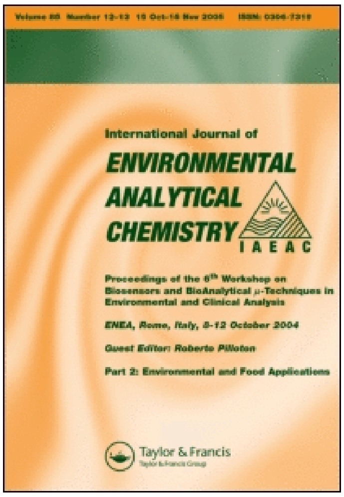

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ANALYSIS OF LONG-CHAIN FATTY ACIDS IN GREY WASTEWATER WITH IN-VIAL DERIVATISATION

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The presence and levels of long-chain fatty acids (C_6–C_{20}) in grey wastewater from bathrooms have been investigated. The acids were purified and concentrated by solid-phase extraction on strong anion exchange discs, in-vial derivatised to their corresponding methyl ester and subsequently analysed by GC-MS. The method was able to quantify the acids at concentration $< 1\,\mu g/L$ with a recovery of 31–97%. The levels of fatty acids were found in the range of < 0.5 to $27\,100\,\mu g/L$ and the highest levels were found for the saturated lauric (C_{12}), palmitic (C_{16}) and stearic (C_{18}) acids. The treatment efficiency of a local treatment plant was evaluated by comparing concentrations of fatty acids at the inlet and the outlet. It was found that the treatability decreases with increasing chain length for the saturated acids (19–100% degradation) whereas the corresponding mono unsaturated acids were more easily degraded.

Keywords: Analysis; Fatty acids; GC-MS; Grey wastewater; In-vial derivatisation; SPE-SAX

INTRODUCTION

There is a growing demand in society for the introduction of integrated decentralised sanitary systems providing opportunities to save water and reuse wastewater for non-potable purposes. One of the possibilities is to reuse grey wastewater, i.e., wastewater produced from bathtubs, showers, hand basins, washing machines and dishwashers as well as kitchen sinks. Suggested applications for reuse are toilet and urinal flushing, laundry, concrete production as well as for irrigation and infiltration. Knowledge of the characteristics of grey wastewater is limited especially in regard to the presence of xenobiotic organic compounds (XOCs). Nine hundred different compounds and compound groups could potentially be present in Danish grey wastewater according to the literature, owing to their presence in laundry detergents, soaps, shampoos, toothpastes and hair conditioners [1]. Both natural and synthetically

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produced fatty acids are present in personal care products as fats and emulsifiers. A screening of grey wastewater from bathrooms confirmed the presence of almost 200 XOCs: surfactants, emulsifiers, fragrances, flavours, preservatives, antioxidants, softeners, plasticizers, UV-filters, solvents and additional compounds of domestic origin [2]. A large number of different long-chain fatty alcohols, acids and esters were detected but the concentrations were only semi-quantitatively assessed, so further investigation into individual concentration levels was needed. The presence of long-chain fatty acids in grey wastewater has previously been shown by Burrows *et al.* [3] who revealed that the even-numbered long-chain fatty acids of n-C_{10} to n-C_{18} were present in shower wastewater from a military facility. In wastewater from Gothenburg, (Sweden) the fatty acids in the inflow to the treatment plant were found to be in the range of 100–500 µg/L (C_3–C_6) and 350–38000 µg/L (C_7–C_{18}) [4]. The fatty acids are generally not very toxic to aquatic organisms and they are relatively easy to degrade. This means that they will not have a significant negative effect on aquatic organisms in a receiving water body. However, in high concentrations they will contribute to BOD and COD and thereby to oxygen consumption. The fatty acids are relatively easy to measure and could therefore act as an indicator for the presence and behaviour of other organic compounds in bathroom grey wastewater as there is no contribution of lipid food residues.

Fatty acids in solid and aqueous samples are often analysed by liquid extraction, which requires high volumes of organic solvents [4–7]. Methods requiring large volumes of organic solvents are generally undesirable owing to the potential exposure of the laboratory technicians as well as the potential environmental impact on, for example, a wastewater treatment plant (WWTP), which receives a solvent-containing wastewater. The extracts may either be analysed by GC directly, after derivatisation or after additional extraction and purification steps. Some extraction methods include solvent reduction or evaporation to dryness followed by redissolution and solvent change before analysis. The evaporation step is a potential source of error as some compounds may evaporate and be lost and should be excluded from the analysis if possible. Fatty acids in liquid and air samples may be extracted by, for example, solid-phase micro-extraction (SPME) [8] followed by derivatisation, which is also a method with low solvent consumption but which cannot be applied to solid samples.

The method for analysis of fatty acids in grey wastewater presented in this study is based on a method developed for analysing nonylphenol carboxylates (NPECs) in surface water by Field and Reed [9]. The method consumes very small volumes of solvent and the extraction procedure is quick compared to many solvent–solvent extraction methods. A similar fast and handy method was desired for analysis of fatty acids and the aim of the present study was to continue the development of that method for analysis of long-chain fatty acids. The method was evaluated by applying it to untreated and treated grey wastewater. In their paper, Field and Reed [9] presented tests determining accuracy, precision, recovery and detection limits with MilliQ-water and surface water samples, whereas the other water fractions analysed in their study (paper mill and WWTP effluents) were only evaluated in recovery tests. Grey wastewater has a complex matrix, with relatively high concentrations of organic matter and that is why the method had to be adapted to and tested throughout the validation process on grey wastewater.

Field and Reed [9] performed the extractions without any pH adjustment before extraction (pH in the range 6 to 8.5). In order to assure that the majority of the long-chain fatty acids are in their unprotonated form the pH should be two units higher than the acid dissociation constants (pK_a). The studied acids have pK_a value in the range 5–6, and that is why a pH adjustment prior to extraction was tested in the development of the method. Furthermore, some work was done regarding the number of discs used for extraction and the washing procedure of these discs. In the method by Field and Reed [9] one Empore anion exchange disc is used for extraction. The discs are soaked in acetonitrile to remove impurities and 5 mL acetonitrile and 5 mL MilliQ-water is used to wash the discs before extraction. Ng *et al.* [10] identified benzoic acid as a manufacturing contaminant in the Empore discs. In the present study, steps were included to further reduce the presence of benzoic acid as well as other contaminants, like long-chain fatty acids.

EXPERIMENTAL

The fatty acids were analysed by solid-phase extraction (SPE) on anion exchange discs and in-vial derivatised with methyl iodide after modification of a method developed for analysis of nonylphenol carboxylates in aqueous samples [9]. The extracts were analysed with GC-MS-SIM.

Reagents and Chemicals

Long-chain fatty acids C_6 to C_{20}, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, 9-octadecenoic acid and eicosanoic acid were purchased from Sigma-Aldrich. 4-Fluoro-benzoic acid (internal standard) and 2-chloro-4-methyl-quinoline (chromatographic standard) were also obtained from Sigma-Aldrich. Methanol and acetonitrile, both HPLC-grade, were purchased from Rathburn Chemicals Ltd and JT Baker, respectively. The methyl iodide came from Merck and the MilliQ-water from a MilliQ Water System from Millipore. Sodium hydroxide (p.a.) and hydrochloric acid (p.a.) came from Merck.

Samples

Grey wastewater samples were taken in BO-90, a tenant owners' society located in the central part of Copenhagen, Denmark. The building has 17 apartments, with 38 inhabitants: 22 adults (ages 18–74) and 16 children (age 2–15). The grey wastewater they produce originates from the showers and hand basins in the bathrooms with a daily production of approximately 750 L. A grey wastewater treatment facility has been installed in the basement to treat the wastewater on-site and reuse it for toilet flushing. The plant consists of a filter bag, two sandfilters fitted with an aeration system and a disinfection system. Grab samples were taken daily (mornings and evenings) at the inlet (P.1) of the treatment facility for eight weeks during 1999 and 2000 and for four weeks at the outlet of filter 1 (P.2) and in the buffer tank (P.3) (see Fig. 1). The retention time in the treatment plant is about 12 h.

Extraction

Before analysis the Empore anion exchange discs (CHROMPACK) were washed overnight with 0.1% HCl in acetonitrile, to remove the interfering benzoic acid derived

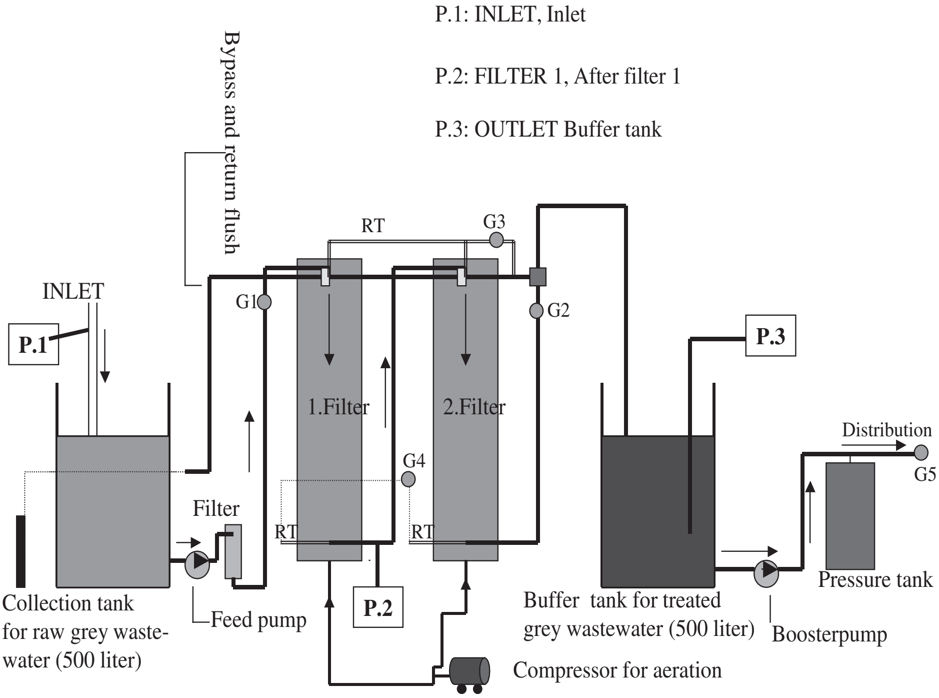

FIGURE 1 Flow diagram for grey wastewater treatment plant at BO-90, Copenhagen, Denmark.

from the anion exchange discs. The discs consist of poly (styrenedivinylbenzene) and the functional group is a quaternary ammonium group [11]. Discs with a diameter of 13 mm were obtained by cutting the commercially available large discs (47 mm) with a tool and they were mounted into 13-mm metal filter holders. The disc holders were attached to a vacuum manifold (VAC ELUT SPS 24$^{\text{TM}}$) from Varian and the discs were conditioned with 10 mL acetonitrile and 15 mL MilliQ-water before extractions. Filter holder funnels were used as sample reservoirs.

Samples with high electrical conductivity were diluted with MilliQ-water prior to extraction and 500 μL of 20 mg/L 4-fluoro-benzoic acid (internal standard) in methanol were added to all samples and standards. The pH was raised to ≥ 9.5 with 10 N NaOH as it was found to increase the yield as the hydroxide ion competes with the water molecules on the solid phase, i.e., less derivatisation agent is used on water and the acids are mainly in their unprotonated form. Two discs were mounted in each filter holder to improve the extraction yield and it was found that the use of two discs increased the recovery by 28–86% for the various acids. The 100-mL samples were centrifuged for 30 min at 8000 rpm to remove any particles and precipitates from the water phase. The supernatant was decanted to a beaker from which the sample was added to the sample reservoir. After the aqueous sample had passed through the discs the precipitate was re-suspended in MilliQ-water and added to the sample reservoir. The centrifuge beakers and decanter glass were rinsed three times with MilliQ-water and the rinsings added to the sample reservoir in order to minimise the losses. The discs were dried in the vacuum manifold for 15–25 min after the extraction.

In-vial Derivatisation

The dried discs were transferred to 2-mL GC-vials, to which 500 µL 2-chloro-4-methylquinoline (chromatographic standard, 20 mg/L) in acetonitrile and 200 µL methyl iodide (derivatisation agent) were added. This total volume (700 µL) was sufficient to cover the discs completely with liquid. The vials were capped and the methylation was carried out at 80°C over 60 min. The disc catalyses the transformation of the acids to their methyl esters and the derivatisation agent is added in excess. The yield of the derivatisation has not been examined. Precipitates are thought to be accessible for derivatisation, as the adsorbed acids ought to be reactive towards the derivatisation agent whether they are adsorbed onto the discs or onto calcium precipitates.

Instrumentation

A Hewlett-Packard 6890 chromatograph coupled with an HP 5973 MS Detector was used for the GC analysis and the injections were performed with a Varian 8200 CX Autosampler. The GC column used for analysis of the methylated fatty acids was a BP21 (15 m × 0.25 mm ID) with 0.25-µm film thickness from SGE. The instrument was operated in the splitless mode with a purge time of 0.16 min with a purge flow of 20.0 mL/min and an injection temperature of 240°C. Helium was used as carrier gas at a flow of 1.3 mL/min. The following temperature program was used: 35°C for 7 min, 15°C/min to 90°C, 5°C/min to 220°C, 30°C/min to 240°C, held 2 min. The MS Quad and MS Source were kept at 150°C and 230°C, respectively. The MS operated in SIM mode with two m/z values for each of the nine fatty acids, m/z 74 for the saturated methylated fatty acids, 55 for the unsaturated acids and the mother ion [M^+] for each of the methylated acids. For the internal standard m/z values of 123 and 154 were used and for the chromatographic standard m/z values of 177 and 179.

Method Validation

Spiked MilliQ and grey wastewater were analysed in order to determine the linear interval for the SPE method. Duplicate tests with concentrations in the range from 0.001 to 1000 µg/L were made in MilliQ water. The results were confirmed with duplicate tests in grey wastewater with concentrations in the range 0.1–200 µg/L. Hexaplicate determinations of spiked MilliQ and grey wastewater samples were used to determine limits of detection (LODs) and limits of quantification (LOQs). Owing to interference with compounds leaching from the discs, the LOQs were increased and recalculated from linear regression. The detection and quantification limits are defined as those concentrations of individual fatty acids needed to produce a signal-to-noise ratio (S/N) of 3 : 1 and 10 : 1, respectively. Before the recovery test, duplicate tests were made on grey wastewater to determine the contribution from the background. Six replicates of grey wastewater were spiked to give concentrations in the range 4–8 µg/L of each fatty acid. Reproducibility, the variance of one sample analysed with the same method, by the same analyst at the same laboratory, were tested over a period of five days. Repeatability, the variance in result analysed with the same method, by different analysts at the same laboratory, were tested with two analysts who each analysed the same sample five times.

RESULTS AND DISCUSSION

Method

The method was linear up to 100–200 μg/L for the various acids. Limits of detection and limits of quantification and are listed in Table I. The practical LOQs were calculated from the linear regression of the standard curve since the discs were found to leach low but measurable quantities of some long-chain fatty acids (Table I). Grey wastewater samples spiked to 4–8 μg/L with their corresponding metal salts dissolved in methanol gave an absolute recovery of 31 to 97% compared to external standards (Table I). The absolute recoveries were low for a few of the acids but the results have shown to be reproducible [standard deviation (SD) < 10] and extracted standards have been used for quantification. Field and Reed found recoveries of 73–170% for the short-chain nonyl phenol carboxylates with SD 1–12% [9]. Decreasing recovery with increasing chain length may be caused by discrimination in the GC-MS and have a greater impact on the large fatty acids compared to the smaller nonyl phenol ethoxylates.

The presence of fatty acids found in blanks was thought to be derived from the discs and this was tested by in-vial derivatisation of freshly cut discs as well as discs that had been washed and conditioned. Measurable quantities of myristic, palmitic and stearic acid were found but a majority of the interferences were found to be removed during the wash procedure used to remove the benzoic acid. The NPECs method of Field and Reed [9] was improved by the use of HCl in the acetonitrile for soaking the discs. Furthermore the volumes of solvent used for washing prior to extraction were by increased from 5 mL acetonitrile and 5 mL MilliQ-water to 10 and 15 mL, respectively.

The samples had to be centrifuged after pH adjustment since the high levels of calcium (100 mg/L) and magnesium (20 mg/L) in the samples caused precipitation of carbonates that clogged the discs during extraction. The pH was raised to ≥ 9.5 as it was found to increase the yield. Furthermore, two discs were used for each sample, which increased the recovery by 28–86% for the various acids.

The robustness of the method was tested by having the same standard analysed over five consecutive days by one analyst (SD $< 2\%$) and then subsequently by another analyst (SD between the two analysts: $< 5\%$).

TABLE I Limits of detection and quantification for the fatty acids

Fatty acid	*Common name*	*Abb.*	*Recovery* (%)[a]	*SD* (*n* = *6*)	*LOD*[b] (ng/L)	*LOQ*[c] (ng/L)	*LOQ*[d] (μg/L)
Hexanoic acid	Caproic	C_6	58	8.8	0.05	0.27	1.3
Octanoic acid	Caprylic	C_8	78	5.1	0.005	0.024	0.86
Decanoic acid	Capric	C_{10}	97	4.1	0.90	4.5	0.38
Dodecanoic acid	Lauric	C_{12}	96	4.4	1.2	6.1	1.3
Tetradecanoic acid	Myristic	C_{14}	84	5.5	0.81	4.1	2.0
Hexadecanoic acid	Palmitic	C_{16}	41	7.1	1.3	6.7	3.4
Octadecanoic acid	Stearic	$C_{18:0}$	31	9.0	1.2	6.0	1.6
9-Octadecenoic acid	Oleic	$C_{18:1}$	57	10.0	0.76	3.8	1.6
Eicosanoic acid	Arachidic	C_{20}	44	6.1	1.0	5.2	0.25

[a] Spiked grey wastewater samples *vs*. external standards.
[b] S/N > 3.
[c] S/N > 10.
[d] By linear regression.

Fatty Acid Characteristics of Grey Wastewater from Bathrooms

It was found that samples taken in the afternoon generally had higher levels of fatty acids than samples taken in the morning. Stearic and lauric acid were present in levels higher than 10 mg/L, capric, myristic, palmitic and oleic acid were found to rise by over 1 mg/L (Table II). In an inventory of 92 household chemicals and personal care products in Denmark, stearic acid was found to be the most frequently listed fatty acid on the declaration of contents [12]. That was confirmed by the results found in this study and stearic acid may potentially be used as an indicator for domestic impact. The lowest concentrations of fatty acids in this study were found for caproic, caprylic and arachidic acids (Table II). For background levels, grab samples of drinking water and hot tap water were taken and analysis showed that levels of these (< 4 μg/L) are generally low.

The treatment efficiency of the grey wastewater treatment plant was evaluated by comparing the levels of fatty acids in the grey wastewater after different treatment steps (effluent of filter 1 and in the buffer tank) with the concentrations found in the inlet, see Table III.

The efficiency of treatment decreases with increasing chain length for the saturated acids. The unsaturated oleic acid is removed completely whereas its saturated analogue, stearic acid, it only reduced by 19%. Other unsaturated acids would have to be tested to see if this is a general trend. The same treatment plant has found to degrade organic matter (NVOC) by 85% [12]. The variation in concentration of stearic acid in the inlet, after filter 1 and in the outlet is shown in Fig. 2.

TABLE II Concentration ranges for fatty acids quantified in the grey wastewater

Compound	*Concentration range* (μg/L)
Caproic acid	41–270
Caprylic acid	< 1–640
Capric acid	< 0.5–1190
Lauric acid	< 1.5–14 500
Myristic acid	< 2–3020
Palmitic acid	< 4–7610
Stearic acid	2–27 100
Oleic acid	15–3580
Arachidic acid	33–240

TABLE III Treatment efficiency (%) for removal of fatty acids in each individual treatment step in the grey wastewater treatment plant

Fatty acid	*Abb.*	*Inlet to effluent of filter 1*	*Filter 1 to effluent of outlet*	*Inlet to outlet*
Caprylic	C_8	91	100	100
Capric	C_{10}	94	41	96
Lauric	C_{12}	99	< 1	99
Myristic	C_{14}	53	39	71
Palmitic	C_{16}	36	51	69
Stearic	$C_{18:0}$	19	< 1	19
Oleic	$C_{18:1}$	84	99	100

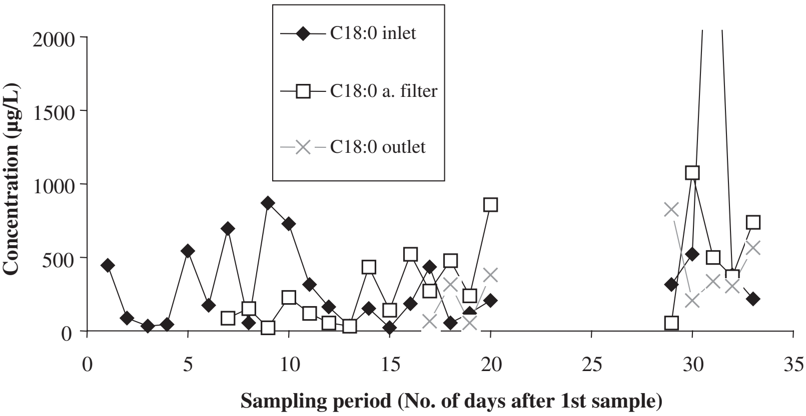

FIGURE 2 Flux of stearic acid in raw and treated grey wastewater.

Several other fatty acids were also found to be present in the grey wastewater samples. The unbranched chains of the small C_4–C_5, the odd-numbered C_{11}, C_{13}, C_{15} and C_{17} and the long-chain C_{22} and C_{24} were identified. A number of mono-unsaturated compounds of C_{16} and mono and di-unsaturated chains C_{18}, as well as a few branched chains of different sizes were also present. The concentrations were semi-quantitatively assessed to be in the range of <1 to 50 μg/L per individual acid where the highest concentrations were found for the mono and di-unsaturated forms of C_{16} and C_{18}.

CONCLUSIONS

The method has proven to be robust and dependable. The strength of in-vial derivatisation is the low consumption of solvent and relatively fast preparation. Low absolute recoveries were found but as the results have proven to be reproducible and as extracted standards have been used for calibration, the results are considered reliable. The discs used were found to leach low but detectable levels of C_{14}, C_{16} and C_{18} besides benzoic acid, which had previously been identified as a pollutant, and the discs require washing prior to use.

Quantitative analyses of fatty acids (C_6 to C_{20}) have shown that the dominating acids in grey wastewater from bathrooms are C_{12} (lauric acid), C_{16} (palmitic) and $C_{18:0}$ (stearic acid). Low levels of the fatty acids were found in the drinking and hot tap water in the building, which indicates that the acids present in grey wastewater from bathrooms originate from the hygiene products, i.e., soap and skin creams, consumed as the grey wastewater was produced. The high levels of long-chain fatty acids found (up to 27 mg/L) indicated that these compounds might significantly contribute to the amount of organic matter in grey wastewater.

A local treatment plant (sandfilters) can be used to reduce the amount of fatty acids in the grey wastewater before reuse or discharge but the efficiency of the treatment decreases with increased chain length.

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