CHARACTERIZATION OF ENHANCED BIOLOGICAL PHOSPHORUS RELEASE AND REMOVAL BY ACTIVATED SLUDGE

Jasna Hrenović,[a] Yüksel Orhan[b] and Hanife Büyükgüngör[b]

[a] *University of Zagreb, Faculty of Science, Department of Botany, Rooseveltov trg 6, 10000 Zagreb, Croatia*
[b] *Ondokuz Mayis University, Engineering Faculty, Environmental Engineering Department, 55139 Samsun, Turkey*

Received 29-07-2003

Abstract

The aim of this study was to investigate the influence of various carbon sources and nitrogen compounds, applied at different initial concentrations of phosphorus, on the phosphorus release and removal in bioaugmented activated sludge system with enhanced biological phosphorus removal characteristics. The system performance for synthetic, as well for fresh municipal wastewater was examined. Successful anaerobic release and aerobic uptake of phosphorus were achieved with acetate and acetate plus propionate plus glucose as the carbon sources, whereas with glucose were significantly lower. Nitrogen compounds inhibited anaerobic release and aerobic uptake of phosphorus only in system with glucose as the carbon source. Phosphorus-accumulating bacteria *Acinetobacter calcoaceticus* presented a good capability of survival, multiplication and incorporation in the activated sludge flocks, in spite of the presence of other microorganisms in the activated sludge system.

Introduction

Enhanced biological phosphorus removal (EBPR) from wastewater, a biological alternative to chemical phosphate (o-P) precipitation, is based on the activity of P-accumulating microorganisms. The dominant bacteria in activated sludge systems are aerobic heterotrophs. P-accumulating bacteria are normally present in activated sludge, but in minority due to the low growth rate.[1] By the process of bioaugmentation, activated sludge is enriched with P-accumulating bacteria, and thus its potential to remove P from wastewater could be improved.

The exposure of sludge to alternating anaerobic and aerobic (or anoxic) conditions is necessary to provoke biological P uptake.[2] Bacteria from the genus *Acinetobacter* has become the model organism for EBPR since it was isolated from a P-removing activated sludge plant.[3] In the absence of oxygen, these bacteria transport volatile fatty acids (VFA, e.g. acetate, propionate) into the cell and subsequently convert and store these as

poly-hydroxy-alkanoates (PHA). The energy for this transport and storage is supplied by hydrolysis of intracellularly stored polyphosphates (poly-P) to o-P, which is released from cell to the liquid. Under aerobic conditions, stored PHA would be catabolised, using oxygen as electron acceptor to generate energy for the cell growth, maintenance, glycogen formation and poly-P synthesis, resulting in the o-P uptake in a quantity greater than the amount previously released.[4-7] Various biochemical models[4-7] have been proposed to explain the EBPR mechanisms, and these models agree that VFA (especially acetate) play a key role as a substrate in EBPR mechanism. In general, anaerobic conditions alone are not able to induce P-release. The P-release phenomenon is primarily dependent on the nature of the feed rather than the anaerobic condition as such. P-accumulating bacteria release phosphorus under anaerobic, anoxic, and aerobic conditions when VFA are present.[8] Chemical oxygen demand (COD) level in wastewater does not have to be the crucial factor in EBPR process if the influent entering the anaerobic zone contains sufficient amount of VFA. EBPR efficiency is significantly affected by the increase of glucose fraction in influent, due to the dominance of glycogen accumulating bacteria labelled as G-bacteria,[9,10] which take up organic substrates in the anaerobic phase without P release. Furthermore, G-bacteria were reported to be able to dominate even in acetate fed sludge.[9] In contrast, good EBPR performance was observed in activated sludge fed with glucose.[11] Except glucose, the inhibitory effect of nitrate[12] and nitrite[13] on the anaerobic P release was reported, due to their conversion to nitric oxide, which inhibits adenylate kinase in the P-accumulating bacteria.[14]

The aim of this study was to investigate the effect of specific carbon sources or their combination (i.e. acetate, propionate and glucose) and nitrogen compounds, applied at different initial concentrations of P, on the P release and uptake in bioaugmented activated sludge system with EBPR characteristics.

Experimental

Microorganism

A strain of P-accumulating bacterium *Acinetobacter calcoaceticus* (DSM 1532), deposited in the German Collection of Microorganisms and Cell Cultures, was received as a lyophilised culture. Strain was maintained on nutrient agar medium, subcultured monthly and stored at 4 °C.

Synthetic wastewater

The compositions of the synthetic wastewaters used to simulate the sewage are reported in Table 1. The concentration of KH_2PO_4, the only sole source of P in tests, was 4, 44, 220 or 440 mg/L, giving a four final total P (P-tot) concentration range: 2.60±1.03, 10.70±3.13, 57.42±4.61 and 111.92±19.52 mg/L. The pH of the synthetic wastewater was adjusted to 7.01±0.01 with 1 M NaOH or 1 M HCl before autoclaving (121 °C/15 min). Some experiments were carried out for fresh municipal wastewater (*H*) - influent from the wastewater treatment plant of Ondokuz Mayis University Campus in Samsun, Turkey. The average composition of municipal wastewater was (in mg/L): P-tot 5.33; NH_4-N 32.96; NO_3-N 31.04; COD 1430; pH 7.02. The fresh municipal wastewater was filtered trough the technical filter paper and without autoclaving used for experiments.

Table 1. Composition of the synthetic wastewaters.

Component (mg/L)	A	B	C	E	F	G
Acetic acid	260	500	-	260	500	-
Sodium propionate	40	-	-	40	-	-
Glucose	40	-	500	40	-	500
Peptone	100	100	100	100	100	100
$MgSO_4$	10	10	10	10	10	10
$CaCl_2$	6	6	6	6	6	6
KCl	30	30	30	30	30	30
Yeast extract	20	20	20	20	20	20
NH_4Cl	-	-	-	38	38	38
KH_2PO_4	Variable (4 – 440)					

Experimental operation

According to the preliminary test of P release and uptake kinetics, laboratory-scale batch experiments were carried out in alternated 24 h anaerobic/24 h aerobic stages in 500-mL Erlenmeyer flask. The fresh activated sludge was obtained from the aeration tank of a municipal wastewater treatment plant and acclimatised for two weeks in the mineral solution with mixing and aerating at room temperature. The wastewater was inoculated with activated sludge bioaugmented with P-accumulating bacteria *A. calcoaceticus*. The flasks, set up as triplicate, were sealed with a gum cap[1] and anaerobically incubated in a water bath controlled with thermostat and shaker at 20.0±0.1 °C. Preliminary dissolved oxygen tests demonstrated that agitation speed of 70

rpm assured complete mixing while, at the same time, avoiding surface turbulence responsible for possible conditions of local aerobiosis. In the following aerobic phase, reactors were shaken at 70 rpm and aerated (4 L/min) at 20.0±0.1 °C.

Analytical methods

All measurements were done according to the APHA et al.[15] pH-values were measured with Crison micro pH 2000 pH-meter. Dissolved oxygen and temperature were controlled with Jenway 9071 dissolved oxygen meter.

The samples were filtered before P-tot and nitrogen forms measurements through the Sartorius nitrocellulose filters pore diameter 0.2 μm. P-tot concentration in water was measured after persulfate oxidation by stannous chloride method in a Cary UV-visible spectrophotometer at 690 nm. P-tot concentration in activated sludge was determined after perchloric acid digestion. A medium pH above 7.8 would have determined that P had precipitated as either calcium or magnesium salts, which could erroneously account for the decrease in P-tot concentration in the sample. Therefore, the pH of such samples was adjusted between 6.8 and 7.5 before P-tot measurement. Ammonia (NH_4-N) concentration in water was measured by nesslerization method in a Hitachi 110-40 spectrophotometer at 425 nm. Nitrate (NO_3-N) concentration in water was measured by ultraviolet spectrophotometric screening method in a Hitachi 110-40 spectrophotometer at 220 nm. COD was determined by open reflux method.

Mixed liquor suspended solids (MLSS) were determined after drying at 105 °C for one hour. Sludge volume index (SVI) was calculated from the settled sludge after 30 min and MLSS. Bacterial number of *A. calcoaceticus* was determined as colony forming units (CFU). Serial dilutions (10^{-1} to 10^{-8}) of the one mL sample were prepared. Dilutions (0.1 mL) were plated (spread plate method) onto nutrient agar to obtain a viable cell count. Plates were incubated at 30.0±0.1 °C for 72 h. After period of incubation, colonies were counted and CFU/L were calculated.

Data analysis

The statistical analyses were done using the program Statistica Version 6.0.[16] The results were set up as variables with different types of wastewaters. Data of this type are independent; therefore ordinary Student's t-test was performed. The null hypothesis

tested by the analysis was that the reactors with different wastewaters showed no difference in performance. Results were taken to be significant at the 5% level (p=0.05). The correlation between variables was estimated using the Pearson linear correlation.

Results

The amount of P-tot anaerobically released and aerobically removed was depended on the wastewater composition and initial P-tot concentrations (Table 2). The amount of P-tot released and removed increased by increasing the initial P concentration. The highest amount of P-tot released and removed was observed for wastewater with the

Table 2. The amount of P-tot anaerobically released and aerobically removed by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent P-tot (mg/L)		
A	1.93	13.24	49.35	98.48
B	3.92	15.25	62.32	114.31
C	3.94	13.02	55.28	91.06
E	1.18	9.40	59.72	112.00
F	2.28	8.40	57.74	108.10
G	2.10	8.90	60.08	147.55
H	2.84	6.70		
		Anaerobic stage, P-tot released (mg/L)		
A	1.59	6.62	28.64	15.33
B	4.43	11.02	26.36	21.89
C	1.99	4.56	11.68	10.19
E	0.97	3.83	17.57	22.80
F	2.01	3.94	36.03	21.22
G	0.47	0.53	1.84	3.43
H	0.89	2.50		
		Aerobic stage, P-tot removed (mg/L)		
A	1.40	7.12	18.28	12.77
B	2.96	8.64	23.16	23.62
C	2.19	6.05	14.80	9.22
E	0.55	4.11	10.83	14.39
F	1.50	4.55	17.55	23.97
G	0.49	1.64	8.50	8.51
H	1.40	2.74		

acetate as the carbon source (*B* and *F*), following acetate plus propionate plus glucose (*A* and *E*) and glucose (*C* and *G*). The released amount of P-tot was significantly ($p<0.05$) lower for wastewater containing glucose (*C* and *G*) in relation to wastewaters containing VFA (*A*, *B*, *E*, *F*). The removed amount of P-tot was significantly ($p<0.05$) higher for wastewater containing acetate (*B* and *F*) in relation to other types of wastewater. The P-tot released was higher for wastewater without nitrogen compounds (as NH_4Cl) addition, but significant ($p<0.05$) just for wastewater containing glucose.

Table 3. MLSS at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent MLSS (g/L)		
A	1.23	1.41	1.11	0.62
B	1.51	1.63	1.46	1.46
C	1.46	1.69	2.00	1.82
E	0.62	0.98	0.70	1.29
F	1.00	0.73	1.37	1.14
G	1.01	1.19	1.64	1.20
H	1.22	1.20		
		Anaerobic stage, MLSS (g/L)		
A	2.91	1.83	2.47	1.69
B	1.77	1.81	1.68	1.62
C	2.08	1.95	1.38	1.21
E	1.06	1.98	1.03	1.74
F	1.10	0.96	1.38	1.45
G	0.96	0.95	1.36	1.06
H	2.01	1.45		
		Aerobic stage, MLSS (g/L)		
A	2.92	2.55	2.66	2.34
B	2.74	2.80	2.82	1.67
C	1.93	2.12	2.17	2.25
E	1.83	2.03	1.55	1.88
F	1.31	1.22	1.42	1.73
G	1.10	0.97	1.50	1.71
H	2.58	1.67		

The P-tot removed was significantly ($p<0.05$) higher for all types of wastewater without nitrogen compounds addition. The amount of P-tot anaerobically released and aerobically removed for the fresh municipal wastewater (*H*), in comparison with the

synthetic wastewaters by lower P concentrations, was in average lower, but higher than with wastewater *G* (Table 2). The losses of P-tot in the systems where in average low (0.89±0.75 mg/L), as indicated by a calculated difference between P-tot removed from water and P-tot accumulated in activated sludge at the end of aerobic phase.

MLSS increased during the anaerobic and aerobic phase (Table 3). The increase during the anaerobic phase was something, and during the aerobic phase significantly ($p<0.05$) higher for wastewater without nitrogen compounds addition. During the anaerobic phase for wastewater *C* (P concentration 3 and 4) and *G* (all P concentrations), and during the aerobic phase for wastewater *G* (P concentration 2 and 3), decay of activated sludge biomass was observed (Table 3).

Table 4. Decrease of SVI at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent SVI (mL/g)		
A	31	34	31	41
B	23	27	25	31
C	23	37	51	51
E	51	50	51	52
F	50	54	34	35
G	41	35	30	42
H	36	47		
		Anaerobic stage, SVI decrease (mL/g)		
A	≤ 5	≤ 5	≤ 5	≤ 5
B	≤ 5	≤ 5	≤ 5	≤ 5
C	≤ 5	≤ 5	≤ 5	14
E	16	11	≤ 5	≤ 5
F	≤ 5	≤ 5	≤ 5	≤ 5
G	9	≤ 5	8	16
H	≤ 5	12		
		Aerobic stage, SVI decrease (mL/g)		
A	≤ 5	≤ 5	≤ 5	11
B	≤ 5	≤ 5	≤ 5	≤ 5
C	≤ 5	8	11	18
E	24	26	20	27
F	12	11	≤ 5	≤ 5
G	10	6	9	17
H	7	17		

SVI decreased during the anaerobic and aerobic phase (Table 4), and just for the wastewater *C* (P concentration 1) a slight increase was observed. Smaller decreasing or increasing of SVI than 5 mL/g was considered negligible when compared to experimental error or measurement uncertainty. Significantly ($p<0.05$) higher SVI decrease was observed for wastewater with nitrogen compounds addition.

A. calcoaceticus cells presented rapid adaptation ability to either anaerobic or aerobic conditions during shifting from one environment to the other. CFU of *A. calcoaceticus* increased for one order of magnitude at the end of anaerobic stage, and one order of magnitude more at the end of aerobic stage (Table 5). Significantly ($p<0.05$) higher increase was observed for wastewater with nitrogen compounds addition.

Table 5. Increase in CFU of *Acinetobacter calcoaceticus* at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
	Influent CFU (10^9/L)			
A	2.39	12.31	3.38	12.31
B	10.80	10.92	11.00	11.10
C	3.61	4.83	5.21	6.15
E	3.60	6.96	4.08	7.44
F	6.20	6.80	7.26	7.07
G	3.82	3.10	5.17	5.80
H	6.04	8.71		
	Anaerobic stage, CFU increase (10^{10}/L)			
A	4.57	5.27	4.67	6.77
B	5.92	5.31	2.10	1.69
C	0.24	1.32	0.48	0.29
E	2.64	7.15	1.40	4.74
F	4.38	8.72	6.28	7.40
G	7.02	6.49	7.19	5.42
H	1.52	0.53		
	Aerobic stage, CFU increase (10^{11}/L)			
A	5.98	5.28	7.17	1.92
B	2.77	1.79	2.69	1.51
C	2.84	2.03	0.85	0.96
E	4.14	6.17	4.86	5.93
F	5.22	5.37	6.29	6.03
G	4.76	5.65	4.19	3.14
H	1.78	4.31		

P-release and uptake rates per MLSS (Table 6) increased with an increase in the initial P-tot concentration. Higher P-release resulted in higher P-uptake rates ($r=0.86$, $p<0.05$). The highest ($p<0.05$) P-release and uptake rates per MLSS were observed for wastewater with the acetate as the carbon source (*B* and *F*), following acetate plus propionate plus glucose (*A* and *E*) and glucose (*C* and *G*). Nitrogen compounds addition resulted in the something higher rates for the wastewater containing VFA (*A*, *B*, *E*, *F*), and significantly ($p<0.05$) lower rates for the wastewater containing glucose (*C*, *G*). P-release and uptake rates for the fresh municipal wastewater (*H*), in comparison with the synthetic wastewaters by lower P concentrations, were in average lower, but higher than with wastewater *G* (Table 6).

Table 6. Phosphorus release and uptake rates per MLSS by different types of wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
	P-release rate (mg P-tot/g MLSS)			
A	0.55	3.62	11.59	9.07
B	2.50	6.09	15.69	13.51
C	0.96	1.31	8.46	8.49
E	0.92	1.93	17.06	13.10
F	1.83	4.10	26.11	14.63
G	0.68	0.56	1.35	3.24
H	0.44	1.72		
	P-uptake rate (mg P-tot/g MLSS)			
A	0.48	2.78	6.87	5.46
B	1.08	3.09	8.21	14.14
C	1.13	2.98	6.82	4.10
E	0.30	2.02	6.99	7.65
F	1.15	3.73	12.36	13.86
G	0.45	0.59	5.67	4.98
H	0.54	1.64		

P-release and uptake rates per CFU of *A. calcoaceticus* (Table 7) increased with an increase in the initial P-tot concentration. The P-uptake rates were lower than P-release rates. Higher P-release resulted in higher P-uptake rates ($r=0.84$, $p<0.05$). The highest P-release and P-uptake rates were achieved for wastewater *C*, which was due to the poor bacterial multiplication. The lowest rates were obtained for wastewater *G*. For the

wastewater containing acetate plus propionate plus glucose (*A*) the nitrogen compounds addition significant ($p<0.05$) increased P-release and uptake rates, but for wastewater containing acetic acid (*B*) and glucose (*C*) by the nitrogen compounds addition rates were lower ($p<0.05$). For the fresh municipal wastewater (*H*), in comparison with the synthetic wastewaters by lower P concentrations, P-release and uptake rates were in the lower range. The amount of P-tot anaerobically released and aerobically removed showed better correlation with P-release and uptake rates per MLSS ($r=0.95$ and 0.94, $p<0.05$) than per CFU of *A. calcoaceticus* ($r=0.60$ and 0.68, $p<0.05$).

Table 7. Phosphorus release and uptake rates per CFU of *Acinetobacter calcoaceticus* by different types of wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
	P-release rate (10^{-10} mg P-tot/CFU)			
A	0.33	1.02	5.73	1.92
B	0.63	1.72	8.24	7.82
C	3.32	2.53	11.68	11.32
E	0.32	0.49	9.76	4.16
F	0.40	0.42	5.15	2.62
G	0.06	0.08	0.24	0.57
H	0.42	1.04		
	P-uptake rate (10^{-11} mg P-tot/CFU)			
A	0.23	0.40	2.45	6.26
B	1.03	4.55	8.27	14.58
C	0.76	2.91	16.45	9.04
E	0.30	2.02	6.99	7.65
F	0.28	0.84	2.76	3.93
G	0.10	0.29	2.00	2.66
H	0.76	0.62		

The amount of COD anaerobically removed (Table 8) was the highest for wastewater containing acetate, following acetate plus propionate plus glucose and only glucose. For the wastewater *G*, increase in COD during the anaerobic stage was observed (Table 8), which was probable connected with the decay of activated sludge biomass. The amount of COD anaerobically removed was significantly ($p<0.05$) higher for wastewater without nitrogen compounds addition. Aerobic COD removal showed the same relationships as in anaerobic stage, but the COD removal was observed also for

wastewater *G* (Table 8). For the fresh municipal wastewater (*H*), COD removal was in range of other synthetic wastewaters (*A*, *B*, *C*, *E*, *F*).

Table 8. Decrease of COD at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent COD (mg/L)		
A	1851	2003	1953	2009
B	2074	2140	2138	2142
C	1753	1756	1758	1749
E	2122	1744	2126	1741
F	1881	1889	1807	1800
G	1750	1756	1841	1777
H	1540	2741		
		Anaerobic stage, COD decrease (mg/L)		
A	684	1448	1153	1244
B	1576	1477	1547	1641
C	112	314	209	321
E	554	270	376	289
F	811	779	725	893
G	-483	-846	-303	-852
H	370	824		
		Aerobic stage, COD decrease (mg/L)		
A	1281	1598	1563	1644
B	1648	2009	1921	1972
C	1312	1296	1362	1341
E	1980	1186	1923	1093
F	1566	1504	1510	1245
G	1109	1049	1291	1010
H	1331	1989		

At the beginning of experiments, the wastewaters without the extra nitrogen compounds addition in a form of NH_4Cl (*A*, *B*, *C*) contained low concentrations of ammonia (1.10 mg NH_4-N/L) and nitrate (0.55 mg NO_3-N/L). The synthetic wastewaters with nitrogen compounds addition (*E*, *F*, *G*), as well as the fresh municipal wastewater (*H*), contained significant amounts of ammonia and nitrate (Table 9,10). At the end of anaerobic phase, high increases of ammonia concentrations with nitrate concentrations near zero suggested that all nitrates were converted to ammonia (Table 9,10).

Table 9. Ammonia (NH_4-N) concentrations at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent NH_4-N (mg/L)		
E	17.82	25.87	17.52	25.88
F	15.53	14.07	11.99	15.86
G	38.48	35.04	17.03	17.18
H	28.74	30.15		
		Anaerobic stage, NH_4-N (mg/L)		
E	62.39	90.81	64.30	87.91
F	75.93	69.41	73.57	71.79
G	63.68	65.72	52.94	60.32
H	69.90	86.89		
		Aerobic stage, NH_4-N (mg/L)		
E	37.92	43.49	44.84	45.56
F	24.31	22.89	25.72	21.46
G	53.14	47.34	47.88	52.41
H	19.25	20.27		

Table 10. Nitrate (NO_3-N) concentrations at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent NO_3-N (mg/L)		
E	45.72	49.26	44.68	53.19
F	44.53	42.02	40.92	42.58
G	45.40	58.30	46.53	45.86
H	34.28	36.27		
		Anaerobic stage, NO_3-N (mg/L)		
E	0.86	0.10	0.20	0.12
F	1.55	0.53	1.14	1.43
G	0.99	1.03	1.73	0.95
H	0.67	0.25		
		Aerobic stage, NO_3-N (mg/L)		
E	18.02	21.89	23.76	25.40
F	11.40	12.17	20.08	17.60
G	15.43	17.40	20.22	24.98
H	30.02	35.61		

Ammonia concentrations at the end of aerobic phase in reactors with synthetic wastewaters were higher than initial concentrations, since in reactors with municipal wastewater were lower (Table 9). Nitrate concentrations at the end of aerobic phase were

lower than initial concentrations for all types of wastewater (Table 10). Decrease of ammonia and increase of nitrate concentrations in relation to the concentrations at the end of anaerobic phase, suggested that during the aerobic phase ammonia was converted to nitrate. In each system, decreases of the pH values during the anaerobic phase (especially with wastewater *C* and *G*) and increases during the aerobic phase were observed (Table 11).

Table 11. pH-values at the end of anaerobic and aerobic phase, in relation to the initial value, by different wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		Influent pH		
A	7.00	7.02	7.01	7.01
B	7.00	7.00	7.01	7.00
C	7.01	7.02	7.01	7.00
E	7.01	7.02	7.00	7.02
F	7.00	7.02	7.01	7.00
G	7.02	7.03	7.01	7.02
H	7.03	7.02		
		Anaerobic stage, pH		
A	6.46	6.51	6.64	6.82
B	6.57	6.56	6.54	6.63
C	5.13	4.93	4.96	5.12
E	6.20	6.34	6.26	6.48
F	6.32	6.27	6.48	6.51
G	5.16	5.20	5.14	5.40
H	6.46	6.47		
		Aerobic stage, pH		
A	8.68	8.52	8.60	8.40
B	8.37	8.45	8.46	8.33
C	8.63	8.38	8.59	8.57
E	8.47	8.75	8.37	8.59
F	8.44	8.50	8.51	8.53
G	8.46	8.41	8.25	8.35
H	8.53	8.55		

Discussion

In this study, significantly lower P-release and uptake were observed with glucose as the carbon source, than with VFA or a combination of VFA and glucose. This was expected, since the activated sludge used was bioaugmented with P-accumulating

bacteria *A. calcoaceticus*. It is generally accepted that poly-P organisms are unable to direct utilize glucose under anaerobic condition in the EBPR system, and moreover, glucose is even detrimental to the EBPR unless it is first converted to VFA by non-poly-P microorganisms (acidogenic bacteria).[17] Glucose cannot serve as a substrate for growth and multiplication of *A. calcoaceticus* in aerobic conditions. This organism can only oxidize glucose to gluconic acid, which accumulates as a dead-end product in the culture media.[18] The tested strain of *A. calcoaceticus* showed significantly poorer cell multiplication with glucose than with VFA as the carbon source. The poor bacterial multiplication was the reason of calculated high P-release and uptake rates per CFU of *A. calcoaceticus*. P-release showed positive correlation with P-uptake rates. In contrast, for the pure culture of other *A. calcoaceticus* strains cultured on acetic, butyric and propionic acid, P-uptake in the aerobic stage was not depended on the P-release rate.[19]

With glucose as the carbon source, poor anaerobic substrate uptake (expressed as COD) accompanied with inhibited P release were observed. These suggest that glucose had an inhibitory effect on the still present P-release and following P-uptake mechanism. From the other unwanted occurrences, with wastewater containing glucose (*C*), during the anaerobic phase by higher initial P concentrations a decay of activated sludge biomass and slight increase of SVI during the anaerobic and aerobic phases by low initial P concentrations were observed. With wastewater containing glucose and added nitrogen compounds (*G*), a decay of activated sludge biomass in the anaerobic and in some cases in aerobic phase, connected with the increase of COD in the supernatant were observed.

For the fresh municipal wastewater, in comparison with synthetic wastewaters, P-release and uptake were in the lower range. Nevertheless, the COD removal was good, suggesting that the municipal wastewater contained enough amount of VFA. *A. calcoaceticus* cells presented a good capability of survival, multiplication and incorporation in the activated sludge flocks, in spite of the presence of other microorganisms in the filtered municipal wastewater.

From the practical point of view, for the wastewater containing VFA by usually P concentrations in municipal wastewater of 10 mg P-tot/L, the final percentages of P-tot removal (which decreased by increasing the initial P-tot concentration) were higher than 50% (Table 12), since by the shock P concentrations of more than 100 mg P-tot/L were

higher than 20%. Treating fresh municipal wastewater, P-tot removal of more than 40% was achieved. The percentages of COD removal were not depended on the initial P-tot concentrations (Table 12). High (80%) COD removals were achieved for all types of wastewaters, and significantly lower were just for wastewater *G* (62%).

Table 12. Treatment efficiency of P-tot and COD removal at the end of aerobic phase by different types of wastewaters and four initial P-tot concentrations.

Wastewater type	Initial P-tot concentration range			
	Concentration 1	Concentration 2	Concentration 3	Concentration 4
		P-tot removal (%)		
A	72.54	53.78	37.04	12.97
B	75.51	56.66	37.16	20.66
C	55.58	46.47	26.77	10.13
E	46.61	43.72	18.13	12.85
F	65.62	54.11	30.36	22.29
G	23.56	18.42	14.15	5.79
H	49.47	41.37		
		COD removal (%)		
A	69.21	79.78	80.03	81.83
B	79.46	93.88	89.85	92.06
C	74.84	73.80	77.47	76.67
E	93.78	68.49	90.45	62.78
F	83.25	79.62	83.56	69.17
G	63.37	59.73	70.12	56.83
H	86.43	72.56		

Activated sludge biomass production per mass of COD removed at the end of aerobic phase was higher in reactors without nitrogen compounds addition, compared with the reactors where nitrogen compounds were added. The mean values for the wastewaters *A*, *B* and *C* amounted 1.01, 0.54 and 0.27 kg MLSS/kg COD, whereas for the wastewaters *E*, *F* and *G* were 0.64, 0.24 and 0.07, respectively. The mean activated sludge biomass production for fresh municipal wastewater amounted 0.64 kg MLSS/kg COD. Carbon incorporation in the biomass was higher with wastewaters without nitrogen compounds addition (61%) than with wastewaters where nitrogen compounds were added (32%). In the system with wastewater *G* carbon incorporation in the biomass was just 7%, which indicated that activated sludge in the aerated stage was in endogenous phase and that decay was predominant on the biomass synthesis.

The inhibitory effect of oxidised nitrogen forms such as nitrate and nitrite on the anaerobic P release and as a consequence, on the aerobic P uptake is reported in the literature.[12-14] In our study, significant inhibitory effect of nitrogen compounds addition on the P-release and uptake was observed only for wastewater containing glucose as the carbon source. The wastewaters tested contained at the beginning of experiments nitrogen compounds in the form of ammonia and nitrate. After 20 min of activated sludge inoculation, no dissolved oxygen was detected in the mixed liquor. After two hours of experiment, nitrate concentrations fell down near zero, suggesting that anaerobic conditions were achieved without using any inert gas. During the anaerobic conditions, nitrates were converted to ammonia by the process of backward ammonification, which was carried out by microorganisms that respire with nitrate as the terminal electron acceptor. No denitrification process observed is explained by the dominance of *A. calcoaceticus* in activated sludge, which are not able to reduce nitrate to the nitrogen gas.[20] In the following aerobic conditions, ammonia was converted to nitrate by the process of nitrification, for which nitrifying bacteria different of *A. calcoaceticus* were responsible.

The pH-values decreased during the anaerobic and increased during the aerobic phase, due to the anaerobic release and aerobic uptake process. A reported explanation for the pH increase during aeration[4] is that air stripping of carbon dioxide took place.

Conclusions

This work highlighted the importance of wastewater composition, such as the presence of the type of carbon source, nitrogen compounds and P concentration, in the estimation of treatment efficiency of activated sludge with EBPR characteristics. The higher the initial concentration of P in the wastewater, the higher the amount of P was finally removed, but taking into consideration that this amount was lower in percent. Successful EBPR was achieved with VFA and VFA plus minor part of glucose as the carbon sources. With the glucose as the sole carbon source, EBPR characteristics of activated sludge were inhibited. As a result of the system efficiency, the final percentages of P removal for wastewater containing VFA or VFA plus glucose were in average 41%, since for wastewater containing glucose were 25%. The system containing VFA or VFA plus glucose as the carbon source was tolerable to the presence of nitrogen

compounds (18 mg NH_4-N/L and 45 mg NO_3-N/L). In the system containing glucose as the sole carbon source, the presence of nitrogen compounds (30 mg NH_4-N/L and 49 mg NO_3-N/L) leaded to the deterioration of EBPR characteristics, resulting in the poorest performance among all reactors. The EBPR activated sludge showed a satisfied performance in the system containing the fresh municipal wastewater of complex composition. P-accumulating bacteria *A. calcoaceticus*, which are crucial in scavenging for P in EBPR process, presented a good capability of multiplication, in spite of the presence of other microorganisms in the activated sludge or fresh municipal wastewater.

Nomenclature

CFU	Colony forming units
COD	Chemical oxygen demand
EBPR	Enhanced biological phosphorus removal
MLSS	Mixed liquor suspended solids
o-P	Orthophosphate
P	Phosphorus
PHA	Poly-hydroxy-alkanoates
poly-P	Polyphosphates
P-tot	Total phosphorus
SVI	Sludge volume index
VFA	Volatile fatty acids

Acknowledgements

This work was supported by a Grant from the Republic of Turkey, Ministry of National Education.

References

1. M. Sidat, F. Bux, H. C. Kasan, *Water SA* **1999**, *25*, 175–179.
2. J. P. Kerrn-Jespersen, M. Henze, *Wat. Res.* **1993**, *27*, 617–624.
3. G. W. Fuhs, M. Chen, *Microb. Ecol*. **1975**, *2*, 119–138.
4. Y. Comeau, B. Rabinowitz, K. J. Hall, W. K. Oldham, *Journal WPCF* **1987**, *59*, 707–715.
5. D. Brdjanovic, M. C. M. van Loosdrecht, C. M. Hooijmans, T. Mino, G. J. Alaerts, J. J. Heijnen, *Wat. Sci. Tech.* **1998**, *37*, 541–547.
6. T. Mino, M. C. M. van Loosdrecht, J. J. Heijnen, *Wat. Res.* **1998**, *32*, 3193–3207.
7. G. J. J. Kortstee, K. J. Appeldorn, C. F. C. Bonting, E. W. J. van Niel, H. W. van Veen, *Biochem.-Moscow* **2000**, *65*, 332–340.
8. A. Gerber, R. H. Villiers, E. S. de Mostert, C. J. Riet, The phenomenon of simultaneous phosphate uptake and release, and its importance in biological nutrient removal. In: R. Ramadori (Ed.), *Biological phosphate removal from wastewaters.* Pergamon Press, Oxford, 1987, p 440.
9. J. S. Cech, P. Hartman, *Wat. Res.* **1993**, *27*, 1219–1225.
10. A. Carucci, M. Majone, R. Ramadori, S. Rossetti, *Wat. Sci. Tech.* **1994**, *30*, 237–246.
11. K. Nakamura, M. Dazai, *J. Ferment. Technol.* **1986**, *64*, 433–439.

12. M. J. Simpkins, A. R. McLaren, *Wat. Tech.* **1978**, *10*, 433–433.
13. J. Meinhold, E. Arnold, S. Isaacs, *Wat. Res.* **1999**, *33*, 1871–1883.
14. E. W. J. Van Niel, K. J. Appeldoorn, A. J. B. Zehnder, G. J. J. Kortstee, *Appl. Environm. Microbiol.* **1998**, *64*, 2925–2930.
15. APHA, AWWA and WEF, *Standard Methods for Examination of Water and Wastewater, 20th edition.* United Book Press, Baltimore, 1998.
16. StatSoft, Inc., *Statistica (data analysis software system) version 6*, 2001.
17. C. O. Jeon, J. M. Park, *Wat. Res.* **2000**, *34*, 2160–2170.
18. C. Hartig, N. Loffhagen, W. Babel, *Arch. Microbiol.* **1999**, *171*, 166–172.
19. E. Rustrian, J. P. Delgenes, R. Moletta, *Curr. Microbiol.* **1997**, *34*, 43–48.
20. M. Merzouki, J. P. Delgenes, N. Bernet, R. Moletta, M. Benlemlih, *Curr. Microbiol.* **1999**, *38*, 9–17.

Povzetek

Cilj študije je bil raziskati vpliv različnih virov ogljika in dušikovih spojin, uporabljenih pri različnih začetnih koncentracijah fosforja, na sproščanje in odstranjevanje fosforja, v sistemu z aktivnim blatom. Preiskovali smo sistema za sintetično, kot tudi komunalno odpadno vodo. Dosegli smo uspešno sproščanje fosforja v anaerobni in porabo fosforja v aerobni stopnji. Dobre rezultate smo dosegli z uporabo acetata in acetata + propionata + glukoze, medtem ko smo dobili slabše rezultate pri uporabi glukoze, kot vir ogljika. Bakterije, ki akumulirajo fosfor, *Acinetobacter calcoaceticum*, so pokazale dobro sposobnost preživetja, razmnoževanja in vključevanja v kosme aktivnega blata, kljub mikroorganizmom, ki so bili prisotnosti v sistemu aktivnega blata.