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Profiling Of Wastewater Effluent From A Beverage Industry By Microdialysis Sampling And A Combination Of Analytical Techniques

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ABSTRACT

Microdialysis sampling in conjuction with other analytical techiques is presented for the monitoring of wastewater from a beverage plant. Microdialysis sampling achieved in situ sampling and sample clean-up for glucose, fructose, sucrose, F^-, Cl^-, NO_3^{2-}, PO_4^{2-}, SO_4^{2-}, Mg^{+2}, K^+, Ca^{+2}, Na^+, Zn^{+2} and Ni^{+2}. High extraction fractions achieved for these analytes demonstrated the potential to apply microdialysis to sample from a complex environmental matrix. Enzymatic assay was employed as a complimentary technique for determining concentration of the saccharides. These results show that profiling of analytes with a combination of analytical techniques is an essential first step to developing a methodology for environmental monitoring. Combining the versatility of microdialysis sampling with chromatography and sensitive electrochemical detection presents a generic approach to achieving real-time monitoring of analytes that are associated with environmental pollution, especially in a wastewater matrix.

KEYWORDS

Microdialysis;
Sampling;
Wastewater;
Beverage industry;
Metals; Anions.

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INTRODUCTION

In order for legislators to enforce regulations regarding the disposal of waste, they need to have rapid, effective, reliable and cost effective analytical methodologies that can be employed to monitor the quality of waste. Monitoring of waste may be carried out using a series or a combination of analytical techniques. Alternatively, methods with a multi-analyte capability may be employed[1]. The monitoring of waste, especially treated wastewater is very important, as there is a renewed trend towards wastewater re-use for various purposes including the irrigation of municipal open grounds or irrigation of horticultural crops[2].

While wastewater can be put to many end-uses, each use will require specific characteristics to be met by such water. It has been shown that the availability of metal ions in different quantities can have varying effects on the growth of plants[3,4]. However, plants show different degrees of accumulation as well as tolerance to high concentrations of ions in irrigation water. The latter is unwelcome when it occurs in the edible parts of plants[5]. Therefore, it is imperative that detailed characteristics of industrial wastewater should be obtained prior to considering any potential end-use. This necessitates the need to develop and employ analytical methodologies that will cope with the complexity of the wastewater effluents.

The method of choice should not only be robust, give precise measurements without any bias, but it is important that it also has a short turnaround time. However, sampling, sample clean-up and sample work-up are the 'side steps' that could introduce uncertainty in the measurements. Such uncertainty may not be unmasked by any statistical treatment of the data. Therefore, the choice of a sample handling technique is as good as the reported precision of measurement. The selectivity of microorganisms as demonstrated in the areas of bioremediation[6] and biomining[7,8] have shown their potential for use in specific assay methodologies, for example[9-11]. In the case of wastewater from a beverage industry, the specificity of enzyme assays[12] can be exploited to reduce sample handling steps. Alternatively, a technique such as microdialysis sampling that achieves in-situ sampling and sample clean-up is well suited to such applications.

Microdialysis has been traditionally applied in neurochemistry, pharmocokinetic studies, and recently its potential was demonstrated by sampling from complex biotechnology matrices[13]. The advantage of microdialysis sampling is that it is easily coupled to separation techniques such as chromatography[13] and capillary electrophoresis[14]. The ease of coupling microdialysis on-line combined with the ability for microdialysis to handle small sample volumes presents a generic approach to designing analytical systems that can reduce loss in time resolution for continuous monitoring.

In this paper, the profiling of wastewater from a beverage industry is reported. Profiling of analytes by combining analytical techniques is an essential first step towards the development of analytical methodology for environmental monitoring. Techniques can thus be chosen or adapted to a particular environment to enable sampling, sample pre-treatment, sample work-up, analyte identification/confirmation as well as quantification. Therefore, a combination of analytical techniques that included microdialysis sampling, enzyme assays, flame emission spectroscopy (FES), atomic absorption spectroscopy (AAS), ion chromatography (IC), as well as microbore high performance anion exchange chromatography with integrated pulsed electrochemical detection (HPAEC-IPED), were used to profile for analytes in wastewater from a beverage industry.

EXPERIMENTAL

Reagents

Saccharides were obtained from Sigma (St. Louis, MO, USA). NaOH of 50 % w/w (J.T. Baker, Denventer, Holland) was used to prepare the 5 mM NaOH mobile phase for micro-HPAEC-IPED. Standards for anions and standards for cations were prepared from the corresponding sodium salts and 1000 ppm metal stock solutions obtained from Saarchem (Muldersdrift, RSA), respectively. All solutions were prepared using ultrapure water.

Sampling and sample clean-up

In figure 1; A, B and C represent different activity levels as described. Given the activities described in figure 1, it is imperative that the sampling of effluent from a beverage plant should be done in a manner that captures the dynamic nature of the activities that take place in it. With the latter in mind, samples were collected from the manhole, where all effluents from the plant (wash room, packaging room and syrup room) combine prior to their disposal into the municipal sewage system.

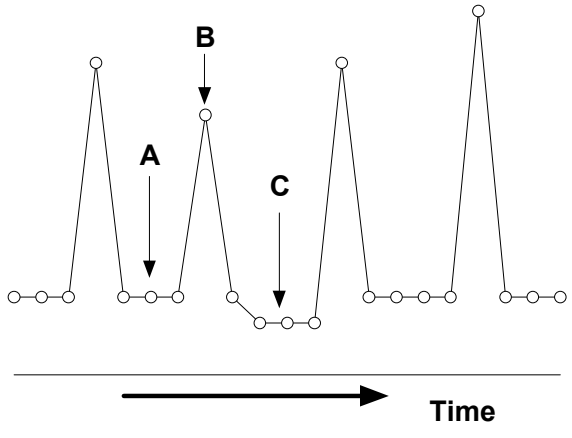

Figure 1: Event-time profile for the studied beverage plant where A, B and C represent normal production, heavy production and no production, respectively.

Two samples were taken everyday for days where the activity was as described by A (see Figure 1). For events described as part B, samples were taken at hourly intervals for 8 h over 2 days. For events described as part C, two samples were taken from the temporary storage tank when activities were as described by B and C respectively (see Figure 1). At all times, samples were handled via appropriate techniques that ensured the maintenance of the original character of each sample.

Microdialysis sampling or a 0.45 μm filter paper was used for sample clean-up before analysis by IC or micro-HPAEC-IPED. Samples for enzymatic assay were concentrated by initially freeze-drying and the solute was recovered in 5 ml of ultrapure water. The respective concentrated samples were separately filtered through a 0.22 μm filter paper. The filtrates were stored at -20 °C until required for analysis.

Analysis of saccharides

A combination of microdialysis sampling coupled to a Dionex 500 micro-HPAEC-IPED system Dionex (Sunnyvale, CA, USA) as reported elsewhere [15], was used for the analysis of glucose, sucrose and fructose. An enzymatic assay was used as a complimentary technique to quantify the saccharides [12].

Analysis of anions

Anions were analysed using a Dionex 100 chromatographic system, also from Dionex. Separation was achieved isocratically using a Dionex Ionpac AG14, 4 x 50 mm pre-column and a Ionpac AS 4A-SC, 4 x 250 mm analytical column, also from Dionex. Detection was achieved using a conductivity detector which is part of the Dionex 100 chromatographic system.

Analysis of metals

Metal analysis was carried out using a Corning Flame Photometer 410 from Ciba Corning Diagnostics (Essex, England) or Varian Spectra AA-10 (Victoria, Australia) flame atomic absorption spectrophotometer or Varian Zeeeman GTA96 PLus electrothermal atomic absorption spectrometer also from Varian.

RESULTS AND DISCUSSION

There are a number of activities that take place in a beverage packaging plant. Such activities include the production of different beverage brands as well as several washing and rinsing steps at different stages of the bottling and canning processes. In order to capture the characteristics of wastewater effluent from these processes, a cost-effective method for sampling and analysis is required. An on-line sensor that can achieve multi-analyte determination, if placed in the effluent wastewater would be the most preferred approach as the composition of the wastewater may be monitored in real time. However such a sensor may only be employed if the component analytes in the wastewater are already known. Such knowledge is essential in order to calibrate the sen-

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sor so that it can cope with the complexity of the matrix. An on-line sensor also requires an on-line sampling and sample clean-up technique such as microdialysis in order to achieve real time monitoring of the effluent.

Microdialysis sampling

The microdialysis probe (see Figure 2) is the sampling device used during microdialysis sampling.

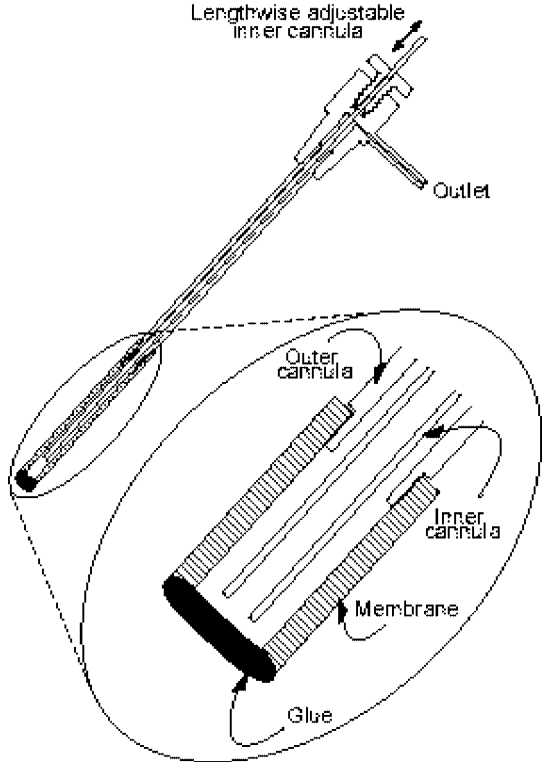

Figure 2: Schematic representation of the in-situ tunable, concentric type of microdialysis probe used in this study.

The probe consists of a hollow fibre membrane of known molecular weight cut-off (MWCO) which is perfused with water. Selectivity and clean-up is dependent on the membrane MWCO and the size of the analyte and matrix molecules. Analytes selectively diffuse through the membrane into the perfusion liquid and are collected either in a vial if one is using a fraction collector or in a sample loop ready for injection into a chromatographic system. The concentration of analytes determined after microdialysis sampling can be evaluated according to eqn 1 below;

$$EF = \frac{C_{det}}{C_{std}} \quad (1)$$

where EF, is the extraction fraction which is more commonly known as relative recovery (RR) as defined in eqn 2;

$$RR = EF \times 100 \quad (2)$$

C_{det} and C_{std} are the concentrations of the detected analyte in the wastewater and standard sample solution, respectively. For every analyte to be sampled using microdialysis, EF data was generated according to eqn 1. The data acquired after microdialysis

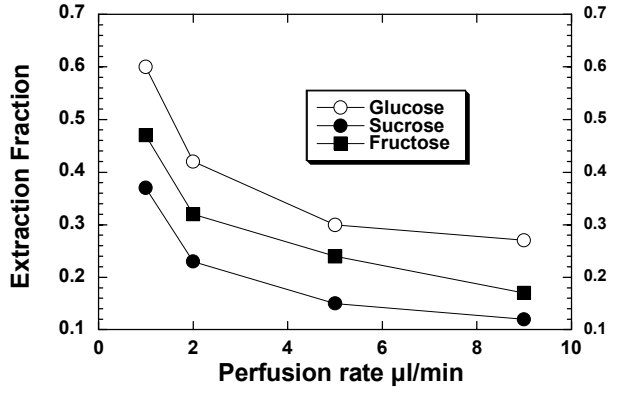

Figure 3A: Extraction fraction plot for glucose, sucrose and fructose evaluated after sampling with a microdialysis probe equipped with a polysulfone membrane having a 30 kDa cut-off and 10 mm effective dialysis length.

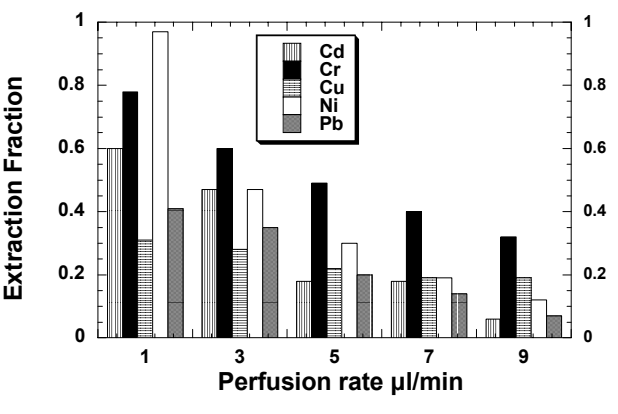

Figure 3B : Extraction fraction plot for metals: Cd, Cr, Cu, Ni and Pb evaluated after sampling with a microdialysis probe equipped with a polysulfone membrane having a 30 kDa cut-off and 10 mm effective dialysis length.

sampling is then evaluated so as to get the exact concentration of analyte in the wastewater effluent. Reports in literature have shown that data evaluated from microdialysis sampling may be affected by the sampling mode, however it is independent of the analyte concentration and the complexity of the sample matrix[16]. Before any real sampling is carried out, EF data for the possible analytes has to be determined so as to enable the evaluation of the concentration in the sample according to eqn 1.

Figure 3 shows the EF values evaluated for saccharides (3A) and metals (3B), respectively. The EF values evaluated for all the analytes show a dependency on the perfusion rate. For the saccharides, glucose shows the highest EF for all perfusion rates. The EF for metal ions in the lower perfusion regime are relatively higher compared to those of the saccharides as exemplified by Ni and Cr with EF values greater than 0.7. However the EF values evaluated for Pb and Cu are below 0.5 for all perfusion rates. Our research group has demonstrated that EF as high as 100% can be achieved for most metals by utilising binding agents[17] such as humic acids, 8-hydroxyquinoline, poly-L-histidine, EDTA and poly-L-aspartic acid, though only pure water was used for this study.

Determination of saccharides

Profiling of saccharides was carried out using an enzyme based assay[12]. As shown in TABLE 1, varying amounts of glucose, fructose and sucrose were detected in the wastewater effluent samples. No other soluble carbohydrates were detected in the wastewater effluent samples. To validate the results obtained through enzymatic assays, analysis was also carried out using a micro-HPAEC-IPED system.

Micro-HPAEC enables the separation of the carbohydrates in their enolate form with subsequent sensitive detection by IPED. Only freshly collected samples that had not been concentrated using freeze-drying were analysed with micro-HPAEC-IPED. This was necessary because the sensitivity of electrochemical detectors such as the IPED requires that they be applied only to small analyte concentrations in order to reduce the possibility of fouling the electrode[18]. Also, high analyte concentrations tend to remain on the stationary phase, and thus require either high concentration of sodium hydroxide or utilisation of sodium acetate as the competing anion in order to facilitate their elution from the anion exchange column. Regeneration of the analytical column and subsequent stabilisation before analysing for the next sample is undesirable for on-line monitoring methodologies as it is time consuming.

To further decrease the possibility of fouling the electrode by analysing highly concentrated samples, a microdialysis probe that has tunable EF to facilitate on-line dilution of the sample[19] and thus maintain the concentration of the sample within the quantification range of the detector was used. A typical

TABLE 1: Concentrations of saccharides detected in wastewater

Concentration range µg/L	Number of detected samples for saccharides		
	Glucose samples	Fructose samples	Sucrose samples
0-50	6	4	6
51-100	6	12	-
101-200	3	5	2
201-400	3	5	-
401-1000	10	3	6
1001-2000	-	-	4
2001-above	2	2	12

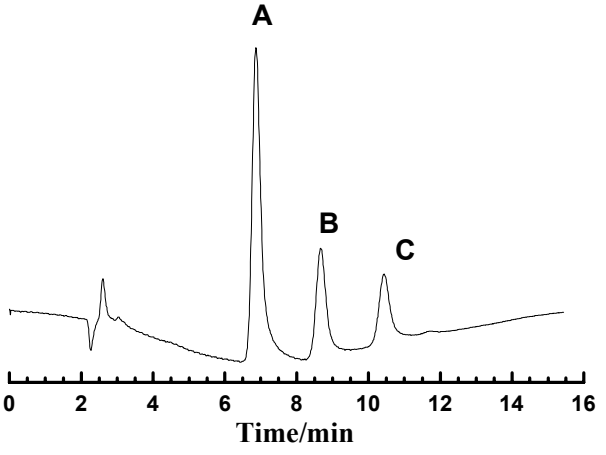

Figure 4: Chromatogram showing saccharides that were separated using microbore high performance anion exchange chromatography with integrated pulsed electrochemical detection (HPAEC-IPED). The letters represent (A) sucrose (B) glucose, and (C) fructose, respectively.

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chromatographic profile obtained through this combination of techniques is presented in figure 4. To confirm the identity of the eluted saccharides, samples were spiked with authentic solutions of the corresponding saccharides.

The detection of glucose, fructose and sucrose in the effluent from a beverage plant is not surprising since sucrose is the main sweetening agent used in the production of beverages. Glucose and fructose may have been contained in the syrup or they may have been derived from the hydrolysis of sucrose in the effluent. The overall quantities that were detected were varied and even occurred at concentrations as high as 1 kg/L (see TABLE 1). The high concentrations of sucrose could be attributed to high sucrose content syrups used in the production of the different beverage brands. Therefore the use of a combination of techniques was a well suited means of profiling for the analytes monitored in this study.

Determination of anions in the wastewater effluent

Ion chromatography (IC) with conductivity detection was used to profile for the anions in the wastewater effluent. The application of IC for the separation of anion is well established[20,21]. Microdialysis sampling was also employed in this aspect of the study. This was necessary because microdialysis can be coupled on-line to chromatographic systems and also produces a chromatographically clean sample ready for injection into the chromatographic system. The profiling of wastewater for anions showed this combination of analytical techniques to be versatile.

As shown in TABLE 2, most of the detected anions were present at concentrations below 20 μg/l. However, some samples had anion concentrations as high as 50 μg/l, particularly for Cl^-, and PO_4^{2-}. These high concentrations were reflective of the chemical composition of some cleaning chemicals used at the plant. The highest concentrations detected for F^-, Cl^-, NO_3^{2-}, PO_4^{2-}, and SO_4^{2-}, were respectively 45, 58, 16, 414 and 32 μg/l.

TABLE 2: Concentrations of anions detected in wastewater

Concentration range (μg/l)	Number of detected samples for anions				
	F	Cl	NO_3	PO_4	SO_4
0.5-10	25	-	34	26	26
10.5-20	5	22	4	9	7
20.5-30	6	9	-	-	2
30.5-40	1	4	-	1	2
40.5-50	1	1	-	-	-
50.5-above	-	2	-	2	-

Determination of metals in the wastewater effluent

TABLE 3 shows the concentration range for the detected metals. For most metals except sodium, all the detected concentrations were in the 0.5-10 μg/l concentration range. The highest concentrations for the detected metal ions, for Na, K, Ca, Mg, Zn and Ni were 92, 12, 7, 4, 2 and 2 μg/l, respectively.

TABLE 3: Concentrations of cations detected in wastewater

Concentration range (μg/l)	Number of detected samples for cations					
	Na	K	Ca	Mg	Zn	Ni
0.5-10	2	37	38	38	38	38
10.5-20	7	1	-	-	-	
20.5-30	11	-	-	-	-	
30.5-40	8	-	-	-	-	
40.5-50	1	-	-	-	-	
50.5-above	9	-	-	-	-	

Overall, the results obtained in this study suggest that the plant operations introduce very little amounts of metal ions into the effluent. These contributions could be reduced further by substituting a component with a low metal ion content for one that is so far used but has a high metal ion concentration. Consequently, the effluent derived from the process may be directly put to use such as irrigation of municipal open spaces without any need for rehabilitation. In general, the metal ion concentrations in wastewater used in this study is within tolerable limits for many plants[22].

CONCLUSION

Profiling of the effluent showed the presence of F^-, Cl^-, NO_3^{2-}, PO_4^{2-}, SO_4^{2-}, Na, K, Ca, Mg, Zn, Ni as well as the saccharides; glucose, fructose and sucrose. The detected concentration of saccharides especially

sucrose, which is the main sweetener for the beverage industry were up to 1 kg/L. There was a tendency for the occurrence of high concentrations of glucose and fructose in samples with pH values of between 6 and 7. These may have arisen from the hydrolysis of sucrose in the syrup by microbial enzymes. Indeed, the presence of coliforms and *E.coli* in the effluent wastewater was confirmed through the use of chromogenic media.

In addition, this study has clearly demonstrated the benefits of combining analytical techniques when dealing with a very complex matrix, as a first step towards development of a system to monitor the effluent. Microdialysis was shown to be a very versatile technique as it provided chromatographically clean samples for the analysis of saccharides and anions using micro HPAEC-IPED and IC, respectively. Microdialysis achieved on-line sampling and sample clean-up, on-line dilution of concentrated saccharide samples by employing the tunable microdialysis probe. The high EF for saccharides, metals and anions by microdialysis sampling, show the potential for employing this in-situ sampling and sample clean-up technique for environmental monitoring of polar analytes. Further studies will involve evaluation of membrane integrity as well as their reusability in the complex wastewater matrix.

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