus removal strategies have been sought.

Biological phosphorus removal by wastewater treatment plant microorganisms in excess of stoichiometric amounts has been noted since the 1950's.[3] Serious research into the phenomenon began in the 1960's. Proprietary processes using some modification of the suspended growth activated sludge process now exist full scale around the world.[3,32]

Fundamental understanding of biological phosphorus removal has not progressed past the theoretical stage. Incorporation of phosphorus into or adsorption of phosphorus onto cell mass in greater than stoichiometric amounts does occur. Environmental stresses created by cycling biological solids through periods of strict anaerobiosis followed by aerobiosis are critical to the induction and perpetuation of the phenomenon. Loading rates, chemical oxygen demand (COD) to phosphorus ratios, and nitrites, nitrates and soluble substrates in the fermentative zone may also affect performance. However, effluent phosphorus limits of 1 mg/L by purely biological removal have yet to be reliably attained.[17,32]

Research has focused on slurry systems. Modifications to the accepted technology of secondary wastewater treatment have been patented and marketed worldwide.[32] Three such processes -- the A/O, Bardenpho, and Phostrip systems -- are represented by full scale plants in the United States.[32] Of these three, the A/O and Bardenpho are mainstream systems, removing phosphorus by sludge wasting.[1,5] The Phostrip process is a sidestream system. Return activated sludge is subjected to conditions that cause massive phosphorus release.[1] This small volume phosphorus rich supernatant is dosed with chemicals to precipitate a cationic - phosphate sludge which is then

separately handled. Economic analysis based on influent characteristics, effluent limits, and retrofitting costs dictate the decision to use any one of these systems over another or any one over conventional chemical treatment on a case by case basis.[1,5,17,31,32]

Another approach to suspended growth biological phosphorus removal has been pioneered by researchers at the University of Notre Dame.[23] The sequencing batch reactor (SBR) is an all in one treatment unit that trades space, inherent in the large basins of conventional treatment trains, for time. A quantity or batch of wastewater is subjected sequentially in time within the same physical space (the reactor) to the anaerobic-aerobic conditions found in conventional biological phosphorus removal schemes. A full scale plant now operating in the midwest has produced effluent consistenly meeting standards.[18] This technology is especially promising for time variant and low loading conditions.

The use of fixed films for wastewater treatment is an old technology now experiencing a revival. From land application, to trickling filters, to the modern permutations of rotating biological contactors, fluidized bed reactors and upflow anaerobic sludge blankets, the idea of fixing microorganisms to a surface and passing

wastewater by and through them has made sense. The pumping of solids is minimized. Longer solids residence times are realized. Resistance to shock loadings, toxicants and washout are achieved, as well as the promise for treatment of some otherwise recalcitrant materials.

Yet, the benefits associated with fixed films do not come without cost. It is unclear whether all organisms attach equally well. Resistance to mass transport inherent in the mixed community and fixed geometry of films can also serve to limit treatment, as films are characterized by gradients of concentration.

The purpose of this study has been to determine the ability of a fixed film to perform biological phosphorus removal. Combining the microbiological and biochemical theory gleaned from the literature with the flexibility inherent in the sequencing batch reactor, a prototype bench scale system was constructed. A fixed film of organisms was grown in the system to reach steady state. Anaerobic and aerobic conditions were cycled sequentially in order to stress the biofilm and select for those phosphorus accumulating organisms. The system was monitored over time to measure phosphorus in the bulk liquid.

CHAPTER II

BACKGROUND

Phosphorus in Wastewater

In normal biological wastewater treatment phosphorus is used in both metabolic and catabolic paths. DNA, RNA, and ATP are some important phosphorus bearing molecules. The high energy bonds formed by phosphorus are critical for energy storage and transfer.

Phosphorus content of normal cell mass, however, is low. COD to phosphorus ratios of 100 to 1 have been suggested by many sources, leading to a cell phosphorus content on a dry weight basis of 2 to 3 percent.[1,6,31] Stoichiometrically, then, phosphorus uptake in a normal activated sludge plant with influent chemical oxygen demand of 200 to 300 mg/L and 85 % removal is limited to the 1.7 to 2.5 mg/L range. With typical influent phosphorus loadings of 5 to 10 mg/L, effluent phosphorus greater than 4 mg/L is common.[1,32]

Early Research in Biological Phosphorus Removal

Levin and Shapiro noted far greater uptake in a laboratory study and coined the term "luxury uptake."[19] Their work focused on the pathways of substrate utilization, especially the Krebs cycle, as the data showed phosphorus uptake to be some function of aeration. Using batch samples of conditioned sludge (already displaying excess uptake behavior) they noted aerobic phosphorus release when the culture entered the endogenous phase of growth and concluded that phosphorus uptake and storage were associated with the active mass of organisms. Batches of sludge with additional inputs of COD were compared with the endogenous phase samples and phosphorus uptake was seen to be enhanced. Levin and Shapiro attributed this to a need for energy during uptake.

By varying oxygen tension and adding specific inhibitors Levin and Shapiro tested the hypothesis that oxidative phosphorylation was the paramount pathway responsible for the luxury uptake of phosphorus. They found a plateau affect for oxygen -- after a minimum value

of dissolved oxygen [DO] was reached no enhanced effect was seen. Addition of 2,4 - dinitrophenol, a known inhibitor of oxidative phosphorylation, stopped all trace of phosphorus uptake behavior.

Shapiro, Levin and Zea noted sudden massive phosphorus release to bulk liquid under anaerobic conditions.[29] They postulated that low DO or low redox potential triggered this release. Using batch tests they time tracked soluble phosphorus release and subsequent uptake upon reaeration. They noted that under normal conditions in a secondary clarifier where solids might be expected to experience anaerobic conditions, excess stored phosphorus would be released back into solution thereby negating any engineering benefit.

Early Theories of Biological Phosphorus Removal

Harold, a microbiologist, examined P storage in a broad range of microorganisms.[14] Identifying the storage and release pathways (the enzymes poly-P Kinase and polyphosphotase), noting metachromatic granules within cells, and pinpointing two stress phenomena leading to this

behavior (luxury uptake and overplus), Harold envisioned poly-P as a "metabolic fossil," a pre-ATP energy and/or storage mechanism. Harold concluded that the behavior was motivated by the need for P in cell building, contrary to the theory that later evolved, but was the first to face the question from the organism's viewpoint.

Menar and Jenkins argued against a strict biological phenomenon, asserting instead that some biologically mediated chemical precipitation was at work.[25] During the course of aeration carbon dioxide produced by substrate utilization would be driven off, raising the pH at or near cell surfaces. This higher pH would favor precipitation of calcium phosphate complexes.

Ferguson and McCarty [11] and Ferguson, Jenkins, and Eastman [10] presented supporting data for this theory based on examination of calcium phosphate precipitates at slightly alkaline pH values.

Arvin argued that the calcium phosphate equilibria in a wastewater stream when combined with the kinetics of the denitrification reaction in a biofilm explained the excess uptake of phosphorus.[1] Again elevated pH due to a biological action was seen as the operative variable.

Carberry and Tenney disputed the gist of this argument

by performance of solubility calculations.[6] They performed additional poisoning tests (with 2,4 - dinitrophenol) and radioactively labelled phosphorus tests to confirm their hypothesis that the phenomenon was biological, arguing that a mechanism was at work that actively transported phosphorus across the cell membrane against a concentration gradient.

Lan, Benefield and Randall tried to quantify the extent of calcium phosphate precipitation as well as purely biological excess uptake in a low calcium wastewater.[18] They found both mechanisms at work, with precipitation accounting for 15 to 27 % of total phosphorus removal, the rest being biological. Of the biological removal at most 30 % was attributed to normal uptake. Thus greater than half of the total phosphorus removal was credited to some uptake and storage mechanism.

Fuhs and Chen approached the problem from an ecological perspective.[12] Examining sludges from excess P uptake plants they found up to 30% of cell mass to be Acinetobacter, an obligate aerobe with a slow growth rate. This finding has been confirmed by numerous researchers.[1,2,4,27] Lotter and Murphy found this organism dominant in the aerobic zone of Bardenpho plants in South Africa.[21]

Fuhs and Chen postulated that the presence of this organism was due to an anaerobic stress followed by a period of aerobiosis and that this stress condition selected for these organisms. Using spike inputs of acetate they determined that the stress served to provide a fermentative zone for the breakdown of higher volatile fatty acids (VFA) and accumulation of lower VFAs, compounds not normally formed by strict aerobic consortia. Their experiments were also noteworthy for their initial failures to duplicate P uptake and release behavior without first obtaining samples of conditioned cultures: specific organisms had to be present at the outset of controlled (often batch) studies.

Malnou *et al.*, using batch experiments, found a slight lag in COD consumption during anaerobiosis consistent with acidogenesis, but then could discern no lower VFAs in solution.[22] They concluded that the production of lower VFAs, then, was the rate limiting step: once produced they were immediately sequestered by the organisms.

Barnard, working with full scale nitrifying - denitrifying plants in South Africa, noticed excess uptake of phosphorus as well.[2] Undertaking a review of the data worldwide to formulate a working hypothesis, Barnard affirmed the direction pointed to by the work of Fuhs and

Chen.[3] A strict anaerobic period during which P was released triggered excess uptake in a subsequent aerobic phase. Attempting to construct a model of this behavior, Barnard sought, as had Levin and Shapiro, a measure of the stress intensity. Anaerobic detention times were increased in full scale plants to good effect, but creation of surrogate parameters linked to nitrates proved misleading.

Biochemical Theory of Biological Phosphorus Removal

Barnard's co-workers in South Africa -- Dold, EKama, Seibritz, von Handel, and Marais -- enlarged upon this idea of a measure of the process by focusing on readily biodegradable COD in the anaerobic phase.[9,24,30] This served to move the research forward from the applied approach towards a theory of the microbiological behavior of the now characterized mixed culture of facultative and obligate aerobic (the poly P storers) organisms.

Why the presence of lower VFAs in the anaerobic zone promoted survival and growth of obligate aerobic phosphorus storers was unclear. The low VFA substrate was not available for use in the substrate level phosphorylation

pathway and in fact was an end product that might inhibit. Oxidative level phosphorylation in an anaerobic zone was a contradiction in terms. Yet all work had shown spike inputs of COD during anaerobiosis to enhance P release and subsequent uptake.

Why slow growing phosphorus storing organisms multiplied to a significant fraction of cell mass in an aerobic process also remained unanswered. Kinetics clearly argued against slow growers outcompeting others for substrate. Obviously, then, both the ability to survive anaerobiosis and thrive because of it upon reaeration escaped theoretical explanation.

Nichols and Osborne examined the survival mechanism of strict aerobes in an anaerobic zone.[27] They postulated two mechanisms by which organisms with a stored poly-P pool might benefit when exposed to anaerobic conditions. Recognizing Harold's work, they proposed an energy advantage to such storage. The high energy bonds of poly-P broken during anaerobiosis might furnish energy for cell maintenance during this period when normal oxidative phosphorylation was quelled. They also proposed a pathway leading to storage of substrate in the form of poly-hydroxy butyrate (PHB) during this anaerobiosis, contrary to the sense of strict aerobic organisms. This storage pathway

utilized poly-P-liberated energy to take the built up end products of the Embden Meyerhoff pathway (EM) -- acetate and reduced NAD -- to PHB and and oxidized NAD, thus regenerating the low yielding yet reliable capacity of the EM path.

The PHB served then as a hydrogen sink. This neatly tied observations of PHB storage to an important mechanism, the regeneration of NAD. Implementation of these findings in the field led to positive results. Addition of a pre-digested acid fraction of substrate to the influent of the anaerobic zone caused increased P release and improved P uptake upon subsequent reaeration.[24]

Rensink et al. took this idea another step.[28] The PHB, they theorized, served as an exclusive energy resource for the poly-P storing organisms once aerobiosis resumed. This was congruent with Fuhs and Chen's work as well. Experiments to show acetate removal during anaerobiosis were performed carefully, measuring both filtered and unfiltered samples from continuous stirred tank reactors [CSTRs] in series. Enhanced P release and uptake followed the anaerobic disappearance of acetate. In contrast to Levin and Shapiro's finding, Rensink provided the conclusive link between active anaerobic acetate (electron donor) consumption and proliferation.

Marais *et al.* went forward from this work to present a biochemical model of poly-P organism behavior during anaerobiosis in the presense of readily biodegradable COD.[24] They proposed three cases: (1) acetate present; (2) glucose or some other higher order VFA present but unavailable for use as a substrate by the poly-P organisms; and (3) glucose present and able to be used.

The first two cases are similar. In case 1 acetate is taken into the cell and converted to a storage product, such as acetoacetate or PHB, at the expense of ATP formed by the breakdown of poly-P. P, here termed "Pi" to denote its stored poly-P source, is released from the cell to solution. The equations:

$$2ADP + 2Pi \Longrightarrow 2ATP$$

$$2Acetate + 4ATP + 2\ Co\text{-}A \Longrightarrow 2\ Acetyl\ Co\text{-}A + 4\ ADP + 4Pi$$

$$2AcetylCo\text{-}A + 2\ ADP + 2\ Pi \Longrightarrow acetoacetate + 2ATP + 2Co\text{-}A$$

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yielding: 2 Acetate ==> acetoacetate

at the expense of 2 poly P radicals

In case 2 the effect of a consortium of organisms is postulated. Facultative organisms degrade glucose to

acetate, gaining 2 ATP. The acetate is then sequestered by the pathway above to stored acetoacetate by the poly-P organsims.

Case 3 is more complex. Glucose is broken down to pyruvate by both facultative and poly-P organisms. Acetate is produced by the facultative organisms, regenerating NAD (ox), yielding 2 ATP, and freeing the EM path to repeat the process (a classic fermentation). The poly-P organisms, lacking this ability, employ a "fossil" pathway to store pyruvate as PHB and regenerate NAD (ox) that once again employs the mechanism of case 1. Thus the presence of a mixed culture or acetate or both is mandated. The reactions are envisioned as follows.

Glucose + 2ADP + 2NADox ==> 2Pyruvate + 2ATP
+2NADred

2Pyruvate + 2NADox +2Co-A ==> 2AcetylCo-A
+ 2NADred +2CO2

2AcetylCo-A ==> 1 Acetoacetate + 2Co-A

Acetoacetate + 1NADred ==> 1PHB + 1NADox

Glucose +2ADP +3NADox ==> PHB +2ATP + 3NADred +2CO2

Obviously NADox must be regenerated. From the consortium 6 acetate molecules are taken into the cell:

6Acetate +12ATP +6Co-A ==> 6AcetylCo-A +12ADP
+ 12Pi

6AcetylCo-A + 6ADP + 6Pi ==> 3 Acetoacetate + 6ATP
+ 6Co-A

3Acetoacetate + 3NADred ==> 3PHB + 3NADox

6Acetate + 6ATP + 3NADred ==> 3PHB + 6ADP + 6Pi
+ 3NADox

The overall reaction:

6Acetate + Glucose + 4ATP ==> 4ADP + 4PHB + 6Pi
+ 2C02

occurs at the expense of 4ATP, but 12 poly-P radicals are used and 6 are released into solution.

This can make sense only in terms of energy realized by the organism during normal oxidative degradation of the stored product. For example, taking the stored product acetoacetate through the Krebs cycle:

Acetoacetate + 4O2 + 8NADred + 22ADP + 22P ==>
4CO2 + 8H2O + 8NADox + 22ATP

Since each acetoacetate is formed from 2 acetate, 11 ATP are realized from each acetate. In case 1 above one acetate was stored at the expense of one poly-P radical.

Therefore a gain in energy equivalent to 10 ATP per acetate stored is the advantage confered to the slow growing poly-P organism to allow it to compete.

Marais' biochemical model, then, codifies a number of concepts or parameters that have been identified by researchers worldwide. Anaerobic stress helps select for those organisms that can not only survive but thrive and profit by that stress at the expense of its competitors. But, a mixed culture must be present. Soluble substrate must be present in a strict fermentative zone. These conditions combine to promote an alleged evolutionary or "fossil" pathway for substrate utilization via sequestration and subsequent degradation, thus yielding an advantage to those organisms capable of storing phosphorus.

Fixed Films

Films are consortia of microorganisms attached to a surface. Explanation of the factors affecting and selecting attachment are not within the scope of this study and may be found elsewhere.[7] Suffice it to say that

systems employing fixed films benefit from this behavior in two general ways.[7,13,15] Long solids residence times are possible. As opposed to collecting and pumping solids, film systems hold solids and pump liquid, saving energy as well. Secondly, films possess a mixed community and fixed geometry. This makeup allows for mixed microbial attack on non-homogeneous substrates and provides a resistance to toxicants. And films provide protection against washout during hydraulic surges.

This resistance however is not without cost. Films offer resistance to the transfer of mass.[13] Unlike slurry systems where bulk homogeneity can reasonably be assumed, films are characterized by gradients of concentration. The fluxes of oxygen, carbon, nitrogen, and phosphorus to and from both the bulk liquid and the biofilm then can greatly influence performance.

Fundamental reactor engineering principles state that overall rates of reaction (from input to output) are a function not only of internal stoichiometry and kinetics but of the flow regime imposed on the process. Thus first order reactions in a plug flow reactor yield a different output than first order reactions in a CSTR. But films also possess thickness, density and variable composition, made up of different species of organisms in different patterns,

interspersed with inert material and exocelluar polymer. Thus the complexity of the overall reactions taking place in a reactor with a fixed film must be illuminated by focusing on three distinct topics: external mass transfer resistance; internal mass transfer resistance; and internal biochemical kinetics.

Various models of fixed films have been proposed, some empirical relating input to output on a gross basis, and some mechanistic, attempting to quantify relationships between all the variables so as best to predict performance. All suffer from lack of knowledge of some fundamental things. Incompletely this list includes film densities and thicknesses, boundary layer thicknesses, diffusion coefficients through non-aqueous matter, double substrate limiting conditions, endogenous growth rates, and mixed culture effects.[13]

From Lewis and Whitman's work on gas diffusion [20] to the present, a simple model has emerged to portray the influence of external mass transfer resistance. In Figure 1.1 a biofilm of thickness L_f is attached to an inert surface. Next to it exists a stagnant liquid layer of thickness L_w. Next to this stagnant liquid layer is the moving bulk liquid, having in it some homogeneous

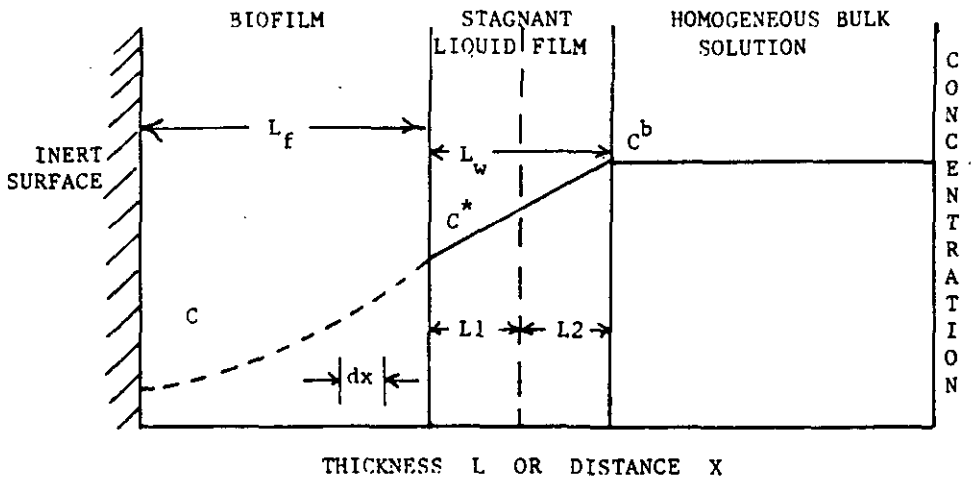


FIGURE 1.1 : Simple fixed film model (after Grady (13)).

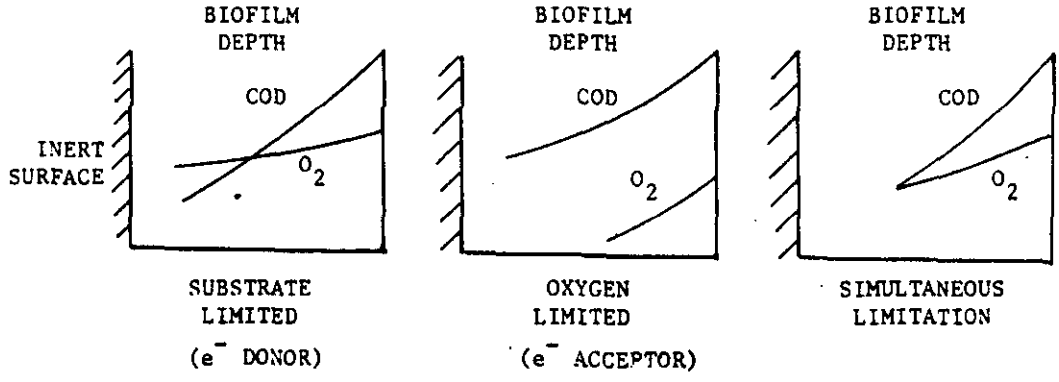


FIGURE 1.2 : Simple mass transfer limitation models in idealized biofilms.

concentration of mass, C^b. All external resistance to the flow of mass from the bulk liquid to the biofilm is contained within the stagnant layer, L_w. The concentration of the mass in question at the biofilm surface is given by C^*. The mass transported across the stagnant layer per unit area per unit time is given by the Fick - Einstein relationship

$$N = D/L_w \, [C^b - C^*] \qquad (1)$$

where N is the flux and D the diffusivity of the mass in question in water.

The flux then is a function of the driving force across some finite distance of stagnant liquid layer. As D is peculiar to the molecule being transported and C^b is determined from influent conditions, an increase in flux is dependent upon decrease in this layer L_w. Williamson and McCarty proposed that this layer is actually composed of two layers such that

$$L_w = L1 + L2 \qquad (2)$$

where L1 is some outer layer dependent on the turbulence of the flow.[33] L1 then can be physically shrunk by increasing flow rate. L2 was thought to be some

irreversible boundary layer, impervious to manipulation.

The idea of flux is also paramount in internal mass transfer resistance. With reference to Figure 1.1 again it is seen that the concentration of mass in question also varies accross the thickness of the biofilm, L_f, from the surface value of C^* to some value C. The resistance to the flow of this mass can also be described by a flux:

$$N_f = D_f \, dC/dx \qquad (3)$$

where the f subscript refers to the film. It is important to note that D_f is not equal to D, i.e. that diffusion through the variable density and composition of a biofilm (a gelatinous matrix) is not the same as diffusion through water. It is also of note that the differential form of the relationship holds here. The depth of the biofilm is an unknown. The depth of the active mass of the biofilm is also unknown. Reduction of the depth of the biofilm, as in the external case, might serve to lessen transfer problems, but in this case might limit overall treatment, as the substrate utilized is a function of the mass of organisms present.

Overcoming internal mass transfer resistance then falls upon increasing the driving force, dC. In turn, this means

an increase in bulk concentration of the mass in question. If that mass is the electron acceptor oxygen then the process becomes expensive, depending upon the mechanical or chemical introduction of a poorly soluble substance.

Internal mass transfer resistance also contributes to limiting the active depth of the biofilm. At some point the concentration of the mass in question falls below a critical value and the microorganisms dependent on that mass for electron donor, electron acceptor or nutrients cease to grow. This leads to endogenous decay. Film begins to slough from the surface and overall reactor performance is diminished. Control over this depth is not available to most reactor configurations. Thus position of the film within the reactor can lead to overgrowth and undergrowth conditions depending on the availability of donor, acceptor or both. Figure 1.2 illustrates this point. Near the influent end of a PFR, bulk concentration would provide more driving force for mass transfer. Plugging, restriction of fluid flow due to overgrowth, is a common problem.[7]

Reactions within a biofilm are governed by potentially limiting fluxes of mass. This limitation may be one of electron donor or electron acceptor or of both. Reaction rates are most often described by Monod kinetics which,

simply put, relate specific growth rate -- or the change in biomass per unit time per unit biomass already present -- to the concentration of some limiting substrate and to some intrinsic affinity of the organism for the substrate. Similarly the specific substrate removal rate is some function of these potentially limited concentrations.

Thus, a biological reaction within a biofilm might also be limited by the kinetics particular to the organism and the substrate. Given the necessity of hydraulic throughput within a reasonable space and time, effective treatment by any biological process, let alone a fixed film of microorganisms, may not be feasible. As films contribute to greater solids residence time within a reactor their potential to offset this intrinsic limitation retains promise.

Proprietary Processes

The Phostrip System

Levin pioneered the Phostrip system for phosphorus removal based upon the release of excess stored phosphorus exposed to anaerobiosis. Either all or a portion of the return activated sludge of a secondary treatment plant is subjected to a phosphorus stripping tank. Simply, this tank exposes the return sludge to anaerobic conditions. Stored phosphorus is released to solution, elutriated and then dosed with a precipitant, usually lime. As the liquid volume to be chemically dosed is small and rich in phosphates, the coagulant dosage is minimal in comparison with chemical treatment of the entire hydraulic throughput. This makes sense when it is realized that coagulant dosage is not a function of phosphate concentration but rather one of alkalinity: calcium must be added in excess to satisfy bicarbonate demand.[1]

The return sludge, now phosphorus poor, is returned to the head end of the aerobic basin, presumably ready to uptake phosphorus once more. The system is inherently

flexible and can be adapted to any flow regime, solids residence time, and aeration rate. But, problems have arisen with the phosphate stripper tank, the crucial part of the system. Anaerobic detention times in the stripper to achieve release must be empirically determined.

The Bardenpho and A/O Systems

The Bardenpho process grew out of the work of James Barnard in South Africa. The A/O process was pioneered by the Air Products & Chemicals Co. Both are mainstream nutrient removal activated sludge processes that rely upon an anaerobic detention basin preceding subsequent anoxic (for denitrification) and oxic basins. Strict control of return activated sludge to the various basins in the treatment train is required. Phosphorus is removed from the bulk liquid by wasting that sludge , now high in phosphorus, subjected to the anaerobic - aerobic sequence. Sludge phosphorus content of 6% on a dry weight basis enhances the value of the sludge as a potential fertilizer.

Sequencing Batch Reactors

Manning and Irvine presented evidence of sequencing batch bench scale reactors operated in various protocols to select for and induce biological phosphorus removal.[23] Their conclusions stated that strict anaerobiosis in the presense of excess substrate hastened phosphorus release and when followed by aerobiosis allowed phosphate accumulating organisms to proliferate. Non ideal operating strategies did not immediately terminate the ability of the reactor to remove phosphorus but casued a gradual decay in performance.

Manning and Irvine used a five part protocol for their sequencing batch reactor: fill, react, settle, draw and idle. Completion of one sequence constituted a cycle. In the "fill" period wastewater entered the reactor, raising the liquid level inside the reactor from a set minimum value to some set maximum. This set minimum liquid level in the reactor prior to wastewater addition contained the active slurry mass of organisms.

The "fill" period could be controlled for time, mixing and aeration and substrate concentration. When the

predetermined volume in the reactor was reached inflow was shunted to another reactor whose "fill" cycle would then commence. This control of conditions during "fill" allowed the authors to create selective pressure for organisms.

During the "react" period further inflow to the reactor ceased. Mixing and aeration were controlled. Following "react" all mixing and aeration ceased allowing the suspended organisms to "settle," as in a clarifier. When sufficient settling had occurred the clear supernatant was drawn ("draw") and the reactor was left "idle" with a concentrated slurry of organisms in the minimum volume left in the reactor. Solids residence time was controlled by wasting a set amount of this concentrated slurry on a daily basis.

The authors employed various strategies to test the known hypotheses promulgated for biological phosphorus removal. These ideas are best represented by the gross parameters of DO, COD concentration, and nitrate concentration. In the author's words: "In particular, a control strategy must be selected which, at a minimum, eliminates oxidized nitrogen and DO during the fill period and allows for aeration during react."[23]

CHAPTER III

MATERIALS AND METHODS

Experimental Overview

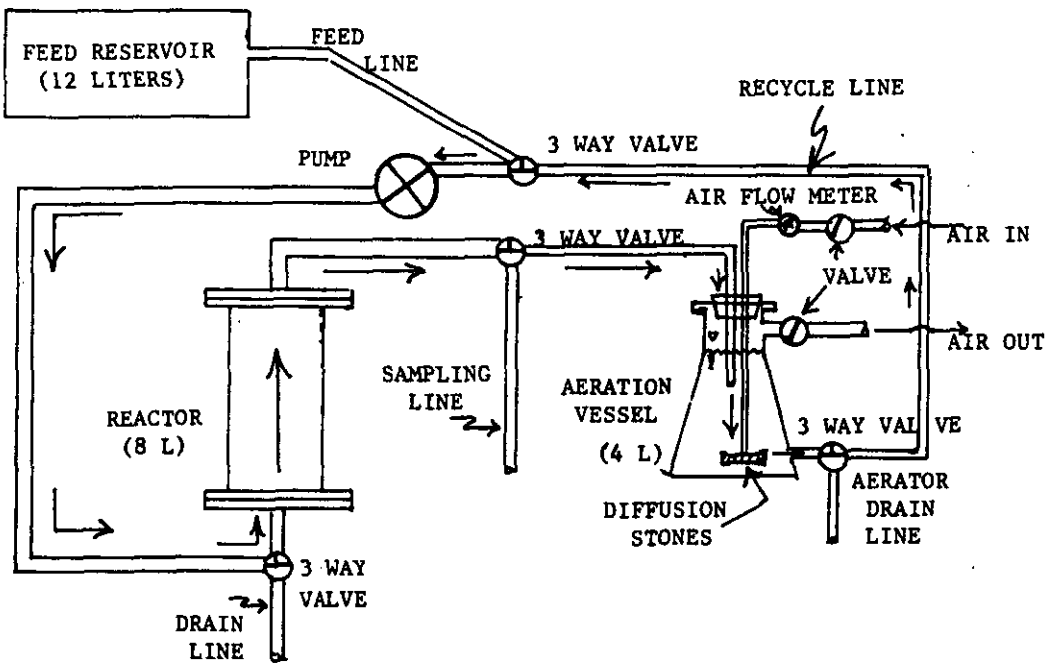
The main research goal of this study was to demonstrate phosphorus removal in greater than stoichiometric amounts required for growth and maintenance by a biofilm. Attention, therefore, was paid to those operational parameters that affected that ability. In particualr utilization of carbon and oxygen by the consortium of organisms was explored. Focus was also directed to the influence of biofilm behavior on performance in relation to similar research performed using a slurry system.

The experiment was conducted in an upflow columnar reactor filled with plastic attachment media. The reactor was run in batch mode -- sequential fill, react and draw periods -- to simplify operation and interpretation of

results. A schematic of this flow pattern and of the reactor set-up is pictured in Figure 2. Effluent from the top of the column was recirculated, during the react period, through an aeration vessel, back to the reactor influent at the bottom. On a fixed schedule during this react period, air was vigorously bubbled into the aeration vessel to transfer oxygen into the system. During anaerobic segments of the react period this air flow was halted and the aeration vessel closed to the atmosphere to insure anoxic or anaerobic conditions. Recirculation was maintained at a constant rate during react, irrespective of gas flow.

At fixed intervals the column was drained and refilled with synthetic wastewater. This wastewater contained soluble carbon, nitrogen, and phosphorus. The phosphorus was in excess of stoichiometric requirements. Trace elements and growth factors were present in the make-up water to avert growth limiting conditions.

A sample port between the reactor effluent and aeration vessel allowed monitoring of the bulk liquid. Samples were tested over time for dissolved oxygen (DO), soluble orthophosphate (PO_4-P), chemical oxygen demand (COD), and pH.



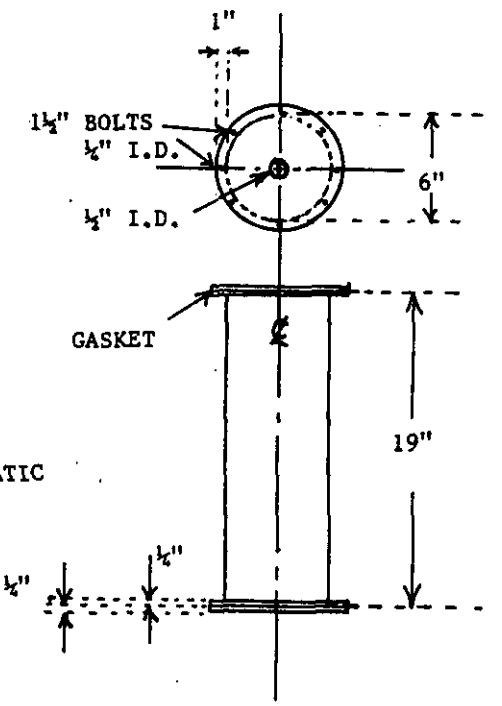


FIGURE 2: REACTOR SCHEMATIC AND FLOW PATH

Once biological phosphorus removal was exhibited, parameters were then varied to observe the sensitivity of the process. These included: carbon to phosphorus ratios in the feed; duration and intensity of the aerobic period; and duration of the anaerobic stress period.

Reactor Set-Up and Analytical Procedures

Experimental Apparatus

Upflow Batch Reactor. A right circular cylindrical reactor, 45 cm. in height and 15 cm. internal diameter, volume 8 liters, was constructed from plexiglas as seen Figure 2. Top and bottom plexiglas plates seated on neoprene gaskets were bolted to the reactor. A center hole was drilled in each plate and plastic screw fittings allowed coupling of top and bottom holes to vinyl tubing.

The reactor was filled with Glitsch Rings, proprietary plastic attachment media. The rings had a packing factor of 32. The specific surface area of the rings was 144 square meters / cubic meter with 91% free space.

Aeration Vessel. The aeration vessel was a glass flask equipped with sidearms at top and bottom. Liquid entered a glass tube threaded through a neoprene stopper in the top of the flask. Air entered another glass tube through the stopper and was diffused through a stone at the end of the tube at the bottom of the flask. Liquid left the vessel through the bottom sidearm. Gas escaped through the top sidearm which also served as an overflow port. The air entry tube and top sidearm were valved so as to prevent transfer of air during the anaerobic/anoxic phase. The capacity of the flask was 4 liters. The aeration rate was 3 liters per minute. Oxygen, when used in place of air, was applied at the rate of 1 liter per minute.

Flow Pattern. Flow was upward from the reactor bottom to the top through the Glitsch Rings. Liquid flowed out of the reactor through the hole in the top plate, entering a vinyl tube. Liquid in the tube was directed through an inline valve and into the glass tube leading down into the aeration vessel. Nominal hydraulic residence time of the aeration vessel was six minutes. During the aerobic phase gas diffusing into the liquid caused intense turbulence. Liquid exited the aeration vessel through the lower sidearm, entered vinyl tubing and flowed to the pump. Once through the pump the liquid was sent to the reactor influent hole in the bottom plate, completing the loop. The volumetric flowrate was 660 ml. per minute.

Pumps. A Masterflex peristaltic pump drive equipped with two heads, size 7018, ran the system.

Reactor Operation

Feed Composition. Twelve liters of feed were prepared daily. Dechlorinated tap water was mixed with sodium acetate, ammonium chloride, yeast extract, and potassium di-hydrogen phosphate in the amounts listed in Table 1. This corresponds to a COD to phosphorus ratio of 27.5 to 1 for the high strength feed, and 13.5 to 1 for the low strength feed. Phosphorus concentration in the bulk feed was 10 mg/L.

TABLE 1

FEED COMPOSITION

COMPONENT	HIGH STRENGTH	LOW STRENGTH
SODIUM ACETATE	3.80 g	1.90 g
YEAST EXTRACT	0.25 g	0.12 g
NH_4CL	0.86 g	0.43 g
KH_2PO_4	0.51 g	0.51 g

Anaerobic and Aerobic Protocols. Various protocols were followed searching for the right combination to encourage excess phosphorus uptake. The most successful one is shown below. Those unsuccessful protocols tried during the experimental period are explicated in Chapter 4 and are discussed in Chapter 5. Those protocols tried and abandoned prior to the start of the experimental period are discussed in the Appendix under "Other Strategies."

Under Protocol 2 the system operated on a single 24 hour cycle. At time 0+00 [hours + minutes] the pump was stopped and the reactor and aeration vessel were drained. At time 0+15 the pump was reconnected and directed feed through the reactor into the aeration vessel until both were filled, the valve on the aeration vessel lower sidearm being closed. At time 0+45 the pump was switched to normal mode, the liquid flow valves were opened and the liquid began to recirculate. The aerator remained closed to the surroundings and no gas was directed into it. The system was anaerobic.

At time 3+15 the closed valves preventing air flow were opened and gas was fed into the aerator. At hour 21+00 airflow was stopped and the valves were closed. At hour 24+00 the pumps were stopped. One cycle was completed.

Measurements and Methods

Chemical Oxygen Demand. COD was measured on 2 ml samples of the bulk liquid. The Closed Reflux Colorimetric Method was used [Standard Methods, 15, 1985: Section 508c]. Both soluble and total COD were measured, the difference being operationally defined by filtration through a 0.45 micron filter. The testing method employed a spectrophotometric determination at 600 nanometers of a change in oxidation state of chromium due to oxygen demanding material in the sample during acid digestion. The technique is a micro-variation of Knechtel's method.

Dissolved Oxygen. A micro-variation of the azide modification of the Winkler method was employed [Standard Methods 15, 1985: Section 421b]. Sixty milliliter BOD bottles were used to minimize system drawdown due to sampling.

pH. A Fisher Accumet pH Meter, Model 630, was employed.

Phosphorus. 0.45 micron filtered effluent was analyzed by the Heteropoly Blue - Ascorbic Acid Spectrophotometric Method at 885 nanometers [Standard Methods 15, 1985: Section 424f].

CHAPTER IV

EXPERIMENTAL RESULTS

Initial start-up and break-in problems were solved over the course of six months. Attention is directed to the Appendix Section "Other Strategies" for discussion of these attempts. Important concepts that emerged from this time period and that were subsequently incorporated into the experiments that followed include the addition of a second final anaerobic period after aerobiosis and the introduction of a COD spike into this second anaerobiosis.

Once the reactor was in place and running smoothly, achieving good COD removal with normal stoichiometric phosphorus uptake, six different strategies were employed. Each strategy consisted of a protocol -- a sequence of anaerobic and aerobic periods -- and a feed composition, either "high" with a COD of 275 mg/l or "low" with a COD of 135 mg/l. Nitrogen varied accordingly but phosphorus was constant at 10 mg/l (see Table 1). A comparison of the parts of the various strategies is made in Table 2.

TABLE 2

COMPARISON OF ALTERNATIVE STRATEGIES

STRATEGY	CYCLES PER DAY	FEED STRENGTH	PRIMARY ANAEROBIC	AEROBIC AIR	AEROBIC OXY	SECONDARY ANAEROBIC
1	2	HIGH	YES	Y	N	NO
2	1	LOW	YES	Y	N	YES
3	1	LOW	YES	Y	N	YES
4	1	LOW	YES	N	Y	NO
5	1	LOW	YES	N	Y	YES
6	1	HIGH	YES	N	Y	YES

Strategy 1 employed a high strength feed and consisted of 2 cycles per day. In cycle 1 a feed period of one half hour was followed by an initial anaerobic period of 2 and one half hours. An aerobic period of four and one half hours was next. At the end of this period the reactor was sampled. A final anaerobic period followed, duration of two and one half hours, during which the reactor was fed an additional input of COD after one half hour. At the end of this second anaerobic period the pumping was stopped and the reactor and aerator were drained. Cycle 2 commenced with introduction of new feed. Once filled, the reactor was run aerobically overnight. At the end of this period the reactor was drained and Cycle 1 was begun again. This

strategy was employed for 7 weeks. No excess biological phosphorus removal was in evidence. Results for this strategy are displayed in Figure 3.

Strategy 2, consisting of one cyle per day, employed a low strength feed. It was operated under a different protocol, called Protocol 2, described in detail in the preceding chapter. Briefly a one half hour feeding period was followed by an initial anaerobic period of two and one half hours. An aerobic period of eighteen and one half hours then occurred, at the end of which the reactor was sampled. A second, final anaerobic period followed during which a spike of COD was introduced one half hour into anaerobiosis. The reactor was then drained and the cycle was complete. This strategy was employed for five weeks. Phosphorus removal in greater than stoichiometric amounts was evident. Results of this strategy are displayed in Figure 4.

Strategy 3, was the same as Strategy 2 except that a high strength feed was used. Phosphorus removal in greater than stoichiometric amounts disappeared within one week. Results for this strategy are displayed contiguosly with results from S1 and S2 in Figure 5, as data points for weeks 12.5 and 13.0.

Strategy 2 was then repeated for a two week period.

FIGURE 3
EFFLUENT SOLUBLE PHOSPHORUS VERSUS TIME
STRATEGY 1

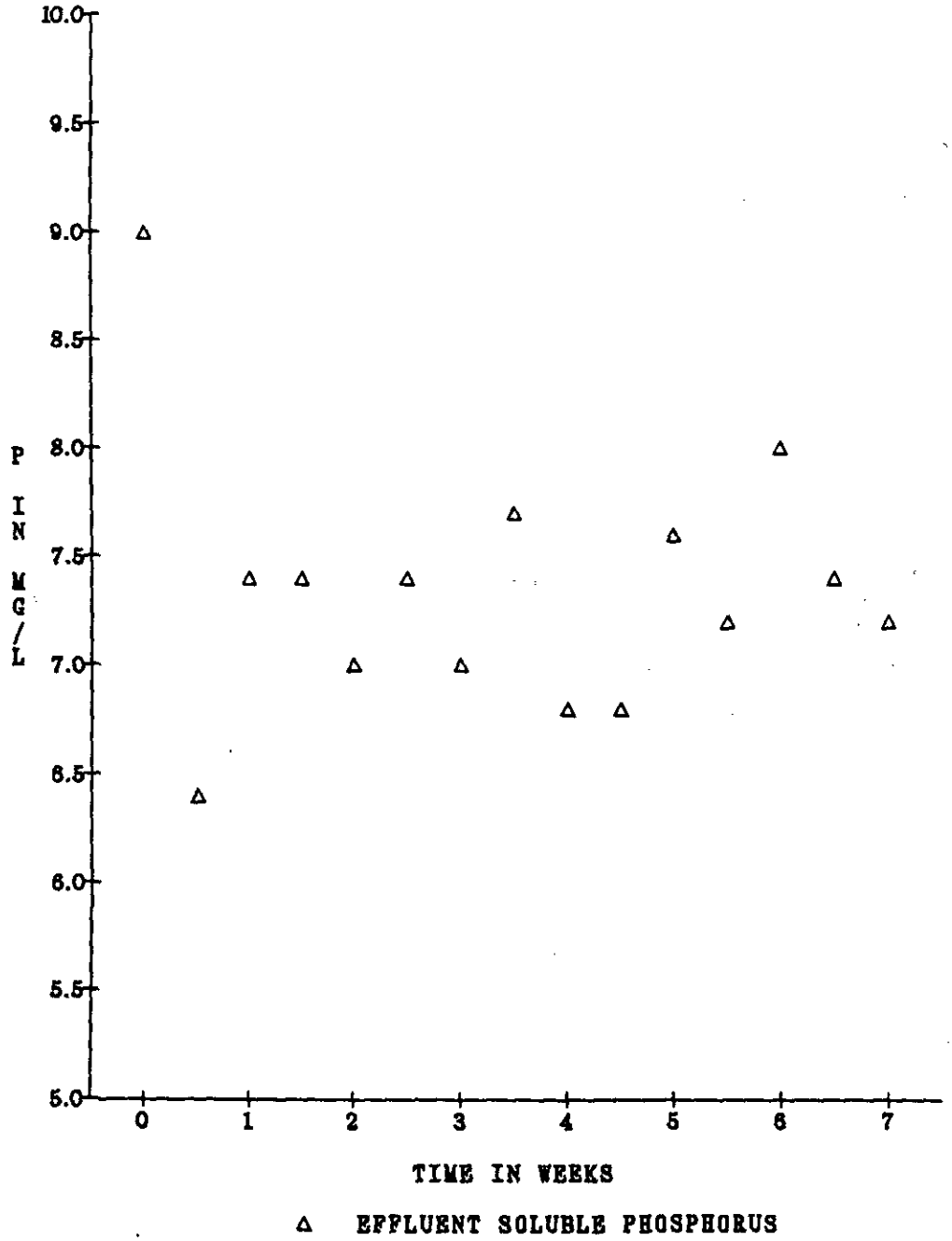


FIGURE 4
EFFLUENT SOLUBLE PHOSPHORUS VERSUS TIME
STRATEGY 2

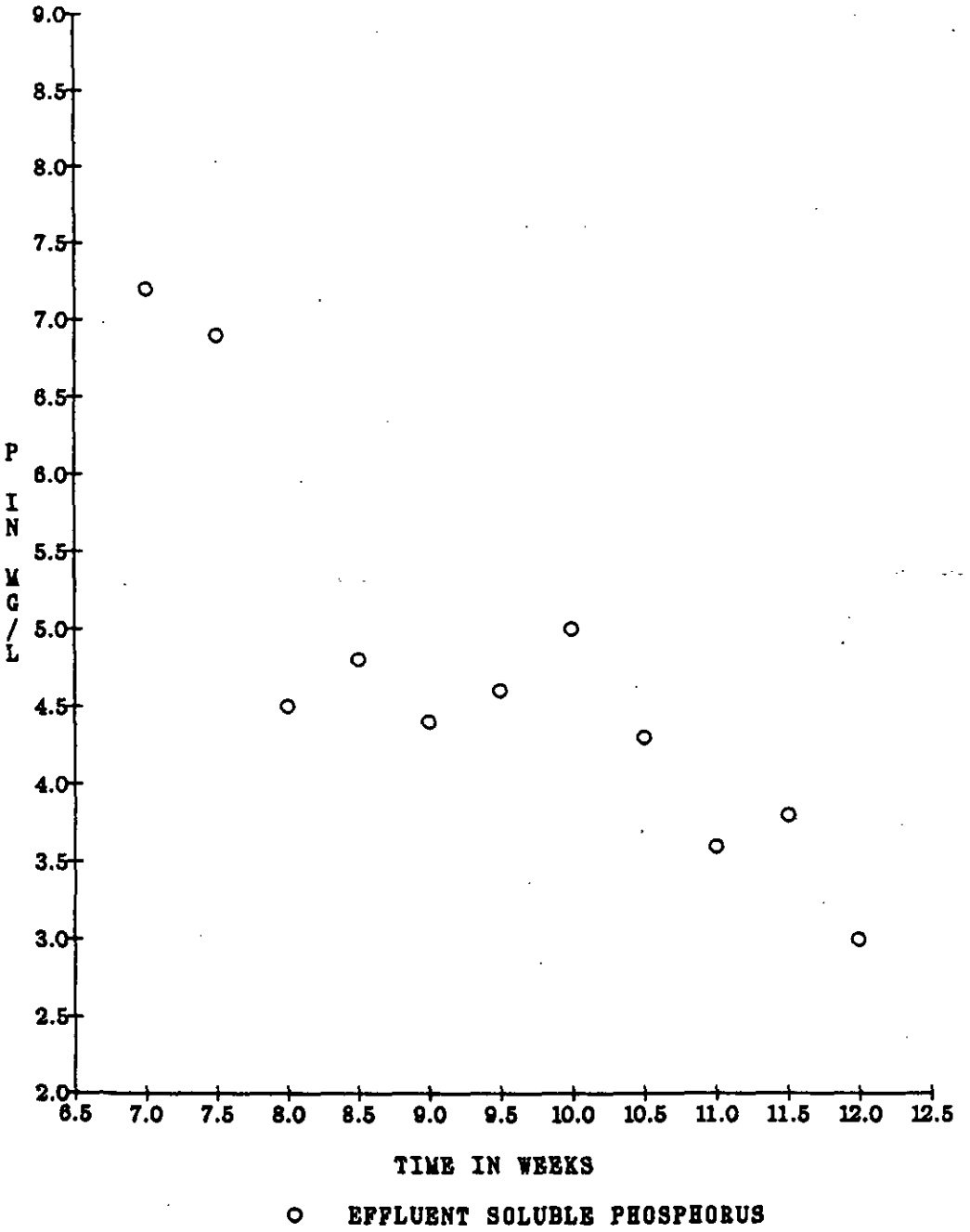
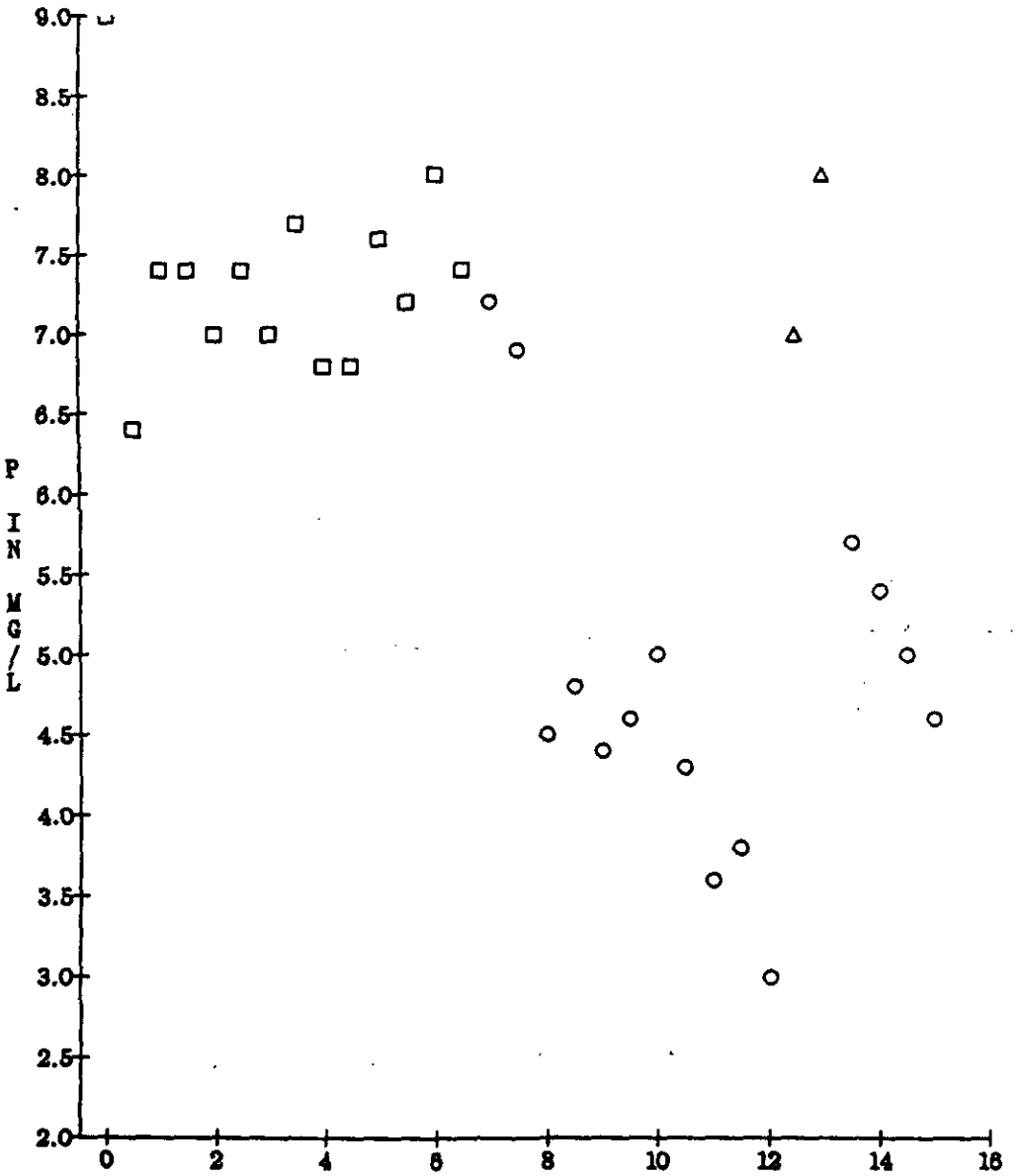


FIGURE 5
EFFLUENT SOLUBLE PHOSPHORUS VERSUS TIME



Excess biological phosphorus removal immediately recommenced. Results are also displayed in Figure 5, as data points for weeks 13.5 -- 15.0.

Strategy 4 differed from Strategy 2 in that pure oxygen was bubbled through the reactor during aerobiosis in place of plain air. No final anaerobic period was included in this strategy. Excess removal disappeared within one week. Strategy 5 was the same as strategy 4 except that a final anaerobic period with spike input of COD was again included. Excess phosphorus removal was restored within two weeks. Strategy 6 replaced only the low feed strength of Strategy 5 with a higher strength feed. Excess uptake of phosphorus was diminished but not eliminated and quickly began to recover. Results for Strategies 4 and 5-6 are displayed in Figures 6 and 7, respectively.

Time tracks of the sampled parameters COD, soluble phosphorus, and dissolved oxygen were performed for Strategies 1,2,4,and 5. These results are displayed in Figures 8,9,10 and 11, respectively, and represent averages of the values recorded at similar times for the three best consecutive operational days.

FIGURE 6
EFFLUENT SOLUBLE PHOSPHORUS VERSUS TIME
STRATEGY 4

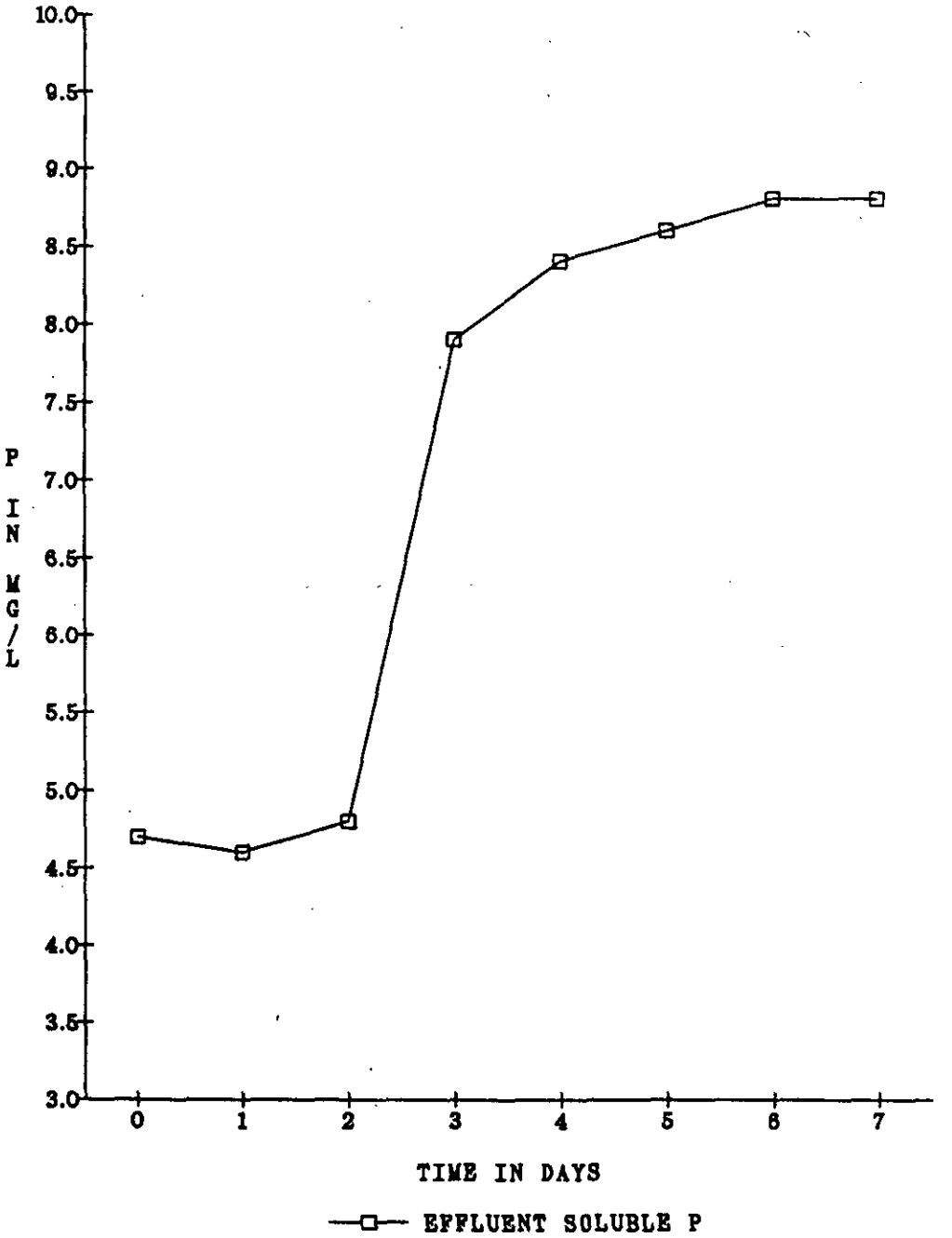


FIGURE 7
EFFLUENT SOLUBLE PHOSPHORUS VERSUS TIME

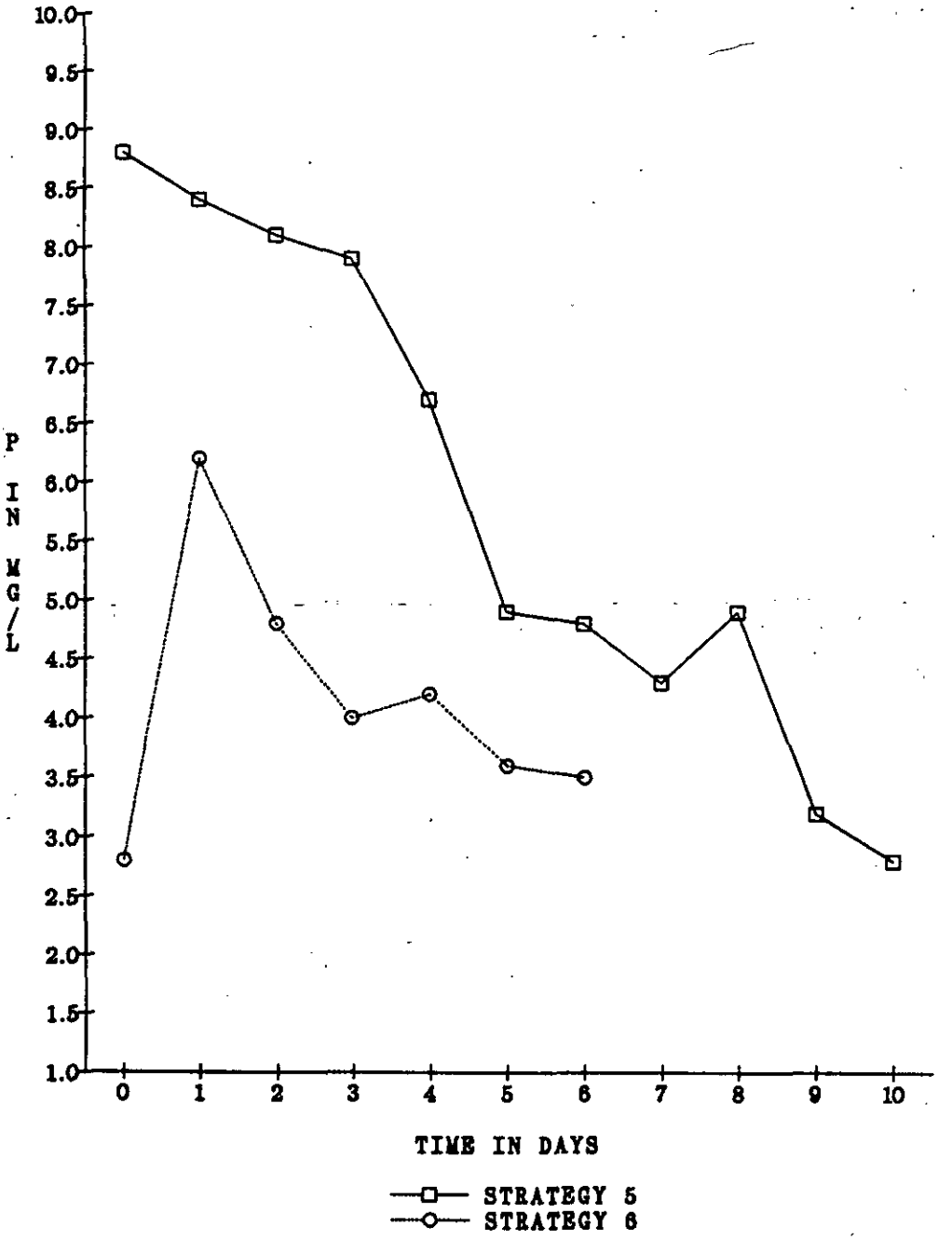


FIGURE 8
TIMETRACK OF PARAMETERS
STRATEGY 1

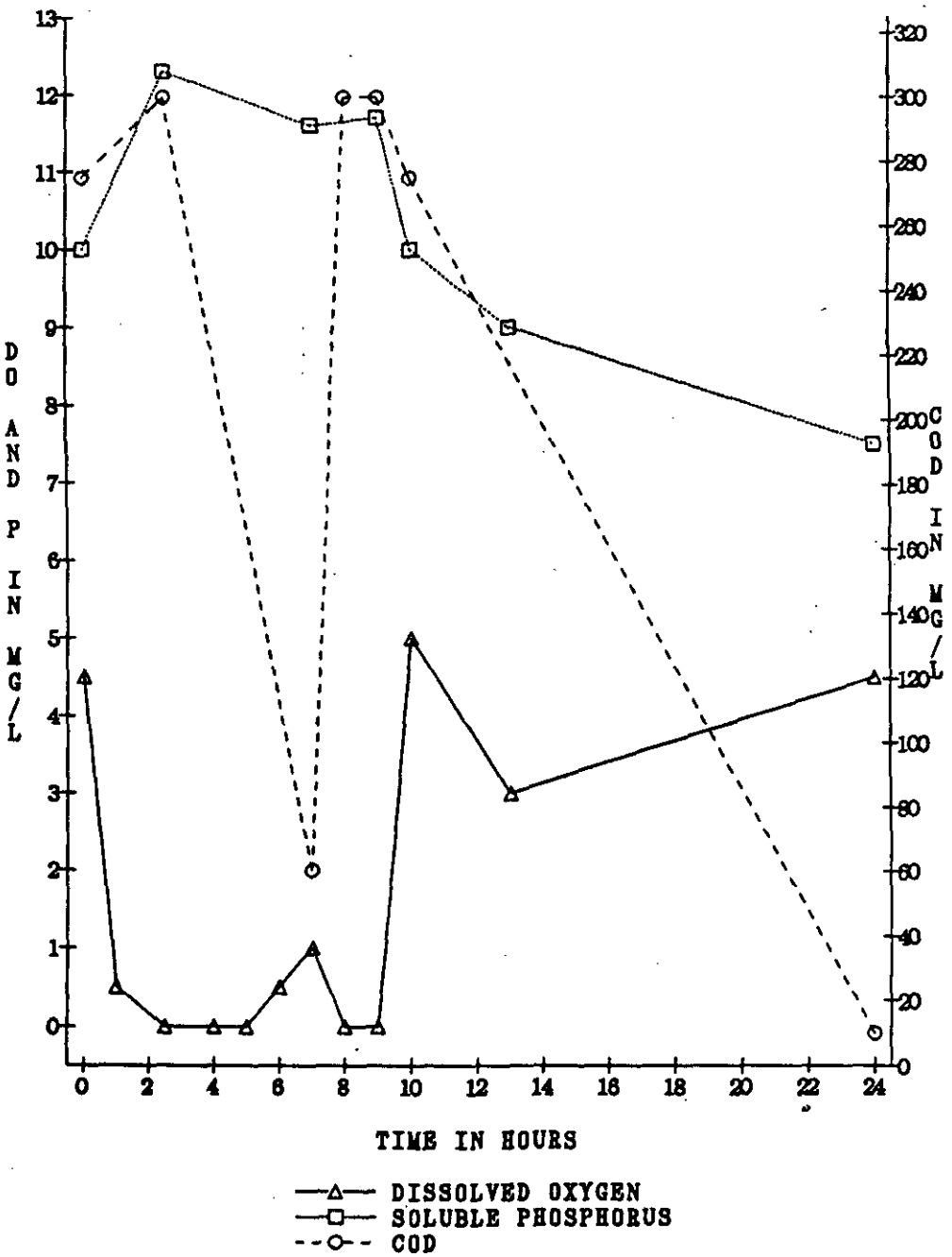


FIGURE 9
TIMETRACK OF PARAMETERS
STRATEGY 2

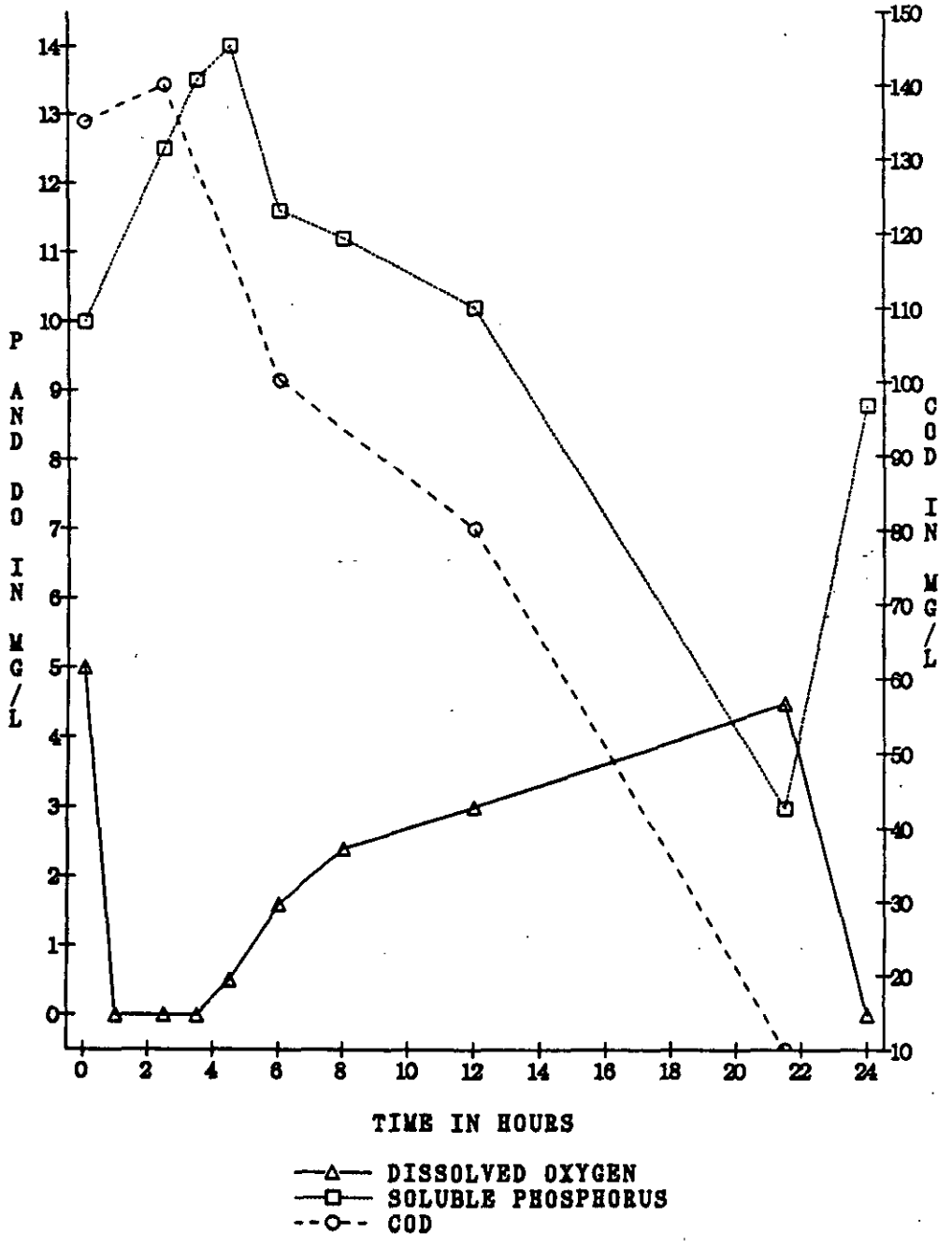


FIGURE 10
TIMETRACK OF PARAMETERS
STRATEGY 4

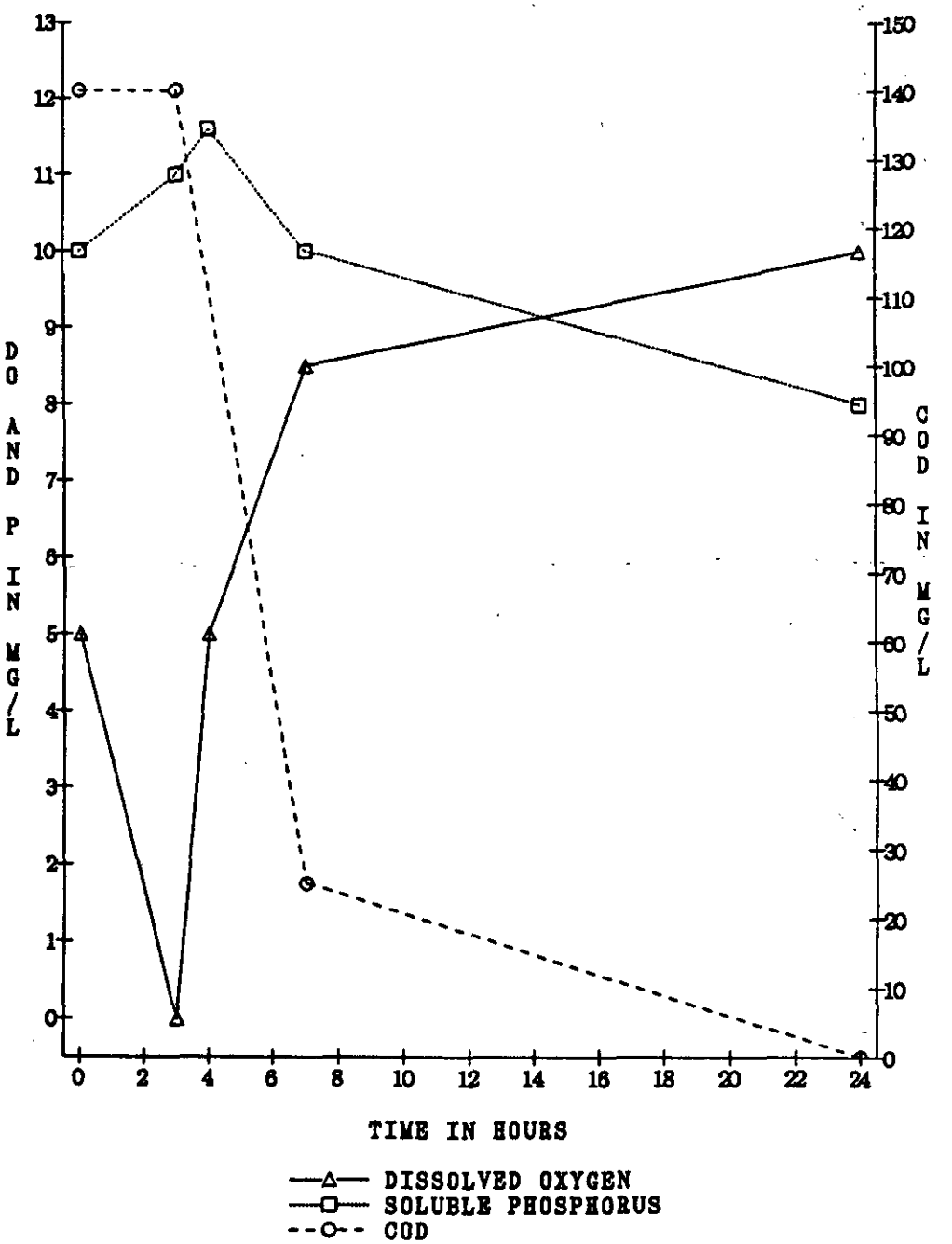
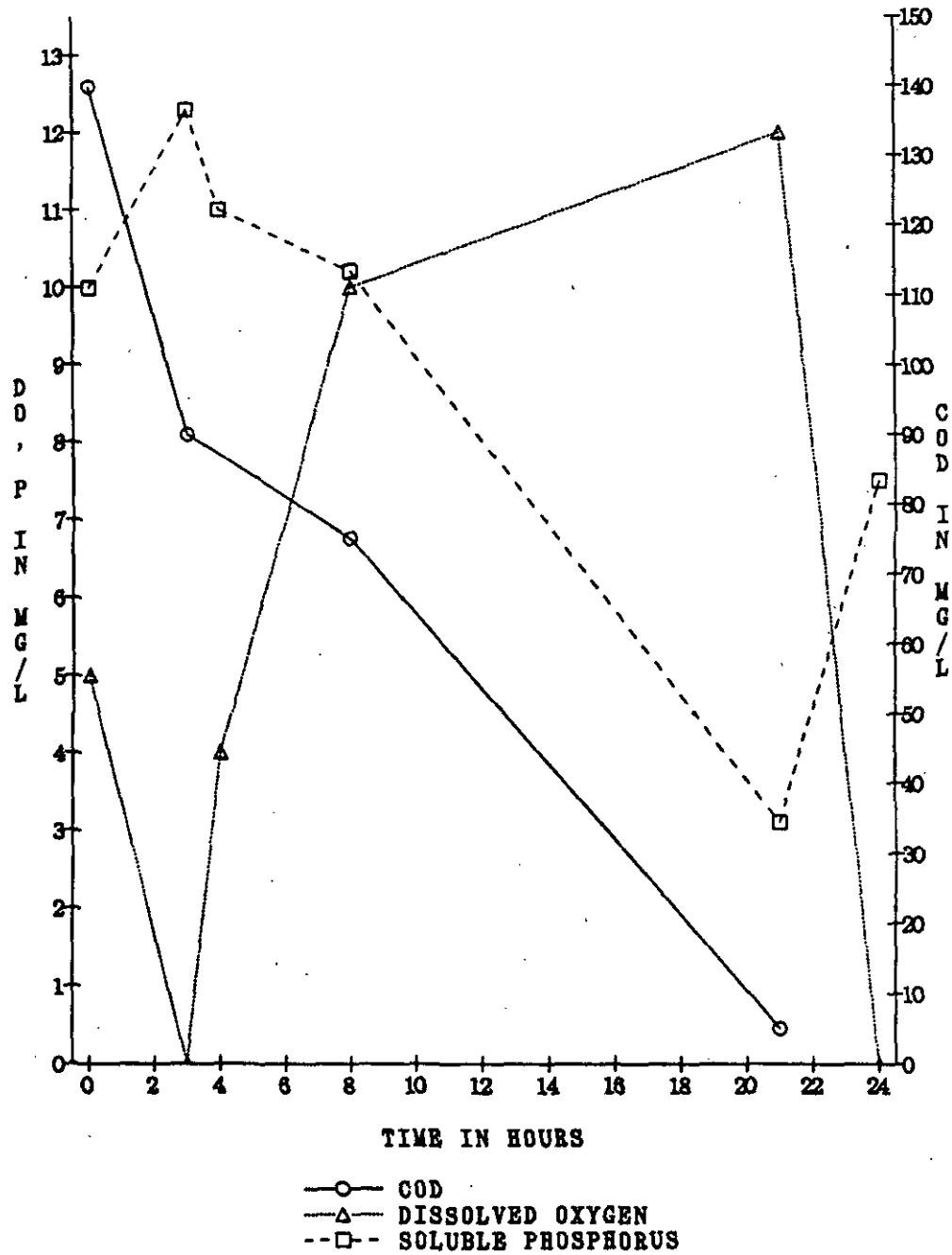


FIGURE 11
TIMETRACK OF PARAMETERS
STRATEGY 5



CHAPTER V

DISCUSSION

Biological phosphorus removal in greater than stoichiometric amounts did develop after time when the anaerobic - aerobic protocol was such that mass transfer limitations were overcome. These successful protocols employed a definitive sequence, variations from which proved fatal either to the induction of the behavior, as conceptualized in Chapter 2 in terms of both a population selection and a triggering mechanism, or to the maintenance of it. This successful sequence, as seen in Strategies 2, 5 and 6 and displayed respectively in Figures 4 and 7, was one of strict primary anaerobiosis,the trigger; primary aerobiosis of sufficient intensity to allow dissolved oxygen greater than 2 mg/l to be present for a period of 8 or more hours; a secondary anaerobiosis with soluble substrate present to induce release of stored P back to solution; and a post release draining of the reactor.

At this point it is useful to note the limits of this study. Consecutive, not simultaneous, experiments were

performed in a single reactor. Thus independence of results cannot be assured. The influence of a previous strategy on a subsequent one should be considered. Nor were experiments performed to measure nitrates and sulfates present in the supposed "anaerobic" period. That sulfate, at least, was present was obvious from olfactory observation during the first half-hour to hour-and-a-half of aerobic running time. A rough estimate of sulfate concentration put the range below 50 mg/l.

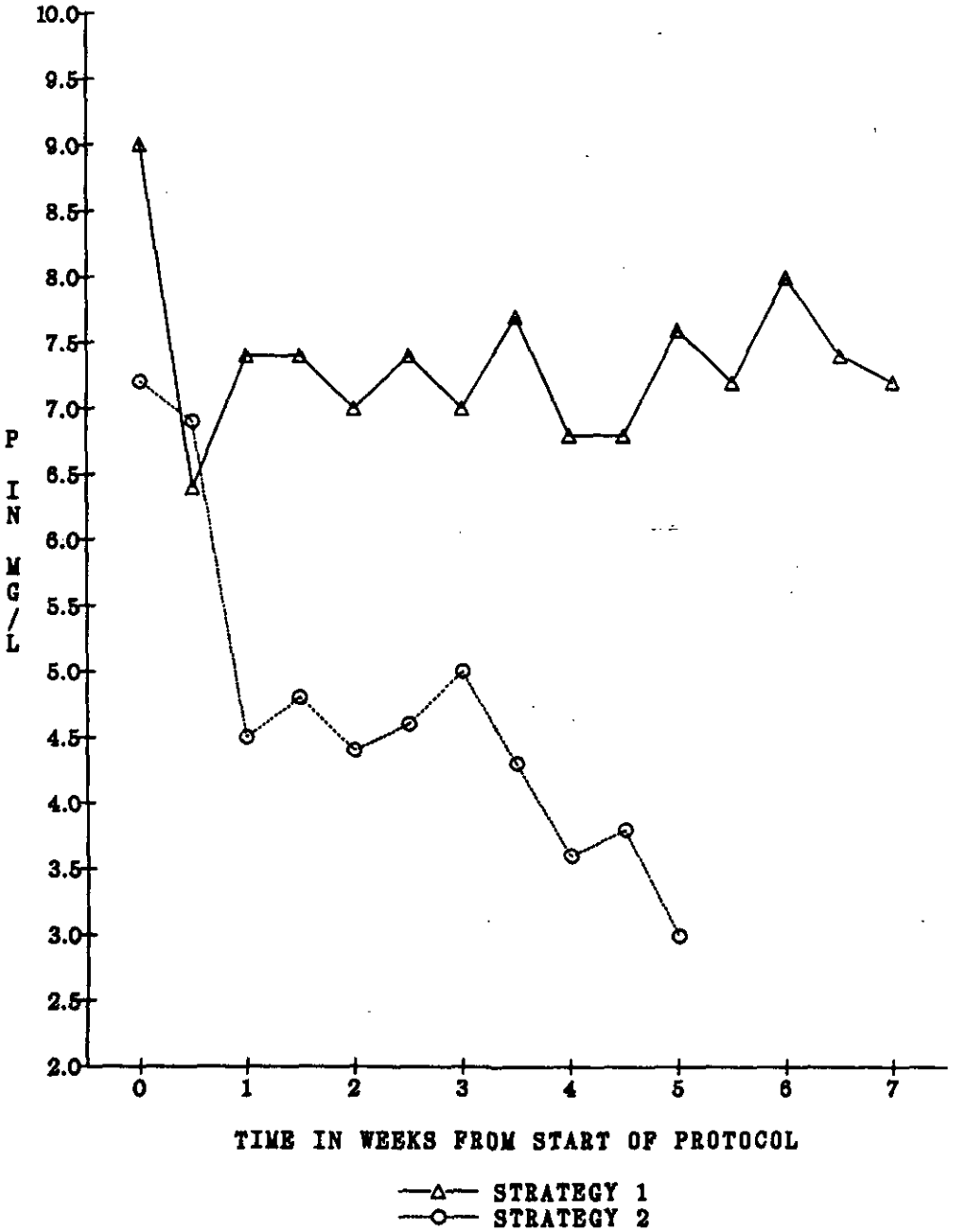
Also, a purely biological phenomenon was assumed to be at work. Thus no attention was paid to possible cationic precipitation of phosphorus. Calcium, magnesium, aluminum and iron levels were not monitored. pH was measured and found to be consistently in the 7.0 to 7.2 range irrespective of time of sampling. These measurements were performed on the bulk liquid, however, and may not be indicative of pH conditions at, near or beneath the surface of the biofilm.

With these caveats in mind a detailed look at the results follows.

Figures 3 and 4 respectively display effluent phosphorus levels over time for the S1 and S2 experiments. In Figure 12 these curves are compared on the same basis: time from start-up of the particular protocol instead of

FIGURE 12
EFFLUENT SOLUBLE PHOSPHORUS VERSUS TIME

STRATEGY 1 AND STRATEGY 2 COMPARED



time from the start of the overall experiment. A linear regression fits a straight line (not shown) to the S1 data in Figure 3 with a y intercept of 7.45, a slope of -0.03 and a regression coefficient of 0.1. Thus no linear trend for greater removal is apparent: at best, a constant horizontal line amidst fluctuating data characterizes S1. Based on a theoretical maximum stoichiometry of 100 parts COD to one part P, expectations for P removal with an influent COD of 275 mg/l are 2.75 mg/l. By taking the y intercept of this imaginary horizontal regression line as some average value it is possible to conclude that this level of P removal (10.0 - 7.45 = 2.55) is stoichiometric.

A regression line (again not shown) fitted to the S2 data in Figure 4 has a downward slope of 0.66 and a regression coefficient of 0.86. Not only is the downward trend visible even after the immediate drop, but stoichiometrically the phosphorus removal is excessive. Based on an influent COD level of 135 mg/l, a removal on the order of 1.4 mg/l would be expected. At week 5 of this strategy P was consistently below 4 mg/l, a 6 mg/l reduction from influent. Compared to stoichiometric uptake then, this removal represents a 330% increase.

A comparison of S1 and S2 on the timetrack graphs, Figures 8 and 9, respectivley, points to several

differences in protocol. The 2 cycle scenario of S1 never allows the biofilm enough time under aerobiosis to assimilate PO_4 from solution. P release continues into the aerobic period to a high of 12.3 mg/l, dropping only to 11.6 mg/l before the advent of the secondary anaerobiosis at hour 7.5. Nor does COD drop during primary anaerobiosis, giving no indication of the storage of soluble substrate.

That biological phosphorus removal (BPR) conditions are latent in the reactor in S1 is apparent from the sudden drop in P levels with the start of S2. Organisms capable of BPR, then, are present but are not being triggered. Nor are they being eliminated.

In Figure 9 accelerated P removal follows the rise in DO. Release of P to solution continues until hour 4.5, 2 hours into the aerobic run, as in S1. At this point with DO at the 2.5 mg/l level P begins to drop from a high of 14.0 mg/l to a low of 3.0 mg/l 17 hours later. Secondary anaerobiosis with a spike of COD induces P release back to the 9 mg/l range.

The other difference between S1 and S2 not apparent here is in feed strength. In S3, phosphorus removal, using the same protocol as S2 but with high strength feed, deteriorated, slowly rising to the normal stoichiometric

range. Figure 5 shows the strategies in their consecutive chronological sequence.

What then is at work here? Obviously an anaerobic stress state with some P release is not enough to guarantee excess uptake using a fixed film. Nor does a higher substrate level during this primary anaerobiosis in itself insure the behavior, contrary to the logic of lowered mass transfer resistance with an increased concentration gradient or driving force. The secondary anaerobic stage, inducing release of stored P prior to draining, is certainly important, as well, but is not in itself conclusive.

Rather, from the information collected,it can be theorized that the lack of dissolved oxygen, as a function of poor mass transfer and exacerbated by the peculiarities of this batch system, acts as the limiting parameter here. Reference is again made to the fixed film model pictured in Figure 1.2. Either a dual substrate or an acceptor limited model might be appropriate. By recalling that in Figure 9 (S2) the P drop mirrored the DO rise in an inverse relationship, it is thus apparent that getting oxygen into the depth of the film where P hoarding organisms may linger is a necessary step. When COD levels are higher, as in S1 and S3, oxygen transfer needs also rise. Those members of

the consortium not involved in P uptake exert a stronger demand, in effect competing for "acceptor" in the presence of higher donor levels.

Therefore, the combined effects of a necessary trigger, sufficient mass transfer of acceptor, secondary anaerobiosis inducing release, and some wasting of P from the system are critical for this fixed film system. This is reinforced by examination of the timetrack studies of the strategies employing pure oxygen.

Despite rapid infusion of oxygen in S4, shown in Figure 10, excess uptake behavior deteriorated over the course of seven days. The protocol used in Strategy 4 mirrored that used in Strategy 1, emphasizing a primary anaerobiosis as a trigger inducing release followed by an oxygen rich period for uptake.

Phosphorus was released into solution during primary anaerobiosis. However no COD uptake during this anaerobic period was evident. With rapid oxygenation P levels in the bulk liquid quickly dropped within the first few hours but levelled off to remain at or above the stoichiometric. COD consumption during this aerobic period was massive, indicative of normal aerobic substrate degradation.

Differences in the protocols and the results of this

strategy and Strategy 2 point to an interesting question: why was there no anaerobic COD drop in Strategy 2? BPR was in evidence. The theory clearly stated that release of stored phosphorus and intake of COD to be sequestered for exclusive use were critical components of this anaerobiosis. Yet in Strategy 2 no rapid oxygen infusion occurred to alter conditions. Rather a slow rise of oxygen following a lag period is evident. By following this on Figure 9 it can be noted that COD did begin to disapppear during this lag period. Was this aerobic degradation of substrate occuring under the aerobic conditions at the influent, oxygen-rich end of the column? Or was this lag period in the rise of oxygen tension indicative of the satisfaction of some other oxygen demand? Certainly sulfides were being volatilized. Was an oxidation occurring as well? Or a nitrogenous demand? These questions were beyond the scope of this study.

If the lag period is treated as an add on to the length of the primary anaerobiosis, then the COD consumed during this lag period fits nicely into the theory: it is being sequestered by the P-storing organisms. Under oxygen conditions later in the cycle this sequestered substrate is converted into valuable high energy stored phosphorus.

It is in Strategies 5 and 6 that theoretical behavior

and empirical observation become consistent. The timetrack study shown in Figure 11 makes this explicit. A COD drop during primary anaerobiosis is accompanied by a P release. Rapid oxygen infusion halts this release and starts P uptake. Over the course of the aerobiosis those P storing organisms within the reactor cause an excess of uptake of approximately 6 mg/l. This stored phosphorus is released back into solution during a second anaerobic period when there is abundant soluble substrate supplied by a spike input. Upon draining, the reactor contains phopshorus poor organisms with stored substrate ready to be cycled again. The use of oxygen rather than air overcomes acceptor mass transfer limitations when the feed strength is high.

The final anaerobic period and the rapid infusion of oxygen during the aerobic period then are the keys to the success of this system. A summary of results is presented in Table 3.

The final anaerobic period is crucial to the mass transfer of substrate or donor to the biofilm. The use of oxygen highlights the mass transfer limitations of acceptor to the biofilm. The combined effects of these mass transfer problems points to the impracticality of this particular system for the removal of phosphorus by biological means.

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missing

With no control of biofilm depth and limited ability to transfer acceptor, the use of an upflow filter style of reactor is especially questionable. These results, however, do point optimistically to the use of an expanded or fluidized bed reactor. These systems exert hydrodynamic control, through shear forces, on biofilm depth. With a thin consistent biofilm depth mass transfer of acceptor can then be idealized.

CHAPTER VI

CONCLUSIONS

The work performed in this study was specific and limited in scope. With this narrow focus in mind, based upon the results of this study, it is possible to conclude that:

1. Biological phosphorus removal using a fixed film is possible.

2. Mass transfer of both donor and especially acceptor substrates limits the feasibility of many fixed film reactors for biological phosphorus removal.

    2.1. Lack of control over biofilm depth exacerbates the mass transfer of these substrates.

    2.2. The hydrodynamic control available in expanded and fluidized bed reactors suggests their suitability for biological phosphorus removal in situations warranting fixed films.

3. Wasting of phosphorus from the reactor must be accomplished in a secondary anaerobiosis which also allows for transfer of donor substrate to the P storing organisms.

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APPENDIX

Other Strategies

A 16 liter reactor was constructed and run in the manner illustrated in Figure 2. A 2.5 liter aerator was used. A high feed strength was employed. The first protocol employed was a one cycle, anaerobic -- aerobic sequence, consisting of 12 hours under anaerobic conditions and 12 hours under aerobic conditions. The reactor was dosed daily for the first week with one liter of mixed liquor from the Amherst Wastewater Treatment Plant. This "seeding" of the reactor was done during the normal feeding period. Growth was immediately noticed near the influent end of the reactor column.

Growth continued to accumulate in the length of the reactor but was excessive near the influent end. Dissolved oxygen levels from the top of the column were slow to rise under aerobiosis. It was concluded that the oxygen became depleted before reaching the sample port at the top.

Growth became excessive at the influent end of the column, causing plugging. After two months of operation the reactor column itself failed, seperating at the joint joining the collar plate to the column. It was concluded that pressure had built up sufficiently in the system due to this plugging at the influent end to overcome the acrylic cement bond.

The film covered Glitsch rings were removed from the broken column and maintained in a simple fill and draw basin until a new reactor was constructed, a period of some two weeks. This reactor was the one pictured in Figure 2. An expanded aeration vessel was also deployed, volume 4 liters. This lowered volume system was seen as a way to improve oxygen transfer at the same time as decreasing oxygen needs.

The 12 hour aerobic, 12 hour anaerobic sequence was continued. When growth appeared consistent in the length of the column, sampling was begun. Less than stoichiometric phosphorus uptake was noted.

Either conditions triggering the excess uptake phenomenon were not being imposed or those organisms capable of this uptake were not present. As the purpose of this study was to investigate whether a film had the capability to exhibit excess uptake behavior, more than one

variable was changed at a time in search of some evidence of the behavior.

A final anaerobic period was added to the one cycle sequence. A six hour anaerobic period was followed by a 10 hour aerobic period. At this point the reactor was again run anaerobically for eight hours. It was theorized that this final anaerobic period would allow stored P to be wasted from the reactor when released to solution. At this time the reactor was again innoculated during the feeding period with mixed liquor.

Phosphorus removal, as measured at the end of the aerobic period, improved to maximum theoretical stoichiometric levels. P release to solution during primary anaerobiosis was also noticed.

It was theorized that the final anaerobiosis had had a positive effect on system performance. To further improve this response a spike input of COD (sodium acetate) into the final anaerobiosis was proposed.