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Influence of sulphate addition on the performance of aerobic wastewater treatment system

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The possibility of the wastewater treatment in an aerobic activated sludge system bioaugmented with a phosphate-accumulating bacteria *Acinetobacter calcoaceticus* was investigated. After establishing the system performance for the conventional and bioaugmented activated sludge, the experiments with the addition of sulphate in the form of ammonium sulphate solution (20 mg L^{-1}) were conducted. By the bioaugmentation of the conventional activated sludge, phosphorus removal and system performance were improved. Sulphate addition in the bioaugmented activated sludge system resulted in a short inhibitory effect (4 h) on the removal of total phosphorus and orthophosphates. Sulphate addition did not influenced mixed liquor suspended solids, bacterial numbers of *A. calcoaceticus* and removal of the chemical oxygen demand in the system, but resulted in the persistent decrease of the medium pH value and dissolved oxygen concentration after six days of feeding. The presented results are important from the practical aspect of the application in the performance of aerobic activated sludge wastewater treatment systems, where the inlet of the wastewater with an increased concentration of sulphate can lead to the disturbances of the system efficiency.

Key words: *Acinetobacter calcoaceticus*, aerobic treatment, phosphorus removal, sulphate acidification, wastewater.

Introduction

It is generally accepted that the exposure of activated sludge to cyclically anaerobic and aerobic (or anoxic) conditions is necessary to achieve enhanced biological phosphorus removal (EBPR) from wastewater (KERRN-JESPERSEN and HENZE 1993, KORTSTEE et al. 2000, MINO et al. 1998). EBPR is based on the presence of P-accumulating bacteria in the activated sludge, which are able to remove P from wastewater in excess and store it intracellularly as polyphosphate.

The strain of P-accumulating bacterium *Acinetobacter lwoffi* was able to remove P in excess when cultivating in the aerobic batch test without the preliminary exposure to anaerobic stress (GHIGLIAZZA et al. 1998). In experiments with aerobic sludge enriched with *A.*

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lwoffi, this bacterium behaved as a P-accumulating bacterium without the necessity of resorting to intermediate anaerobiosis (CONVERTI et al. 1999).

Phosphorus-accumulating bacterium *A. calcoaceticus* in the pure culture was able to remove P from wastewater in aerobic batch and semi-continuous test without the necessity of preliminary resort to the anaerobic stage (HRENOVIĆ 2001). In the alternated anaerobic/aerobic sequencing batch experiments, this strain presented a good capability of survival, multiplication, incorporation in the activated sludge flocs and EBPR characteristics (HRENOVIĆ et al. 2003).

The aim of this study was to investigate the possibility of the wastewater treatment and the influence of sulphate addition in an aerobic activated sludge system bioaugmented with a P-accumulating bacteria *A. calcoaceticus*.

Material and methods

Activated sludge

The fresh activated sludge was obtained from the aeration tank of a municipal wastewater treatment plant, settled in laboratory during 30 min, and mixed with distilled water at ratio 1:1 in a 10 L reactor. A 1% of the mineral solution (K_2HPO_4 1600 mg L^{-1}; KH_2PO_4 400 mg L^{-1}; $MgSO_4 \times 7H_2O$ 200 mg L^{-1}; NaCl 100 mg L^{-1}; $CaCl_2$ 20 mg L^{-1}) was added in reactor, and activated sludge was acclimatised for two weeks with mixing (70 rpm) and aerating (4 L min^{-1}) at room temperature. Every day a 1% of the mineral solution was added and volume was kept constant by adding the distilled water. Such acclimatised sludge was used as a control activated sludge without EBPR characteristics. By the process of bioaugmentation with the P-accumulating bacteria *A. calcoaceticus* (HRENOVIĆ 2003), activated sludge with EBPR characteristics was achieved.

Synthetic wastewater

The composition of the synthetic medium used to simulate the sewage was: acetic acid 260 mg L^{-1}; sodium propionate 40 mg L^{-1}; glucose 40 mg L^{-1}; peptone 100 mg L^{-1}; $MgSO_4$ 10 mg L^{-1}; $CaCl_2$ 6 mg L^{-1}; KCl 30 mg L^{-1}; yeast extract 20 mg L^{-1}; KH_2PO_4 44 mg L^{-1}. The pH of the synthetic wastewater was adjusted to 7.0 ± 0.1 with 1 M NaOH or 1 M HCl before autoclaving (121 °C, 15 min).

Experimental operation

The biomass of the conventional or bioaugmented activated sludge was centrifuged (200 rpm, 10 min) and 0.5 L was resuspended in synthetic wastewater. Experiments were carried out in 3 L reactor with mixing (70 rpm) and aerating (4 L min^{-1}) at 20.0 ± 0.1 °C. Reactors were performed by means of semi-continuous test; every 24 h 300 mL of mixed liquor was taken from the reactor and 300 mL of fresh wastewater was added into the reactor. In order to examine the influence of sulphate on the performance of bioaugmented activated sludge, after 48 h of the semi-continuous system operation, a 100 ml of 300 mL of wastewater volume was changed with the ammonium sulphate solution (600 mg L^{-1}), giving the final ammonium sulphate concentration in reactor of 20 mg L^{-1}. The addition of sulphate in the form of ammonium sulphate was continued by means of semi-continuous test every next 24 h of experiment.

Analytical methods

All measurements were done in triplicates according to the Standard Methods for the Examination of Water and Wastewater (APHA 1992). pH-values were measured with a Crison micro pH 2000 pH-meter. Dissolved oxygen and temperature were controlled with a Jenway 9071 dissolved oxygen meter. The samples were filtered before orthophosphate, total phosphorus and nitrogen forms measurements through Sartorius nitrocellulose filters pore diameter 0.2 μm. Orthophosphate concentration in water was measured immediately after filtration by the stannous chloride method in a Cary UV-visible spectrophotometer at 690 nm. Total phosphorus concentration in water was measured after persulfate oxidation. Total phosphorus concentration in activated sludge was determined after perchloric acid digestion. The pH of samples having a pH above 7.8 was adjusted between 6.8 and 7.5 before total phosphorus and orthophosphate measurement (HRENOVIĆ et al. 2003). Ammonium nitrogen (NH_4-N) concentration in water was measured by the nesslerization method in a Hitachii 110-40 spectrophotometer at 425 nm. Nitrate nitrogen (NO_3-N) concentration in water was measured by the ultraviolet spectrophotometric screening method in a Hitachi 110-40 spectrophotometer at 220 nm.

Chemical oxygen demand (COD) was determined by the open reflux method. Mixed liquor suspended solids (MLSS) were determined after drying at 105 °C for 1 h. Sludge volume index (SVI) was calculated after 30 min sludge settlement and MLSS. Bacterial number of *A. calcoaceticus* in mixed liquor was determined as colony forming units (CFU) on the nutrient agar. Serial dilutions (10^{-1} to 10^{-8}) of 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 a period of incubation, the colonies were counted and CFU per L was calculated.

Data analysis

The results were set up as reactor with conventional versus reactor with bioaugmented activated sludge. Independent data of this type were tested statistically using ordinary Student's *t*-test, and program Statistica Version 6.0 (StatSoft 2001). The null hypotheses was that reactors with conventional and bioaugmented sludge showed no difference in performance, and that there was no change in performance between reactors during time. 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 and discussion

Conventional and bioaugmented system

The experiments with conventional and bioaugmented sludge were conducted in order to examine the possibility of application of activated sludge bioaugmentation with a P-accumulating bacteria *A. calcoaceticus*, and thus increase the P removal in the conventional aerobic wastewater treatment systems.

Total phosphorus removal during the experiments was significantly ($p < 0.05$) higher in the bioaugmented than in the conventional activated sludge system (Tab. 1). The losses of total phosphorus in the systems, explained by the P precipitation and adsorption on the reactors surfaces, were something higher in conventional (2.37 mg L^{-1}) than in bioaugmented system (1.65 mg L^{-1}), as indicated by a calculated difference between total phosphorus removed from water and total phosphorus accumulated in the activated sludge biomass.

The amount of mixed liquor suspended solids (MLSS) increased during experiments in both systems (Tab. 1). At the end of experiments, the increment of MLSS with regard to the initial value was something higher in the bioaugmented (73.45%) than in conventional (71.32%) system. The mean P-uptake rates per MLSS (Tab. 2) during the experiment were higher ($p > 0.05$) in the bioaugmented (2.71 mg T-P g^{-1} MLSS) than in conventional (1.70 mg T-P g^{-1} MLSS) activated sludge system.

The *A. calcoaceticus* cells in the aerobic bioaugmented activated sludge system showed a good capability of survival, multiplication and incorporation in the activated sludge flocs (Tab. 1), although its multiplication in the anaerobic/aerobic bioaugmented activated sludge system was better (HRENOVIĆ et al. 2003). The P-uptake rates per CFU of *A. calcoaceticus* (Tab. 2) averaged 6.74×10^{-11} mg T-P CFU^{-1}, similar to those in the anaerobic/aerobic bioaugmented activated sludge system (HRENOVIĆ et al. 2003).

Tab. 1. Performance of the conventional and bioaugmented activated sludge system versus time.

Parameter	Time	
	Conventional	Bioaugmented
	0 hr (Influent)	
T-P (mg L^{-1})	16.40	17.60
COD (g O_2 L^{-1})	2.01	2.09
MLSS (g L^{-1})	1.11	1.41
CFU (10^9 L^{-1})		10.20
pH	7.18	7.16
	24 hr	
T-P-removed (%)	24.02	40.63
COD (g O_2 L^{-1})	1.59	1.51
MLSS (g L^{-1})	2.43	1.76
CFU (10^9 L^{-1})		140.04
pH	8.07	7.96
	48 hr	
T-P-removed (%)	24.23	40.76
COD (g O_2 L^{-1})	0.58	0.82
MLSS (g L^{-1})	3.24	2.53
CFU (10^9 L^{-1})		70.11
pH	8.22	8.19
	72 hr	
T-P-removed (%)	25.39	53.33
COD (g O_2 L^{-1})	0.54	0.68
MLSS (g L^{-1})	3.51	3.82
CFU (10^9 L^{-1})		180.23
pH	7.95	7.16

Tab. 1. – continued

Parameter	Time	
	Conventional	Bioaugmented
	96 hr	
T-P-removed (%)	44.74	53.61
COD (g O_2 L^{-1})	0.41	0.50
MLSS (g L^{-1})	4.13	4.44
CFU (10^9 L^{-1})		82.37
pH	8.14	7.01
	120 hr	
T-P-removed (%)	51.97	56.25
COD (g O_2 L^{-1})	0.43	0.48
MLSS (g L^{-1})	3.62	4.00
CFU (10^9 L^{-1})		210.82
pH	8.20	7.48
	144 hr	
T-P-removed (%)	52.60	68.82
COD (g O_2 L^{-1})	0.30	0.45
MLSS (g L^{-1})	3.87	5.31
CFU (10^9 L^{-1})		60.94
pH	8.05	7.34

Tab. 2. Phosphorus uptake rates per mixed liquor suspended solids (MLSS) and per colony forming units (CFU) of *Acinetobacter calcoaceticus* for the conventional and bioaugmented activated sludge system versus time.

Time / hr	P-uptake rate (mg T-P g^{-1} MLSS)		P-uptake rate (mg T-P CFU^{-1})
	Conventional	Bioaugmented	Bioaugmented
0	0.00	0.00	0.00
24	1.62	4.06	5.11×10^{-11}
48	1.23	2.83	1.02×10^{-10}
72	1.01	2.46	5.22×10^{-11}
96	1.78	2.16	1.15×10^{-10}
120	2.35	2.46	6.39×10^{-11}
144	2.23	2.28	2.02×10^{-10}

The amount of total phosphorus removed in the conventional activated sludge system showed significant positive correlation with the P-uptake rates per MLSS ($r = 0.954$, $p < 0.05$). For the bioaugmented activated sludge system, the amount of total phosphorus removed showed no significant correlation with the P-uptake rates per MLSS ($r = 0.618$, $p > 0.05$), but correlation was significant positive with the P-uptake rates per CFU of *A. calcoaceticus* ($r = 0.759$, $p < 0.05$). These suggest different mechanisms of the P uptake in the conventional and in the bioaugmented activated sludge system. In the conventional system, total phosphorus removed from wastewater was used by the activated sludge biomass. In the bioaugmented system, total phosphorus uptake was the result of the activity of P-accumulating bacteria.

The COD decreased during the experiments in both systems, especially during the first and second day (Tab. 1). At the end of experiments, COD removal was 85.07% in the conventional, and 78.47% in the bioaugmented system. Activated sludge biomass production per mass of COD removed at the end of experiments was higher in the bioaugmented (2.38 kg MLSS kg^{-1} COD) system, compared with the conventional (1.61 kg MLSS kg^{-1} COD) system.

The pH values increased during the first and second day of experiments above 8 in both csystems (Tab. 1). Until the end of experiments, pH stayed around 8 in the conventional

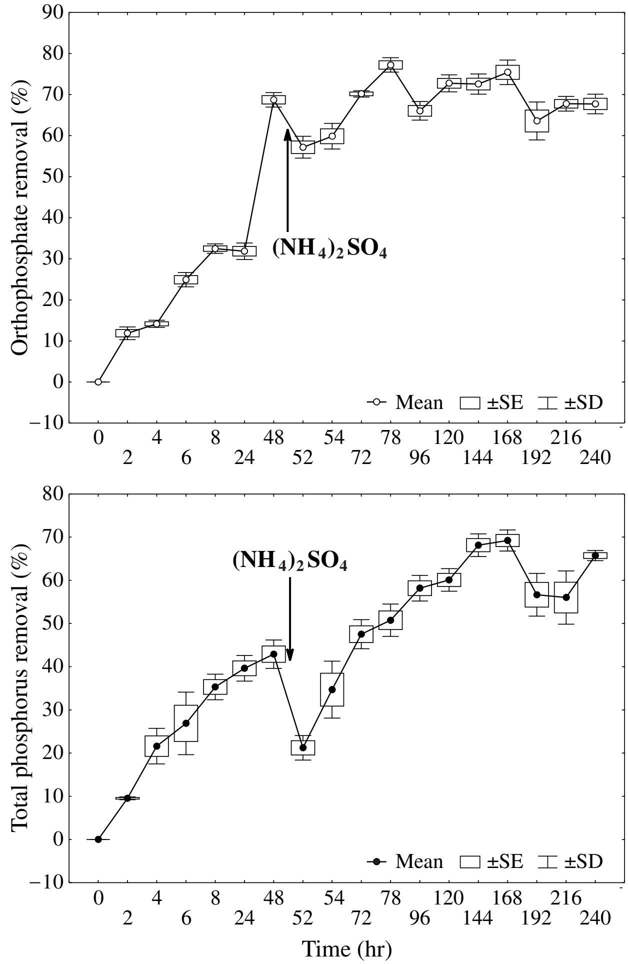

Fig. 1. Influence of the ammonium sulphate addition in the bioaugmented activated sludge system on the orthophosphate and total phosphorus removal. γ_0(total phosphorus) = 10.54 ± 0.07 mg L^{-1}, γ_0(orthophosphate) = 8.42 ± 0.06 mg L^{-1}.

system and around 7 in the bioaugmented system. The variations of the pH did not show any influence on the total phosphorus removal process.

The results showed that by the bioaugmentation of conventional activated sludge better total phosphorus removal and better system performance were achieved.

Bioaugmented system with sulphate addition

In order to examine the influence of sulphate on the P removal in the aerobic bioaugmented activated sludge system, after 48 h of system operation, ammonium sulphate was added and the system performance was continued to follow.

In the aerobic bioaugmented activated sludge system the P removal was evident just 2 h after the start of experiment (Fig. 1). It was reported (GERBER et al. 1987) that P-accumulating bacteria release P under anaerobic, anoxic and aerobic conditions when acetate or propionate is present. This occurrence was not observed in this study. The bioaugmented activated sludge system showed satisfied removal of total phosphorus (44.96%) and orthophosphate (69.45%) after a 48 h of operation (Fig. 1). Sulphate addition in the bioaugmented activated sludge system resulted in a short inhibitory effect of a 4 h on the removals of total phosphorus and orthophosphate. A 6 h after the sulphate addition P removal started to increase, and after 24 h reached the values higher than before the sulphate addition (Fig. 1). Removals of total phosphorus and orthophosphate stayed stabile until the end of experiment (Fig. 1). The total phosphorus removal during the experiment positively ($r = 0.857$, $p < 0.05$) correlated with the orthophosphate removal. The loss of total phosphorus in the system was 2.24 mg L^{-1}, as indicated by a calculated difference between total phosphorus removed from water and total phosphorus accumulated in the activated sludge biomass. Something higher loss of total phosphorus than in the bioaugmented activated sludge system without sulphate addition is probable due to the longer duration of experiment.

COD removal (Fig. 2) at the end of experiment was high (92.61%). COD removal was especially high during the first 24 h of experiment (Fig. 2), which correspond to the high in-

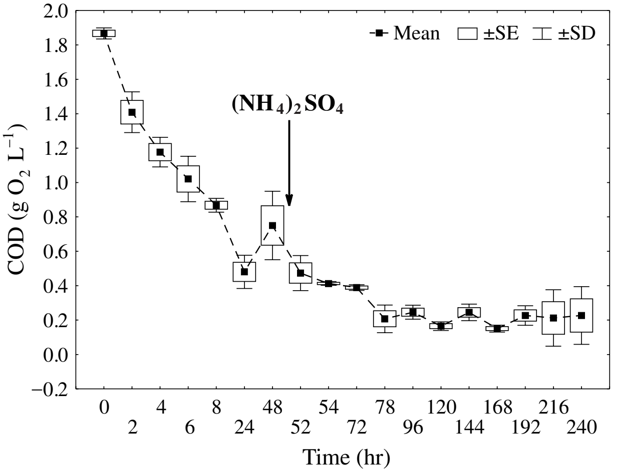

Fig. 2. Influence of the ammonium sulphate addition in the bioaugmented activated sludge system on the chemical oxygen demand (COD) removal.

creases of MLSS (Fig. 3) and CFU of *A. calcoaceticus* (Fig. 4). An increase of COD at 48 h (Fig. 2) was probable due to the decay of bacterial biomass (Fig. 4). MLSS after an increase during the first 48 h, stayed constant until the end of experiment (Fig. 3). The initial value of SVI (53 mL g^{-1}) decreased negligible (< 5 mL g^{-1}) during first 48 h of system operation, and stayed constant until the end of experiment (41 mL g^{-1}).

During the system operation, oscillations in CFU of *A. calcoaceticus* were observed (Fig. 4), but without influence on the total phosphorus and orthophosphate removal in the system. The first peak was measured at 24 h which corresponded to the end of logarithmic growth of *A. calcoaceticus* (HRENOVIĆ et al. 2003). The peak obtained 4 h after ammonium

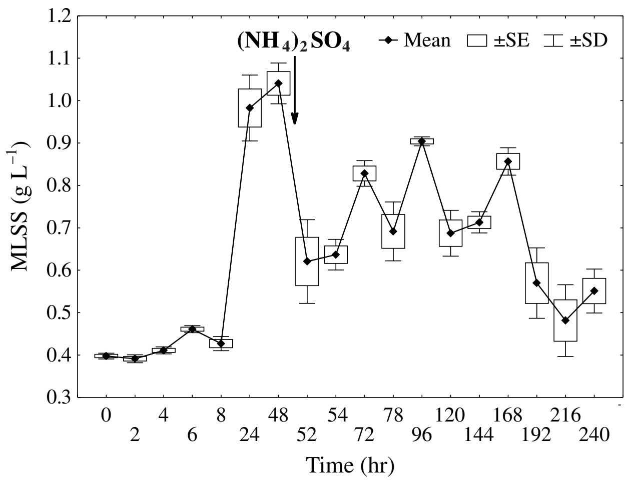

Fig. 3. Influence of the ammonium sulphate addition in the bioaugmented activated sludge system on the mixed liquor suspended solids (MLSS) concentration.

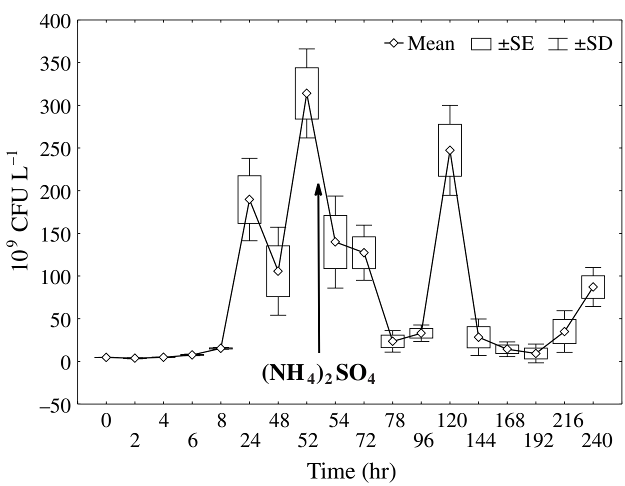

Fig. 4. Influence of the ammonium sulphate addition in the bioaugmented activated sludge system on the number of colony forming units (CFU) of *Acinetobacter calcoaceticus*.

sulphate addition was probable connected with increase of nutrient concentration in the system, since *A. calcoaceticus* is able to use ammonium as a source of nitrogen. This peak was followed by the decrease in CFU of *A. calcoaceticus*, probable due to the products of metabolism of very dense bacterial population. The third peak was again obtained at 120 h of system operation (Fig. 4).

Generally, the sulphate addition showed no influence on the P-uptake rates per MLSS and per CFU of *A. calcoaceticus* (Fig. 5). A high calculated P-uptake rate per CFU of *A. calcoaceticus* at 192 h of experiment (Fig. 5) was due to the low measured bacterial number (Fig. 4). At the end of experiment P-uptake rates amounted 13.32 mg T-P g^{-1} MLSS and 8.57×10^{-11} mg T-P CFU^{-1}. The average P-uptake rates per MLSS (7.84 mg T-P g^{-1} MLSS) were much higher ($p < 0.05$) than in the bioaugmented system without sulphate addition

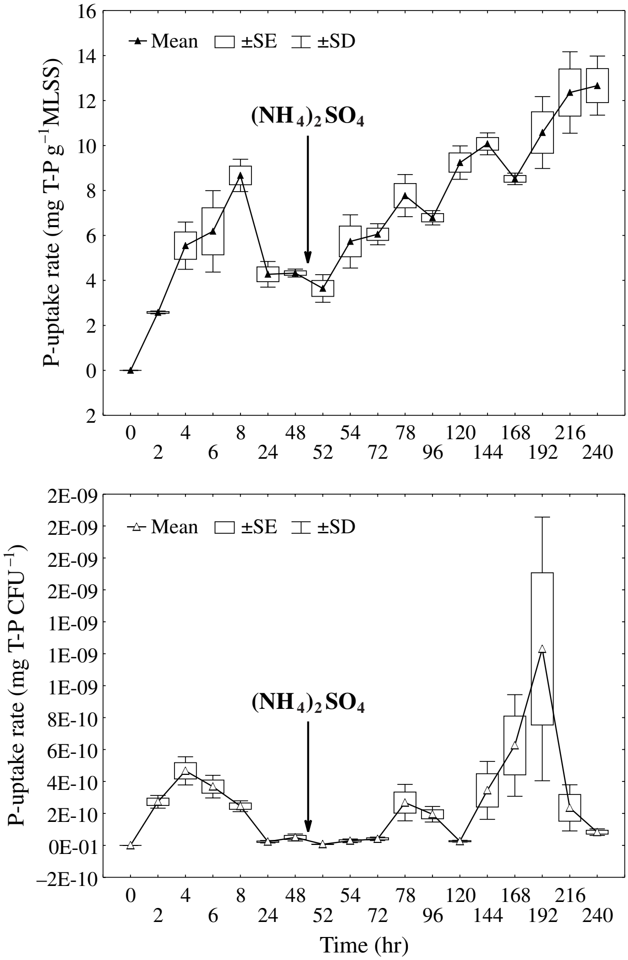

Fig. 5. Influence of the ammonium sulphate addition in the bioaugmented activated sludge system on the total phosphorus (T-P) uptake rates per mixed liquor suspended solids (MLSS) and per colony forming units (CFU) of *Acinetobacter calcoaceticus*.

(2.63 ± 0.71 mg T-P g^{-1} MLSS), which is due to the very high concentration of MLSS in the system without sulphate addition. The average P-uptake rates per CFU of *A. calcoaceticus* were higher (3.42×10^{-10}) than in the bioaugmented system without sulphate addition (9.77×10^{-11}), but not significantly ($p > 0.05$). The P-uptake rates per MLSS and CFU of *A. calcoaceticus* achieved after a 24 h of aerobic operation of the bioaugmented systems, were comparable to the P-uptake rates achieved in alternated 24 h anaerobic/24 h aerobic system (HRENOVIĆ et al. 2003). It can be concluded that by the process of activated sludge bioaugmentation with P-accumulating bacteria *A. calcoaceticus* P removal can be significantly improved in the aerobic system, without the necessity of activated sludge restoring to intermediate anaerobiosis. These results showed a good agreement with the behaviour of P-accumulating bacteria *A. lwoffi* (GHIGLIAZZA et al. 1998, CONVERTI et al. 1999).

The pH reached 8.38 in the first 48 h of experiment, but after the sulphate addition started to decrease until the end of experiment (Fig. 6). The irreversible decrease of the medium pH was clearly influenced by the sulphate addition. Unchangeable efficiency of the total phosphorus and orthophosphate removals suggests that the system was tolerable to low pH (5.52).

Significant decrease of the dissolved oxygen concentration was measured after eight days of operation (Fig. 6), but the minimum dissolved oxygen of 2.8 mg L^{-1} was in excess of the typical oxygen half-saturation coefficients (0.75 mg L^{-1}) for activated sludge (GRADY et al. 1999). At the end of experiment ammonium nitrogen (NH_4-N) concentration was 0.1 mg L^{-1} and nitrate nitrogen (NO_3-N) concentration 1.3 mg L^{-1}, which indicate nearly completely consumption of the added ammonium. The probable explanation for the pH and dissolved oxygen decrease is ammonium nitrogen (from the added ammonium sulphate addition) oxidation to nitrate ion. This process can be conducted in the aerobic conditions by microorganisms different than *A. calcoaceticus* (nitrifying bacteria) present in the activated sludge. During the nitrification kinetics ($2NH_4^+ + 4O_2 \rightarrow 2NO_3^- + 2H_2O + 4H^+ +$ new cells) the H^+ is originated. In this case pH value decreases when complete hardness is

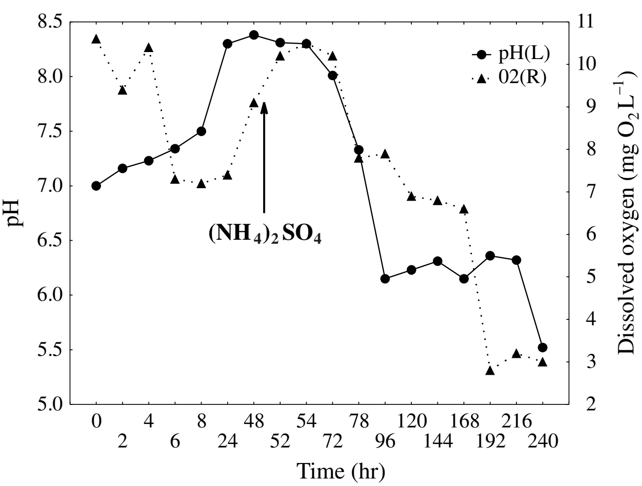

Fig. 6. Influence of the ammonium sulphate addition in the bioaugmented activated sludge system on the pH and dissolved oxygen concentration.

used up. In the same time dissolved oxygen decreases because of higher oxygen uptake for nitrification (for oxidation of 1.0 g of ammonia about 4.6 g of oxygen is needed).

Conclusions

Total phosphorus removal and system performance were improved by the bioaugmentation of the conventional activated sludge.

Sulphate addition in the bioaugmented activated sludge system resulted in a short (4 h) inhibition of the removal of total phosphorus and orthophosphate. Sulphate addition did not influenced the MLSS, SVI, CFU of *A. calcoaceticus* and removal of the COD in the system, but led to the persistent increase of acidity of the medium and decrease of dissolved oxygen concentration after six days of feeding. The presented results are important from the practical aspect of the application in the performance of aerobic activated sludge wastewater treatment systems, where the inlet of the wastewater with an increased concentration of sulphate can lead to the disturbance of the system efficiency.

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