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Influence of ammonium, nitrate and nitrite on the performance of the pure culture of *Acinetobacter junii*

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Abstract: The influence of different concentration ranges (0–500 mg/L) of ammonium, nitrate and nitrite presence in the wastewater, on the performance of the pure culture of phosphate-accumulating bacterium *Acinetobacter junii* in the anaerobic and aerobic conditions, was investigated. *A. junii* was able to use ammonium and nitrate salts as the source of nitrogen, unlike in the case of nitrite salt. Comparing to the control reactors with the peptone and yeast extract as the sources of nitrogen, at the lowest tested concentration of ammonium and nitrate the performance of the system was inhibited due to the nitrogen deficit in the wastewater, while at the highest concentration it was positively influenced. Nitrite in all concentrations detrimentally affected the phosphate release and uptake rates, chemical oxygen demand uptake rates, nitrogen uptake rates, as well as multiplication of *A. junii*. The higher the nitrite concentration, the more pronounced was the effect. At the highest nitrite concentration tested a complete failure of the system was observed.

Key words: *Acinetobacter*; ammonium; bacteria; nitrate; nitrite; enhanced biological phosphorus removal.

Abbreviations: CFU, colony forming units; COD, chemical oxygen demand; EBPR, enhanced biological phosphorus removal.

Introduction

Varieties of wastewater treatment systems with enhanced biological phosphorus removal (EBPR) characteristics include combined removal of carbon, nitrogen and phosphorus. In the case where the biological reactors are loaded with high levels of ammonium, as a result of nitrogen removal process (nitrification and denitrification), various nitrogen forms such as ammonium, nitrate and nitrite were present during the phosphate release and uptake cycle in different extents.

Research indicated the negative influence of different nitrogen forms on the EBPR process (Kortstee et al. 2000). In the presence of nitrate, EBPR fails because the denitrifying bacteria are able to respire anaerobically and deplete the supply of organic substrates, required by the phosphate-accumulating bacteria (Kortstee et al. 2000). Nitrite concentration of 8 mg/L and higher inhibits anoxic and aerobic phosphate uptake completely and severely, respectively (Meinhold et al. 1999). Nitrate and nitrite present in the activated sludge could be converted to nitric oxide, which has inhibitory effect on the phosphate release mechanism at the level of adenylate kinase (Van Niel et al. 1998).

The lack of pure culture studies hampers a basic understanding of the influence of nitrogen forms on the phosphate-accumulating bacteria, which are crucial in scavenging for phosphate in EBPR process. Weon et al. (2002) suggested the toxicity of nitrite on the aerobic growth and phosphate uptake in a pure culture of the phosphate-accumulating bacterium *Acinetobacter* sp. However, when phosphate-accumulating bacteria are applied in the operating scale of the EBPR, they will be exposed to the alternated anaerobic/aerobic conditions (Rustrian et al. 1998; Mino 2000).

The aim of this study was to determine the influence of ammonium, nitrate and nitrite presence in the wastewater on the performance (phosphate release and uptake rates, chemical oxygen demand uptake rates, nitrogen uptake rates, multiplication) of the pure culture of phosphate-accumulating bacterium *Acinetobacter junii*.

Material and methods

Experimental culture
Lyophilised culture of *A. junii* DSM1532, which has been described as a phosphate-accumulating bacterium, was obtained from Deutsche Sammlung von Microorganismen und Zellkulturen GmbH (Hrenovic et al. 2003).

Synthetic wastewater
The composition of the control synthetic medium used to simulate the sewage was (mg/L distilled water): Na-propionate 1000; KH_2PO_4 44; $MgSO_4$ 10; $CaCl_2$ 6; KCl 30;

peptone 200; yeast extract 40. The composition of the experimental medium was the same as control medium, except that peptone and yeast extract as the sources of nitrogen were replaced with NH_4Cl, $NaNO_3$ or $NaNO_2$ to obtain a concentration range of 5, 50 and 500 mg/L as nitrogen. The pH of the synthetic wastewater was adjusted to 7.0±0.1 with 1 M NaOH or 1 M HCl before autoclaving (121 ℃, 15 min).

Experimental methods

The experiments were carried out as triplicate sequencing batch tests in alternating 24 h anaerobic / 24 h aerobic stages. The bacteria were pre-grown in a nutrient broth for 24 h at 30.0 ± 0.1 ℃ and then cultivated aerobically for 24 h in the control synthetic medium. The biomass was centrifuged (7,000 rpm, 15 min), washed with sterile distilled water, centrifuged, and re-suspended in Erlenmeyer flasks containing 300 mL of synthetic wastewater. The flasks were sealed with a sterile gum cap and anaerobically incubated (70 rpm, 30.0 ± 0.1 ℃) in a water bath controlled with thermostat and shaker. In the following aerobic phase, reactors were shaken at 70 rpm, aerated (1 L/min) with sterile air and incubated at 30.0 ± 0.1 ℃.

Analytical methods

The pH-value, temperature and dissolved oxygen in the water were measured with WTW 330 SET equipped with pH-electrode, temperature sensor and dissolved oxygen electrode. The samples were filtered before measurements of the chemical parameters through Sartorius nitrocellulose filters, pore diameter 0.2 μm (according to Standard Methods for the Examination of Water and Wastewater, 20th edn., 1998, United Book Press, Baltimore). The phosphate (PO_4-P) concentration in water was measured spectrophotometrically in a DR/2500 Hach spectrophotometer by the ascorbic acid method. Ammonium (NH_3-N) concentration in water was measured spectrophotometrically by the ammonium salicylate method (Hach method 8155 and 10031). Nitrate (NO_3-N) concentration in water was measured spectrophotometrically by the cadmium reduction method (Hach method 8039). Nitrite (NO_2-N) concentration in water was measured spectrophotometrically by the diazotization (Hach method 10019) or ferrous sulphate (Hach method 8153) method. Chemical oxygen demand (COD) was determined spectrophotometrically after reactor digestion method (Hach method 8000). Bacterial numbers of *A. junii* were determined as colony forming units (CFU) (Hrenovic et al. 2003) per one mL of sample. All measurements were done in triplicate and the mean values were presented. Neisser stain was performed to confirm poly-phosphate granules in cells of *A. junii*.

Data analysis

Results were statistically analysed using the Statistica program (Data Analysis Software System, Version 7.1., 2005, StatSoft, Inc., Tulsa). The results obtained for the triplicate media containing ammonium, nitrate, nitrite and control medium were compared. Since the data were independent ordinary Student's *t*-tests were performed. The null hypothesis tested by the analysis was that media with different sources of nitrogen showed no difference in performance. Results were considered significant at the 5% level ($p = 0.05$).

Table 1. Performance of the pure culture of *Acinetobacter junii* in relation to ammonium concentrations.

Period[a]	Control	Range of NH_3-N concentration (mg/L)		
		5	50	500
Influent				
COD (g/L)	1.48	1.59	1.48	1.62
NH_3-N (mg/L)	27.66	8.79	53.40	503.00
NO_3-N (mg/L)	28.0	1.3	1.0	1.2
NO_2-N (mg/L)	0.018	0.018	0.010	0.012
PO_4-P (mg/L)	11.20	13.00	12.82	13.41
CFU (10^9/L)	2.55	2.77	2.62	2.68
pH	6.97	7.01	7.03	6.98
Anaerobic phase				
COD (g/L)	0.77	0.92	0.78	0.56
NH_3-N (mg/L)	25.00	3.48	45.81	484.60
NO_3-N (mg/L)	24.4	0.7	0.8	1.0
NO_2-N (mg/L)	0.049	0.007	0.006	0.005
PO_4-P (mg/L)	13.25	15.23	15.25	16.50
CFU (10^9/L)	2.82	3.10	3.19	3.41
pH	6.37	6.75	6.72	6.61
COD-uptake rate (mg COD/CFU)	2.52×10^{-7}	2.16×10^{-7}	2.19×10^{-7}	3.11×10^{-7}
N-uptake rate (mg N/CFU)	2.21×10^{-9}	1.91×10^{-9}	2.44×10^{-9}	5.46×10^{-9}
P-release rate (mg PO_4-P/CFU)	7.27×10^{-10}	7.19×10^{-10}	7.62×10^{-10}	9.06×10^{-10}
Aerobic phase				
COD (g/L)	0.62	0.85	0.60	0.44
NH_3-N (mg/L)	0.20	0.30	29.30	431.98
NO_3-N (mg/L)	7.2	0.4	0.6	1.2
NO_2-N (mg/L)	0.024	0.010	0.014	0.013
PO_4-P (mg/L)	4.79	10.71	7.43	4.94
CFU (10^9/L)	54.20	14.43	32.21	49.11
pH	7.66	7.14	7.38	7.54
COD-uptake rate (mg COD/CFU)	1.60×10^{-8}	5.13×10^{-8}	2.73×10^{-8}	2.40×10^{-8}
N-uptake rate (mg N/CFU)	8.90×10^{-10}	6.51×10^{-10}	7.61×10^{-10}	1.45×10^{-9}
P-uptake rate (mg PO_4-P/CFU)	1.18×10^{-10}	1.59×10^{-10}	1.67×10^{-10}	1.72×10^{-10}
P removal (%)	57.23	17.62	42.04	63.16

[a] N, nitrogen, P, phosphate.

Table 2. Performance of the pure culture of *Acinetobacter junii* in relation to nitrate concentrations.

Period[a]	Range of NO_3-N concentration (mg/L)		
	5	50	500
Influent			
COD (g/L)	1.47	1.38	1.51
NH_3-N (mg/L)	0.17	1.11	0.76
NO_3-N (mg/L)	6.8	55.8	529.6
NO_2-N (mg/L)	0.008	0.012	0.010
PO_4-P (mg/L)	10.22	10.81	9.60
CFU (10^9/L)	2.00	2.50	2.25
pH	7.02	7.01	6.98
Anaerobic phase			
COD (g/L)	0.94	0.68	0.72
NH_3-N (mg/L)	0.04	0.02	0.10
NO_3-N (mg/L)	2.2	46.2	509.8
NO_2-N (mg/L)	0.045	0.099	0.054
PO_4-P (mg/L)	12.10	13.75	13.00
CFU (10^9/L)	2.72	3.33	3.50
pH	6.62	6.39	6.34
COD-uptake rate (mg COD/CFU)	1.95×10^{-7}	2.10×10^{-7}	2.26×10^{-7}
N-uptake rate (mg N/CFU)	1.73×10^{-9}	3.18×10^{-9}	5.83×10^{-9}
P-release rate (mg PO_4-P/CFU)	6.91×10^{-10}	8.83×10^{-10}	9.71×10^{-10}
Aerobic phase			
COD (g/L)	0.90	0.61	0.57
NH_3-N (mg/L)	0.02	0.01	0.01
NO_3-N (mg/L)	0.7	40.0	481.3
NO_2-N (mg/L)	0.032	0.036	0.038
PO_4-P (mg/L)	8.96	7.64	3.89
CFU (10^9/L)	10.61	24.10	42.65
pH	7.48	7.53	7.68
COD-uptake rate (mg COD/CFU)	5.37×10^{-8}	3.20×10^{-8}	2.20×10^{-8}
N-uptake rate (mg N/CFU)	5.87×10^{-10}	7.00×10^{-10}	1.15×10^{-9}
P-uptake rate (mg PO_4-P/CFU)	1.19×10^{-10}	1.32×10^{-10}	1.34×10^{-10}
P removal (%)	12.33	29.32	59.48

[a] N, nitrogen, P, phosphate.

Results and discussion

Two hours after the start of experiments the oxygen concentration was reduced to 0.0 mg/L and remained at this level until the end of the anaerobic phase, which indicates that anaerobic conditions were successfully achieved due to oxygen consumption by *A. junii*, without the necessity of using any inert gas. Two hours after the beginning of aeration, the oxygen concentration increased to 5.5–5.8 mg/L and remained high (6.0–6.6 mg/L) until the end of experiment in all the reactors. The pH values decreased during the anaerobic phase and increased during the aerobic phase in relation to initial values, without difference between the reactors with various sources of nitrogen. Only at the highest tested concentration of nitrite the pH values progressively decreased in relation to initial value (Tables 1–3).

The tested strain of *A. junii* in control reactors (Table 1) showed a typical behaviour of phosphate-accumulating bacterium. The phosphate was released during the anaerobic phase and taken up during the aerobic phase at rates 7.27×10^{-10} and 1.18×10^{-10} mg PO_4-P/CFU, respectively. The strains that accumulate more than 10^{-12} mg PO_4-P/CFU are defined as the phosphate-accumulating bacteria (Sidat et al. 1999). The observed phosphate uptake rates were comparable to the reported 1.84×10^{-10}, 1.65×10^{-10}, 6.00×10^{-11}, 4.26×10^{-11} and 3.43×10^{-11} mg PO_4-P/CFU for the *A. calcoaceticus* var. *lwoffi*, *Aeromonas hydrophila*, *Pseudomonas putrefaciens*, *P. mendocina* and *P. fluorescens*, respectively, which were isolated from EBPR-activated sludge (Sidat et al. 1999). Neisser stain at the end of the experiments confirmed the presence of polyphosphate granules in cells of *A. junii*. The COD and nitrogen compounds decreased more rapidly during the anaerobic than during the aerobic phase. Ammonium was consumed in a higher extent than nitrate. Peptone and yeast extract are rich in nitrogen and would have also been a good source of this element for uptake and growth. In relation to initial numbers, CFU of *A. junii* increased slightly at the end of anaerobic phase and for one order of magnitude at the end of aerobic phase.

Addition of three ascending (5, 50 and 500 mg/L as nitrogen) concentration ranges of ammonium (Table 1), nitrate (Table 2) or nitrite (Table 3) to the wastewater composition affected the performance of the pure culture differently. No reduction of nitrate or nitrite during the anaerobic phase was observed, since *A. junii* is not capable to use the oxidised nitrogen forms as the electron acceptor as confirmed in a preliminary experiment.

A. junii was able to use ammonium and nitrate salts as the source of nitrogen (Tables 1,2), unlike in the case of nitrite salt (Table 3). The lowest ammonium, nitrate and nitrite input was 5 mg/L whereby the nitrogen/COD ratio indicates a nitrogen deficiency for bacterial growth. In these cases ammonium and nitrate

Table 3. Performance of the pure culture of *Acinetobacter junii* in relation to nitrite concentrations.

Period[a]	Range of NO_2-N concentration (mg/L)		
	5	50	500
Influent			
COD (g/L)	1.34	1.43	1.61
NH_3-N (mg/L)	0.03	0.03	0.03
NO_3-N (mg/L)	1.5	1.6	1.5
NO_2-N (mg/L)	6.69	50.80	511.42
PO_4-P (mg/L)	9.73	10.67	10.11
CFU (10^9/L)	2.20	2.29	2.09
pH	7.01	7.07	7.02
Anaerobic phase			
COD (g/L)	1.21	1.36	1.57
NH_3-N (mg/L)	0.01	0.01	0.01
NO_3-N (mg/L)	1.0	1.3	1.5
NO_2-N (mg/L)	6.98	52.97	518.85
PO_4-P (mg/L)	12.54	12.76	12.34
CFU (10^9/L)	1.74	1.16	0.82
pH	6.64	6.71	6.91
COD-uptake rate (mg COD/CFU)	7.14×10^{-8}	6.16×10^{-8}	5.45×10^{-8}
N-uptake rate (mg N/CFU)	1.68×10^{-10}	-1.46×10^{-9}	-8.91×10^{-9}
P-release rate (mg PO_4-P/CFU)	1.61×10^{-9}	1.81×10^{-9}	2.75×10^{-9}
Aerobic phase			
COD (g/L)	1.13	1.33	1.68
NH_3-N (mg/L)	0.00	0.01	0.01
NO_3-N (mg/L)	0.2	0.3	0.8
NO_2-N (mg/L)	7.16	52.93	525.30
PO_4-P (mg/L)	9.46	10.58	10.80
CFU (10^9/L)	3.63	1.45	0.14
pH	7.34	7.09	6.85
COD-uptake rate (mg COD/CFU)	6.03×10^{-8}	6.87×10^{-8}	-7.04×10^{-7}
N-uptake rate (mg N/CFU)	2.15×10^{-10}	-6.12×10^{-10}	-9.40×10^{-8}
P-uptake rate (mg PO_4-P/CFU)	7.49×10^{-11}	6.14×10^{-11}	-7.25×10^{-9}
P removal (%)	2.77	0.84	−6.82

[a] N, nitrogen, P, phosphate.

Table 4. Inhibitory effect of ammonium on the CFU of *Acinetobacter junii*, COD-uptake, nitrogen-uptake, phosphate-release, phosphate-uptake rates and percentage of phosphate removal.[a]

Parameter	Inhibition by range of NH_3-N concentration (mg/L)		
	5	50	500
Anaerobic phase			
CFU	−1.06	−9.57	−14.89
COD-uptake rate	14.29	13.10	−23.41
N-uptake rate	13.57 *	−10.41	−147.06 *
P-release rate	1.10	−4.81	−24.62 *
Aerobic phase			
CFU	75.50 *	42.36 *	13.93
COD-uptake rate	−220.63 *	−70.63 *	−50.00
N-uptake rate	26.85 *	14.49 *	−62.92 *
P-uptake rate	−34.75	−41.53	−45.76
% P removal	69.21 *	26.54 *	−10.36

[a] N, nitrogen, P, phosphate, * statistically significant ($p = 0.05$). The inhibitory effect is in % of control.

were completely consumed at the end of experiments, but nitrite was not consumed.

The performance of the reactors with the ammonium as the source of nitrogen was compared with control (Table 4). During the anaerobic stage at the starting concentration of 5 mg/L NH_3-N all determined parameters except CFU were slightly inhibited, while at the highest concentration of 500 mg/L NH_3-N were positively influenced. During the aerobic stage at lower concentrations of 5 and 50 mg/L NH_3-N the calculated COD and phosphate-uptake rates were higher than control, but the key parameters such as CFU and the percentage of phosphate removal were significantly inhibited. At the concentration of 500 mg/L NH_3-N all determined parameters except CFU were positively influenced, but not statistically significant. These results suggest that although the ammonium in a sufficient concentration was a suitable source of nitrogen for *A. junii*, it is not better than peptone and yeast extract.

When comparing the performance of reactors containing nitrate as the source of nitrogen with control, similar observation can be seen (Table 5). At the initial concentration of 5 and 50 mg/L NO_3-N the overall system performance during the anaerobic and aerobic stage was poorer than control. At the sufficient concentration of 500 mg/L NO_3-N the overall performance of the system was better than the control, but also not statistically significant. The nitrate as the source of nitrogen can be classified close to ammonium.

Although in the case with the 500 mg/L of ammonium and nitrate as the source of nitrogen the overall performance (carbon, nitrogen and phosphate-uptake rates, percentage of phosphate removal) was positively influenced, lower CFU of *A. junii* at the end of aerobic phase, if again returned to the anaerobic conditions,

Table 5. Inhibitory effect of nitrate on the CFU of *Acinetobacter junii*, COD-uptake, nitrogen-uptake, phosphate-release, phosphate-uptake rates and percentage of phosphate removal.

Parameter	Inhibition by range of NO_3-N concentration (mg/L)		
	5	50	500
Anaerobic phase			
CFU	−23.40	−20.57	−28.79 *
COD-uptake rate	22.62	16.67	10.32
N-uptake rate	21.72 *	−43.89 *	−163.80 *
P-release rate	4.95	−21.46 *	−33.56 *
Aerobic phase			
CFU	74.94 *	54.65 *	11.09
COD-uptake rate	−235.63 *	−100.00 *	−37.50
N-uptake rate	34.04 *	21.35 *	−29.21
P-uptake rate	−0.85	−11.86	−13.56
% P removal	78.46 *	48.77 *	−3.93

[a] N, nitrogen, P, phosphate, * statistically significant ($p = 0.05$). The inhibitory effect is in % of control.

Table 6. Inhibitory effect of nitrite on the CFU of *Acinetobacter junii*, COD-uptake, nitrogen-uptake, phosphate-release, phosphate-uptake rates and percentage of phosphate removal.

Parameter	Inhibition by range of NO_2-N concentration (mg/L)		
	5	50	500
Anaerobic phase			
CFU	28.13 *	54.25 *	63.59 *
COD-uptake rate	71.67 *	75.56 *	78.37 *
N-uptake rate	92.40 *	166.06 *	503.17 *
P-release rate	−121.46 *	−148.97 *	−278.27 *
Aerobic phase			
CFU	92.18 *	97.03 *	99.68 *
COD-uptake rate	−276.88 *	−329.38 *	4500.00 *
N-uptake rate	75.84 *	168.76 *	10661.80 *
P-uptake rate	33.72 *	45.66 *	6515.93 *
% P removal	95.16 *	98.53 *	111.92 *

[a] N, nitrogen, P, phosphate, * statistically significant ($p = 0.05$). The inhibitory effect is in % of control.

can result in a lower EBPR efficiency. The multiplication of *A. junii* mainly occurred in aerobic conditions. In the EBPR process, among a variety of factors, surely the bacterial biomass also plays a role; less phosphate-accumulating bacteria can remove less phosphate and if bacteria multiply poorly during the process, it is obvious that less phosphate will be removed. Hence it can be considered that ammonium and nitrate were suitable sources of nitrogen for *A. junii*, but not better than peptone and yeast extract. This is in agreement wit the previous experiences where the KNO_3 (Sidat et al. 1999), NH_4Cl (Rustrian et al. 1997) and peptone and yeast extract (Hrenovic et al. 2003) served as the suitable sources of nitrogen for phosphate-accumulating *Acinetobacter* species.

In the experiments with nitrite as the nitrogen source (Tables 3,6) during the anaerobic stage decay of cells was evident as a decrease of CFU. The decay of cells probable resulted in the extra carbon, nitrogen and phosphate in the bulk solution, but in the same time live bacterial cells took up nutrients for their metabolism. Therefore, the calculated rates of carbon, nitrogen and phosphate metabolism can be masked at the moment of screening. The decay of cells with the poly-phosphate reserves which entered the experiment can explain the calculated phosphate-release rates more than 100% higher comparing to the control. Carbon-uptake rates were about 70% lower than control, whereas the nitrogen-uptake rates were unusually low or negative. This low nitrogen-uptake can be explained with the hypothesis that live cells took up the residual ammonium, nitrate and nitrogen compounds formed during the decay of cells. Decay of cells resulted in the accumulation of nitrite which was not a suitable nitrogen source for *A. junii*. During the aerobic stage, when comparing to the control, a poor multiplication of cells was observed in the nitrite concentration range of 5 and 50 mg/L, but in the nitrite concentration range of 500 mg/L decay of cells determined as CFU was continued (Tables 3,6). In the concentration range of 5 and 50 mg/L NO_2-N the carbon-uptake rates were quite high, but relatively small amount of COD was removed in relation to anaerobic stage. The calculated nitrogen and phosphate-uptake rates were statistically significantly lower than control. The average percentages of phosphate removal were very low (below 3%) and very few amount of phosphate was removed from influent. In the concentration range of 500 mg/L NO_2-N all calculated parameters were negative and no stoichiometric evaluation can be established.

From the above-mentioned it can be seen that nitrite in all concentrations detrimentally affected the performance of the key EBPR parameters (such as phosphate-uptake rates, percentage of the phosphate removal and CFU of the phosphate-accumulating bacteria) of the system when compared to the control (Table 6). The higher the nitrite concentration, the more pronounced was the effect. At the lowest nitrite concentration examined (5 mg/L) all parameters were inhibited at the lowest extent, which could be estimated close to the reported (Meinhold et al. 1999) severely inhibition of aerobic phosphate uptake in the EBPR-activated sludge system at the nitrite concentration of 8 mg/L and higher. At the highest nitrite concentration (500 mg/L) tested complete failure of the system was observed. The results obtained are consistent with the nitrite concentration of 520 mg/L at which there was no phosphate uptake by the pure culture of *Acinetobacter* sp. and aerobic growth was inhibited to around 90% (Weon et al. 2002). It was reported (Yarbrough et al. 1980) that nitrite inhibits the energy generation in aerobic bacteria. Nitrite presence inhibited in a high degree the anaerobic COD uptake by the tested strain of *A. junii*. The nitrite inhibition of energy generation led probably to the limited intracellular poly-hydroxy-alkanoate storage (Mino et al. 1998; Kortstee et al. 2000) which resulted as a consequence in the lower phosphate uptake and cell multiplication during the aerobic stage. It

can be summarised that the exposure to all examined nitrite concentrations (5–500 mg/L) was damaging to the EBPR characteristics and multiplication of *A. junii*.

Conclusions

The pure culture of phosphate-accumulating bacterium *A. junii* was able to use ammonium and nitrate salts as the source of nitrogen. Comparing to the control reactors with the peptone and yeast extract as the sources of nitrogen, at the lowest tested concentration of ammonium and nitrate (5 mg/L) the performance of the system was inhibited due to the nitrogen deficit in the wastewater, while at the highest concentration (500 mg/L) it was slightly positively influenced. Nitrite in all concentrations detrimentally affected the phosphate release and uptake rates, COD uptake rates, nitrogen uptake rates, as well as the multiplication of *A. junii*. The higher the nitrite concentration, the more pronounced was the effect. At the highest nitrite concentration tested, a complete failure of the system was observed. Hence, the nitrite presence or accumulation either during the anaerobic or aerobic stage should be obligatory avoided in order to ensure a good EBPR performance of *A. junii*.

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