CHAPTER II: IMPACT OF THE FEED COD/TP RATIO ON THE INTRACELLULAR STORAGE MATERIALS AND SYSTEM PERFORMANCE OF BIOLOGICAL PHOSPHORUS REMOVAL

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Abstract

A UCT type pilot plant fed with synthetic wastewater was operated with influent COD/TP ratios of 18, 12 and 8 to investigate the role of intracellular storage products on EBPR mechanisms. Acetate was the sole carbon source and other nutrients were provided in excess during plant operation at an SRT value of 10 days and at 20^0C temperature. COD utilization, P-release and uptake, PHA and glycogen storage and utilization of the system were monitored. The steady state data showed that PHA storage, glycogen production and system performance were influenced by the COD/TP ratio. System P removal performance decreased as COD/TP ratio decreased and some deterioration in overall performance occurred at the lowest COD/TP ratio. Very good stoichiometry was obtained between both organic storage and its utilization, and P release and P uptake. Glycogen production and P-uptake were observed in the absence of extracellular organics and in the presence of NO_3-N. The consumption and production of glycogen in anaerobic and aerobic reactors favored the Mino model over the Comeau/Wentzel model for EBPR. At the lowest COD loading, glycogen was not generated and energy obtained through PHA utilization apparently was used for maintenance energy needs.

Keywords: COD/TP ratio, intracellular storage, glycogen, PHA, biological phosphorus removal, stoichiometry.

INTRODUCTION

Biological nutrient removal (BNR) is an effective and economical way to remove phosphorus along with nitrogen and organic materials from wastewater. Biological phosphorus removal (BPR) is one of the important elements of a complete BNR system. It is a process in which alternation of anaerobic and aerobic cycles favors the phosphorus accumulating organisms (PAO), and provides them with the required substrates and opportunity to compete with other groups of active microorganisms. PAO can store inorganic phosphorus as intracellular poly-phosphate (poly-P) to a greater extent than is needed for growth metabolism. In anaerobic zones, short chain fatty acids (VFAs) are utilized and stored as intracellular poly-hydroxy-alkanoates (PHA) and orthophosphorus is simultaneously released to the medium as poly-P is de-polymerized.

As discussed in Erdal and Randall (2002a), during the past two decades, numerous researchers have investigated different aspects of BPR, and different biochemical models have been developed to explain the mechanisms of BPR. The most widely accepted of these models have been the Comeau/Wentzel model (based on Comeau *et al.*, 1986), the Mino model (Mino *et al.*, 1987) and the Adapted Mino model (Wentzel *et al.*, 1991). In addition, modifications of these models and new mechanisms have been proposed by Pereira *et al.* (1996); Louie *et al.* (2000); Maurer *et al.* (1997); and Sudiana *et al.* (1999). However, a complete picture of BPR has not yet been drawn and it needs further investigation.

Different from the Comeau/Wentzel model, the Mino and Adapted Mino models predict that glycogen, an intracellular carbohydrate reserve, serves as a supplemental electron donor for PHA production. Thus, it is degraded in the anaerobic stage (Mino *et al.*, 1987 and Smolders *et al.*, 1994) for this purpose, and in the following aerobic stage, PHA is broken down as a carbon and energy source to: synthesize new cells, produce the reducing equivalents (NADH) needed for ATP production and restore the depleted glycogen reserves. The newly generated ATP is used by cells for energy or to store poly-phosphate granules, which are later used as an energy source for acetate uptake and PHA storage in the anaerobic zone. The cations, potassium and magnesium, are essential for BPR (Pattarkine, 1991), and are released with phosphorus in the anaerobic zone and taken-up in the aerobic zone. Besides the released phosphorus, some of the

phosphorus initially present in the wastewater is removed, resulting in net removal when sludge is wasted from the system. The overall process is known as excess biological phosphorus removal (EBPR).

Comparison of the two major biochemical models, the Comeau/Wentzel and the Mino models, indicate that the main difference between them is related to the mechanism of reducing power generation within the anaerobic zone, which is necessary for PHA production. In the Comeau/Wentzel model, acetate or other VFAs are taken up by the cells and directed through the TCA cycle. In the simple case, a portion of the acetyl-CoA is directed to the TCA cycle and NADH generated from the cycle interacts with the remaining acetyl CoA to produce PHB. In the Mino Model, reducing power is generated through breakdown of stored glycogen, which is recycled from the aerobic stage. Glucose-1-phosphate is then directed through the Embden-Meyerhoff-Parnas (EMP) pathway to create reducing power. The Adapted Mino model is similar to the Mino model in the general outlook of events, but the former one considers the Entner-Doudoroff (ED) pathway as the NADH source. Researchers agree on the storage of organics in the form of polyhydroxyalkanoate polymer, which is a complex polymer of poly-hydroxy-butyrate, polyhydroxyvalerate and their methylated forms (Satoh *et al.*, 1992). PHA oxidation and intracellular phosphate storage in the aerobic stage also are defined in all models. However, the Mino and Modified Mino models additionally suggest glycogen formation in the aerobic stage.

The effect of different types of VFAs on EBPR was investigated by Abu-ghararah and Randall (1991) in a continuous type UCT process using municipal sewage supplemented with VFAs, and a similar study using an SBR was recently performed by Lemos *et al.* (1998). Best PHA production was obtained when the reactors were fed with acetate, as compared to propionate, butyrate and valerate. In addition, the effects of the COD/TP ratio on EBPR performance as proposed by Randall *et al.* (1992) from full-scale and pilot-plant research have been investigated by several. The EBPR process is considered to be COD limited when the COD/TP ratio is low (<<20:1 for settled domestic sewage), whereas it is P limited when the COD/TP ratio is high. Low COD/TP ratios are thought to result in EBPR failure because PHA storage possibly becomes too low to encourage intracellular poly-P storage and glycogen replenishment in the aerobic stage. Thus, very low P removal will be achieved in such situations. This idea is

well supported by Stephens and Stensel (1998), and Brdjanovic *et al.* (1998). When the COD/TP is high, very low effluent P concentrations can be maintained. However, excessive acetic acid concentrations (COD = 800 mg/L) were found to act as an inhibitor to PAOs (Randall and Chapin, 1995). Therefore, it is clear that the applied COD/TP ratio should be within a range in which neither one of the upper or lower limits are exceeded. In early studies by Punrattanasin and Randall (1998), EBPR performance under a series of applied COD/TP ratios (20, 30, 40 and 60) was investigated using a UCT type system fed with domestic wastewater. These ratios were achieved by keeping the COD value constant at 400 mg/L throughout the study and changing the TP value. The above mentioned behavior of P-limited versus excess-P fed systems were well proven in that study. Jespersen and Henze (1993) have shown that much lower ratios such as 12 are still applicable to EBPR systems before a deterioration of the effluent quality occurs, when acetate is used as the primary organic substrate instead of domestic sewage. In most of these studies, however, glycogen was not measured. Thus, without glycogen data it is hard to obtain a complete picture of the EBPR mechanisms.

In this study, COD/TP ratios of 18, 12 and 8 were examined using a UCT type lab-scale BNR system operated at 20°C. A major objective of this study was to investigate the role of glycogen in EBPR biochemistry. Other objectives of this study were to determine how the COD/TP ratio affects the performance of the system, especially under COD limited conditions.

MATERIALS AND METHODS

A UCT type BNR plant consisting of two 2-L anaerobic, two 2-L anoxic and three 3.5-L aerobic completely mixed reactors in series was operated in a constant temperature room kept at 20±1°C (Figure 1). The mean cell residence time (MCRT) of 10 days used throughout the study was achieved through wastage out of the third aerobic reactor. Synthetic feed flow rate was maintained at 40 L/day. Internal system recycles (i.e. nitrate recycle from third aerobic reactor to first anoxic reactor, return activated sludge to first anoxic reactor, and anoxic recycle from second anoxic reactor to first anaerobic reactor) were also kept at 40 L/day. Air diffuser

stones were used for both aeration and mixing purposes in the aerobic zone of the system. Non-aerated zones of the system were mixed with motors installed at the top of each reactor. Seed sludge was obtained from the Stroubles Creek Wastewater Treatment Plant located in Blacksburg, Virginia. Synthetic feed water was deoxygenated by purging with N_2 gas until the dissolved oxygen concentration in the influent, which enters the system through the first anaerobic reactor, fell below 1 mg/L. Synthetic feed was prepared daily to contain acetate as the sole COD and VFA source, $(NH4)_2SO_4$ 20 mg/L as N, K_2HPO_4, 125 mg/L $CaCO_3$ alkalinity as $NaHCO_3$, 210 mg/L $MgSO_4$, 44.4 mg/L $CaCl_2$, 1.11 mg/L $FeCl_3$, 0.66 mg/L $MnCl_2 \cdot 6H_2O$ 0.44 mg/L $ZnSO_4 \cdot 7H_2O$, 0.14 mg/L $CuSO_4 \cdot 5H_2O$, 0.14 mg/L $CoCl_2 \cdot 6H_2O$, 0.05 mg/L KI, 0.12 mg/L H_3BO_4. COD/TP ratios of 18, 12 and 8 were achieved by keeping the feed PO_4-P concentration constant at 20 mg/L and changing the feed COD accordingly.

The system was operated for three months after it was placed into operation before steady state was assumed and data collection started. The plant was monitored for six months, thereafter, through the measurement of soluble and total COD, MLSS, MLVSS, NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{-3}-P, PHB, PHV, and glycogen on samples taken from the influent, all seven reactors, the recycle lines, and the system effluent. Three MCRTs (30 days) were allowed after each change in system feed composition before steady state data were collected. A series of anaerobic-aerobic batch tests were performed to determine the anaerobic and aerobic production and utilization rates for glycogen, PHA and poly-P. Anoxic sludge was mixed with an equal volume of synthetic feed solution at the exact composition of the system feed and the 2-L beaker was immediately sealed. Anaerobic conditions were maintained by continuous N_2 sparging. Sampling was performed through a sampling tube by suction with a peristaltic pump. At the end of the anaerobic period, air was used instead of N_2 sparging to establish aerobic conditions.

The cation and anion profiles of the filtered samples were determined using a DIONEX Ion Chromatograph according to APHA (1995). Soluble and total COD, MLSS and MLVSS analyses were performed as outlined in APHA (1995). PHA content of the sludge was analyzed and expressed as the poly (3-hydroxybutyrate-co-3-hydroxyvalerate) co-polymer according to Hart (1994). Mixed liquor samples taken from each reactor of the UCT system were centrifuged for 5 minutes at 10 000*g*, the supernatant was removed, and the remaining solids

were dried at 103°C for two days. Solids were weighed into 5-mL Wheaton V-vials. Eight to ten external PHA standards were prepared by weighing different quantities of (0 to 15 mg) PHB-HV copolymer standard (12%HV) obtained from Sigma Chemicals. Methanol-sulfuric acid-benzoic acid solution was then added (2 mL) to each vial. Benzoic acid served as an internal standard, and the solution was prepared freshly by solubilizing 50 mg of benzoic acid in 100 mL of 3% sulfuric acid in a methanol solution (v/v). Before vials were tightly sealed, 2 mL of chloroform was also added to each vial. The vials were incubated in an oven at 100^{o}C for 3.5 hours. Following digestion, the vials were cooled to room temperature and 1 mL of distilled water was added to each vial. The vials were shaken for 10 minutes to separate methanol and chloroform layers. For the measurement of the HB and HV units, 1μL of the chloroform phase was manually injected to a 3-meter 2% Reoplex 400 Chromosorb GAW GC column. The injector temperature was 160^{o}C. The FID detector and the oven temperatures were 200^{o}C and 130^{o}C, respectively. The air, hydrogen and nitrogen gas flow rates were 428, 30 and 29 mL/min, respectively. The detention times of HB and HV were 3.5 min and 5 min, and the benzoic acid came out at 6.5 min.

Glycogen measurements were performed using the anthrone method according to Murray (1981) and Jenkins *et al.* (1993). Mixed liquor samples taken from each reactor of the UCT system were centrifuged for 5 minutes at 10 000*g*, the supernatant was removed, and the remaining solids were dried at 103°C for two days. Solid samples were weighed into screw-cap plastic centrifuge tubes. 1 mL of 30% KOH (wt/v) was added into each tube, tubes were sealed and heated at 100°C for 3 hours. At the end of three hours, the tubes were taken out and cooled to room temperature. Then, 3 mL of water and 8 mL of ice-cold ethanol was added to precipitate glycogen. Tubes were then centrifuged at 10 000*g* for 15 minutes. The pellet was left to dry at 60°C. Glucose standard was prepared by adding 100 mg of glucose and 150 mg benzoic acid to 100 mL of distilled water. The standard was diluted 1:10 with distilled water for daily use. The anthrone reagent was prepared by adding 200 mg anthrone to 5 mL absolute ethanol, and making up to 100 mL with 75% sulfuric acid (v/v). Both solutions were stored at 5°C, and the anthrone reagent was replaced when the greenish-yellow color turns brownish-yellow. The pellets were re-suspended in distilled water to solubilize the glycogen. A dilution ratio of 1:10 was found to be appropriate for the levels of glycogen in MLSS samples. Thus, 0.1

mL of sample solution and 0.9 mL distilled water was transferred to Pyrex boiling tubes. Same was done to the glucose standards prepared to yield a glucose concentration range of 0 to 100 mg/L. Tubes were chilled on ice water bath, while 5 mL of chilled anthrone reagent was added to each tube, mixed thoroughly. All tubes were transferred to a boiling water bath for exactly 10 min, and they were then returned to ice-water bath. Absorbance of each anthrone-sample solution was measured at 625 nm. Standard curve prepared for the glucose standards were used to calculate glucose content of each sample, and reported as % dry weight of sample.

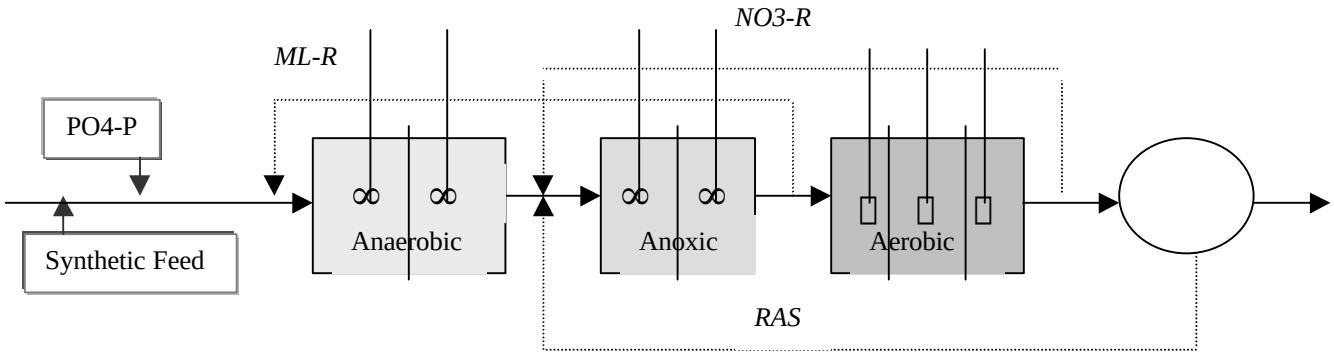

Figure 1. UCT type lab-scale plant operated during the study

RESULTS AND DISCUSSION

The model plant performed well and accomplished good EBPR throughout the experimental period. Complete nitrification and at least 90% reduction of organic matter were experienced for all cases. The plant achieved complete denitrification of the recycles at all times except when the COD/TP ratio was 8. The average VSS/TSS ratio varied from 0.8 in the anaerobic zone to 0.6 in the aerobic zone. A summary of the average substrate removal and storage data for the three COD/TP periods, with the standard errors, is given in Table 1. The PHA and glycogen data clearly show storage of PHA and utilization of glycogen in the anaerobic zone, and generation of glycogen and utilization of PHA in the aerobic zone. The data strongly indicate a linkage between the two. PHA and glycogen changes in the anoxic reactors depended upon the COD/TP ratio of the experiment. Combined steady state data were evaluated for the differences in system response observed during these periods.

The utilization of COD throughout the reactor train was similar for all experiments. In all cases, a major part of the COD was removed in the first anaerobic tank. However, the anaerobic COD utilization rates decreased as the COD/TP ratios decreased. The rates of storage and utilization in each BNR unit were found to increase as the COD/TP ratio increased in all cases (Table 2). Furthermore, significant relationships were observed between the internal storage products PHA and glycogen, and between the storage products and phosphate. These relationships are illustrated in Figures 2 - 5. These figures illustrate the dependency between the key parameters as observed in both the anaerobic and the aerobic zones of the BNR plant. The overall stoichiometry of storage and utilization taking place in all three zones (anaerobic, anoxic, and aerobic), for all three experiments is given in Table 3. The standard errors of the mean for the average values reported in Tables 2 and 3 are given as ± values for each measured value.

Table 1. Summary of substrate removal and storage during the COD/TP experiments

COD/TP = 18	(Influent COD = 402±13 mg/L; TP = 22±1.5 mg/L) n = 8				
Location	COD (mg/L)	PO_4-P (mg/L)	MLSS (mg/L)	Glycogen(mg/L)	PHA (mg/L)
Anaerobic 1	101±7.6	116±8.4	1597±51	102±7.4	132±6.52
Anaerobic 2	76±5.6	182±10.0	1675±36	89±6.5	173±10.4
Anoxic 1	42±4.7	69±5.2	3012±98	299±11.9	101±4.3
Anoxic 2	37±4.2	85±6.3	3020±72	297±7.2	114±6.2
Aerobic 1	27±3.2	27±1.7	3168±113	330±6.8	74±3.9
Aerobic 2	26±2.5	7.8±1.2	3075±97	337±16.9	61±1.5
Aerobic 3	25±2.1	0.7±0.2	3150±99	337±5.1	60±2.5
Effluent	22±2.2	0.8±0.3	95±14	NM	NM
RAS	22±3.9	0.7±0.2	5046±224	618±21.3	72±3.2

COD/TP = 12	(Influent COD = 306±5 mg/L; TP = 26±0.3 mg/L) n = 6				
Location	COD (mg/L)	PO_4-P (mg/L)	MLSS (mg/L)	Glycogen(mg/L)	PHA (mg/L)
Anaerobic 1	65±1.6	111±2.5	1606±64	76±4.9	111±5.6
Anaerobic 2	48±1.7	174±5.8	1654±43	64±8.2	138±5.8
Anoxic 1	31±2.4	67±1.0	3280±81	214±14.1	97±6.0
Anoxic 2	26±2.7	80±2.4	3603±92	210±13.5	107±6.9
Aerobic 1	22±2.1	40±2.9	3817±127	229±20.9	82±5.4
Aerobic 2	20±1.6	15±1.9	3645±80	235±18.0	74±2.9
Aerobic 3	20±1.4	2±0.6	3724±91	235±17.4	69±3.1
Effluent	19±1.0	2±0.5	42±8.1	NM	NM
RAS	22±1.1	2±0.5	6746±209	459±20.9	74±3.8

COD/TP = 8	(Influent COD = 214±5 mg/L; TP = 26±0.2 mg/L) n = 2				
Location	COD (mg/L)	PO_4-P (mg/L)	MLSS (mg/L)	Glycogen(mg/L)	PHA (mg/L)
Anaerobic 1	49±3.5	79±2.6	1802±53	57±5.3	57±7.5
Anaerobic 2	33±0.7	108±2.3	1845±40	51±5.9	73±2.4
Anoxic 1	21±1.4	45±2.7	3190±255	148±12.7	46±6.2
Anoxic 2	19±0.8	42±1.0	3338±167	152±17.4	42±2.3
Aerobic 1	17±2.1	27±2.3	3508±172	152±19.1	40±3.4
Aerobic 2	19±2.2	16±0.6	3454±159	151±20.6	39±1.6
Aerobic 3	19±1.4	11±0.6	3676±171	152±15.1	40±1.4
Effluent	21±0.8	11±1.0	71±32	NM	NM
RAS	19±0.6	10±0.8	6081±139	297±14.5	49±4.3

Table 2. Observed kinetics of the intracellular storage products under different COD loadings

	COD/TP = 18	**COD/TP = 12**	**COD/TP = 8**
Anaerobic P-release, P-mmol/gVSS-min	0.092	0.087	0.053
Anaerobic acetate uptake, C-mmol/gVSS-min	0.187	0.156	0.102
Anaerobic PHA storage, C-mmol/gVSS-min	0.072	0.063	0.029
Anaerobic glycogen utilization, C-mmol/gVSS-min	0.020	0.017	0.004
Aerobic P-uptake, P-mmol/gVSS-min	0.027	0.019	0.007
Aerobic PHA utilization, C-mmol/gVSS-min	0.013	0.008	0.001
Aerobic glycogen production, C-mmol/gVSS-min	0.030	0.021	0.001

Table 3. Overall stoichiometry between the key parameters of the BNR system

	COD/TP = 18	**COD/TP = 12**	**COD/TP = 8**
P-released/PHA stored, P-mmol/C-mmol	0.77	0.95	0.95
P-uptake/PHA stored, P-mmol/C-mmol	0.82	1.03	1.04
P-uptake/PHA utilized, P-mmol/C-mmol	0.99	1.48	1.45
Glycogen utilized/PHA stored, C-mmol/C-mmol	0.36	0.37	0.39
Glycogen produced/PHA utilized, C-mmol/C-mmol	0.61	0.58	0.61

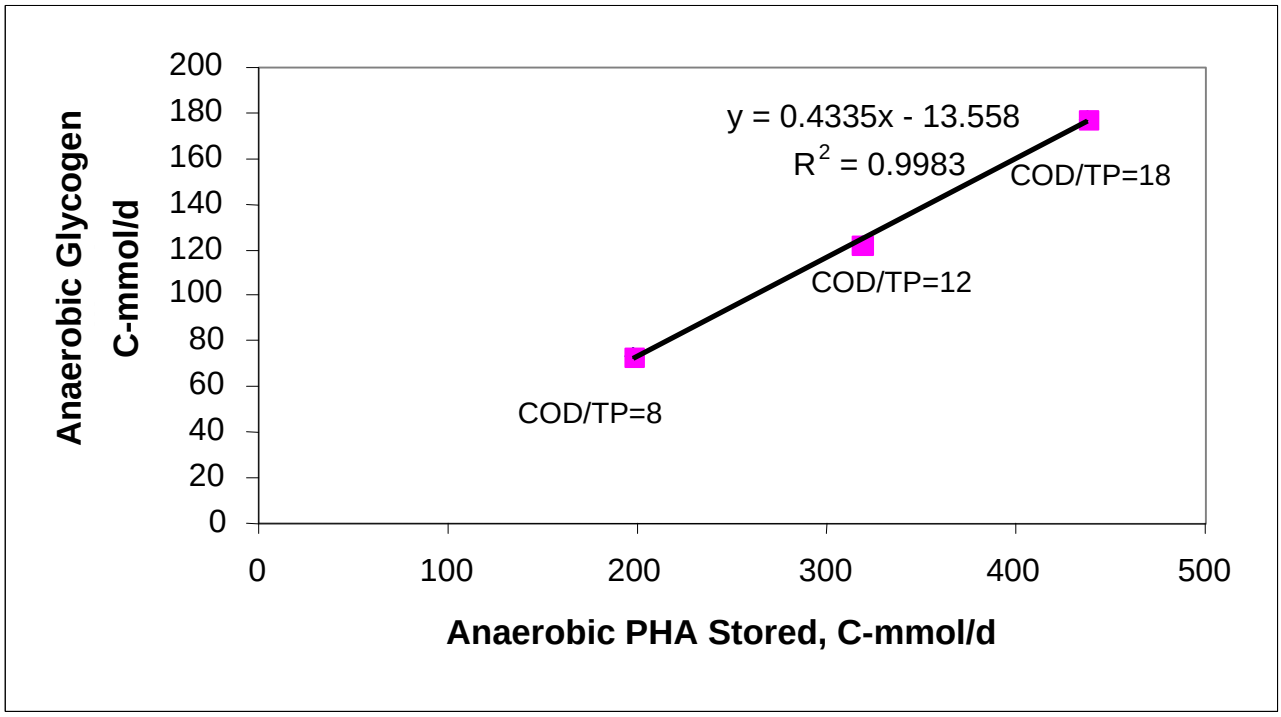

Figure 2. Observed stoichiometry between anaerobic PHA storage and anaerobic glycogen utilization.

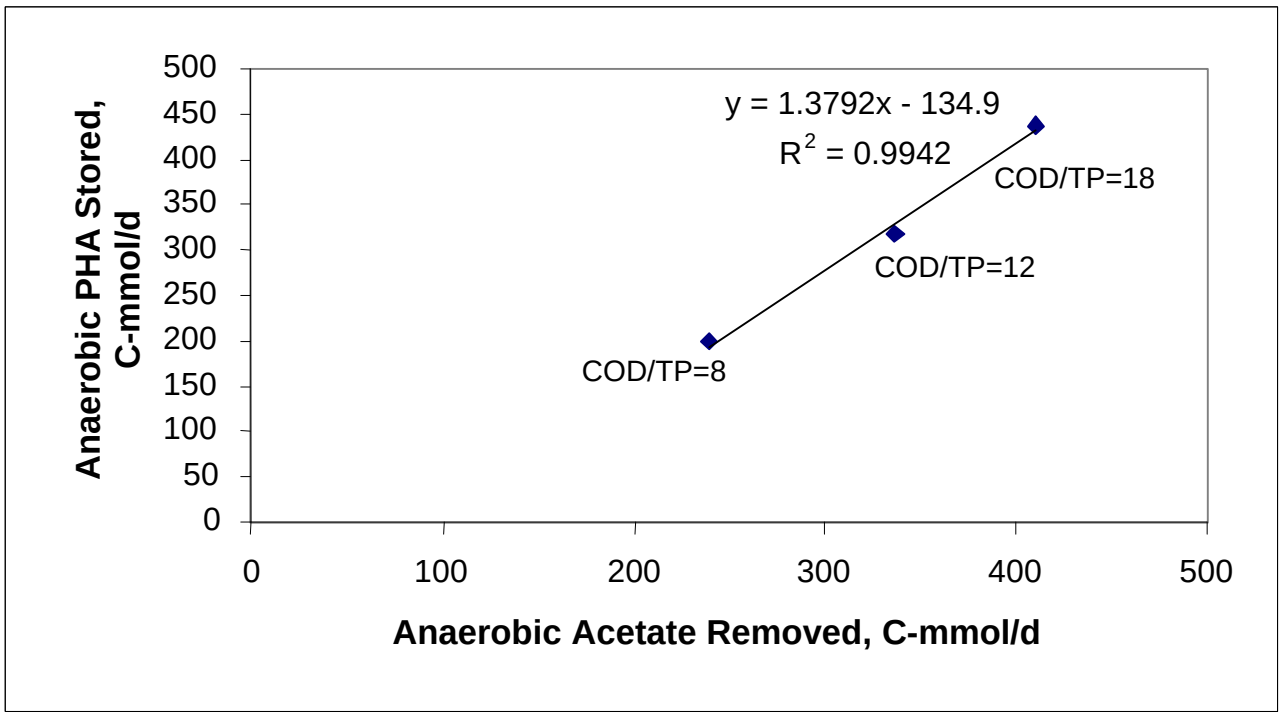

Figure 3. Observed stoichiometry between anaerobic acetate removed and anaerobic PHA stored.

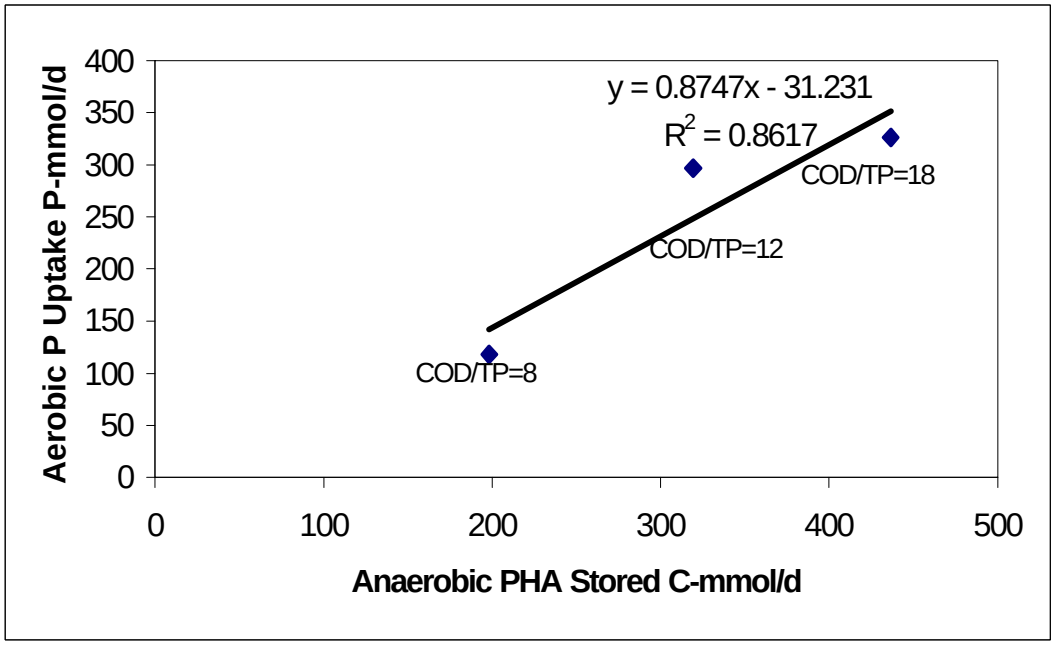

Figure 4. Observed stoichiometry between anaerobic PHA storage and aerobic P-uptake.

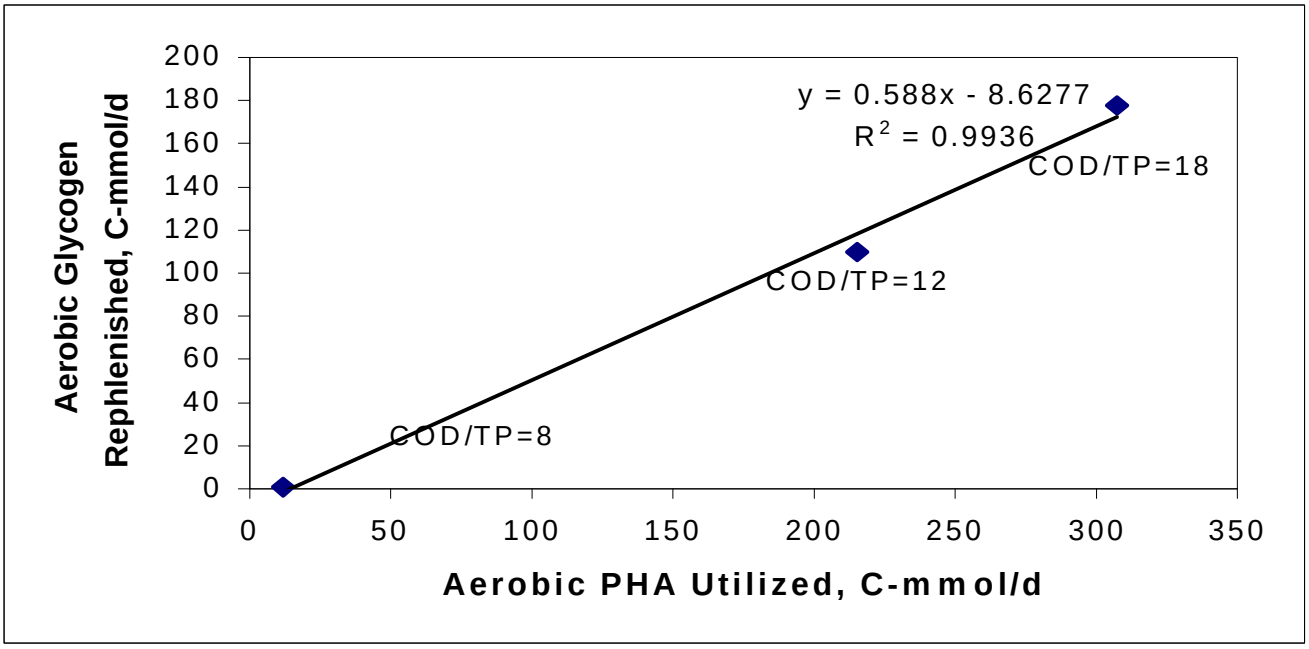

Figure 5. Observed stoichiometry between aerobic PHA utilized and aerobic glycogen replenished.

The regression line of aerobic P uptake versus anaerobic PHA stored, in Figure 4, indicates that there is a minimum amount of PHA that must be stored in the cells for aerobic P-uptake to take place. Similarly, there is a minimum amount of PHA that must be stored below which its aerobic utilization does not lead to glycogen replenishment. This can be deduced from Figure 5 in which at the lowest COD/TP ratio this minimum value (15 C-mmol/d) was reached and all the energy from aerobic PHA utilization was channeled into maintenance requirements. Due to this insufficient PHA storage and the absence of glycogen replenishment, the PAOs would be washed out of the system and eventually PO_4-P removal would be lost. In the case of the lowest COD/TP ratio, some glycogen production was observed in the anoxic zones (Figure 11).

The higher COD/TP ratios (18 and 12) produced lower effluent phosphorus concentrations and overall performance that was consistent with the literature. However, the results with the lowest COD/TP were somewhat different. The behavior of the intracellular storage products, PHA and glycogen, was different in the anaerobic, anoxic and aerobic reactors when compared to the two higher COD/TP ratios. Figures 6 and 7 present the changes in PHA and glycogen concentrations expressed as mg/L, and Figures 8 through 11 show the mass balances. These values were calculated by multiplying PHA (% TSS) and Glycogen (%TSS) with the corresponding TSS values. The storage of PHA was the lowest in the system when the ratio was 8. Unlike the high COD/TP ratios, the PHA concentrations decreased in the anoxic reactors. In addition, no P-release was observed in the second anoxic tank although it was observed at the higher COD/TP ratios (Figure 9). Moreover, a slight increase in glycogen concentration through the first to the second anoxic reactor was found according to Figure 7. These graphs, unfortunately, did not explain the increase of glycogen in the anoxic zone. However, the results of the NO_3-N measurements do explain the increase. The NO_3-N data for the three ratios are presented in Figure 13. This graph clearly shows that incomplete denitrification occurred in the anoxic reactors for the lowest COD/TP ratio. This resulted from the presence of insufficient COD in the feed wastewater. With incomplete denitrification, nitrate was available as an electron acceptor (NO_3-N) in the second anoxic reactor and the PAOs used the nitrate to form and store glycogen. It has been observed that nitrate can be utilized as an electron acceptor by PAOs for the oxidation of stored PHAs and simultaneous P-uptake. Accordingly, the decreased PHA

concentration in the second anoxic tank as shown in Figure 6 and the increase in glycogen (Figure 7) strongly indicate that PHA can serve as an electron donor in the absence of external substrate. This implies that de-polymerization of PHA in the absence of an external organic substrate is also possible in an anoxic environment. This reaction caused the PHA concentration to be lower in the medium and P-uptake was achieved simultaneously.

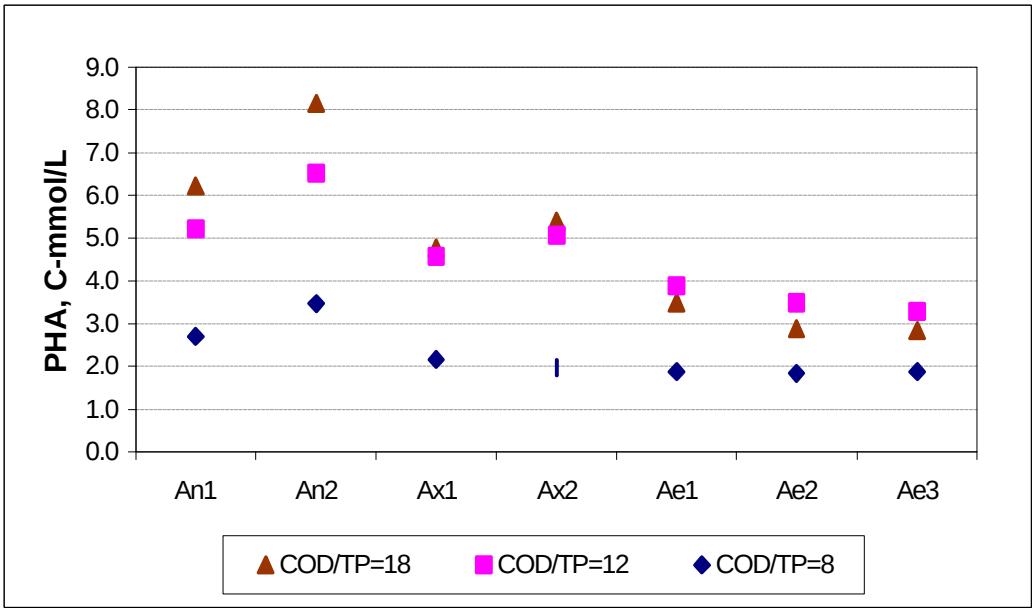

Figure 6. Observed PHA concentrations through the system at steady state.

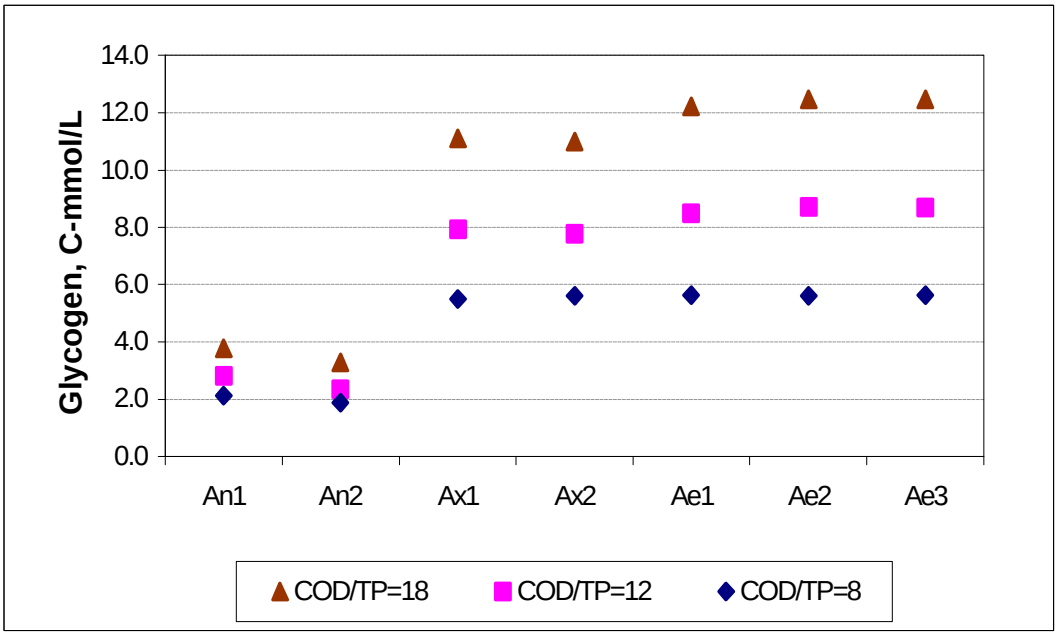

Figure 7. Observed glycogen concentrations through the system at steady state.

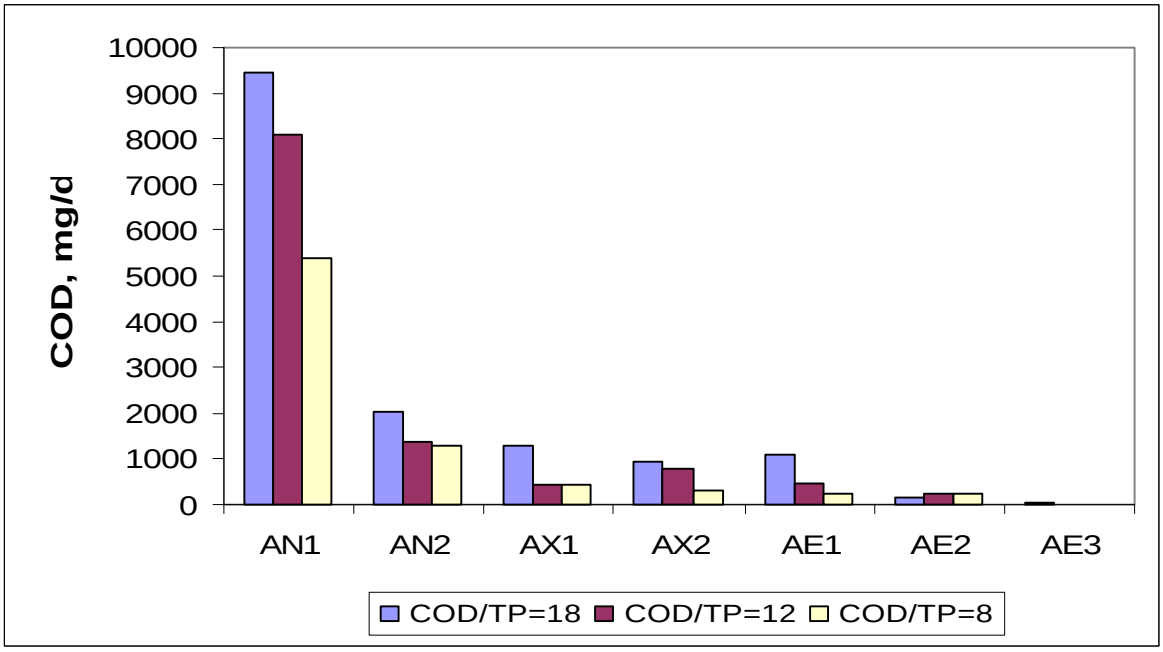

Figure 8. COD mass balance through the system at steady state.

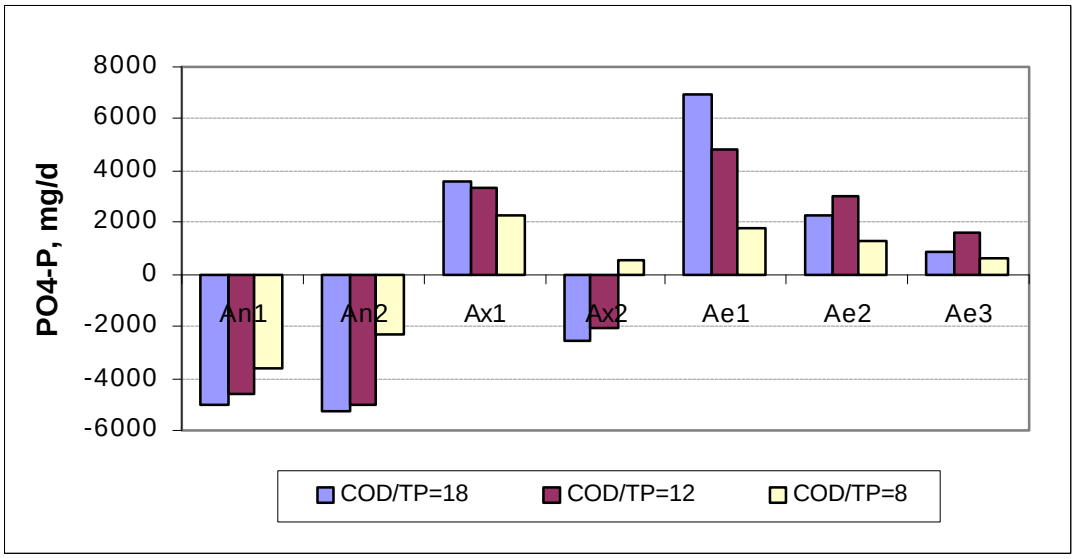

Figure 9. Phosphorus mass balance through the system at steady state.

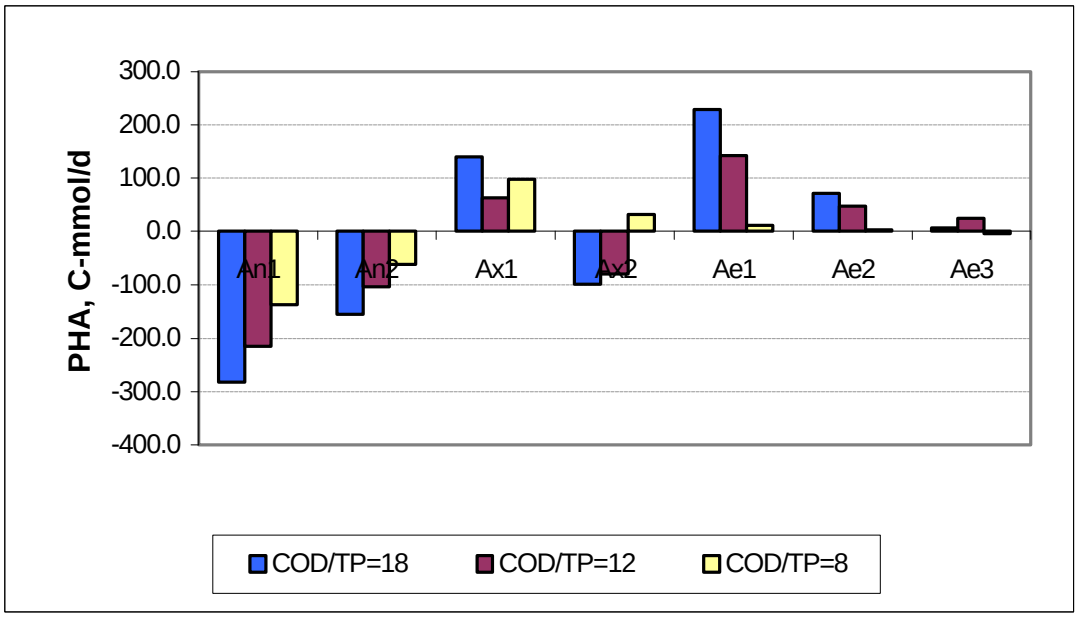

Figure 10. PHA mass balance through the system at steady state.

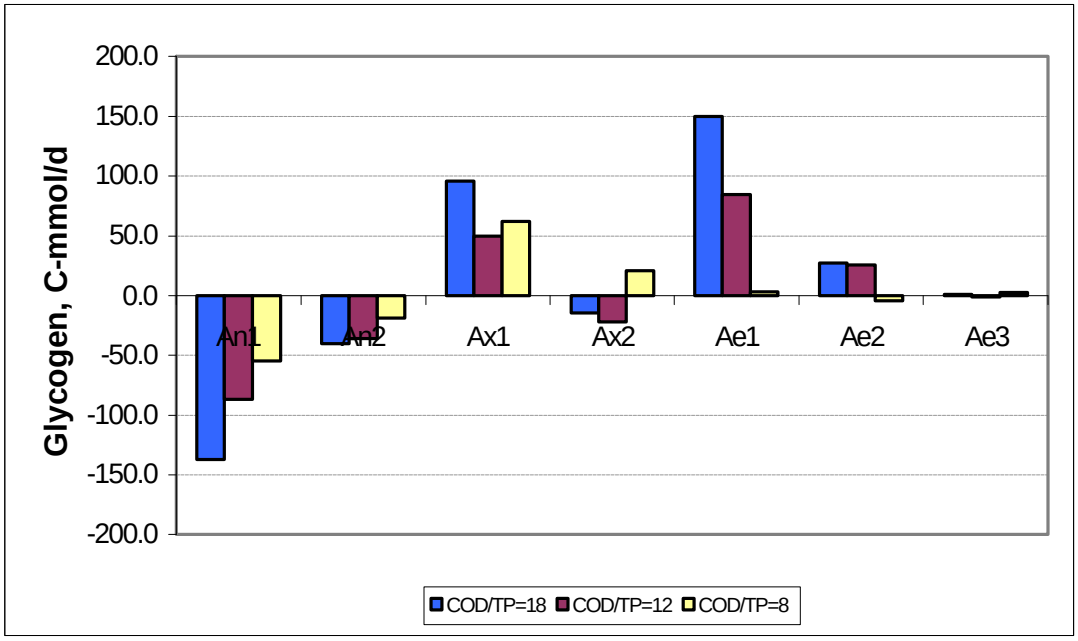

Figure 11. Glycogen mass balance through the system at steady state.

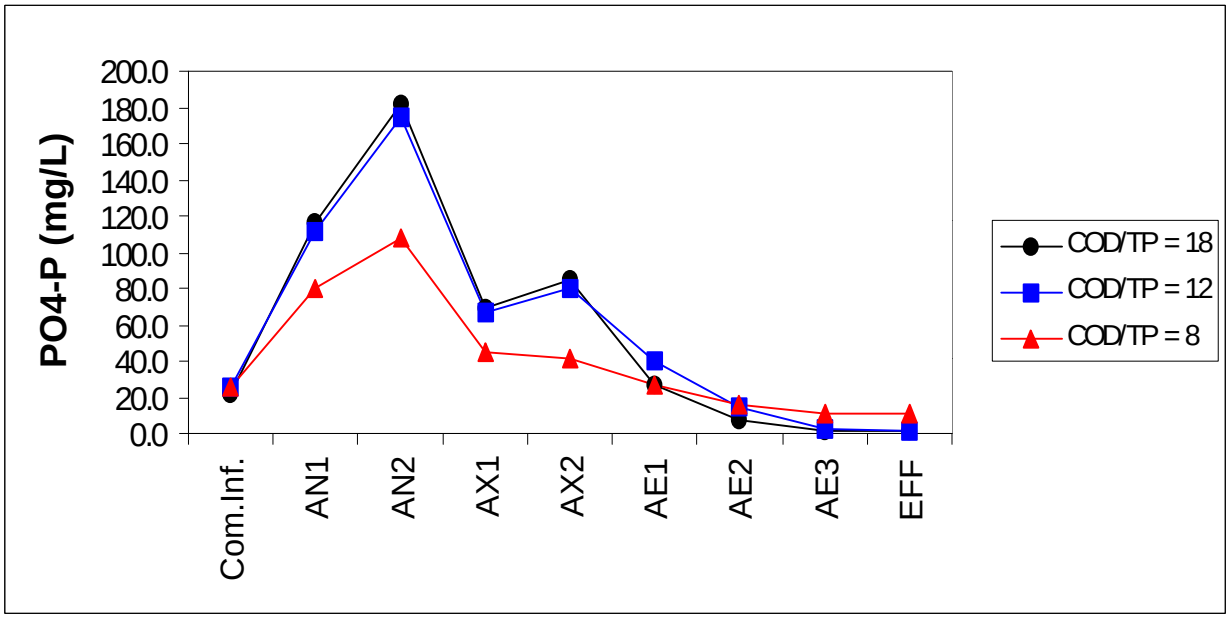

Figure 12. Average phosphate concentrations for the three periods of operation

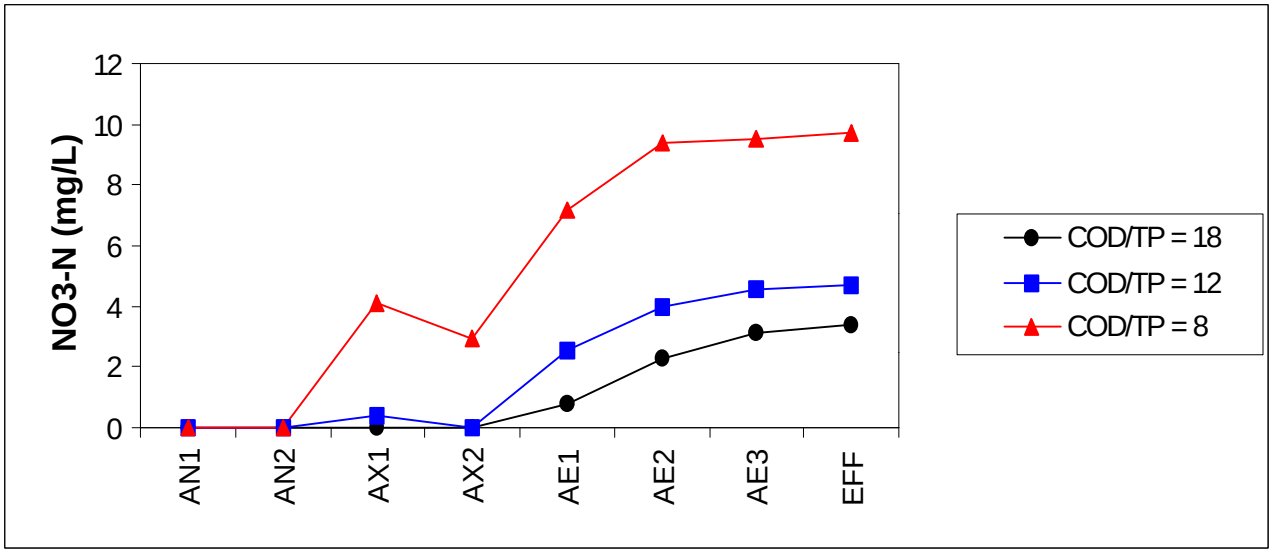

Figure 13. Average NO3-N concentration in the system

Mino *et al.* (1987) and Arun *et al*. (1988) first proposed the regeneration of glycogen in the aerobic zone. This idea was further supported by Smolders *et al.* (1994) and other researchers. The data obtained in this study clearly favors the Mino model and all others that consider glycogen as a key parameter involved in the biochemical metabolism of EBPR. At the lowest COD/TP ratio, the glycogen concentrations remained almost the same throughout the aerobic zone. This, however, does not bring any doubt on the Mino model. The lower COD case is representative of extended aeration conditions wherein the biomass generated by growth is destroyed by endogenous decay. During endogenous respiration, glycogen cannot be regenerated from PHA, as PHA breakdown was only sufficient to support maintenance energy needs of the cells as mentioned earlier. It is also known that during extended aeration microbial cells will maintain a minimum amount of intracellular storage material. Thus a minimum value exists for PHA which was found to be around 2.0 C-mmol/L during this investigation.

Table 4. P-release and Intracellular storage products produced per acetate taken up (C-mmol/C-mmol) observed in this study and those given in other published studies

	P-Release	**PHA Stored**	**Glycogen Utilized**
Mino *et al.* (1987)	0.145	0.48	0.99
Comeau *et al.* (1987)	0.70 - 0.80	1.26 - 1.40	-
Mino *et al.* (1987)	0.39	0.55	1.02
Arun *et al.*(1988)	0.21 - 0.39	0.32 - 0.74	0.06 - 0.48
Satoh *et al.*(1992)	0.44	1.36	0.60
Smolders *et al.*(1994)	0.26 - 0.76	1.26 - 1.36	-
Pereira *et al.* (1996)	0.16	1.48	0.70
Liu *et al.*(1994)	0	-	1.08 - 1.47
Christensson *et al.* (1998)	0.44	1.12	0.56
This study			
COD/TP = 18	0.81	1.06	0.43
COD/TP = 12	0.93	0.95	0.36
COD/TP = 8	0.80	0.83	0.31

The results obtained in this study compared reasonably well with the results obtained by other researchers (Table 4). One has to keep in mind that the values listed in Table 4 were observed in systems operated with different feed, influent COD and influent TP values. This has resulted in different biomass compositions in terms of poly-P and non-poly-P organisms, which can both store PHA, and those that rely on glycogen storage for P-storage metabolism and those that do not. Different sludges enriched with different microbial populations most definitely have different energy needs and different means for its supply. As mentioned in Maurer *et al*. (1997) anaerobic use of carbohydrates is not entirely due to the demand for reducing equivalents, but also for energy production. According to Table 4, the sludge enriched during this study relied more on poly-P break down for energy than on glycogen metabolism.

CONCLUSIONS

When the results of this study are examined, the internal carbon storage product glycogen appears to be directly involved in excess biological phosphorus removal metabolism. As the feed COD/TP ratio was lowered through decreasing influent COD concentration, direct relationships between the stored PHA, consumed glycogen and released phosphorus amounts were found. Under COD limiting conditions (COD/TP = 8) insufficient PHA storage was accompanied with decreased P-release and glycogen consumption. Such a strong relationship between the storage units suggests a direct involvement of glycogen. However, the question that is raised by these results is whether the observed response is directly related to phosphorus removal metabolism, or is it the net sum of different responses of different microbial groups. In other words, is it the combined response of poly-P storers and glycogen storers, each of which can store PHAs under anaerobic conditions, but utilize different forms of energy to polymerize acetate? It can be that glycogen is still needed for poly-P storers to some extent. However, more research is needed to answer these questions. Another, conclusion of this study, linked to the results of Punrattanasin and Randall (1998), is that besides the feed composition in terms of the distribution and quantity of the present volatile fatty acids, the ratio of COD/TP bears a significant role in dictating the performance of the treatment system..

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