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CHEMICAL FACULTY

DEPARTMENT OF ANALYTICAL CHEMISTRY

MASTER THESIS

„Determination of volatile fatty acids in municipal wastewater by gas chromatography-mass spectrometry preceded by headspace- solid phase microextraction with PDMS-CAR fibre”

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LIST OF ABBREVIATIONS

APT	Activated Primary Tank
DI-SPME	Direct Immersion - Solid Phase Micro Extraction
EBPR	Enhanced Biological Phosphorus Removal
FID	Flame Ionisation Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography - Mass Spectrometry
GC–MS–O	Gas Chromatography – Mass Spectrometry - Olfactometry
HPLC	High Performance Liquid Chromatography
HS- SPME	Headspace - Solid Phase Micro Extraction
LLE	Liquid- Liquid Extraction
LLME	Liquid- Liquid Micro Extraction
LOD	Limit of Detection
LOQ	Limit of Qunatitation
MS	Mass Spectrometry
MSW	Municipal Solid Waste
ND	Not Detected
PAO	Phosphorus-Accumulating Organisms
PDAM	1-Pyrenyldiazomethane
PDMS	Polydimethylsiloxane
PDMS - CAR	Polydimethylsiloxane - Carboxen
PEG	Polyethylene glycol
PHB	Polyhydroxybutyrate
PST	Primary Settler Tank
PT	Purge and Trap
PTFE	Polytetrafluorethylene
SDE	Single Drop Extraction
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
UV	Ultraviolet
VFAs	Volatile Fatty Acids

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ABSTRACT

An analytical procedure based on headspace – solid phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS) has been developed for the determination of free volatile fatty acids in municipal wastewater samples. Polydimethylsiloxane-Carboxen (PDMS-CAR) fibre was found to have greater affinity to extract lower chain fatty acids. Extraction and gas chromatographic parameters of importance such as desorption time, sample volume, amount of salt to be added and extraction time have been optimized. Detection limits were in the low to medium mg/L levels. To evaluate the applicability of the developed HS-SPME procedure on real samples, municipal wastewater treatment plant samples were analysed.

Keywords: Headspace-solid phase microextraction, municipal wastewater, VFAs

1. INTRODUCTION

Environment is of great concern with the evolution of human life. Human is getting all resources from environment and making it polluted in return by various ways. So, it is essential for the sustainable utilization of natural resources and to safeguard them for the future generation. Sources of pollution arise from petroleum refining, food and paper industries, chemical industries as well as from transportation, municipal waste and agriculture. With industrial development and household activities large amount of waste is generated which in turn is disposed of to landfill or sent to sewage when it is in liquid phase. During storage, solid waste decomposes by physical, chemical and biological processes which can lead to bad smell to the environment. The main components that are responsible to bad odour in the environment are volatile fatty acids (VFAs).

Volatile organic substances in the atmosphere can move significant distances, they undergo transformations, processes of sedimentation and leaching by rainfall. The excessive accumulation of pollutants in nature leads to the depletion of the regenerative capacity of the biosphere and leads to increased morbidity and a reduction in the comfort of human beings and animals.

Volatile fatty acids (VFAs) are organic substances composed of alkyl chain and carboxylic group. Volatile fatty acids (VFAs) are low-molecular-mass organic acids with a strong hydrophilic character [1]. They originate from anaerobic biodegradation of carbohydrates, proteins and fats. In addition, VFAs constitute one of the chemical classes responsible for unpleasant odour generation in wastewaters, together with volatile amines and sulphur compounds [2]. Volatile fatty acids (VFAs) comprise a variety of low-molecular weight carboxylic acids containing up to six carbon atoms [3].

Volatile fatty acids (VFAs) are the by-products of rumen digestion. Hence, the wastewater from pig and cattle farms contains a high proportion of VFAs. Volatile fatty acids accumulate in the wastewater and in the activated sludge in the process of anaerobic fermentation [4]. Low-molecular-mass carboxylic acids (aliphatic short chain C_2 –C_5) are important intermediates and metabolites in biological processes. The presence of VFAs in a sample matrix is often indicative of bacterial activity [5]. VFA determination is of significance in studies of health and disease in the intestinal tract [6].

2. THEORETICAL PART

2.1 Physical and chemical properties of VFAs

Depending on their structure organic acids can differ in their physical and chemical properties which are shown in Table 1.

Table 1: Physical and chemical properties of VFAs

Common name	IUPAC name	Structure	Density (g/cm3)	Boiling point	Solubility in water (g/dm^3)	Pka at 25 ^oC	Mol.wt (g/mol)	Odour	Colour
Acetic acid	Ethanoic acid	CH_3-COOH	1.05	117.9 ^oC	Fully miscible	4.76	60.05	Vinegar smell	Colourless liquid
Propionic acid	Propanoic acid	CH_3-CH_2-COOH	0.933	140.99 ^oC	Miscible	4.88	74.08	Pungent	Colourless liquid
Butyric acid	Butanoic acid	CH_3-CH_2-CH_2-COOH	0.958	163.53 ^oC	Miscible	4.82	88.12	Sweat smell	Colourless liquid
Isobutyric acid	2-methyl butanoic acid	CH_3-CH (CH_3)-COOH	0.947	155 ^oC	210	4.84	88.12	Fusty smell	Colourless liquid
Valeric acid	Pentanoic acid	CH_3-CH_2-CH_2-CH_2-COOH	0.938	186.05 ^oC	40	4.82	102.13	Unpleasant	Colourless liquid
Isovaleric acid	3-methyl pentanoic acid	CH_3-CH(CH_3)-CH_2-COOH	0.925	176 ^oC	25	4.78	102.13	Mould cheese smell	Clear colourless liquid
Caproic acid	Hexanoic acid	CH_3- CH_2-CH_2-CH_2-CH_2-COOH	0.927	205 ^oC	10	4.88	116.16	Sharp smell	Oily colourless liquid
Enanthic acid	Heptanoic acid	CH_3-CH_2- CH_2-CH_2-CH_2-CH_2-COOH	0.917	223 ^oC	2.6	4.89	130.19	Unpleasant rancid	Oily colourless liquid
Caprylic acid	Octanoic acid	CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOH	0.910	239.3 ^oC	0.7	4.89	144.22	Slightly unpleasant rancid like	Oily colourless liquid

2.2 Sources of VFAs

Volatile fatty acids play an important role in metabolism of many living organisms and can be excreted by microorganisms, higher plants and animals. Photochemical and biochemical degradation of anthropogenic and natural organic matter can also be a source of VFAs. There are numerous sources of natural (biological, geological, transformation of other pollutants) and anthropogenic (agriculture, livestock farming, food processing, waste disposal) origin and VFAs can be detected in many environmental compartments including air, water and soil. They can be present in air, rain, groundwater, soil pore water, wastewater, drinking water, and landfill leachates. They are present in larger amounts in activated sludge, waste and landfill leachates and wastewaters [7].

Major sources of organic acids in troposphere are direct biogenic and anthropogenic emissions but there are indirect contributions through photochemical formation from organic precursors [8]. Biogenic sources include emissions from soil and especially vegetation, whereas anthropogenic emissions can be traced back to biomass combustion (e.g.; forest fires, agricultural burnings) and incomplete combustion of fuel and fuel additives [7].

VFAs are produced by microbial oxidation of dissolved and particulate organic carbon and commonly accumulate in anaerobic environments. VFAs are microbial breakdown products of anaerobic fermentation; therefore, they are present in high concentrations in anoxic waters. The major volatile fatty acid in natural waters is acetic acid. Because acetic acid is a key intermediate in oxidative degradation of dissolved carbon by bacteria, it is also the most important volatile fatty acid as a biochemical intermediate in metabolism [9].

Thus main sources of VFAs can be defined as:

Geological processes

Biological processes - compounds excreted by animals, higher plants, bacteria, fungi,

Biochemical processes - transformations of compounds forming pollution

Biogeochemical processes

Agriculture

Pigs, cattle

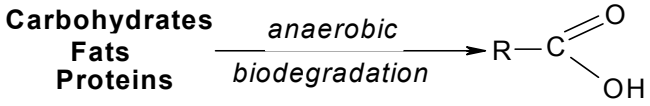
Landfills

Food Processing

Wastewater and sewage sludge

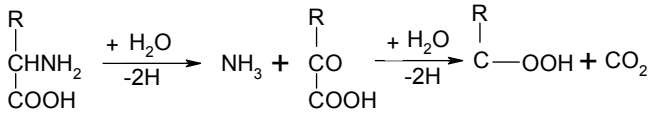
2.3 Formation of VFAs

In the process of anaerobic biodegradation volatile fatty acids are formed by the breakdown of larger molecules, such as fats, carbohydrates and proteins (Reaction 1).



$R = C_1$ to C_7

A wide variety of bacteria strains can produce one or more VFAs from dietary carbohydrates in the gut by anaerobic bacterial fermentation. The most abundant are acetic, propionic and n-butyric acid but iso-butyric, n-valeric, iso-valeric and n-caproic are also present though in smaller quantities. Some fraction of the acids formed is excreted [10]. Bacteria capable of fermenting of amino acids to VFAs are limited to anaerobes and facultative anaerobes. Bacterial activity can influence the flavour and taste of cheese and fermented milk products. Some lactic acid bacteria can produce acetic acid from alanine and glycine, iso-butyric acid from valine, iso-valeric acid from leucine and valeric acid from isoleucine in the process of oxidative deamination and decarboxylation [11]. The proposed reaction (Reaction 2) is as follows:



VFAs are also by-products of rumen digestion and livestock farm wastewater contains a large proportion of VFAs [12]. VFAs produced in the above processes can participate in pollution of domestic, diary industry and livestock farm wastewaters. VFAs are formed by fermentation of organic matter in aqueous media, such as wastewater, sanitary landfill leachate, etc. Generally anaerobic degradation of organic matter proceeds via hydrolysis, acidogenesis, acetogenesis and finally methanegenesis in which biogas is formed. The anaerobic biodegradation finds increasingly wide application in disposal of municipal solid waste (MSW) [13]. Moreover, it is of great importance in livestock manure and liquid swine manure combined with energy production.

Photochemical processes in the gaseous phase and to some extent in the aqueous phase lead to organic acid formation via radical reactions involving, for example, oxidation of hydrocarbons and aldehydes by free radicals and other oxidants. They are formed in drinking water as by-products by ozone based purification of water. Organic acids in

soil are formed by excretion from plant roots, release by microorganisms and degradation of organic matter.

2.4 General scheme for the formation of VFAs in wastewater treatment plant

VFAs are source of nutrients for bacteria which are responsible for bio-degradation of organic matter in wastewater. There are several schemes of VFA formation from primary sludge fermentation. One of the simplest configurations for fermentation and elutriation of primary sludge consists in allowing settled raw sludge solids to accumulate at the bottom of the primary settler tank (PST) and recycling partially this sludge to elutriate the fermentation products out of the sludge (Fig. 1(a)). This operation scheme is usually known as activated primary tank (APT). When the required VFA concentration is not able to be produced in the PST itself, a separated fermentation unit can be used to increase the VFA production (Fig. 1(b)). In this case, the settled sludge is conducted to the fermentation unit and the fermented sludge is returned to the primary settler to elutriate the VFA with the influent wastewater. This operation scheme is known as side-stream process [14].

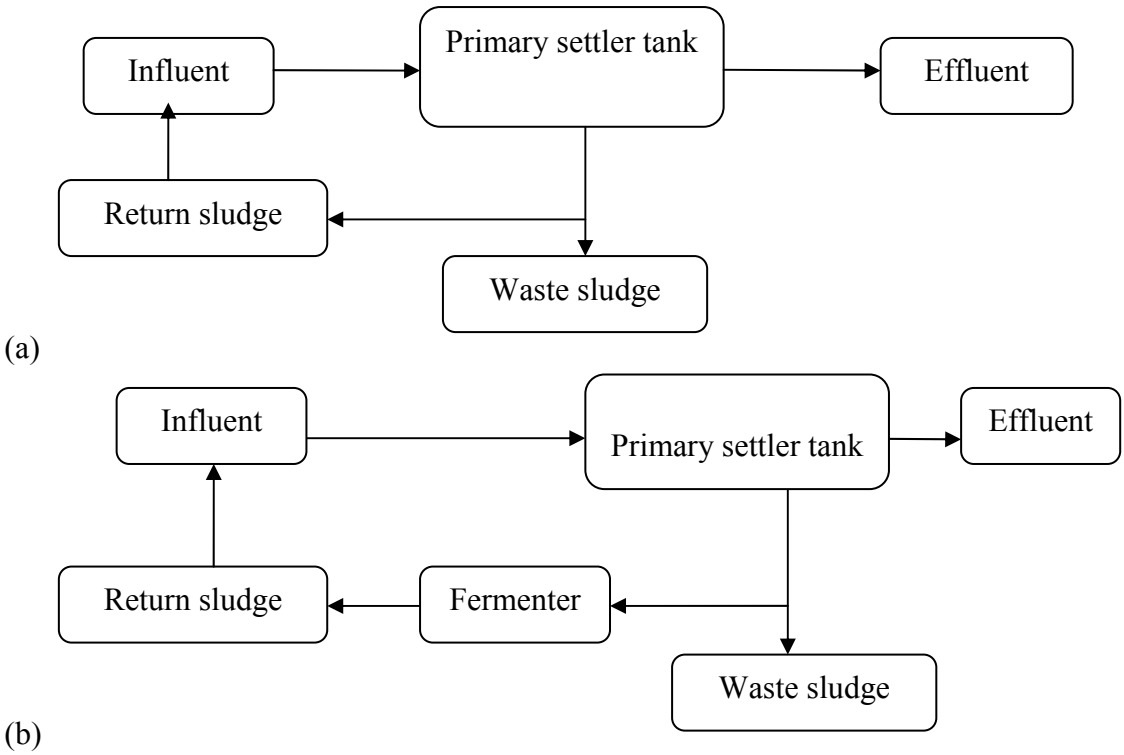


Figure 1: Fermentation–elutriation schemes: (a) activated primary tank and (b) side stream fermentation process

2.5 Role of VFAs in biological phosphorous removal from municipal wastewater

Phosphorus is a key pollutant in municipal wastewater. Phosphorus enters municipal wastewater treatment facilities from both domestic and industrial sources. Domestic contributions come from human waste and detergents while the remaining portion comes primarily from agriculture, through run-off and animal husbandry. Phosphorus in municipal wastewater occurs in dissolved and particulate forms. Most occurs as dissolved phosphate and consists of ~50% orthophosphate, 35% condensed phosphates and 15% organic phosphates. Typically, total phosphorous concentration in municipal wastewater is between 6 and 8 mg L^{-1}[15].

Excess phosphorous leads to eutrophication of lakes and rivers, adversely affecting biological diversity and water quality. To minimize eutrophication, treatment facilities must often reduce phosphorus levels to less than 1 mg L-1. Increased phosphorous levels in the sludge and sludge liquors can lead to formation of magnesium ammonium phosphate hexahydrate, more commonly known as the mineral struvite. Struvite formation in pipes reduces the pipe diameter. The composition of struvite in particular, where nitrogen (N), phosphorus (P) and magnesium (Mg) occur in equal molar concentrations, makes it a potentially interesting product in the fertiliser industry. It has been found to be as good a fertiliser as commercial mono calcium phosphates in plant-growth trials. A benefit is struvite's low solubility, which prolongs nutrient release during the growing season, minimising the danger of burning crop roots [15].

Phosphorus can also be removed biologically from wastewater by incorporation into cells; these cells are then removed as sludge. Conventional biological treatment typically removes only 20% of the phosphorous present, whereas encouraging the establishment of bacteria that can take up and store more phosphorous than they need for their normal metabolic requirements can increase this to 90%. This process is termed enhanced biological phosphorus removal (EBPR) and relies on establishing a community of phosphorus-accumulating organisms (PAO) that take up 20–30% of their dry weight as P compared to 2% for conventional organisms. To establish PAO requires both a change in process and also a sufficient quantity of readily biodegradable organic matter in the influent wastewater. A typical process flow-sheet and the mechanisms involved in EBPR are shown in Figure 2. The process requires the combination of both anaerobic and aerobic stages to encourage phosphorous uptake and release. Sufficient quantities of readily biodegradable matter, specifically volatile fatty acids (VFAs), are required during this process. Wastewater contains VFAs, but from the experience of

many operating EBPR plants, the addition of chemicals such as acetate is required before they achieve good removal rates [15].

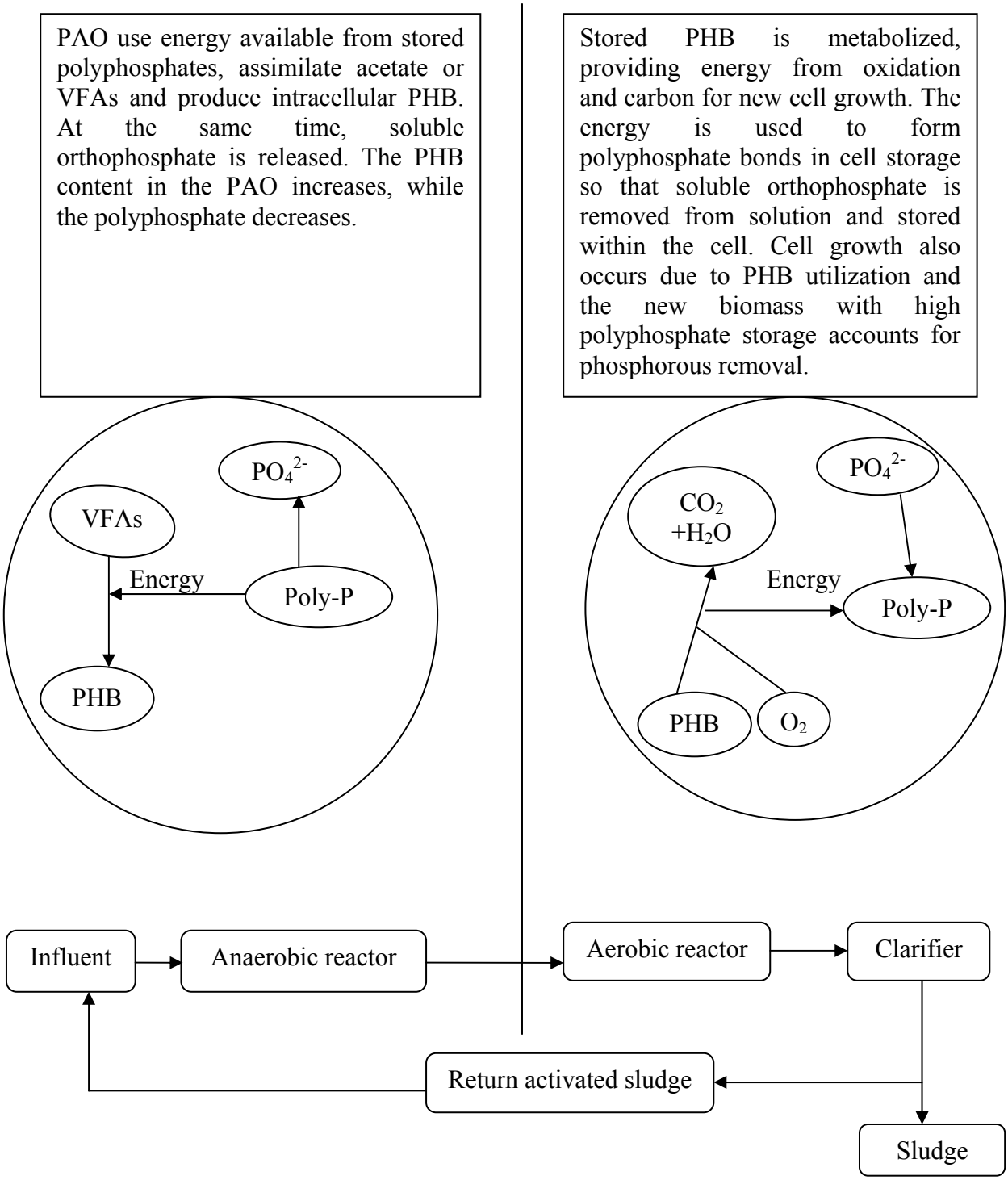


Figure 2: The process and configuration of a typical reactor for enhanced biological phosphorous removal

2.6 The role of VFAs in the environment

VFAs are one of the key constituents in the biological removal of phosphorus from waters or nitrification–de-nitrification in activated sludge. Volatile fatty acids not only affect the storage stability of waste incineration residues by reducing the pH value but also by increasing the mobility of heavy metals and radio nuclides.

The determination of fatty acids in different matrices is of concern because they are important metabolites and intermediates in biological processes. They are widely dispersed in nature and are often produced from humic substances during water treatment processes [16]. Volatile fatty acids (VFAs) are important intermediate compounds in the metabolic pathway of methane fermentation and cause microbial stress if present in high concentrations. This results in a decrease of pH, ultimately leading to failure of the digester. Therefore, the monitoring of VFA concentrations is very important for the operation performance of an anaerobic digester. The intermediates produced during the anaerobic bio-degradation of an organic compound are mainly acetic acid, propionic acid, butyric acid, and valeric acid. Amongst these, acetic and propionic acids are the major VFAs present during anaerobic bio-degradation and their concentrations provide a useful measure of digester performance. VFAs indicate the metabolic state of the obligate hydrogen producing acetogens and the acetoclastic methanogens, which are the most delicate microbial groups [17].

Monitoring of VFAs is of growing interest since they are, together with sulphur compounds and volatile amines, responsible for odour formation in wastewater treatment or during composting operations. On the other hand, they may have some profitable effects since they act as a source of carbon for microorganisms involved in the removal of phosphorus from waters [18].

Nitrates and orthophosphates removal is more effective if wastewater contained higher amount of volatile fatty acids. Wastewater rich in VFA may improve high poly-beta-hydroxybutryrate (PHB) storing by polyphosphate-accumulating bacteria [19].

The VFA potential of wastewater, i.e. the sum of VFA originally present in the wastewater and the amount of VFA that can be formed through fermentation of the other organic wastewater constituents, is a crucial parameter for the performance of enhanced biological phosphorous removal. The VFA potential represents the maximum amount of VFA, the principal substrate for polyphosphate accumulating bacteria; that can be made available to these bacteria in the anaerobic stage of an enhanced biological phosphorous removal (EBPR) process [20].

Monitoring of a content of VFAs can help control the process of efficient methane production out of organic matter. An additional advantage of the controlled conversion of organic waste matter is reduction of malodorous VFAs release to air, water and soil. This also needs monitoring of VFAs at various steps of the process.

2.7 Determination of VFAs

Many wastewater treatment and environmental applications require determination of VFAs concentrations in the range of 1–5000 mg/L and involve a variety of matrices. These matrices often contain components that can result in the degradation of sensitive equipment such as elements of a gas chromatograph and high-performance liquid chromatograph. Traditionally, the VFA content in wastewater has been determined by titrimetric methods and direct aqueous injection GC or GC preceded by solvent extraction. More recently, ion chromatography and HPLC have been applied to VFAs determination [18].

Controlling and determination of the concentration of volatile fatty acids in wastewater and leachates is of great importance and therefore, there is demand for universal, selective, sensitive and possibly quick methods of VFAs determination.

Titration techniques are characterized by low accuracy, selectivity, and high limit of quantitation. Titrimetric procedures are not sufficient to determine the particular species of VFAs and results are commonly reported as total VFAs content. High performance liquid chromatography and ion chromatography often require complicated cleaning procedures for the sample and use of derivatisation technique or reagent in order to reduce interference and achieve an appropriate limit of detection. Gas chromatography is a technique that can be successfully applied for the determination of VFAs on very low concentration levels [21].

The determination of VFAs in environmental samples must be preceded by pre-treatment, generally based on their selective (without interfering matrix components) isolation and enrichment using an appropriate technique. For determination of individual VFAs, chromatographic techniques are generally used; gas chromatography coupled with flame ionization detection (GC-FID) using direct aqueous injection. Such methods can lead to contamination of the GC injection port and column with complex sample matrix component that interfere with analysis and can degrade chromatographic performance.

Sample clean up by solvent extraction can prevent the introduction of sample matrix components but it includes extended sample preparation time and costly disposal of used solvents. To avoid this problem HS-SPME technique is used for sample preparation which is a relatively rapid and solvent free technique.

2.8 Preparation of samples for analysis

Sampling and sample preparation are key steps in analytical procedure. In many cases sample preparation generally involves much time and labour; and can introduce large error to the final results. If sampling is not done properly, we cannot get right results although we use sophisticated instruments and right method. Each analytical procedure consists of several basic steps that must be carried out very carefully and in a certain order. For this reason, it is essential to have appropriate samplers and appropriate containers to ensure stability during transportation to the place of testing, storage under appropriate conditions, to analyze by using the appropriate procedures and equipment, and interpret the results obtained.

As our main focus is to determine the volatile fatty acids in environmental samples, sampling should be carried out in such a way as to prevent loss of volatile compounds. Wastewater samples should be collected in PTFE bottles and it is essential to acidify the samples with concentrated inorganic acid to pH 2 to inhibit biodegradation processes. Sample bottles should be stored in the refrigerator at 4 ºC in order to avoid losses. Reversion to the bottom of the container closure leads to a reduction of losses of analytes and it is necessary to analyze sample in the shortest possible time after sampling.

2.9 Techniques of isolation and / or enrichment

Extraction or separation of dissolved chemical component X from liquid phase A is accomplished by bringing the liquid solution of X into contact with a second phase, B, given that phases A and B are immiscible. Phase B may be a solid, liquid, gas, or supercritical fluid. A distribution of the component between the immiscible phases occurs. After the analyte is distributed between the two phases, the extracted analyte is released and/or recovered from phase B for subsequent extraction procedures or for instrumental analysis.

The theory of chemical equilibrium leads us to describe the reversible distribution process as

$$X_A \rightleftharpoons X_B$$

and the equilibrium constant expression, referred to as the Nernst distribution law is,

$$K_D = \frac{[X]_B}{[X]_A}$$

The equilibrium constant, named partition coefficient is independent of the rate at which it is achieved. Higher the value of K_D, higher would be extraction from phase A into phase B. Conversely, if K_D is small, less chemical X is transferred from phase A into phase B. If K_D is equal to 1, equivalent concentrations exist in each phase [22].

Extraction techniques play a key role at the stage of the isolation and enrichment of analytes from environmental samples. During the extraction process, there is the transport of analytes from the sample (primary matrix) to collecting matrix (secondary matrix) in a simple and clearly defined chemical composition. The introduction of the extraction operation facilitates the transfer of analytes to the secondary matrix with a much simpler composition than the primary matrix, reduces interference at later stages of analysis and increases the concentration of the analyte in the secondary matrix. The choice of the extraction technique used depends on many factors such as: the cost of equipment, the unit cost of operation, the amount of organic solvent consumed and related environmental risks, the required sample size, degree of automation and number of sample preparation steps. Any additional step can be a source of errors. The commonly used methods for the extraction of volatile fatty acids from water samples are:

- Liquid - Liquid Extraction (LLE)
- Purge and Trap (PT))
- Solid Phase Micro extraction (SPME)
- Headspace extraction (HS)

In our research solid phase microextraction (SPME) coupled with HS was used as sample preparation technique.

2.10 Solid Phase Microextraction (SPME)

Solid phase microextraction (SPME) is a relatively novel extraction technique which is inexpensive and time efficient and environment friendly. This technique eliminates the use of organic solvents in sample preparation [23]. This method was first reported by Arthur and Pawliszyn in 1990 [24].

The main principle behind SPME is the partitioning of analytes between sample matrix and the fibre. The amount of analytes extracted depends on the partition coefficient between the fibre coating and the sample matrix. The amount of analyte absorbed by the fibre at equilibrium, n_s, depends on the initial sample concentration, c_2^o, the volume of the stationary phase, V_1, the sample volume, V_2, and the distribution constant between the sample matrix and the stationary phase, K. The relationship is described by

$$n_s = \frac{KV_1V_2c_2^0}{KV_1 + V_2}$$

If K is large or the sample volume is very small, the sample can be significantly depleted after one injection and the concentration of analyte changes from injection to injection.

If the KV_1 term is small relative to V_2, the relationship is described by,

$$n_s = K\ V_1\ c_2^o$$

This situation would normally be encountered when K is small (<l000) or the sample volume is large (>100mL). The independence from the sample volume is very attractive when doing field sampling, as the fibre can simply be placed in the air or a lake or a flowing stream [25].

Sampling with an SPME device involves a few simple steps. Sampling time of SPME is usually <l h and the procedure is significantly simpler than conventional methods, thereby reducing the potential for analyte loss during the extraction process [23]. The fibre is withdrawn into the syringe needle and the needle is pierced through the septum of a sample container. The fibre is then lowered into the sample by depressing the plunger. Organic analytes partition into the stationary phase of the fibre until an equilibrium has been reached. The plunger is then withdrawn until the fibre is within the needle and the needle is removed from the sample container. The syringe needle is then used to pierce the septum of the injector of a gas chromatograph and the fibre is lowered into the injector by depressing the plunger. The analytes are thermally desorbed from the fibre and measured by gas chromatography (GC) or GC-MS [25].

An SPME device consists of a fused-silica fibre coated with an organic stationary phase contained in a Hamilton syringe, which makes the fibre easy to handle and the device portable. A 1 cm length of fibre is coated with stationary phase, typically a cross-linked methylsilicone. Schematic representation of SPME device has been shown in Figure 3. The SPME fibre of interest in this study was PDMS-CAR.

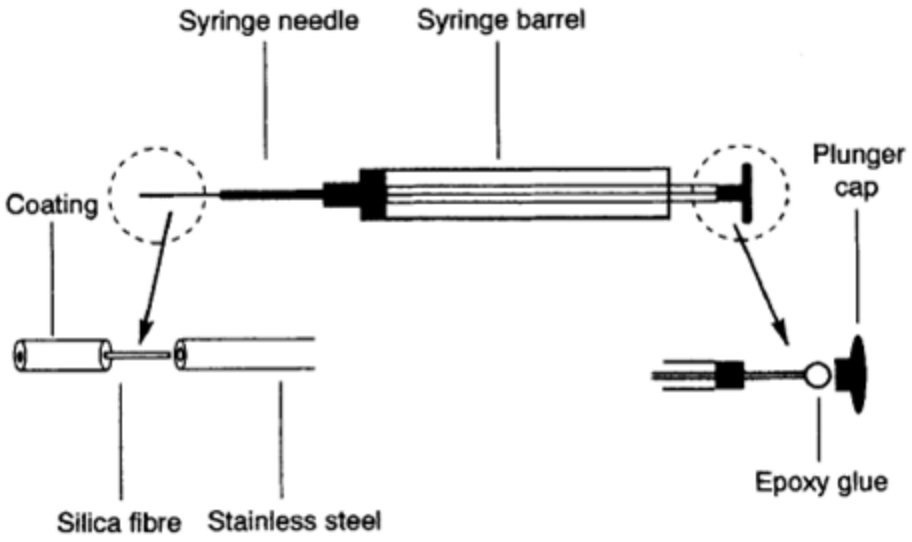


Figure 3: SPME device constructed by adapting a Hamilton 7005 syringe [25]

2.10.1 PDMS-CAR SPME fibre

Carboxen/PDMS is a multiple-component bipolar sorbent (75 or 85 µm thickness) used for SPME of gases and low-molecular-weight compounds with GC analyses [26]. Carboxen is suspended in the PDMS phase. Carboxen is a trademark for porous synthetic carbons; Carboxen 1006 used in SPME has an even distribution of micro-, meso-, and macropores. Carboxens uniquely have pores that travel through the entire length of the particle, thus promoting rapid desorption [27]. Among the SPME fibres currently available, the 85 µm Carboxen/PDMS sorbent is the best choice for extracting analytes having molecular weights of less than 90, regardless of functional groups present with the exception of isopropylamine [28]. The Carboxen particles extract analytes by adsorption.

2.10.2 Methods of application

Depending on mode of fibre introduction in extraction procedure, SPME can be divided into two modes: Direct Immersion SPME (DI-SPME) and Headspace SPME (HS-SPME). With direct immersion (DI-SPME) sampling, the fibre is inserted into a liquid. In headspace (HS-SPME) sampling, the fibre collects compounds from the headspace above the sample. HS-SPME reduces interferences from the sample matrix and is often used for analysis of wastewater and other complicated matrices.

The amount of sample collected on the SPME fibre depends on a number of factors. As with GC columns, a fibre coated with a polar polymer is more sensitive to polar compounds, and non-polar coatings are more suitable for extracting non-polar compounds. Thicker polymer coatings are generally preferable for collecting more volatile compounds. The temperature and exposure time during sample collection also affect the results. With liquid samples the sensitivity may be influenced by the pH, the addition of salts, and the agitation of the fluid. For HS-SPME, the headspace volume for a given sample volume is an important variable. Finally, desorption of compounds from the fibre depends on the temperature of the GC injector, the time, the gas flow, and the depth at which the fibre is inserted in the port [29].

2.10.3 Advantages and disadvantages of SPME procedure

SPME is a technique extensively used for isolation and enrichment of organic pollutants in environmental samples. It allows determination of organic pollutants in water at very low concentration levels [30]. One of the major advantages of SPME is that it is a solvent less sample preparation procedure, so solvent disposal is eliminated [31, 32]. SPME is a relatively simple, straightforward procedure involving only sorption and desorption [33]. SPME is compatible with chromatographic analytical systems, and the process is easily automated [32, 34]. SPME sampling devices are portable, thereby enabling their use in field monitoring.

SPME has the advantages of high concentrating ability and selectivity. SPME has the potential for analyses in living systems with minimal disturbance of chemical equilibria because it is a nonexhaustive extraction system [23].

The main drawback of this technique is relatively high cost of fibre and a limited duration of use of fibre associated with the pollution during the extraction and eventually degradation. Partial degradation of the stationary phase of fibre, especially if the desorption is carried out at high temperature, and/or thermally unstable components of the sample

may lead to the emergence of additional peaks in the chromatogram, which may impair the accuracy and precision of the analysis [35]. Matrix effects can be a major disadvantage of a sample preparation method that is based on equilibration rather than exhaustive extraction [36]. Changes in the sample matrix may affect quantitative results, due to alteration of the value of the distribution constant relative to that obtained in a pure aqueous sample [31, 36].

2.11 Final Determination of VFAs

GC is the premier analytical technique for the separation of volatile compounds. It combines speed of analysis, resolution, ease of operation, excellent quantitative results, and moderate cost. Unfortunately, GC systems cannot confirm the identity or structure of any peak. GC data alone are not generally sufficient to identify peaks.

Mass spectrometry on the other hand is one of the most information- rich detectors. It requires only micrograms of sample, but it provides data for both the qualitative identification of unknown compounds (Structure, elemental composition and molecular weight), as well as their quantitation. In addition, it is easily coupled with a GC system [37].

Due to the high resolving power and the possibility of work with a wide range of selective and sensitive detectors, gas chromatography plays an important role in the final determination of VFAs occurring at low concentration levels in complex matrix (wastewaters and leachates). In this technique, analytes are partitioned between the stationary phase and mobile gas phase. GC has great ability to separate complex mixtures, is relatively inexpensive, fast, reliable and sensitive. It requires small samples and the mobile phase used is a pure inert gas which is environment friendly.

Coupled with mass spectrometer gas chromatographic system is often used in the analysis of environmental samples due to the high selectivity, high sensitivity, good reproducibility and a relatively simple structure and high identification potential.

2.12 Application of HS – SPME and GC combination for the determination of VFAs

HS – SPME coupled with GC is quite popular in the analysis of different matrixes for VFAs content. Published applications are presented in Table 2. The table contains the sample preparation methods, method of final determination, type of VFAs, different matrixes and their limit of detection.

Table 2: Literature review presenting different methods for extraction and determination of VFAs

Determined Volatile Fatty Acids	Matrix	Sample preparation technique	Final Analysis	LOD / Concentration range	Publication date	Reference
C_4, C_6	Fruit juice beverage	SPME	GC - MS	-	1994	38
C_3, C_5, C_6, C_8	Spanish red and white wines	LLME	GC - FID	C_3 - 2.02 mg/L C_5 - 1.63 mg/L C_6 - 1.53 mg/L C_8 - 1.73 mg/L	2001	39
C_2 - C_{10}	Water	SPME	GC - FID	C_2 - 760 ng/mL C_3 - 280 ng/mL C_4 - 122 ng/mL C_5 - 3.1 ng/mL C_6 - 0.5 ng/mL C_7 - 0.11 ng/mL C_8 - 0.04 ng/mL C_9 - 0.03 ng/mL C_{10} - 0.02ng/mL	1995	14
C_2 - C_7	Landfill leachate	LLE	GC - FID	C_2 - 1442 mg/L C_3 - 160.7 mg/L iC_4 - 226.3 mg/L C_4 - 220.5 mg/L iC_5 - 290.7 mg/L C_5 - 290.1 mg/L iC_6 - 379.1 mg/L C_6 - 381.9 mg/L	1995	40

				C_7 - 446.4 mg/L		
C_2 - C_7	Faecal	HS - SPME (PDAM)	GC - MS	-	1999	41
C_2 - C_7	Urban wastewater	HS - SPME	GC - FID	C_2 - 675 µg/L C_3 - 54 µg/L C_4 - 6 µg/L C_5- 46 µg/L C_6- 19 µg/L C_7- 38 µg/L	2000	2
C_2 - C_7	Water Sample	HS - SPME	GC - CI - MS	C_2 - 150µg/L C_3 - 5 µg/L C_4 - 2 µg/L C_5 - 2 µg/L C_6 - 6 µg/L C_7 - 5 µg/L	2000	3
C_2 - C_5	Urban Wastewater	HS - SPME	GC - FID	1- 5000 mg/L	2002	18
C_2	Wastewater from cellulose industry	LLE	LC - MS	3 µg/L	2003	42
C_2 - C_8	Bioreactor effluent	-	GC - FID	1586 - 126 mg/L	2007	43
C_2 - C_8	Landfill leachate	Acidification of ethylene glycol	Colorimetric method	19560 mg/L	2005	44
C_2 - C_6	Odor from the pig farm	SPME	GC - FID	C_2 - 0.5 ng/L C_3 - 0.6 ng/L	2004	45

				C_4 - 4.2 ng/L C_5 - 4.3 ng/L C_6 - 5.4 ng/L		
C_2 - C_7	Air from the pig farm	HS - SPME	GC - MS - O	-	2006	46
C_2 - C_7	Sewage from pig farm	SPME	GC - MS	C_2 - 16 nM C_3 - 17 nM C_4 - 16 nM C_5 - 13 nM C_6 - 12 nM C_7 - 10 nM	1999	4
C_2 - C_6	Wastewater from constructed wetlands	HS - SPME	GC - FID	C_2 - 467 µg/L C_3 - 62 µg/L C_4 - 19 µg/L C_5 - 12 µg/L iC_5 - 3 µg/L C_6 - 3 µg/L	2008	47
C_2 - C_7	Cow slurries	PT	GC - FID	C_2 - 780 µg/L C_3 - 230 µg/L iC_4 - 220 µg/L C_4 - 770 µg/L iC_5 - 1260 µg/L C_5 - 590 µg/L C_6 - 460 µg/L C_7 - 520 µg/L	2008	48

3. AIM OF THE RESEARCH

The main objective of the study is:

(1) To develop a method for the determination of VFAs in municipal wastewater using HS – SPME with PDMS-CAR SPME fibre for VFAs isolation and gas chromatographic technique coupled with mass spectrometry for the analysis proper. The study focuses to optimize the important extraction and gas chromatographic parameters: temperature and time of desorption in GC injection port, sample volume for extraction, amount of salt (NaCl) to be added, extraction temperature and extraction time.

(2) Initial validation of some important parameters for thus developed method.

(3) Application of the method to determine VFAs in real samples of municipal wastewater.

4. EXPERIMENTAL PART

4.1 Chemicals and reagents

- Butyric acid (99.5%, Fluka, Switzerand)
- Ethanoic acid (99.8%, Fluka, Switzerand)
- 2-ethyl butyric acid (99.0%, Aldrich, Germany)
- Helium gas
- Heptanoic acid (99.0%, Fluka, Switzerand)
- Hexanoic acid (98.0%, Fluka, Switzerand)
- Iso-butyric acid (99.5%, Fluka, Switzerand)
- Iso-pentanoic acid (98.0%, Fluka, Switzerand)
- Methyl-tert-butyl ether (MTBE) (Merck, Germany)
- Millipore water
- Octanoic acid (98.5%, Fluka, Switzerand)
- Pentanoic acid (99.0%, Fluka, Switzerand)
- Propionic acid (99.5%, Fluka, Switzerand)
- Sulphuric acid (95%, Polskie Odczynniki Chemiczne POCH, Poland)
- Sodium chloride (99.9%, Polskie Odczynniki Chemiczne POCH, Poland)
- Water (PESTANAL, Wasser, Fluka, Germany)
- Volatile acid standard mix in water (C_1-C_7 10 mM, Supelco, USA)

4.2 Apparatus and laboratory equipments

- Analytical balance (RADWAG WAX 110, Poland)
- Capillary chromatographic column: Stabilwax - DA (polyethylene glycol) with a length of 30 m and 0.32 mm internal diameter, coated with a stationary phase film thickness of 0.5 μm
- Device for microextraction (Supelco, Bellefonte, PA, USA)
- Disposable pipette tips (Eppendorf, Germany)
- Gas chromatograph Trace GC Thermo FINNINGAN coupled with the Trace DSQ mass spectrometer from Thermo FINNINGAN
- Laboratory glassware (volumetric flask, measuring cylinder, beaker)
- Magnetic stirring bar
- Microliter Syringe 500 μL (Hamilton, USA)

- Micro pipettes, capacity: 0.5-10 μL, 10 - 100 μL, 100 - 1000 μL, 500 - 5000 μL (BRAND, Germany)
- MR3000D magnetic stirrer with adjustable speed of rotation (Heidolph) and stir (Aldrich)
- Millipore equipment for de-ionized water
- Refrigerator (MNHCK 16, Poland)
- SPME fibre PDMS-CAR with a film thickness of 85 μm and length 1cm
- Thermostat with temperature control
- Vials, 15 mL capacity, caps, diaphragms PTFE / silicone (Supelco)

4.3 Analytical procedure

4.3.1 Preparation of stock and working standard solutions

5 mL of 5000 mg/L VFAs standard stock solution containing 9 VFAs (ethanoic acid, propionic acid, butyric acid, iso-butyric acid, pentanoic acid, iso-pentanoic acid, hexanoic acid, heptanoic acid and octanoic acid) and internal standard (2-ethyl butyric acid) was prepared in MTBE. From that stock solution, 5 mL of each 500 mg/L, 100 mg/L, 10 mg/L and 1 mg/L standard solutions were prepared by successive dilution of respective higher concentration solution with Milli Q water.

From 500 mg/L VFAs mixture solution, 25 mg/L of 2 mL working solution of VFAs mixture was prepared in Milli Q water. Similarly, 2 mL each of 0.1 mg/L, 0.5 mg/L and 1.0 mg/L working VFAs mixture solutions were prepared from 10 mg/L stock solution by dilution method using Milli Q water and 2 mL each of 1.5 mg/L, 2.0 mg/L, 2.5 mg/L, 3.0 mg/L, 3.5 mg/L, 4.5 mg/L and 5.0 mg/L working VFAs mixture solutions were prepared from 100 mg/L stock solution by dilution method using Milli Q water.

Likewise, from volatile acid standard mix (10 mM in deionized water) containing acetic acid, formic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, n-valeric acid, isocaaproic acid, n-caproic acid and heptanoic acid, 20 mg/L and 10 mg/L stock solutions were prepared in deionized water. On preparing such solution, mass of acetic acid was taken into account and later concentration of other acids was calculated based on that concentration. 0.05 mg/L working solution was prepared from 1.0 mg/L working solution. 0.1 mg/L, 0.5 mg/L, 1.0 mg/L and 2.0 mg/L working solutions were prepared from 10.0 mg/L stock solution and 2.5 mg/L, 3.0 mg/L, 3.5 mg/L, 4.0 mg/L, 4.5 mg/L, 5.0 mg/L working solutions were prepared from 20.0 mg/L stock solution using dilution

method. Similarly, 7.5 mg/L, 10.0 mg/L, 25.0 mg/L, 50.0 mg/L, 75.0 mg/L, 100.0 mg/L, 125.0 mg/L, 150.0 mg/L and 175.0 mg/L working solutions were prepared from an original VFAs mixture solution (10 mM). 25. 0 mg/L internal standard solution of 2-ethyl butyric acid was prepared from 1000.0 mg/L of stock solution.

4.3.2 Sample preparation and extraction procedure

To each of 2 mL of working sample solution, 0.75 g of NaCl, one drop of concentrated sulphuric acid was added and one magnetic stirring bar was also put into a vial. Extraction temperature was set to 25 ^{o}C and agitation was set to 1200 rpm. After 3 min agitation the previously conditioned PDMS-CAR SPME fibre (One hour at 300 ^{o}C) was pierced through the septum into the vial and it was allowed to stay for 40 min for extraction. After 40 min, the fibre was withdrawn and desorbed into heated GC injector.

4.3.3 Gas Chromatographic conditions

Gas chromatograph	Thermo Finnigan Trace GC, USA
Temperature of injection port	300 ^{o}C
Carrier gas	Helium
Mode of injection	Splitless
Flow rate of carrier gas	1.5 mL/min
Length of GC column	30 m
Inner diameter of GC column	0.32 mm
Film thickness of stationary phase	0.5 µm
Stationary phase	Stabilwax- DA (PEG)
Desorption time	3 min

GC oven temperature program

The initial oven temperature, final maximum temperature and ramp temperature with holding time of GC system has been shown in Table 3.

Table 3: Oven temperature program for VFAs separation

	Rate (^{o}C/Min)	Temp (^{o}C)	Hold time (Min.)
Initial	-	40	3
Ramp 1	40	180	1
Ramp 2	10	230	1

4.3.4 Mass Spectrometric conditions

Mass analyser	Quadrupole mass spectrometer
Ion source temperature	240 ^{o}C
Electron ionization energy	70eV
Operating mode	SIM/SCAN

4.3.5 SIM/SCAN mode

SIM mode was used for quantitative determination of VFAs content. Mass ions selected for monitoring are given in Table 4. In a SCAN mode all ions from the range (m/z 40 - 400) were monitored. The mass spectrums obtained were used to identify the particular peaks in chromatograms.

Table 4: Mass ions selected for monitoring the particular VFAs

VFAs	Chemical formula	Mass
Ethanoic acid	CH_3COOH	43, 45, 60
Propionic acid	CH_3CH_2COOH	45, 73, 74
Iso-butyric acid	$(CH_3)_2CHCOOH$	60, 73
Butyric acid	$CH_3(CH_2)_2COOH$	43, 73
Iso-pentanoic acid	$(CH_3)_2CHCH_2COOH$	60, 73
Pentanoic acid	$CH_3(CH_2)_3COOH$	41, 60, 87
Iso-hexanoic acid	$(CH_3)_2CHCH_2CH_2COOH$	60, 73
Hexanoic acid	$CH_3(CH_2)_4COOH$	60, 73
Heptanoic acid	$CH_3(CH_2)_5COOH$	60, 73, 87
Octanoic acid	$CH_3(CH_2)_6COOH$	43, 60, 73

4.3.6 Selection of SPME fibre

PDMS-CAR SPME fibre has already been proposed for HS-SPME extraction of some lower chain fatty acids to determine them with GC-FID [2]. In this study the similar approach has been tested to determine larger group of VFAs in more complex matrix by means of GC-MS preceded by HS-SPME.

4.3.7 Optimization of extraction and gas chromatographic parameters

4.3.7.1 Selection of desorption temperature and desorption time in GC injector

Injector temperature and desorption time are important parameters that have to be optimized in SPME analysis in order to avoid the carryover effect. Since adsorption

capacity of solid sorbents is much higher than capability of liquid stationary phases, injector temperature was chosen 300 ^{o}C, maximum allowable temperature for PDMS–CAR fibre recommended by manufacturer. For the selection of desorption time, the fibre with VFAs trapped was exposed in the GC injector for 3 and 5 min. To 2 mL of 25 mg/L 9 VFAs solution in water containing internal standard was put in 15 mL vials and 0.75 g NaCl and 1 drop of concentrated sulphuric acid was added. The fibre was exposed to HS of agitated sample for 40 min and the extracted substances were then desorped in GC injector for 3 and 5 min. The chromatograms were recorded. The areas of the same peaks on both chromatograms were compared.

4.3.7.2 Selection of sample volume

For the selection of sample volume, three different types of volume were chosen. First one was 2mL of 25 mg/L 9 VFAs and internal standard solution was prepared in 15 mL vials and to this 0.75 g of NaCl and 1 drop of concentrated sulphuric acid was added and then one magnetic stirring bar was put. Second one was 4 mL of 25 mg/L 9 VFAs and internal standard solution was prepared in 15 mL vials and to this 1.50 g of NaCl and 1 drop of concentrated sulphuric acid was added and then one magnetic stirring bar was put. The third one was 8 mL of 25 mg/L 9 VFAs and internal standard solution was prepared in 15 mL vials and to this 3.0 g of NaCl and 1 drop of concentrated sulphuric acid was added and then one magnetic stirring bar was put. These samples were allowed to agitation for 3 min and extraction for 40 min and desorped in GC injector for 3 min and respective chromatographic peak areas were noted.

4.3.7.3 Selection of amount of sodium chloride

2 mL of 25 mg/L 9 VFAs and internal standard solution was introduced into vials with capacity of 15 mL, sample was acidified and then agitated. Different amount of NaCl (0.25 g, 0.5 g, 0.75 g and without salt) was added to check the salting out effects. These samples were allowed to agitation for 3 min and then to extraction for 40 min and desorped in GC injector for 3 min and respective chromatographic peak areas were compared.

4.3.7.4 Selection of extraction time

2 mL of 25 mg/L 9 VFAs and internal standard solution was prepared in 15 mL vials and to this sample solution 0.75 g NaCl and 1 drop of concentrated sulphuric acid was

added and then one magnetic stirring bar was put. These samples were allowed to extraction for 10, 20, 30, 40 and 50 min preceded by agitation for 3 min and desorped in GC injector for 3 min and respective chromatographic peak areas were noted.

4.3.7.5 Selection of extraction temperature

Extraction temperature was selected based on the research carried out previously.

4.3.8 Validation of parameters

4.3.8.1 Determination of LOD and LOQ

Using volatile acid standard mix (10 mM in deionized water) containing acetic acid, formic acid, propionic acid, isobutyric acid, butyric acid, iso-valeric acid, n-valeric acid, iso-caproic acid, n-caproic acid and heptanoic acid, 20 mg/L and 10 mg/L stock solutions were prepared in deionized water. On preparing such solution, mass of acetic acid was taken in account and later concentration of other acids was calculated based on that concentration. 0.05 mg/L of working solution was prepared from 1.0 mg/L of working solution. 0.1 mg/L, 0.5 mg/L, 1.0 mg/L and 2.0 mg/L of working solutions were prepared from 10.0 mg/L stock solution and 2.5 mg/L, 3.0 mg/L, 3.5 mg/L, 4.0 mg/L of working solutions were prepared from 20.0 mg/L stock solution using dilution method.

Then to each 2 mL of sample solution 0.75 g of NaCl and one drop of concentrated sulphuric acid was added and then one magnetic stirring bar was put, allowed to agitation for 3 min and then allowed to extraction for 40 min and desorped for 3 min and respective chromatographic peak areas were noted.

4.3.8.2 Preparation of calibration curve

Using volatile acid standard mix (10 mM in deionized water) containing acetic acid, formic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, n-valeric acid, isocaaproic acid, n-caproic acid and heptanoic acid, 20 mg/L and 10 mg/L stock solutions were prepared in deionized water. On preparing such solution, mass of acetic acid was taken in account and later concentration of other acids was calculated based on that concentration. 0.05 mg/L of working solution was prepared from 1.0 mg/L of working solution. 0.1 mg/L, 0.5 mg/L, 1.0 mg/L and 2.0 mg/L of working solutions were prepared from 10.0 mg/L stock solution and 2.5 mg/L, 3.0 mg/L, 3.5 mg/L, 4.0 mg/L, 4.5 mg/L, 5.0 mg/L of working solutions were prepared from 20.0 mg/L stock

solution using dilution method. Similarly, 7.5 mg/L, 10.0 mg/L, 25.0 mg/L, 50.0 mg/L, 75.0 mg/L, 100.0 mg/L, 125.0 mg/L, 150.0 mg/L and 175.0 mg/L working solutions were prepared from original VFAs mixture solution (10 mM).

Then to each 2 mL of aqueous solution 0.75 g of NaCl and one drop of concentrated sulphuric acid was added and then one magnetic stirring bar was put, allowed to agitation for 3 min and then allowed to extraction for 40 min and desorped for 3 min and respective chromatographic peak areas were noted. Using these data calibration curves were prepared for all volatile fatty acids.

4.3.9 Sampling and analysis of real municipal wastewater samples

Real wastewater samples of 4 bottles from one sampling point of each site were collected in plastic bottles from the municipal wastewater treatment plant Debogorze, Gdynia and Wschod, Gdansk. The samples collected were excessive sludge, pre-concentrated sludge, raw wastewater, wastewater after secondary settlement and treated wastewater. Those samples were acidified to pH 2 for their preservation and stored in refrigerator at 4^{o}C. Samples from excessive sludge and pre-concentrated sludge were diluted to half in deionised water before doing analysis. Then 2mL of acidified samples were taken into 15 mL of glass vials and internal standard (2-ethyl butyric acid), one drop of concentrated sulphuric acid, 0.75 g of NaCl was added and then one magnetic stirring bar was put. The sample vial was allowed to agitation for 3 min and then extraction procedure was followed for 40 min and fibre was desorped in GC injector and respective chromatographic peak areas were noted. Sampling pictures from two wastewater treatment plants are shown in Figure 4 and 5.

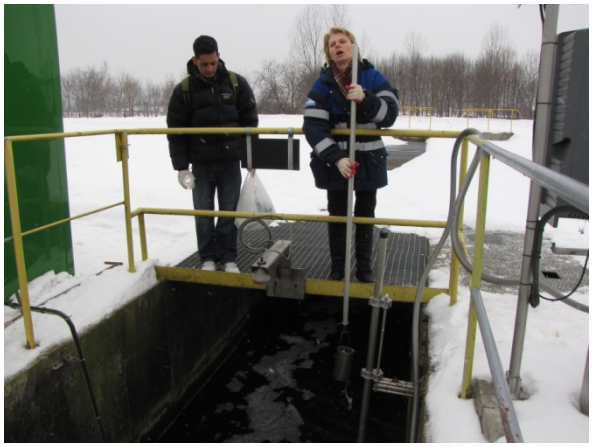


Figure 4: Sampling in Debogorze wastewater treatment plant, Gdynia

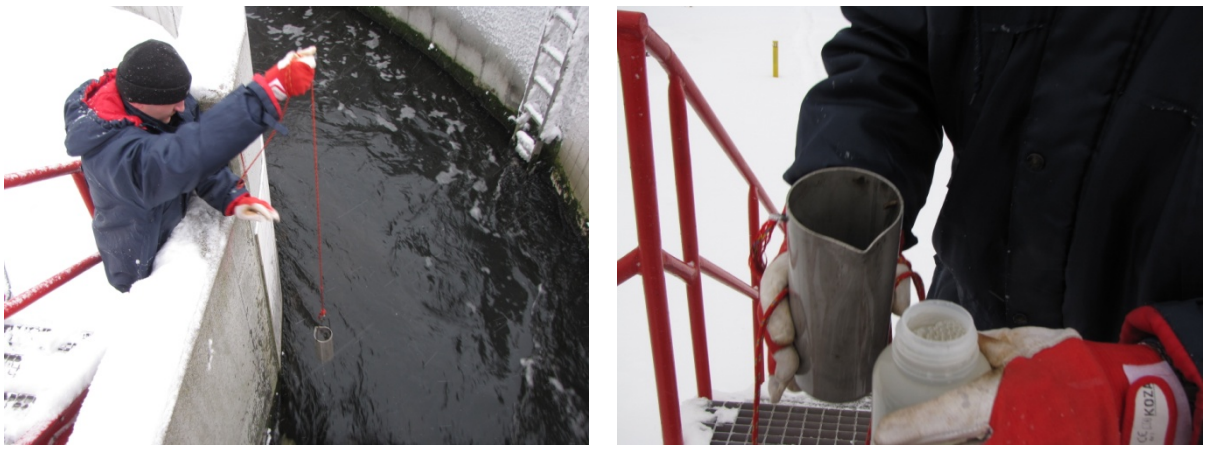


Figure 5: Sampling in Wschod wastewater treatment plant, Gdansk

5. RESULTS AND DISCUSSIONS

5.1 Optimization of extraction and gas chromatographic parameters

5.1.1 Selection of desorption temperature and desorption time of injector

Desorption temperature and desorption time are critical parameters for the study of carryover effects. Higher the desorption temperature, greater will be desorption of analytes from the fibre. Since maximum temperature allowed for the PDMS-CAR fibre was 300 ^{o}C, it was chosen as desorption temperature for the injector. For the selection of desorption time, desorption time of 3 min and 5 min were used and respective peak areas obtained were plotted in histogram. From the histogram it was found that desorption time of 3 min was better for all 9 VFAs and there was no carryover effects as well. So, desorption time of 3 min was chosen on the basis of visual observation of symmetrical peaks and histogram obtained during this work.

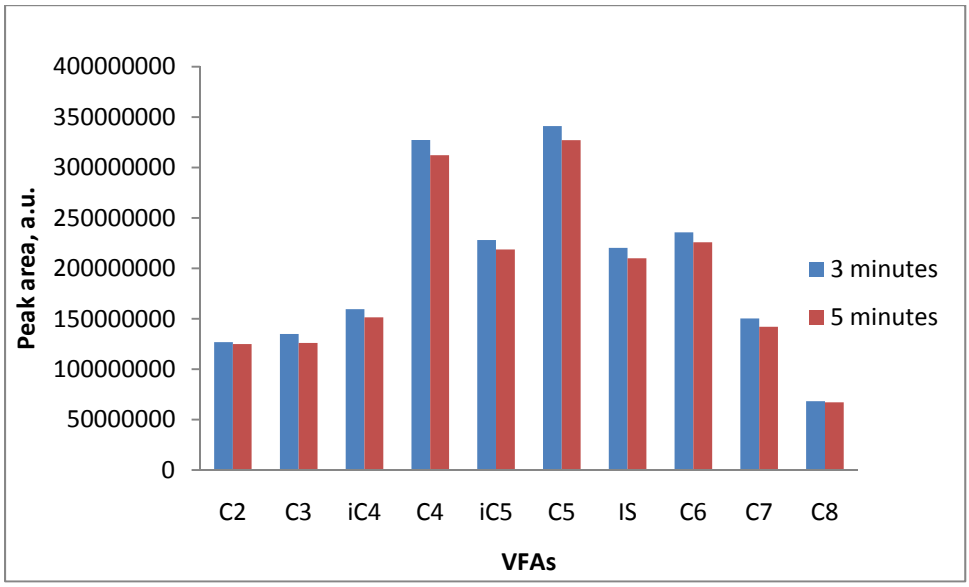


Figure 6: Selection of desorption time

5.1.2 Selection of sample volume

Sample volume of 2 mL, 4 mL and 8 mL was tested. On plotting histogram, it was found that 2 mL sample volume was better for extraction of all fatty acids as compared to a larger sample volume. For a given extraction system (fibre type and size, extraction time and temperature, matrix, vial etc.), the sensitivity is the best when the amount extracted is the largest. It depends, among others, on the ratio of HS and liquid sample and on the fact if equilibrium is reached or how far from equilibrium the system is. On

checking the chromatographic peak areas of three different volumes on identical vials of 15 mL volume, it appeared that the largest peaks were seen for 2 mL sample volume and it was selected for final procedure.

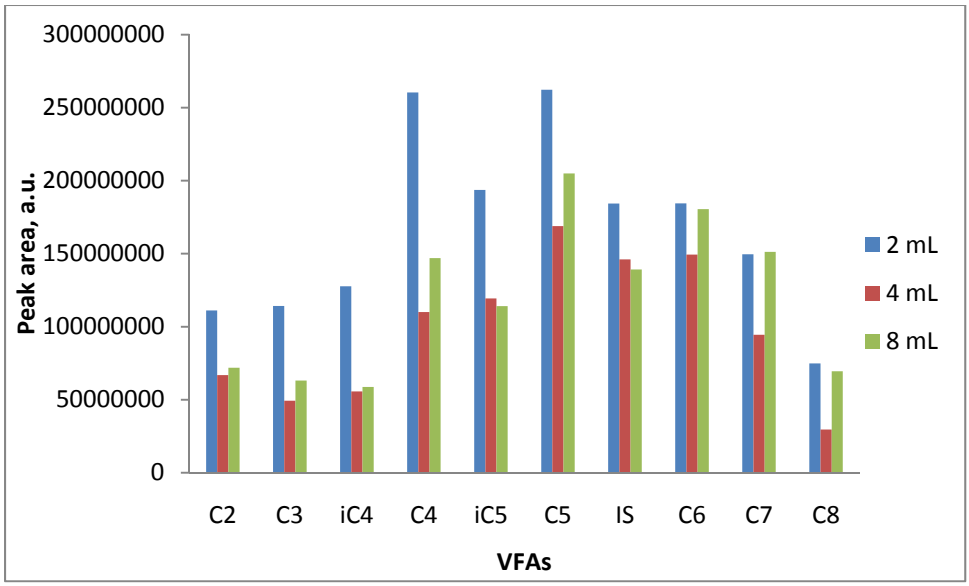


Figure 7: Selection of sample volume

5.1.3 Selection of amount of sodium chloride

Different amount of NaCl was used to check the salting effect. The possible reason of addition of the salt into the sample matrix is to increase the amount of acids extracted into fibres. Water molecules prefer to solvate the salt ions, thus the addition of salt into sample matrix should decrease the solubility of acids, which results in an increase in the amount extracted into HS and in this way into the fibre. From the histogram obtained 0.75 g salt was best for the experiments as our concern is to determine lower chain fatty acids and amount of salt for this study was chosen as 0.75 g.

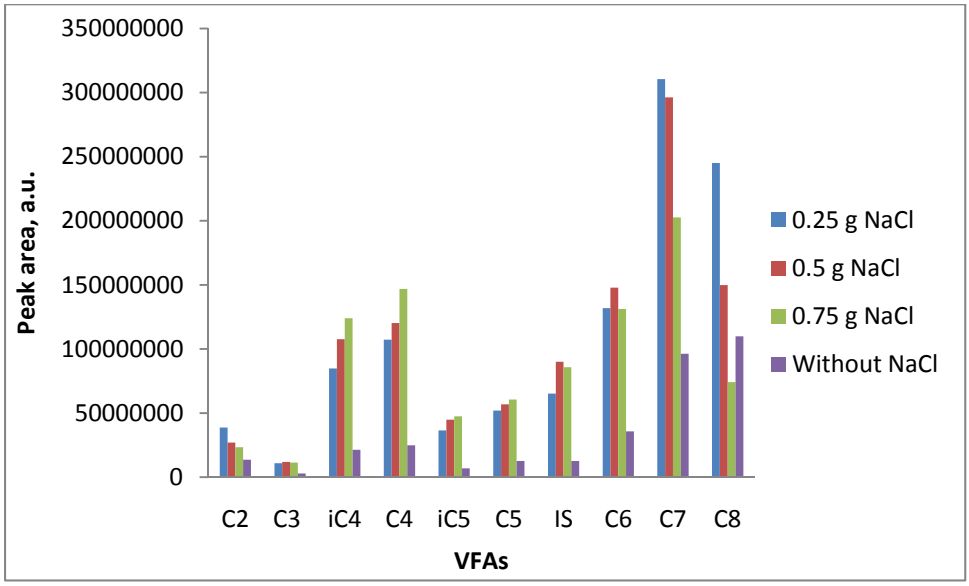


Figure 8: Selection of amount of sodium chloride added to the sample

5.1.4 Selection of extraction time

Generally amount of solute extracted should increases until equilibrium is reached.In this study there was no significant change in the peak areas of acids above 40 min as can be seen comparing 40 and 50 min extraction. If changes are smaller than an experimental error , then there is no need to increase extraction time further. So, 40 min was chosen as extraction time which allows us to save time for analysis of samples.

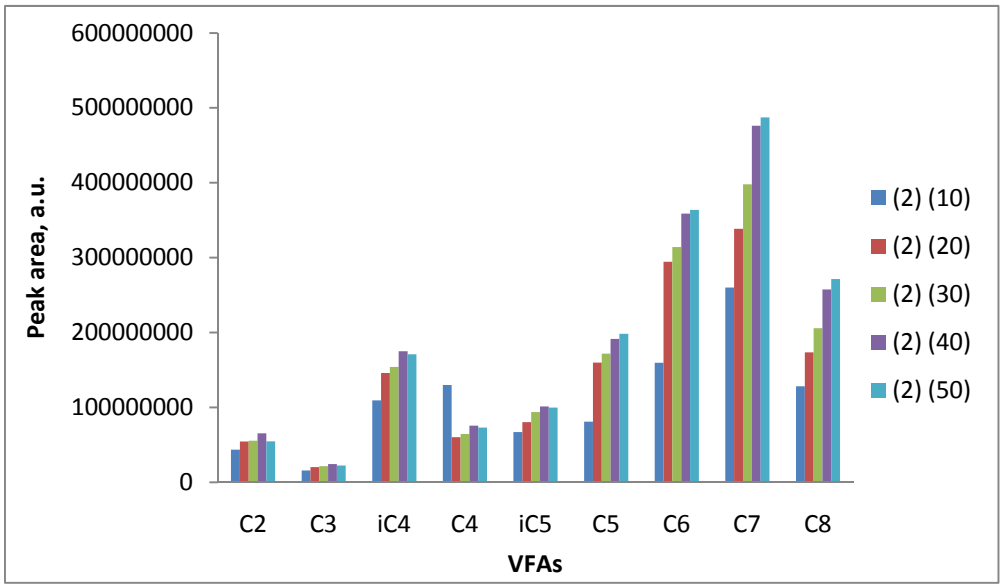


Figure 9: Selection of extraction time

5.1.5 Selection of extraction temperature

Extraction temperature was selected on the basis of previous studies. Generally increase of temperature may have a noticeable effect on the sensitivity enhancement since extraction kinetics is temperature dependent. But higher extraction temperature can lead to damage of fibre coating due to the presence of acid in the headspace. Due to this reason, 25 ^{o}C was chosen as the extraction temperature for this study.

5.2 Validation of parameters

5.2.1 Determination of LOD and LOQ

LOD is the lowest concentration (smallest quantity) of an analyte that can be detected with statistically significant certainty while LOQ is the quantity or the smallest concentration of a substance that can be determined using a given analytical procedure with an assumed accuracy, precision and uncertainty [49].

LOD value was calculated based on parameters of the determined calibration graph using standard deviation of the intercept.

The calculated LOD was checked by using the following condition:

(I) $10 \times LOD > C_{min}$

(II) $LOD < C_{min}$

The calculated LOD fulfilled the above conditions.

LOQ value was calculated as: $LOQ = 3 \times LOD$

Table 5: LOD and LOQ values for different VFAs

Volatile fatty acids	LOD (mg/L)	LOQ (mg/L)
Acetic acid	0.09	0.27
Propionic acid	0.03	0.09
Butyric acid	0.08	0.24
Iso-butyric acid	0.05	0.15
Valeric acid	0.04	0.12
Iso-valeric acid	0.06	0.18
Caproic acid	0.04	0.12
Iso-caproic acid	0.04	0.12
Enanthic acid	0.05	0.15
Caprylic acid	-	-

Lower sensitivity was obtained for all fatty acids studied. These values are also comparable to the studies carried out earlier.

5.2.2 Calibration curve for different VFAs

Calibration curve expresses the relationship between the peak area of the labelled substance and its concentration in the sample. For the purposes of quantitative analysis it is required to have the calibration curves linear and smaller scatter of points (i.e. a high correlation coefficient).

Different concentrations of standard solutions of volatile fatty acids were used to prepare calibration curves. Regression parameters were calculated using following equation:

$$y = ax + b$$

Where, "y" is the peak area of analyte, "x" is concentration of the analyte, "a" is slope and "b" is intercept of regression line.

"r^2" is the coefficient of correlation

The calibration curves were found linear for lower and higher concentration ranges with higher value of coefficient of correlation which are tabulated in Table 6. The calibration curves are presented in the Figure 10-18.

Table 6: Linearity range and r^2 values for VFAs

VFAs	Linearity range (mg/L)		r^2 value	
	Lower concentration	Higher concentration	Lower concentration	Higher concentration
Acetic acid	0.500 – 10.0	10.0 – 200	0.9999	0.9992
Propionic acid	0.620 – 12.3	12.3 - 154	0.9917	0.9991
Butyric acid	0.140 – 3.66 3.66 – 14.7	14.7 – 183 -	0.9986 0.9947	0.9905 -
Isobutyric acid	0.140 – 1.46 0.73 – 14.7	14.7 – 183 -	0.9853 0.9943	0.9937 -
Valeric acid	0.160 – 16.9	16.9 – 255	0.9937	0.9959
Isovaleric acid	0.160 – 16.9	16.9 – 212	0.9978	0.9924
Isocaproic acid	0.190 – 19.3	19.3 – 290	0.9909	0.9979
Caproic acid	0.190 – 19.3	19.3 – 241	0.9963	0.9920
Enanthic acid	0.220 – 16.2	16.2 – 271	0.9990	0.9975

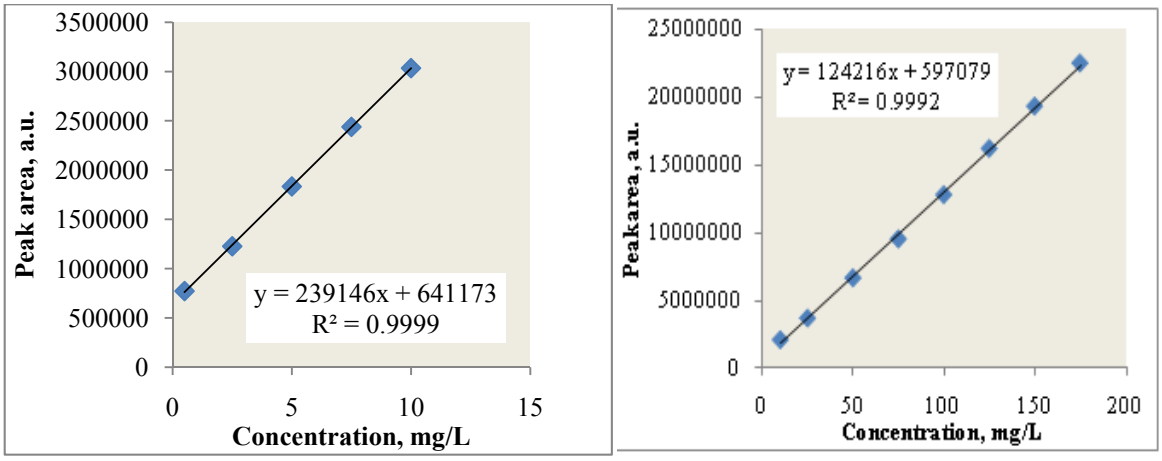


Figure 10: Calibration curve for acetic acid for lower and higher concentration

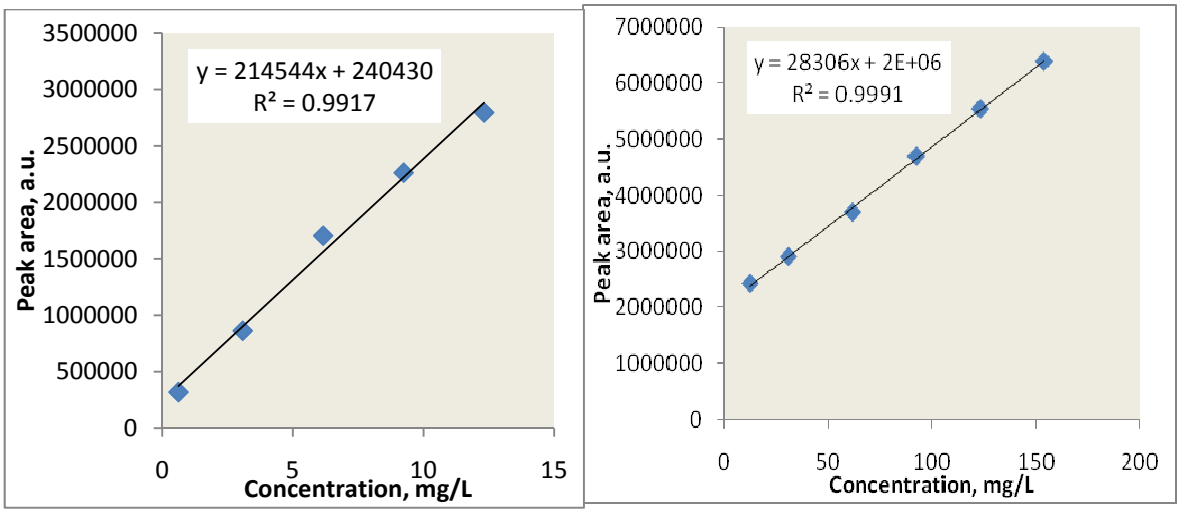
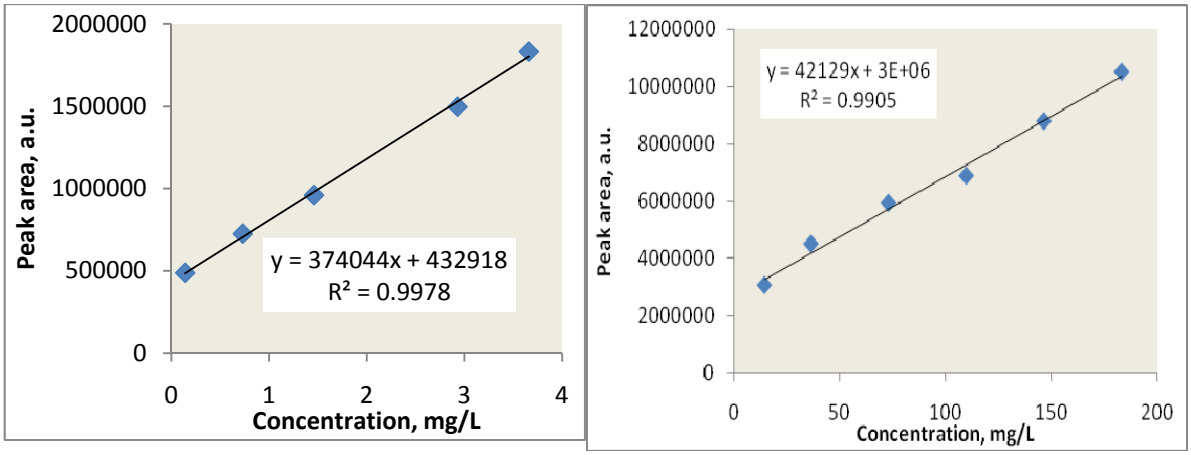


Figure 11: Calibration curve for propionic acid for lower and higher concentration



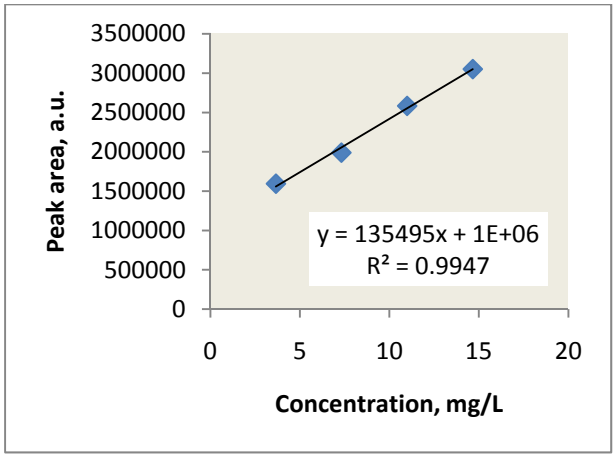
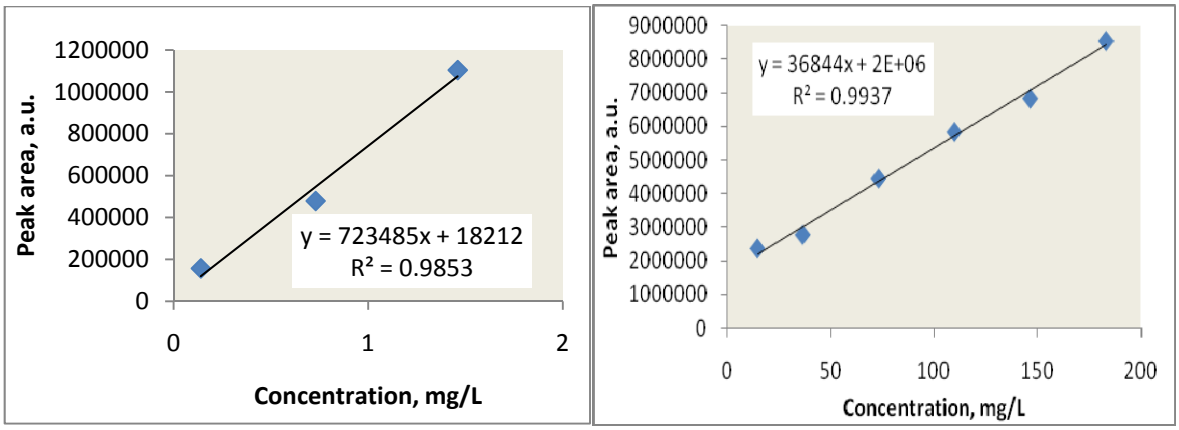


Figure 12: Calibration curve for butyric acid for lower and higher concentration



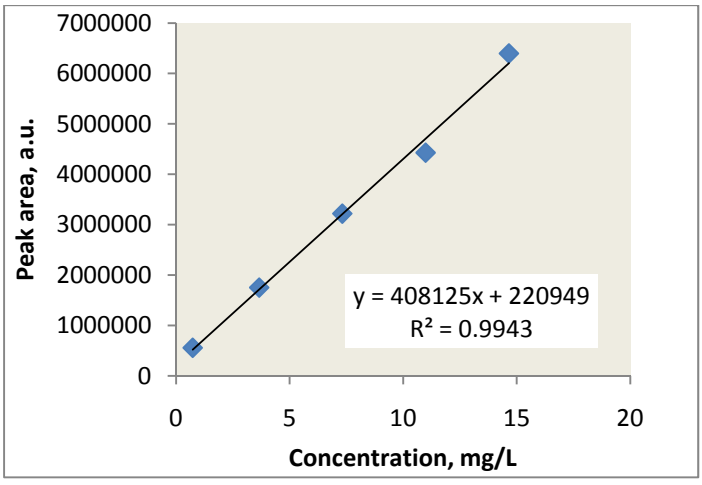


Figure 13: Calibration curve for iso-butyric acid for lower and higher concentration

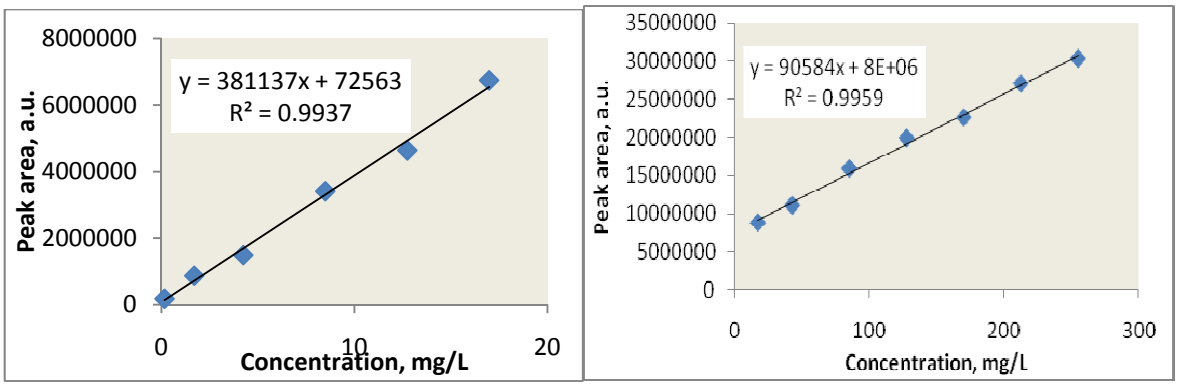


Figure 14: Calibration curve for valeric acid for lower and higher concentration

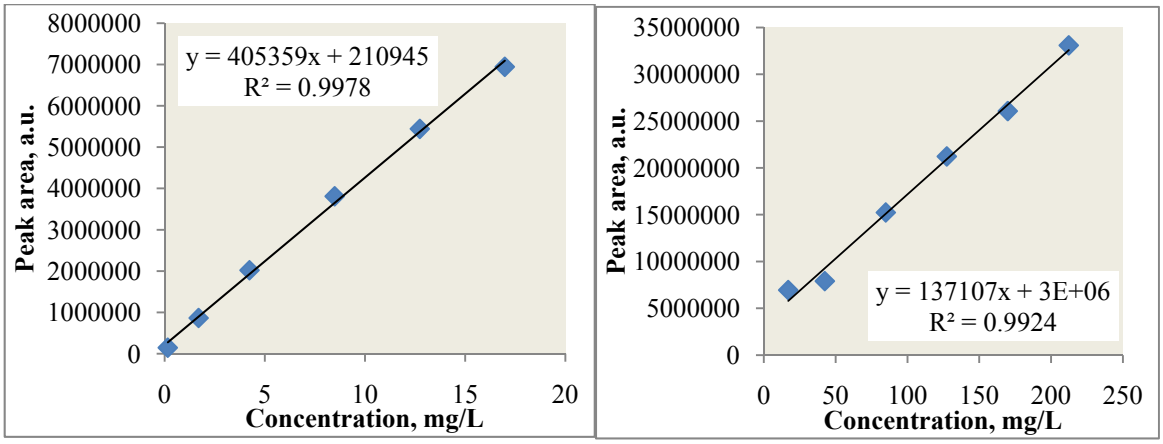


Figure 15: Calibration curve for iso-valeric acid for lower and higher concentration

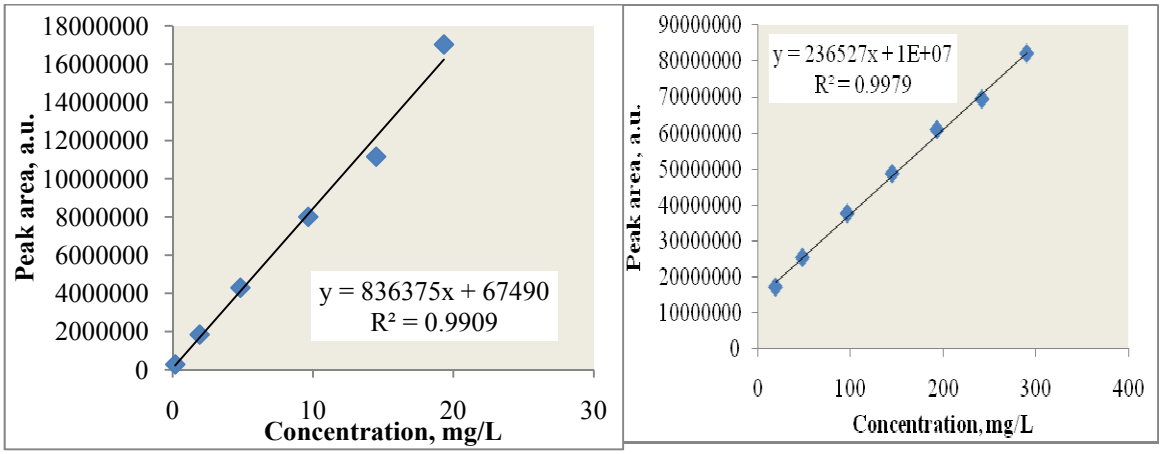


Figure 16: Calibration curve for iso-caproic acid for lower and higher concentration

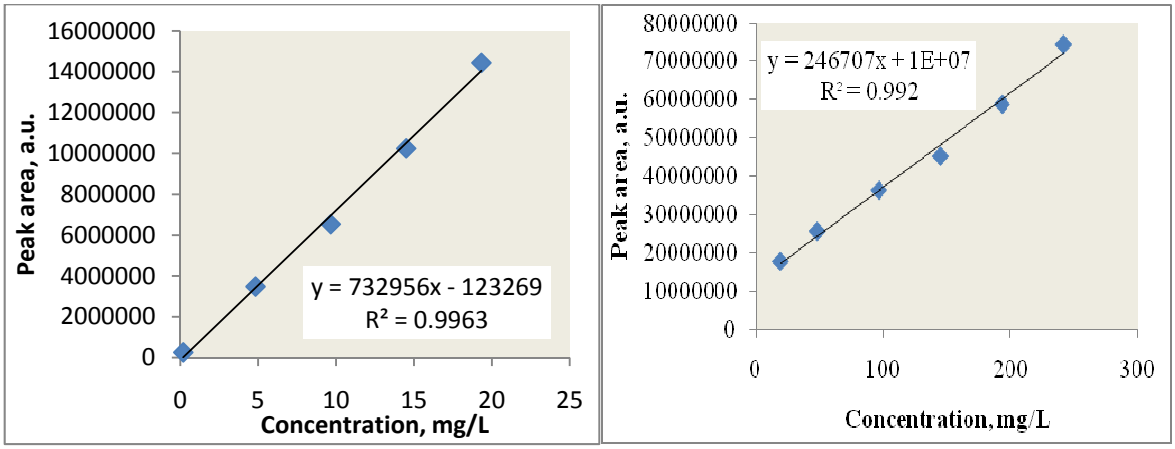


Figure 17: Calibration curve for caproic acid for lower and higher concentration

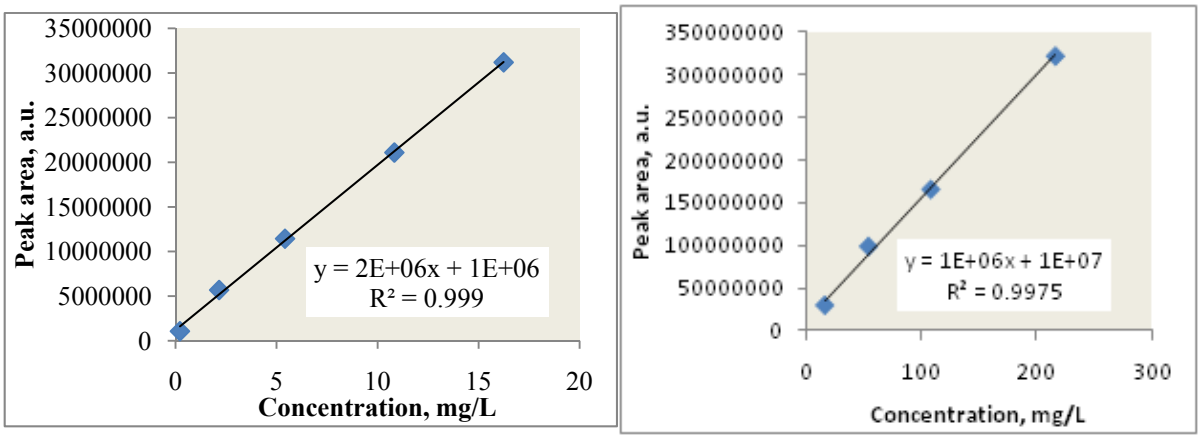


Figure 18: Calibration curve for enanthic acid for lower and higher concentration

5.3 Analysis of real municipal wastewater samples for VFAs content

Different wastewater samples were analyzed for the determination of VFAs content. The wastewater samples were treated wastewater, excessive sludge, pre concentrated sludge, raw sewage and wastewater after secondary settlement. The concentration of different VFAs were determined by plotting the peak area obtained in the calibration curve prepared for lower and higher concentrations which are tabulated in Table 7. The chromatograms of wastewater after secondary treatment, excessive sludge wastewater and raw wastewater samples are shown in Figure 20, 21 and 22 respectively. Acetic and propionic acids were found in higher concentration than other acids. This can be due to fact that lower molecular weight acids have higher affinity to adsorb to carboxen sorbent. The VFAs content in two different wastewater treatment plants were found similar.

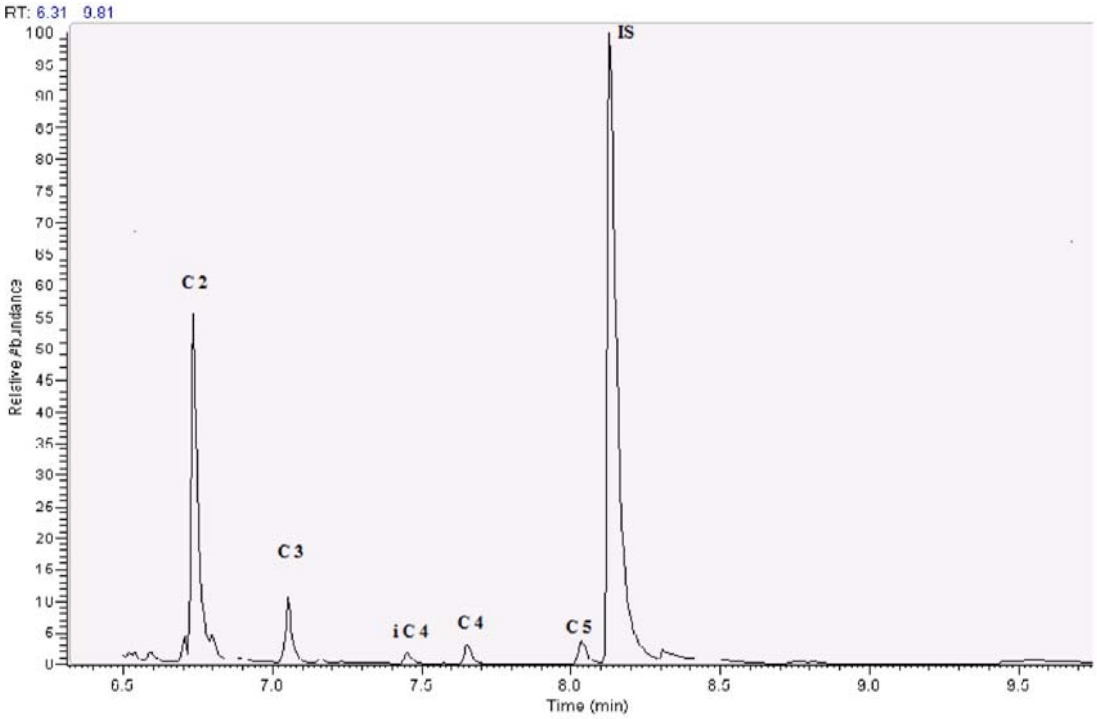


Figure 19: Chromatogram for wastewater after secondary settlement

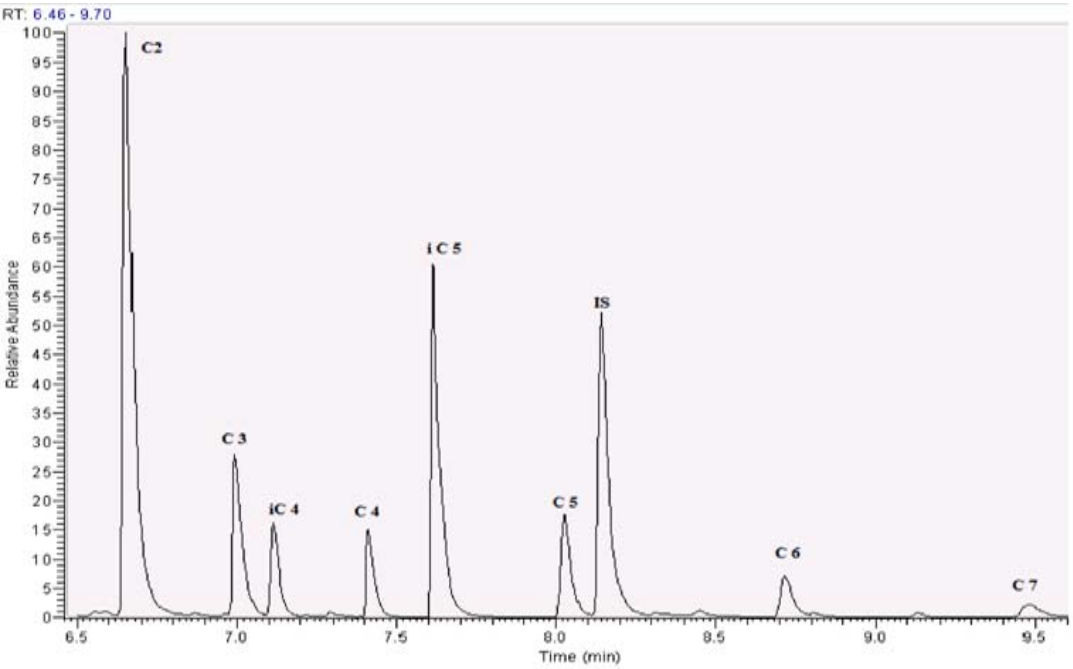


Figure 20: Chromatogram for excessive sludge wastewater

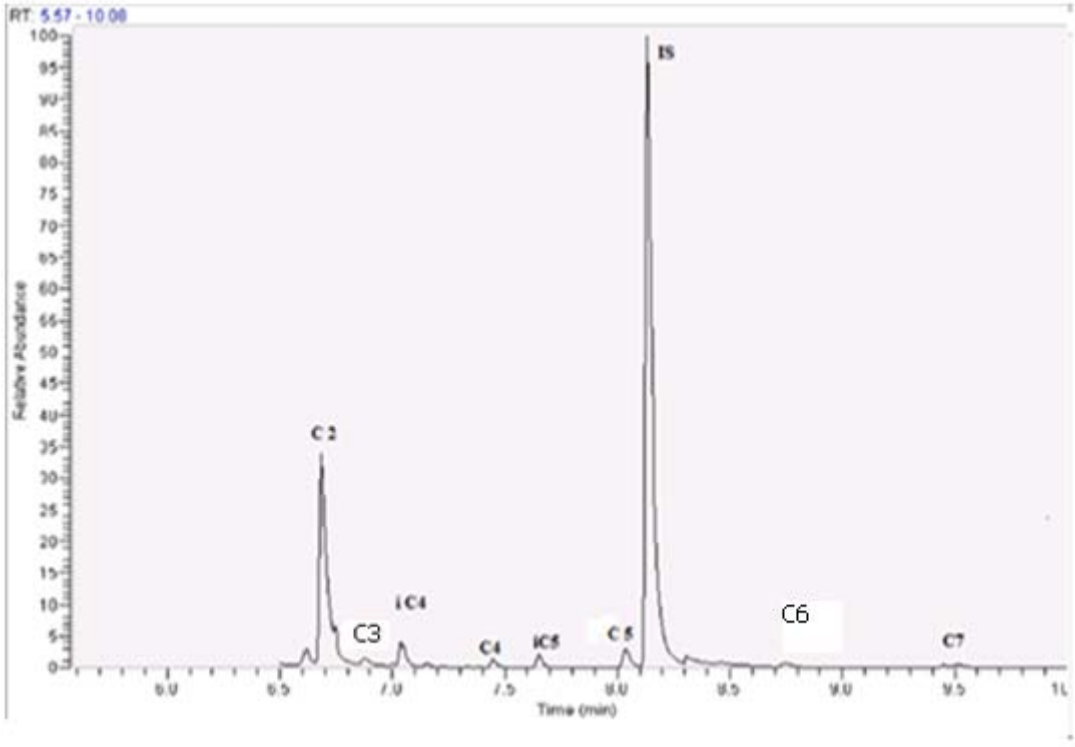


Figure 21: Chromatogram for raw wastewater

Table 6: VFAs content in real wastewater samples from Debogorze and Wschod Wastewater Treatment Plants

VFAs	Concentration of VFAs (mg/L)							
	Excessive sludge	Pre-concentrated sludge	Raw wastewater		Wastewater after secondary settlement		Treated water	
	Debogorze		Wschod	Debogorze	Wschod	Debogorze	Wschod	Debogorze
Acetic acid	304 - 309	256 - 259	55.8 - 56.7	44.7 - 45.8	69.8 - 72.9	64.2 - 66.7	< LOD	< LOD
Propionic acid	244 - 246	179 - 181	2.58 - 3.16	1.32 - 1.67	5.91 - 7.24	5.11 - 5.48	< LOD	< LOD
Butyric acid	9.27 - 10.30	4.78 – 5.09	1.89 - 2.56	1.17 - 1.62	1.94 – 2.67	1.26 – 1.89	ND	ND
Isobutyric acid	9.86 - 11.6	0.851 – 1.97	< LOD	< LOD	< LOD	< LOD	ND	ND
Valeric acid	16.3 - 17.5	11.4 – 13.7	< LOD	< LOD	1.87 - 2.49	1.38 – 2.06	ND	ND
Isovaleric acid	110 - 118	5.45 - 6.39	< LOD	< LOD	0.851 - 1.09	ND	ND	ND
Isocaproic acid	< LOD	< LOD	< LOD	ND	< LOD	ND	ND	ND
Caproic acid	3.72 - 4.48	< LOD	< LOD	< LOD	< LOD	ND	ND	ND
Enanthic acid	< LOD	< LOD	< LOD	< LOD	< LOD	ND	ND	ND

**ND: Not detected*

6. CONCLUSION

The headspace - solid phase micro extraction with PDMS-CAR SPME fibre combined with gas chromatography - mass spectrometry was successfully applied to determine VFAs in wastewaters. Chromatographic and extraction parameters affecting the method performance were optimized. Extraction was carried out for 40 min taking 2 mL of sample volume and 0.75 g of salt (NaCl). Desorption in the GC injection port was kept for 3 min at 300 ^{o}C. The LOD values ranged from 0.03 mg/L to 0.09 mg/L. PDMS-CAR fibre was found more suitable to extract lower chain fatty acids as compared to higher chain fatty acids. VFAs content in two different wastewater treatment plants were found similar. The optimized HS-SPME procedure using PDMS-CAR fibre was found to be reliable for isolation of VFAs from aqueous sample and monitoring of VFAs in municipal wastewater.

7. REFERENCES

1) N. Narkis, S. Henfeld-Furie, *Water Res.,* 12 (1978) 437

2) M. Abalos, J.M. Bayona, J. Pawliszyn, *J. Chromatogr. A,* 873 (2000) 107–115

3) M. Abalos, J.M. Bayona, *J. Chromatogr. A, 891* (2000) 287–294

4) S.P. Yo, *Chemosphere,* 38 (1999) 823

5) J.A. Cruwys, R.M. Dinsdale, F.R. Hawkes, D.L. Hawkes, *J. Chromatogr. A,* 945 (2002) 195–209

6) A. Tangerman, F.M. Nagengast*, Anal. Biochem.,* 236 (1996) 1

7) L.M.L. Nollet, *Chromatographic Analysis of the Environment*, CRC Taylor and Francis, (2006) 456-459

8) A. Chebbi and P. Carlier, *Atmos. Environ*, 30 (1996) 4233-4249

9) E.M. Thurman, *Organic Geochemistry of Natural Waters,* Kluwer Academic Publishers, 116-119

10) J.T.Bjork, K.H.Soergel, C.M.Wood, *Gastroenterology,* 70 (1976) 864

11) T. Nakae, J.A. Elliott, *J. Dairy Sci.,* 48, 3 (1965) 293-299

12) C.L Kien, R.D. Murray, A. Ailabouni, P. Powers, H. Brunengraber, *Journal of Nutrition,* 126 (1996) 3069–3076

13) S. Renou, J.G. Givaudan, S. Poulain, F. Dirassouyan, P. Moulin, *J. Hazard. Mater.,* 150 (2008) 468–493

14) J. Chanona, J. Ribes, A. Seco, J. Ferrer, *Water Res*., 40 (2006) 53 – 60

15) S. A. Parsons and J. A. Smith, *Elements* , 4 (2008) 109–112

16) L. Pan, M. Adams, J. Pawliszyn, *Anal. Chem.,* 67 (1995) 4396-4403

17) N. Buyukkamaci, A. Filibeli, *Process Biochemistry,* 39 (2004) 1491–1494

18) M.C. Wentzel, G.A. Ekama, G. Marais, *Water Sci. Technol*., 23 (1991) 555.

19) W. Janczukowicz, M. Dębowski, M. Zieliński, J. Pesta 2007, *Electronic Journal of Polish Agricultural Universities,* http://www.ejpau.media.pl/volume10/issue1/art-31.html

20) E. Lie, T. Welander, *Water Res.* 31 (1997) 1269-1274

21) J. Światłowska, A. Zaborowska, B. Zygmunt, *Analityka*, 2 (2006) 4

22) S. Mitra, *Sample Preparation Techniques in Analytical Chemistry*, John Wiley & Sons, Inc. (2003) 37-38

23) H. Lord, J. Pawliszyn, *J. Chromatogr. A,* 885 (2000) 153–193

24) C.L. Arthur, J. Pawliszyn, *Anal. Chem.,* 62 (1990) 2145

25) M. Chai, C. L. Arthur, J. Pawliszyn, R. P. Belardi, K. F. Pratt, *Analyst* 118 (1993) 1501-1505

26) Supelco, Inc., *Product literature* (2002), www.supelco.com

27) R. E. Shirey, *SPME fibers and selection for specific applications, in S. A.Scheppers Wercinski, ed., Solid Phase Microextraction: A Practical Guide,* Marcel Dekker, New York, (1999) 59–110

28) Supelco, Inc., *How to Choose the Proper SPME Fibre, product literature,* T499102, 1999/2000, www.supelco.com

29) M. Ormsby, *Journal of American Institute for Conservation,* 44 (2005) 13-26 http://cool.conservation-us.org/jaic/articles/jaic44-01-002.html

30) E. Psillakis, N. Kalogerakis, *J. Chromatogr. A,* 999 (2003) 145-153

31) J. S. Fritz and M. Macka, *J. Chromatogr. A,* 902 (2000) 137

32) N. H. Snow, *J. Chromatogr. A*, 885(2000) 445

33) J. Beltran, F. J. Lopez, and F. Hernandez, *J. Chromatogr. A*, 885 (2000) 389

34) G. Theodoridis, E. H. M. Koster, and G. J. de Jong, *J. Chromatogr. B,* 745 (2000) 49

35) E. Psillakis, N. Kalogerakis, *J. Chromatogr. A*, 907 (2001) 211-219

36) Z. Penton, *Method development with solid phase microextraction, in S. A. Scheppers Wercinski, ed., Solid Phase Microextraction: A Practical Guide,* Marcel Dekker, New York, (1999) 27–57

37) H.M. McNair, J.M. Miller, *Basic Gas Chromatography*, John Wiley & Sons, Inc, 1997

38) X. Yang, T. Peppard, *J. Agric. Food Chem.* 41 (1994) 1925-1930

39) C. Ortega, R. Lopez, J. Cacho, V. Ferreira, J. *Chromatogr. A,* 923 (2001) 205-214

40) G. Manni, F. Caron, *J. Chromatogr. A,* 690 (1995) 237

41) G. A. Mills, V. Walker and H. Mughal, *J. Chromatogr. B, 730* (1999) 113–122

42) A. Latorre, A. Rigol, S. Lacorte, D. Barcel*o, J. Chromatogr. A,* 991 (2003) 205

43) J. Jianguo, Y. Guodong, D. Zhou, H. Yunfeng, H. Zhonglin, F. Xiangming, Z. Shengyong, Z. Chaoping, *Waste Manage.,* 27 (2007) 893

44) R. He, D- s. Shen, J- q. Wang, Y- h. He, Y- m. Zhu, *Process Biochem., 40* (2005) 3660

45) S. Willing, M. Lacorn, R. Claus, *J. Chromatogr. A,* 1038 (2004) 11

46) L. Cai, J. A. Koziel, Yin-Cheung Lo, S. J. Hoff, *J. Chromatogr. A,* 1102 (2006) 60

47) L. Feng, Y. Huang, H. Wang, *J. Chromatogr. Sci.,*46 (2008) 577-584

48) J. Laretta, U. Bilbao, A. Vallejo, A. Usobiaga, G. Arana, O. Zuloaga, *Chromatographia,* 67 (2008) 93-99

49) P. Konieczka and J. Namiesnik, *Quality Assurance and Quality Control in the Analytical Laboratory*, CRC Press, (2009) 143-150