

ELECTROCHEMICAL SPECIATION OF IRON OXIDATION STATES IN WASTEWATER TREATED USING A CONSTRUCTED WETLAND

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Abstract

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Differential pulse voltammetry (DPV) was tested for the determination of iron in wastewater samples from a constructed wetland used for improving water quality. The concentration of total iron was determined in an oxalate working electrolyte. The electrode process was electrochemically irreversible in a sodium pyrophosphate working electrolyte and the iron oxidation states were separated. Iron concentrations determined using DPV were compared with results attained for a traditional spectrophotometric determination with 1,10-phenanthroline. Attained limits of detection were 0.03 mg l⁻¹ for the voltammetric determination of total iron and 0.02 mg l⁻¹ for Fe^{II} and 0.01 mg l⁻¹ for the spectrometric determination. DPV determination of iron expands the possibilities of electrochemical methods for wastewater analysis. The content of individual iron oxidation states serves as an indicator of constructed wetland aeration and redox properties.

Key words: environmental analytical chemistry, differential pulse voltammetry, spectrophotometry, redox properties, municipal wastewater treatment, *Phragmites australis*

Introduction

It is well known that wetland systems reduce many contaminants, including organics, suspended solids, nitrogen, phosphorus, trace metals and pathogens. This reduction is accomplished by diverse treatment mechanisms (Mitsch, Gosselink, 2000; Vymazal et al., 1998) being mainly physical (sedimentation, filtration, volatilisation), chemical (precipitation, adsorption) and biological (bacterial metabolism, plant metabolism, plant absorption, decomposition of organic matter and natural die-off). Natural wetlands have been used

for wastewater treatment for centuries. However constructed wetlands represent specially designed and engineered wetland systems for wastewater treatment.

Constructed wetland systems utilize natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater. They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment. A constructed wetland is actually an ecosystem which is manipulated in order that the water flowing out is cleaner, mainly without organic, suspended solids and partial nutrients (nitrogen, phosphorus) contamination (Pícek, Dušek, 2003; Šantrůčková et al., 2001). A constructed wetland should include zones with oxidation and reduction properties in its profile in order to attain optimal functioning with respect to its ability to treat wastewater. Wastewater treatment using constructed wetlands is simple and is characterized with less power (and also financial) requirements than wastewater treatment using traditional technologies. Constructed wetlands are very suitable mainly for small villages.

Wetlands are dominated by anaerobic conditions induced by soil saturation and flooding. The most significant result of flooding is the isolation of the soil system from atmospheric oxygen which activates biological and chemical processes that change the system from aerobic and oxidizing to anaerobic and reducing (Čížková et al., 2001; Faulkner, Richardson, 1989; Vymazal et al., 1998). The reduced layer is metabolically anaerobic with low redox potential values and contains reduced ions such as NH_4^+ , Fe^{2+} and Mn^{2+} ions, S^{2-} and the products of the anaerobic decomposition of organic matter (aldehydes, alcohols, organic acids and mercaptanes) (Ponnamperuma, 1972). However, there may be small oxidized pockets in the reduced soil matrix in many flooded systems with oxidized streaks corresponding to root channels. The oxidized zones are metabolically aerobic and contain oxidized ions and species such as Fe^{III} , Mn^{IV} , NO_3^- , SO_4^{2-} .

Iron is found in wetlands primarily in its reduced (ferrous, Fe^{II}) form. This form is much more soluble and available to organisms than Fe^{III} . The direct enzymatic oxidation of Fe^{II} is confined to a restricted range of organisms. The majority of bacteria indirectly cause precipitation of Fe^{III} by altering the redox potential or pH value, which in turn leads to chemical oxidation and precipitation. Thus the oxidation of ferrous ions may proceed almost completely chemically under conditions found in constructed wetlands. Wetland plants oxygenate rhizosphere sediments by radial oxygen loss from their roots (Armstrong et al., 1990; Armstrong et al., 1991; Brix, 1993). There are very steep redox potential gradients at the root/sediment interface, which can result in the oxidation and precipitation of iron as complex ferric oxyhydroxides.

The concentration ratios of individual iron oxidation states (Fe^{II} and Fe^{III}) can serve as an indicator of constructed wetland redox properties. Therefore, it is important to know the ratio of Fe^{II} and Fe^{III} concentrations in the reed bed wastewater treatment plant. This information allows for a close description and evaluation of the processes affecting wastewater treatment efficiency.

Spectrophotometry is the most popular method of iron determination in the relevant concentration range. This technique commonly uses 1,10-phenanthroline as the reagent for

the determination of Fe^{II} . This represents a very sensitive and robust analytical method. Total dissolved iron is usually determined after reduction of Fe^{III} with hydroxylamine hydrochloride solution, with total iron (dissolved + precipitated) determined following conversion of the precipitate into soluble forms. The direct determination of both iron oxidation states by spectrophotometry, based on the reaction with tiron, has been described (Abe, 1986).

Electrochemical analytical methods are very popular among ecologists and they are often used for studying the chemical properties of wetland systems. Many important parameters are determined using these methods. Potentiometric determination of redox potential, amperometric determination of dissolved oxygen, determination of conductivity (the measure of dissolved ionic species), determination of pH, NH_4^+ ions or nitrates using ISE, are often performed. The aim of this work is to test the possibilities of differential pulse voltammetry (DPV) for the determination and speciation of oxidation states of iron, in order to expand the use of electrochemical methods in the determination of this analyte – important from the ecological point of view.

The polarographic determination of iron is rather problematic when using common working solutions. In principle Fe^{II} can be determined by its electrochemical reduction to the metal at potentials between -1.4 and -1.6 V versus a saturated calomel electrode at the mercury drop electrode (Ugo et al., 2002; Vydra et al., 1976). However, for many frequent working solutions this potential range lies near the potentials where a working electrolyte starts to decompose making the determination difficult or impossible. Moreover, Fe^{II} complexation by natural ligands can hinder accurate quantification of the analyte concentration when using this determination.

Therefore, the polarographic determination of iron uses the redox reaction between the two positive oxidation states (Fe^{III} and Fe^{II}) (Beyer et al., 1975; Bond et al., 1993; Heyrovský, Zuman, 1968; Pournaghi-Azar, Fatemi, 2000). The polarographic half-wave potential for this process in non-complexing media appears at more positive potentials than the oxidation of mercury; thus the determination is impossible using a mercury electrode. However, this half-wave potential can be shifted into the potential range using suitable complex media (citrate, tartrate, oxalate), where the mercury electrode can be used to perform a highly sensitive determination of iron using differential pulse polarography or voltammetry. Using these electrolytes, the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ couple is electrochemically reversible; half wave potentials for oxidation of Fe^{II} and for reduction of Fe^{III} are identical and the iron oxidation states cannot be separated in the differential pulse voltammetry mode because they provide only one peak. The spontaneous reduction of Fe^{III} can take place in oxalic solution; however this reaction should not influence the analysis if total iron is determined.

If both iron oxidation states (Fe^{II} and Fe^{III}) are to be determined simultaneously by differential pulse voltammetry, then the electrode process has to be electrochemically irreversible (Parry, Anderson, 1973; Perämäki et al., 2000; van Staden, Matoetoe, 1998). Both DPV peaks (for Fe^{II} and Fe^{III}) are separated sufficiently (the potential ranges of cathodic reduction and anodic oxidation are separated) under the conditions of the irreversible electrode process. As the working electrolyte pyrophosphate solution can be used at a pH of about 10.

Several published papers have already dealt with iron determination and oxidation states speciation in constructed wetlands (Goulet, Pick, 2001; Wang, Peverly, 1996). These papers were mainly focused on iron determination in constructed wetland water and also on determination of iron present in plaque deposited on the surface of reed roots. However, these published papers preferred spectrometric analytical methods for iron determination. So far voltammetric methods were seldom utilized for constructed wetland water sample studies. Their use was focused on the determination of heavy metal contents in constructed wetland systems (Lim et al., 2003). The use of differential pulse voltammetry for iron determination represents a novel approach to the study of constructed wetland chemical properties.

Experimental part

Constructed wetland

The studied system was a constructed wetland with horizontal sub-surface flow located in Slavošovice, 15 km east of České Budějovice (South Bohemia, Czech Republic). This constructed wetland began operating in August 2001. The system consists of a storm overflow, a pretreatment (screens, horizontal sand trap and sedimentation basin) and two vegetated beds planted with common reeds (*Phragmites australis* (Cav.), Trin. ex Steudel). The size of each reed bed is 17 m (length), 22 m (width) and 0.9 m (depth). The reed bed is filled with gravel of a 1–2 cm size. The studied constructed wetland is projected for 150 person equivalents (PE); the area for one PE is about 5 m².

Water sampling

Water for iron determination was sampled at the inflow to the wastewater treatment plant, in the inflow zone of the reed bed and at the outflow from the constructed wetland. During sampling and sample storage, contact with air was minimized in order to avoid oxidation of Fe^{II}. Water was sampled into plastic bottles; every sampling bottle was completely filled with water so that no air remained between the liquid level and the plug. All bottles were carefully closed airtight. The samples were analysed in the laboratory as soon as possible after the sampling. Wastewater was sampled on 13th January 2005.

Instrumentation

An Eco-Tribo Polarograph (Polaro-Sensors, Prague, Czech Republic) voltammetric analyser, equipped with a hanging mercury working electrode, calomel reference electrode (3.0 mol l⁻¹ KCl) and platinum wire auxiliary electrode, was used for the electrochemical measurements. It was operated in the three-electrode arrangement in the differential pulse voltam-

metry mode. A Jenway 6300 spectrophotometer (Jenway, Felsted, Great Britain), equipped with a 10.0 millimetre glass cuvette, was used for spectrophotometric measurements. It was operated at a 510.0 nm wavelength for iron determination.

Reagents

All reagents used were either analytical reagent grade or higher purity. A standard stock solution of Fe^{III} (1000 mg l^{-1}) was prepared from $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Sigma-Aldrich, St. Louis, USA), while a standard solution of Fe^{II} was prepared from $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (Lach-Ner, Neratovice, Czech Republic). These were prepared by weighing an appropriate amount of the appropriate compound, diluting, acidifying with 1.0 ml of H_2SO_4 (2.0 mol l^{-1}) and filling to a 100 ml volume. The concentrations of prepared iron standard solutions were verified using Certipur[®] standard solution (Merck, Darmstadt, Germany). The following working solutions were used for the differential pulse voltammetric determinations: $0.2 \text{ mol l}^{-1} \text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ (Sigma – Aldrich, St. Louis, USA), 0.2 mol l^{-1} solution of ammonium oxalate (Lach-Ner, Neratovice, Czech Republic) pH adjusted with diluted H_2SO_4 (Lach-Ner, Neratovice, Czech Republic), 2.0 mol l^{-1} KCl (Lach-Ner, Neratovice, Czech Republic), $2.0 \text{ mol l}^{-1} \text{NH}_4\text{OH} / 2.0 \text{ mol l}^{-1} \text{NH}_4\text{Cl}$ (both Lach-Ner, Neratovice, Czech Republic) and 4.0 mol l^{-1} acetic acid / 4.0 mol l^{-1} ammonium acetate (both Lach-Ner, Neratovice, Czech Republic). A 1,10-phenanthroline monohydrate solution (Lach-Ner, Neratovice, Czech Republic, 0.5 g of the reagent in 100 ml of solution) was used for the spectrophotometric determination. A hydroxylamine hydrochloride solution (Penta, Chrudim, Czech Republic, 100 g l^{-1}) was used as a reducing agent. HCl (Lach-Ner, Neratovice, Czech Republic), diluted 1:9 with distilled deionized water, was added to the samples in order to dissolve them during boiling of the precipitated iron species. An acetate buffer solution, as a medium for spectrophotometric iron determination, was prepared from 200 g of ammonium acetate and 250 ml of acetic acid (both Lach-Ner, Neratovice, Czech Republic), filling up to a volume of 500 ml.

Differential pulse voltammetry (DPV)

It is essential to use the most sensitive, non-stationary voltammetric methods for the determination of iron species expected to be found in the constructed wetland. Differential pulse voltammetry (DPV) with a hanging mercury drop electrode is the suitable electrochemical method. The following selected conditions of the DPV determination were always set. The height of the potential pulse was 50 mV, pulse duration was 100 ms with a polarization rate of 10 mV s^{-1} . Prior to the analysis, all samples were deaerated with helium (99.99%) for ten minutes. Differential pulse voltammograms were recorded in a negative direction. Sensitivity was 0.1 nA. All voltammograms were recorded three times repeatedly. The working solution was not stirred during the measurement.

Differential pulse voltammetry, in an oxalate working solution, was used for the determination of total iron. An ammonium oxalate solution was prepared in distilled deionized water and pH adjusted by H_2SO_4 addition. Voltammograms were recorded from +100 to -1100 mV against a reference calomel electrode.

DPV, with the irreversible electrode process, was used for speciation of iron oxidation states. A 0.2 mol l^{-1} working solution of $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ (pH 10, adjusted with NaOH solution) was used for this determination. A working electrode was polarized from 0 mV to -1600 mV against the calomel electrode.

Ten ml samples were always taken for the voltammetric determinations, to which 10.0 ml of working solution were added. The appropriate dilution was taken into account during the evaluation of the results. For the measurement, the standard addition method was always used. Standard additions (of iron) were 0.01 mg (1.0 ml of the standard solution of 10.0 mg l^{-1} concentration) and 0.02 mg (2.0 ml of the standard solution of 10.0 mg l^{-1} concentration) respectively, if not otherwise stated.

Spectrophotometric determination of iron

The spectrophotometric determination of iron was based on the formation of a characteristic red-orange complex of Fe^{II} with 1,10-phenanthroline. Total iron was determined after the conversion of precipitated iron forms into the solution and the reduction of Fe^{III} with hydroxylamine hydrochloride. Fifty ml of the sample were always taken for analysis. One milliliter of HCl (diluted 1:9) was added and the sample was then boiled to a 10–20 ml volume. In the case of total iron determination, 1.0 ml of hydroxylamine hydrochloride solution was then added to the sample treated in this manner. After that the pH value was adjusted to about 4.5 with acetate buffer solution. Then the colored complex forming reaction between Fe^{II} and 1,10-phenanthroline took place and the volume was refilled again to 50.0 ml with deionized water. The calibration curve was calculated for all spectrophotometric iron determinations.

Results and discussion

Traditional polarographic working solutions

KCl, HCl, KNO_3 , NaOH, $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$ and acetate solutions are traditional polarographic working electrolytes. However these solutions are not usually suitable for iron determination. Selected working electrolytes (KCl, $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$ and acetate) were tested and the corresponding voltammograms were recorded during this study.

The differential pulse voltammogram attained in KCl solution for water from the inflow zone of the constructed wetland is shown in Fig. 1. It is evident from this figure that KCl electrolyte solution is not suitable for the voltammetric study of constructed wetland water

samples. No depolarizer peaks were observed on the voltammogram. Standard additions of iron did not provide a signal in the KCl working electrolyte under these experimental conditions. Similar observations were attained for $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$ and acetate solutions.

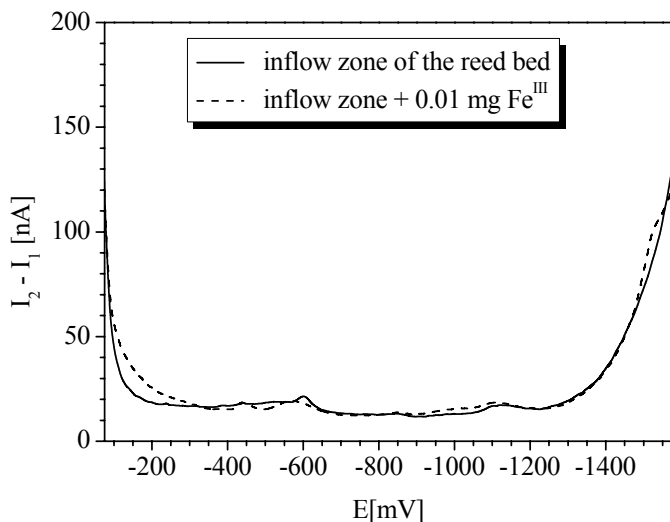


Fig. 1. Differential pulse voltammogram of wastewater sampled from the inflow zone of the reed bed on 13th January 2005, attained in KCl solution. A hanging mercury drop electrode was used as the working electrode. Pulse height 50 mV, pulse duration 100 ms and polarization rate 10 mV s^{-1} were set.

Determination of total iron in oxalate working solution

Oxalate solution proved to be the most suitable electrolyte for total iron determination during this study. The $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was completely electrochemically reversible, and one well developed peak was observed on the differential pulse voltammogram.

The influence of oxalate electrolyte pH on the iron signal was tested. The signal systematically decreased as pH was increased from acidic ($\text{pH} = 2$) to neutral. The peak height when using an oxalate solution with $\text{pH} = 6$ reached only about 30% of the value attained for an acidic working solution ($\text{pH} = 2$). Moreover, the peak was less developed and the peak shape was not as reproducible using the oxalate solution with $\text{pH} = 6$, in comparison with the acidic solution. Therefore, all analyses of real samples were conducted using the acidic oxalate solution ($\text{pH} = 2$).

Using the acidic oxalate solution ($\text{pH} = 2$), the electrode process electrochemical reversibility was tested by additions of Fe^{II} and Fe^{III} solutions (signals of the same amounts of Fe^{II} and Fe^{III} were compared). The reversibility verification was performed for the sample from the inflow to the wastewater treatment plant. The differential pulse voltammogram of the sample was recorded

first in the oxalate working solution. After that Fe^{III} standard additions (twice $0.01 \text{ mg Fe}^{\text{III}}$) were added and the polarization curves recorded. Then a $0.01 \text{ mg Fe}^{\text{II}}$ standard addition was also added and the resulting voltammogram recorded. These additions confirmed that the same peak height increased and that the increases of this peak were the same for the same amounts of both iron oxidation state additions. Thus, the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was electrochemically reversible and the oxalate working electrolyte ($\text{pH} = 2$) could be used for total iron determination in water from the constructed wetland using differential pulse voltammetry. Differential pulse voltammetry under these conditions did not separate the oxidation states of iron.

Iron was determined in three real samples from the Slavošovice constructed wetland. These samples represented wastewater from the inflow into the system, the inflow zone of the reed bed and the outflow from the constructed wetland. The highest concentration of iron was measured at the outflow from the constructed wetland. This may result from iron release from the clay serving as the bottom of the constructed wetland. Differential pulse voltammogram of water from the inflow zone is shown in Fig. 2. The results are summarized in Table 1.

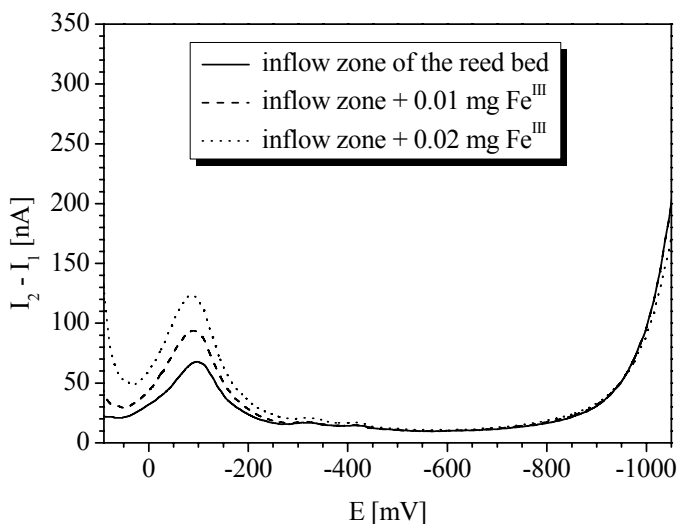


Fig. 2. Differential pulse voltammogram of wastewater sampled from the inflow zone of the reed bed on 13th January 2005, attained in oxalate ($\text{pH} = 2$) solution. Other parameters were the same as for Fig. 1.

Table 1. Determination of iron in the Slavošovice constructed wetland.

	$\text{Fe}_{\text{total}} [\text{mg l}^{-1}]$		$\text{FeII} [\text{mg l}^{-1}]$		$\text{Fe}^{\text{II}} [\%]$	
	DPV	Spectrometry	DPV	Spectrometry	DPV	Spectrometry
Inflow	0.80	0.81	0.82	0.81	100	100
Inflow zone	1.22	1.05	1.23	1.04	100	99
Outflow	6.7	8.0	4.6	4.4	68	55

Speciation of iron oxidation states using DPV in pyrophosphate medium

Electrochemical speciation of the iron oxidation states was attained using sodium pyrophosphate as the working electrolyte, as described previously (Parry, Anderson, 1973; Perämäki et al., 2000). The $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ redox couple was electrochemically irreversible and two signals were observed on the voltammogram, one for reduced (Fe^{II}) and the other for oxidized (Fe^{III}) iron. The differential pulse voltammogram of water from the outflow is shown in Fig. 3. It was shown previously (Kennedy, 1990; Perämäki et al., 2000) that both reductive and oxidative processes might occur in the polarograph cell during the determination, making it inaccurate. During this study, it was observed that the signal (peak) for Fe^{II} did not decrease or increase when it was recorded ten times repeatedly. However, the Fe^{III} determination was

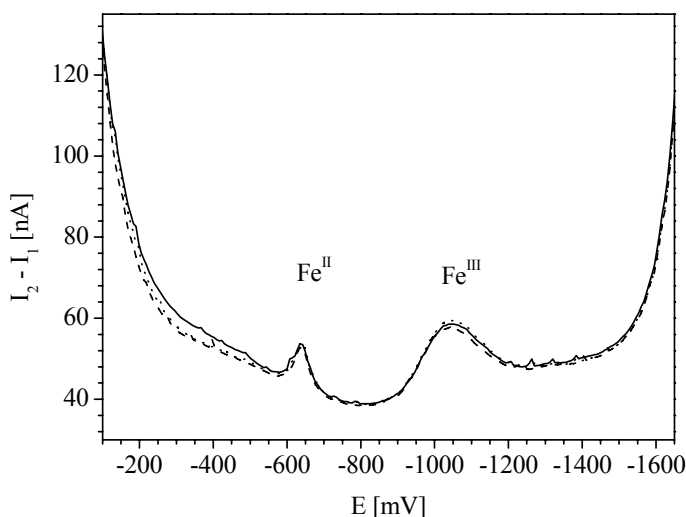


Fig. 3. Differential pulse voltammogram of water sampled at the outflow from the constructed wetland on 13th January 2005, attained in a pyrophosphate electrolyte solution. The voltammogram is recorded three times repeatedly. Other parameters were the same as for Fig. 1.

not sufficiently repeatable. Therefore, the Fe^{III} content in the samples was calculated as the difference between total and reduced iron contents. The concentrations of Fe^{II} determined in the constructed wetland are summarized in Table 1. Almost all of the total iron in the inflow to the wastewater treatment plant and at the inflow zone of the reed bed was reduced to Fe^{II} . This is in agreement with the reduction properties of wetland soils, and when the pore water contains a high amount of degradable organic pollutants. On the other hand, about 32% of the iron was oxidized to Fe^{III} at the outflow from the reed bed wastewater treatment plant. This is due to the presence of aerobic zones in the profile of the constructed wetland.

Spectrophotometric determination and speciation of iron oxidation states

Spectrophotometric determination of iron oxidation states was performed as a comparative determination beside the voltammetric analysis. A good agreement between the results obtained using both methods was attained for samples collected at the inflow to the wastewater treatment plant and the inflow zone of the reed bed (Table 1). Fe^{II} concentration values obtained by both methods corresponded well for the sample from the outflow of the constructed wetland. However, Fe^{III} concentration values varied to some extent, with the concentration determined using differential pulse voltammetry being lower than the value attained for the 1,10-phenanthroline method. This can be explained by the different types of sample pretreatment before iron determination. The sample was boiled with added diluted HCl prior to the spectrophotometric determination, converting the insoluble Fe^{III} species to a soluble form. On the other hand, before the differential pulse voltammetric determination, the sample was only diluted (1:1) with oxalate working electrolyte acidified with diluted H₂SO₄ to pH = 2, then stirred and deaerated for 10 minutes.

Characteristics of analytical methods

Basic characteristics were determined for the methods used for the analysis and speciation of iron oxidation states. These characteristics are summarized in Table 2. It is evident that the attained limits of detection (LOD), quantification (LOQ) and repeatability values are similar and comparable for both the differential pulse voltammetry and spectrophotometry methods. The 1,10-phenanthroline method is well established in analytical laboratories and serves as the routine method for iron determination. However, the water samples from the constructed wetland are often turbid and colored, which can result in inaccurate results using spectrometric determination. The advantage of differential pulse voltammetry is that its efficacy is not reduced under these conditions. Therefore, it can serve as a suitable alternative to spectrophotometric determination.

Conclusion

The present paper shows that differential pulse voltammetry with a hanging mercury drop electrode can be used for iron determination and its oxidation states speciation in real wastewater samples. The described determination of oxidation states of iron in samples from the

Table 2. Characteristics of methods used for iron determination.

	DPV - oxalate (total Fe)	DPV - Na ₄ P ₂ O ₇ (Fe ^{II})	Spectrophotometry (Fe ^{II} and total Fe)
LOD [mg.l ⁻¹]	0.03	0.02	0.01
LOQ [mg.l ⁻¹]	0.08	0.07	0.03
Repeatability [%]	1.53	0.90	0.86

Notes: LOD – limit of detection, LOQ – limit of quantification

constructed wetland represents a novel approach to the determination of this ecologically important element and simultaneously to the study of constructed wetland redox properties. Although differential pulse voltammetry is not used often for iron determination, it can provide valuable information and can contribute to the elucidation of constructed wetland chemical properties. Determination of iron, using differential pulse voltammetry, expands the possibilities of electrochemical approaches to the study of wetland systems. In comparison with other published papers (Perämäki et al., 2000), the data presented here were attained using a hanging mercury drop electrode, which is a more advantageous version of a working electrode, due to a lower consumption of toxic mercury. In addition, the analysis presented here is more repeatable and less time consuming than for differential pulse anodic stripping voltammetry using a carbon electrode (van Staden, Matoetoe, 1998).

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