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Development of flow injection potentiometric methods for the off-line and on-line determination of fluoride to monitor the biodegradation of a monofluorophenol in two bioreactors

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ABSTRACT

Water treatment has become a source of concern as new pollutants and higher volumes of waste water must be treated. Emerging biological approaches, namely the use of bioreactors, for cleaning processes have been introduced. The use of bioreactors requires the development of efficient monitoring tools, preferably with real-time measurements. In this work, a couple of flow injection systems were developed and optimized for the potentiometric determination of fluoride to monitor a rotating biological contactor (RBC) bioreactor and a sequencing batch reactor (SBR) with off-line and on-line sampling. Both the RBC and the SBR bioreactors were set up for the biodegradation of the halogenated organic compound 2-fluorophenol and, as fluoride was a degradation byproduct, the process was monitored by following up its concentration.

The described flow injection potentiometric methods enabled the fluoride determination within the required quantification range 0.10–100 mM. The possible interferences from the growth medium were minimized in-line. The determination rate was 78 h^{-1} for the off-line monitoring of RBC and 50^{-1} h for the on-line monitoring of the SBR, with a sample consumption of 0.500 mL and 0.133 mL per determination, respectively. Furthermore, the overall reagent consumption was quite low. The accuracy of the system was evaluated by comparison with a batch procedure. The SBR efficiency was monitored both on-line by the flow system and off-line by HPLC, for comparison purposes.

1. Introduction

The awareness of water pollution as a significant environmental problem has led to a tighter control of water quality. The new regulations and more strict limits have created awareness of the importance of cleaning effluents to prevent water contamination. Treatment plants are required to carry out more efficient cleaning procedures both in terms of new parameters and higher effluent volume. Due to the exponential increase of the volume of waste water to be treated, treatment plants must become more efficient, avoiding the need to increase their operation area. So, bioremediation processes are becoming quite common nowadays, namely involving the use of microorganisms and plants. The use of microorganisms for waste water treatment is very promising and provides an effective solution for the treatment of new pollutants. In this scenario, bioreactors have been the aim of intensive study as new methods for cleaning water effluents.

To assure efficient working conditions, it is crucial to monitor key parameters, otherwise the entire process can be lost. The monitoring process should be in real-time, preferably on-line, enabling immediate action when and if necessary. Parameters that can be assessed with probes such as pH, oxygen level and temperature, are fairly easy to monitor but normally they do not provide information upon the bioprocess itself.

The aim of this work was to devise automatic flow analysis methods to monitor the efficiency of a rotating biological contactor (RBC) bioreactor and a granular sequencing batch reactor (SBR), with off-line and on-line sampling, respectively. Both the SBR and the RBC bioreactors were set up for the biodegradation of 2-fluorophenol, a halogenated organic micropollutant of some industries that can be present in the waste waters at low (but worrying) concentrations (unpublished results). At the polishing level of cleaning waste waters effluents the aim is to remove minimal amounts of highly toxic pollutants, such as the 2-fluorophenol.

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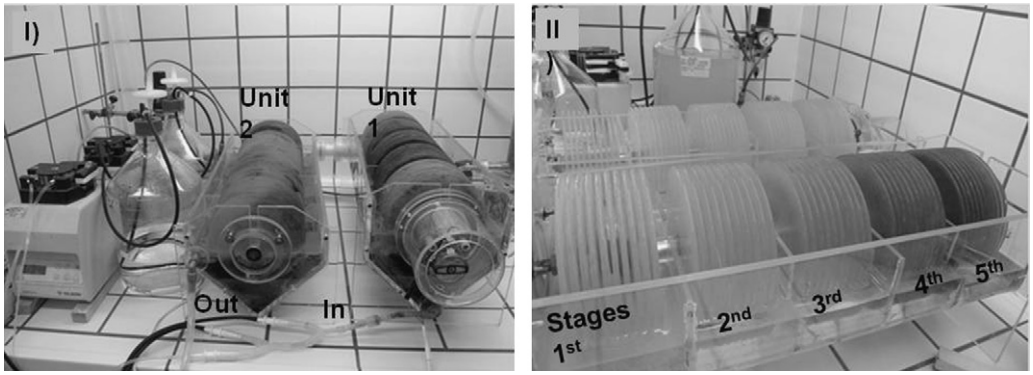

Fig. 1. RBC bioreactor, (I) the two units with the locations of feeding inlet (In) and outlet (Out); (II) the different stages of unit 1.

To monitor the 2-fluorophenol removal, chromatographic techniques are generally employed. But these techniques imply expensive equipment (as well as high maintenance costs) and also involve time-consuming sample preparation and analysis. So, an alternative strategy would be to measure the by-products of the biodegradation process.

The overall mineralization process of the 2-fluorophenol in aerobic conditions is the following:

$$2C_6H_5FO + 13O_2 \Rightarrow 12CO_2 + 4H_2O + 2H^+ + 2F^-$$

So, measuring fluoride concentration may be a straightforward method to monitor the bioprocess efficiency. However if the determination of the by-products, such as fluoride, is carried out in a batch mode, it will remain a time consuming and laborious process. So, a reliable, real-time, preferably on-line alternative should be aimed.

Flow injection analysis (FIA) [1] can be a very useful tool for on-line bioprocess monitoring [2–9]. If the determination of fluoride is aimed, potentiometry with a fluoride ion-selective electrode is the obvious choice for its measurement. Actually, the use of flow injection potentiometry has been previously reported for drinking waters [10], waters and toothpaste [11] and tap-water [12] and it proved to be an effective combination.

In this scenario, two flow injection potentiometric methods were developed for the determination of fluoride to monitor the two aforementioned bioreactors, set up for the 2-fluorophenol biodegradation. The FIA method devised for the RBC monitoring involved an off-line determination using a commercial combined fluoride. The operation conditions of the fluoride electrode in the aimed dynamic range were studied and an extensive interference study, due to the use of growth medium in the bioreactor, was carried out.

To monitor the SBR, the developed FIA system enabled the on-line determination of fluoride using a laboratory made tubular fluoride selective electrode, previously developed and studied by Santos et al. [11]. The manifold configuration, the operation conditions, the in-line interference minimization, together with the sampling location and procedure, were studied. In the end, the developed FIA method enabled an automated, on-line, reliable solution with a simple manifold assembly to monitor the biodegradation process of the 2-fluorophenol.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals and boiled deionized water (specific conductance of less than 0.1 μS/cm).

The stock solution of 0.1 M sodium fluoride was prepared after weighing 1.05 g of the solid in 250 mL of deionized water. Working standards were obtained by proper dilution of the stock solution in the range 1.0×10^{-4} to 1.0×10^{-1} M.

The carrier solution was also prepared by dilution of the fluoride stock solution to a final concentration of 2.0×10^{-6} M.

The inner solution of the tubular fluoride electrode was obtained by dissolution of 500 mg of sodium fluoride and 584 mg of sodium chloride in 100 mL of deionized water to a final concentration of 0.1 M each.

The total ionic strength adjuster buffer solution (TISAB) was prepared by dissolving, in 1 L: 58.4 g sodium chloride, 61.5 g sodium acetate, 588 mg sodium citrate, 3.0 g ethylene glycol-bis[β-aminoethylether]-N,N,N′N′-tetraacetic acid (EGTA); and adding 14.5 mL of concentrated acetic acid (100%, $d = 1.05$); to the final concentrations of 1.0 M NaCl, 0.75 M $NaCH_3COO$, 2.0 M $Na_3C_6H_5O_7{\cdot}2H_2O$, 7.9 mM $C_{14}H_{24}N_2O_{10}$ and CH_3COOH 0.25 M.

2.2. Sample collection and preparation

2.2.1. Rotating biological contactor (RBC)

For the RBC bioreactor, the samples were collected from the "In" of Unit 1 (Fig. 1I), the stages 1, 3 and 5 of Unit 1 (Fig. 1II) and also the "out" of Unit 2 (Fig. 1I). The collected samples were centrifuged at 8000 rpm for 10 min at 4 °C before introduced in the developed FIA system.

2.2.2. Sequencing batch reactor (SBR)

The SBR consists of a column where the biomass grows without any physical support and works in cycles of 4 h. Each cycle consists of four different phases: feeding, aeration (reaction), settling and effluent withdrawal, as shown in Fig. 2I.

Due to the on-line sampling process, no sample pretreatment was necessary. The sampling point was at the top of the bioreactor, as shown in Fig. 2.

In this way, the collected sample had less biomass interference (Fig. 2II). Two tubes were placed inside the SBR in order to enable the recirculation of the sample in a loop of the injection valve (Fig. 2III).

2.3. Apparatus and electrodes

The flow injection systems comprised a Gilson Minipuls 3 peristaltic pump and a Rheodyne Type 5020 six-port rotary injection valve. The flow channels were assembled with Gilson PTFE tubing for propulsion and Teflon tubing from Omnifit (0.8 mm) for the remaining conduits.

As detection system, either a Crison pH meter GLP 21 potentiometer equipped with a combined fluoride electrode (Thermo

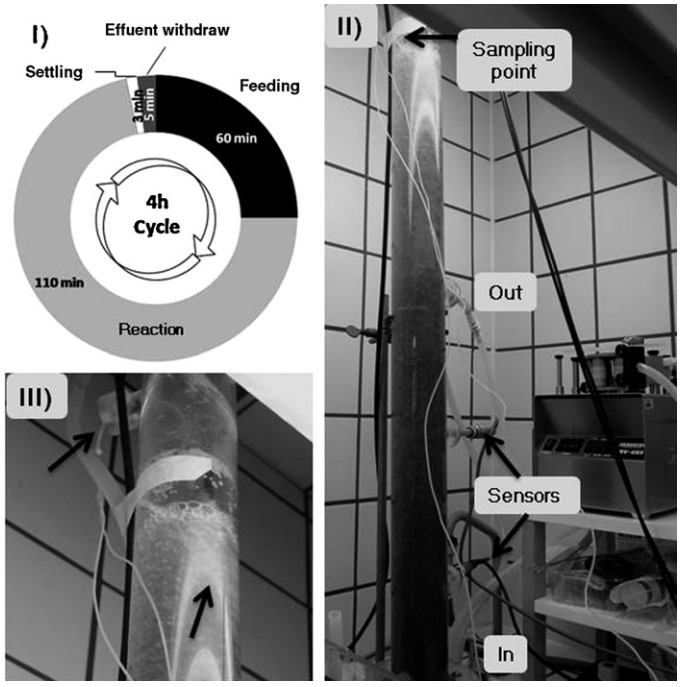

Fig. 2. Granular sequencing batch reactor (SBR), (I) schematic representation of the cycle profile with the duration of each phase; (II) SBR manifold: the inlet of air and medium with the pollutant (In), the sensors of dissolved oxygen and pH (sensors), the out channel for medium renewal (Out) and the sampling point for the FIA system (sampling point); (III) detail of the connection of the sample loop and location of the ends of the sample loop inside the SBR.

Orion 96-09), or a Crison micropH 2002 potentiometer equipped with a laboratory made tubular fluoride electrode [11], and an Orion 90-02 double junction reference electrode, were used. A stainless-steel ground electrode was used and the potentiometric signal was recorded in a Kipp & Zonnen BD 111 chart recorder.

To accommodate the combined fluoride electrode in the flow system, an option for a wall-jet arrangement was made. A Perspex device was used for a more robust arrangement and the flow inlet was from the bottom of the Perspex device in the so called "wall-jet" arrangement (Fig. 3I).

To carry out the in-line sampling in the SBR bioreactor, an additional peristaltic pump, Ismatec mini S-640, was used.

2.4. Flow injection manifold and procedure

2.4.1. Off-line monitoring of a RBC reactor, using a combined fluoride electrode

The devised flow system is depicted in Fig. 4. The sample plug was injected through a six port injection valve (IV) in a carrier

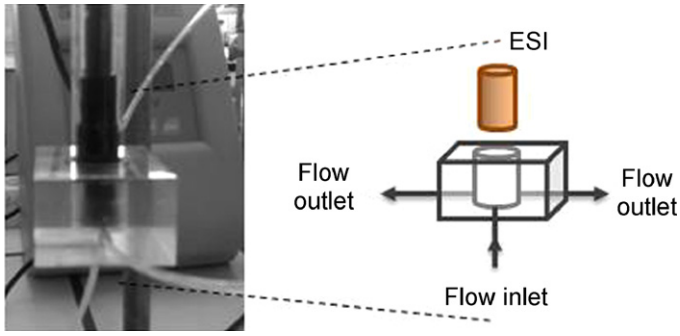

Fig. 3. Picture of the acrylic device used to incorporate the combined fluoride electrode (ESI) in the flow system and the respective schematic representation to show the configuration of the flow inlet and outlet.

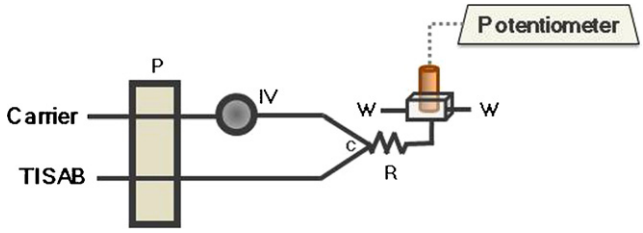

Fig. 4. FIA manifold with the combined fluoride electrode: P, peristaltic pump; IV, injection valve; c, confluence; R, reaction coil (17 cm); carrier, fluoride solution 1×10^{-6} M; TISAB, total ionic straight adjusting buffer; W, waste.

stream (2×10^{-6} M of fluoride) and then merged with the TISAB solution at the confluence (c). This way, both the adjustment of the ionic strength and the pH was obtained. The reactor (R) was placed before the electrode to promote an efficient mixing, thus resulting in a stable baseline.

2.4.2. On-line monitoring of a SBR, using a tubular fluoride electrode

The proposed flow system is depicted in Fig. 5, with the schematic representation of the sampling of the bioreactor. The sample loop of the injection valve was connected to the SBR reactor in a large recirculation loop. For the automatic recirculation procedure, a second peristaltic pump was added to the manifold.

This extra peristaltic pump was manually activated for the sampling process and it could operate in two directions. This feature enabled the reversal of the flow, effectively avoiding the possible clogging along the connection tubes.

The sample plug was injected through a six port injection valve (IV) in a carrier stream (2×10^{-6} M of fluoride) and then merged in the confluence (c) with the TISAB solution. This way, both the adjustment of the ionic strength and the pH were obtained. A ground electrode was positioned before the tubular fluoride electrode to eliminate electrical background noise.

3. Results and discussion

3.1. Potentiometric flow injection system for the off-line monitoring of a RBC reactor with a combined fluoride electrode

To carry out some preliminary studies of the potentiometric determination and the detailed study of the possible interferences of the growth medium, a simple manifold using the combined fluoride electrode set up, as shown in Fig. 4, was used. After these studies, samples were collected from the RBC bioreactor and analyzed using this manifold.

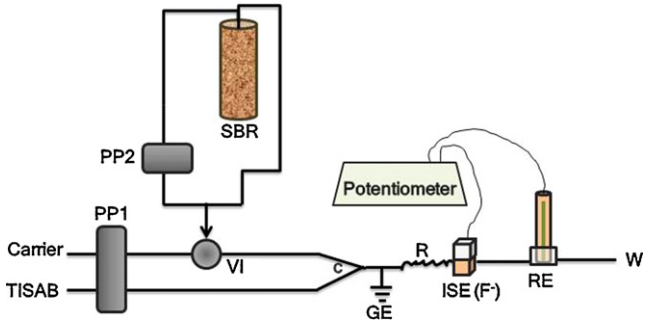

Fig. 5. Flow injection manifold for the in line potentiometric determination of fluoride in a SBR bioreactor: SBR, sequencing batch reactor; PPi, peristaltic pumps; VI, 6 six port injection valve; GE, ground electrode; ISE, ion selective tubular electrode for fluoride; RE, reference electrode; R, reaction coil (52 cm); c, Perspex Y shaped confluence; carrier, fluoride solution 2×10^{-6} M; TISAB, total ionic straight adjusting buffer; W, waste.

Table 1
TISAB solutions composition.

Solution	NaCl (mol/L)	CH_3COOH (mol/L)	$NaCH_3COO$ (mol/L)	$Na_3C_6H_5O_7{\cdot}2H_2O$ (mol/L)	CDTA (mol/L)	pH
I	1.0	0.1	–	–	–	5.0–5.5
II	0.10	0.25	0.75	0.020	–	–
III	1.0	1.0	1.0	–	0.010	5.0–5.5
IV	1.0	0.25	0.75	0.0020	–	–

3.1.1. Physical parameters: injection volume, mixing reactor and flow rates

Having set the design of the FIA manifold, the operation parameters were studied. Injection volumes of 80.0 μL, 245 μL and 500 μL were tested. The volume of 500 μL was selected because it resulted in the highest sensitivity and better linearity, so higher volumes were not tested.

Reactor lengths of 17, 25 and 100 cm were tested, but minor influence on the calibration curves slopes was noticed. The chosen reactor, length 17 cm, corresponds to the minimal length between the confluence and the electrode and was enough to ensure a good repeatability.

Before testing different flow rates for the carrier and TISAB solutions, the influence of the proportion between them was assessed. A proportion of 1:1 carrier:TISAB was compared to a proportion of 2:1 carrier:TISAB. Although no significant difference was observed, the 2:1 carrier:TISAB proportion was selected to minimize sample dispersion.

Flow rates of 3.6, 1.7 mL/min; 5.4, 2.6 mL/min and 7.2, 3.5 mL/min for carrier and TISAB solutions, respectively, were tested. The flow rates of 2.6 mL/min for the TISAB solution and 5.4 mL/min for the carrier were chosen as compromise between linearity, sensitivity and reagent consumption.

3.1.2. Carrier solution

For obtaining a faster return to the baseline, different fluoride concentrations were tested. For this study, solutions with no fluoride (deionized water), 1×10^{-7} M and 2×10^{-6} M of fluoride, were prepared. The return to the baseline with deionized water as carrier took about 2.5 min. Although the solution 1×10^{-7} M of fluoride provided a faster return, it took more than 1 min. By using the solution 2×10^{-6} M of fluoride, a decrease to 0.75 min was achieved, therefore it was the concentration chosen. Higher concentrations of fluoride were not tested to avoid the increase of the detection limit.

3.1.3. TISAB composition

The TISAB solution composition was studied in order to obtain a good adjustment of both the ionic strength and pH.

Four TISAB solutions were tested (Table 1): Solution I, based on the indicated procedure of the commercial electrode [13]; Solution II, from the previously described method by Conceição et al. [14]; Solution III, from the previously described method by Santos et al. [11]; and Solution IV, a combined arrangement of Solutions I and II.

Calibration curves were established for all the solutions and, although the slopes obtained were very similar, the TISAB solution IV provided a better sensitivity and linearity and so this was the composition chosen. This was the basic composition used for further studies.

The application of the developed FIA method to the bioreactor required an extensive interferences study. The pollutant (2-fluorophenol) is added to the RBC and SBR bioreactors together with the minimal growth medium (synthetic waste water) so the possible interferences from this medium had to be assessed. The minimal growth medium composition is presented on Table 2 and the possible interference from different components was assessed.

Table 2
Minimal growth medium composition for the two bioreactors: rotating biological contactor (RBC) and sequencing batch reactor (SBR).

Minimal growth medium component	Concentration in the:	
	RBC	SBR
$NaCH_3COO$	0.201 g/L	0.484 g/L
$MgSO_4{\cdot}7H_2O$	0.086 g/L	0.083 g/L
KCl	0.034 g/L	0.033 g/L
Na_2HPO_4	0.268 g/L	0.056 g/L
NH_4Cl	0.151 g/L	0.177 g/L
KH_2PO_4	0.128 g/L	0.027 g/L
Vischniac trace element solution (pH 6) 63.7 g/L $EDTA{\cdot}2H_2O$, 22.0 g/L $ZnSO_4{\cdot}7H_2O$, 5.54 g/L $CaCl_2$, 3.81 g/L $MnCl_2{\cdot}4H_2O$, 4.99 g/L $FeSO_4{\cdot}7H_2O$, 1.10 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1.57 g/L $CuSO_4{\cdot}5H_2O$, 0.879 g/L $CoCl_2$	0.944 ml/L	

The concentrations of the interfering agents were obtained by dissolving the appropriate solid in the fluoride standard solution. The studied concentrations of interfering agent, the respective solids used and the interfering percentages are summarized in Table 3.

The percentage of interference was calculated by comparing the potential of a 1.0×10^{-3} M fluoride standard with an equimolar standard also containing the possible interfering agent.

To the basic TISAB composition, two different chelating agents, EDTA and EGTA, were added in three different concentrations (1, 2, 3 g/L) to minimize the possible interferences. The concentrations of interfering agent, the respective solids and the percentages of interferences obtained for the different TISAB solutions are summarized in Table 3.

The results obtained showed that some ions did interfere even in the presence of lower concentrations of chelating agents. These interferences were effectively minimized with higher concentration of both chelating agents, EGTA and EDTA. When the two chelating agents are compared, EGTA proved to be more effective, reducing all interferences to <5% (Table 3), with a concentration of 3 g/L.

3.1.4. Features of the developed system

The determination of the detection limit (DL) and lower limit of linear response (LLLR) of the commercial electrode was assessed both in batch (1.4×10^{-7} M for DL and 4.8×10^{-7} M for LLLR) and in the developed FIA method (7.2×10^{-5} M for DL and 1.0×10^{-4} M for LLLR).

A typical electrode response (in the range 1.0×10^{-4} M to 1.0×10^{-2} M) was calculated as a result of 4 calibration curves obtained along four months of work: E (mV) = −58.7 (±0.9) log[F^-] − 276.8 (±34.3).

The set operation parameters were: an injection volume of 500 μL, a reactor length of 17 cm, a flow rate of 2.6 mL/min for the TISAB solution and 5.4 mL/min for the carrier. With these conditions, a determination rate of 78 h^{-1} with a waste volume of 10.4 mL per determination was obtained. The overall consumption values per determination were: 0.295 μg of NaF (carrier); 187 mg of NaCl, 208 mg of $NaCH_3COO$, 50.7 mg of CH_3COOH, 1.99 mg of $Na_3C_6H_5O_7{\cdot}2H_2O$, 10.1 mg of EGTA (TISAB solution).

Table 3
Interference values of the different ions with different amounts of chelating agents. The 3 g/L EGTA concentration was chosen due to the lower interference percentages obtained (in bold).

Solid/standard solution	Tested ion	Concentration (mM)	% interference					
			TISAB	TISAB with EDTA			TISAB with EGTA	
				1 g/L	2 g/L	3 g/L	1 g/L	2 g/L	**3 g/L**
$MnCl_2{\cdot}2H_2O$	Mn^{2+}	1.80×10^{-2}	−2.4	0.5	−3.5	4.1	4.3	−2.1	**−2.6**
$(CH_3COO)_2Zn{\cdot}2H_2O$	Zn^{2+}	1.40×10^{-2}	−7.1	−1.0	−4.5	3.6	5.8	−2.5	**0.0**
$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$	Fe^{2+}	7.20×10^{-2}	−10.3	−5.3	−4.2	2.6	8.6	−0.7	**0.5**
$(NH_4)_2SO_4$	NH^{4+}	7.57	−12.2	−3.6	−4.9	5.5	8.2	−2.1	**−0.5**
Cu(II) (1000 ppm)	Cu^{2+}	1.00	−2.0	−7.6	−0.3	6.0	11.8	−2.7	**3.9**
Na_2SO_4	$SO_4{}^{2+}$	5.40	−6.4	−4.2	−1.4	5.5	10.0	2.9	**0.5**
K_2HPO_4	$HPO_4{}^{2-}$	15.0	−13.2	−9.3	−9.2	−0.5	−1.1	−10.0	**−4.2**
$NaH_2PO_4{\cdot}H_2O$	$H_2PO_4{}^{-}$	10.3	−5.3	−8.2	−8.0	−1.1	−1.1	−9.3	**−1.5**
$CoCl_2$	Co^{2+}	6.39×10^{-3}	–	–	–	–	–	–	**−0.6**

3.1.5. Application to bioreactor samples – rotating biological contactor (RBC)

In order to assess the efficiency of the developed methodology, six samples were collected from a RBC bioreactor. To evaluate the accuracy, the results obtained by the developed FIA method, C_{FIA} (mM), were compared with those provided by the reference potentiometric batch method (method 4500 F^- C.) [15], $C_{Ref.Met.}$ (mM); the equation found was: $C_{FIA} = 0.760\ (\pm 0.457) C_{Ref.Met.} + 1.5 \times 10^{-5}$ ($\pm 1.4 \times 10^{-4}$), where the values in parenthesis are 95% confidence limits. These figures show that the estimated slope and intercept do not differ statistically from values 1 and 0, respectively. Therefore, there is no evidence for systematic differences between the two sets of results [16].

The degradation of 2-fluorophenol was assessed by the correspondent increase in fluoride concentration along the RBC bioreactor. Samples were collected from the feeding inlet (In), stages 1, 3 and 5 from unit 1 and the outlet (Out), according with the (Fig. 1) shown in Section 2.2.1. The samples were then analyzed with the developed method in order to follow the biodegradation process Table 4.

The concentration of the 2-fluorophenol fed in the inlet was about 0.3–0.4 mM and expected to decrease along the bioreactor. So, the reverse was expected from the fluoride concentration and, in fact, it increased from a value close to zero in the inlet up to a maximum in the 5th stage. The small decrease obtained in the "out" location may result from the dilution in the 2nd unit as almost all the pollutant had been degraded by the end of the 1st unit.

3.2. Potentiometric flow injection system for the on-line monitoring of a SBR with a tubular fluoride electrode

For the on-line monitoring of the SBR bioreactor, a tubular electrode was used. Some of the previous studies were revisited to readjust the determination conditions.

3.2.1. Physical parameters: injection volume, mixing reactor and flow rates

Injection volumes of 80.0 μL, 133 μL, 200 μL, 250 μL, 325 μL, 500 μL and 575 μL were tested. The volume of 133 μL was selected because it resulted in better linearity than with the volume of 80.0 μL and higher volumes did not show any significant improvement. Reactor lengths of 12 cm, 52 cm and 100 cm were experimented, with no significant influence on the calibration curves slopes. The reactor of 52 cm was chosen as a compromise between repeatability and response time.

From the studied flow rates of 2.73, 3.52, 4.46, 5.01, 5.90 mL/min, 3.52 mL/min was chosen as a compromise between linearity, sensitivity and reagent consumption.

3.2.2. Re-evaluation of interferences

The composition of the TISAB solution had been previously studied in Section 3.1.3. The evaluation of the possible interferences was reassessed according to the procedure described in the mentioned Section 3.1.3 (Table 5).

3.2.3. Features of the developed system

The chosen parameters of an injection volume of 133 μL, a reactor length of 52 cm and a flow rate of 3.52 mL/min were used for further characterization of the tubular electrode. With these conditions a determination rate of 50 h^{-1} was obtained resulting in a sample consumption of <400 μL (3 replicas) and a waste volume production of 4.2 mL per determination.

The determination of the detection limit (DL) and lower limit of linear response (LLLR) was carried out by injecting fluoride standard solutions within the concentration range from 6.6×10^{-6} M to 1.0×10^{-1} M. The values obtained were 8.1×10^{-5} M for DL and 1.0×10^{-4} M for LLLR. A typical calibration curve was: E (mV) = −58.9 (±5.5) log[F^-] − 246.4 (±28.9), the values in brackets

Table 4
Monitorization of the fluoride concentration in different sampling points of the RBC bioreactor for two consecutive days; SD, standard deviation, RSD, relative standard deviation.

Sample point	Sample ID	[F^-] ± SD, mM	RSD, %
In	I0807	0.029 ± 0.002	9.0
1st stage	1s0807	0.250 ± 0.026	10.5
3rd stage	3s0807	0.243 ± 0.020	8.5
5th stage	5s0807	0.360 ± 0.009	2.5
Out D	D0807	0.276 ± 0.021	7.5
Out E	E0807	0.246 ± 0.012	4.8
1st stage	1s0707	0.211 ± 0.016	7.5
3rd stage	3s0707	0.227 ± 0.014	6.1
5th stage	5s0707	0.239 ± 0.014	5.7
Out D	D0707	0.231 ± 0.009	3.9
Out E	E0707	0.220 ± 0.010	4.7

Table 5
Percentages of interference for several possible interfering ions with the FIA tubular electrode manifold.

Solid/standard solution	Tested ion	Concentration (mM)	% interference
$MnCl_2{\cdot}2H_2O$	Mn^{2+}	2.42×10^{-1}	0.7
$(ZnCH_3COO)_2){\cdot}2H_2O$	Zn^{2+}	8.39×10^{-2}	1.1
$(NH_4)_2SO_4$	$NH_4{}^{+}$	5.72	0.6
$NaH_2PO_4{\cdot}H_2O$	$H_2PO_4{}^{-}$	1.19	2.0
Cu(II) (1000 ppm)	Cu^{2+}	6.30×10^{-3}	−0.1
$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$	Fe^{2+}	1.59×10^{-2}	0.8
Na_2SO_4	$SO_4{}^{2-}$	5.07×10^{-1}	0.7
K_2HPO_4	$HPO_4{}^{2-}$	2.07	0.2

The percentage of interference of all possible interfering ions was inferior to 3%, thus ensuring the effective minimization of interferences.

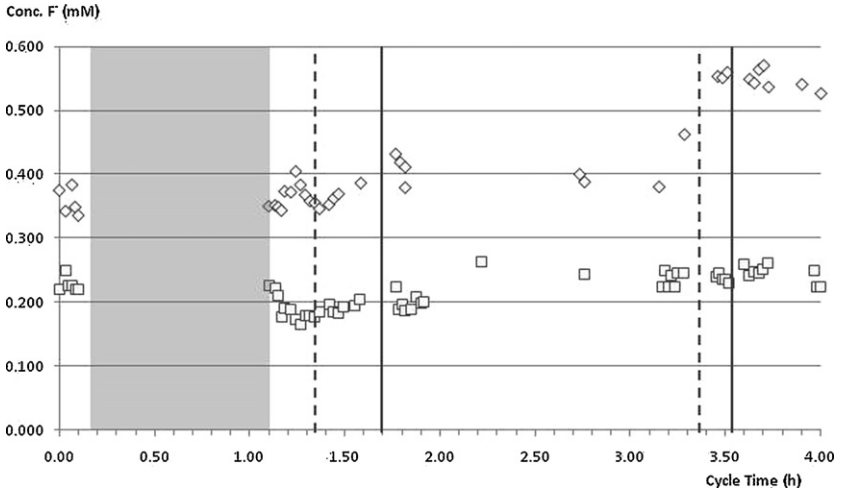

Fig. 6. Study of the efficiency of the on-line sampling of the SBR to monitor complete cycles in two days: ◊, 18.09.09; □, 26.10.09. Changing the flow direction of the sample loop: filled line, flow forward; dashed line, flow reversed. The shadowed area represents the feeding phase.

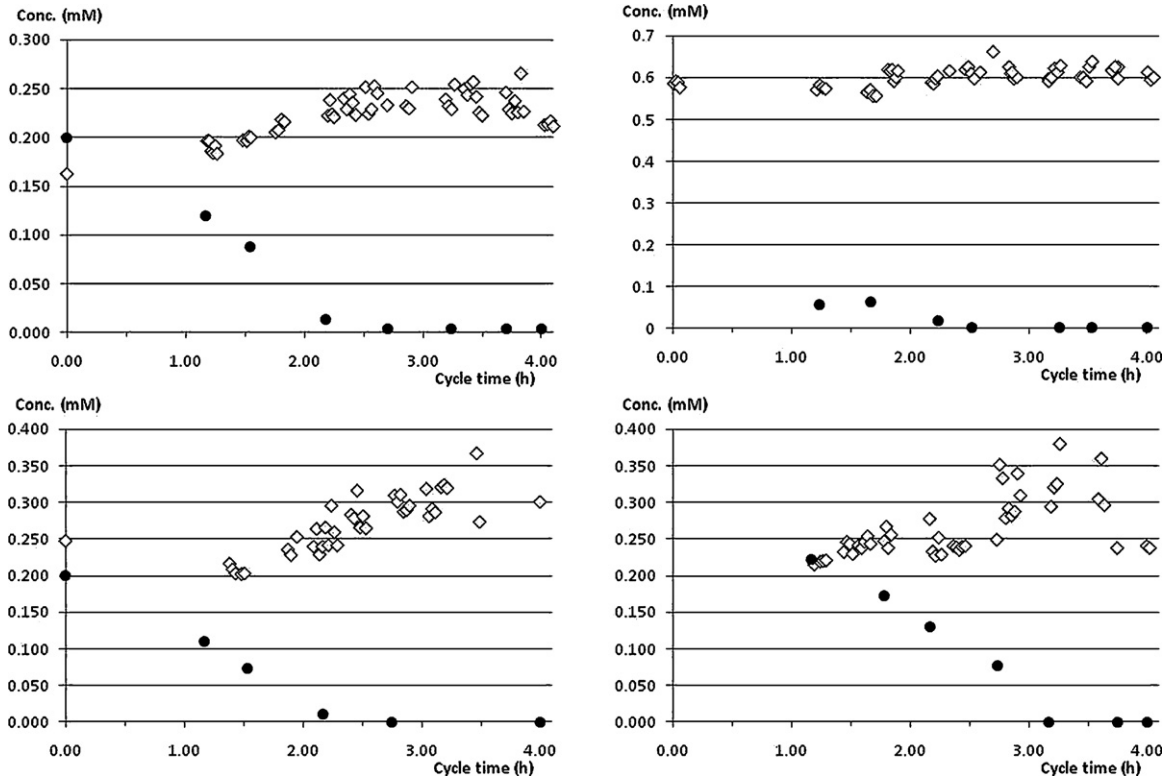

Fig. 7. Monitoring of a complete cycle of the SBR in four different days (I–IV), the ◊ represents the fluoride concentration and ● the 2-fluorophenol concentration; (I) day 28.10.09; (II) day 02.11.09; (III) day 25.11.09; (IV) day 14.12.09.

are the standard deviation of 4 calibration curves obtained along a month work.

The overall consumption values per determination were: 252 μg of NaF (carrier); 74.2 mg of NaCl, 78.2 mg of $NaCH_3COO$, 19.1 mg of CH_3COOH, 747 μg of $Na_3C_6H_5O_7{\cdot}2H_2O$, 3.80 mg of EGTA (TISAB solution).

3.3. Application to the on line SBR bioreactor monitoring

For the on-line determination, several aspects had to be considered: how and where to establish the connection between the FIA system and the SBR.

3.3.1. Sampling point

To achieve the on-line monitoring, a physical connection between the FIA assembly and the bioreactor was required. The ideal approach was to use one of five bioreactor sampling points already in place for off-line sampling, but three were already occupied. Two were used for the pH and the oxygen probes, and one was used for draining out the synthetic water at the end of the cycle. One possibility was to use the same exit used for empting the bioreactor between cycles, but then the fluoride determination could only be carried out during the empting procedure. Another possibility was at the bottom of the bioreactor, but there would be a problem with the biomass accumulation.

The chosen location was at the top of the bioreactor. This way, there would be less biomass accumulation and the sampling could be carried out through most of the cycle. At the end of the effluent withdraw, to renew the minimal growth medium (synthetic waste water), and the feeding phase, sampling cannot be carried out. This is due to the low medium level inside the SBR bioreactor.

3.3.2. Follow up of the SBR cycle

In order to ensure that the sampling loop was an effective approach and that the chosen location for sampling was appropriate, two complete cycles of the SBR were monitored. A complete cycle (Fig. 6) of the bioreactor starts with the stop of the air flow for the sedimentation of the biomass, then the medium is drained out and new synthetic water with pollutant is fed.

The on-line determination of fluoride was carried out starting just before the stop of the air flow until the next stop of the air flow (Fig. 6). The direction of the flow of the sampling loop was used in both forward and backwards to ensure that it did not affect the determination.

The results obtained proved that the choice of the sampling point was suitable for the fluoride monitoring and that the flow direction of the sample loop did not affect the determination. The flow reversal was important to ensure that there was no clogging in the sample loop.

3.3.3. Combined monitoring of fluoride and 2-fluorophenol

The increase of the fluoride concentration in the SBR results from the degradation of the 2-fluorophenol, so the degradation profile should be the mirror image of the fluoride concentration profile. This was proved by following both profiles (Fig. 7).

The 2-fluorophenol concentration was determined by HPLC, and the samples were manually collected at the indicated hours. The preparation of the sample included centrifugation at 8000 rpm for 10 min at 4 °C. As for the fluoride determination it was carried out with the developed method, with on-line sampling.

The results obtained proved that the developed method was effective in the on-line determination of the fluoride along the degradation of the 2-fluorophenol, enabling a real time follow up of the process. Even when there was a problem in the bioreactor, affecting the biodegradation process, the fluoride determination also showed an altered profile suggesting that there was in fact something wrong (Fig. 7II).

4. Conclusions

The developed methods proved to be an efficient alternative for fluoride determination as a monitoring procedure of the 2-fluorophenol biodegradation process. The on-line monitoring of the SBR resulted in avoiding the laborious process of manually taking a sample and pre determination steps such as centrifugation and/or filtration. Even with the off-line method for the RBC bioreactor, a faster determination time and real time analysis was obtained. As the fluoride formation correlates directly with the efficiency of the biodegradation process, the real-time fluoride determination, provided by the developed methods, enables a better control of the bioreactors. Therefore, it proved to be an effective alternative to monitoring 2-fluorophenol removal, using chromatographic techniques.

The possible interferences from the minimal growth medium were assessed and minimized with the presence of an additional chelating agent, the EGTA. Despite the requirement of an extra reagent an overall reduction in reagent consumption was obtained with the developed method.

For the on-line procedure, the use of a sampling loop with a secondary peristaltic pump, enabling the change of the flow direction (forward and backwards), was a successful solution to prevent clogging of biomass along the tubes connected to the bioreactor. Also the location chosen for the connection enabled a cleaning procedure to be carried out between working days. Because the tubes were connected to the top of the bioreactor, at the time of replacing the growth medium, the tubes could be filled with air to improve cleaning and avoid bacterial growth.

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