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OPTIMIZATION OF ISEs FOR SIMULTANEOUS NH_4^+, NO_3^- AND NO_2^- MONITORING IN SYNTHETIC WASTEWATER USING *SOLVER*

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Abstract

The adaptation of commercially available ion-selective electrodes (ISEs) for use in wastewater matrices was demonstrated using multivariate analysis and optimization with the Microsoft Excel *Solver* tool. The electrodes were characterized in pure analyte solutions, and their parameters were estimated using multivariate analysis and *Solver* optimization. The ammonium, nitrate, and nitrite ions were measured in model systems that were designed to simulate wastewater. The accuracy of the measurements (95.3% - 101.0%) was satisfactory for the determination of ammonium, nitrate, and nitrite in complex matrices. The adapted ISEs were then successfully used for bioprocess (synthetic wastewater treatment) dynamics and efficiency studies in a horizontal rotating tubular bioreactor (HRTB) by monitoring the concentrations of the substrates and intermediates in the samples collected from the bioreactor. The simultaneous determination of ammonium, nitrate, and nitrite in the samples through the use of adapted ISEs considerably shortened the time required for ion determination.

Key words: ammonium, ion-selective electrodes, nitrate, nitrite, wastewater

1. Introduction

Wastewater may contain several different forms of nitrogen, including organic nitrogen, ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen. Nitrogen compounds are some of the most common pollutants in aquatic environments and pose a threat to both aquatic organisms and humans (Sumino et al., 2006). Therefore, it is important that these compounds are monitored and removed from wastewater to an appropriate concentration prior to its discharge into the environment (Seifi and Fazaelipoor, 2012).

Biological nitrogen removal is an effective method for reducing the concentrations of nitrogen compounds in wastewaters, which is typically accomplished in two sequential steps: nitrification and denitrification. During nitrification by autotrophic nitrifying bacteria, the oxidation of ammonium into nitrite is followed by the oxidation of nitrite into nitrate. Under anoxic conditions, the nitrate is then reduced to N_2 (or denitrification intermediates) by denitrifying bacteria. In simultaneous nitrification and denitrification (SND), the nitrification and denitrification occur simultaneously in the same reactor under aerobic conditions (Guo et al., 2013; Holman and Wareham, 2005; Pochana and Keller, 1999; Walters et al., 2009). The pure microorganism cultures capable of performing SND are *Paracoccus denitrificans* and *Nitrosomonas europaea* (Ahn, 2006; Helmer and Kunst, 1998). Different types of bioreactors can be used for wastewater treatment, especially those containing microbial biofilms, e.g., trickling filters,

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biodisc bioreactors, packed bed bioreactors, fluidized bed bioreactors (Nicolella et al., 2000) or moving bed biofilm reactors (Quan et al., 2013).

The horizontal rotating tubular bioreactor (HRTB), which was developed by Šantek et al. (1996a, 1996b), combines the characteristics of thin layer and biodisc reactors. HRTBs have been used to conduct both aerobic (Slavica et al., 2004) and anaerobic (Ivančić et al., 2004) bioprocesses. In further studies, the HRTB was used for the heterotrophic cultivation of *P. denitrificans* bacteria, i.e., the SND of synthetic wastewater (Rezić et al., 2007), and for the monitoring and removal of heavy metals from textile wastewaters (Zeiner et al., 2012; Zeiner et al., 2010).

The on-line monitoring of the primary bioprocess variables, such as the concentrations of the substrates, the metabolites and the suspended cells, is often difficult or expensive; thus, off-line measurements must be employed. This is even more evident in the case of the HRTB, where on-line monitoring is further enabled by the design and construction of the bioreactor. Therefore, the development of simple, accurate, and fast methods of measuring these variables off-line and preferably on-site is of vital importance. The most commonly used methods for the determination of ammonium, nitrate, and nitrite are colorimetric (spectrophotometric) techniques that require tedious sample pre-treatment steps (Michalski and Kurzyca, 2006). Several other methods for the simultaneous determination of ammonium, nitrate and nitrite include ion chromatography (IC), high-performance liquid chromatography (HPLC), sequential injection analysis (SIA), flow injection analysis (FIA), capillary electrophoresis (CE), fluorimetry, chemiluminescence, and direct potentiometry by ion-selective electrodes (ISEs) (Bouvier et al., 2008). ISEs are a promising approach because of their small size, rapid response, simplicity of use, low cost, portability, and ability to directly measure the analyte across a wide range of concentrations (Kim et al., 2007). Recently, research efforts have been dedicated to the development of multisensory systems, such as voltammetric electronic tongues, for monitoring the above-mentioned ions in waters (Campos et al. 2012; Nuñez et al., 2013).

Incorporated into Microsoft Excel for Windows, *Solver* is a tool used for mathematical simulation, optimization, and modeling and has the capability to solve an extensive range of linear, nonlinear, and integer problems. It is an affordable substitute to expensive commercial software packages because it can yield the same results when applied to the same sets of data (Sak-Bosnar et al., 2011). In this investigation, *Solver* was used for fitting of the data with nonlinear functions via an iterative algorithm.

The aim of this investigation was to adapt commercially available ISEs using multivariate analysis and *Solver* for the detection of ammonium, nitrate, and nitrite in wastewater matrices.

Furthermore, the overall aim was to shorten the time required to monitor the bioprocess in the HRTB by developing a method for the simultaneous determination of these ions using ISEs.

2. Materials and methods

2.1. Simultaneous determination of ammonium, nitrate, and nitrite by ISEs

2.1.1. Standard solutions and calibrations

Stock solutions (c=1 mol L^{-1}) of NaNO$_2$, NaNO$_3$, and NH$_4$Cl were used in the calibration procedure. A conditioning solution (CS), which consists of CH$_3$COONa × 3H$_2$O (c=7.351 × 10^{-2} mol L^{-1}), KH$_2$PO$_4$ (c=2.204 × 10^{-3} mol L^{-1}), K$_2$HPO$_4$ (c=4.650 × 10^{-3} mol L^{-1}), and MgSO$_4$ × 7H$_2$O (c=1.623 × 10^{-3} mol L^{-1}), was prepared to adjust the ionic strength.

The chemicals were reagent grade quality and were supplied by Kemika (Croatia), except for CH$_3$COONa × 3H$_2$O, which was supplied by Mallinckrodt Baker (Holland).

Deionized water was used in the preparation of all solutions. The synthetic wastewater (SW), and trace elements solution (TES) were prepared as described below in section 2.2. A synthetic wastewater blank (SWB) was also prepared, which contained all of the chemicals listed below except for the ions to be determined.

2.1.2. Electrode

Ammonium (ELIT 8051), Nitrite (ELIT 8071), and Nitrate (ELIT 8021) solid ISEs were used in the measurements. A silver/silver(I) chloride maintenance-free Single Junction Reference Electrode (ELIT 001, with gel 4 mol L^{-1} solution of KCl saturated with AgCl) was used as a reference. All of the electrodes were supplied by NICO2000 Ltd. (UK). Between measurements, the electrodes were stored in the preconditioning/standard solutions that were recommended by the manufacturer.

2.1.3. Apparatus

The potentiometric measurements were performed on an EA 168 Quad pH/mV Amp 4 channel amplifier connected to an e-corder 821, which is an 8 channel, high-resolution, high-speed, computer-based data recording system that uses Chart software for data acquisition and analysis (supplied by eDAQ Pty Ltd., Australia). The solutions were magnetically stirred during the measurements (801 Stirrer; Metrohm, Switzerland).

2.1.4. Procedure

The electrodes were calibrated in solutions containing four different concentrations of the analytes, ranging from c=10^{-5} - 10^{-2} mol L^{-1}. Each solution was diluted in 25 mL of the CS. The electrodes were recalibrated every 30 measurements. The sample aliquots were diluted in 25 mL of the CS, and the electrode potentials were recorded at room

temperature under constant stirring. Stable values of the potential (± 0.1 mV) were achieved within 5 minutes, although the exact time varied for each sensor depending on the concentration of the species being determined.

2.1.5. Optimization strategy using Solver

Microsoft Excel (2007) for Windows contains a spreadsheet optimization modeling system called *Solver*. The activation of *Solver* is simple and can be selected by choosing *Add ins* in the *Tools* menu. *Solver* was used to predict the results of the model for an initial set of parameters over a range of values of the dependent variables and to compare these results with the experimental data. The sum of the squared residuals between the two arrays was calculated, and the error between the two data sets was minimized by varying the values of the parameters according to an iterative search algorithm.

2.2. Simultaneous nitrification and denitrification experiment in the HRTB

2.2.1. Microorganism, SW and growth conditions

The working microorganism was a pure culture of *P. denitrificans* DSM 413 (obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) cultivated at room temperature (20±1 ºC) in the SW, which was composed of $CH_3COONa \times 3H_2O$ (c=7.351 × 10^{-2} mol L^{-1}), KH_2PO_4 (c=2.204 × 10^{-3} mol L^{-1}), K_2HPO_4 (c=4.650 × 10^{-3} mol L^{-1}), NH_4Cl (c=5.613 × 10^{-2} mol L^{-1}), $NaNO_3$ (c=3.235 × 10^{-2} mol L^{-1}), $MgSO_4 \times 7H_2O$ (c=1.623 × 10^{-3} mol L^{-1}), and 3 mL L^{-1} of TES. TES was prepared according to Robertson and Kuenen (1992) and was composed of the following trace elements: $ZnSO_4$ (c=1.362 × 10^{-2} mol L^{-1}), $CoCl_2 \times 6H_2O$ (c=6.767 × 10^{-3} mol L^{-1}), $CuSO_4 \times 5H_2O$ (c=6.288 × 10^{-3} mol L^{-1}), $C_{10}H_{16}N_2O_8$ (EDTA) (c=0.171 mol L^{-1}), $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (c=8.899 × 10^{-4} mol L^{-1}), $FeSO_4 \times 7H_2O$ (c=1.798 × 10^{-2} mol L^{-1}), $MnCl_2 \times 4H_2O$ (c=2.557 × 10^{-2} mol L^{-1}), and $CaCl_2$ (c=4.956 × 10^{-2} mol L^{-1}). The medium was sterilized at 121 ºC for 20 minutes. The chemicals were reagent grade quality and supplied by Kemika (Croatia), except for $CH_3COONa \times 3H_2O$, which was supplied by Mallinckrodt Baker (Holland).

2.2.2. HRTB and experimental set-up

The HRTB design and construction used herein, has been described elsewhere (Rezić et al., 2007). The microbial culture was first cultivated on a rotary shaker (72 h, 20±1 ºC, 150 min^{-1}, eccentricity 50 mm) and further propagated by batch cultivation (inoculum, 7.5% v/v) in a stirred tank bioreactor. The bacterial biomass obtained by batch cultivation (7.5 L) in the stirred tank bioreactor was used for the inoculation of the HRTB (liquid volume, 15 L; total volume, 98 L). A constant airflow rate of 152 L h^{-1} was used throughout the experiment, except for the periods when the aeration was turned off to enhance the denitrification process. The bioprocess dynamics

were studied at different combinations of the process parameters: the medium inflow rate was varied from 0.5 to 2 L h^{-1} and the HRTB rotation speed was varied from 5 to 20 min^{-1}. Samples were collected in duplicate at five positions along the length of the HRTB. After establishing a new set of process parameters, five residence times (37.5-150 h, depending on the medium inflow rate) were allowed to pass before the samples were collected.

2.2.3. Determination of biomass, acetate, ammonium, nitrate, and nitrite concentrations

To determine the concentration of the biomass in the SW/biomass suspension, a 35-mL sample was centrifuged for 20 minutes at 4500 min^{-1}, washed twice with demineralized water, dried at 105 ºC for 48 h, cooled and weighed. The supernatant was stored at −20 °C. The liquid samples were thawed and homogenized before the acetate, ammonium, nitrate, and nitrite concentrations were determined. The acetate concentration was determined spectrophotometrically (UV/Vis spectrophotometer model 1700, Shimadzu, Japan) using Boehringer Mannheim/R-Biopharm enzymatic test kits (Cat. No. 10 148 261 035).

The concentrations of ammonium, nitrate, and nitrite were simultaneously determined using ISEs, as described above. All values were expressed as the average of 4 measurements.

2.2.4. Bioprocess efficiency parameters

The removal efficiencies of acetate, ammonium, and nitrate (substrate, S) were calculated using Eq. (1), where γ_0 is the concentration of the substrate in the inflow to the HRTB, and γ_{out} is the concentration of the substrate in the outflow from the HRTB.

$$RE_S = [(\gamma_0 - \gamma_{out}) / \gamma_0] \times 100 \tag{1}$$

The volumetric consumptions of acetate, ammonium, and nitrate were estimated using Eq. (2), where τ is the hydraulic residence time of the liquid in the HRTB.

$$Q_S = (\gamma_0 - \gamma_{out}) / \tau \tag{2}$$

3. Results and discussion

3.1. Characterization and estimation of the electrode parameters

Before determining the ammonium, nitrate, and nitrite content in the synthetic wastewater samples that were withdrawn from the HRTB, it was necessary to adapt commercially available ISEs for use in complex wastewater matrices. Additionally, before estimating the electrode parameters with multivariate analysis and *Solver* optimization, the response of each ISE in pure analyte solutions was characterized. The electrodes responded to the ammonium, nitrate, and nitrite ions according to the

Nernst equations (Eqs. 3 - 5), where E is the potential difference between the sensing and reference electrodes, E^0 is a constant potential, S is the electrode slope and $a_{NH_4^+}$, $a_{NO_3^-}$ and $a_{NO_2^-}$ are the activities of the ions.

$$E = E^0 + S \times \log a_{NH_4^+} \qquad (3)$$

$$E = E^0 - S \times \log a_{NO_3^-} \qquad (4)$$

$$E = E^0 - S \times \log a_{NO_2^-} \qquad (5)$$

The response characteristics and the corresponding statistics of the electrodes in the pure ion solutions are summarized in Table 1. The electromotive force of the cell consisting of each particular electrode and the reference cell was measured in the series of corresponding ion solutions, covering the concentration range of 1×10^{-6} - 1×10^{-1} mol/L. The correlation between the measured E values and logarithm of the concentrations, defined by Eqs. (3) - (5), was confirmed by using the linear regression analysis. Therefore, the slope value (S) and constant potential term (E^0) were determined. The high correlation coefficient (R^2) values indicated the strong linear relation between the variables.

The confidence intervals for S and E^0 were calculated from the corresponding standard deviation values. In real systems, which are typically more complex, ISEs can be affected by numerous analytical interferents, which can either increase or decrease the detected analyte concentration. The response of the ISE to the primary ion and the interferents is described by the Nikolsky-Eisenman equation (Eq. 6). Here, a_i is the activity of the

primary (measured) ion (I), K_{ij} is the selectivity coefficient of the electrode against the interfering ion, a_j is the activity of the interfering ion (j), and z_i and z_j are the charges of the primary and the interfering ions, respectively. The activity coefficients were calculated according to the Davies equation.

$$E = E^0 + S \times \log(a_i + \sum K_{ij} a_j^{z_i/z_j}) \qquad (6)$$

Potassium ions can seriously interfere with the determination of ammonium using ISEs (Jin et al., 2004) and they are present at a high concentration in the SW formulation that was used in this investigation. The mixed solution method was used for the determination of the potentiometric selectivity coefficients.

A multivariate calibration of this electrode set (ammonium, nitrate, and nitrite) was performed by varying the concentrations of the important interferents, including potassium. This procedure allowed for the calculation of the selectivity coefficient, electrode slope, and constant potential term of each electrode. The Nikolsky-Eisenman parameters for each electrode were modeled using a matrix of the potentials that were measured by the electrodes in the calibration solutions. A set of potentials was predicted based on the model and calibration solution parameters, and the validity of the model was estimated by examining the residuals between the predicted and observed potentials.

Table 2 shows a portion of the spreadsheet that displays the potentiometric calibration data and the model parameters for the ammonium electrode after optimization using *Solver*.

Table 1. The response characteristics and the corresponding statistics of the ammonium, nitrate, and nitrite electrodes in the pure analyte solutions

ISE	$\dfrac{S}{\text{mV/decade}}$	E^0 / mV	R^2	*Linearity range* c / mol L^{-1}	*Detection limit** c / mol L^{-1}
Ammonium	53.2 ± 0.8	420.4 ± 4.8	0.9997	5×10^{-5} - 5×10^{-1}	2×10^{-5}
Nitrate	55.8 ± 1.9	205.0 ± 3.4	0.9977	5×10^{-6} - 1×10^{-1}	2×10^{-6}
Nitrite	55.5 ± 2.3	12.5 ± 4.2	0.9973	1×10^{-5} - 1×10^{-2}	4×10^{-6}

*DL (detection limit) = 3 SD (standard deviation) number of measurements, n = 5

Table 2. Potentiometric calibration data and model parameters for the ammonium electrode after optimization using *Solver* (with an initial analyte volume of 25.0 mL and an initial ammonium concentration of 1.0 mol L^{-1})

Model parameter	
E^0	425.433
S	57.197
K_{ij}	1.14×10^{-1}
$\log K_{ij}$	-0.9427

E/mV	$c_{NH_4^+}$ /mol L^{-1}	$^a I^*$/mol L^{-1}	$a_{NH_4^+}$ /mol L^{-1}	c_{K^+} /mol L^{-1}	$^b E_{model}$	^{c}res.	^{d}SR
288.86	3.98×10^{-3}	9.98×10^{-2}	3.07×10^{-3}	1.15×10^{-2}	288.79	0.0710	0.0050
314.32	1.38×10^{-2}	1.09×10^{-1}	1.06×10^{-2}	1.14×10^{-2}	314.66	-0.3310	0.1095
338.64	3.85×10^{-2}	1.31×10^{-1}	2.90×10^{-2}	1.11×10^{-2}	338.29	0.3510	0.1232
385.45	2.86×10^{-1}	3.54×10^{-1}	2.00×10^{-1}	8.22×10^{-3}	385.54	-0.0911	0.0083
						$^e S_{sr} = 0.2461$	

$^a I^*$ = ionic strength, $^b E_{model}$ = calculated value obtained by the Nikolsky-Eisenman equation after optimization, ^{c}res. = difference between measured E and E_{model} values, ^{d}SR = square of residuals (res.), $^e S_{sr}$ = sum of squares of residuals

The experimental data have been compared to an appropriate theoretical model in which the unknown parameters (E^0, S, K_{ij}) were optimized. By using *Solver*, the values for those variables that would minimize the sum of the squares of the differences between the theoretical and the experimental data were determined. In other words, the least-squares criterion was used to fit a theoretical model to the experimental data using the entire data set.

3.2. Determination of the ammonium, nitrate, and nitrite concentrations in the model systems

An SWB solution was prepared that contained all of the ingredients except the analytes (ammonium, nitrate, and nitrite), thus simulating a real system. Known amounts of the analytes were added to this solution to verify the impact of the SWB ingredients on the quantitation of the analytes. The measured electrode parameters and the corresponding statistics are given in Table 3. The generated potentiometric experimental data were compared to the appropriate theoretical model in which the sensor response parameters were optimized.

Solver was used to determine the values for those variables that would minimize the sum of the squares of the differences between the theoretical model and experimental data, using the same methodology as previously described. The SWB solutions contained the three analytes at two concentrations over two orders of magnitude that covered the expected concentration range of the bioprocess.

The calculated K_{ij} value is in good accordance with the literature data. No other interfering ions were present in the system investigated herein. The results of these investigations are given in Table 4. The accuracy of the measurements (95.3% - 101.0%) is satisfactory for the determination of ammonium, nitrate, and nitrite in complex matrices. A good fit of the theoretical models to the experimental values was obtained for all the electrodes and calibration parameters that were tested. Using these methodologies, the values of the selectivity coefficient, K_{ij}; the slope, S; the constant potential term, E^0, (Table 3) and the sample concentration, expressed as the recovery (Table 4) were calculated.

The estimates of the standard errors of the parameters, the standard error of the output variable, and the correlation coefficients proved the validity of the model.

3.3. Bioprocess dynamics and efficiency studies

The investigation in the HRTB was a continuation of a previous study by Rezić et al. (2007), which confirmed that acetate and ammonium removal in the HRTB by *P. denitrificans* in a one-step process can be achieved. The commercial ISEs adapted to wastewater matrices were used for bioprocess dynamics and efficiency studies by monitoring the concentrations of the substrates and the intermediates/products in samples that were collected from the HRTB.

Table 3. The parameters and corresponding statistics of the electrodes in the model SWB solutions containing known concentrations of ammonium, nitrate, and nitrite

Parameter	Electrode investigated		
	Ammonium	*Nitrate*	*Nitrite*
^aS/mV/decade	53.9 ± 1.4	52.6 ± 0.1	51.4 ± 0.1
$^aE^0$/mV	376.2 ± 2.3	210.0 ± 0.3	-11.7 ± 0.2
$logK_{ij}$*	-0.80 ± 0.04	-3.90 ± 0.02	-3.60 ± 0.01
R^2	0.9999	1.000	1.000
$^bSE(y)$	0.494	0.126	0.041

^{a}*value ± standard deviation;* ^{b}*SE(y) = standard error of measurement*

Table 4. The results of the direct potentiometric measurements of ammonium, nitrate, and nitrite in the model SW solutions

Analyte used	c_{added}/mol L^{-1}	$^ac_{found}$/mol L^{-1}	$a_{recovery}$ / %	RSD / %
Ammonium	5.61×10^{-2}	5.43×10^{-2}	96.8	1.22
	5.61×10^{-4}	5.35×10^{-4}	95.3	1.67
Nitrate	3.24×10^{-2}	3.27×10^{-2}	101.0	0.49
	3.24×10^{-4}	3.21×10^{-4}	99.2	0.78
Nitrite	4.00×10^{-3}	3.89×10^{-3}	97.2	1.32
	4.00×10^{-5}	3.82×10^{-5}	95.5	1.63

^{a} *Average of 5 measurements*

Ammonium, nitrate, and nitrite, the latter of which is an intermediate of the bioprocess, were simultaneously determined in the samples, thus shortening the ion determination time. This is of vital importance when employing off-line/on-site analysis of bioprocess variables. The obtained data were then compared to data obtained by Rezić et al. (2007), in which the spectrophotometric determination of ammonium and nitrite was applied.

In the investigation by Rezić et al. (2007), the SW contained ammonium as a source of N and acetate as a source of C; however, in the current study, nitrate was included in the SW as an additional source of N. Furthermore, the aeration regime was changed during the cultivation to enhance the denitrification process within the microbial biofilm. The concentrations of the substrates in the inlet feed were 4.3 g L^{-1} acetate, 1.0 g L^{-1} ammonium and 2.0 g L$^-$nitrate. After a stable microbial biofilm had formed, the effect of the bioreactor process parameters on the bioprocess dynamics in the HRTB was studied. The process parameters that were investigated included the medium inflow rate (F=0.5 – 2 L h^{-1}) and the HRTB rotation speed (n = 5 - 20 min^{-1}), as well as changes in the aeration regime (Fig. 1). The concentration profiles of acetate, ammonium, and suspended biomass concentration as well as pH along the length of the HRTB were similar to those obtained by Slavica et al. (2004) and Rezić et al. (2007) and are presented in Fig. 2.

A bacterial culture of *P. denitrificans* grew in a suspension of single cells and cell aggregates and attached to the inner surface of the bioreactor as a biofilm. The preliminary results of biofilm thickness measurements (data not shown) as well as the characteristics of the formed biofilm were similar to the previous study conducted by Rezić et al. (2007). The concentration profile of the suspended biomass, shown in Fig. 2, exhibits a gradual increase along the length of the HRTB, which agrees with the results of the investigation conducted by Rezić et al. (2007). A similar pattern was observed for all other combinations of the process parameters (data not shown).

An increase in the pH was observed along the length of the HRTB (Fig. 2) for all combinations of process parameters because of acetate degradation. Tubular bioreactors are characterized by a liquid plug flow that leads to the formation of concentration and/or temperature gradients along the length of the bioreactor (Moser, 1985). The concentration gradients were also observed for ammonium, nitrate, and nitrite ions. The ammonium ion concentration profile and range were in agreement with those obtained by Rezić et al. (2007), as previously mentioned. As a result of dilution, the concentrations of all the substrates were lower at the place of medium inflow to the HRTB (0% l_{HRTB}) then their initial concentrations in the inlet feed. The nitrite ion in the output flow of the HRTB, however, was significantly higher (100 to 1000 times) than that measured by Rezić et al. (2007). This disparity is expected considering that the cultivation medium in this investigation contained nitrate at a concentration of 2.0 g L^{-1}, which is subsequently reduced to nitrite by *P. denitrificans*.

The removal efficiency and volumetric consumption of acetate, ammonium, and nitrate for different combinations of bioprocess parameters are presented in Table 5.
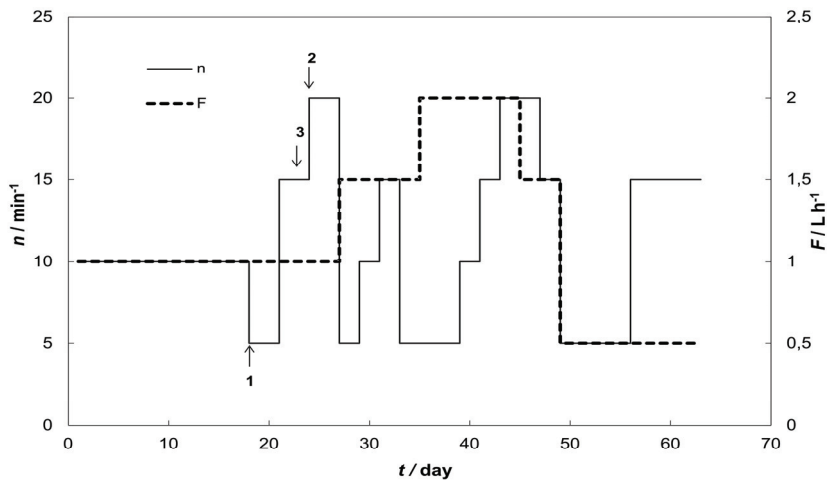

Fig. 1. The changes in the medium inflow rate (*F*, ---) and the bioreactor rotation speed (*n*, —) of the HRTB during the investigation. Arrows 1, 2 and 3 indicate the changes in the aeration regime
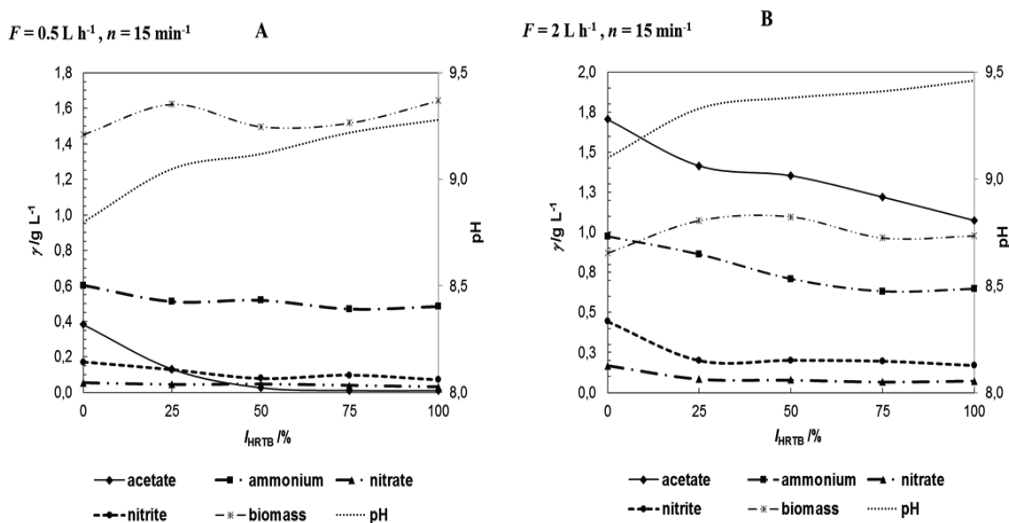

Fig. 2. The biomass, acetate, ammonium, nitrate, and nitrite concentrations as well as the pH along the length of the HRTB (*l*) at medium inflow rates of $F = 0.5$ L h^{-1} (A) and 2 L h^{-1} (B) and at rotation speed of $n = 15$ min^{-1}

Table 5. The removal efficiency (RE_S) and volumetric consumption (Q_S) of acetate, ammonium, and nitrate at different combinations of the process parameters

F/L h⁻¹	τ /h	n/min⁻¹	Aeration regime	$\dfrac{U_{Ac}}{\%}$	$\dfrac{U_{NH_4^+}}{\%}$	$\dfrac{U_{NO_3^-}}{\%}$	$\dfrac{Q_{Ac}}{\text{g L}^{-1}\text{h}^{-1}}$	$\dfrac{Q_{NH_4^+}}{\text{g L}^{-1}\text{h}^{-1}}$	$\dfrac{Q_{NO_3^-}}{\text{g L}^{-1}\text{h}^{-1}}$
0.5	30	5	aerobic	99.78	62.00	96.91	0.143	0.021	0.065
		15	aerobic	99.75	51.48	98.41	0.143	0.017	0.066
1	15	5	anaerobic	62.04	16.80	99.31	0.178	0.011	0.132
		10	aerobic	98.31	25.70	0.00	0.282	0.017	0.000
		15	aerobic/anaerobic*	97.72	25.34	80.31	0.280	0.017	0.107
		20	anaerobic	69.30	19.00	97.62	0.199	0.013	0.130
1.5	10	5	aerobic	93.74	52.70	98.99	0.403	0.053	0.198
		10	aerobic	92.45	44.40	99.05	0.398	0.044	0.198
		15	aerobic	95.75	61.56	97.94	0.412	0.062	0.196
		20	aerobic	96.15	35.40	99.12	0.413	0.035	0.198
2	7.5	5	aerobic	81.73	41.80	0.00	0.469	0.056	0.000
		10	aerobic	81.36	58.40	94.12	0.466	0.078	0.251
		15	aerobic	75.02	35.40	96.35	0.430	0.047	0.257
		20	aerobic	84.91	39.60	96.01	0.487	0.053	0.256

* 4 residence times aerobic, and 1 residence time anaerobic

A complete consumption of acetate was achieved at an inflow rate of 0.5 L h⁻¹. At an inflow rate of 1.0 L h⁻¹, the aeration regime was changed more than once (Fig. 1), and the acetate consumption decreased. The volumetric consumption of acetate ranged from 0.143 to 0.282 g L⁻¹ h⁻¹ at medium inflow rates between 0.5 and 1.0 L h⁻¹. When the medium inflow rate was increased to 1.5 – 2.0 L h⁻¹, the volumetric acetate consumption increased by a factor of two (0.398 – 0.487 g L⁻¹ h⁻¹). This increase can be attributed to a higher volumetric acetate overload. The calculated efficiencies and volumetric consumption rates for ammonium and nitrate were based on data obtained by using adapted ISEs and were calculated for all combinations of selected bioprocess parameters.

In comparison to the results based on the spectrophotometric determination of ammonium ion obtained by Rezić et al. (2007), the removal efficiency and volumetric consumption of ammonium obtained in this study using adapted ISEs were similar. The ammonium consumption efficiency ranged from 35.4 to 62.0% for medium inflow rates of F=0.5, 1.5 and 2.0 L h⁻¹ at all bioreactor rotation speeds. The volumetric consumption of ammonium (for the same process parameters) ranged from 0.017 to 0.078 g L⁻¹ h⁻¹. The minimum ammonium consumption efficiency (0.011 – 0.017 g L⁻¹ h⁻¹) and the minimum volumetric consumption of ammonium (0.011 – 0.017 g L⁻¹ h⁻¹) were observed at an inflow rate of 1 L h⁻¹ for all bioreactor rotation speeds. The low efficiency and consumption of ammonium are results of the multiple changes in the aeration regime that were observed for these combinations of the process parameters (Fig. 1).

The nitrate consumption efficiency was high (> 94%) for almost every combination of the process parameters. A decrease in the nitrate concentration was observed along the length of the HRTB, which indicates that denitrification occurred. For F=1.0 L h⁻¹ and n=10 min⁻¹ as well as for F=2.0 L h⁻¹ and n=5 min⁻¹, the efficiency was 0% and the nitrate concentrations along the bioreactor were higher than the input concentrations. Sudden environmental changes occurred in both cases: for F=1.0 L h⁻¹ and n=10 min⁻¹ there was an anaerobic-aerobic transition, and for F=2.0 L h⁻¹ and n=5 min⁻¹ there was a biofilm regeneration preceded by a sudden drop in the pH and a peeling of the biofilm. Therefore, the 0% efficiency is a result of an insufficiently rapid metabolic adjustment to the changes in the conditions.

Nitrite was detected at concentrations ranging from 17.54 to 443.74 mg L⁻¹ along the length of the HRTB. The lowest nitrite concentrations in the bioreactor occurred at a flow rate of F=1.0 L h⁻¹ and rotation speeds of n=5 and 20 min⁻¹ during the anaerobic regime and a rotation speed of n=15 min⁻¹ during the final working bioreactor volume when the aeration was turned off. The lowest input and output concentrations were determined under these same conditions.

These conditions favor the denitrification process; therefore, nitrite was reduced to its gaseous intermediates and no nitrite accumulated in the medium. The consumption of acetate, ammonium, and nitrate and the presence of nitrite confirmed that SND processes occurred in the HRTB.

4. Conclusions

Multivariate analysis and *Solver* optimization were used to adapt and optimize commercially available ISEs for the detection of ammonium, nitrate, and nitrite in wastewater matrices.

Apart from being cheaper and simpler compared with ion chromatography and spectrophotometry, this technique reduced the time required for the off-line analysis of these ions in synthetic wastewater samples during and after treatment in the HRTB.

The simultaneous determination of the consumption of ammonium and nitrate and the presence of nitrite by adapted commercial ISEs

confirmed that SND processes occurred in the HRTB. Thus, it can be concluded that the adaptation and optimization of commercial ISEs by using *Solver* was performed successfully.

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