FATE OF NONYLPHENOL COMPOUNDS IN AEROBIC BATCH REACTORS

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SEÇİL ÖMEROĞLU

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submitted by **SEÇİL ÖMEROĞLU** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Göksel N. Demirer _____
Head of Department, **Environmental Engineering**

Prof. Dr. F. Dilek Sanin _____
Supervisor, **Environmental Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Celal. F. Gökçay _____
Environmental Engineering Dept., METU

Prof. Dr. F. Dilek Sanin _____
Environmental Engineering Dept., METU

Prof. Dr. Gürdal Tuncel _____
Environmental Engineering Dept., METU

Prof. Dr. Ufuk Alkan _____
Environmental Engineering Dept., Uludağ University

Assoc. Prof. Dr. İpek İmamoğlu _____
Environmental Engineering Dept., METU

Date : 28.05.2012

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Seçil Ömeroğlu

Signature:

ABSTRACT

FATE OF NONYLPHENOL COMPOUNDS IN AEROBIC BATCH REACTORS

Ömeroğlu, Seçil

MSc., Department of Environmental Engineering

Supervisor: Prof. Dr. F. Dilek Sanin

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Today, numerous studies indicate the presence of synthetic organics such as nonylphenol (NP) compounds in wastewater. NP compounds are a group of chemicals including nonylphenol, nonylphenol polyethoxylates (NPnEO) and nonylphenoxy polyethoxy acetic acids (NPnEC). Since NP compounds have significant industrial, commercial and domestic use, they enter environmental systems and reach human beings from various pathways. Their presence is of concern because they are toxic, carcinogenic and endocrine disrupting due to their ability to mimic oestrogen hormone.

The information available on the degradation of NPnEOs, is such that degradation starts with the reduction of ethylene oxide units, resulting in the formation of

nonylphenol, nonylphenol mono- or diethoxylate (NP1EO and NP2EO) and nonylphenoxy acetic acid (NP1EC). Although their fate during wastewater treatment was investigated in the past, not many research investigating their fate in sludge treatment can be found. Therefore, the objective of this study is first to come up with reliable extraction and measurement methods for NP compounds and then to investigate the fate of NP2EO in aerobic digesters.

After the development of techniques for the extraction and measurement of NP compounds, aerobic reactors spiked with NP2EO were operated. The samples were analyzed for solids content, COD, pH and NP compounds. The results showed that NP2EO degrades rapidly under aerobic conditions. As time proceeded, NP1EC formation was observed with the degradation of NP2EO, and NP1EC became the dominant specie. The solids concentration measurements showed that concentration of NP compounds did not affect the efficiency of aerobic digesters.

Key words: Aerobic digestion, nonylphenol, nonylphenol diethoxylate, nonylphenoxy acetic acid, sewage sludge

ÖZ

NONİLFENOL BİLEŞİKLERİNİN AEROBİK KESİKLİ REAKTÖRLERDEKİ AKIBETİ

Ömeroğlu, Seçil

Yüksek Lisans, Çevre Mühendisliği Bölümü

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Günümüzde birçok çalışma, atıksularda nonilfenol bileşikleri (NP) gibi sentetik organik kirleticilerin varlığına işaret etmektedir. NP bileşikleri, nonilfenol, nonilfenol polietoksilat (NPnEO) ve nonilfenoksi polietoksi asetik asitlerden meydana gelen bir grup kimyasaldır. Bu bileşikler çok yoğun endüstriyel, ticari ve evsel kullanıma sahip olduklarından doğaya karışır ve birçok farklı yolla insanoğluna erişirler. Toksik, kanserojen ve östrojen hormonunu taklit edebilmelerinden kaynaklanan endokrin bozucu özellikleri sebebiyle, doğadaki varlıkları endişe uyandırmaktadır.

NPnEO'ların parçalanmasına ilişkin bilgilere göre, parçalanma etilen oksit ünitelerinin indirgenmesi ile başlamaktadır ve bunun sonucunda nonilfenol, nonilfenol mono-/dietoksilat (NP1EO ve NP2EO) ve nonilfenoksi asetik asit

(NP1EC) oluşmaktadır. Bu bileşiklerin arıtma sistemlerindeki akıbetine ilişkin geçmişte çalışmalar yapılmış olmasına rağmen, çamur arıtma sistemlerindeki akıbetine ilişkin çok fazla bilgiye rastlanılmamaktadır. Buna binaen, bu çalışmanın amacı öncelikle NP bileşikleri için güvenilir ekstraksiyon ve ölçüm metotları geliştirmek ve daha sonra da NP2EO'nun aerobik çürütücülerde akıbetini incelemektir.

Ekstraksiyon ve ölçüm metotlarının geliştirilmesini takiben, NP2EO ile aşılanmış aerobik reaktörler işletilmiştir. Alınan numunelerde katı madde, KOİ, pH ve NP analizleri gerçekleştirilmiştir. Elde edilen sonuçlar incelendiğinde NP2EO'nun aerobik koşullar altında hızlı parçalanmaya uğradığı görülmüştür. Zaman ilerledikçe, NP2EO parçalanmış ve NP1EC oluşumu gözlenmiş ve NP1EC reaktör içerisindeki baskın madde haline gelmiştir. Katı madde giderim ölçümleri ortamdaki NP bileşiklerinin aerobik çürütücülerin verimini etkilemediklerini göstermiştir.

Anahtar Kelimeler: Aerobik çürütme, arıtma çamuru, nonilfenol, nonilfenol dietoksilat, nonilfenoksi asetik asit

To my family…

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LIST OF ABBREVIATIONS

BSTFA:	N,O-Bis(trimethylsilyltrifluoroacetamide)
CEPA:	Canadian Environmental Protection Agency
COD:	Chemical Oxygen Demand
DS:	Dissolved Solids
EDC:	Endocrine Disrupting Compound
EU:	European Union
EC:	European Commission
GC:	Gas Chromatography
GC/MS:	Gas Chromatography-Mass Spectrometry
JEA:	Japan Environmental Agency
LOD:	Limit of Detection
LOQ:	Limit of Quantification
MS:	Mass Spectrometry
NP:	Nonylphenol
NP1EC:	Nonylphenoxy acetic acid
NP2EC:	Nonylphenoxyethoxy acetic acid
NP1EO:	Nonylphenol monoethoxylate
NP2EO:	Nonylphenol diethoxylate

OSPAR:	Oslo and Paris Commission
QA/QC:	Quality Assurance/Quality Control
%RSD:	Relative Standard Deviation
S/N:	Signal to noise
TIC:	Total Ion Chromatogram
TMCS:	Trimethylchlorosilane
TS:	Total Solids
TSS:	Total Suspended Solids
UK:	United Kingdom
UKEA:	United Kingdom Environmental Agency
US:	United States
USEPA:	United States Environmental Protection Agency
VS:	Volatile Solids
VSS:	Volatile Suspended Solids
WAS:	Waste Activated Sludge

CHAPTER 1

INTRODUCTION

Nowadays, xenobiotic substances are of great concern due to the adverse effects they create on environment and human beings. Xenobiotic substances are not produced naturally, but rather produced by anthropogenic activities. These synthetic compounds are generally resistant to degradation and that's why they can be easily detected in wastewater and sewage sludge. Alkylphenols are a group of synthetic organic chemicals that are used in many industrial and commercial formulations. Nonylphenols (NP) and nonylphenol polyethoxylates (NPnEOs) are members of the alkylphenol family and they form about 80% of this group (Ying et.al., 2002). Due to their surface active properties, nonylphenols are known to be excellent detergents, surface cleaners and lubricants. They have extensive industrial, commercial and domestic use. Industrial and commercial production/consumption of NP compounds include uses in wetting and dispersing agents, pulp and paper production, textile production, metal finishing, etc. (CEPA, 1999). Even though in most of the countries NP in domestic detergents has been banned, NPs still exist in personal care products such as shampoos, anti-wrinkle creams, moisturizers and deodorants. Their use in these personal care products makes direct human contact with these compounds possible. Since they have widespread use, they eventually end up in the wastewater treatment systems and environment. Most of the NP compounds are not fully degraded in the wastewater treatment systems. Therefore, once they are discharged into the environment via treatment plant effluents, it is inevitable that they reach soils, sediments, surface waters, etc. Many of the research showed their presence in environmental systems at a range of concentrations. Although these concentrations do not seem high, their effects are severe.

NP compounds have some characteristics that make them popularly investigated among scientists. These compounds are known to be toxic, even at short term exposures they cause adverse effects such as skin/eye irritation, respiratory diseases, or damage in vocal cords (Cox, 1996). NP compounds are also carcinogenic. This fact was accidentally discovered in a study carried out on human breast cancer cells, where NP compounds leaking from the material of plastic tubes resulted in the multiplication of cancer cells abruptly (Soto et. al., 1991). Actually, the main reason behind this is the oestrogenic property of NPs, specifically, due to the structural similarity between NP and 17-β-ostradiol. For this reason, NP compounds can mimic the oestrogen hormone (Warhaust, 1995). Compounds that can mimic natural hormones are named as endocrine disrupting compounds (EDCs). By mimicking hormones they interfere with the endocrine system and result in the malfunctioning of hormones (Holbrook et.al., 2002 and Soares et.al., 2008). So the carcinogenicity of NP compounds is linked with their endocrine disrupting property and this characteristic possibly is the one that creates the highest concern for these chemicals. In addition, these compounds are very persistent, hydrophobic and lipophilic, meaning if they are carried along the food chain they will accumulate in the fat tissues of living organisms (Cox, 1996 and Soares et.al., 2008). NP compounds have high biconcentration factors measured between 3 and 10,000 (Cox, 1996).

As underlined before, these compounds are extensively used and discharged, reaching treatment systems. Since they are resistant to degradation and hydrophobic, NPs tend to accumulate on soil, sediment and sewage sludge. Many studies showed the presence of NP compounds in environmental systems and wastewater (Ahel et.al., 1994; Corsi et.al., 2003; Soares et.al., 2008; Ying et.al., 2002). Even if they degrade in wastewater treatment, they lead to metabolites some of which are even more hazardous and persistent. A full understanding of their transformation and fate in environmental systems is critically important.

NP compounds are generally found in the form of polyethoxylates in detergents and similar formulations. The limited information on the biodegradation pathway of NP

compounds in environment and wastewater treatments systems suggest; degradation starts with the shortening of the ethylene oxide chain of nonylphenol polyethoxylates (NPnEO). Once the ethylene oxide unit is reduced, NPnEOs are converted into NP2EO, NP1EO and NP. However, the degradation pathway differs under aerobic and anaerobic conditions. Under aerobic conditions, biodegradation is much faster and there is an extra metabolite formation which is nonylphenoxypolyethoxy acetic acid (NPnECs) in addition to NP, NP1EO and NP2EO. Whereas, under anaerobic conditions the rate of degradation is slower and there is no such metabolite formation such as NPnECs (Ahel et.al.,1994; CEPA, 1999;Soares et.al., 2008).

The scientific findings and concerns about NP compounds have alarmed the governments to take some preventive measures considering the environmental levels given by studies. First measure on NP compounds was the ban on the use of NPs in detergents in 1976, in United Kingdom (La Guardia et.al., 2003). Following this, many countries started to limit the concentration of these compounds in industrial formulations. These substances were included in the hazardous substances list of many commissions (i.e. EU and OSPAR) and banned in most of the European countries. Even though most countries banned their use, these substances are still used in South American and Asian countries.

The problem with NP compounds does not end with the ban on their use. As stated before, these compounds accumulate on organic phase including sewage sludge. So once sewage sludge is used for beneficial purposes in agriculture, these chemicals rise in the food chain easily. So, prior to the application of sewage sludge to soil, NP compounds must satisfy the limits stated by the regulations. The current limit value in many countries is set for the sum of NP, NP1EO and NP2EO, represented as NPE. European Union has set the limit as 50 mg/kg NPE on dry mass basis in their Working Document on Sludge, 3rd Draft, and this value has been internalized by most of the member countries with few exceptions. In Turkey, the limit value is also stated as 50 mg/kg NPE on dry mass basis in the Regulation on Land Use of Domestic and Urban Sludges (August, 2010). Both the health concerns and the

issued regulations make it critical to correctly measure concentration of NPE and control their levels in the environment.

The motivation of this study is the combination of what has been discussed so far. These compounds have very important properties: toxic, carcinogenic and endocrine disrupting. They are found in environmental systems due to their extensive use. Sewage sludge which is a by-product of wastewater treatment can most easily be disposed by agricultural applications. When NP concentrations are beyond the limits enforced, sludge becomes inappropriate for land use. Therefore, their degradation levels should be investigated in details so that actions can be taken. Unfortunately, information on degradation pathways is limited. The motivation of the study is to fill this gap in the literature and analyze the degradation kinetics under aerobic digestion conditions. The objective of this study is to develop reliable methods of extraction and measurement of these compounds in sludge and monitor the degradation of NP2EO in laboratory scale aerobic batch reactors. 3.2 L reactors dosed with 3 mg/L NP2EO were analyzed both in solid and liquid phases. Besides, solids content, chemical oxygen demand (COD) and pH were monitored. The NP compounds analyzed were: NP, NP1EO, NP2EO and NP1EC. The analyses were carried out using GC-MS.

CHAPTER 2

LITERATURE SURVEY

2.1 Sludge

Sludge (biosolid) is a semi-solid material produced as a residual during wastewater treatment. Sludge is a by-product that needs to be disposed into the environment properly. The reuse of sludge seems sustainable, however this issue is not simple as it seems since sludge contains harmful substances like toxicants and synthetic organic chemicals together with the beneficial ingredients like nutrients and organics.

Quality and quantity of sludge is directly related with the type/level of treatment applied. For instance, with the increasing levels of treatment of wastewater the amount of sludge produced is multiplied. Or, if chemical treatment is applied rather than biological, together with the increasing amount of sludge, the constituents also vary. Today, amount of sludge produced across the world is increasing rapidly due to the increase in population, in other words, increase in the boundaries of sewer network. Therefore, sludge needs to be properly managed in order to avoid the harm it may create (Sanin et.al., 2011).

As stated at the very beginning, sludge needs to be properly disposed into environment due to some unwanted properties. Sludge is unaesthetic, contains pathogens therefore poses danger to human health and contains moisture. Aesthetic

concerns and pathogenic content can be solved via stabilization and water content can be reduced by thickening and dewatering of sludge before disposal (Weiner and Matthew, 2003).

The aim of stabilizing the sludge is to reduce the putrefaction and odor problems together with the pathogens so that it won't pose a risk to environment and human health. Stabilization of sludge can be achieved by three main approaches:

- Physical stabilization: Pasteurization (stabilization via heat), irradiation
- Chemical stabilization: stabilization via the use of chemicals such as lime ($Ca(OH)_2$ or CaO). The aim is to reduce the pathogens and related hazards by increasing the pH up to 11. However this is a temporary solution to the problem since as soon as the pH falls the microorganisms may flourish again.
- Biological stabilization: Examples of this stabilization method includes anaerobic and aerobic digestion, and composting (Sanin et.al., 2011). Since this study involves aerobic stabilization, the brief description of this process is given in the section 2.2.

2.2 Aerobic Digestion of Sludge

Even though the volume of sludge in a wastewater treatment plant makes up maximum 3% of the wastewater treated, due to its unappealing properties, sludge creates as much concern as wastewater does (Hartman et.al., 1979). As mentioned before, removal of the putrescible and odorous portion of the sludge is the aim of sludge stabilization. One of the methods is aerobic digestion which is like an extension of the activated sludge treatment used for sludge stabilization (Benefield and Randall, 1978). Aerobic digestion is generally applied for small communities due to extensive energy requirements along continuous operation (Koers and Mavinic, 1977).

But when compared to anaerobic digestion, aerobic digestion:

- Consumes organic portion of the sludge much more efficiently resulting in a highly oxidized product,
- Is less sensitive to operational conditions
- Produces extensively nitrified sludge which is suitable for land application.

Although anaerobic digestion is not energy intensive like aerobic digestion, it produces a supernatant that contains concentrated nutrients and organics since all the organic material is solubilized for methanogens. Also it is very sensitive to environmental conditions. For these reasons, aerobic digestion seems better in terms of operation (Rein, 1977 and Benefield and Randall, 1978).

Once sewage sludge enters aerobic digestion, the microorganisms present in the reactor attack organics. They consume the organic matter in order to produce new cells (biomass) and energy for their metabolism (Benefield and Randall, 1978). The reaction proceeds as follows:

$$\text{Organic Matter} + O_2 + NH_3 \rightarrow \text{Biomass} + CO_2 + H_2O \qquad \text{……. Reaction (1)}$$

If the amount of organics is vast, Reaction (1) proceeds smoothly since the microbial activity is at maximum. But as time goes by, organic matter will be oxidized and microorganisms follow declining growth meaning microbial activity is below maximum. Therefore, to satisfy the energy requirement for their metabolism, microorganisms start to oxidize their own cellular material. In other words, once the organic matter is depleted at maximum, endogenous respiration takes place.

Then Reaction (1) becomes (Kim and Hao, 1990):

$C_5H_7NO_2 + 5O_2 \rightarrow 4CO_2 + NH_4HCO_3 + H_2O$ ……. Reaction (2)

$C_5H_7NO_2$ in Reaction (2) represents the biomass. Ammonia formed in the reaction above is then converted into NO_3^- by the nitrifying organisms (Mavinic and Koers, 1979):

$C_5H_7NO_2 + O_2 \rightarrow 5CO_2 + 3H_2O + H^+ + NO_3^-$ ……. Reaction (3)

Once these reactions are completed, the rate of microbial activity is minimized and the sludge becomes biologically stable.

Aerobic digesters can be continuous, semi-continuous (fill and draw) or batch. In batch systems, since there is no entrance and exit, the amount of organic matter can only change with the microbial activity. This is best represented by the equation below:

$dC/dt = -k_d \cdot t$ ……..…….. Equation (1)

where,

dC/dt: rate of change of degradable organic matter with respect to time

C: concentration of degradable organic matter

t: time

k_d: decay coefficient (first order)

Equation (1) can be also represented as:

$C_t/C_0 = e^{-k_d.t}$ …… Equation (2)

where,

C_t: concentration of degradable organic matter at time t

C_0: initial concentration of degradable organic matter

As can be understood from Equation (1) and (2), the amount of degradable organics, in other words degradable solids, within a batch aerobic reactor will follow an exponential decay until it reaches a stable state.

The objective of aerobic digestion is to produce a more stabilized, smaller volume (reduced solids content) and good settling sludge. It is easier in operation when compared to anaerobic systems, but it is affected by certain parameters:

- **Temperature:** Temperature and decay rate are proportional to each other. As temperature rises, the decay rate also increases. This is best shown by the Arhenius Equation:

  $k_{d,1} = k_{d,2}\Theta^{(T1-T2)}$ …….. Equation (3)

  where,

  $k_{d,1}$ and $k_{d,2}$: the decay rate coefficients at temperatures T_1 and T_2

  T_1 and T_2: tempearature (°C)

  Θ: thermal coefficient

However, the increase in decay rate coefficients does not infinitely increase with increasing temperature. In the study carried out by Hartman et.al., 1979, it has been shown that the maximum decay rate was observed at 30°C but at temperatures lower/greater than 30°C, the rate constant dropped.

- **Solids Concentration:** Solids concentration within a digester also affects the efficiency of stabilization. According to Rein, 1977, the amount of solids by weight affects the type of microorganism and as a result the extent of digestion. The results of the study indicate that certain microorganisms do not develop until the optimum solids concentration is reached and therefore the efficiency will decrease. For instance, according to Ganczarczyk et.al., 1980, the degree of decomposition increases with increasing solids concentration.
- **pH:** pH is also an important parameter affecting the stabilization efficiency. During aerobic digestion, as ammonia is produced and nitrified, the pH will decreases together with alkalinity. If pH is kept around neutral (6-8), the digesters will work at better efficiencies (Anderson and Mavinic, 1984). Therefore, pH controlled digesters will result in higher degrees of stabilization.
- **Dissolved Oxygen (DO):** As the name implies, aeration is vital in aerobic digestion process. Generally it is recommended to keep the DO level greater than 2-3 mg/L for microbial activity to proceed. Beyond this DO, the microorganisms necessary for stabilization will not flourish (Rein, 1977 and Koers and Mavinic, 1977).

In addition to the factors listed above, sludge age, type of microorganisms and sludge and mixing conditions also affect the performance of aerobic digestion. Generally the degree of solids reduction expected in an aerobic digester is around 40-60% (Bernard and Gray, 2000, Rein, 1977 and Sanin et.al., 2011). But this number may change depending on the composition of sludge together with other parameters mentioned.

2.3 Description of Nonylphenol Compounds

Nonylphenol compounds (NP, NPnEO and NPnECs) belong to a larger group of non-ionic surfactants named as alklyphenol compounds. Their consumption in industrial and commercial formulations is vast; therefore their releases into the natural and engineered systems are high. These compounds can degrade in nature to a certain extent; however, most metabolites are even more dangerous than the parent compound. For instance, nonylphenol itself is a breakdown product of higher ethoxylated forms and a highly lipophilic compound leading to its persistence and bioconcentration abilities in organisms. As a group, nonylphenol compounds are toxic to most organisms, persistent in nature, bioaccumulate and carcinogenic for humans, additionally they are endocrine disrupting, in other words they can mimic hormones and interfere with the proper functioning of the endocrine system. For this reason, great concern has been raised over their production, consumption and presence in the environment (Warhurst, 1995).

2.3.1 Molecular Structures of Nonylphenol Compounds

In this study, under the name of nonylphenol compounds, four different chemicals have been investigated. These can be listed as: nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO) and nonylphenoxy acetic acid (NP1EC).

Nonylphenol ethoxylates are a part of alkylphenol ethoxylates, representing approximately 80% of this group of chemicals (USEPA, 2010). NPnEOs consist of a nonyl chain attached to a phenol ring, but coupled with an ether linkage with ethylene oxide units as shown in Figure 2.1. NPnEO is described by the number of its ethylene oxide units (length of ethylene oxide chain). The general formula of NPnEOs is $C_{15}H_{24}O(C_2H_4)_n$, where n indicates the number of ethylene oxide units.

For instance, NP2EO has two ethylene oxide units and its formula is $C_{15}H_{24}O(C_2H_4)_2$.

NP is one of the last metabolites of NPnEOs. It is formed from the attachment of straight or branched nonyl group to a phenol ring as shown in Figure 2.2. The reason for naming these chemical as nonylphenols (NPs, in plural) is that they are formed by a number of different isomers. NPs all have the same general formula $C_{15}H_{24}O$. Commercially produced NPs are mostly composed of branched nonyl groups whereas straight chain forms are less likely to be found. Isomeric structure depends on the point of attachment of the nonyl group as well as the structure of branching (CEPA, 1999 and Nielsen et.al., 2000).

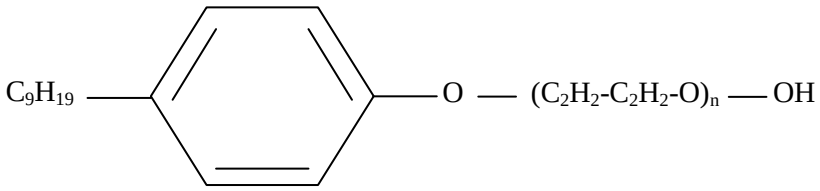


Figure 2.1 Molecular structure of NPnEO

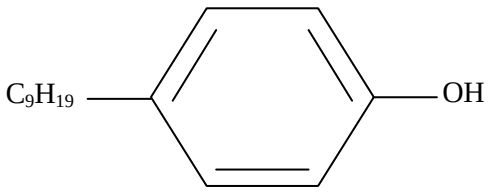


Figure 2.2 Molecular structure of NP

The last group of chemicals governed under the scope of this study is nonylphenoxypolyethoxy acetic acids (NPnECs). These compounds are formed via

the oxidation of the ethylene oxide group of NPnEOs and the structure is given in Figure 2.3.

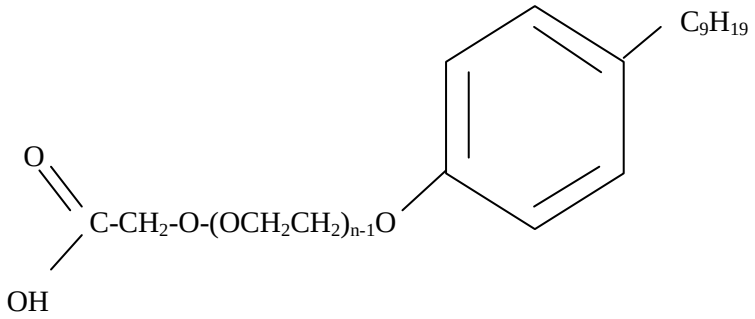


Figure 2.3 Molecular structure of NPnEC

The letter "n" in NPnEC refers to the number of ethoxy chain associated with one acetate group and identifies the type (Warhurst, 1995).

2.3.2 Sources of Nonylphenol Compounds

NP compounds have no known natural sources, meaning that these compounds are released into the environment via anthropogenic activities (CEPA, 1999). As a whole, alkylphenol ethoxylates are the most commonly used group of non-ionic surfactants and nonylphenol ethoxylates constitute almost 80% of the broad group (Nielsen et.al., 2000 and Ying et.al., 2002). Since 1940, when NP was synthesized for the first time, the production and consumption of these compounds have been increasing (Soares et.al., 2008). Industrial, commercial and even domestic applications have been developed for them. Industrial and commercial uses govern the manufacture of antioxidants, wetting/dispersing agents, demulsifiers, lubrication, paints/dyes, metal finishing and pulp and paper production (Birkett and Lester, 2003; Soares et.al.,2008; CEPA, 1999). The domestic use of nonylphenol compounds are

mostly in household detergents, deodorants, hair products, moisturizers, make up and many other personal care products (CEPA, 1999; Birkett and Lester, 2003). These domestic products may contain NP and related compounds in the range of concentration as small as 0.2-3% or as high as 30-100%. Through the use of these cosmetic or cleaning products, direct human exposure to these chemicals becomes possible (CEPA, 1999).

Industrial applications (i.e. pulp and paper, textile, metal, etc.) form approximately 55% of the total market; the rest 30 and 15% being the industrial and institutional cleaning products and domestic applications, respectively (Ying et.al., 2002). The annual production increased recently and exceeded approximately 154,200 tons in United States, 73,500 tons across the Europe and 16,500 tons in Japan (Soares et.al., 2008). Since most of these products are eventually discharged to treatment works and are not degraded fully, they find their way to reach different sectors of the environment. The discharge of treated effluent into receiving water bodies will introduce these partially degraded compounds into the aquatic life and application of sludge in agriculture will result in their presence in the terrestrial environment (Birkett and Lester, 2003).

2.3.3 Physicochemical Properties of Nonylphenol Compounds

The fate and existence of NP compounds are governed by their physical and chemical properties. Physicochemical properties are the main reason behind their persistency and bioaccumulation. Therefore, before describing their fate and degradation pathways, physicochemical properties must be examined first (Birkett and Lester, 2003). Unfortunately, number physicochemical properties are not available in literature.

Some physicochemical properties of interest NP and NPnEO are given in Table 2.1. But since there is not much physicochemical data available, especially for nonylphenol higher ethoxylates, the ones given for NP1EO, NP2EO and NP4EO will be assumed to be representative of the whole family (CEPA, 1999). The physicochemical properties of NPnEOs depend on the number of ethylene oxide units. As can be seen from Table 2.1 and Figure 2.4, solubility increases with increasing number of ethylene oxide units. This is due to the presence of hydrophilic moiety of NPnEOs which is the ethylene oxide unit. Therefore, as the length of hydrophilic part increases, in other words when the number of ethylene oxide units increases, solubility also rises. These compounds are said to have higher solubility once the number of ethylene oxide unit is above 6 (Nielsen et.al., 2000). There is not much data on the Henry's constant of these chemicals, but they are also not detected in air, therefore, it can be concluded that Henry's constant and vapor pressure values are small for NPnEOs (CEPA, 1999).

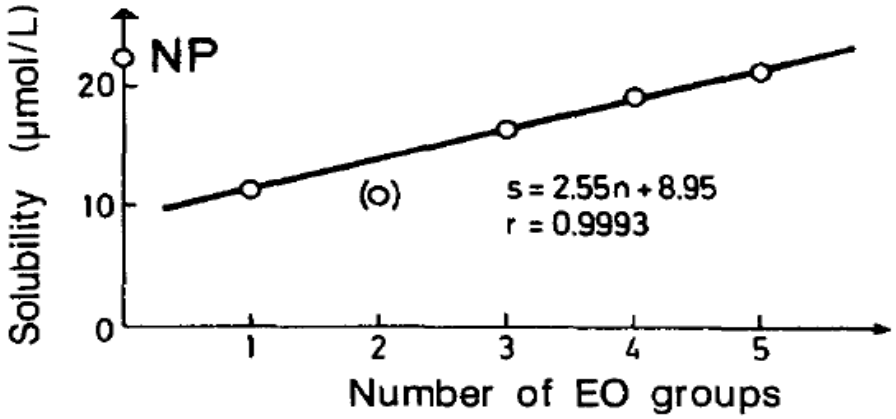


Figure 2.4 Correlation between the aqueous solubility of NPnEOs and the number of ethylene oxide units (Ahel and Giger, 1993a)

As can be seen in Table 2.1, solubility of NP is not high and can be said to be practically insoluble due to presence of hydrophobic structure (Nielsen et.al., 2000) and tends to be in the undissociated form in water (CEPA, 1999). NP, generally tends

to stay on sediment surfaces due to this low solubility and high octanol/water partition coefficient (K_{ow}). Due to its small Henry's constant, NPs do not tend to stay airborne and it has not been measured in atmosphere so far (Nielsen et.al., 2000). NPnEOs show similar physicochemical properties when compared to NPs, they are also not airborne and tend to stick on organic surfaces due to their high K_{ow} values. The only difference is that these properties vary numerically when the number of ethylene oxide unit changes as stated before. As can be seen in Table 2.1, as the ethylene oxide unit increases in number, solubility also increases from 3.38 to 7.65 mg/L.

The carboxylated metabolites of NP, which are NPnECs, are found mostly in ionized form in the water due to their pKa values around 5.12. The octanol/water partition coefficients of these metabolites are lower than those of NP or NPnEO, therefore, these compounds tend to stay in aqueous phase not in sedimentary phase or biota (CEPA, 1999).

Table 2.1. Physicochemical properties of NP and some NPnEOs

	NP	NP1EO	NP2EO	NP4EO
Synonyms	4-nonylphenol, p-nonylphenol, monoalkylphenol[5]	2-(2-nonylphenoxy)ethanol[4]	2-[2-(4-nonylphenoxy)ethoxy]ethanol[4]	Nonoxynol-4[3]
CAS Number	84852-15-3	104-35-8 or 27986-36-3[5]	20427-84-3 or 27176-93-8[5]	7311-27-5[5]
Molecular Formula	$C_{15}H_{24}O$	$C_{15}H_{24}O(C_2H_4)$	$C_{15}H_{24}O(C_2H_4)_2$	$C_{15}H_{24}O(C_2H_4)_4$
Molecular Weight (g/mole)	220.3	264.4	308.5	396.2
LogKow	4.48[2]	4.17[2]	4.21[2]	4.3[2]
Solubility (mg/L) at 20.5 °C	5.43[1]	3.02[1]	3.38[1]	7.65[1]
Henry's Constant ($Pa.m^3/mole$)	1.02[5]	-	-	-

1. Ahel and Giger 1993a
2. Ahel and Giger 1993b
3. CEPA, 1999
4. Chemindustry, 2012
5. Nielsen et.al., 2000

2.4 Effects of Nonylphenol Compounds on Living Organisms

NP compounds are toxic xenobiotic compounds (xeno means “strange” in Greek and biotic means “related to living organisms”, therefore xenobiotic means foreign to living organisms) that have extensive use and production, therefore introduced into environment through many pathways (Ying et.al. 2002, Soares et.al., 2008). As stated before, NPnEOs are the most commonly used group of surfactants and once they are discharged into the environment they degrade into either shorter chain ethoxylates or more persistent metabolites such as NP and carboxylated forms (Ying et al. 2002). As a whole family, NP compounds are known to be toxic to living organisms, but just like physicochemical properties, toxicity of ethoxylated forms also change with the number of ethylene oxide units, but this time as the number of ethylene oxide units increases, toxicity decreases. Therefore, toxicity is inversely proportional to the length hydrophilic structure and it is correct to say that NP is more toxic than NPnEOs (Warhaust, 1995). Even in short term exposures severe effects can be observed. For instance, if NP is somehow swallowed or inhaled, skin, eye or even severe respiratory irritation occurs. These effects have been tested on rabbits and guinea pigs and by which they are proven (Cox, 1996).

Beyond toxicity, NP compounds have one important feature that brings them to the top of latest concerns in scientific world. This is their endocrine disrupting property. NP compounds are listed among a group of chemicals named as endocrine disrupting compounds/chemicals (EDCs) (Gonzalez et.al., 2010). EDC is an emerging concept and has many definitions declared by different institutions. For instance, the definition that Kavlock et.al., 1996 holds forth is:

“An endocrine disruptor is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior”

Another definition is given by European Workshop (European Commission, 1997) as:

"An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function."

There are other definitions on EDCs and all of them point out the same concerns actually. Simply, EDCs are chemicals with the ability to mimic hormones and unfortunately interfere with the proper functioning of endocrine system (Holbrook et.al., 2002 and Soares et.al., 2008). Since endocrine system is responsible for the functioning of the body in terms of growth, reproduction and maintenance, once EDCs step in, they directly attack the hormone receptors. EDCs can either bind to the hormone receptor to directly mimic the hormone, or they just bind but do not activate the hormone and produce no response. First case is defined as agonistic effect and the second one is antagonistic effect (Birkett and Lester, 2003). The effect of EDCs on hormone receptors is shown in Figure 2.5.

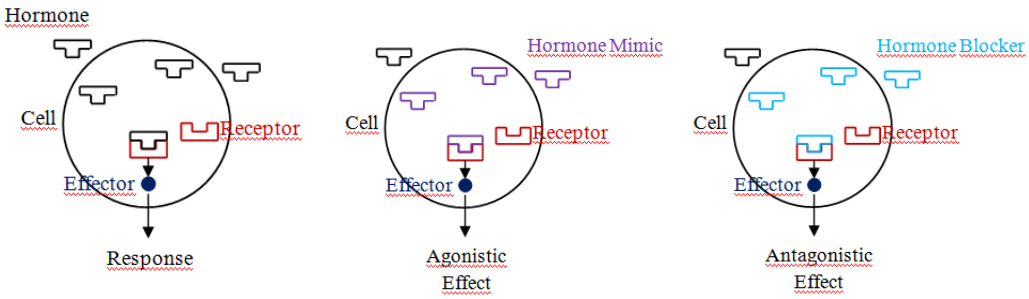
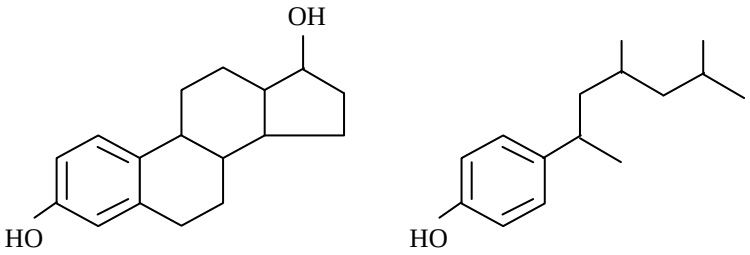


Figure 2.5 Natural hormone mechanism and endocrine disruption (Birkett and Lester, 2003)

Alkylphenol ethoxylates and their metabolites, including NP compounds, are known to show oestrogenic effect since 1940s (Ashfield et.al., 1998 and Warhaust, 1995). How NP mimics oestradiol, the hormone that regulates female sex characteristics, can be best demonstrated with structural similarity as given in Figure 2.6 (Warhaust, 1995). NP has been identified as EDC by many organizations such as UK Environmental Agency (UKEA), USEPA, Oslo and Paris Commission (OSPAR), Japan Environmental Agency (JEA) and World Wildlife Foundation (WWF) (Birkett and Lester, 2003).



(i) (ii)

Figure 2.6 Structural similartity between (i) 17-β-oestradiol and (ii) 4-nonylphenol (Warhaust, 1995)

Since NP compounds can mimic the sex hormone oestradiol, the adverse effects of these interference is observed as reproductive diseases. For instance, according to the tests conducted on laboratory animals, it has been shown that the NPnEOs result in the inflammation of the vagina in rabbits, decrease in the amount of viable embryos, decline in sperm counts, etc. (Warhaust, 1995 and Cox, 1996). These compounds result in the inhibition of growth, and this has been shown as the decline in body weight of female juvenile rainbow trout (Ashfield et.al., 1998).

Endocrine disrupting effect of NP compounds lead to another more severe effect; NP compounds (NPs, NPnEOs, NPnECs) are also able to cause cancer. This has been

understood after a study conducted on human breast cancer cells, since breast cancer is related to the oestrogen exposure. Therefore, once these cells are exposed to NPs, NPnEOs or NPnECs, the oestrogenic effect of these compounds resulted in the multiplication of breast cancer cells. Also, ethoxylated forms of NPs are known to be co-carcinogenic, meaning these chemicals can increase the cancer effect of any other carcinogenic chemical (Cox, 1996 and Soto et.al., 1991).

To sum up, NP compounds are toxic, carcinogenic, persistent but more importantly endocrine disrupting compounds. Even at short term exposures they have detrimental effects. They inhibit the growth and reproduction, and these effects can last for long periods of time since they accumulate in the fat tissues of organisms. The bioconcentration factors are as high as 10,000 for algae and between 3 and 1300 for fish. These high bioconcentration factors make the situation even worse since it means the amount of NP compounds will be higher in plants, animals and human beings when compared to the environmental systems or lower organisms (Cox, 1996).

2.5 Preventive Measures against Nonylphenol Compounds

The concerns regarding EDCs did not alert scientific world only, but also forced governments to take precautions against the production and use of these chemicals. Because of the risk they pose, United Kingdom (UK) has a voluntary agreement with industries on not to use NP compounds in domestic detergents since 1976. However, other uses (industrial, institutional, commercial) still continue. Since domestic consumption is significant and eventually ends up in the sewerage system, the aim of this ban was to protect natural waters and aquatic environment. European Union also banned the use of NP for many purposes and restricted to certain extent in some industrial formulations. For instance, REACH (Registration, Evaluation, Authorization and Restrictions on Chemicals Regulation) Regulation in 2006 has been arranged to set the rules on the supply and use of NPnEOs. This is a set of

regulation that is applicable to all EU member states. In Annex 17 of this regulation (amended as EC 552/2009 in 2009), there is a list of hazardous substances that are restricted both in terms of manufacture and market supply. NP and NPnEOs are in line 46 of this list and the use of these compounds in the market at concentrations greater than 0.1% by weight including: industrial/institutional cleaning, domestic cleaning, textile/leather processing, agricultural uses, pulp and paper industry, cosmetics, pesticides and herbicides formulations (with certain exceptions) have been strictly prohibited. This regulation however does not govern the materials imported from other countries.

NP compounds have been included in the list of *"priority hazardous substance"* of EU Water Framework Directive (Directive 2000/60/EC). Other than this, these compounds are also included in the list of hazardous substances of OSPAR and HELCOM. Actually in 1992, the Paris Commission (PARCOM 92/8) recommended that the domestic and industrial use of NPnEOs should be phased out in 1995 and 2000, respectively.

Even though many EU member states adopted the restrictions on NP compounds, some of the countries are more stringent and faster on the application of these regulations. For instance, Danish Government proposed the ban of alkylphenols in general in 2000 and this resulted in the removal of all alkylphenols from soaps and detergents (WWF Canada, 1997). Switzerland also banned the use of all alkylphenol ethoxylates (WWF Canada, 1997). Germany didn't only ban the use of NP compounds in domestic applications but also in industrial formulations in 2004 (EPHA, 2005). The bans and restrictions of European countries made United States to take actions like them and USEPA started a research on the contamination levels of contamination in surface waters and sewage works. At first sight, they thought that Europe has overreacted since the degradation efficiencies are acceptable but then with further studies it was understood that the levels of contamination cannot be ignored. The USEPA decided set a guideline on the NP concentrations in water

resources: below 6.6 µg/L in freshwater and below 1.7 µg/L in saline water (USEPA, 2006).

Even though many countries banned the use and supply of NP compounds, there are countries who haven't taken any precautions. For instance, in China, India and in several other South American countries, the extensive use and production of NP compounds still continue (Soares et.al., 2008).

The restrictions on NP compounds are not just about their marketing and use but there are also restrictions on their discharge in treatment works. Due to their physicochemical properties these compounds favor organic systems such as soil, sediment and sludge. Once these chemicals are used and discharged, they eventually reach treatment works and end up accumulated in sludge. Among other sludge handling methods, land application is the most favorable due to its low cost and beneficial recycle of nutrients in sludge. However, sludge does not only contain nutrients but also contaminants such as NP compounds; they must monitored properly to judge about their suitability for land application (Aparicio et.al., 2007). To test the suitability, European Union came up with a limit value for the sum of NP compounds as NPE (NP+NP1EO+NP2EO) and set it as 50 mg/kg dry mass (dm) in its draft regulation (Working Document on Sludge, 3rd Draft, 2000). While most of the European countries are using this limit, some made it much more stringent like Denmark. In Denmark, the limit value for NPE is 10 mg/kg dm, which forces researchers/engineers to look for alternative sludge handling methods (Knudsen et.al., 2000). In Turkey also the limit value is set as 50 mg/kg dm in the Regulation on Land Use of Domestic and Urban Sludges (August, 2010).

2.6 Degradation Mechanisms of Nonylphenol Compounds

The degradation mechanism of NP compounds is not very well defined in the literature but it is generally believed that the degradation starts with the shortening of ethylene oxide units of NPnEOs. This transformation results in the formation of NP1EO and NP2EO at the end. However, degradation mechanisms and the products formed are different for aerobic and anaerobic conditions (Ying et.al., 2002). The difference between two mechanisms is clearly shown in Figure 2.7.

The reason why degradation mechanisms under aerobic and anaerobic conditions are evaluated separately can be clearly understood from Figure 2.7. The mechanism of transformation, also the end products differ from each other in each condition. But even if there are differences in the later steps of degradation, the common step in both aerobic and anaerobic transformation of NPnEOs is the attack of microorganisms to shorten of the ethylene oxide chain at the very beginning. As can be seen in Figure 2.7, under both conditions, the first step is the shortening of ethylene oxide chain, e.g. the conversion of NP2EO into NP1EO. NP1EO is the final ethoxylated metabolite formed during biodegradation. Under aerobic conditions, following the formation of NP1EO with the oxidation of ethylene oxide chain, the carboxylated forms such as NP2EC and NP1EC are formed (Soares et.al., 2008). However, under anaerobic conditions NPnECs are never formed, the only metabolites formed are: NP, NP2EO and NP1EO. Similar to anaerobic conditions, under aerobic conditions, NP is formed finally. Since in most of the legislations, the sum of NP, NP1EO and NP2EO (NPE) is taken into account and NPECs are not included, aerobic treatment processes are better in complying with the regulations when compared to anaerobic treatment.

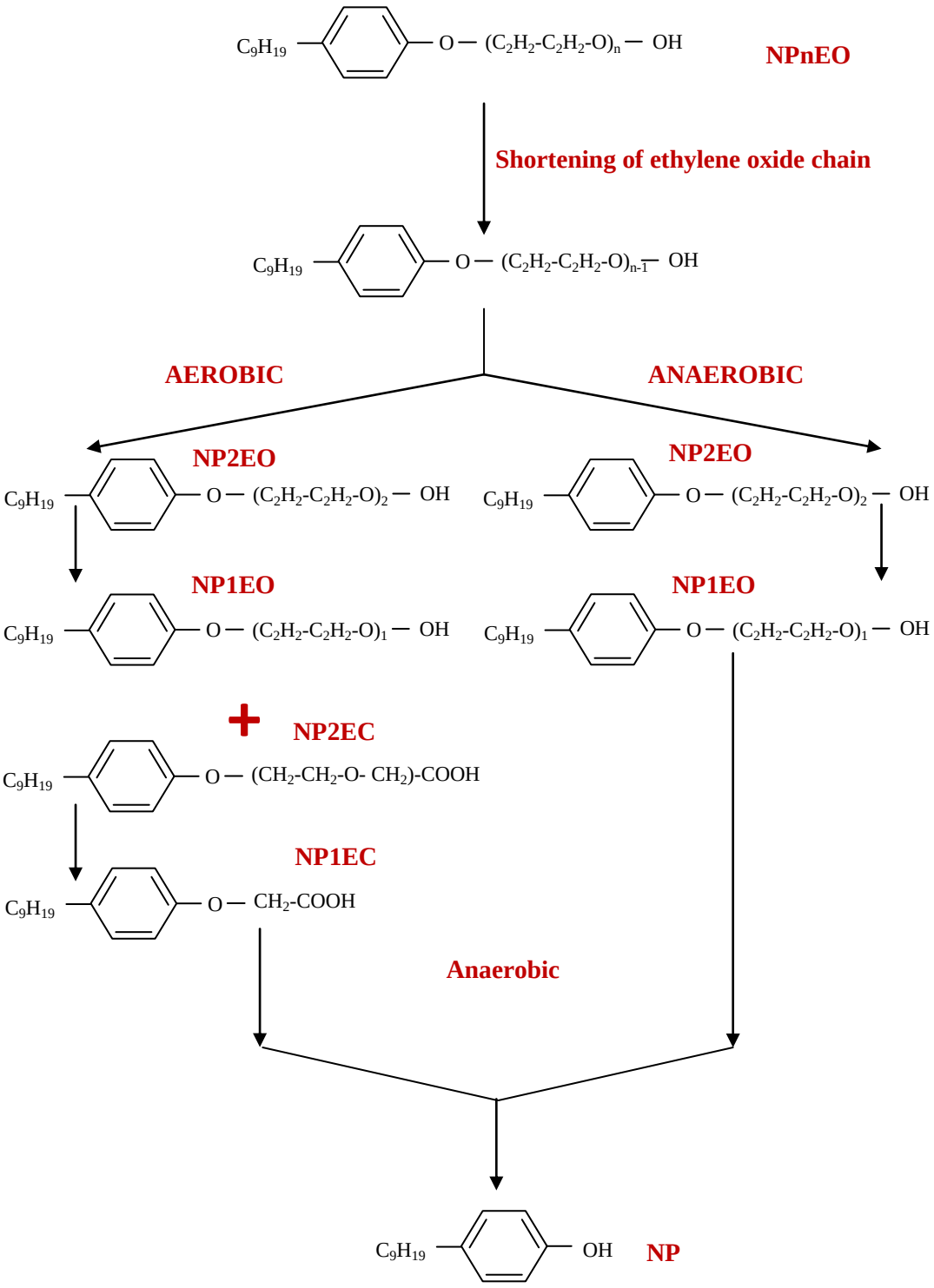


Figure 2.7. Biological degradation mechanisms of NPnEOs under aerobic and anaerobic conditions (Ahel et.al.,1994; CEPA, 1999)

Under anaerobic conditions, the speed of degradation is much slower and this is another difference between two conditions in addition to the type of metabolites formed. NPnEOs are degraded much faster under aerobic conditions, but still under both aerobic and anaerobic conditions, these compounds are not fully degraded.

2.7 Presence of Nonylphenol Compounds in Natural Systems

Fate and occurrence of any chemical is determined by its physicochemical properties and this statement is same for the NP compounds, too. For NPnEOs (with number of ethylene oxide unit less than 6) and NP, octanol/water partition coefficients are above 4 (see Table 2.1), indicating once these compounds reach surface waters they tend to stay on organic phase like sediments (John et.al., 2000). These compounds are recalcitrant meaning they persist in the environment and resist degradation. NP is the least soluble metabolite when compared and during its transport along groundwater supplies, it has been proven that the transport is especially being retarded by its sorption to sediments and soil (Barber et.al., 1988). The main degradation mechanism for NP compounds is biological; therefore compounds must be free for microbial breakdown. That's why degradation metabolites of NPnEOs are recalcitrant, they bind to organic phases and become unavailable to microorganisms for breakdown (John et.al.,2000). It has been shown that once NPnEOs stick onto sediment surfaces, they resist degradation so intense that the half life for these chemicals exceed well beyond 60 years (Shang et.al., 1999). Especially, if soil/sediment surface is contaminated with organic pollutants for a prolonged period of time, then the bioavailability of the compounds is decreased even further. Due to ageing or weathering effect, the sorbed organics establish some sort of bond with the organic fraction of soil/sediment. This eventually results in the slow mass transfer of the compound to be degraded to the microorganisms in charge, even in nutrient rich medium (Bosma et.al., 1997).

The metabolites of NPnEOs, with K_{ow} higher than 4, are not the only ones that stick onto sediment surfaces. Hydrophilic NPnEOs are also found to stay on sediments but this time obviously not due to the organic content and hydrophobic interactions but mineral rich content of sediments. Through multiple interactions with the organic free but mineral rich sediments like clay, NPnEOs with higher ethylene oxide units can bind to sediment surfaces (John et.al., 2000).

Even though, the dominant mechanism for the breakdown of NPnEOs is biological degradation, Faust and Hoigne, 1987, claimed that a significant portion of NPs, approximately 30%, can be degraded photochemically in the top layers of water. The results of this study indicated that under a clear sky with summer sunlight, the half life of NP is about 10-15 days at the surface. When the depth increases, photochemical transformation becomes slower due to lower light attenuation. Also, it has been demonstrated that the photolysis rate of NPnEOs is much slower when compared to NP.

2.7.1 Levels of Nonylphenol Compounds in Surface Waters

Like other surfactants, NPnEOs are present in water bodies, such as rivers, lakes and groundwater supplies, at considerable concentrations due to municipal or industrial discharges or surface runoffs (Ahel et.al., 1994). Other than NPnEOs, NP and NPnECs are also found to exist in environmental systems mainly due to the fact that these compounds are the metabolites of widely used NPnEOs (Voutsa et.al., 2006). Unfortunately, literature on the analysis of these compounds in the nature is limited since measurement is a challenge due to two things: (i) each NP compound has several isomers with different properties and (ii) environmental systems are complex matrices (Arditsoglou and Voutsa, 2008). Even limited, there still exist some measurements of NP compounds in natural waters and sediment. Some examples of the studies conducted on the levels of NP compounds in water resources are given in Table 2.2.

Most of the studies given in Table 2.2 are done to show whether the levels of NP compounds pose a threat to the aquatic life. For instance, in the analysis done by Bennie et.al. 1997, it was claimed that concentrations of NP compounds increase at the points close to industrialized areas and sewage discharge, but even at those points the concentrations are much less than the lowest concentration to induce plasma vitellogenin (the concentration necessary to mature male rainbow trout). In the study carried out by Field and Reed in 1996, it was found that the highest concentrations for NPECs have been observed in points close to the sewage treatment plant, since these compounds are intermediate compounds and formed as a result of degradation of NPnEOs during treatment. Unfortunately, since limited amount of data is available on the bioaccumulation and toxicity levels of NPECs, it is difficult to make a judgment on the impact of NPEC concentrations.

Table 2.2. Levels of NP compounds in the surface waters of Europe, USA and Japan

Compound	Sampling Point	Concentration (µg/L)	Reference
NP	Glatt River,	<0.5-3	
∑NPnEO (n=1-2)	Switzerland	<0.5-20	Ahel et.al., 1987
∑NPnEC (n=1-2)		2-50	
NP	Thermaikos Gulf,	0.112	Arditsogluou and
NP1EO	Thessaloniki, Greece	0.113	Voutsa, 2008
NP2EO		0.05	
NP	Louidias River,	0.277	Arditsogluou and
NP1EO	Thessaloniki, Greece	0.147	Voutsa, 2008
NP2EO		0.029	
NP		<0.01-0.92	
NP1EO	Great Lakes Basin, Canada	<0.02-7.8	Bennie et.al., 1997
NP2EO		<0.02-10	
NP		1.8-10	
∑NPnEO (n=1-3)	River Waters of Taiwan	2.8-25.7	Ding et.al., 1999
∑NPnEC (n=1-3)		16.4-292	
NPEC	Fox River,	nd-13.5	Field and Reed,
	Green Bay, WI		1996
NP	Tokyo, Japan	0.051-1.08	Isobe et.al., 2001
NP	Glatt River, Switzerland	0.029-0.195	Jonkers et.al., 2009
NP	Han River, Korea	23.2-187.6	Li et.al., 2004b
∑NPnEO (n=1-2)	Venice Lagoon,	1.1-38.5	Marcomini et.al.,
∑NPnEC (n=1-2)	Italy	0.5-102	2000
NPEO	River Po, Italy	0.6-1.8	Pojana et.al., 2004
NP	Glatt River, Switzerland	0.068-0.326	Voutsa et.al., 2006

Another important point to be discussed is the seasonal variations of NP compounds in surface waters. For instance, in the study carried out by Fries and Püttman in 2003, NP concentration has been measured to be between 28-1220 ng/L and this huge variation was due to the seasonal changes (i.e. temperature, rainfall, use and consumption, etc.). It is not surprising to hear that NP concentrations change with the

seasonal parameters. For example, when temperature rises, the biological activity of microorganisms degrading NP compounds also increase, resulting in extensive breakdown. As a result of this, the concentrations of metabolites like NP and NPECs may rise, whereas NPnEO concentrations shall fall. Another seasonal parameter that results in the variation of NP concentrations is rainfall. Rainfall has two different effects on concentration change: (i) with the increased rainfall amount surface runoff will carry more organic contaminants to the surface waters (ii) when rainfall increases surface waters will be diluted, in other words, concentrations will be diluted.

As can be seen from Table 2.2 clearly, the concentrations of NP compounds in surface waters vary from country to country. In Japan and USA, the levels are much higher than that in the European countries. The reason is the consumption habits and legislative actions taken against these compounds. In most of the European countries, there are strict legislations on the production and consumption of NP containing products and even in some of them NP compounds has been banned altogether. However, in USA, the importance of the issue is very well understood and USEPA has revealed documents on the levels of these chemicals but there are no strict bans on the levels or production of NP compounds when compared to European countries.

2.7.2 Levels of Nonylphenol Compounds in Groundwater

Just like other natural systems, the occurrence of NP compounds in groundwater systems is solely due to human activities such as wastewater treatment plant effluent discharge, leachate, surface runoff from agricultural activities, etc. Fate and occurrence of NP compounds in groundwater sources are more critical since groundwater is used as drinking water supplies in many places. Therefore, if NP compounds are present in groundwater supplies at high concentrations, it would mean direct intake of these contaminants by ingestion. The degradation of contaminants in subsurface layers is not as fast as it is in surface waters since the

microbial population is limited and degradation is governed by: (i) microbial breakdown, (ii) chemical oxidation/reduction, (iii) sorption, (iv) filtration and (v) volatilization (Tchobanoglous, 2004). Among these mechanisms, biological degradation and sorption are the dominant ones that determine the fate of organic contaminants such as NPnEOs in subsurface layers (Barber et.al., 1988).

Organic contaminants, including NP compounds, can easily enter aquifer systems since the top layer is rich with minerals, carbon content and microorganisms but beyond a certain km of depth (depending on the type of aquifer) degradation is limited. Since NPnEO, NP and NPECs are recalcitrant compounds, they tend to persist in aquifers and the extent of degradation and sorption becomes limited. In the study carried out by Barber et.al., 1988, the concentration of NP isomers in the aquifers in Boston, Massachusetts has been found to be close to 1 μg/L. It has been underlined that, since NP is a persistent compound the biological breakdown is limited and the subsurface transport is generally controlled by sorption mechanism. However, sorption is also limited due to the low organic carbon content of subsurface sediments. Unfortunately, since NP persists for long years of time in groundwater supplies, it may contaminate larger areas via subsurface transport in the future even if its transport is limited to its solubility.

In another study carried out by Montgomery-Brown et.al., 2003, the parameters crucial for the degradation of NPnEOs in subsurface layers have been discussed. It has been found out that presence of oxygen and depth of aquifer are two important parameters in the degradation of NPnEOs. It has been demonstrated that the degree of degradation of NPnEOs is directly proportional to the amount of oxygen present. At a certain aquifer depth, the conditions shift from aerobic to anoxic and at that point the rate of degradation starts to decline.

2.7.3 Levels of Nonylphenol Compounds in Soil and Sediment

Data on the levels of NP compounds in soil and sediment is limited when compared to natural waters. However, it is certain that these compounds are introduced into soil via anthropogenic activities like land application of sludge, accidental spills and leachate. Lately, land application of sludge is becoming more popular and that's why understanding the fate and occurrence of these compounds in soil is crucial. According to a Danish study carried out in the year 2002, it has been found out that the percent of sludge being recycled by farmers was almost 66% of the total production. This fact underlines the critical importance of monitoring of NP compounds in soil systems is important (Soares et.al., 2008).

It has been reported that once soil is exposed to sewage sludge, almost 80% of NP is degraded within the first 3 weeks whereas 10% of the initial concentration was found to persist in soil even after a year (Hesselsoe et.al., 2001). In the study carried out by Gibson et.al., 2005, the level of NP in sludge amended soil was found to be higher than 1 mg/kg dry weight but NP wasn't present in the unamended soil. The level in the sewage sludge was determined as 238 mg/kg dry weight providing the evidence of NP occurrence in sludge amended soil for this study and many other similar cases of land application of sludge.

NPs have been identified in soil samples not only amended with sludge but close to runoff points. NP compounds have also been analyzed in landfill leachate under aerobic conditions. Once NP compounds enter soil environment, their concentrations vary with the mechanisms such as: (i) biological breakdown, (ii) sorption and (iii) volatilization. Due to their high $\log K_{ow}$ values, these compounds tend to stick onto soil particles and that's why the rate of breakdown or volatilization is limited (Soares et.al., 2008). But other than their strong interactions with the soil matrix, environmental circumstances also affect the rate of biodegradation and this has been

best shown by the study of Hesselsoe et.al., 2001. Here, oxygen has been found out to be a limiting factor in the degradation of NPnEOs.

Once NP compounds enter aquatic system, similar to soil, they generally tend to stick onto sediment surfaces due to their high K_{ow} coefficients and low solubility. Again due to the recalcitrant nature and their strong sorption interactions, extent of degradation is limited in sediments. It is claimed that the half life of these compounds in sediments could be as long as 60 years due to these interactions (Shang et.al., 1999). The levels of these compounds in sediments are given in Table 2.3.

Table 2.3. Levels of NP compounds in sediment

Compound	Sampling Point	Unit	Level	Reference
NP			0.17-72	
NP1EO	Great Lakes Basin, Canada	μg/g	<0.015-38	Bennie et.al.,
NP2EO			<0.015-6	1997
NP	Jamaica Bay, Long Island,		6.99-3700	
NP1EO	NY	ng/g	26.4-13300	Ferguson et.al.,
NP2EO			16.1-3580	2001
NP	Lake Shihwa	ng/g	11-624	Li et.al., 2004a
NP	Han River, Korea	ng/g	25.4-932	Li et.al., 2004b

Similar to the findings in natural waters, the highest values for NP compounds in sediments were observed in the points close to treatment plant runoffs. Again the concentration profile changes between sampling points in each of the studies given in Table 2.3 depending on the distance to the contaminated zones. Seasonal change in the levels of NP compounds is also observed possibly due to microbial breakdown, and the dependence of the activity of microorganisms on temperature. One final point is that, the isomers of NP compounds at each sampling point is different from

each other, and that is also a reason why the concentration profile differs that much (Li et.al., 2004a).

2.8 Presence of Nonylphenol Compounds in Engineered Systems

Domestic, industrial and urban wastewater treatment plants contain many different synthetic organic contaminants like NP compounds. These chemicals are not removed effectively during wastewater treatment and eventually detected in the receiving bodies (Conn et.al., 2006) or accumulate in sewage sludge. Sludge is a by-product of wastewater treatment and concentrates the pollutants present in the wastewater. Therefore, once land application of sludge is in question, then the fate of these contaminants should also be considered (Gibson et.al., 2005).

2.8.1 Levels of Nonylphenol Compounds in Wastewater Treatment Plant Effluents

The principal system responsible for the presence of NP compounds in surface waters is the effluents of the wastewater treatment plants. Generally, NPnEOs in higher forms pose less endocrine disruption threat, but the degradation by-products are more dangerous. What treatment processes typically do is to convert NPnEOs into more persistent compounds and NP. The fate of NP compounds in wastewater treatment is determined by the mechanical, chemical and biological processes applied during the treatment (Ahel et.al., 1987; Esperanza et.al., 2004; Lian et.al., 2009). Unfortunately, under any condition the degradation is incomplete and the most oestrogenic metabolites like NP has the potential to accumulate (Sole et.al., 2000).

There are a number of studies on the levels of NP compounds in wastewater treatment plant influents and effluents. These studies provide information on which compounds face biodegradation and which ones accumulate in the effluents and sludge. Table 2.4 summarizes some of these studies.

Table 2.4. Levels of NP compounds detected in the influent/effluent of wastewater treatment plants

Compound	Sampling Point	Raw/Treated	Concentration	Reference
NP		Raw	12.0 μg/L	
	Rome, Italy	Treated	1.0 μg/L	DiCorcia
NPnEO		Raw	203.0 μg/L	et.al., 1994
(n=11-18)		Treated	9.9 μg/L	
NP			1.6 μg/L	
NP1EO			9.6 μg/L	
NP2EO	Taipei, Taiwan	Treated	15.3 μg/L	Ding and
NP1EC			19.2 μg/L	Tzing, 1998
NP2EC			99.2 μg/L	
NP	Green Bay, WI,	Treated (primary)	2.8-30 μg/L	Field and
	USA	Treated	0.8-15 μg/L	Reed, 1996
		(postchlorination)		
NP		Raw	545-3022 ng/L	
		Treated	126-1965 ng/L	
NP1EO	Northern Greece	Raw	466-4025 ng/L	Pothitou and
		Treated	13-573 ng/L	Voutsa, 2008
NP2EO		Raw	490-2670 ng/L	
		Treated	26-216 ng/L	

As can be seen from the concentrations stated in the studies above, NPnEOs decrease significantly once the wastewater faces treatment. This is explained by biodegradation most of the time. One should note that, the levels in the treated

effluents are not that low either, meaning the formation rates of NP compounds (or production and consumption) exceed the degradations rates during treatment (Pothitou and Voutsa, 2008).

However, if the single mechanism responsible for the disappearance of NP compounds was biodegradation, NP levels should increase in the treated effluents as NPnEOs are converted. But as shown in Table 2.4, that is not the case due to adsorption. As stated, these compounds have higher affinity to organic surfaces like biota and as a result, during treatment, NP accumulates on sludge surface and that's why its concentration drops in the secondary effluent (Scrimshaw et.al., 2004).

These compounds have complex nature and their degradation rates depend on the process applied. But other than the nature of the process, seasonal variations and changes in the influent characteristics also affect the removal rates (Esperanza et.al., 2004). Especially, for biological systems seasonal variations mean change in temperature and rainfall, which will affect the microbial activity. Change in the influent characteristics means shock loading, variations in the NP compounds in the raw sewage. As the loading of NP compounds varies and exceeds the capacity of treatment plant, it is not surprising to hear that efficiency of treatment declines immediately. However, regardless of the treatment efficiency it is inevitable that carboxylated forms or NP are formed at the end of treatment.

2.8.2 Levels of Nonylphenol Compounds in Sludge

Production of sludge has increased over the years significantly. Today the most common sludge handling methods are landfilling, incineration and agricultural use of sludge. Among all these three methods, the cheapest and most beneficial option is the land application of sludge (Aparicio et.al., 2007; Santos et.al., 2007). Sludge is rich in nutrients and that is why it is suitable for land use as a soil conditioner or fertilizer

(Gibson et.al., 2005). However, since it is a by-product of wastewater treatment, it also contains most of the contaminants being removed, like heavy metals, pathogens and organic chemicals like NPnEOs (Abad et.al., 2005). Once sludge is used as a fertilizer for agricultural purposes without testing its suitability, these contaminants may enter the food chain, taken up by the roots of plants and pose both environmental and human health risk (Gibson et.al., 2007; Pryor et.al., 2002). Therefore, these contaminants, including NP compounds, must be monitored properly to prevent the risk.

Due their high consumption and production, NPnEOs are the dominant compounds in the influent sewage, however, once treatment is applied, the situation is reversed and the metabolites start to accumulate. They do not accumulate solely in the treatment effluent but the majority prefers to accumulate on sludge surface due to their physicochemical properties underlined before. A summary of recent research studies evaluating the levels of NP compounds in sludge is given in Table 2.5.

Table 2.5. Levels of NP compounds in sludge

Compound	Sampling Point	Type of Sludge	Concentration (mg/kg dm)	Reference
NPE (NP + NP1EO + NP2EO)	Catalonia, Spain	Raw Composted Thermally Treated	48.6-2668 17.9-363.4 14.3-3150.3	Abad et.al., 2005
NPE (NP + NP1EO + NP2EO)	Seville, Spain	Primary Secondary Anaerobically Digested	16.87-405.87 12.44-207.26 30.93-1700.00	Aparicio et.al., 2007
NPE (NP + NP1EO + NP2EO)	Andalusia, Spain	Composted Anaerobically Digested Lagoon Aerobically Digested	44-962 8-669 27-319 61-282	Gonzalez et.al., 2010
NPE (NP + NP1EO + NP2EO)	Different regions of USA	Composted Lime Treated Heat Treated Anaerobically Digested	6.1-176 529-932 544 721-981	La Guardia et.al., 2001
NP	NY, USA	Anaerobically Digested	1100-1800	Pryor et.al., 2002

As given in Table 2.5, each study compared the concentrations of NP compounds at different sludge treatment alternatives. In most of the studies, most of the times the sum of NP, NP1EO and NP2EO which is indicated by NPE, is greater than 50 mg/kg dm stated in the European Union Sludge Directive (Working Document on Sludge). Even though, in all of the sludge stabilization methods the limit value is exceeded, the methods are compared based on which one produces less contaminated sludge in terms of NPE.

For instance, in the study of Abad et.al., 2005, composting was suggested to be a better method when compared to thermal treatment. But this does not mean that composted sludge always produces the least contaminated sludge. In another study done by Gonzalez et.al., 2010, aerobic digestion was found to be the best method in terms of NPE degradation. Actually, in this study not only aerobic digestion, but lagoon and anaerobic digestion were also found better than composting. Since the sum of three NP compounds is considered in legislations, it is not surprising that aerobic methods yield sludges in better conformity with regulations, since under aerobic conditions, carboxylated forms are also produced decreasing the sum NPE.

In some studies primary and secondary sedimentation sludges are also compared (Gonzalez et.al., 2010). It has been observed that in primary and secondary sludge, NP is less abundant when compared to NP1EO and NP2EO. In primary and secondary sedimentation NP is not abundant because the extent of degradation into metabolites is much less when compared to sludge stabilization techniques. From primary to secondary treatment, the concentrations also change due to degradation during aerobic treatment of wastewater.

Since in sludges examined in most of the studies the 50 mg/kg dm is found to be exceeded and the limits are more stringent in some countries like Denmark, alternative methods of sludge treatment are being searched. Knudsen et.al., 2000, started to work on methods to decrease the sum under 50 mg/kg dm. They have worked on post-aeration technique following anaerobic digestion. The aim of this study is actually not the 50 mg/kg dm but to satisfy the 10 mg/kg dm limit of Denmark. According to the results of the study, sludge stabilization methods combined with aerobic techniques yield NPE values lower than 50 mg/kg dm and even 10 mg/kg dm.

CHAPTER 3

MATERIALS AND METHODS

3.1 Waste Activated Sludge

Waste activated sludge (WAS) samples used during the experimental work were supplied from Ankara-Tatlar Wastewater Treatment Plant, which has a current flowrate 765,000 m^3/day (ASKI, 2012).

The samples were taken from the return activated sludge line. The collected samples were left to settle so that a certain solids concentration could be achieved by removing the supernatant from the top of sample bottles. During the settlement of solids, samples were kept at 4°C in the refrigerator so that microbial activity could be minimized.

3.2 Chemicals

NP compounds used during the calibration of gas chromatography-mass spectrometry device (GC-MS) were: nonylphenol solution (analytical standard, 5µg/L in acetone), nonylphenol monoethoxylate solution (analytical standard, 5µg/L in acetone), nonylphenol diethoxylate solution (analytical standard, 5µg/L in acetone) were purchased from Fluka, Sigma Aldrich Chemie GmbH and

nonylphenoxy acetic acid (10 ng/L in acetone) was supplied from Dr. Ehrenstorfer GmbH. 4-n-nonylphenol (10 ng/µL in cyclohexane) was used as surrogate during extraction studies and was purchased from Dr. Ehrenstorfer GmbH. In order to spike the aerobic batch reactors, solid nonylphenol diethoxylate (purity 99,0%, 10 mg) was used and obtained from Dr. Ehrenstorfer GmbH.

During derivatization of the chemicals prior to GC-MS analysis, BSTFA+TMCS (N,O-Bis(trimethylsilyltrifluoroacetamide) + trimethylchlorosilane), 99:1 (Sylon BFT) Kit was used and it was supplied from Supelco Analytical, Sigma Aldrich Chemie GmbH. The chemical used for the derivatization of NP1EC was boron trifluoride (BF_3, in methanol) and supplied from Merck KGaA, Germany.

Following the extraction of the chemicals from sludge samples several chemicals were used prior to GC injection. One of these chemicals was sodium sulfate (anhydrous granulated for organic trace analysis) which was used to remove the residual moisture of extracts to protect GC-MS and supplied from Merck KGaA, Germany. During extraction copper (fine powder GR particle size<63 µm) was added to remove sulfate and obtained from Merck KGaA, Germany. For the extraction of liquid samples, SEP-PAK Vac C18 (6 cc/500 mg) cartridges were used and they were purchased from Waters Co.

All throughout the study, solvents like acetone, methanol, hexane and petroleum ether were used for various purposes and they were gas chromatography grade and supplied from Merck KGaA, Germany.

The chemicals used for the preparation of COD were: high purity (95-97%) H_2SO_4, $K_2Cr_2O_7$, $AgSO_4$, and $HgSO_4$. All of the chemicals used for the preparation of COD solution were supplied from Merck KGaA, Germany.

3.3 Experimental Set-up

3.3.1 Set-up of Preliminary Laboratory Scale Aerobic Batch Reactors

In the first part of the study, laboratory scale aerobic batch reactors without NP2EO were operated. The objective was to determine the systematic and possible problems that may be faced during the operation of future NP2EO dosed aerobic batch reactors so as to take the necessary precautions beforehand. For this purpose two sets of reactors were operated with different solids concentrations. The only data monitored was the change in solids concentration throughout the operation.

First Set Reactors

Two liter aerobic laboratory scale batch reactors were set up using WAS. Without any external force applied, the WAS added to the reactors were let to settle under gravity for one day to obtain a more concentrated sludge. Once the supernatant was removed, 2 L of WAS was placed into the 2.5 L reactors and then the operation was started. Initially, TSS and VSS values of WAS used in the set up of both reactors were 8540 mg/L and 6985 mg/L, respectively. The duration of operation was 32 days. Reactors were kept at 25°C (constant temperature) in a water bath. pH was not adjusted to a certain value since sludge contains enough buffering materials. The reactors were aerated using air pumps connected via 2 mL pipettes dipped into them. Maximum amount of oxygen was supplied into system.

Second Set Reactors

After the operation of first set of reactors, it was decided to operate another set of aerobic batch reactors. The idea behind this set was to observe the performance of reactors in terms of solids destruction if the solids concentration is increased. For this purpose, in addition to the settling of sludge for one day, the WAS added to the reactors were centrifuged at 2200 rpm for 1 min. The initial TSS and VSS values for WAS used in the set up of second set of reactors were 21850 and 17050 mg/L, respectively. Similar to the first set, the reactors were established in 2.5 L glass bottles with an effective volume of 2 L. This set of reactors was operated for 30 days. Reactors were kept at 25°C (constant temperature) in a water bath. The reactors were aerated using air pumps connected with 2 mL pipettes.

In both sets, daily sampling was applied. Since there was only one exit from each reactor, the samples were poured through the tubulure into a graduated cylinder after shaking. All the analyses were conducted in duplicate and the average of the duplicate results are presented.

3.3.2 Set-up of Nonylphenol Diethoxylate Spiked Laboratory Scale Aerobic Batch Reactors

The third set of aerobic laboratory scale batch reactors were set up in order to observe the degradation mechanisms of NP compounds under aerobic conditions. For this purpose, two sets of 5 L aerobic batch reactors were operated with a working volume of 3.2 L. Head space was increased to overcome the overflow problem that was encountered in the previous sets. If not prevented, the overflow could result in the loss of compounds to be analyzed and the results could have been misleading.

Each set was operated in duplicates, making four reactors in total. The details are given in Table 3.1.

Table 3.1 Details of the reactor set-up

Reactor	WAS (L)	Acetone addition	NP2EO addition
Control-1 (C-1)	3.2	5 mL	
Control-2 (C-2)	3.2	5 mL	
Reactor-1 (R-1)	3.2		3 mg/L
Reactor-2 (R-2)	3.2		3 mg/L

One set of these reactors was composed of two control reactors containing WAS and acetone (the solvent which is used in spiking NP2EO) only, whereas, the other two reactors were dosed with 3 mg/L NP2EO in the same volume of acetone. All four reactors were filled with WAS, concentrated (TS~2%, VS 1.4%) by settling.

The 5 L glass reactors used were graduated in order to follow the volume change along the operation. The configuration of the reactors used is shown in Figure 3.1. As can be seen from the Figure, there are two outputs at the top, one used for inserting aeration pipettes (connected to an air pump) and the other is closed. At the bottom of the reactors there is a sampling port used to withdraw sludge and closed with a hose clip after each sample is taken.

Figure 3.1 3.2 L laboratory scale aerobic batch reactors

Instead of water bath, this time all four reactors were kept at constant temperature room which was at 25 °C. The reason for placing this set of reactors in constant temperature room was to assure constant temperature in the whole reactor. Because in water bath, only a portion of the reactor is within the constant temperature water and as a result uniform temperature distribution within the reactor cannot be achieved. The pHs of the reactors were 6.9±0.1 and were not buffered at the day of set up. Mixers were placed under the reactors to make sure complete mixing is achieved.

For the first 4 days, the reactors were operated without any external NP2EO addition. At the 4^{th} day of reactor operation, one set of reactors (two reactors) were spiked with 3 mg/L NP2EO dissolved in 5 mL acetone. At the same time 5 mL of acetone (which did not contain NP2EO) was added to the other set (two control reactors). Before spiking NP2EO (in the first four days of reactor operations) samples were taken once a day. Since under aerobic conditions biodegradation is faster, it was decided to take samples more frequently in the following couple of days of NP2EO spike. Starting from the 4^{th} day, samples were taken twice a day until the 8^{th} day of

operation. As the degradation of NP2EO became more stationary, the sampling schedule was switched to daily basis, this schedule was kept going until the 13th day of reactor operation which was time at which reactors were thought to stable both in terms of solids concentration and concentration of NP compounds. Starting from 13 days, the sampling schedule was widened and the samples were taken once in two days. The sampling schedule was decided dynamically based on previous samplings and the measurements done upon them. In each sampling, 40 mL of sludge was withdrawn from the reactor. On these samples, duplicate analysis of TS, VS, TSS, VSS, COD and NP compounds (NP, NP1EO, NP2EO and NP1EC) were carried out.

3.4 Analytical Methods

Parameters measured all throughout the study were total solids (TS), volatile solids (VS), total suspended solids and volatile suspended solids (VSS) concentration, pH and COD. For the determination of NP compounds using GC-MS device, first the extraction of these compounds from liquid and solid phases were achieved. All of these analyses are carried out on the samples collected from the laboratory scale aerobic batch reactors being operated. In this section, each of the methods listed above will be described.

3.4.1 Solids Determination

Determination of TS and VS concentrations within the sludge samples were carried out according to the Standard Methods, 2540B and 2540E, respectively. Whereas, for the determination of TSS and VSS, Standard Methods 2540D and 2540E were followed, respectively (APHA, AWWA, WEF, 2005).

3.4.2 Chemical Oxygen Demand

One of the analyses done on sludge samples was chemical oxygen demand (COD) determination. For this purpose, one of the widely used COD method which was Hach USEPA approved COD test was followed. The COD kits were not purchased from Hach Company. They were prepared in the laboratory according to the Hach Water Analysis Handbook (2011, 5^{th} Ed.). Since the kits were prepared in the laboratory, a calibration curve was prepared and used for the spectrophotometric analysis and this curve is given in Appendix-A. Potassium acid phthalate (KHP) was used and on weight basis, the theoretical oxygen demand of KHP was 1.175 mg O_2/mg KHP.

Once the COD kits were prepared and calibrated, sludge samples taken from the reactors were diluted and added to the kits to react. The spectrophotometric analysis of this experiment was carried out using a Hach DR 2400 portable spectrophotometer.

3.4.3 pH Measurements

The pH measurements were conducted according to Standard Method 4500H^+ using a pH meter of model 510 with the pH probe (EC-PH 510/21S) purchased from Eutech Instruments (APHA, AWWA, WEF, 2005). The pH meter was calibrated prior to analysis using standard solutions of pH 4, 7 and 10.

3.4.4 Determination of Nonylphenol Compounds

The compounds of concern (NP, NP1EO, NP2EO and NP1EC) were analyzed using GC-MS (Agilent Technologies 7890 GC system coupled with 5975C inert MSD with triple axis detector). The methods which are explained in the following sections were used in analysis. However, before deciding which method to use, an extensive study was conducted which is also explained in the coming up sections of this thesis.

3.5 Development of a GC-MS Procedure for the Measurement of Nonylphenol Compounds

3.5.1 General Principles of Gas Chromatography-Mass Spectrometry

GC-MS is an analytical device used for the separation, identification and quantification of volatile or semi-volatile compounds in mixtures. Besides its well-known pharmacological applications, GC-MS is also used in organic trace analysis in water or wastewater samples and forms a basis for many USEPA drinking and wastewater analysis methods (Hites, 1997).

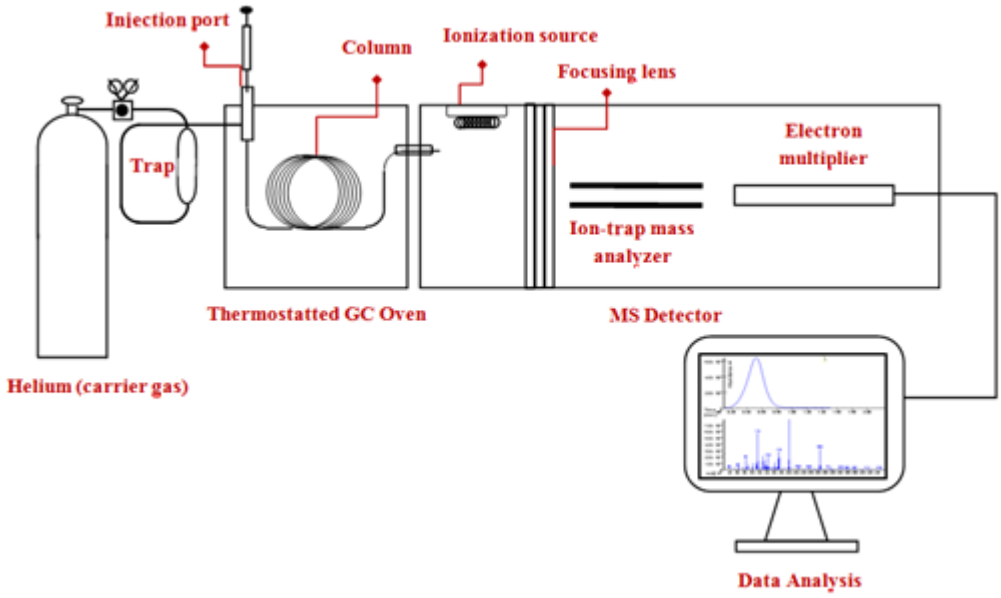


Figure 3.2 A general overview of GC-MS device (Whitman College, 2008)

As the name implies, GC-MS is composed of two different devices, each having its own responsibilities. Gas chromatography (GC), is mainly responsible for separating the chemical mixtures into individual components. Once the sample is injected, it is volatilized at high temperatures. The carrier gas, mostly helium, transports the compounds on the stationary phase, in other words column as shown in Figure 3.2. The gas is supplied from a cylindrical tank coupled with a reducing valve to keep the pressure at a constant value. As the sample flows through the column, each compound within the sample interacts with the coating of the column. According to the speed of interaction, each compound leaves the column at different time intervals. This time interval between the sample injection and column elution is called retention time (Douglas, 2012). Within this time interval each component of the mixture is separated based on their physicochemical properties. For instance, if the compound of interest has a low molecular mass it interacts with the column much quickly and leaves before other molecules. Not only the physicochemical properties but also the rate and extent of interaction with other molecules within the mixture also affect the retention time (Douglas, 2012).

GC is coupled with a detector in order to measure the compounds eluted from the column (Douglas, 2012). In this study, mass spectrometer (MS) detector has been used. Once the gas molecules enter the MS via transfer line, they are bombarded by high energy electron beam, accelerated through a magnetic field and disintegrated into charged fragments. Then these fragments travel towards the MS detector, whose responsibility is to identify the different charges with respect to the mass of each fragment and obtain an output. The output produced by MS detector is named as mass spectrum which is a chart of peaks. The height of a peak changes with the number of fragments detected for a certain mass, however, according to the sensitivity of the spectrum this may also change. Mass spectrums are unique; each compound is distinguished from the other based on this uniqueness (Douglas, 2012).

Since mass spectrums are unique for substances, compounds can be identified by comparing the spectrum obtained at the end of the analysis with the spectrum of known substances. Mass spectrums generally present one great peak which is the parent mass. Once the parent mass is identified, the molecular mass and structure of the molecule can be guessed and the compound will be determined. For confirmation, one can compare the spectrum with a reference spectrum of the compound (Douglas, 2012).

Even though, GC-MS is a helpful analytical tool, it has its own limitations also. These limitations can be listed as:

- Depending on the duration of the GC-MS program, the analysis of certain compounds may be time-consuming.
- Only the compounds with vapor pressure greater than 10^{-10} torrs can be analyzed using GC-MS.
- Determination of the positional substitution of aromatic rings is difficult.
- Even if a compound is separated by GC-MS, it may not be identified by MS detector or vice versa (Douglas, 2012; Hites 1997).

3.5.2 Selection of Proper Solvent for Sample Preparation

The measurements of the chemicals (NP, NP1EO, NP2EO, NP1EC) included in this thesis were done using GC-MS device. The first stage in the analysis of these compounds in GC-MS is the selection of the solvent proper for both the compound and GC-MS. For the injection of the samples, they must be in solution first. Just like the compound to be analyzed, the solvent must also be volatile and organic. But more importantly, while calibrating the device, the compounds must be dissolved homogenously in the stock solutions prepared so that accurate measurements can be done. To prevent contamination, high quality solvents were used throughout this study.

To select the proper solvent, different solvents like acetone, methanol, hexane, dichloromethane were tested and whether the chemicals could be dissolved properly or not were determined based on GC-MS analysis. According to the analysis done, acetone and methanol showed the highest responses whereas hexane and dichloromethane showed the lowest responses. Acetone was selected for the preparation of stock solutions due to two reasons: (i) the chemicals used were dissolved in acetone originally as they were purchased, (ii) methanol was thought to harm GC-MS device in long run.

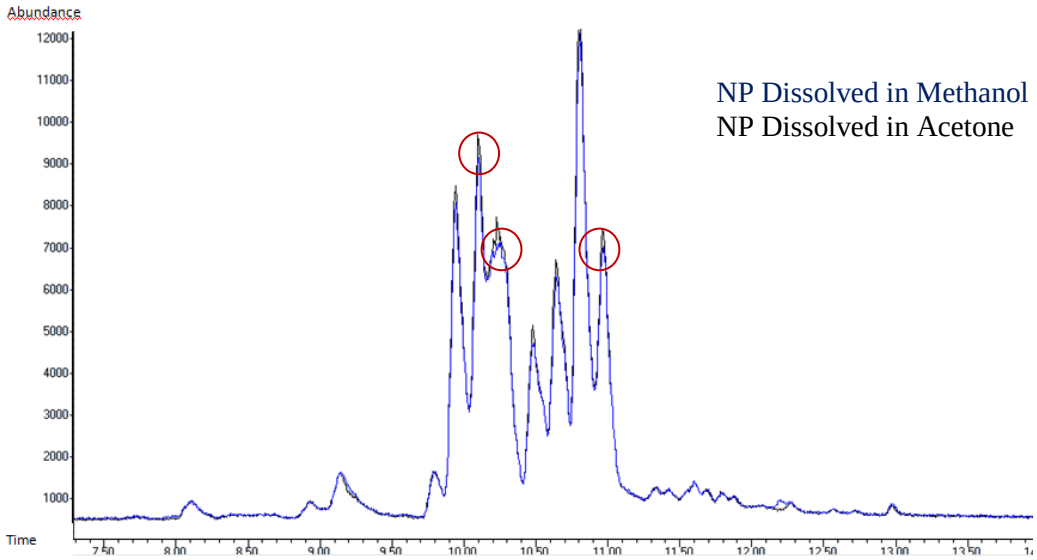


Figure 3.3 Total ion chromatogram (TIC) for NP dissolved in methanol and acetone

3.5.3 Optimization of a Derivatization Method for Nonylphenol Compounds

As mentioned above, the mixture of compounds injected into GC is volatilized and separated at the high temperature column. The compounds must be volatile and thermally stable at high temperatures. The NP compounds analyzed during this thesis study have rather low volatility and thermal stability, meaning these compounds cannot be volatilized without thermal decomposition at high temperatures within the column. As a result the compounds cannot be separated properly in the GC and MS cannot detect them.

To preserve the thermal integrity of the compounds, a well-known chemical process called “derivatization” was applied. Derivatization converts highly polar materials into volatile compounds that could be resolved at high temperatures without thermal or molecular decomposition. This procedure makes the analysis of this group of compounds even at low concentrations with improved GC efficiency.

To derivatize these chemicals, there are several methods underlined in the literature. Derivatization agents and time of derivatization reaction is different for each method. In this study, derivatization methods underlined in Gatidou et.al., 2007 and Barber et.al., 2000 were tested, modified and compared. Chemicals to be analyzed were reacted with chemicals in Table 3.2 at the given temperatures and times.

Table 3.2 Details of the derivatization methods derived from Gatidou et.al., 2007 (Method-1) and Barber et.al., 2000 (Method-2)

Parameter	**Method**	
	Method-1	**Method-2**
Name of the chemical used	BSTFA + Pyridine	BSTFA + Pyridine
Volume of the chemical	50 µL BSTFA	50 µL BSTFA
	50µL pyridine	50µL pyridine
Derivatization temperature (°C)	65 °C	90 °C
Time for derivatization	20 min	5 hours

The derivatives obtained with both of the methods were analyzed by the following GC oven programs shown in Table 3.3. The chromatograms given in Figures 3.4 and 3.5 were obtained. These Figures and the ones coming after were similar for NP1EO and NP2EO tested together with NP.

Table 3.3 GC oven programs used in the analysis of derivatives obtained by Method-1&2

	Barber et.al., 2000	**Gatidou et.al., 2007**
Initial Temperature	40°C	80°C
Ramps	6°C/min to 300°C, hold for 15 min	15°C/min to 220°C, and then 5°C/min to 280°C
Carrier Gas	Helium (constant pressure)	Helium (constant flow)
Column Type	HP-5MS Phenyl Methyl Siloxane	HP-5MS Phenyl Methyl Siloxane

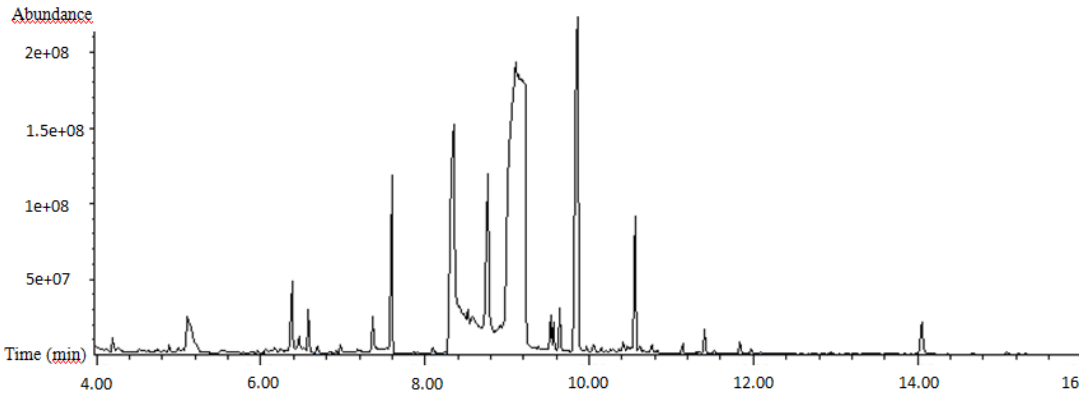


Figure 3.4 TIC for NP derivatized using Method-1

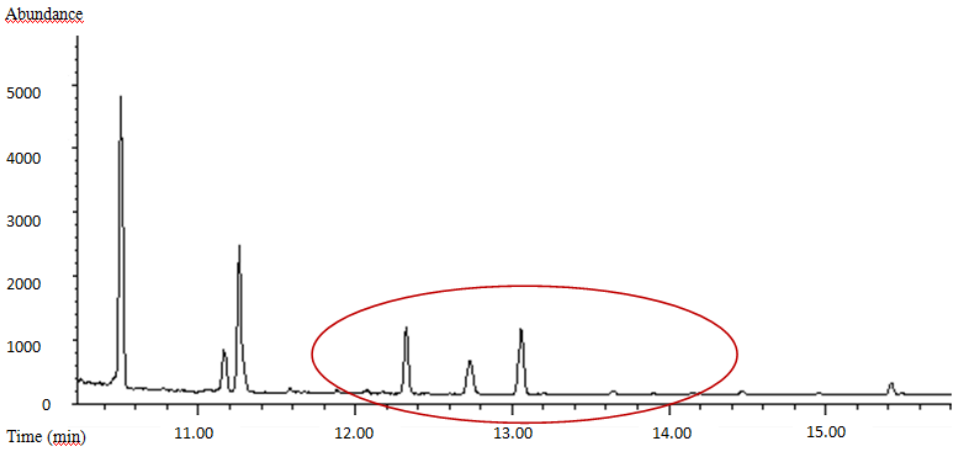


Figure 3.5 TIC for NP derivatized using Method-2

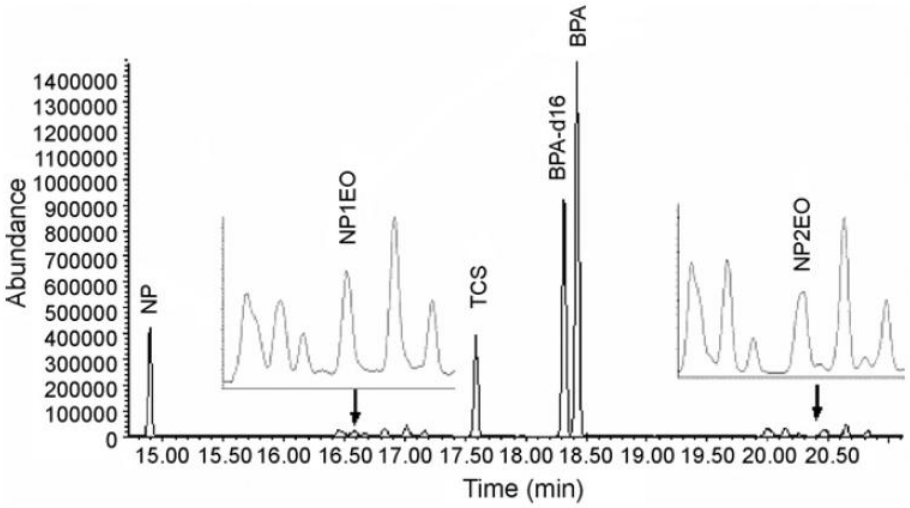


Figure 3.6 TIC presented in Gatidou et.al., 2007

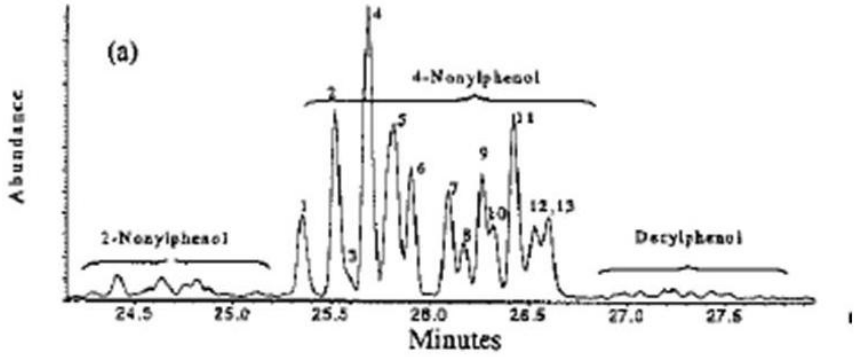


Figure 3.7 TIC presented in Barber et.al., 2000

As can be seen in Figure 3.4 and 3.5 the family of peaks obtained were not acceptable when compared to Figures 3.6 and 3.7. The abundances were unacceptably low. Therefore, modifications were done on these methods. The parameters that were altered are given in Table 3.4.

In the application of these methods, water bath and drying oven were used and the results were compared. The results were close to each other (in terms of abundances in TIC), and due to this drying oven was selected for more uniform distribution of heat since in water bath only the bottom of vials were submerged to prevent water intrusion into the vials.

Table 3.4 The modifications in the derivatization methods of Gatidou et.al., 2007 (Method-1) and Barber et.al., 2000 (Method-2)

	Method-1	**Method-2**
Temperature	65°C and 70°C	75°C and 90°C
Volume	50 µL BSTFA; 50µL pyridine	50 µL BSTFA; 50µL pyridine
	100 µL BSTFA; 100µL pyridine	100 µL BSTFA; 100µL pyridine
Duration	60 min, 90 min, 120 min	5 hours

When these two methods were compared, Method-2 yielded the same results as it was before modification. Therefore, the derivatization studies were concentrated on Method-1 at this point. However, since pyridine causes background concentration to rise and a decrease in signal to noise (S/N) ratio, alternative reagents have been investigated. Instead of pyridine it was decided to use TMCS. The idea behind the addition of TMCS or pyridine is to speed up the derivatization reaction, in other words, these two chemicals act as catalysts. Since they serve for the same purpose, the solutions of BSTFA+TMCS have been prepared at different volumetric ratios (see Table 3.5).

Table 3.5 Details of the modifications of Method-1 using TMCS as catalyst

	Method-3	**Method-4**
Reagents	BSTFA+TMCS	BSTFA+TMCS +Solvent
	BSTFA+1%TMCS	BSTFA+1%TMCS
% Volume	BSTFA+10%TMCS	BSTFA+10%TMCS
	BSTFA+33.3%TMCS	BSTFA+33.3%TMCS
Total Volume	50 µL, 100 µL, 200 µL	50 µL, 100 µL, 200 µL
Temperature	70°C	70°C
Duration	30 min	30 min

The only difference between Method-3 and Method-4 is that in Method-4 solvent is added to the reaction vial after the same derivatization procedure. The purpose of solvent addition is to increase the effectiveness of derivatization reaction. In this context, acetone, hexane and dichloromethane have been used. The example TIC for acetone, hexane and dichloromethane is given Figure 3.8, Figure 3.9 and Figure 3.10.

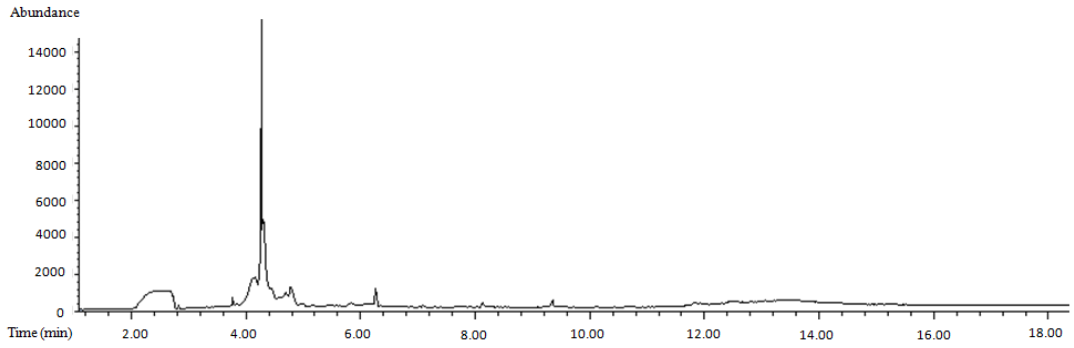


Figure 3.8 TIC for NP derivatized according to Method-4 using acetone

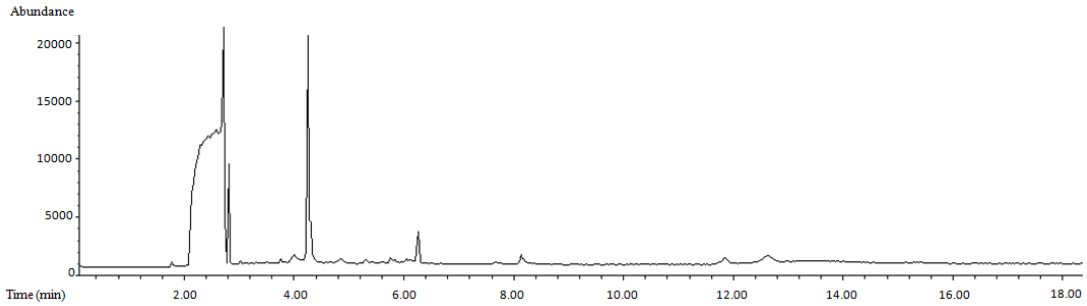


Figure 3.9 TIC for NP derivatized according to Method-4 using hexane

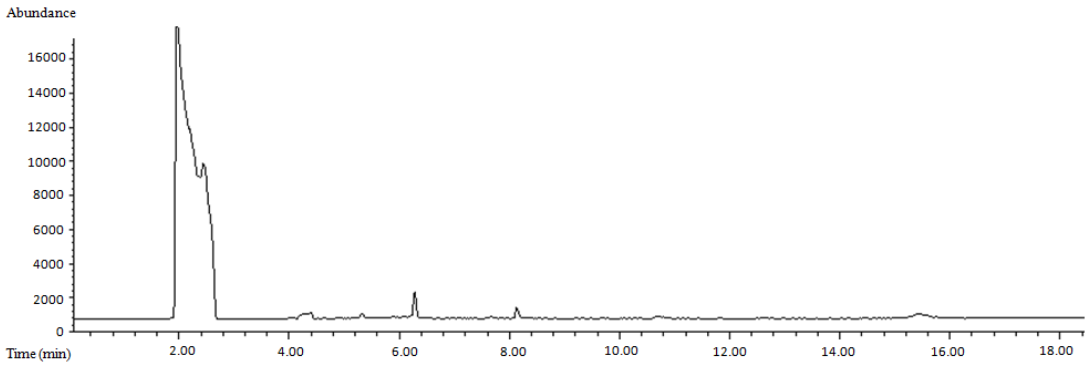


Figure 3.10 TIC for NP derivatized according to Method-4 using dichloromethane

As can be seen from the Figures 3.8, 3.9 and 3.10, addition of solvent didn't enhance the effectiveness of derivatization but rather retarded the process. That is why, Method-4 is eliminated and the modifications of Method-3 according to the values given in Table 3.5 were continued. One of the parameters was the volume of BSTFA+TMCS solution added to derivatize the sample. 50 µL, 100 µL and 200 µL were the volumes of BSTFA+TMCS solution being experimented. As can be seen from Figure 3.11, addition of 200 µL didn't improve the derivatization efficiency. Both in terms of peak quality (resolution of peaks) and integrated area, 100 µL BSTFA+10%TMCS addition was better than 200 µL.

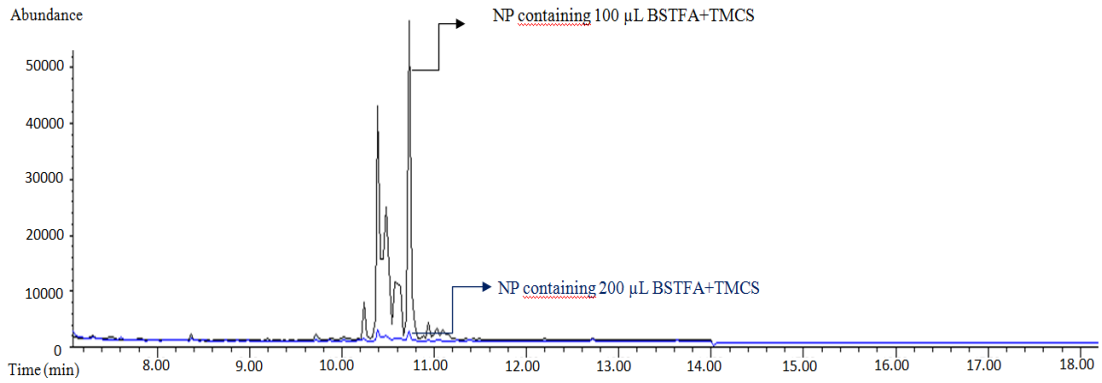


Figure 3.11 TIC for NP derivatized with 100 µL and 200 µL BSTFA+TMCS

Therefore, the volume 200 µL BSTFA+TMCS was eliminated. Even though 50 µL of BSTFA+TMCS solution gave close abundances to 100 µL volume (see Figure 3.12), the integrated area for 50 µL (23721903) was lower than that for 100 µL (33179081). Besides, for the homogeneity of sample, it is better to inject it at higher volumes. As a result, 100 µL BSTFA+TMCS solution was selected as the optimum volume for derivatization.

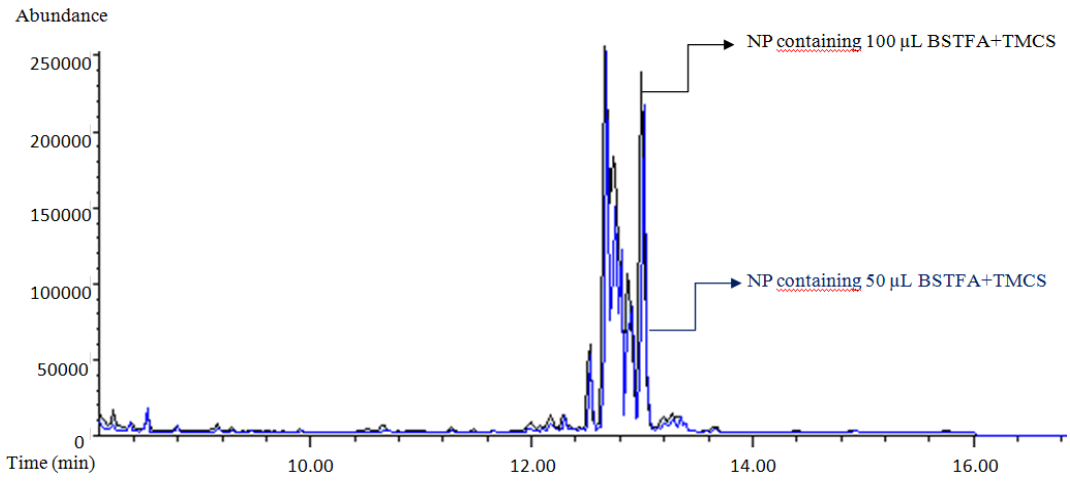


Figure 3.12 TIC for NP derivatized with 50 µL and 100 µL BSTFA TMCS

The next step in the establishment of the derivatization method is to decide on the volumetric ratio of the BSTFA+TMCS solution mix. As stated in Table 3.5, 1%, 10% and 33.3% of BSTFA+TMCS solutions on volumetric basis have been prepared and experimented for 100 µL volume. As the percentage of TMCS increases, the effectiveness of derivatization declines and this is best shown in Figure 3.13.

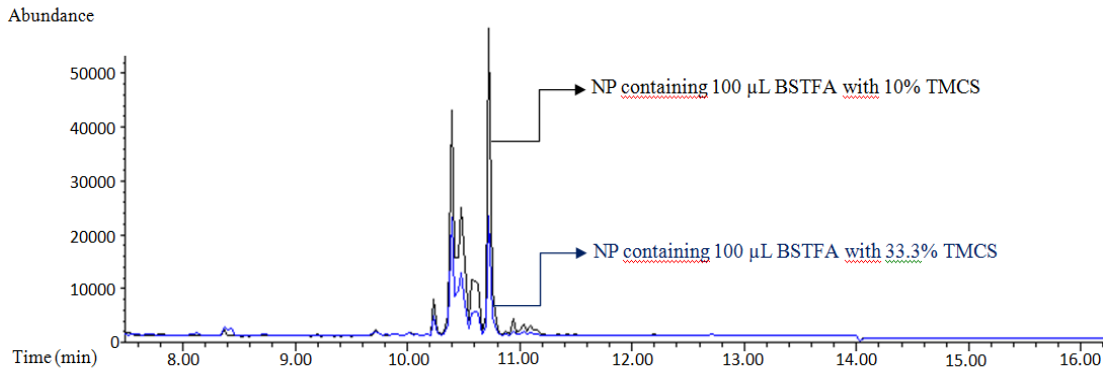


Figure 3.13 TIC for NP derivatized with 100 µL BSTFA+10%TMCS and BSTFA+33.3% TMCS

As can be seen in Figure 3.13, the abundance and resolution of the peaks for 10% TMCS is better than 33.3% TMCS. Also the integrated area of BSTFA+10%TMCS is 4265590 and it is greater than that of BSTFA+33.3%TMCS which is 1927378.

When BSTFA+1%TMCS and BSTFA+10% TMCS were compared, it was seen that they were close to each other both in terms of integrated areas and peak resolutions (see Figure 3.14). In order to save chemicals it has been decided to use BSTFA+1%TMCS. Besides this, rather than preparing the 10% TMCS solution in the laboratory, it is a better decision to use the prepacked 100 µL BSTFA+1%TMCS ampoules to exclude the experimental errors from the picture.

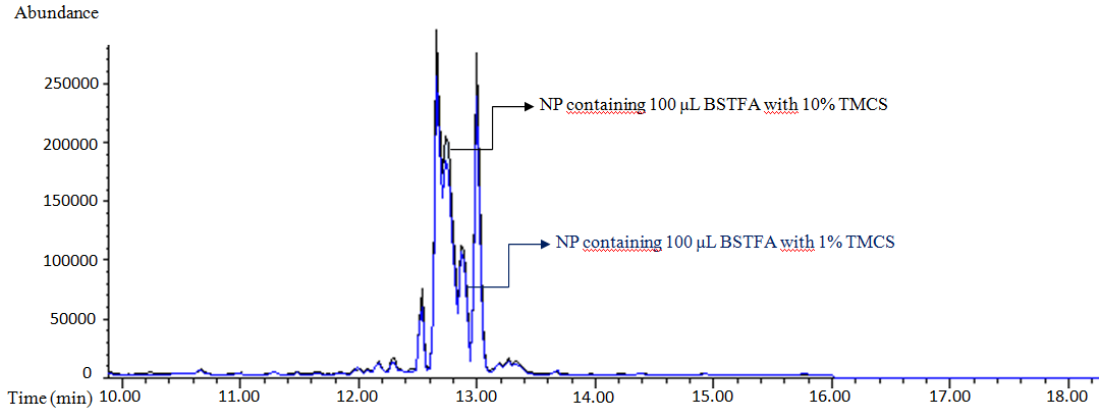


Figure 3.14 TIC for NP derivatized with 100 µL BSTFA+1%TMCS and BSTFA+10% TMCS

As a result of all the trials made on the components of the derivatization method, the reagent selected was the mixture of BSTFA and TMCS. The volume of the solution has been decided to be 100 µL with 1%TMCS by volume. Drying oven has been decided to be used as heating device at 70°C. The time of derivatization was 30 min. The details of the ultimate derivatization method are given in Figure 3.15.

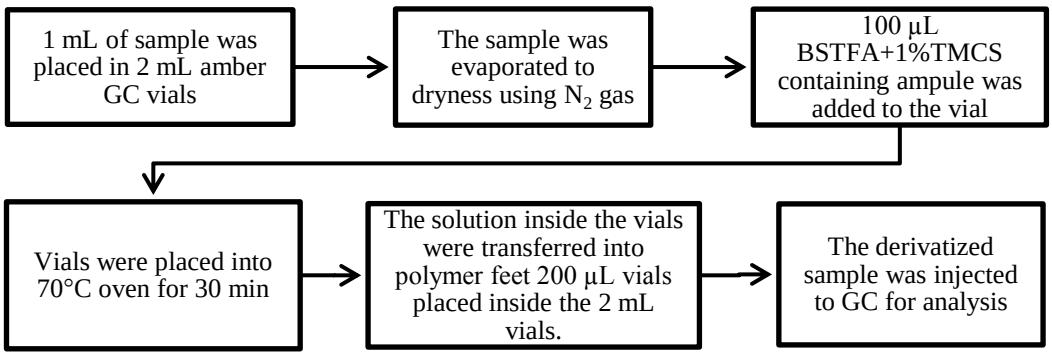


Figure 3.15 Flow scheme of the selected derivatization method

Since the characteristics of carboxylated forms of NPs are fairly different, the method of derivatization employed also differs from the previous method. NP1EC is the only carboxylated form of NP compounds that is under research in this study. At the very beginning of the experiments, the silyl derivatization of NP1EC was investigated, and this method was not satisfactory. Besides, silylation of NP1EC as a derivatization method is not commonly seen in the literature.

In order to derivatize NP1EC, three different methods have been experimented. The first method is the one applied by Diaz et.al., 2002. In Figure 3.16, the details of the procedure are presented.

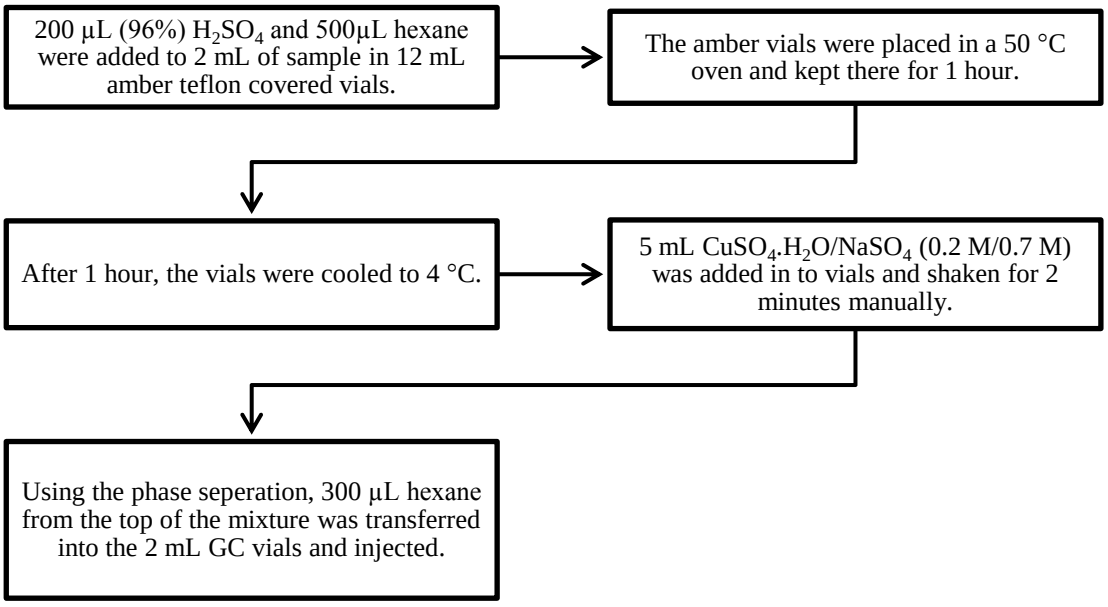


Figure 3.16 Derivatization procedure applied by Diaz et.al., 2002

The method given in Figure 3.16 has been applied without a modification. The GC program given in the article has been used for GC/MS analysis. The chromatogram

obtained and the one given by Diaz et.al., 2002 in their study are given in Figure 3.17 and Figure 3.18 for comparison.

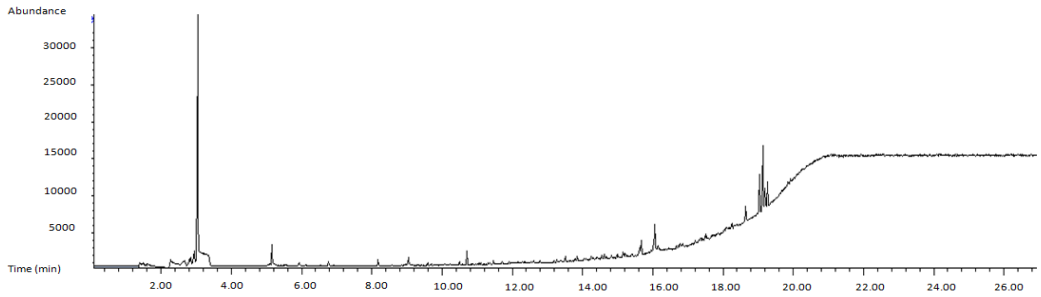


Figure 3.17 TIC for NP1EC derivatized according to Diaz et.al., 2002

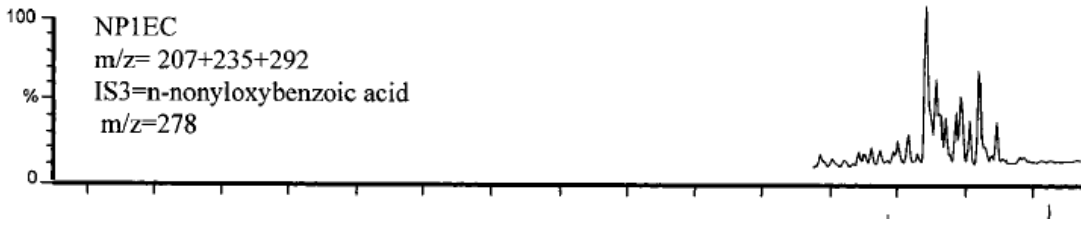


Figure 3.18 TIC given by Diaz et.al., 2002

As can be seen from Figures 3.17 and 3.18, the method proposed by Diaz et.al., 2002, was not successful in the derivatization of NP1EC. As a result, other methods of derivatization were investigated. The second method experimented was the one proposed by Ding and Tzing, 1998. The details of the methods are given in Figure 3.19.

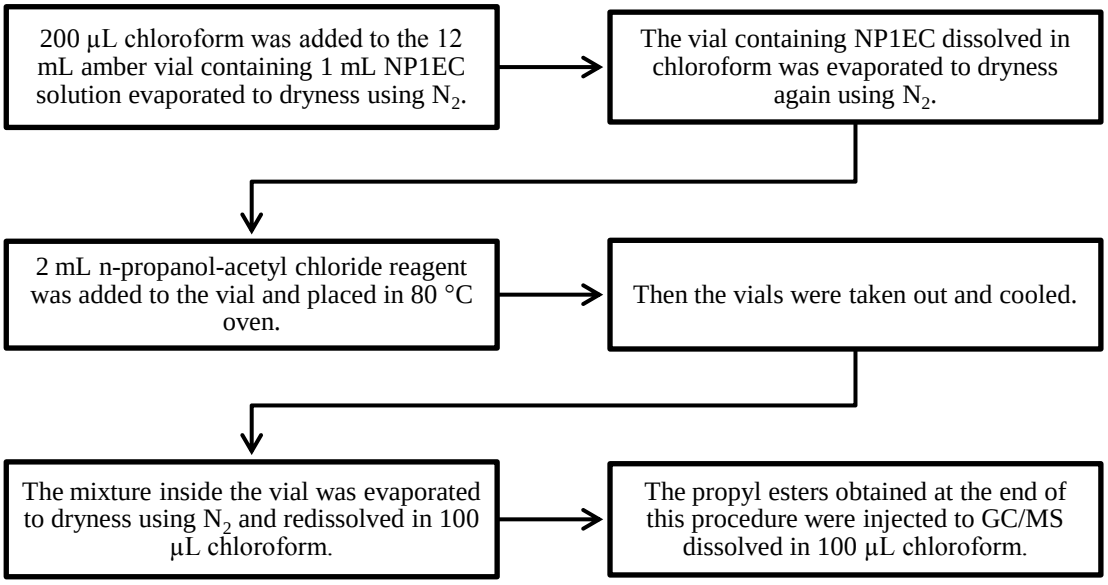


Figure 3.19 Derivatization procedure applied by Ding and Tzing, 1998

The derivatized samples obtained according to the method given above were analyzed using the GC/MS method given by Ding and Tzing, 1998 (see Figure 3.20).

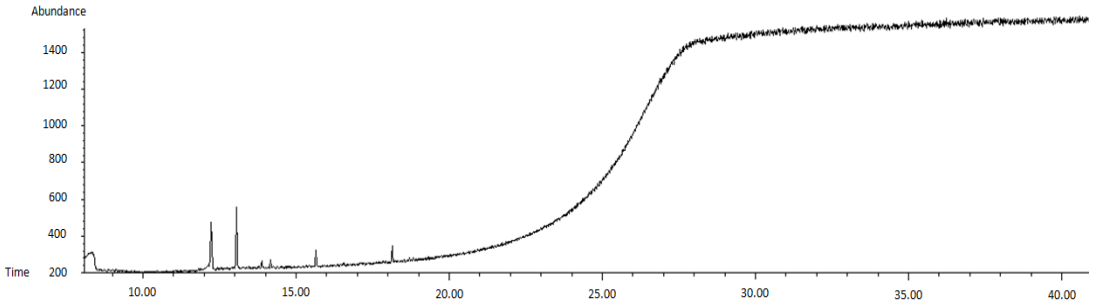


Figure 3.20 TIC for NP1EC derivatized according to Ding and Tzing, 1998

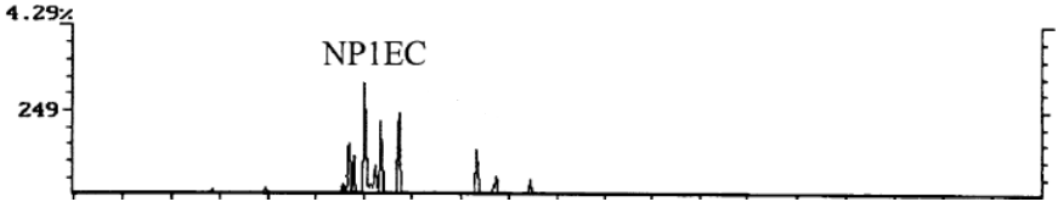


Figure 3.21 TIC given by Ding and Tzing, 1998

As can be seen from Figures 3.20 and 3.21, the second derivatization method experimented did not work either. Since both of the methods did not yield satisfactory results, the derivatization method applied by Lee et.al., 1997, was experimented with some modifications in the solvent used and volumes added. The details of the method are given in Figure 3.22.

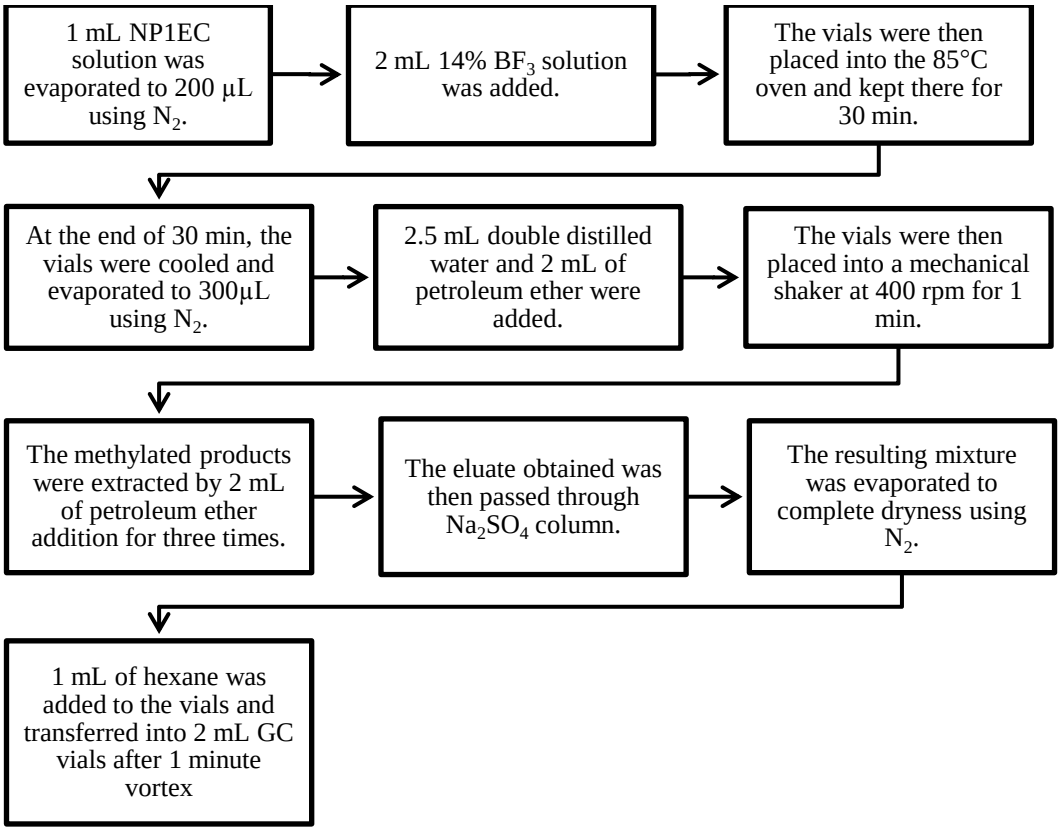


Figure 3.22 Derivatization procedure applied by Lee et.al., 1997

Like in the previous methods, the derivatized samples obtained are injected into the GC/MS using the program applied in the study of Lee et.al., 1997. The result of the analysis is given in Figure 3.23.

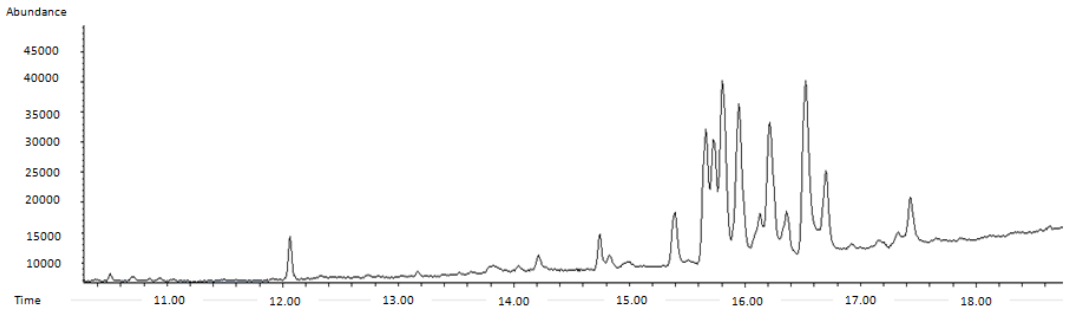


Figure 3.23 TIC for NP1EC derivatized according to Lee et.al., 1997

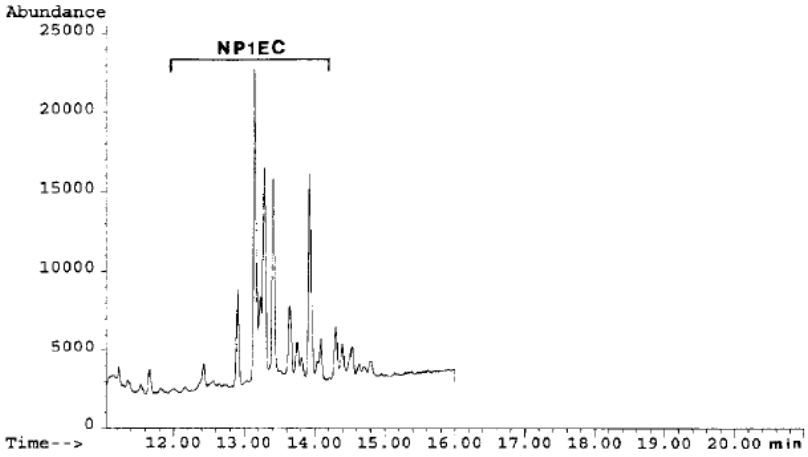


Figure 3.24 TIC given by Lee et.al., 1997

When the TICs given in Figure 3.23 and 3.24 are compared to each other, it is obvious that this last method applied was successful in the derivatization NP1EC. This way, the derivatization method applied by Lee et.al., 1997 was selected as the method to be used.

3.5.4 Development of a Gas Chromatography-Mass Spectrometry Method for the Determination of Nonylphenol Compounds (NP, NP1EO and NP2EO)

In order to determine the GC-MS program for the analysis of NP compounds, a detailed literature research was conducted to benefit from the experiences in this field. The details of the programs being experimented in the first place are given in Table 3.6. The programs given in this table include some modifications (i.e. helium flow, initial temperature, etc.), when compared to the original studies, according to the solvent used in this study and the features of the GC-MS device used. In all of these programs the carrier gas was helium and the mode of injection was splitless. The type of column was Agilent 19091S-433E HP-5MS 5% phenyl methyl siloxane (30 mx0.25mmx0.25μm). The mass spectrometry scanning was done by selective ion monitoring (SIM) mode, meaning the compounds were identified using the mass to charge (m/z) ratios given specifically for each. The reason for that is SIM mode increases the sensitivity and eventually the accuracy of the analysis.

Since NP is the most commonly analyzed chemical in the literature and thermally stable among the other NP compounds, programs given in Table 3.6 were compared on NP, and then the ones found satisfactory were verified with other compounds.

All these programs in Table 3.6 were applied to the compounds without derivatization. Unfortunately at low concentrations, these methods did not make satisfactory readings in the analysis. Since these low concentrations are critical for the analysis in reactors derivatization methods have been investigated as presented in the previous part. Then according to the derivatization method, other methods have been searched. Since the derivatization method used was very close to the one presented by Gatidou et.al., 2007, their method of analysis was used along with other methods in the literature and the ones developed in our laboratory.

Table 3.6 GC-MS programs experimented (1)

Name of Method	Agilent Technologies, 2007	Aparicio et.al., 2007	Barber et.al., 2000	Diaz et.al., 2002
Injection Volume	1 µL	1 µL	1 µL	1 µL
Injection Temperature	250°C	250°C	280°C	250°C
Gas Flow (mL/min)	1.2	1.0	1.5	1.5
MS Interphase Temperature	280°C	280°C	280°C	280°C
MS Source Temperature	230°C	230°C	230°C	230°C
MS Quadrupole Temperature	150°C	150°C	150°C	150°C
Initial Temperature	60°C	70°C	100°C	70°C
Ramps	60°C for 1 min 10°C/min to 120°C 5°C/min to 150°C 10°C/min to 240°C	70°C for 2 min 25°C/min to 150°C 3°C/min to 200°C 8°C/min to 280°C 280°C for 15 min	100°C for 1 min 6°C/min to 300°C 300°C for 15 min	70°C for 3 min 20°C/min to 160°C 10°C/min to 285°C 285°C for 7 min
Duration	22.0 min	57.0 min	49.3 min	27.0 min

Table 3.6 GC-MS programs experimented (1-Cont'd)

Name of Method	Field and Reed, 1996	Gatidou et.al., 2007	Gibson et.al., 2005	ISO 18857-2, 2009
Injection Volume	2 µL	2 µL	1 µL	2 µL
Injection Temperature	280°C	250°C	250°C	280°C
Gas Flow (mL/min)	1.5	0.9	1.0	1.0
MS Interphase Temperature	280°C	280°C	280°C	280°C
MS Source Temperature	230°C	230°C	230°C	230°C
MS Quadrupole Temperature	150°C	150°C	150°C	150°C
Initial Temperature	70°C	40°C	60°C	60°C
Ramps	70°C for 1 min 20°C/min to 300°C 300°C for 1 min	40°C for 1 min 15°C/min to 220°C 5°C/min to 280°C	60°C for 1 min 10°C/min to 280°C 280°C for 7 min	60°C for 1 min 10°C/min to 280°C 280°C for 10 min
Duration	12.5 min	25.0 min	30.0 min	25.0 min

Table 3.6 GC-MS programs experimented (1-Cont'd)

Name of Method	Isobe et.al., 2001	Li et.al., 2001	Lian et.al., 2009	Lu et.al., 2008	Richter et.al., 2009
Injection Volume	1 µL	1 µL	1 µL	1 µL	1 µL
Injection Temperature	300°C	280°C	300°C	280°C	250°C
Gas Flow (mL/min)	1.5	1.5	0.9	1.2	1.0
MS Interphase Temperature	280°C	280°C	280°C	280°C	280°C
MS Source Temperature	230°C	230°C	230°C	200°C	230°C
MS Quadrupole Temperature	150°C	150°C	150°C	150°C	150°C
Initial Temperature	70°C	50°C	50°C	130°C	100°C
Ramps	70°C for 2 min 30°C/min to 180 °C 2°C/min to 200°C 30°C/min to 310°C 310°C for 10 min	50°C for 2 min 20°C/min to 100°C 10°C/min to 200°C 20°C/min to 290°C 290°C for 2 min	50°C for 1 min 20°C/min to 200 °C 200°C for 2 min 5°C/min to 235°C 235°C for 5 min 25°C/min to 280°C 280°C for 5 min	5°C/min to 280°C 280°C for 20 min	100 °C for 1min 10°C/min to 280°C 280°C for 3 min
Duration	22.0 min	21.0 min	29.3 min	50.0 min	22.0 min

The programs applied by Field and Reed, 1996, Isobe et.al., 2001, and ISO 18857-2 yielded no peaks in the total ion chromatogram and therefore failed in the analysis of NP compounds. Some examples of TIC obtained for the programs given are presented in Figures 3.25, 3.26 and 3.27.

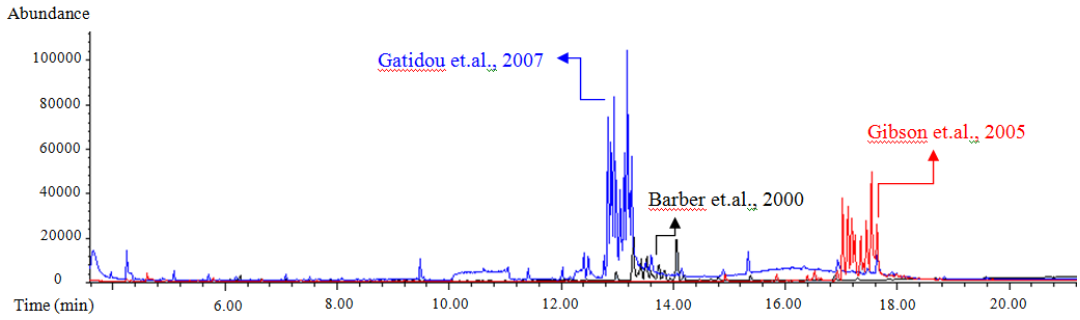


Figure 3.25 Example TIC: Comparison of NP (1ppm) analyzed via Gatidou et.al., 2007, Gibson et.al., 2005 and Barber et.al., 2000

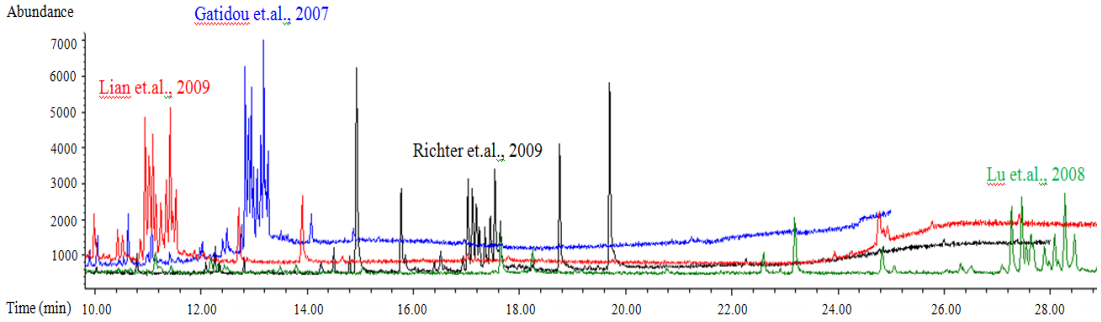


Figure 3.26 Example TIC: Comparison of NP (0.1 ppm) analyzed via Gatidou et.al., 2007, Lian et.al., 2009, Lu et.al., 2008 and Richter et.al., 2009

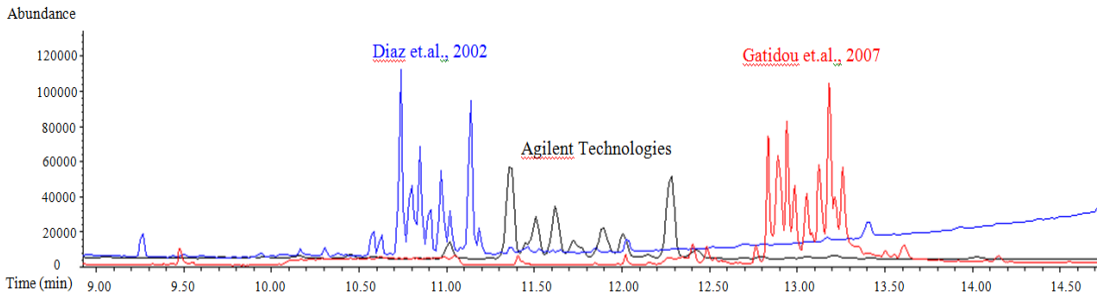


Figure 3.27 Example TIC: Comparison of NP (1 ppm) analyzed via Gatidou et.al., 2007, Diaz et.al., 2002, and Agilent Technologies, 2007

As can be seen from Figure 3.25, 3.26 and 3.27, the GC-MS methods Gatidou et.al., 2007 and Diaz et.al., 2002 were the ones that showed the best TIC for the analysis of NP. When these two methods were considered for the other chemicals, the results were again too close to each other both in terms of resolution of the peaks and abundances. But in order to analyze all NP compounds in this study, each one has to appear on the chromatogram on a different retention time, each of which come as a number of isomeric peaks, if possible. In these two programs, there was the risk that NP compounds would be close to each other. That's why, it was decided to develop a GC-MS method based on these two sources but obtain peaks with relatively different detention times. The methods experimented are presented in Table 3.7. In all four methods, the column used was Agilent 19091S-433E HP-5MS 5% phenyl methyl siloxane (30 mx0.25mmx0.25µm) and the samples were derivatized according to the method given in Figure 3.15. Splitless injection was carried out and the sample volume was 2 µL to assure homogenous injection into the device and the injection temperature was 250 °C. The carrier gas was helium, but rather than changing the flow, this time constant pressure (10.152 psi) mode was applied. The ions of the compounds were detected via SIM mode. The Interphase, MS source and MS quadrupole temperatures were the same for all four programs as 280°C, 230°C and 150°C.

Table 3.7 GC-MS programs experimented (2)

Name of the program	Program-1 (P1)	Program-2 (P2)	Program-3 (P3)	Program-4 (P4)
Initial temperature	100°C		100°C	
Ramps	100°C for 5 min 25°C/min to 160°C 10°C/min to 200°C 8°C/min to 240°C 10°C/min to 250°C 250°C for 5 min 35°C/min to 285°C 285°C for 7 min		100°C for 5 min 25°C/min to 160°C 10°C/min to 260°C 260°C for 5 min 35°C/min to 285°C 285°C for 7 min	
EM Volts	1400	1494	1400	1494
Duration (min)	30.4		30.114	

The TICs obtained when the programs given in Table 3.7 were applied are presented in Figure 3.28 to Figure 3.30.

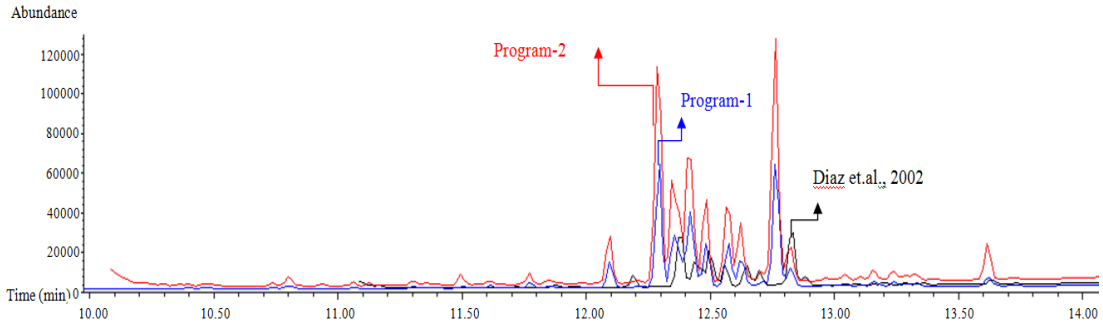


Figure 3.28 Comparison of TICs for NP analyzed using Diaz et.al.,2002, Program-1 and Program-2

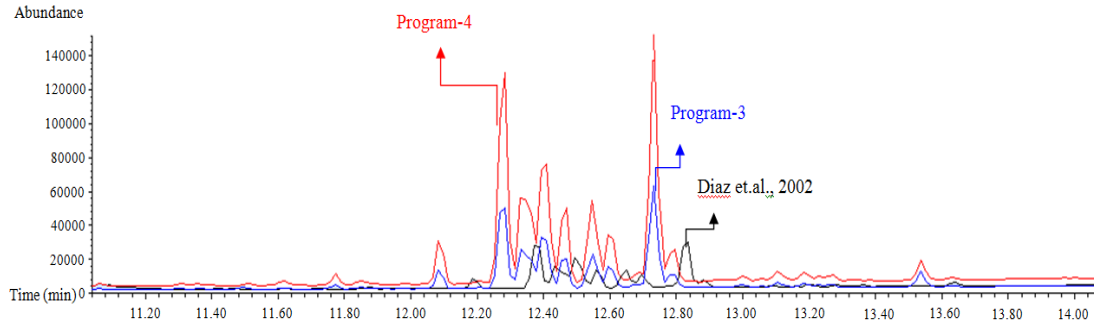


Figure 3.29 Comparison of TICs for NP analyzed using Diaz et.al.,2002, Program-3 and Program-4

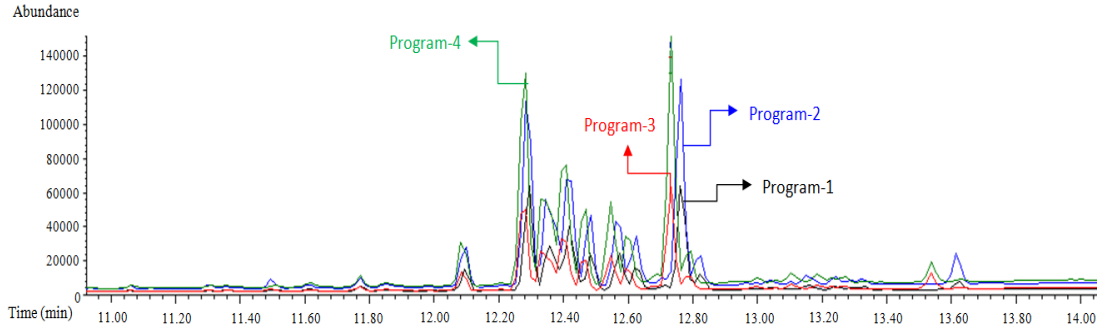


Figure 3.30 Comparison of TICs for NP analyzed using Program-1, Program-2, Program-3 and Program-4

When all four GC-MS programs given in Table 3.7 were evaluated based on the chromatograms presented, it was obvious that Program-4 yielded the best results in terms of peak quality (resolution and abundance) and integrated area under the family of peaks obtained. Therefore, it was decided to use Program-4. The injection volume was decreased to be 1 µL since the BSTFA+TMCS mix may affect the column negatively if injected at high volumes. And since the volume of injection was decreased, EM volts have been increased to ~1800 EM volts. As a result, the final

GC-MS program used in all analysis during the reactor operation is given in Table 3.8.

Table 3.8 GC-MS program used for the analysis of NP, 4-nNP, NP1EO and NP2EO

Column	Agilent 19091S-433E HP-5MS 5% phenyl methyl siloxane (30 mx0.25mmx0.25μm)
Carrier Gas	Helium
Mode	Constant Pressure (10.152 psi)
Flow	1.0043 mL/min
Injection Temperature	280 °C
Injection Mode	Splitless
Injection Volume	1 μL
MS Interphase Temperature	280°C
MS Source Temperature	230°C
MS Quadrupole Temperature	150°C
EM Volts	1718
Initial Temperature	100°C
Ramps	100°C (hold for 5 min), 25°C/min to 160°C, 10°C/min to 260°C (hold for 5 min) and 35°C/min to 285°C (hold for 7 min)
Final Temperature	285°C
Duration	30.114 min

When chemicals NP, 4-nNP, NP1EO and NP2EO were all silyl derivatized and analyzed using the program given in Table 3.8, the resulting TIC is given in Figure 3.31.

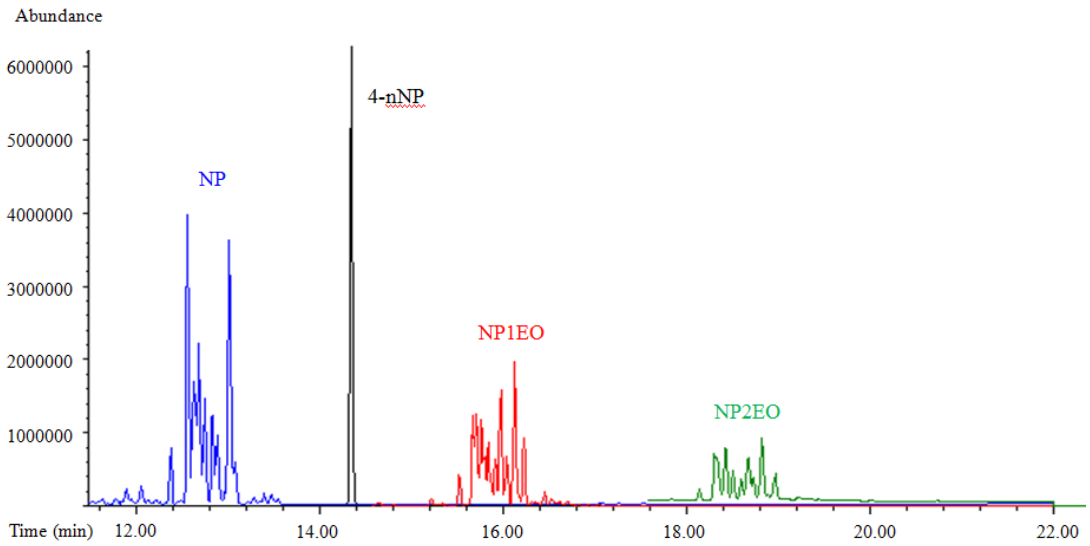


Figure 3.31 TIC obtained for NP, 4-nNP, NP1EO and NP2EO analyzed by the selected GC-MS program

As stated before, the compounds were analyzed using SIM mode. NP compounds have numerous isomers and each isomer have its characteristic target and quantification ions. For each chemical (NP, 4-nNP, NP1EO and NP2EO) these ions are listed in Table 3.9 to Table 3.12 together with their retention times.

Table 3.9 Target and quantification ions of silyl derivatized NP according to their retention times

Isomer Number	Retention Time (min)	Target Ion	Quantification Ions
NP-I1	12.384	193	107, 135, 150, 179, 207, 235, 277, 292
NP-I2	12.570	207	107, 135, 150, 179, 193, 235, 277, 292
NP-I3	12.632	207	107, 135, 150, 179, 193, 235, 277, 292
NP-I4	12.694	207	107, 135, 150, 179, 193, 235, 277, 292
NP-I5	12.756	235	107, 135, 150, 179, 193, 207, 277, 292
NP-I6	12.848	207	107, 135, 150, 179, 193, 235, 277, 292
NP-I7	12.895	235	107, 135, 150, 179, 193, 207, 277, 292
NP-I8	13.034	207	107, 135, 150, 179, 193, 235, 277, 292
NP-I9	13.081	207	107, 135, 150, 179, 193, 235, 277, 292

*NP-I: Nonylphenol isomer

Table 3.10 Target and quantification ions of silyl derivatized 4-nNP according to their retention times

Isomer Number	Retention Time (min)	Target Ion	Quantification Ions
4-nNP-I1	14.364	179	292

*4-nNP-I: Nonylphenol isomer

Table 3.11 Target and quantification ions of silyl derivatized NP1EO according to their retention times

Isomer Number	Retention Time (min)	Target Ion	Quantification Ions
NP1EO-I1	15.524	279	237, 251, 265, 293, 307
NP1EO-I2	15.670	251	237, 265, 279, 293, 307
NP1EO-I3	15.711	265	237, 251, 279, 293, 307
NP1EO-I4	15.763	251	237, 265, 279, 293, 307
NP1EO-I5	15.803	251	237, 265, 279, 293, 307
NP1EO-I6	15.847	265	237, 251, 279, 293, 307
NP1EO-I7	15.919	279	237, 251, 265, 293, 307
NP1EO-I8	15.982	265	237, 251, 279, 293, 307
NP1EO-I9	16.044	279	237, 251, 265, 293, 307
NP1EO-I10	16.128	251	237, 265, 279, 293, 307
NP1EO-I11	16.232	265	237, 251, 279, 293, 307

*NP1EO-I: Nonylphenol monoethoxylate isomer

Table 3.12 Target and quantification ions of silyl derivatized NP2EO according to their retention times

Isomer Number	Retention Time (min)	Target Ion	Quantification Ions
NP2EO-I1	18.144	323	281, 295, 309
NP2EO-I2	18.306	295	281, 309, 323
NP2EO-I3	18.334	309	281, 295, 323
NP2EO-I4	18.426	295	281, 309, 323
NP2EO-I5	18.510	309	281, 295, 323
NP2EO-I6	18.595	323	281, 295, 309
NP2EO-I7	18.672	309	281, 295, 323
NP2EO-I8	18.729	323	281, 295, 309
NP2EO-I9	18.820	295	281, 309, 323
NP2EO-I10	18.961	309	281, 295, 323

*NP2EO-I: Nonylphenol diethoxylate isomer

Once the isomers and their retention times are set, the calibration curves for each chemical were constructed. For the compounds NP, 4-nNP, NP1EO and NP2EO, the calibration curves were prepared for the concentrations between 10 ppb and 1000 ppb, making 8 points in total. Calibration curves require revision whenever a change occurs in the device (change of liner, column, etc). The calibration curves used for the analysis of chemicals during the operation of reactors is given in Appendix-A. Each point on curve is the average of duplicate injections and duplicate GC-MS readings making four data per point for each concentration. The integrated areas and their standard deviations for calibration curve are given Appendix-C. The R^2 values in these curves show how closely the data fits to a linear relationship. If the number is close to 1, as in all calibration curves obtained here, it means the data set shows almost perfect fit and good correlation. Therefore, the calibration curves obtained were reliable and can be used in the data computation.

3.5.5 Gas Chromatography-Mass Spectrometry Method for the Determination of Nonylphenoxy Acetic Acid

In the beginning of the laboratory studies on GC-MS method development to analyze NP compounds, the target was to analyze all NP compounds using single GC-MS program, if possible. Unfortunately, this didn't hold for NP1EC. Early on, when setting up the calibration curves silylation was being applied to derivatize NP1EC and the GC-MS program was the one stated in Table 3.8 and the TIC obtained as a result is given in Figure 3.32. However, together with the extraction studies, it was understood that silylation is not the correct method to derivatize NP1EC and search for other methods became obligatory. The methods used for GC-MS analysis of NP1EC (besides the one given in Table 3.8) are presented in Table 3.13.

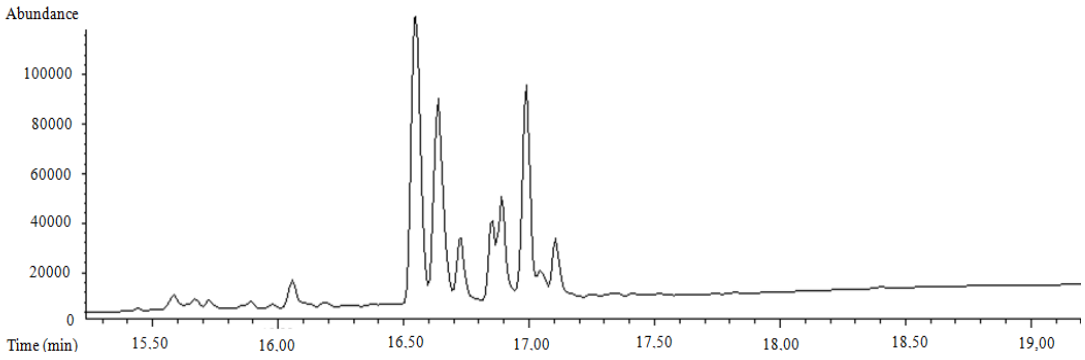


Figure 3.32 TIC for silyl derivatized NP1EC analyzed by the GC-MS program given in Table 3.8

For the analysis of NP1EC, type of column, mode of injection and mass spectrometry were the same as the ones for NP, NP1EO and NP2EO analysis given in the previous section.

Table 3.13 GC-MS methods tested in the analysis of NP1EC

Name of the program	Diaz et.al., 2002	Ding and Tzing, 1998	Lee et.al., 1997
Injection Volume	1 µL	1 µL	1 µL
Injection Temperature	250°C	250°C	250°C
Gas Flow	1.2 mL/min	1.0 mL/min	1.2 mL/min
MS Interphase Temperature	280°C	280°C	280°C
MS Source Temperature	230°C	230°C	230°C
MS Quadrupole Temperature	150°C	150°C	150°C
EM Volts	1718	1718	1800
Initial Temperature	70°C	100°C	70°C
Ramps	70°C for 3 min	100°C for 5 min	70°C for 1 min
	20°C/min to 160°C	8.5°C/min to 280°C	30°C/min to 160°C
	10°C/min to 285°C	280°C for 15 min	5°C/min to 290°C
	285°C for 7 min		290°C for 5 min
Duration	27.0 min	41.18 min	35 min

When all four programs (3 given in Table 3.13 and the one selected for NP, 4-nNP, NP1EO and NP2EO) were experimented the best methods for the analysis of NP1EC was found to be the one given by Lee et.al., 1997. For each GC-MS program, the original derivatization methods given in the articles were also applied. The chromatograms obtained were given in Figure 3.17, 3.20 and 3.23. As can be seen from the Figures, when the method proposed by Diaz et.al., 2002 was applied, no peak was detected and this is the same for Ding and Tzing, 1998. However, as can be seen from Figure 3.23 (in the previous sections), by Lee et.al., 1997 method, the peak quality (resolution and abundance) obtained for NP1EC was satisfactory. Therefore, it was decided to use GC-MS method proposed by Lee et.al., 1997, which is given in Table 3.13.

SIM mode was used for the analysis of NP1EC like the other four NP compounds. Just like the other NP compounds, NP1EC also has numerous isomers and the m/z ratios for each isomer according to their retention times are given in Table 3.14.

Table 3.14 Target and quantification ions of methyl derivatized NP1EC in accordance with their retention times

Isomer Number	Retention Time (min)	Target Ion	Quantification Ions
NP1EC-I1	14.767	179	193, 207, 221, 292
NP1EC-I2	14.871	179	193, 207, 221, 292
NP1EC-I3	14.968	179	193, 207, 221, 292
NP1EC-I4	15.116	207	107, 193, 221, 292
NP1EC-I5	15.212	179	193, 207, 221, 292
NP1EC-I6	15.300	221	107, 193, 207, 292
NP1EC-I7	15.405	207	107, 193, 221, 292
NP1EC-I8	15.544	207	107, 193, 221, 292
NP1EC-I9	15.684	207	107, 193, 221, 292
NP1EC-I10	15.973	179	193, 207, 221, 292
NP1EC-I11	16.104	193	107, 207, 221, 292
NP1EC-I12	16.261	207	107, 193, 221, 292
NP1EC-I13	16.427	221	107, 193, 207, 292

*NP1EC-I: Nonylphenoxy acetic acid isomer

Once the GC-MS method is set, calibration of the device was done so as to quantitate the TICs obtained. Just like the calibration curves obtained for NP, 4-nNP, NP1EO and NP2EO, again the R^2 value obtained for the curve has been checked for the reliability of the curve. For NP1EC, different from the rest, 6 points between 50 ppb and 1000 ppb were used for calibrating the device. The calibration curve obtained for NP1EC is also given in Appendix-A and the details of calibration curve data are given in Appendix-C.

3.6 Development of Extraction Methods for Nonylphenol Compounds

Extraction is an analytical technique applied in order to isolate a certain compound from a complex matrix. It is generally applied to prepare a sample prior to chromatographic analysis. Just like the GC-MS method determination, extraction procedure should be established based on the characteristics of the compound in question. That's why first possible methods used to extract NP compounds were searched and then solvent selection based on the polarity of compounds was carried out.

3.6.1 Extraction of Nonylphenol Compounds from Solid Phase

There are several methods in the literature for the extraction of NP compounds from solid phase (i.e. sludge, soil, sediment, etc.). Some of the methods applied are soxhlet extraction, sonication, mechanical shaking (or combination of sonication and mechanical shaking), microwave assisted extraction, pressurized liquid extraction or accelerated/enhanced solvent extraction. A summary of the methods applied to extract NP compounds are given in Table 3.15.

Table 3.15 Extraction methods used to isolate NP compounds from solid phase

Sample	Method	Solvent	Reference
Sludge	Sonication+mechanical shaking	Dichloromethane	Abad et.al., 2005
Sludge	Mechanical shaking+sonication	Hexane	Aparicio et.al., 2007
Sludge	Soxhlet	Methanol	Fountoulakis et.al., 2005
Sludge	Sonication	Hexane:acetone (1:1) or methanol:dichloromethane (7:3)	Fountoulakis et.al., 2005
Sludge	Sonication+solid phase extraction	Methanol:distilled water (5:3)	Gatidou et.al., 2007
Sludge	Sonication+mechanical shaking	Hexane	Gonzalez et.al., 2010
Sludge	Enhanced solvent extraction	Dichloromethane	La Guardia et.al., 2001
Sludge	Liquid-liquid extraction	Ethyl acetate:dichloromethane	Lu et.al., 2008
Sludge	Ultrasonication+solid phase extraction	Methanol:acetone (1:1) + dichloromethane:acetone (7:3)	Nie et.al., 2009
Sludge	Sonication	Methanol:dichloromethane: (7:3)	Petrovic and Barcelo, 2000
Sludge	Sonication	Acetone:methanol (1:1)	Pothitou and Voutsa, 2008
Sludge	Soxhlet extraction	Ethyl acetate	Pryor et.al., 2002
Sludge	Sonication	Hexane	Santos et.al., 2007

Since methods like soxhlet extraction and accelerated/enhanced solvent extraction are time and solvent consuming, first sonication and mechanical shaking methods were applied and compared for the extraction of NP compounds.

In sonication, the compound of interest is mainly extracted via the sound waves travelling in the solvent used. With the help of sound waves, the compound will be detached from the solid phase and transferred into the solvent. But whether it is sonication or mechanical shaking applied (regardless of the type of force being applied), solvent selection is critical. Without the proper solvent, extraction recovery will not be satisfactory. The hydrophobicity and affinity of the solvent to the chemical to be extracted must be suitable. In most of the studies given in Table 3.15, acetone, methanol and mixtures of these two solvents were commonly used, so in the extraction tests of NP compounds acetone and methanol were included. Dichloromethane is also another organic solvent used, however, since it is very toxic and dangerous to work with it was not considered as a major option. Besides it did not yield satisfactory results at the very beginning when stock solutions were prepared and injected into GC-MS. Rather than using dichloromethane, hexane was preferred and compared with acetone and methanol. TIC obtained for NP with these three solvents is given in Figure 3.33.

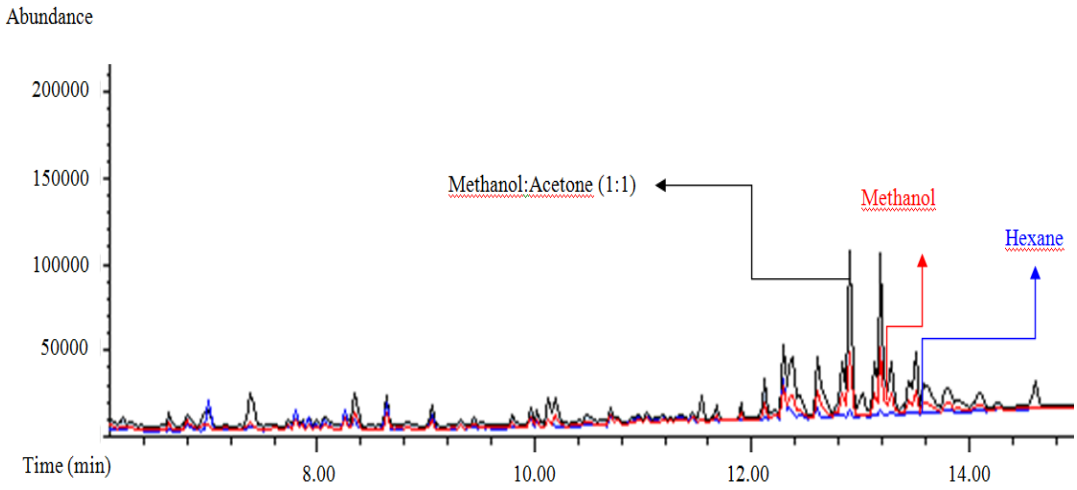


Figure 3.33 Comparison of different solvents for the extraction of NP

As can be seen from Figure 3.33, it is obvious that hexane is not a proper solvent for the extraction of NP compounds from sludge. According to the TIC given in this Figure, it was decided to consider three solvents in the extraction recovery tests: acetone, methanol and acetone:methanol (1:1, v/v) mix. The parameters that were tested during the extraction of NP compounds from sludge are given in Table 3.16. During the extraction studies clean soil was used since it was thought soil and sludge have similar characteristics (i.e. affinity for NPEs). Clean soil was obtained by soxhlet extraction (acetone:hexane-1:1, v/v for 16 hours) followed by sonication and drying in the oven (105°C). The resulting dry matter was then sieved through 1 mm sieve and stored at room temperature in a dessicator.

Table 3.16 Parameters tested in the extraction experiments of NP compounds

Solvent	Acetone
	Acetone:methanol (1:1)
	Hexane
	Methanol
Duration of sonication	5 min
	8 min
	10 min
	20 min
	30 min
Duration of mechanical shaking	16 hr
	48 hr
Duration of sonication + mechanical shaking	1 min + 16 hr
	1 min + 24 hr

The details of the extraction methods tested are presented in Figure 3.34 and 3.35.

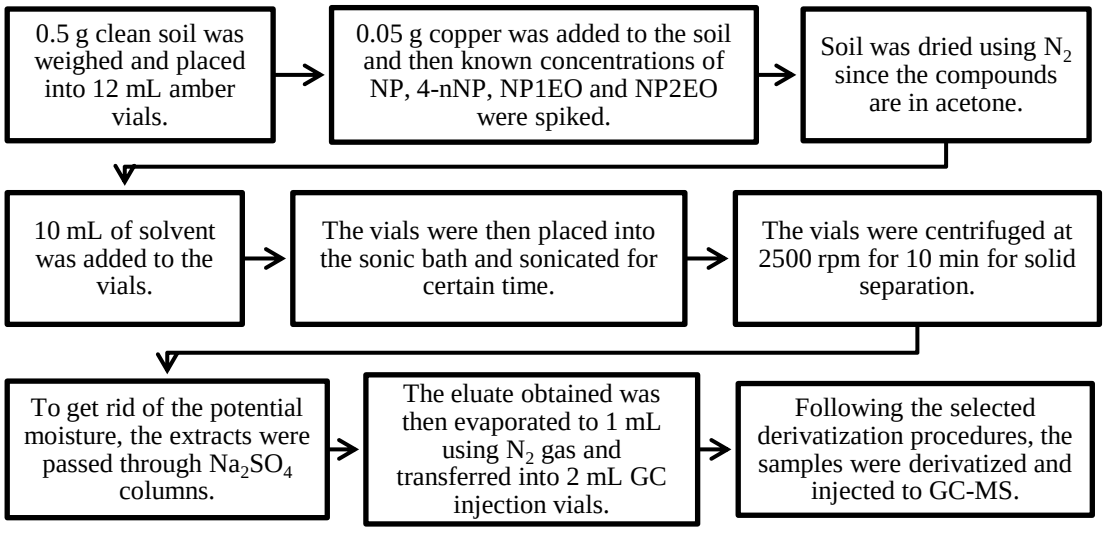


Figure 3.34 Details of the sonication based extraction method

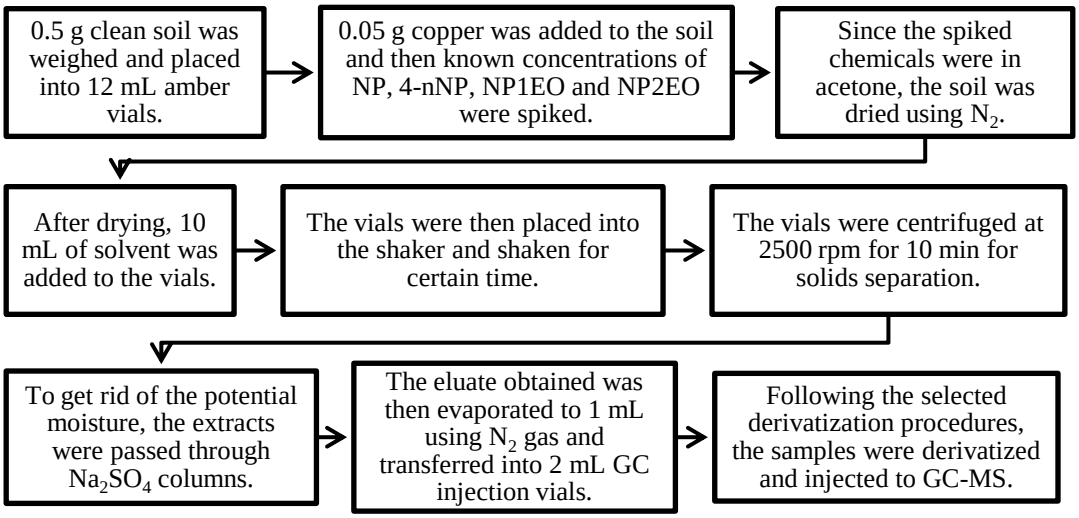


Figure 3.35 Details of the mechanical shaking extraction method

Using the methods given in Figures 3.34 and 3.35 and the GC-MS program selected in the previous section, the spiked samples were extracted in triplicate samples each time to calculate recovery and make comparisons of the solvents and methods. In all extraction experiments, soil blanks were also subjected to extraction procedures given to consider possible NP contribution from the non-spiked soil sample (even if it was cleaned). The results obtained from the spiked samples were used to calculate recoveries. Even though, all the glassware and syringe used during the studies were cleaned meticulously, soil-free 12 mL amber vials were also filled with solvent and subjected to extraction. These were called clean blanks and they were used to test whether there was any experimental contamination. Since clean blanks were free of NP compounds, they were not considered during the recovery calculations, however every once in a while during the experiments they were injected to make sure there was no contamination from the equipments used. On the other hand soil blanks which contained the same amount of unspiked soil going through the same extraction procedure were included with every extraction experiment. While calculating the recoveries, the results from soil blanks were subtracted from the original findings. The recoveries calculated for combined sonication and mechanical shaking extraction are given between Table 3.17 and Table 3.21.

Table 3.17 Extraction efficiencies obtained for 3 min and 5 min sonication

Extraction Efficiency (% Recovery)									
Time	**3 min**				**5 min**				
Solvent	**Acetone**				**Acetone**				
Sample	**NP**	**4-nNP**	**NP1EO**	**NP2EO**	**Sample**	**NP**	**4-nNP**	**NP1EO**	**NP2EO**
S1 (1)	106.1	105.3	126.8	177.9	S1 (1)	105.0	-	140.6	86.4
S1 (2)	103.0	103.1	122.1	170.3	S1 (2)	106.9	-	142.5	86.0
S1 (3)	104.6	104.2	124.5	174.1	S1 (3)	107.5	-	139.0	81.2
-	-	-	-	-	S2 (1)	112.9	109.7	129.9	161.5
-	-	-	-	-	S2 (2)	95.7	108.1	123.4	147.7
-	-	-	-	-	S3 (1)	104.3	108.9	126.7	154.6
-	-	-	-	-	S3 (2)	107.7	115.3	127.6	145.0
-	-	-	-	-	S3 (3)	109.6	120.9	132.7	163.8
-	-	-	-	-	S3 (4)	103.1	108.5	125.1	155.7
-	-	-	-	-	S3 (5)	111.9	116.8	126.6	169.1
Ave	104.6	104.2	124.5	174.1	**Ave**	106.5	112.6	131.8	134.8
SD	1.6	1.1	2.4	3.8	**SD**	4.9	5.0	6.5	35.5
%RSD	1.5	1.1	1.9	2.2	**%RSD**	4.6	4.5	4.9	26.4

S1 (1) indicates sample 1, parallel measurement 1 and "-" means at this condition experiment was not done

Table 3.18 Extraction efficiencies obtained for 10 min, 20 min and 30 min sonication

Extraction Efficiency (% Recovery)											
Time	**10 min**			**20 min**				**30 min**			
Solvent	**Acetone**			**Acetone**				**Acetone**			
Sample	**NP**	**NP1EO**	**NP2EO**	**Sample**	**NP**	**NP1EO**	**NP2EO**	**Sample**	**NP**	**NP1EO**	**NP2EO**
S1 (1)	80.6	126.4	142.7	S1 (1)	61.1	116.1	138.3	S1 (1)	161.3	55.9	ND
S1 (2)	81.9	122.8	141.4	S1 (2)	63.0	115.9	136.4	S1 (2)	139.0	58.1	ND
S1 (3)	74.0	125.4	137.6	S1 (3)	63.3	112.6	132.5	S1 (3)	233.7	76.8	ND
S2 (1)	116.5	183.2	212.0	-	-	-	-	-	-	-	-
S2 (2)	121.3	198.0	219.2	-	-	-	-	-	-	-	-
S2 (3)	112.5	193.5	231.8	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
Ave	97.8	158.2	180.8	**Ave**	62.5	114.9	135.7	**Ave**	178.0	63.6	-
SD	21.1	36.9	44.5	**SD**	1.2	2.0	3.0	**SD**	49.5	11.5	-
%RSD	21.6	23.3	24.6	**%RSD**	1.9	1.7	2.2	**%RSD**	27.8	18.1	-

ND means not detected

Table 3.19 Extraction efficiencies obtained for 8 min sonication

Extraction Efficiency (%Recovery)												
Time	**8 min**											
Solvent	**Acetone**				**Methanol**				**Acetone:Methanol (1:1)**			
Sample	**NP**	**4-nNP**	**NP1EO**	**NP2EO**	**NP**	**4-nNP**	**NP1EO**	**NP2EO**	**NP**	**4-nNP**	**NP1EO**	**NP2EO**
S1 (1)	73.5	-	119.1	109.8	181.8	-	280.8	275.1	81.0	-	135.0	155.3
S1 (2)	75.6	-	122.6	114.7	187.7	-	286.2	272.6	94.4	-	140.6	152.5
S1 (3)	72.6	-	125.5	122.5	185.4	-	283.2	273.4	85.4	-	140.4	151.4
S2 (1)	107.3	110.6	132.9	163.9	-	-	-	-	-	-	-	-
Ave	82.3	-	125.0	127.7	184.9	-	283.4	273.7	86.9	-	138.7	153.1
SD	16.7	-	5.9	24.6	3.0	-	2.7	1.3	6.8	-	3.2	2.0
%RSD	20.4	-	4.7	19.3	1.6	-	1.0	0.5	7.9	-	2.3	1.3

Table 3.20 Extraction efficiencies obtained for 16 hr, 24 hr and 48 hr mechanical shaking

Extraction Efficiency (%Recovery)													
Time	**16 hr**			**24 hr**				**48 hr**					
Solvent	**Acetone**			**Acetone**				**Acetone**			**Acetone:Methanol (1:1)**		
Sample	**NP**	**NP1EO**	**NP2EO**	**NP**	**4-nNP**	**NP1EO**	**NP2EO**	**NP**	**NP1EO**	**NP2EO**	**NP**	**NP1EO**	**NP2EO**
S1 (1)	71.9	91.3	38.7	100.6	97.7	103.4	161.9	75.6	149.1	165.2	71.7	145.2	158.7
S1 (2)	72.9	90.7	39.5	135.8	122.1	146.9	204.3	87.9	151.8	180.6	77.4	139.0	149.0
S1 (3)	71.5	93.0	42.3	-	-	-	-	69.9	148.3	175.2	71.4	140.5	153.6
S2 (1)	-	-	-	-	-	-	-	41.8	130.9	142.4	-	-	-
S2 (2)	-	-	-	-	-	-	-	48.9	144.1	140.5	-	-	-
S2 (3)	-	-	-	-	-	-	-	42.6	134.5	140.9	-	-	-
Ave	72.1	91.7	40.2	118.2	109.9	125.2	183.1	61.1	143.1	157.5	73.5	141.6	153.8
SD	0.7	1.2	1.9	24.9	17.3	30.8	30.0	19.3	8.5	18.4	3.4	3.2	4.9
%RSD	1.0	1.3	4.7	21.1	15.7	24.6	16.4	31.6	5.9	11.7	4.6	2.3	3.2

Table 3.21 Extraction efficiencies obtained for 1 min sonication combined with 16 hr and 24 hr mechanical shaking

Extraction Efficiency (% Recovery)								
Time	**1 min + 16 hr**				**1 min + 24 hr**			
Solvent	**Acetone**				**Acetone**			
Sample	**NP**	**4-nNP**	**NP1EO**	**NP2EO**	**NP**	**4-nNP**	**NP1EO**	**NP2EO**
S1 (1)	36.9	52.5	37.5	95.3	138.1	118.7	134.5	253.0
S1 (2)	69.1	66.8	156.6	198.9	127.7	117.4	129.6	184.8
Ave	53.0	59.7	97.1	147.1	132.9	118.1	132.1	218.9
SD	22.8	10.1	84.2	73.3	7.4	0.9	3.5	48.2
%RSD	43.0	17.0	86.8	49.8	5.5	0.8	2.6	22.0

As can be seen in Table 3.18, as extraction time increases NP2EO seems to degrade into NP and NP1EO. For instance, when sonication time was 30 min, NP2EO was not detected in the sample. Therefore, it was concluded that increasing sonication times would deflect the results obtained. In Table 3.19, when the solvent used was methanol, the recoveries obtained were higher than the ones obtained for acetone and mixture of acetone and methanol. Higher does not always means better, in extraction recoveries there are ranges for chemicals and this range is generally between 70-140 % for NP compounds. That's why, methanol was eliminated. The results obtained for mechanical shaking (Table 3.20) were not repeatable as can be seen from the % RSDs obtained. Also the recoveries obtained were not satisfactory; recoveries such as 40-50% were obtained.

When sonication based extraction and mechanical shaking were compared to each other, it was found out that as shaking time increased, recovery also increased. Unfortunately, repeatability of recoveries obtained for mechanical shaking were not satisfactory and even if they were satisfactory, mechanical shaking was thought to be a time consuming method which would raise difficulties during samplings throughout reactor operation.

According to the recoveries obtained and given in Tables 3.17-3.21, it has been decided to use sonication for 5 min using acetone as solvent as the extraction method. There were several reasons for this judgment. When compared to other methods/durations of extraction, 5 min sonication based extraction yielded the most repeatable results among others. In addition, when the duration increased during sonication, it was observed that NP2EO recoveries dropped significantly, and this indicated that increasing sonication times possibly led to the degradation of NP2EO. For instance, as the duration of sonication increased from 10 min to 30 min, NP2EO disappeared and NP1EO also dropped, whereas NP increased almost 2 fold. This indicated that as the time of sonication increased, sound waves might be resulting in the destruction of ethylene oxide units and converting ethoxylated forms into NP. Therefore, it was decided to keep sonication time as short as possible and when compared to 3 min and 8 min, 5 min yielded the most accurate and repeatable results. Acetone has been selected as the extraction solvent since high and consistent recoveries were obtained for extraction tests carried out with acetone.

The extraction efficiencies obtained for 5 min sonication based extraction using acetone were generally between 100-140%, on the average (specifically 106%, 132% and 135% for NP, NP1EO and NP2EO, respectively). Those recoveries were considered acceptable considering 70-130% range stated by USEPA (USEPA, 2003). Also in several other studies made on NP compounds the recovery ranges were found out to be within the range 90-140% (Arditsoglou and Voutsa, 2008) or %70-120 (Lian et.al., 2009). In another study, recoveries above 65% were also stated to be acceptable (Gatidou et.al., 2007).

An extensive study was not conducted for NP1EC. Since from the reactors samples would be collected at once, application of the same procedure would be preferred. So for NP1EC, recoveries only for 5 min sonication assisted extraction were calculated for NP1EC. The recoveries range between 96% and 99%, which were again acceptable according to the values stated in the previous paragraph.

The details of the extraction procedure applied during reactor operation can be summarized as follows:

- 10 mL of sludge samples taken from the reactors were centrifuged at 3000 rpm for 20 min in order to separate solid and liquid phase.
- Solid part of the sample was freeze dried for 24 hours.
- 0.05 g copper and 10 mL acetone was added to the freeze-dried sludge samples and put into sonic bath for 5 min sonication.
- After 5 min sonication, the samples were then centrifuged once again at 3000 rpm for 20 min.
- Once acetone and dry solids were separated from each other, the solvent part was passed through anhydrous sodium sulfate column.
- For NP, NP1EO, NP2EO and 4-nNP, the eluate collected from the column was then evaporated to 1mL using N_2 gas and transferred into 2 mL GC vials for derivatization.
- For NP1EC, the eluate collected was evaporated to 200 µL for derivatization.
- Derivatized samples were injected into GC-MS for analysis.

Since these extraction studies were done on clean soil samples, prior to reactor operation, these compounds were also spiked into sludge samples to see whether there would be any matrix effect that would block the future analysis. Example TICs obtained for both sludge blanks and spiked sludge samples are given in Figures 3.36 and 3.37.

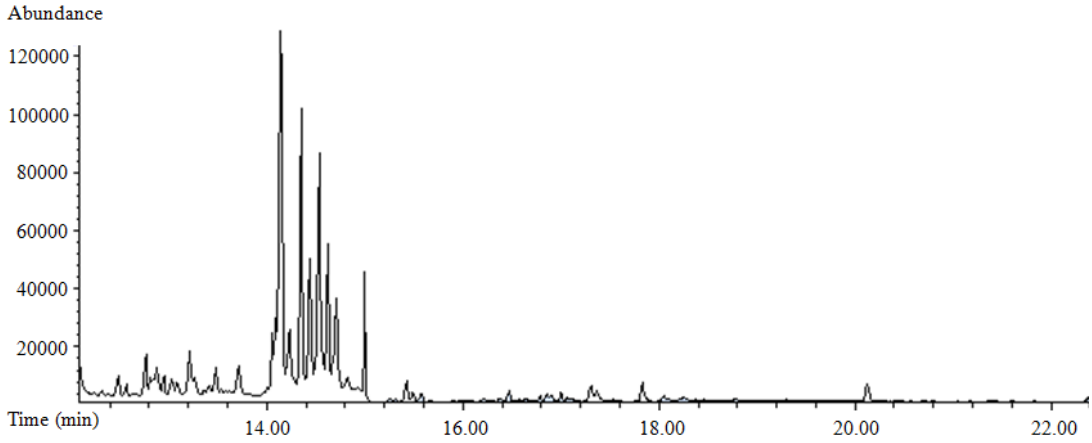


Figure 3.36 TIC obtained for NP spiked sludge extracted via 5 min sonication based extraction

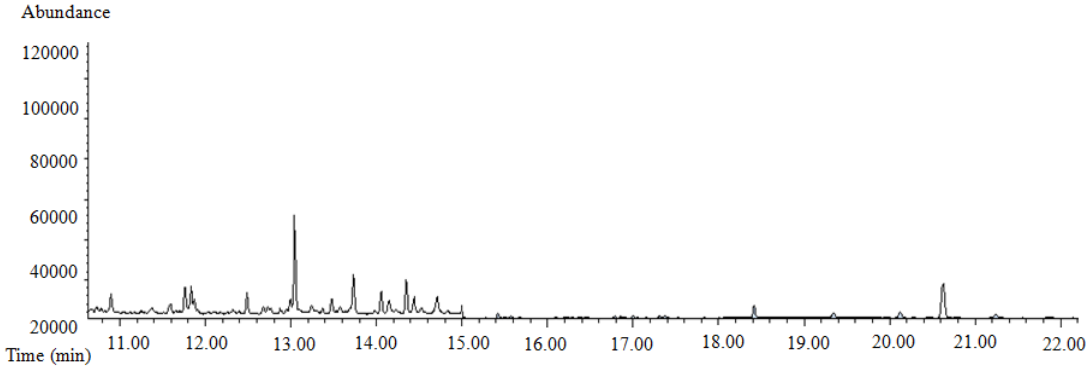


Figure 3.37 TIC obtained for sludge blank extracted via 5 min sonication based extraction

As can be seen from the Figures 3.36 and 3.37, the matrix effect was minimum, in other words, there was no interference that would block the future analyses. There were couple of peaks between 12 min and 15 min, but when these were analyzed based on the target and quantification ions of NP, it was understood that they did not belong to NP molecule. This was valid for NP1EO, NP2EO and NP1EC also. The

recoveries obtained also reflect the same results. They were repeatable, and close to the recoveries obtained for soil spiked samples. The recoveries obtained are presented in Table 3.22.

Table 3.22 Extraction efficiencies obtained for spiked sludge samples using 5 min sonication as extraction method and acetone as solvent

Extraction Efficiency (% Recovery)				
Sample	**NP**	**4-nNP**	**NP1EO**	**NP2EO**
S1 (1)	95.3	103.1	131.4	142.6
S1 (2)	95.3	102.6	132.9	145.5
S1 (3)	93.4	99.9	135.3	140.3
S2 (1)	91.1	106.2	136.5	135.3
S2 (2)	90.5	100.8	139.3	138.7
S2 (3)	89.9	104.3	142.5	135.9
S3 (1)	98.9	99.2	129.8	136.0
S3 (2)	94.9	100.8	132.0	130.4
S3 (3)	92.0	97.6	127.6	135.2
Ave	93.5	101.6	134.1	137.8
SD	2.9	2.7	4.7	4.5
%RSD	3.1	2.6	3.5	3.3

3.6.2 Extraction of Nonylphenol Compounds from Liquid Phase

Sludge is a semi-solid substance, including both solid and liquid phases. Even though, most of the NP compounds are hydrophobic and tend to stay on solid phase, to carefully monitor degradation of these compounds during reactor operation, it is crucial to analyze NP compounds in liquid phase. Just like in the extraction from the solid phase, there are several methods stated in the literature for the extraction of NP compounds from liquid phase. Until recently, mainly liquid-liquid extraction was

applied however, it is a time and solvent consuming method, so nowadays, liquid-liquid extraction started to lose its popularity. Besides, during liquid-liquid extraction, phase separation cannot be achieved with 100% efficiency all the time, resulting in emulsion phase formation. Methods that are far more effective, cheaper and applicable are being searched now. Today the most commonly applied methods are liquid-liquid extraction, solid phase extraction (SPE) and solid phase microextraction (SPME) (Gatidou et.al., 2007). The summary of the methods applied in the studies conducted on NP compounds are given in Table 3.23.

SPE is an extraction method that has extensive use lately since it uses low volumes of solvent, results in higher recoveries and saves time. SPE is mainly the isolation of the compound of interest on the surface of a cartridge and elution using solvent under vacuum. There are two important parameters while carrying out SPE: (i) selection of the proper cartridge for extraction (ii) selection of proper solvent for elution. The type of cartridges that have extensive use during SPE are HLB (hydrophilic-lipophilic balanced reversed phase sorbent) cartridges, GCB (graphitized carbon black) cartridges and C-18 cartridges. Among these three, C-18 is the most commonly used one, and in this study all the extraction tests were carried out using C-18 type of SPE cartridges. The solvents tested were ethyl acetate, dichloromethane, hexane, acetone, methanol or volumetric mixtures of them. The details of the SPE method applied are given in Figure 3.38.

Table 3.23 Extraction methods used to isolate NP compounds from liquid phase

Sample	Method	Solvent	Reference
Surface water	Liquid-liquid extraction	Dichloromethane	Bennie et.al., 1997
Wastewater	Liquid-liquid extraction	Dichloromethane	Conn et.al., 2006
Wastewater	Solid phase extraction	Methanol followed by Dichloromethane:methanol (4:1)	DiCorcia et.al., 2000
Surface water	Solid phase extraction	Dichloromethane:methanol (9:1)	Ding et.al., 1999
Surface water	Solid phase extraction	Methanol:acetonitrile (1:1)	Fries and Püttmann, 2003
Wastewater	Solid phase extraction	Dichloromethane:hexane (4:1)	Gatidou et.al., 2007
Wastewater/Surface water	Solid phase extraction	Methanol	Isobe et.al., 2001
Wastewater	Solid phase extraction	Methanol:diethylether (1:9)	Jeannot et.al., 2002
Wastewater	Solid phase extraction	Dichloromethane:methanol (8:2)	Lian et.al., 2009
Wastewater	Solid phase extraction	Dichloromethane:acetone (7:3)	Nie et.al., 2009
Surface water	Solid phase extraction	Dichloromethane:ethyl acetate (1:1), hexane:dichloromethane (1:1), methanol and water	Navarro et.al., 2010
Wastewater	Solid phase extraction	Acetone	Pothitou and Voutsa, 2008
Wastewater	Solid phase extraction	Dichloromethane:hexane	Stasinakis et.al., 2008
Surface water	Mechanical shaking	Dichloromethane	Watanabe et.al., 2007

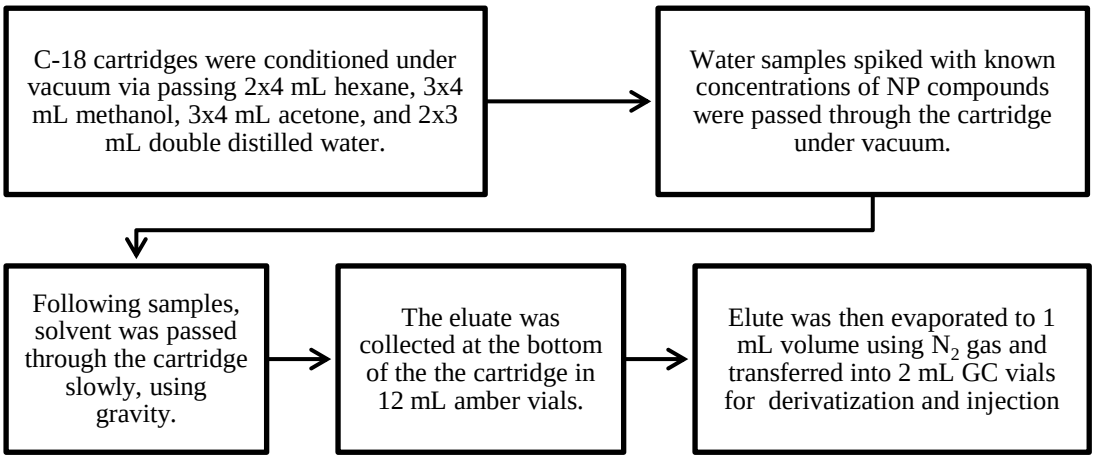


Figure 3.38 Details of SPE applied to extract NP compounds from liquid phase

The first step in Figure 3.38 is called conditioning and it is a critical step for the isolation of the compound to be extracted. A vacuum manifold (AGT-5892-9110, Agilent Technologies) was used to insert the cartridges and a vacuum pump was connected to this device. The only parameter that was subjected to change in the method given in Figure 3.38 was the type of solvent used for elution. The solvents tested based on their recoveries were acetone, methanol, acetone:methanol (1:1, v/v) and hexane. The recoveries obtained are presented in Table 3.24. It should be noted that the results given in this table were the preliminary results without the use of the vacuum manifold, but even though they were abrupt, they were helpful in the comparison of solvents. After this first trial, the vacuum pump was replaced. The first solvent that was eliminated was hexane, since no NP2EO was detected in the eluate. Once hexane was eliminated, one more trial was made using the solvents acetone, methanol and acetone:methanol (1:1, v/v). In this set, 4-nNP was also included as the surrogate and the average recoveries obtained for NP, 4-nNP, NP1EO and NP2EO were: 60.3, 96.8, 70.0 and 71.7 for acetone, 198.6, 85.8, 94.0 and 72.3 for methanol and 113.9, 95.2, 103.7 and 90.3 for acetone:methanol (1:1, v/v). Among these results the most reasonable ones were obtained for SPE with acetone: methanol (1:1, v/v) mixture. Finally, one last set of SPE was done using acetone:methanol and the results are given in Table 3.25.

Table 3.24 Extraction efficiencies obtained for SPE of NP compounds from liquid phase

Extraction Efficiency (%Recovery)												
Solvent	Acetone			Methanol			Acetone:Methanol (1:1)			Hexane		
Sample	**NP**	**NP1EO**	**NP2EO**	**NP**	**NP1EO**	**NP2EO**	**NP**	**NP1EO**	**NP2EO**	**NP**	**NP1EO**	**NP2EO**
S-1	18.3	47.7	59.2	32.4	26.9	34.9	63.7	30.0	41.8	397.1	105.5	ND
S-2	19.9	49.5	62.5	30.6	26.6	36.9	67.2	30.3	49.0	403.3	125.1	ND
S-3	18.9	51.3	67.2	28.5	25.7	35.9	66.7	31.5	50.5	400.2	115.3	ND
Ave	19.0	49.5	63.0	30.5	26.4	35.9	65.9	30.6	47.1	400.2	115.3	-
SD	0.8	1.8	4.0	2.0	0.6	1.0	1.9	0.8	4.6	3.15	9.8	-
%RSD	4.4	3.6	6.3	6.5	2.4	2.7	2.9	2.5	9.9	0.8	8.5	-

ND: Not Detected

Table 3.25 Extraction efficiencies obtained for SPE using acetone:methanol (1:1, v/v) as solvent

Extraction Efficiency (%Recovery)				
Sample	**NP**	**4-nNP**	**NP1EO**	**NP2EO**
S-1	119.4	100.0	80.5	81.8
S-2	103.7	92.6	75.8	78.9
S-3	119.7	96.2	88.5	88.6
S-4	95.1	108.2	89.6	94.6
Ave	109.5	99.3	83.6	86.0
SD	12.2	6.7	6.6	7.0
%RSD	11.1	6.7	7.9	8.2

As can be seen from Table 3.24 and 3.25, SPE method using acetone:methanol (1:1, v/v) has been the best solvent among the others for the extraction of NP compounds from liquid phase.

As one may recall from the physicochemical properties, NP1EC is not as hydrophobic as the other NP compounds. Therefore, it should be carefully extracted from the liquid media. At this point, SPE with C-18 cartridges was again selected as the method of extraction. But to be on the safe side, elution solvent was reevaluated for NP1EC. The results are given in Table 3.26. According to these results, acetone:methanol (1:1, v/v) yielded again the best results like in the case of other four NP compounds.

For all NP compounds the recoveries range between 70-130%, which is acceptable according to USEPA (USEPA, 2003). Also in other studies, recoveries between %90-120 (Field and Reed, 1996) and %90-120 (Arditsoglou and Voutsa, 2008) were stated as acceptable.

Table 3.26 Extraction efficiencies obtained for NP1EC using SPE

Extraction Efficiency (%Recovery)			
Sample	**Acetone**	**Methanol**	**Methanol:Acetone**
S-1	110.6	92.0	102.9
S-2	107.3	88.4	111.8
S-3	126.3	109.7	104.9
S-4	123.8	108.0	101.0
Ave	117.0	99.5	105.1
SD	9.4	10.9	4.7
%RSD	8.1	10.9	4.5

3.7 Quality Assurance/Quality Control

The definition of quality assurance and quality control (QA/QC) are given as (ISO 9000):

"Quality control involve the operational techniques and activities that are used to fulfill the requirements for quality, whereas, quality assurance involves all those planned and systematic activities implemented to provide adequate confidence that and entity will fulfill requirements for quality."

Therefore, for the results to be reported within the safe boundaries and accurately, QA/QC is an important step. So by QA, one can make sure that the objectives of an analysis are met and by QC, one can make sure that the results of an analysis can be evaluated properly and accurately.

QA includes cleaning of all the glassware being used during the experiments, setting up calibration curves, establishing the detection and quantification limits according to the calibration curves, etc.. For instance, blanks performed during extraction experiments were a part of QA. Most of the isomers of the NP compounds were not detected in the blanks. Clean blanks performed were also free of NP compounds and showed that all the equipments and glassware were free of contamination. Cleaning of glassware was achieved via the use of a detergent named Alconox. Immediately after use, the samples were rinsed with technical grade ethanol and then soaked into a hot water containing Alconox. Then the glassware was rinsed to flush the detergent from the surface. Once they are rinsed, chromic acid (prepared in the laboratory) was added to destroy organics. To remove the oxidizing agent from the surfaces, they were washed with water and then passed through double distilled water to remove any kind of trace compound. Finally, they were covered with aluminum foil and put into 105°C oven. This procedure applied also matches with the one proposed by USEPA (USEPA, 2007).

Another QA measure was the preservation of the samples. In order to keep the sample's integrity (physical and chemical properties) and representativeness, samples and extracts were stored in amber vials to prevent photodegradation and kept at -18°C to minimize microbial activity. Teflon caps were always used to cover the 12 mL amber vials in which samples were stored for a maximum time of 7 days.

Another QA measure was the freeze-drying of the samples and use of anhydrous sodium sulfate to make sure that the extracts were moisture free. Even a small drop of water can result in the destruction of GC-MS. This would result in the misevaluation of the samples.

Calibration curves were set for all five compounds (NP, NP1EO, NP2EO, 4-nNP and NP1EC) using stock solutions prepared in acetone. As stated before, R^2 values

obtained from these curves showed that the analyses of these compounds were achieved successfully. During reactor operation, to check the accuracy of the device, stock solutions of known concentrations were injected into the device once in every four days and the results were compared with the calibration curves.

For QC, calculations were made based on certain formula and the repeatability of the results was checked based on standard deviations and relative standard deviations (%RSD). Especially, during extraction tests, 4-nNP was used as surrogate and the recoveries were calculated based on the following formula:

$$\% \text{ Recovery} = \frac{\text{Concentration of amount measured}}{\text{Concentration of amount spiked}} \times 100 \quad \text{.....Equation (4)}$$

To calculate the recoveries of the samples spiked with NP compounds were calculated based on the given formula:

$$\% \text{ Recovery} = \frac{Cs\text{-}Cu}{Cn} \times 100 \quad \text{.....Equation (5)}$$

where,

Cs is the measured concentration of the spiked sample

Cu is the measured concentration of the blank sample

Cn is the concentration spiked into the sample

During the operation of NP2EO spiked reactors, in order to make sure that the recoveries obtained in the extraction tests were applicable and see the effects of

chemical aging, spiked samples were kept for 10 days in a dessicator and then extracted. The recoveries obtained are given in Table 3.27.

Table 3.27 Extraction efficiencies obtained for 10 day soil spiked sample

	Extraction Efficiency (%Recovery)			
Sample	**NP**	**4-nNP**	**NP1EO**	**NP2EO**
S1 (1)	122.4	98.3	123.3	144.7
S1 (2)	121.6	98.6	124.4	142.2
S1 (3)	114.1	101.3	129.6	148.8
S1 (4)	114.4	100.5	125.5	147.8
Ave	118.1	99.7	125.7	145.9
SD	4.5	1.5	2.8	3.0
%RSD	3.8	1.5	2.2	2.1

To test the accuracy and the repeatability of the samples, especially during the recovery calculations, relative standard deviations were calculated based on the formula:

$$\text{Relative standard deviation (\%RSD)} = \frac{|C1-C2|}{[(C1+C2)/2]} \times 100 \quad \text{.....Equation (6)}$$

where,

C_1 is the amount measured in the first sample

C_2 is the amount measured in the second sample

For the proper reporting and documentation of the data obtained, volume correction was applied as a QC measure. Since in every sampling a certain volume was

withdrawn from the reactors, the volume of the reactors decreased together with the samplings done. Therefore, in order to base the data obtained on a standard volume, volume correction was applied to each parameter except for pH despite differences between corrected and uncorrected concentrations were small (around 1.8%). Volume correction was done as follows:

$C_0 = C_0$ (at t=0 days, since volume is the initial working volume)

$C_n = [(C_n x V_n) + (C_{n-1} x V_s)]/V_n$ ……..…….Equation (7)

where,

C_n is the concentration at t = n days

C_{n-1} is the concentration at t = n-1 days

V_n is the volume of sludge in the reactor at t = n days

V_s is the volume of sample withdrawn from the reactor

Limit of detection (LOD) and limit of quantification (LOQ) are also QA/QC tools. In this study, the LOD and LOQ values of the compounds are given in Table 3.28. Values of LOD and LOQ were determined based on the S/N ratios through repetitive analysis.

Table 3.28 Limits of detection and quantification for NP, 4-nNP, NP1EO and NP2EO

Compound	LOD	LOQ
NP	3 ppb	10 ppb
4-nNP	15 ppt	50 ppt
NP1EO	3 ppb	10 ppb
NP2EO	6 ppb	20 ppb
NP1EC	15 ppb	50 ppb

3.8 Reaction Rate Calculation

One of the objectives of this study was to monitor the degradation kinetics and products of NP2EO under aerobic conditions. Therefore, reaction rate constants must be calculated to report the degradation and formation rates of NP2EO and NP1EC, respectively. For this purpose, first of all the reaction order must be identified.

In zeroth order reactions, rate is independent of the concentration of the reactant. This can be best represented by the following equation:

k (reaction rate coefficient) = r (reaction rate) ……...…Equation (8)

In other words,

$$r = -\frac{dC}{dt} = k \rightarrow C_t = -kt + C_0$$ …….....Equation (9)

where,

C_t is the concentration of the reactant at time t

C_0 is the initial concentration of the reactant

t is time

A reaction is said to be zeroth order if the C vs t plot yields a straight line.

In first order reactions, reaction rate depends on the concentration of one reactant.

$$k = rC \quad \text{.....Equation (10)}$$

In other words,

$$r = -\frac{dC}{dt} = kC \rightarrow \ln C_t = -kt + \ln C_0 \quad \text{.....Equation (11)}$$

where,

C_t is the concentration of the reactant at time t

C_0 is the initial concentration of the reactant

t is time

A reaction is first order if the plot lnC vs t yields a straight line.

The third type is the second order reactions. Here, rate is dependent on the concentration of a second order reactant or two first order reactants.

$$r = -\frac{dC}{dt} = kC^2 \rightarrow 1/C_t = 1/C_0 + kt \quad \text{.....Equation (12)}$$

where,

C_t is the concentration of the reactant at time t

C_0 is the initial concentration of the reactant

t is time

A reaction is first order if the plot 1/C vs t yields a straight line.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary Operation of Laboratory Scale Aerobic Batch Reactors

The purpose of setting up aerobic batch reactors that did not contain any NP2EO was to understand the possible problems that may occur in the future reactors dosed with NP2EO so as to come up with the most proper operation. These reactors were operated during the development of extraction and measurement methods for NP compounds which was long before the NP2EO spiked reactors were started. Two sets were operated each with different solids concentration. These reactors were set up in 2.5 L amber glass reactors with a working volume of 2 L filled with WAS. They were placed in water baths at a temperature of 25°C. The duration of operation was between 30-40 days, depending on the solids content of the reactors. The parameters analyzed were TSS and VSS. These parameters were measured at certain time intervals during reactor operation.

First Set Reactors

TSS and VSS values are represented graphically in Figure 4.1 and 4.2. These are the averages of the replicate analysis done (two replicas).

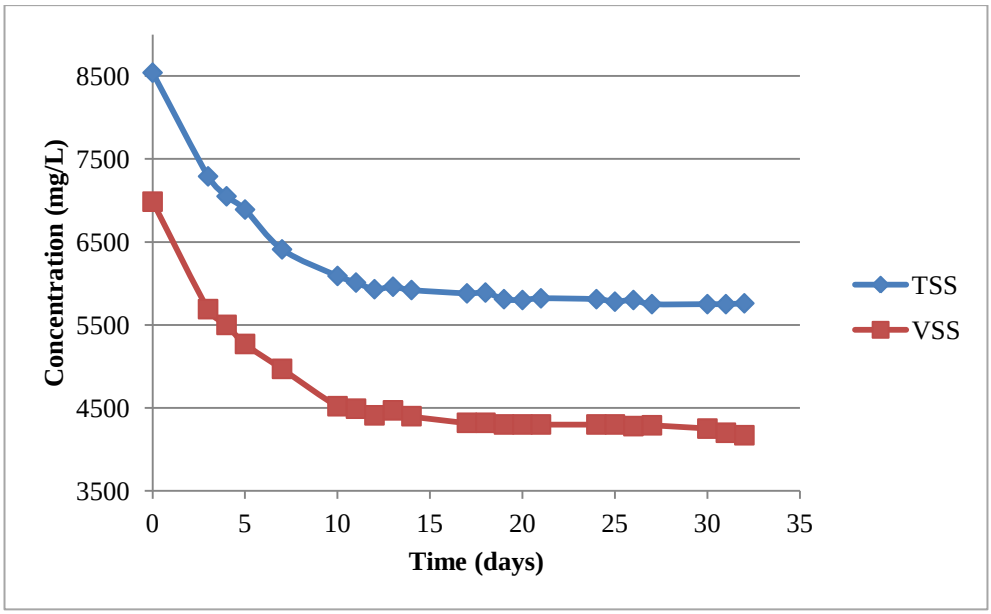


Figure 4.1 TSS and VSS change in the first set of reactors (Reactor-1)

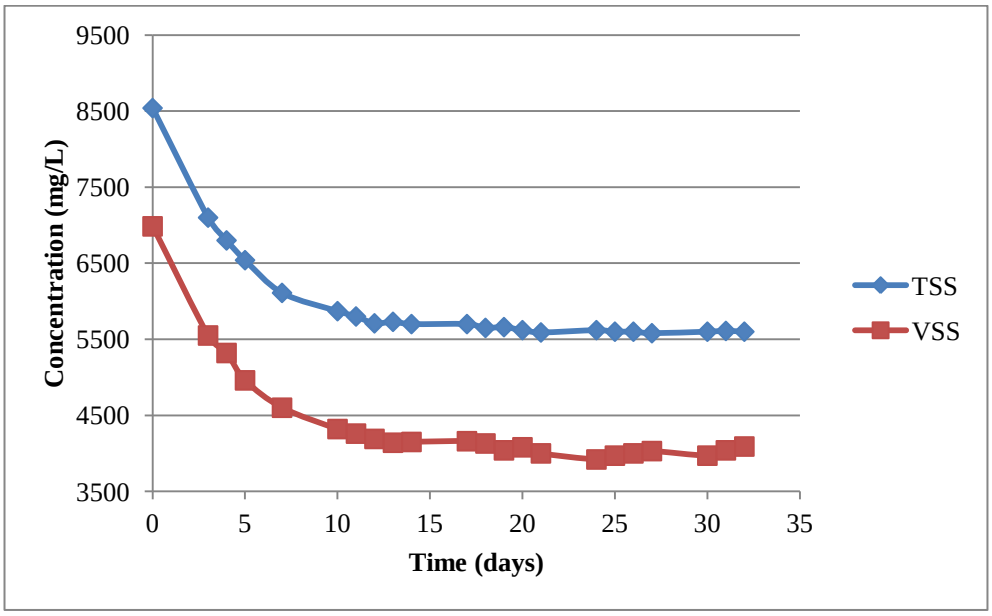


Figure 4.2 TSS and VSS change in the first set of reactors (Reactor-2)

As one may understand from the graphs given, the reduction in the organic matter (VSS) continued until when the energy of the biomass decreased to a certain level. From that point on, there was no change in the organic content of sludge, in other words, the reactors reached a stable state.

In this set, the initial TSS and VSS concentrations are around 0.85% and 0.70%, respectively. The initial and final concentrations together with the removal rates are given in Table 4.1.

Table 4.1 VSS concentrations and %removals in first set

	Reactor-1	**Reactor-2**
VSS at t=0 days (mg/L)	6985	6985
VSS at t=32 days (mg/L)	4170	4090
% Removal	40.3	41.4

Second Set Reactors

Another set of 2 L aerobic laboratory scale batch reactors were set up using WAS. This time, the aim was to observe the change in organic matter reduction at a higher solids concentration, to understand the effect on digesters efficiency. For this reason, the initial solids concentration was doubled this time and solids destruction was monitored all throughout operation. Unfortunately, the one of two replica reactors was terminated at the end of 8 days due to overflow of sludge. Therefore, TSS and VSS values given in Figure 4.3 are for one reactor only.

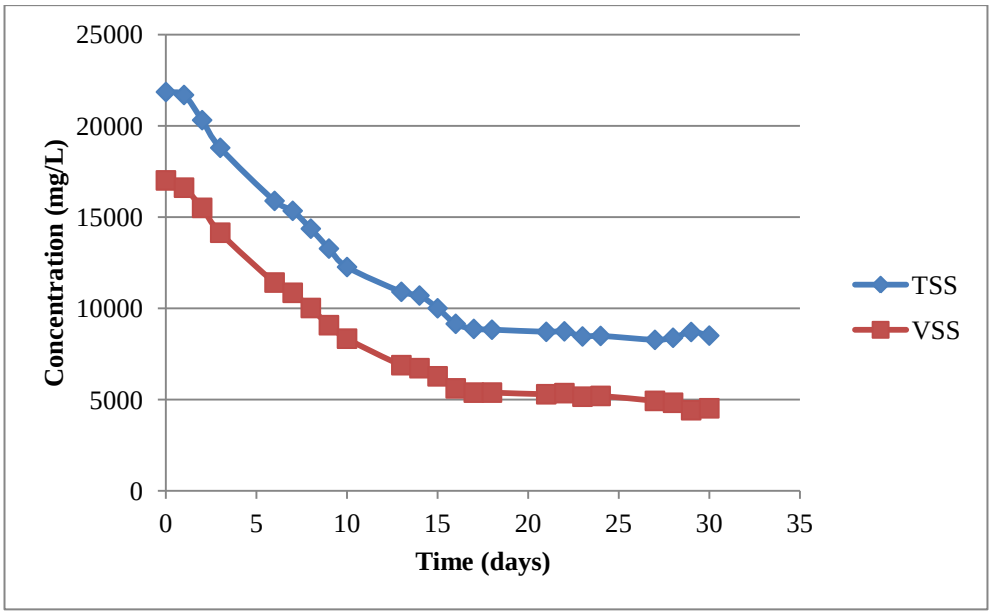


Figure 4.3 TSS and VSS change in the second set of reactors (Reactor-1)

In this set, the % removal of organic matter was about 73%. When the removal rates for both sets were compared it was understood that as the solids concentration increases, the percentage of organic matter reduction also increases and this is a parallel finding analogous to a study carried out by Hartman et.al., 1979. Other than percentage of reduction, the rate of reduction is also dependent on the solids concentration. Therefore, based on the light of this information, it was decided to keep the solids content of the NP2EO spiked aerobic batch reactors as high as possible.

4.2 Nonylphenol Diethoxylate Spiked Laboratory Scale Aerobic Batch Reactors

The objective of these reactors was to monitor the degradation patterns, kinetics and metabolites of NP2EO. The reactors were filled with WAS that was settled for one day at 4°C (inside a refrigerator) in the laboratory. The results of the initial analyses for control (C) and NP2EO dosed (R) reactors are given in Table 4.2. The replica control reactors (C-1 and C-2) were operated without any NP2EO spike, whereas the other two replica reactors (R-1 and R-2) had NP2EO spiked.

Table 4.2 Characteristics of WAS used in the set up

		mg/L			µg/L			
Reactor	**pH**	**TS**	**VS**	**COD**	**NP**	**NP1EO**	**NP2EO**	**NP1EC**
C-1	6.8	20860	14360	45424	83	159	468	ND
C-2	6.82	20860	14310	43263	82	157	457	ND
R-1	7.03	20670	14240	44049	84	163	440	ND
R-2	6.77	20350	13980	45817	57	137	482	ND

ND: Not Detected

pH

pH is an important parameter in microbial systems since it affects the activity of microorganisms. Aerobic microorganisms can operate on a wide range of pH. During aerobic stabilization, since oxygen is continuously added to the system, CO_2 is produced and ammonia is nitrified, pH is expected to decrease. In these set of reactors, pH started around seven at the reactor initiation. No buffer was added due to sludge containing enough buffering materials. After about 2-3 days, pH started to increase and by the end of 5 days pH was about 8 in all reactors. Until about 12 days pH increase continued to values close to 9 and then pH started to decrease sharply

and then stayed very stable after 15 days until 30 days at around 6.5. The reason behind the increase observed was thought to be the rate of oxygen supply into the system. The air pumps used to aerate the reactors were operated at maximum rate, supplying oxygen possibly more than required. In order not to limit the oxygen in the reactors, it was decided to supply excess. Therefore, a portion of the supplied oxygen was used by the microorganisms in degradation and converted into CO_2. The excess amount of oxygen is thought to possibly strip some of the CO_2 from the system and caused an initial increase in pH. With passage of time, nitrification starts and with the added CO_2 and H^+ production, pH decreased. In addition, acetic acid compounds of NP started to form and started to show up in the reactors. Their production and dominance in the reactors is thought to be one of the factors that cause this decrease in pH. As can be seen in Figure 4.4, there is a difference in pH values of C-1/C-2 and R-1/R-2 reactors. pH of control reactors start to drop before NP2EO spiked reactors. The only difference between these two reactors was the presence of NP2EO in R-1 and R-2, therefore, the reason behind the time of pH change was thought to be the presence of NP compounds within the reactors.

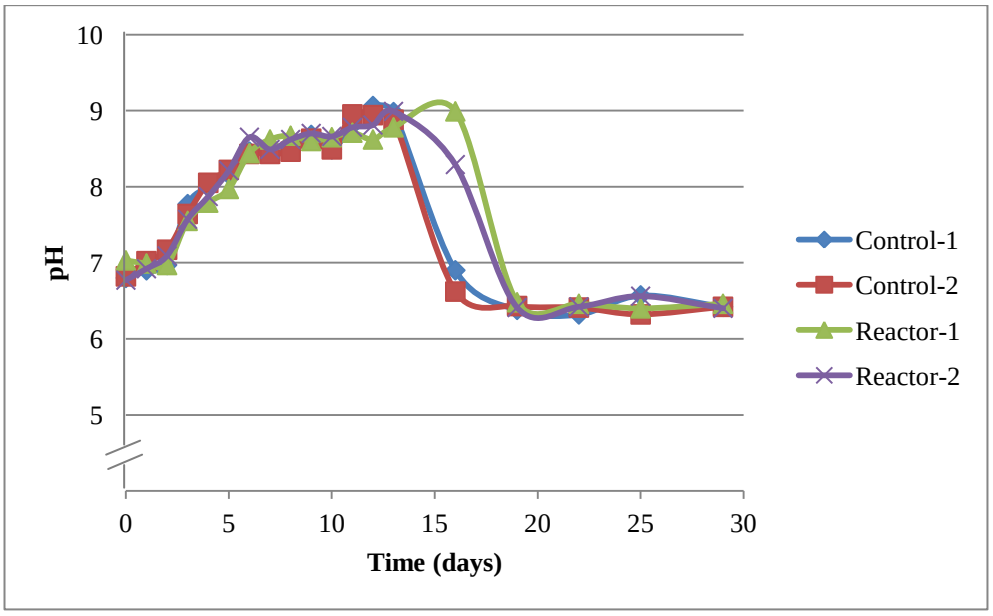


Figure 4.4 pH vs time for control and NP2EO spiked aerobic batch reactors

All throughout the reactor operation, even if the pH increased/decreased, it was at most of the times between ~6 and 8, which was considered as an acceptable pH range for an efficiently operating aerobic digester (Anderson and Mavinic, 1984). The reactor operation was not affected in any negative way because of this pH change.

Total Solids and Volatile Solids

One of the important parameters in aerobic stabilization is the solids concentration, especially the volatile solids concentration since it is an indicator of organic matter. Aerobic digesters aim to degrade organics in sludge so as to stabilize the sludge. Therefore, VS should be carefully monitored. The reactors were set up with TS and VS at about 20,000 and 14,000 mg/L, respectively, and these values decreased steadily throughout the reactor operation. The results of this analysis are given in Figure 4.5 and Figure 4.6 for TS and VS, respectively.

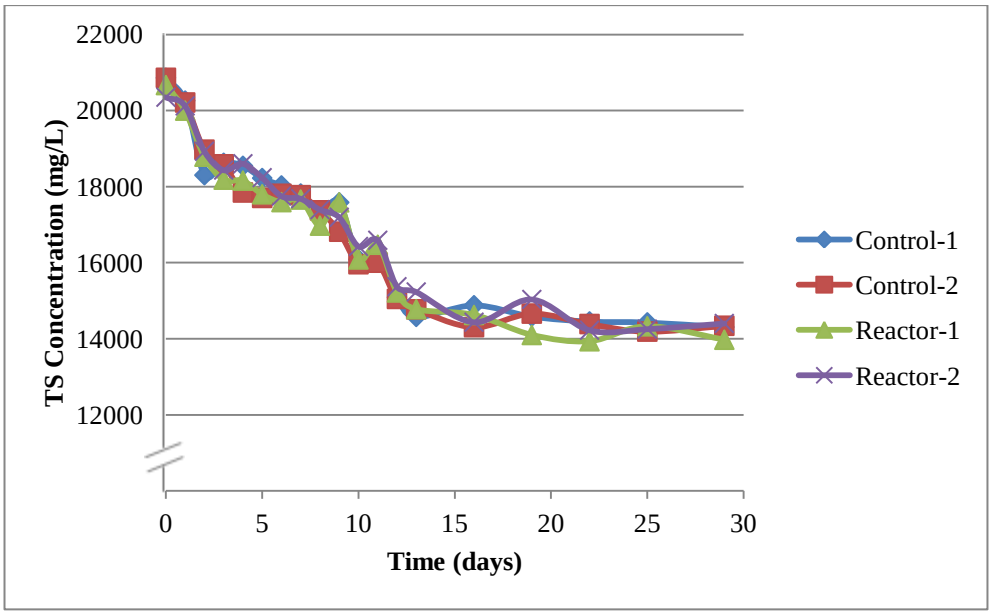


Figure 4.5 TS (mg/L) vs time for control and NP2EO spiked aerobic batch reactors

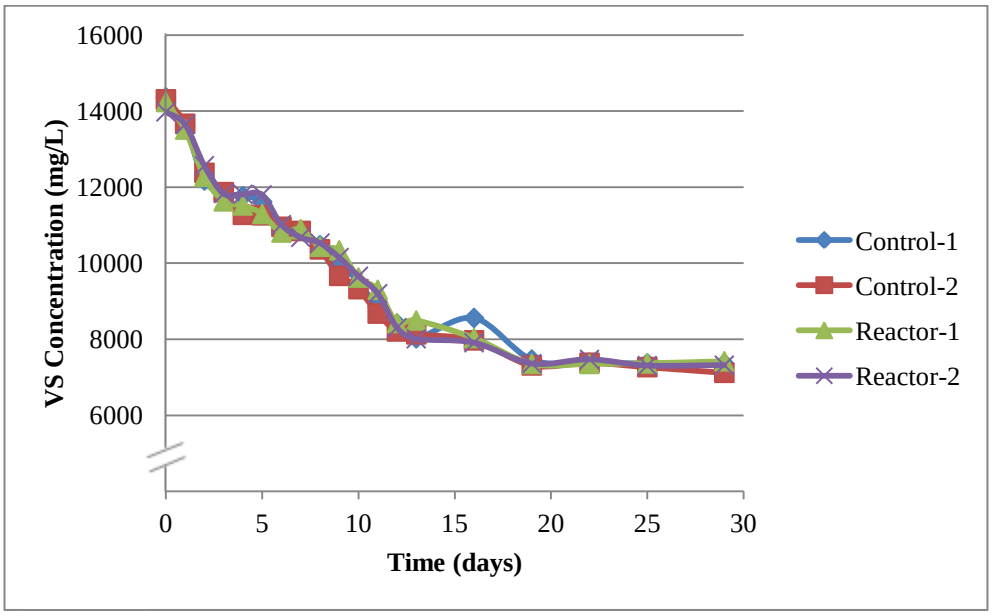


Figure 4.6 VS (mg/L) vs time for control and NP2EO spiked aerobic batch reactors

From the Figures 4.5 and 4.6, there seems to be no effect of NP2EO spike onto the reactor performance for the reduction of TS and VS. It is obvious that most of the easily oxidizable mass was already oxidized by about 20 days and the system became stable after that.

The VS reductions in the reactors are calculated and given in Table 4.3.

Table 4.3 VS concentrations and % removals

	C-1	**C-2**	**R-1**	**R-2**
Initial VS (mg/L)	14360	14310	14240	13980
Final VS (mg/L)	7361	7119	7421	7320
% Removal	48.7	50.3	47.9	47.6

As can be seen the % removals vary between 48-50%, and all four reactors represent each other both in terms of pattern of solids destruction and the removal percentages. Generally, in aerobic digesters, the removal of organic matter is between 40-50% (Sanin et.al., 2011), which shows us that the operation of the reactors was successful and degradation of organic matter was efficient. From these data too, no negative effect of spiking the reactors with NP2EO was observed on solids destruction.

Total Suspended Solids and Volatile Suspended Solids

TSS and VSS were the two other parameters that were analyzed. TSS and VSS show the same pattern with TS and VS; the concentrations are smaller as expected due to the presence of dissolved solids in TS and VS. The graphical representations are given Figure 4.7 and Figure 4.8.

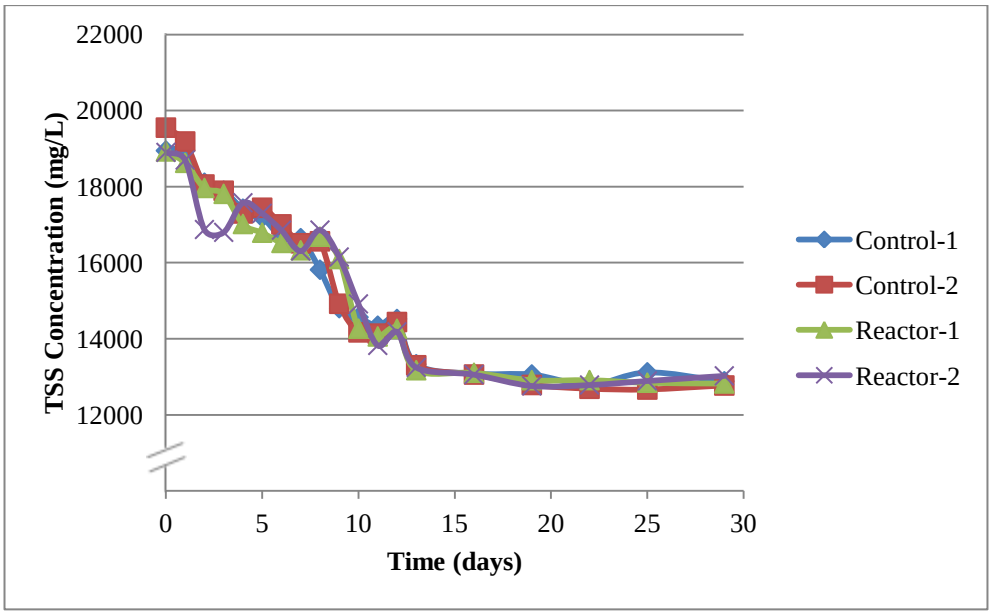


Figure 4.7 TSS (mg/L) vs time for control and NP2EO spiked aerobic batch reactors

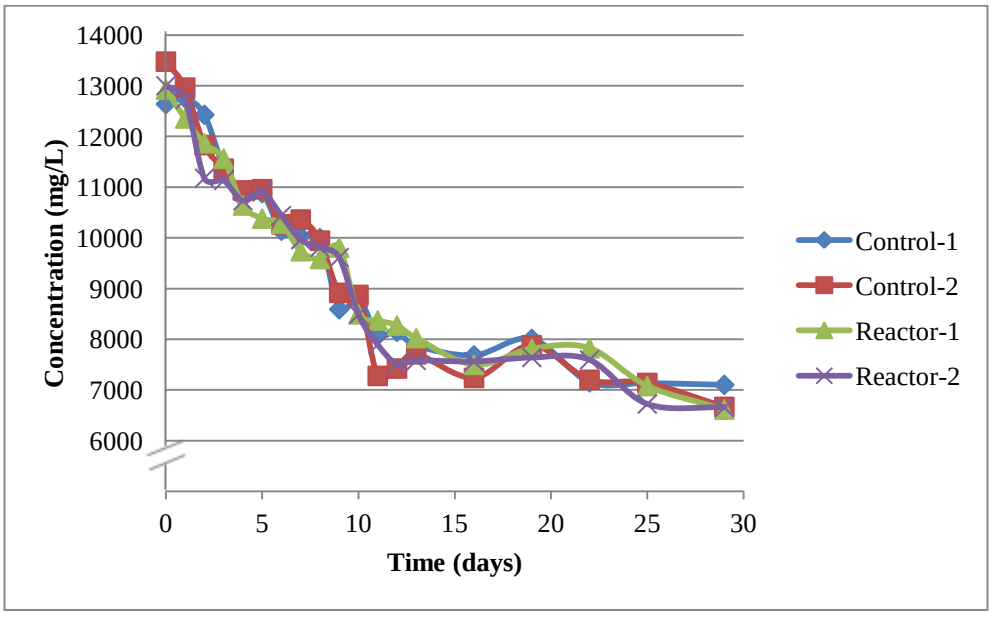


Figure 4.8 VSS (mg/L) vs time for control and NP2EO spiked aerobic batch reactors

The VSS removals for all four reactors are given in Table 4.4.

Table 4.4 VSS concentrations and %removals

	C-1	**C-2**	**R-1**	**R-2**
Initial VS (mg/L)	12639	13475	12925	13000
Final VS (mg/L)	7101	6666	6614	6656
% Removal	43.8	50.5	48.8	48.8

As can be seen in Table 4.4, the removals are between 44-50%, which is between the % removal calculated for the NP free reactors. Since the solids concentration is also almost the average of the previous two sets, the % removals obtained were in the range expected.

Chemical Oxygen Demand

COD change with respect to time was also monitored during reactor operation. The results are presented in Figure 4.9. As can be seen from the graph, with the addition of NP2EO in acetone or acetone alone into all four reactors, the COD values increase on day 4. However, the COD values for the NP2EO dosed reactors were slightly higher on the day of spike possibly due to the presence of NP2EO. Then COD started to decrease in all reactors and at the point where the reactors reached stationary operation, COD also became stationary. The approximate overall removal of COD was 70% for all four reactors.

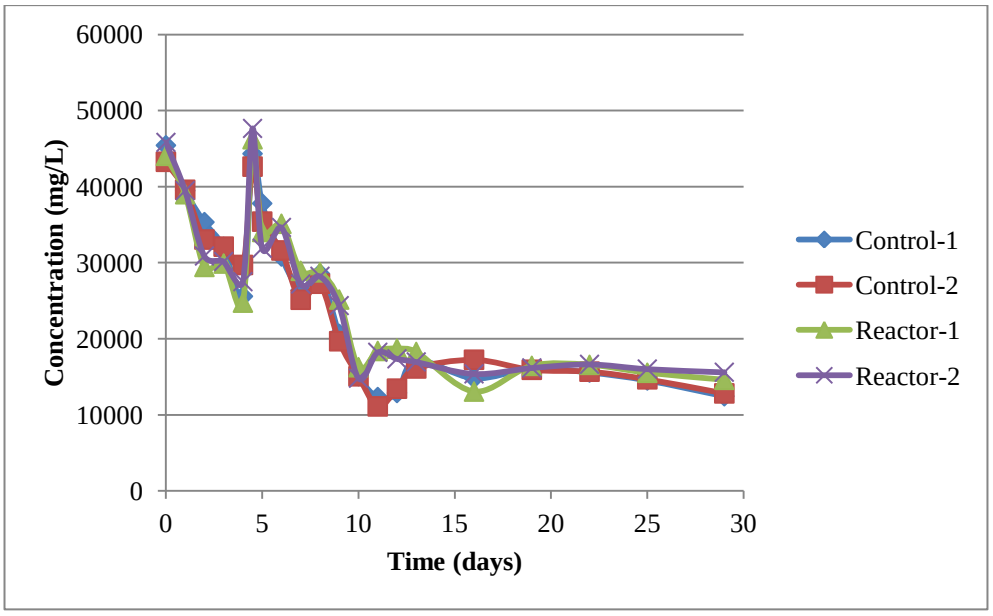


Figure 4.9 COD (mg/L) vs time for control and NP2EO spiked aerobic batch reactors

Nonylphenol Compounds

The purpose of this study was to monitor the fate of NP compounds in aerobic batch reactors. So their concentrations were closely followed both in solid and liquid phases. The concentrations extracted and measured in solid phase, liquid phase and in the whole reactor (solid + liquid) are represented graphically in this section.

The graphs present the 29 days results. Some fluctuations were observed but those were not significant to affect the mass balance of the system and were thought to be tolerable.

Control-1 Reactor

The graphs obtained for solid phase, liquid phase and whole reactor are given in Figures 4.10, 4.11 and 4.12.

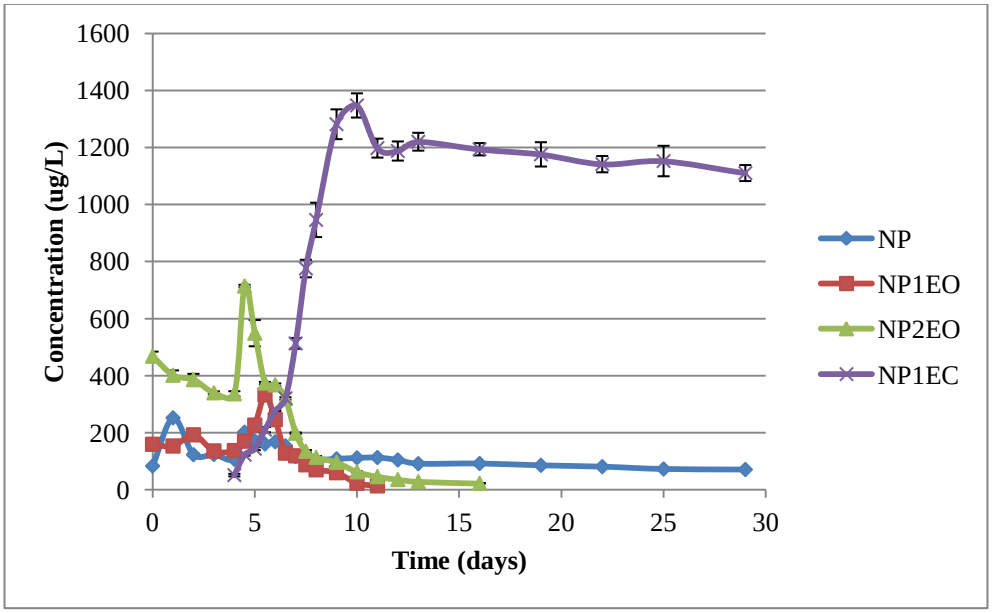


Figure 4.10 NP compounds (solid phase) vs time for C-1

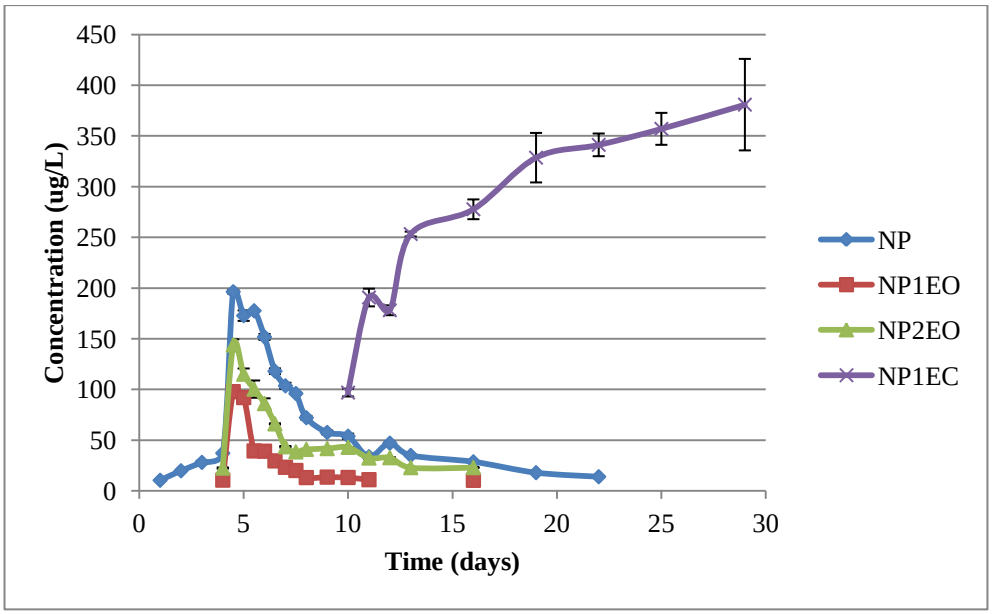


Figure 4.11 NP compounds (liquid phase) vs time for C-1

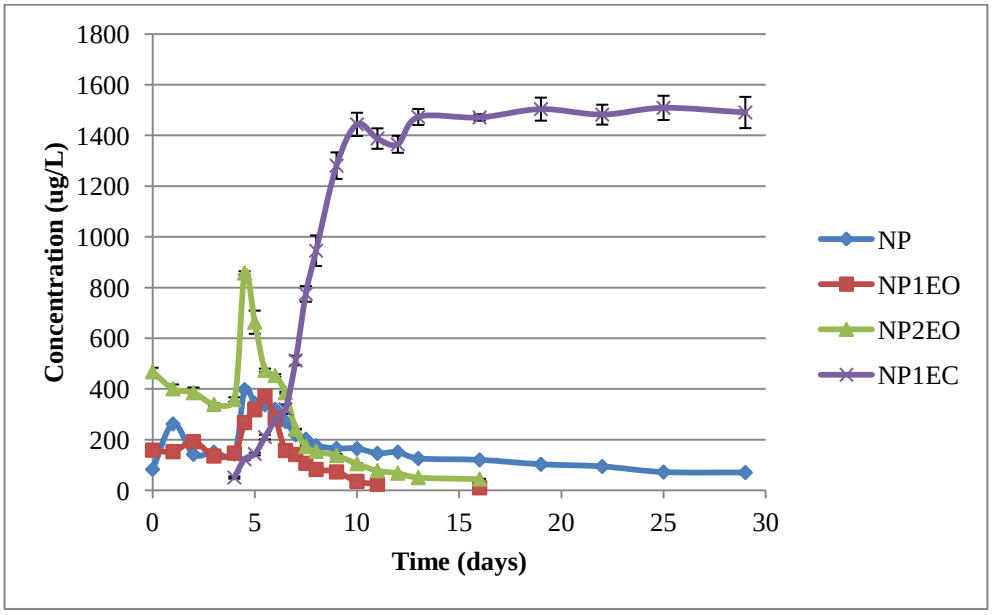


Figure 4.12 NP compounds vs time (whole reactor) for C-1

As can be seen in Figure 4.10, the concentration of NP compounds (ppb or mg/L) started from about 80, 150 and 450 ppb for NP, NP1EO and NP2EO, respectively at day zero, in solid phase. Initially, the concentrations of NP compounds were small. During the first 4 days (before spike), NP2EO showed a decrease in concentration whereas concentrations of NP and NP1EO showed slight changes indicating biodegradation of NP2EO into its metabolites. However, on the day of acetone spike, the concentration of NP2EO showed an increase and reached a concentration almost twice of its initial value, even though only acetone was added. There could be several reasons behind this increase. Firstly, the higher forms of ethoxylates are not measured (i.e. NP4EO or NP9EO), and these compounds are constantly converted into NP2EO as discussed before. Therefore, it is possible that these compounds were converted into NP2EO and NP1EO in the first couple of days and resulted in such increase in concentration. Another more likely reason for this could be contamination of the syringe used for addition of acetone. Even if the glassware and equipment used were checked for contamination via clean blanks, still the 10 mL syringe used for the addition of acetone could be contaminated since it was not checked for contamination on the day of spike.

NP1EC was not detected till the 4^{th} day of operation (in solid phase) and increased steadily and reached a stationary concentration around 9 days. NP2EO degradation is very fast but the conversion into carboxylated metabolites takes some time since there are other metabolites formed in between. First NP2EO should be converted into NP1EO and then NP1EO should be degraded into NP2EC. NP2EC was then degraded to form NP1EC. Since there were various steps in between NP2EO degradation and NP1EC formation, it is not surprising to observe NP1EC formation few days after NP2EO degradation.

As can be seen from the Figures 4.10, 4.11 and 4.12, NP did not accumulate within the reactor. One would expect NP accumulation if this were an anaerobic system, however, under aerobic conditions NP1EC is the dominating metabolite. NP2EO and

NP1EO were degraded into NP1EC rather than NP under aerobic conditions, and that's why the sum NPE yields smaller values when compared to anaerobic systems.

Once NP1EC concentration reached a stationary level, the other NP compounds (NP, NP1EO and NP2EO) also showed stable patterns for almost 20 days. This is actually an indicator of NP2EO being converted into its metabolites successfully.

The patterns of degradation in both phases (solid and liquid) were similar to each other. However, in liquid phase, concentrations were smaller or below detection limits, especially at the beginning of reactor operation since NP compounds are hydrophobic and tend to accumulate on sludge solids. The degradation patterns of solid phase and the whole reactor were very close to each other since most of the compounds stayed on solid phase and the only NP compound detected at high levels was NP1EC which is less hydrophobic when compared to the rest of NP family as stated before.

The graphical representations for solid and liquid phases (Figure 4.10 and 4.11) contain error bars based on the standard deviations of the measurements done. The standard deviations are so small when compared to the scale of y-axis and not visible for NP, NP1EO and NP2EO. However, for NP1EC standard deviations are higher. The reason is that while the derivatization method for NP, NP1EO and NP2EO was standard (100 μL ampoules of BSTFA+TMCS were used) and simple, the derivatization method for NP1EC was more error prone. Even the separation of petroleum ether from the top of water could lead to certain errors if the whole volume was not withdrawn. The manual work involved in NP1EC derivatization affects the accuracy and therefore the analysis results. However, this does not mean that the standard deviations were unacceptable, actually they were small, too. Standard deviations were relatively high when compared to NP, NP1EO and NP2EO results.

Control-2 Reactor

The graphs obtained for solid phase, liquid phase and whole reactor are given in Figures 4.13, 4.14 and 4.15.

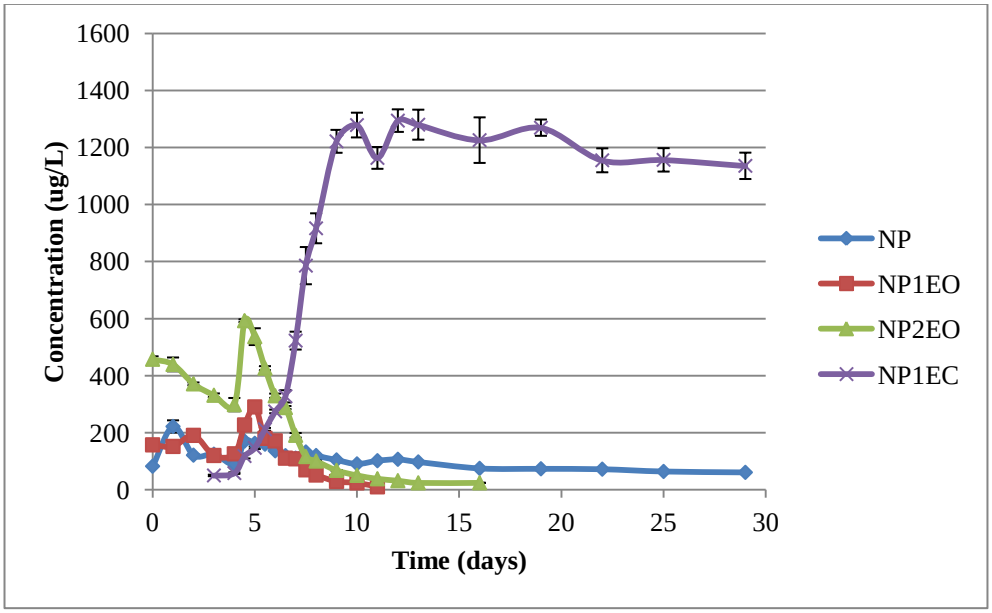


Figure 4.13 NP compounds (solid phase) vs time for C-2

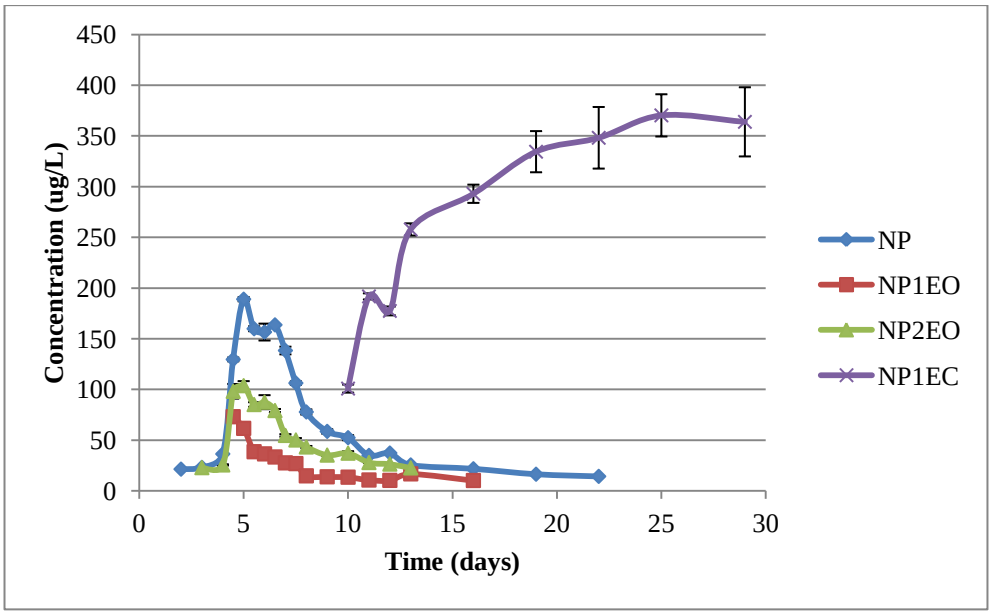


Figure 4.14 NP compounds (liquid phase) vs time for C-2

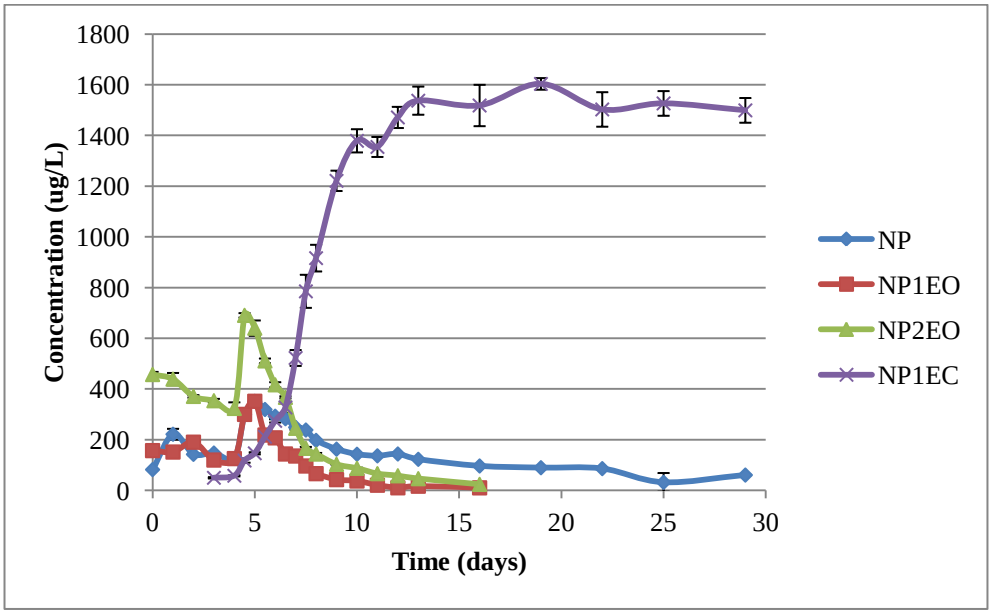


Figure 4.15 NP compounds vs time (whole reactor) for C-2

When the concentration of NP compounds and the degradation patterns of C-2 reactor were analyzed, it was found to be very similar to C-1 reactor, which shows the repeatability of both the reactor operation and analyses done. In fact this degree of repeatability can rarely be achieved in most biologically operated reactors. Therefore, this can be taken as the indicator of the reliability of the extraction and measurements methods for NP compounds and diligent operation of the reactors. As can be seen from Figure 4.13, the concentration of NP, NP1EO and NP2EO were about 80, 150 and 450 ppb for NP, NP1EO and NP2EO, respectively, in solid phase, initially. These values are almost the same with the ones obtained for C-1 reactor. Again, the initial concentrations of NP compounds were not significant. NP1EC was not detected for the first couple of days. During the first 4 days of reactor operation, NP2EO was degraded and decreased in concentration resulting in a slight increase in the NP and NP1EO concentrations. Just like in C-1 reactor, in C-2 reactor also NP2EO concentration increased on the day of acetone addition. As stated before, the reason could be the degradation of higher form of ethoxylates into NP2EO and NP1EO (both compounds show an increase on the day of acetone addition). The glassware and equipment used were checked for contamination (during extraction), still the 10 mL syringe used for the addition of acetone could be contaminated since it was not checked for contamination on the day of spike. Another reason could be the related with the sampling, even though the reactors were mixed via aeration, the samples were not taken homogenously.

NP1EC was not detected till the 3rd day of operation (in solid phase) and increased steadily together with the degradation of NP2EO into NP1EO and NP2EC first and then finally into NP1EC.

As can be seen from the Figures 4.13, 4.14 and 4.15, NP did not accumulate within the reactor similar to C-1 reactor. The reason is same as before, under aerobic conditions NPnEC compounds are the dominating metabolites in the degradation of NPnEOs.

After 10 days of reactor operation, NP1EC concentration reached stationary phase and showed this pattern for the rest of 20 days. When NP1EC became stable, other compounds (NP, NP1EO and NP2EO) also became stationary. This showed that degradation was limited to a certain extent and stopped on the 10^{th} day of operation.

The degradation patterns for both phases were similar to each other. The only difference was the concentration levels. Since NP compounds are hydrophobic in nature, they accumulated on solid surfaces, as a result the concentrations in sludge were always higher and the majority of the concentration within the whole reactor was due to the solid phase. In liquid phase, initially, most of the compounds were below detection. NP1EC was detected only after 8 days; while it was detected at 3 days in solid phase. When the degradation was completed, parent compound was not detected in liquid phase for most of the samples, however, it was still detectable for solid phase.

Again, the figures for solid and liquid phase (Figures 4.13 and 4.14) involve error bars that were not visible for NP, NP1EO and NP2EO. Due to the same reasons stated in C-1 reactor (error prone nature of the derivatization method applied to NP1EC) the standard deviations obtained were higher for NP1EC.

Reactor-1

The graphs obtained for solid phase, liquid phase and whole reactor are given in Figures 4.16, 4.17 and 4.18.

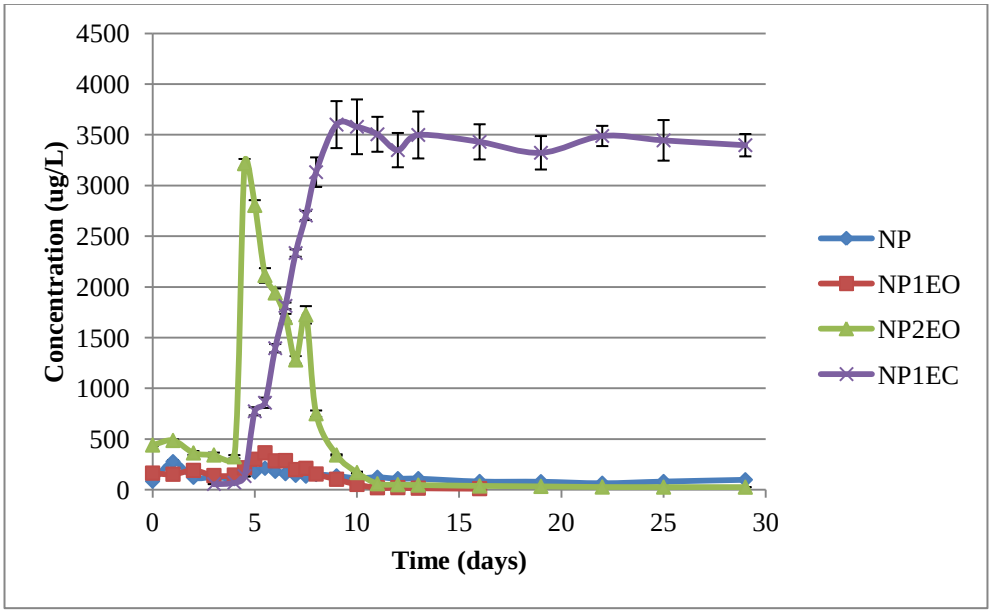


Figure 4.16 NP compounds (solid phase) vs time for R-1

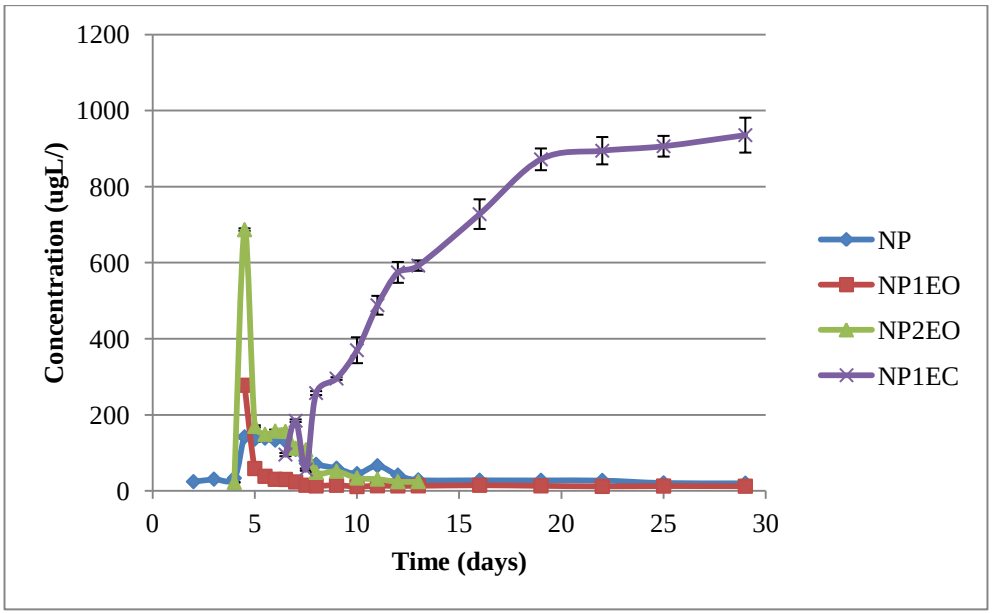


Figure 4.17 NP compounds (liquid phase) vs time for R-1

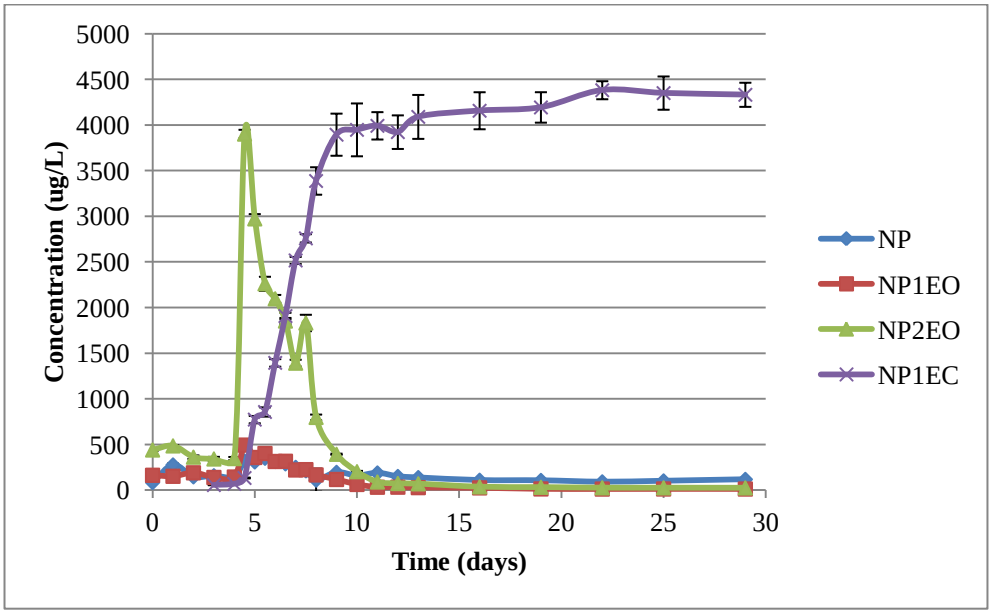


Figure 4.18 NP compounds vs time (whole reactor) for R-1

Initially, on the first four days before NP2EO spike, the concentrations of NP compounds were very similar to both of the control reactors C-1 and C-2. This shows the homogenous addition of WAS in all four reactors. Until the 4^{th} day of operation, NP2EO decreased in concentration due to degradation. On the day of spike, as can be seen from Figures 4.16, 4.17 and 4.18, NP2EO concentration increased sharply to a concentration almost 10 times of the concentration before the spike. This showed that NP2EO was spiked successfully; an increase of about 3 mg/L was observed which was the target concentration. After the day of spike, NP2EO concentration dropped sharply, and at this point frequent sampling allowed for the observation of degradation pattern better. After 4 days, the metabolites of NP2EO started to form, but the dominating specie among all others was NP1EC. While NP2EO was degraded, NP1EC was formed as can be seen in Figures 4.16, 4.17 and 4.18. Also, NP1EO was converted into NP1EC as expected and decreased in concentration. The reactors were terminated when these concentrations became stationary.

The degradation pattern and concentrations of NP compounds discussed above for solid samples taken from the reactors well-represented the reactor since the compounds are hydrophobic. The compounds were not easily detected in liquid phase since they tend to stay on solid surfaces.

Again, the figures for solid and liquid phase (Figures 4.16 and 4.17) involve error bars that were not visible for NP, NP1EO and NP2EO. Due to the same reasons stated in C-1 and C-2 reactors (more standard and simple derivatization method for NP, NP1EO and NP2EO and error prone nature of the derivatization method applied to NP1EC) the standard deviations obtained were higher for NP1EC.

Reactor-2

The graphs presenting the results obtained from the spiked replica of the reactor for solid and liquid phase and the whole reactor are given in Figures 4.19, 4.20 and 4.21.

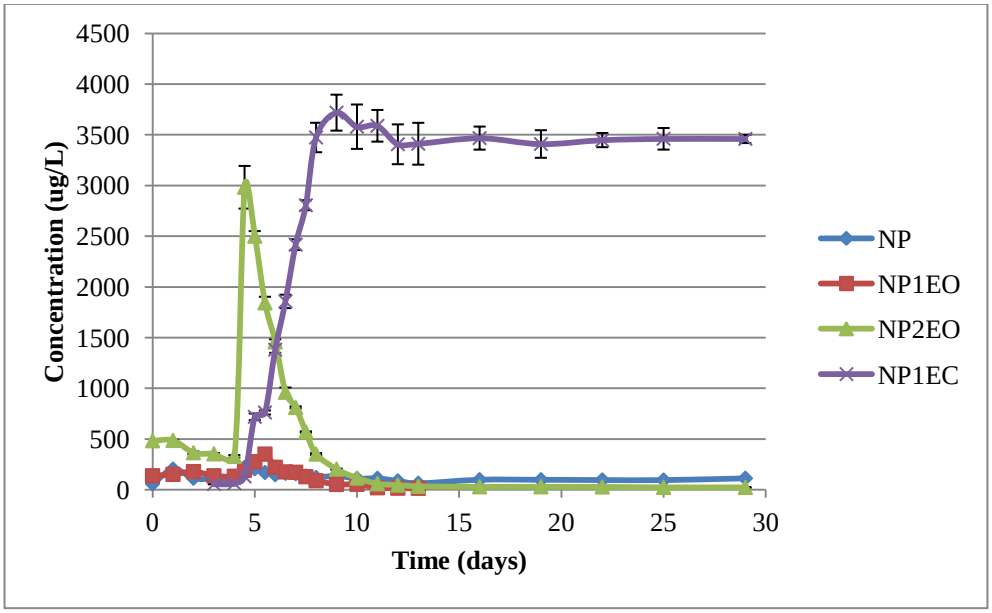


Figure 4.19 NP compounds (solid phase) vs time for R-2

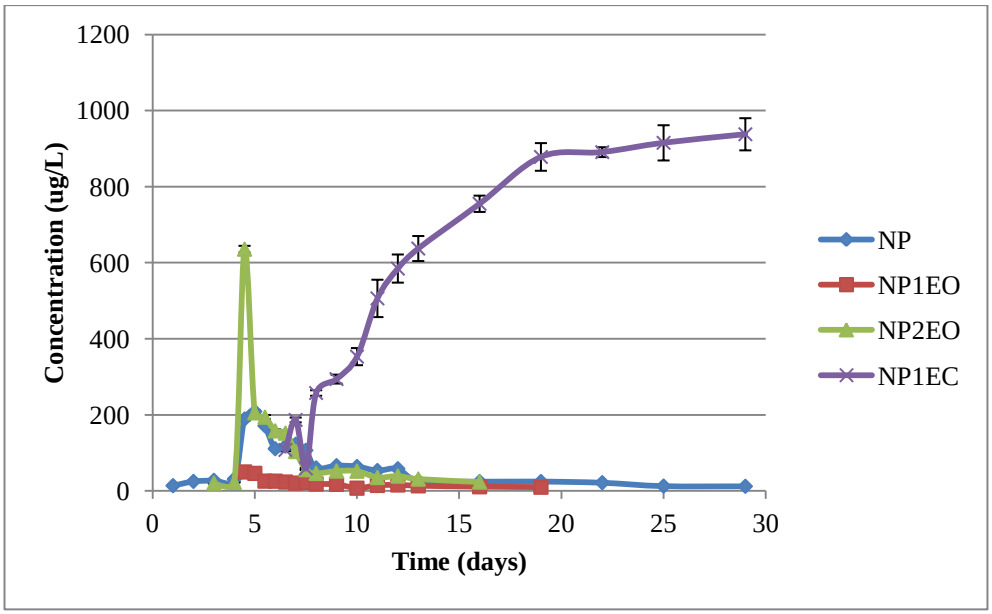


Figure 4.20 NP compounds (liquid phase) vs time for R-2

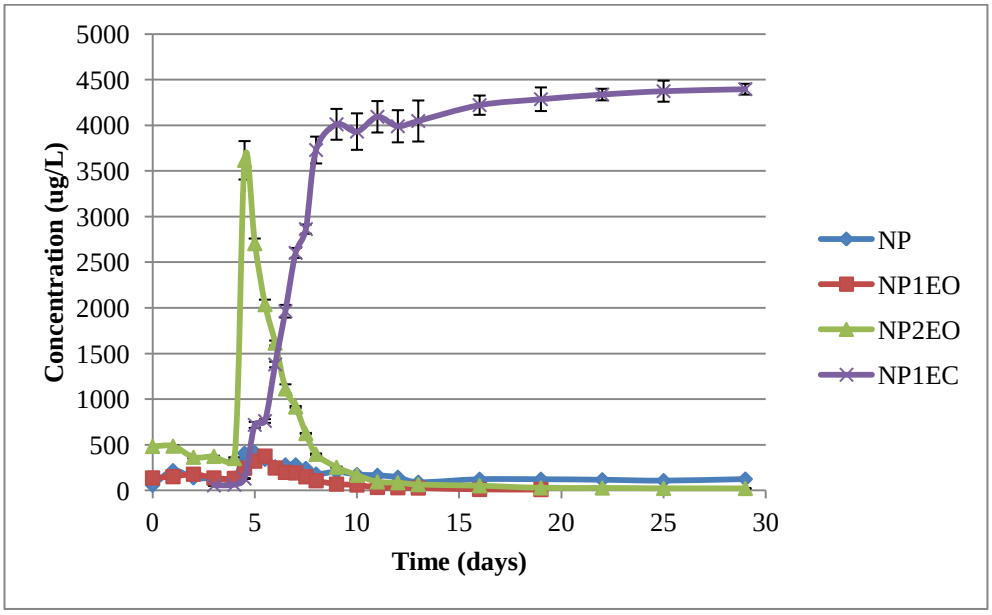


Figure 4.21 NP compounds (whole reactor) vs time for R-2

Similar to the control reactors, NP species in R-2 reactor showed almost the same concentrations with the R-1 reactor. In fact the initial concentration in all four reactors which were measured all separate after the reactor set up showed almost the same concentration. Until the day of NP2EO spike, NP2EO decreased in concentration and there was a slight change in the concentration of metabolites. On the day of spike (see Figures 4.19, 4.20 and 4.21), NP2EO level in the R-2 reactor increased sharply due to injection of 3 mg/L NP2EO dissolved in acetone showing that spike was successful. Following the day of spike, NP2EO concentration starts to decrease very fast. The dominant specie between the metabolites formed was NP1EC. As NP2EO was degraded, NP1EC started to form at 10 days, all of the species reached stationary phase and after monitoring for a while, the reactors were terminated.

The degradation pattern and concentrations of NP compounds in solid phase represented the whole of the reactor. The patterns were also similar for both phases, however, the compounds were not easily detected in liquid phase since they tend to stick on solid surfaces due to their hydrophobic nature.

Again, the figures for solid and liquid phase (Figures 4.19 and 4.20) involve error bars that were not visible for NP, NP1EO and NP2EO. Due to the same reasons stated in C-1 and C-2 reactors (more standard and simple derivatization method for NP, NP1EO and NP2EO and error prone nature of the derivatization method applied to NP1EC) the standard deviations obtained were higher for NP1EC.

Rate of NP2EO Degradation and NP1EC Formation

In all four reactors (C-1, C-2, R-1 and R-2), there was a decrease in NP2EO where NP1EC formation was observed. At these data points, it was decided to calculate the rate of NP2EO degradation and NP1EC formation within the reactors. For this purpose, first the order of reaction should be determined. There are three main types rate relations: zeroth order reaction, first order reaction and second order reaction.

When the graphs C vs t (zeroth order), lnC vs t (first order) and 1/C vs t (second order) were plotted for both NP2EO and NP1EC. R^2 values for both NP2EO degradation and NP1EC formation were found to be 0.95, 0.99 and 0.93 for zeroth, first and second order, respectively, therefore the highest R^2 values for both NP2EO degradation and NP1EC formation were obtained for the lnC vs t plot and the reactions were found to follow first order kinetics. The reaction rate constants obtained for all four reactors (C-1, C-2, R-1 and R-2) are given in Table 4.5.

Table 4.5 NP2EO degradation and NP1EC formation rate constants calculated for the reactors

	Rate constant (day^{-1})	
Reactor	**NP2EO degradation**	**NP1EC formation**
C-1	0.50	0.63
C-2	0.48	0.63
R-1	0.42	0.53
R-2	0.57	0.59

As can be seen in Table 4.5, degradation and decay rates for all four reactors were very close to each other indicating that the degradation/formation rates were independent of the concentrations of NP compounds.

Mass Balance Calculations

In order to further control the data obtained and see the transformation between the parent compounds and its metabolites, a mass balance should be done on the species present. The mass balance results for all four reactors are given in Table 4.6 to Table 4.9. All the calculations were done for the whole reactor (summation of liquid and solid phases). The first sample after spike was taken 3 hours after the injection of 3 mg/L NP2EO in acetone. Following this, samplings were done twice a day with 12 h intervals.

Table 4.6 Results of mass balance calculations for C-1

Time (days)	NP (mg)	NP1EO (mg)	NP2EO (mg)	NP1EC (mg)	Total (mg)
0	0.27	0.51	1.50	<LOD	2.27
1	0.84	0.49	1.28	<LOD	2.61
2	0.46	0.61	1.23	<LOQ	2.30
3	0.48	0.43	1.09	<LOQ	2.00
4	0.46	0.47	1.15	0.16	2.24
4.5*	1.27	0.86	2.75	0.39	5.27
5	1.10	1.02	2.12	0.46	4.70
5.5	1.08	1.19	1.52	0.67	4.46
6	1.02	0.91	1.45	0.89	4.27
6.5	0.87	0.50	1.23	1.02	3.63
7	0.70	0.45	0.77	1.64	3.57
7.5	0.65	0.34	0.55	2.48	4.02
8	0.56	0.26	0.49	3.03	4.35
9	0.53	0.23	0.44	4.10	5.30
10	0.53	0.11	0.34	4.62	5.60
11	0.47	0.08	0.25	4.44	5.24
12	0.48	<LOQ	0.22	4.37	5.07
13	0.40	<LOQ	0.16	4.71	5.28
16	0.38	0.03	0.14	4.71	5.27
19	0.33	<LOQ	<LOQ	4.81	5.14
22	0.30	<LOQ	<LOQ	4.74	5.05
25	0.23	<LOQ	<LOQ	4.83	5.06
29	0.23	<LOQ	<LOQ	4.77	5.00

*Time of acetone addition

Table 4.7 Results of mass balance calculations for C-2

Time (days)	NP (mg)	NP1EO (mg)	NP2EO (mg)	NP1EC (mg)	Total (mg)
0	0.26	0.50	1.46	<LOD	2.23
1	0.71	0.48	1.40	<LOD	2.60
2	0.46	0.61	1.19	<LOQ	2.25
3	0.47	0.38	1.13	0.16	2.15
4	0.40	0.40	1.04	0.18	2.03
4.5*	0.96	0.96	2.21	0.38	4.50
5	1.12	1.13	2.05	0.47	4.77
5.5	1.02	0.70	1.63	0.68	4.03
6	0.94	0.66	1.33	0.88	3.81
6.5	0.90	0.46	1.17	1.05	3.59
7	0.80	0.43	0.78	1.67	3.69
7.5	0.76	0.31	0.53	2.51	4.11
8	0.63	0.21	0.46	2.93	4.23
9	0.52	0.14	0.33	3.91	4.89
10	0.46	0.12	0.28	4.41	5.27
11	0.44	0.07	0.21	4.33	5.05
12	0.46	0.03	0.19	4.71	5.39
13	0.39	0.05	0.15	4.92	5.51
16	0.31	0.03	0.08	4.86	5.28
19	0.29	<LOQ	<LOQ	5.13	5.42
22	0.28	<LOQ	<LOQ	4.81	5.08
25	0.20	<LOD	<LOQ	4.88	5.09
29	0.19	<LOD	<LOQ	4.80	4.99

*Time of acetone addition

Table 4.8 Results of mass balance calculations for R-1

Time (days)	NP (mg)	NP1EO (mg)	NP2EO (mg)	NP1EC (mg)	Total (mg)
0	0.27	0.52	1.41	<LOD	2.20
1	0.88	0.49	1.55	<LOD	2.92
2	0.47	0.61	1.16	<LOQ	2.23
3	0.50	0.44	1.09	0.18	2.22
4	0.45	0.46	1.11	0.22	2.24
4.5*	1.02	1.58	12.48	0.43	15.51
5	0.99	1.15	9.51	2.47	14.12
5.5	1.12	1.28	7.23	2.74	12.38
6	1.00	1.00	6.71	4.46	13.18
6.5	0.92	1.01	5.93	6.10	13.97
7	0.80	0.71	4.45	8.05	14.01
7.5	0.68	0.72	5.86	8.83	16.09
8	0.35	0.53	2.55	10.84	14.27
9	0.62	0.37	1.26	12.46	14.72
10	0.51	0.20	0.65	12.63	13.99
11	0.60	0.10	0.29	12.77	13.76
12	0.48	0.10	0.23	12.55	13.37
13	0.44	0.09	0.22	13.09	13.84
16	0.35	0.08	0.12	13.30	13.84
19	0.34	0.04	0.10	13.42	13.90
22	0.29	0.04	0.08	14.02	14.43
25	0.32	0.04	0.08	13.92	14.36
29	0.38	0.04	0.08	13.86	14.35

*Time of NP2EO spike

Table 4.9 Results of mass balance calculations for R-2

Time (days)	NP (mg)	NP1EO (mg)	NP2EO (mg)	NP1EC (mg)	Total (mg)
0	0.18	0.44	1.54	<LOD	2.16
1	0.69	0.48	1.55	<LOD	2.73
2	0.44	0.57	1.16	<LOQ	2.17
3	0.43	0.44	1.19	0.17	2.24
4	0.42	0.42	1.12	0.20	2.16
4.5*	1.30	0.78	11.57	0.42	14.07
5	1.33	1.03	8.65	2.30	13.31
5.5	1.09	1.20	6.51	2.43	11.24
6	0.83	0.79	5.17	4.42	11.19
6.5	0.89	0.63	3.56	6.28	11.37
7	0.89	0.61	2.93	8.33	12.76
7.5	0.77	0.48	2.00	9.16	12.41
8	0.57	0.34	1.27	11.93	14.12
9	0.65	0.22	0.81	12.84	14.52
10	0.56	0.19	0.53	12.58	13.86
11	0.53	0.10	0.30	13.10	14.03
12	0.46	0.10	0.27	12.77	13.59
13	0.29	0.08	0.20	12.95	13.53
16	0.39	0.03	0.16	13.51	14.10
19	0.39	0.03	0.09	13.72	14.23
22	0.37	<LOQ	0.09	13.88	14.34
25	0.34	<LOQ	0.07	14.00	14.41
29	0.40	<LOQ	0.07	14.07	14.53

*Time of NP2EO spike

As can be seen from the tables given for mass balance calculations, the results match the day spike was done. The total mass in the reactors at days 1, 2,3 and 4 can nicely be matched to the total mass at day zero. This good match can be seen for all the four reactors. At day 4.5 and onwards, the spiked masses can be seen in the tables. Therefore, the data after 4.5 days should be compared to the data taken at 4.5 days. This data too shows a very good match between themselves for all reactors. The only time interval that the data deviated was the time between 5.5 to 9 days. The reason why between the days 5.5-9 the results does not match with each other is the missing intermediate metabolite in between which is NP2EC. The mass balances were also carried out on molar percentage basis and the results are given in Appendix-D.

As NP2EO was converted into NP1EO, NP1EO was being degraded into NPnECs and NP on one side. This can be easily seen in the mass balance tables given (see Tables 4.6, 4.7, 4.8 and 4.9). As time proceeded, NP1EO mass/concentration decreased and became almost stable at the end. As NP2EO and NP1EO were degraded NP1EC formation increased.

Suggested Mechanism for NP2EO Degradation in Aerobic Batch Digesters

One may recall from Chapter 2 that under aerobic circumstances the rate of degradation and type of metabolites are different. As described before, degradation of NP compounds starts with the attack of microorganisms to ethylene oxide unit of NPnEOs. The metabolites formed are NP, NP1EO, NP2EO, NP1EC and NP2EC. The formation of NP1EC at rate close to the degradation of NP2EO was the evidence that NP2EO degraded to form NPnECs and other metabolites such as NP1EO. The sharp decrease in the plotted graphs showed that the rate of degradation was fast. Unlike anaerobic systems, under aerobic conditions accumulation of NP is not expected and this can also be seen from the results obtained. The dominant metabolite was NP1EC, since NP is not the only daughter product in aerobic

systems. Instead, NP1EC formation was observed and NP1EC accumulated within the system. In terms of NPE sum underlined in the legislations, NP1EC formation is favored. The degradation of NP2EO into NP1EO and then into NP1EC was very fast that when one checks the molar percentages it seems like NP1EC formation occured before NP1EO was completely degraded. But this was not the case, the rate of NP1EO degradation into NPnECs was not as fast as the rate of NP2EO transformation into NP1EO. From here, it could be concluded that not every intermediate step has the same reaction rate. It could be said that as the number of ethylene oxide chain decreases, the rate of degradation also decreases. Unfortunately, the intermediate metabolite NP2EC was not measured throughout the study due to some limitations. But the presence of NP2EC was for sure and this was the reason for the gap in mass balance between days 5.5 and 9.

From the results obtained, it can be easily claimed that under aerobic conditions, degradation is faster as stated in the literature (CEPA, 1999), but still the extent of degradation is limited. The good thing about aerobic degradation is that the sum NPE becomes lower and the chance of complying with the regulations is higher. That is why, nowadays, the systems are generally combined with aerobic systems to enhance NPnEO degradation.

CHAPTER 5

CONCLUSION

This study was formed from three main parts: establishment of methods for the analysis of NP compounds, operation of NP free laboratory scale preliminary aerobic batch reactors and operation of NP2EO spiked laboratory scale aerobic batch reactors to observe the degradation kinetics and products of NP2EO degradation.

The NP compounds (NP, NP1EO, NP2EO and NP1EC) were analyzed using GC-MS. Unfortunately, NP compounds are challenging chemicals due to their many properties. These compounds need derivatization for sensitive and more accurate analysis. The first thing done in this study was the determination of a proper derivatization method for the NP compounds. Many methods of derivatization (i.e. acetylation, propylation, methylation, etc.) are present in the literature. From a number of methods tested, two methods were selected for two different groups of compounds. For NP, 4-nNP, NP1EO and NP2EO, silylation as a method of derivatization yielded the best results. Since NP1EC has totally different characteristics when compared to the other four chemicals, methylation yielded the best results.

Once the derivatization method was set GC-MS program was improved to detect the chemicals with better accuracy. Several methods given in the literature were listed and applied. It became necessary that a GC program was developed in this work. Different temperature ramps and GC parameters were tested and the best method was

selected for the analysis of NP, 4-nNP, NP1EO and NP2EO. For NP1EC, however, the GC-MS program originally given by Lee et.al., 2007 was used with minor modifications done due to the difference in the devices used.

The study continued with the development of extraction methods for the five chemicals used from solid and liquid phases. The most commonly applied methods for the extraction of NP compounds from solid phase were sonication and mechanical shaking procedures. Both methods and their combinations were tested with different solvents for different time intervals. The results obtained were compared based on the recoveries and the repeatabilities of the results. The method satisfying these requirements was 5 min sonication based extraction using acetone as solvent. For all five chemicals investigated in this study, this method yielded recoveries between 90-130%. For the liquid phase extraction, an SPE method was developed using C-18 cartridge. Mixture of acetone and methanol (1:1, v/v) was selected as the most appropriate extraction solvent. This method of extraction was applied for all chemicals.

In the preliminary set of aerobic digesters, two sets of reactors with different solids concentrations were operated to find out the typical performance for solids reduction, understand and overcome the possible operational problems that may affect the future operation of NP2EO dosed reactors. It was observed that as the solids concentration increased, the effectiveness of digestion, in other words, reduction of organic matter increased. This was important information used in the set up of NP2EO dosed reactors. Another point that was helpful for the future reactors was the overflow of the reactors due to aeration. As air was pumped into the system, air bubbles started to form requiring a large head space for the reactors to prevent the overflow.

NP2EO dosed aerobic batch reactors were set up to observe the change in NP compounds and degradation along aerobic digestion. The parameters TS, VS, TSS and VSS showed expected patterns and the percentages of organic matter reduction that was within the ranges stated in the literature and parallel with the previous set reactors. pH showed increase after some point and then decreased sharply as the reactors became steady.

NP2EO was dosed into the reactors R-1 and R-2 on the 4^{th} day of operation. Together with the spike of NP2EO, the concentration in the reactor showed an increase similar to the amount added. After the spike, NP2EO showed a very fast degradation and was converted into NP, NP1EO and NP1EC. NP1EC was observed after a couple of days and after the spike the concentrations increased steadily. For all chemicals mass balance calculations were done and the results were satisfactory. Following couple of days after the NP2EO spike, the sum of the compounds did not match with each other due to the formation of unmeasured NP2EC compound. Other than that the rate of degradation and the metabolites formed were parallel to the degradation details given in the literature for other types of aerobic studies showing the success of the analysis and operation in this work and also the validity of similar reactions in aerobic sludge digesters.

As can be seen from the results, NP did not accumulate. This is the reason why aerobic systems are more successful in complying with the regulatory values. Since under anaerobic conditions NP accumulates significantly, NPE (the sum of NP, NP1EO and NP2EO) measured results in values greater than 50 mg/kg dry mass. On the other hand, aerobic conditions make NPnEC formation possible and since carboxylated forms are not included in the NPE sum that is used in regulations a wrong opinion can be obtained. For a total removal of the NP compounds aerobic and anaerobic systems need to be combined and operated in sequence.

CHAPTER 6

FUTURE WORK AND RECOMMENDATIONS

The results of this study indicate the rapid degradation of NP2EO under aerobic conditions. However, aerobic treatment is energy intensive and expensive method of stabilization, it is not feasible to apply it to large volumes of sludge. But it can be used as a pretreatment method before other methods of stabilizations to enhance NPnEO degradation, considering the regulations on sludge for the sum NPE.

There are some but limited information degradation mechanisms of NPnEOs, however, when it comes to the degradation of NPnECs, not much is provided in the literature. Therefore, as a new area of research, the degradation mechanisms of NPnECs could be taken into consideration.

The reactors in this study were operated in mesophilic range. Using thermophilic aerobic digesters, the effect of temperature on the degradation of these compounds can be understood. Since the information on the degradation of NP compounds is limited and treatment cannot be achieved 100%, thermophilic aerobic digesters could enhance the extent of degradation.

During the determination of the extraction method for solid phase, it was found out that sonication has a degrading effect on NP2EO. This could be analyzed in detail and sonication can be used as a pretreatment method. Sonication is not an

extraordinary method for sludge treatment, and if using it during pretreatment enhances the extent of degradation, especially for land application of sludge (in order to satisfy the NPE limit), it could be an option.

In conclusion, aerobic digestion does not only reduce the NP content of sludge rapidly but also produces highly oxidized product when compared to anaerobic digestion. Considering land application of sludge becoming popular lately, aerobic digestion could be a solution for small communities to overcome the NPE limit of governments. For larger systems, combining the stabilization techniques with aerobic methods could be a solution.

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APPENDIX A: CALIBRATION CURVES

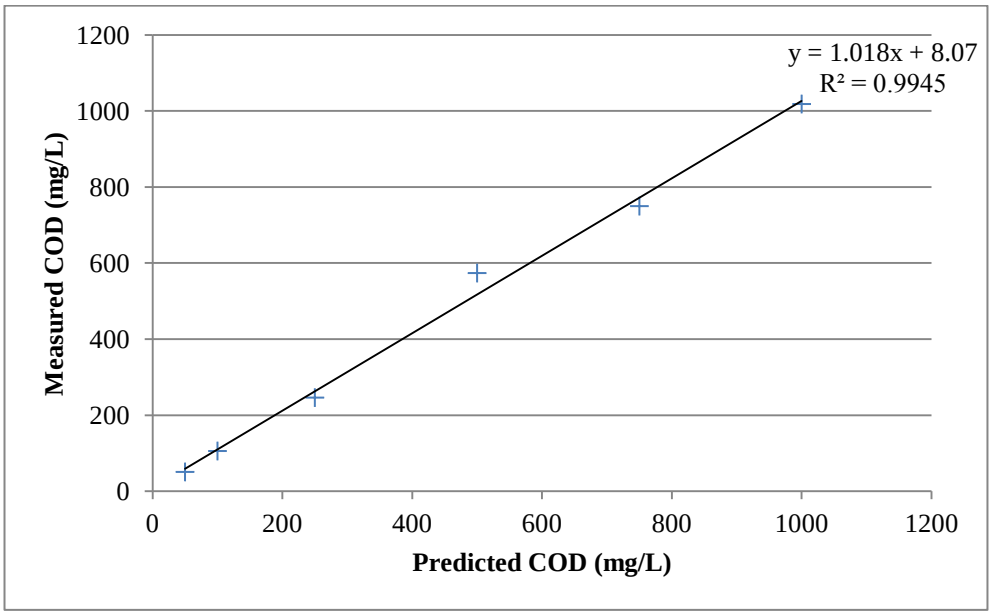

Figure A.1 Calibration curve for the COD solution prepared manually

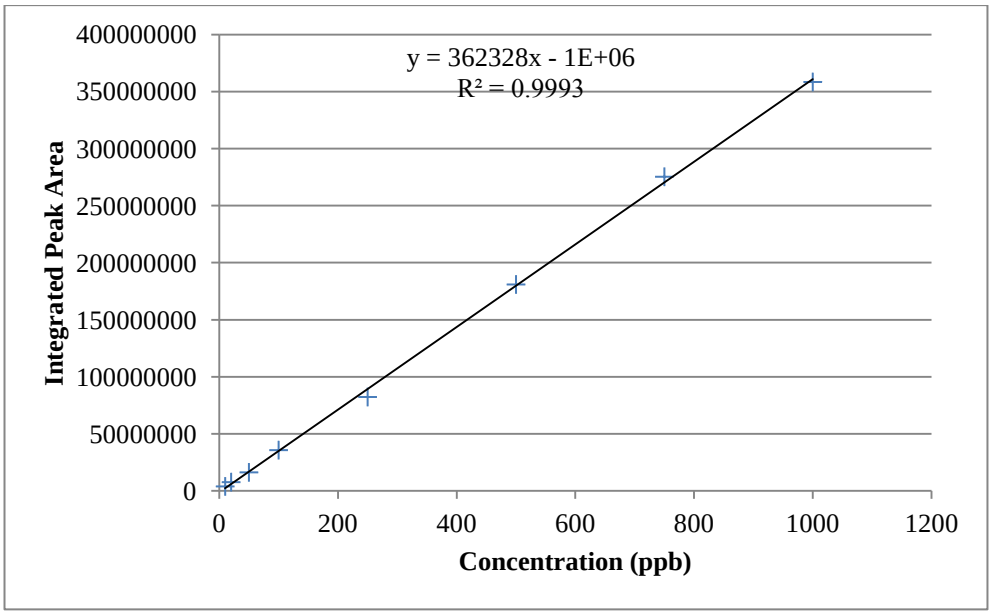

Figure A.2 Calibration curve for silyl derivatized NP

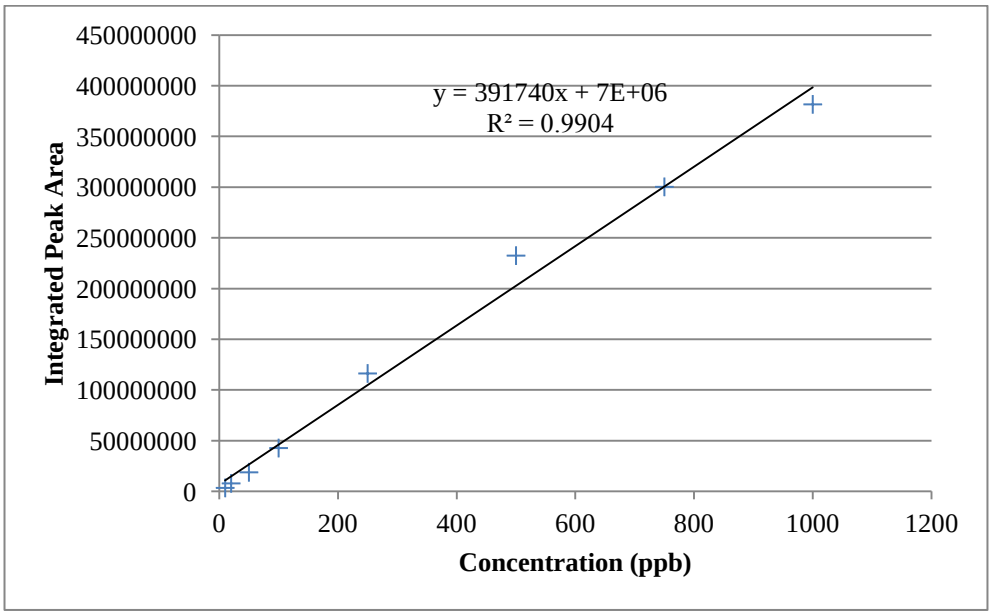

Figure A.3 Calibration curve for the silyl derivatized 4-nNP

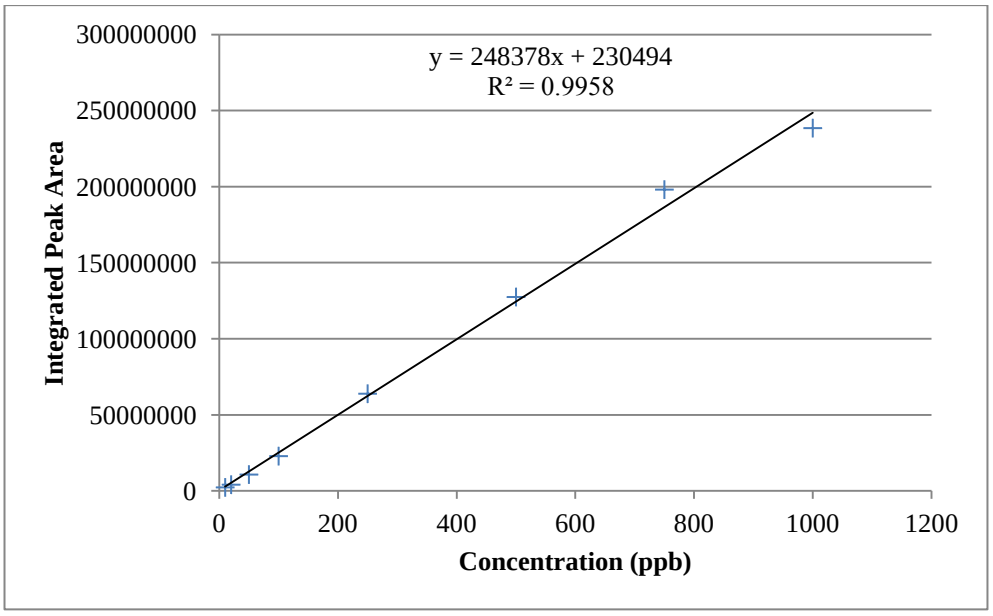

Figure A.4 Calibration curve for the silyl derivatized NP1EO

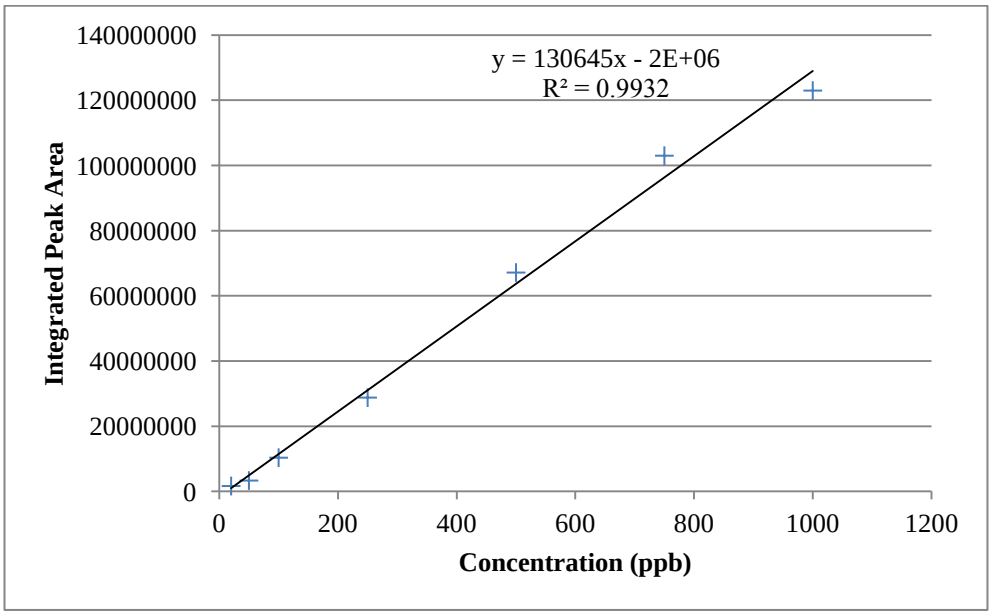

Figure A.5 Calibration curve for silyl derivatized NP2EO

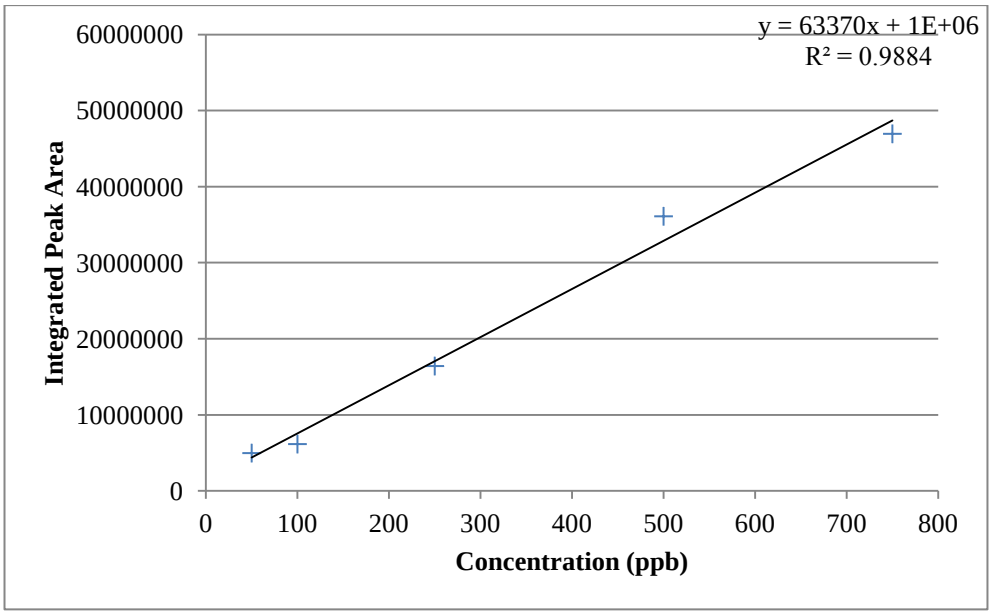

Figure A.6 Calibration curve for methylated NP1EC

APPENDIX B: EXAMPLE TOTAL ION CHROMATOGRAMS

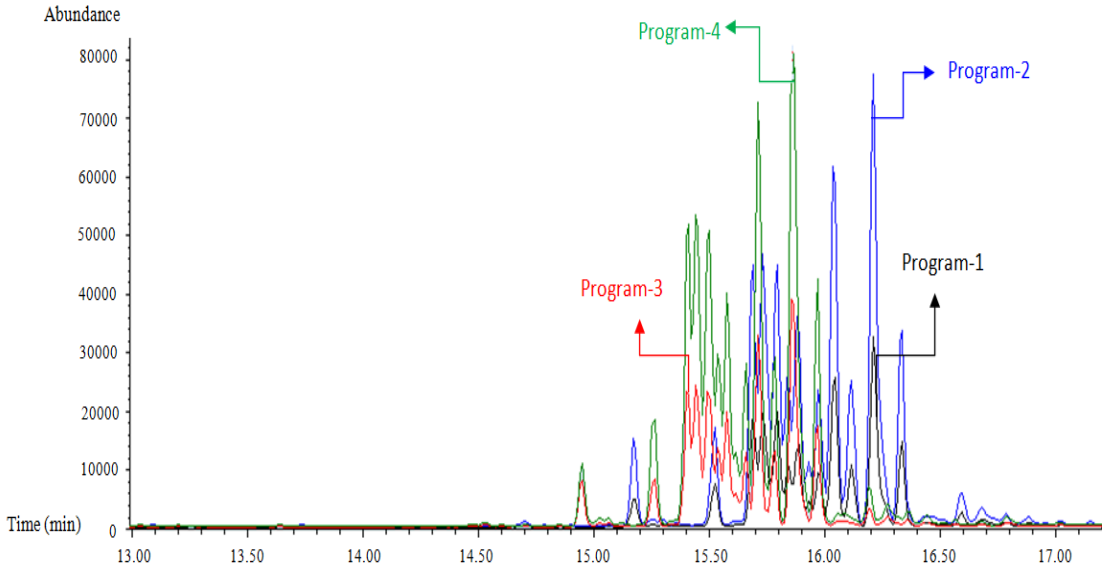

Figure B.1 Comparison of TICs for NP1EO analyzed using Program-1, Program-2, Program-3 and Program-4

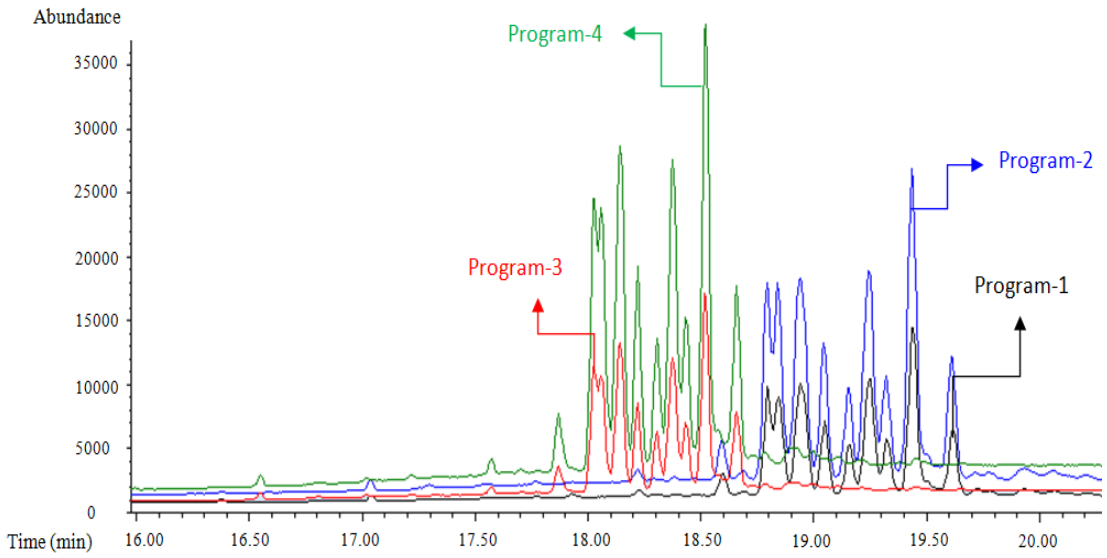

Figure B.2 Comparison of TICs for NP2EO analyzed using Program-1, Program-2, Program-3 and Program-4

APPENDIX C: DETAILS OF CALIBRATION CURVES FOR NP COMPOUNDS

Table C.1 Calibration curve data for NP, 4-nNP, NP1EO, NP2EO and NP1EC

Compound	Concentration (ppb)	Integrated Peak Area	% RSD
NP	10	3861536.50 ± 30568.93	0.79
	20	7599687.00 ± 116266.74	1.53
	50	16178595.50 ± 409343.41	2.53
	100	35654839.50 ± 199595.74	0.56
	250	82233150.00 ± 1194663.98	1.45
	500	180878003.00 ± 1810744.90	1.00
	750	275301145.50 ± 3717217.22	1.35
	1000	358362333.00 ± 364077.97	0.10
NP1EO	10	2239002.50 ± 57379.59	2.56
	20	4040509.00 ± 3115.51	0.08
	50	10677187.00 ± 222720.25	2.09
	100	22804030.00 ± 22804030.00	3.08
	250	63843006.50 ± 166880.74	0.26
	500	127419710.00 ± 785502.30	0.62
	750	198024761.50 ± 3767403.41	1.90
	1000	238448657.50 ± 476041.96	0.20

Table C.1 Calibration curve data for NP, 4-nNP, NP1EO, NP2EO and NP1EC (cont'd)

Compound	Concentration (ppb)	Integrated Peak Area	% RSD
	20	1653453.50 ± 14589.73	0.88
	50	3330467.50 ± 15787.57	0.47
	100	10352355.50 ± 131034.66	1.27
NP2EO	250	28778795.00 ± 544419.90	1.89
	500	67136540.00 ± 320134.11	0.48
	750	102997782.00 ± 16533.57	0.02
	1000	122974694.50 ± 4289174.68	3.49
	10	3458803.50 ± 31230.79	0.90
	20	7903868.00 ± 38227.61	0.48
	50	18824834.50 ± 15586.75	0.08
4-nNP	100	42753179.00 ± 211349.97	0.49
	250	116325387.50 ± 1445095.04	1.24
	500	232518298.00 ± 2748826.70	1.18
	750	300339482.50 ± 218657.92	0.07
	1000	381666443.00 ± 5201398.29	1.36
	50	59913996.00 ± 1212791.37	2.02
	100	46939229.00 ± 655028.37	1.40
NP1EC	250	36092768.67 ± 1125606.06	3.12
	500	16405803.94 ± 507318.08	3.09
	750	6145025.23 ± 352695.29	5.74
	1000	4958540.00 ± 117249.62	2.36

APPENDIX D: MASS BALANCE TABLES

Table D.1 Results of mass balance calculations (molar percentage) for C-1

Time (days)	NP (%M)	NP1EO (%M)	NP2EO (%M)	NP1EC (%M)	Total (%M)
0	15.1	24.1	60.8	<LOD	100.0
1	38.8	18.9	42.4	<LOD	100.0
2	24.6	27.7	47.7	<LOQ	100.0
3	29.8	22.3	47.9	<LOQ	100.0
4	25.5	21.8	45.5	7.2	100.0
4.5*	29.9	16.8	46.0	7.3	100.0
5	28.7	22.2	39.6	9.5	100.0
5.5	29.3	26.9	29.4	14.4	100.0
6	29.1	21.6	29.5	19.9	100.0
6.5	29.2	14.1	29.6	27.2	100.0
7	24.0	12.9	18.8	44.3	100.0
7.5	19.7	8.7	12.0	59.7	100.0
8	16.0	6.2	10.0	67.8	100.0
9	12.4	4.5	7.3	75.8	100.0
10	11.7	2.1	5.3	80.9	100.0
11	11.1	1.5	4.2	83.2	100.0
12	11.8	<LOQ	3.8	84.4	100.0
13	9.5	<LOQ	2.7	87.8	100.0
16	9.1	0.7	2.4	87.9	100.0
19	8.0	<LOQ	<LOQ	92.0	100.0
22	7.4	<LOQ	<LOQ	92.6	100.0
25	5.7	<LOQ	<LOQ	94.3	100.0
29	5.6	<LOQ	<LOQ	94.4	100.0

*Time of acetone addition

Table D.2 Results of mass balance calculations (molar percentage) for C-2

Time (days)	NP (%M)	NP1EO (%M)	NP2EO (%M)	NP1EC (%M)	Total (%M)
0	15.2	24.2	60.5	<LOD	100.0
1	33.5	19.1	47.4	<LOD	100.0
2	25.2	28.0	46.8	<LOQ	100.0
3	27.3	18.5	46.9	7.3	100.0
4	24.9	20.6	45.5	9.0	100.0
4.5*	26.4	22.0	43.4	8.2	100.0
5	28.9	24.1	37.6	9.5	100.0
5.5	30.9	17.6	35.3	16.2	100.0
6	29.9	17.6	30.4	22.1	100.0
6.5	30.6	13.0	28.4	28.0	100.0
7	26.2	11.9	18.4	43.5	100.0
7.5	22.5	7.6	11.2	58.7	100.0
8	18.3	5.1	9.5	67.1	100.0
9	13.1	2.9	5.9	78.1	100.0
10	10.7	2.3	4.7	82.2	100.0
11	10.7	1.3	3.7	84.2	100.0
12	10.6	0.6	3.0	85.7	100.0
13	8.8	1.0	2.4	87.8	100.0
16	7.3	0.6	1.3	90.8	100.0
19	6.6	<LOQ	<LOQ	93.4	100.0
22	6.7	<LOQ	<LOQ	93.3	100.0
25	5.0	<LOD	<LOQ	95.0	100.0
29	4.9	<LOD	<LOQ	95.1	100.0

*Time of acetone addition

Table D.3 Results of mass balance calculations (molar percentage) for R-1

Time (days)	NP (%M)	NP1EO (%M)	NP2EO (%M)	NP1EC (%M)	Total (%M)
0	15.7	25.4	58.9	<LOD	100.0
1	36.8	16.9	46.3	<LOD	100.0
2	25.9	28.1	46.0	<LOQ	100.0
3	28.1	20.6	43.5	7.8	100.0
4	25.1	21.3	43.9	9.6	100.0
4.5*	8.8	11.4	76.9	2.9	100.0
5	9.3	8.9	63.5	18.3	100.0
5.5	11.7	11.2	54.2	22.8	100.0
6	9.9	8.2	47.1	34.7	100.0
6.5	8.5	7.8	39.1	44.6	100.0
7	7.3	5.4	29.1	58.3	100.0
7.5	5.5	4.8	33.6	56.1	100.0
8	3.1	4.0	16.3	76.6	100.0
9	5.3	2.7	7.7	84.3	100.0
10	4.6	1.5	4.2	89.8	100.0
11	5.5	0.8	1.9	91.9	100.0
12	4.5	0.8	1.6	93.1	100.0
13	4.0	0.7	1.4	93.9	100.0
16	3.1	0.6	0.8	95.5	100.0
19	3.1	0.3	0.7	96.0	100.0
22	2.5	0.3	0.5	96.7	100.0
25	2.8	0.3	0.5	96.4	100.0
29	3.3	0.3	0.5	95.9	100.0

*Time of NP2EO spike

Table D.4 Results of mass balance calculations (molar percentage) for R-2

Time (days)	NP (%M)	NP1EO (%M)	NP2EO (%M)	NP1EC (%M)	Total (%M)
0	11.1	22.1	66.8	<LOD	100.0
1	31.4	18.2	50.4	<LOD	100.0
2	25.2	27.1	47.6	<LOQ	100.0
3	24.1	20.4	47.7	7.7	100.0
4	24.4	20.3	46.2	9.0	100.0
4.5*	12.4	6.1	78.4	3.1	100.0
5	13.0	8.4	60.7	17.9	100.0
5.5	12.6	11.6	53.7	22.2	100.0
6	9.5	7.6	42.6	40.3	100.0
6.5	10.0	5.9	28.4	55.6	100.0
7	8.9	5.1	20.7	65.4	100.0
7.5	7.8	4.0	14.5	73.6	100.0
8	5.1	2.5	8.1	84.3	100.0
9	5.6	1.6	5.0	87.8	100.0
10	5.0	1.4	3.4	90.1	100.0
11	4.7	0.8	1.9	92.6	100.0
12	4.3	0.7	1.8	93.2	100.0
13	2.7	0.7	1.4	95.3	100.0
16	3.5	0.3	1.0	95.2	100.0
19	3.5	0.2	0.6	95.8	100.0
22	3.3	<LOQ	0.5	96.2	100.0
25	3.0	<LOQ	0.4	96.6	100.0
29	3.4	<LOQ	0.4	96.2	100.0

*Time of NP2EO spike