FATE OF NONYLPHENOL COMPOUNDS IN AEROBIC SEMI-CONTINUOUS REACTORS

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FATE OF NONYLPHENOL COMPOUNDS IN AEROBIC SEMI-CONTINUOUS REACTOR

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ABSTRACT

FATE OF NONYLPHENOL COMPOUNDS IN AEROBIC SEMI-CONTINUOUS REACTOR

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In the last few decades, numerous studies have been conducted on xenobiotic compounds due to their vast use in industries, households, etc. and consequently high exposure of these compounds. The main focus of this study is nonylphenol compounds such as nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxy acetic acid (NP1EC) and nonylphenol (NP), which are among the harmful xenobiotic compounds that can cause endocrine disruption, cancer and other health problems and which are used widely in the production of surfactants and personal care products. In this study, laboratory scale aerobic semi continuous reactors containing Waste Activated Sludge (WAS) spiked with NP2EO were operated for a period of 91 days, to inspect the decomposition of NP2EO in solid and liquid phases. The results obtained on the final day of operation (91st day) showed that NP2EO degraded into product compounds among which NP1EC contributed to 90% of molar mass. In general, NP2EO showed a sharp degradation while NP1EC was produced rapidly. NP1EO also showed a steady degradation. However, NP was accumulated in the reactor. During the study, TS, VS, TSS and VSS degradation was also monitored and the percentage

removals were found to be between 40-60%. COD removal on the other hand was between 64-66%.

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

ÖZ

NONİLFENOLLÜ BİLEŞİKLERİNİN AEROBİK YARI SÜREKLİ REAKTÖRLERDEKİ AKIBETİ

Ahmad, Muneer

Yüksek Lisans, Çevre Mühendisliği Bölümü

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Ağustos 2012, 111 Sayfa

Endüstri ve evlerde geniş kullanımı ve bu nedenle de yarattıkları yüksek maruziyet sebebiyle ksenobiyotik bileşikler üzerin`de son birkaç on yılda çok sayıda çalışma yapılmıştır. Bu çalışmanın ana odağı, endokrin bozulmasına, kansere ve başka sağlık problemlerine sebep olabilen zararlı ksenobiyotik bileşikler olan ve deterjan ve kişisel bakım ürünlerinin üretiminde yaygın olarak kullanılan nonilfenol monoetoksilat (NP1EO), nonilfenol dietoksilat (NP2EO), nonilfenoksiasetik asit (NP1EC) ve nonilfenol (NP) gibi bileşikleridir. Bu çalışmada, NP2EO'nun katı ve sıvı fazlardaki ayrışmasının incelenmesi için NP2EO katılmış aktif çamur içeren laboratuvar ölçekli yarı sürekli aerobik reaktörler 91 gün süreyle işletilmiştir. İşletimin son gününde (91. gün) elde edilen sonuçlar NP2EO'nun, aralarında NP1EC'nin molar kütlenin %90'ını oluşturduğu ürün bileşiklere ayrıştığını göstermiştir. Genel olarak, NP1EC hızlı bir şekilde üretilirken NP2EO ani bir degradasyon göstermiştir. NP1EO da devamlı bir degradasyon göstermiştir. Bununla birlikte, NP reaktörde birikmiştir. Çalışma süresince, Toplam Katı Madde (TKM), Toplam Uçucu Madde (TUM), Toplam Askıda Katı Madde (TAKM), Uçucu

Askıda Katı Madde (UAKM) degradasyonları da izlenmiştir ve yüzde giderimleri %40-60 arasında bulunmuştur. Bunun yanısıra, KOİ (Kimyasal Oksijen İhtiyacı) giderimi %64-66 arasında gerçekleşmiştir.

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

To my family

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LIST OF ABBREVIATIONS

BSTFA: N, O-Bis (trimethylsilyltrifluroacetamide)

CEPA: Canadian Environmental Protection Agency

COD: Chemical Oxygen Demand

EDS: Endocrine Disrupting Compounds

EU: European Union

EC: European Commission

GC: Gas Chromatography

GC-MS: Gas Chromatography-Mass Spectrometry

IUPAC: International Union of Pure and Applied Chemistry

LOD: Limit of Detection

LOQ: Limit of Quantification

MS: Mass Spectrometry

MWWTP: Municipal Wastewater Treatment Plant

4-NP/NP: Nonylphenol

NP1EC: Nonylephenoxy acetic acid

NP2EC: Nonylephenol diacetic acid

NP1EO: Nonylphenol monoethoxylate

NP2EO: Nonylphenol diethoxylate

NPnEO: Nonylphenol polyethoxylates

NPEC: NP1EC+ NP2EC

NPnEC: (nonylphenol polycarboxylates)

NPEs: (NP+NP1EO+NP2EO)

OSPAR: Oslo and Paris Commission

STP: Sewage Treatment Plant

TMCS: Trimethylchlorosilane

TS: Total Solids

TSS: Total Suspended Solids

UK: United Kingdom

US: United States

USEPA: United States Environmental Protection Agency

VS: Volatile Solids

VSS: Volatile Suspended Solids

WAS: Waste Activated Sludge

WWTP: Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

The use of surfactants is employed in several industrial and domestic applications, such as cleaning, degreasing and formulating detergents. Surfactants can be classified in four different groups: anionics, nonionics, cationics and amphoterics (Ying et.al., 2006). In Western Europe, the quantity of surfactants produced was 2.5 million tons in the year 2002 which consisted of 50% anionic and 40% nonionic surfactants (European Committee of Organic Surfactants and their Intermediates, 2007).

Alkylphenol polyethoxylates (APnEOs), in specific nonylphenol polyethoxylates (NPnEOs) and octylphenol polyethoxylates (OPnEOs), are generally used as non-ionic surfactants (Ying et.al., 2006). Since these compounds are not produced naturally, they are considered xenobiotic in nature. Their use is frequently employed as pesticides, wetting and foaming agents, emulsifants, and detergents and in various industrial, agricultural, and household applications (Ying et.al., 2002). Around 650,000 tons of APEOs is produced annually worldwide (Guenther et.al., 2002). Almost 60% of used APEOs are dumped into water bodies (Porte et.al., 2000; Renner, 1997). These chemicals subsequently decompose into shorter and tenacious alkylphenol polyethoxylates (AP2EO, AP1EO, etc) and further down to nonylphenol (NP) and octylphenol (OP) (Giger et.al., 1984; Jonkers et.al., 2001). Besides these, alkylphenol carboxylates (APnECs) are also produced. A research conducted in Europe over 100 rivers has shown the presence of 35 organic compounds among which APnEOs degradation products were the most frequently detected (Loos et.al., 2009). Since it is

known that these compounds are used extensively in household and industrial applications, it is reasonable to assume that they will emerge frequently and excessively in wastewater treatment plants (WWTPs). The study by González (2004) is the only example to serve as evidence to this statement where hundreds of µg/L concentrations of APnEOs degradation products were detected in the influents of a number of sewage treatment plants. In all the studies NPnEO products have been detected more often compared to OPnEO products, due to the higher share of NPnEOs (80%) in APnEOs.

The molecular and chemical characteristics of nonylphenol compounds are a reason of tremendous curiosity among scientific society. Their toxicity, endocrine disruption and carcinogenic and oestrogenic effects lead to a significant number of health risks. A brief exposure to these compounds is enough to trigger irritation in the eye or skin, headaches, breathing difficulties, vocal cords impairments, and other health problems (Cox, 1996). The NP compounds mimic the natural hormones leading to a failure in the proper functioning of the endocrine system. The carcinogenic nature of these compounds is also linked to the endocrine disruption ability, the property which serves as a root to breast cancer (Soto et.al., 1991). Likewise, their analogous properties with the 17-β-ostradiol enable them to imitate the oestrogen hormone, which consequently leads to a disorder in the reproductive system. Besides the effects mentioned above, their physiochemical properties such as hydrophobicity and lipophilic nature, cause them to be persistent and pile up in tissues and organic matter such as sludge (McLeese et.al., 1981, Soares et.al., 2008). Due to the extensive usage, these compounds are present in a considerable amount in the sewage systems, subsequently being part of rivers, oceans, lakes, etc. As a consequence, they integrate into the food chain and climb up to the top harming human beings. These horrendous effects of NP compounds discouraged the European Union and other nations around the world of their use. As an environmental protection policy, the EU put forward various laws to

limit or ban the use of these compounds. In spite of the bans, these compounds are still being used in many regions around the world such as South America and Asia.

All the studies till the present day have focused mainly on the concentrations of NP compounds in various environments such as rivers, oceans, seas and WWTPs. However, the studies on the fate of these compounds in controlled laboratory scale aerobic reactors are very seldom. This fact provided a motivation to investigate the transformations of nonylphenols in aerobically operated semi-continuous reactors. In this study, laboratory scale reactors containing Waste Activated Sludge (WAS) spiked with NP2EO are operated to inspect the decomposition of NP2EO in solid and liquid phases. The study aims to investigate the formation of NP, NP1EO, and NP1EC along with the disappearance of NP2EO to find out the possible degradation mechanisms of these compounds under aerobic conditions.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Nonylphenol Compounds

2.1.1. Description

Nonylphenol (NP) is an organic compound of the wider family of alkyl-phenols (AP). AP consists of a phenol ring which is mono- or poly-substituted by alkyl chains of variable length. During the alkylation process of phenols, particularly in the synthesis of polyethoxylate detergents, the product formed is NP as a result of industrial synthesis. Nonylphenols are classified as xenobiotics because they are synthesized. Furthermore, linear either *n*-alkyl chains or complex branched chains can be found in existence for alkyl chains. It is produced industrially under conditions of acidic catalysis by the alkylation of phenol with nonene. The product is a viscous liquid in ambient conditions with a light pale colour immiscible with water. There are more than 22 isomers of 4-substituted monoalkyl phenols in the composition of the final mixture (Thiele et.al, 2004). Nonylphenol polyethoxylates (NPnEO) and octylphenol polyethoxylates (OPnEO) are two main comercial compounds of Alkylphneol polyethoxylates (APnEO). APnEO are produced as a result of a reaction between alkyphenols and ethylene oxide. NPnEO contributes to 80% of total APnEO usage (Ying et.al., 2002). Degradation of nonylphenol polyethoxylates (NPnEO) is the main contributer of nonylphenol to the environment (Langford et.al., 2002). Ethoxylate chain having a length that follows a Poisson distribution is contained in nonylphenol ethoxylates (Hager CD, 1998) where if the number of ethoxylate groups decreases, the recalcitrance increases (Soares, 2005).

The presence of NP was reported extensively in surface water, groundwater, atmosphere, pristine and sludge amended soils and foods (Giger et.al., 1984, 1987) as NP was a persistent pollutant in sewage sludge as reported in studies conducted in the early eighties (Saito 2004a, 2004b). The chemical NP is composed of a phenol ring with a lipophilic straight or branched nonyl group, for most industrial formulation it is principally attached in the para position (4-NP). Para NP (4-NP) and some other chemical structures of NP and related products are shown in Figure 2.1 and 2.2 respectively. (USEPA, 1990). NP can exist in the form of different isomers depending on the structure of side alkyl chain. A practical numbering system based on all possible isomers has been developed by International Union of Pure and Applied Chemistry (IUPAC) to rationalize the identification of individual NP isomers in scientific examinations. 4-NP is an isomer of nonylphenol and it is highly abundant in technical NP mixture and in natural environmental matrices (Zhang et.al., 2007)

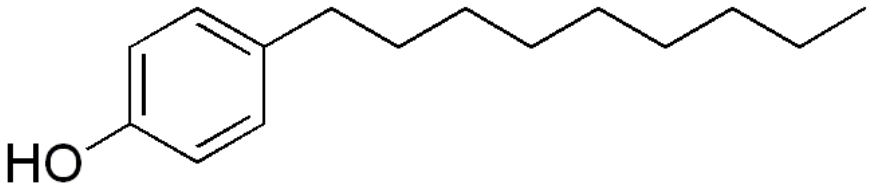
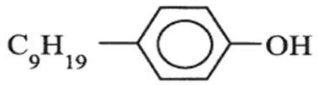
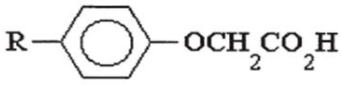


Figure 2.1: Nonylphenol (4-NP) (para-position)

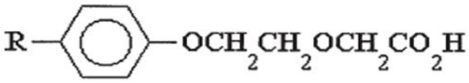


4-nonylphenol (NP)

nonylphenol ethoxylates (NPE), R = C_9H_{19}



nonylphenoxyacetic acid (NP1EC), R= C_9H_{19}



nonylphenoxyethoxyacetic acid (NP2EC), R = C_9H_{19}

Figure 2.2: Chemical structures of nonylphenols and related compounds

2.2. Uses of Nonylphenol Ethoxylate

Nonylphenol (NP) is a primary component in the production of surfactants (cleaning agents) that are used in domestic and commercial products. The surfactant produce of NP is mainly "nonylphenol polyethoxylates" (NPnEO). Since long NPnEO has its application in the production of wide variety of consumer goods (e.g., personal care, laundry products and cleaners), commercial products (e.g., floor and surface cleaners), and in various industrial cleaning processes (e.g., textile scouring and preparation). NP

and NPnEO also have a direct application in assisting to retain other slightly soluble and insoluble materials in solution.

The main use of NP is to generate NPnEO surfactants (65%) and it is also used for manufacture and production of antioxidants, greasing oil additives (USEPA, 1990). According to findings of Giger (1984), the distribution of nonylphenol compounds in the digested sludge is 95 % NP and 5% NP1EO + NP2EO, due to the hydrophobic nature of NP and the anaerobic digestion of the sludge resulted in other NPnEO being transformed into NP. NPnEO undergo biodegradtion in aerobic and anaerobic conditions. The degradation process involves stepwise loss of ethoxy group from higher polyethoxylates to form lower polyethoxyaltes such as NP2EO and NP1EO and ultimately to NP. Moreover, acidic metabolites of NPnEO such as nonylphenol diacetic acid (NP2EC) and nonylphenoxy acetic acid (NP1EC) are also formed under aerobic environment (CEPA, 1999). It is shown in other works by Giger and his coworkers (1984) that NP concentrations in anaerobically digested sludge range from 0.45 to 2.53 g/kg dry weight, at the same time as in aerobically stabilized sludge levels were lower, at 0.08 to 0.5 g/kg dry weight (Giger et.al., 1987) .

The production of NPnEO has a vast usage for industrial sector consumption including industrial laundering, textile processing, pulp and paper processing, paint and resin formulation, oil and gas recovery, steel manufacturing, pest control and power generation. Other common commercial uses of NPnEO include, industrial and commercial detergent, as an emulsifier in wax for fruit and vegetables, as a polymer resin in plastic food packaging and polyethylene plastic, in cosmetic products (such as skin cream, deodorant, makeup, hair dye, and shampoo), and even in spermicides (CEPA, 1999). The major usage of NPnEO is in cleaning products, especially detergents which is evident from the fact that, of the 260 million pounds of NP used in 2004 in US, 80% was used as a surfactant (Sierra Club, 2005). In general, 37% of NPnEO metabolites enter the aquatic ecosystem (Sierra Club, 2005). It is apparent from the data that,

nearly 77 million pounds (35000 ton) of NPnEO based cleaning agents entered U.S. waterways in 2004 (World wildlife federation, 1999). According to literature study conducted by Soares (2008) and his coworkers, the annual production of nonylphenol is found to be 154,200 tons in the USA, 73,500 tons in Europe, 16,500 tons in Japan and 16,000 tons in China.

2.3. Physical and Chemical properties of NP and NPnEO

Physical and chemical properties that have a bearing on the environmental persistence of NP and NPnEO with average chain lengths of one (NP1EO), two (NP2EO), four (NP4EO) and nine (NP9EO) are shown in Table 2.1. The properties of NP4EO and NP9EO are considered to be representative of NPnEO and are given below because the available data set of these two compounds is the most comprehensive.

Table 2.1: Properties on NP and NPnEO

Properties	NP	NP1EO	NP2EO	NP4EO	NP9EO
Synonyms	4-nonylphenol[1] p-nonylphenol[1]			Nonoxynol-4[1]	Nonoxynol -9[1] Tergitol NP-9[1]
Molecular Formula	$C_{15}H_{24}O$	$C_{17}H_{28}O_2$	$C_{19}H_{32}O_3$	$C_{25}H_{40}O_5$	$C_{33}H_{60}O_{10}$
Molecular Weight (g/mol)	220,3	281,4	308,46	396,2	617,6
Melting Point (^{0}C)	-8[2,3]	-9[4]	-4[5]	-40[6]	2,8[6]
Boiling Point (^{0}C)	295-320[7,3]	-	-	-	-
Physical Characteristics	colorless (liquid)[7,3]	colorless (liquid)[	colorless (liquid)[9]	White to light amber (liquid)[9]	colorless (liquid)[10]
Specific Gravity	0,953[10]	-	-	1,020-1,030(25^{0}C)[9]	1,057 (25^{0}C)[6]
pKa	10,7[11]	-	-	-	-
Vapor Pressure (Pa)	0,00455±0,0035[12]	245,3 hPa at 20°C	245,3 hPa at 20°C	-	-
Solubility in Water (mg/L)	5,4[12]	3,02[12]	3,38[12]	7,65[12]	slouble[8]
Log K_{ow}	4,2-4,48[13,14,15]	4,17[15]	4,21[13]	4,24[13]	3,59[13]
Henry's Constant (Pa.m^3/mol)	11,02[3]	-	-	-	0,000 24[8]
Flash Point (^{0}C)	140[16]	-17 (closed cup)	-17	-	-

1 *U.S. EPA (1985).*
2 *Hüls, AG (1994).*
3 *OECD (1997).*
4 *Huntsman (1999a)(verilen değer NP1,5EO içindir)*
5 *Huntsman (1998b) (verilen değer NP3EO içindir)*
6 *Weinheimer ve Varineau (1998).*
7 Reed (1978)
8 *CIR (1983).*
9 *WHO (1998).*
10 *Enyeart (1967)*
11 *Romano (1991).*
12 *Ahel ve Giger (1993a).*
13 *Ahel ve Giger (1993b).*
14 *McLeese vd. (1981).*
15 *World Wildlife Fund Canada (1996)*
16 *URL-1*

NP is a hydrophobic compound with low solubility in water and log Kow value of 4.48. Therefore, it partitions positively to organic matter and has low mobility which restricts its capacity for spread in aqueous phase of soil and sediments. The vapor pressure and the Henry's law constant of NP are 2.07×10^{-2} Pa and 8.39×10^{-1} $Pa.m^3/mol$, respectively (Soares et.al., 2008). These properties show that NP is a semi-volatile organic compound capable of water air exchange.

Specific gravity, viscosity and aqueous solubility are directly proportional to ethoxylate chain length and increase with increase in ethoxylate chain length (CEPA, 1999). If the chain length of NPnEO is greater then 6 then they are readily soluble in water. On the other hand, hydrophobicity of these molecules is inversely proportional to ethoxyalte chain length i.e. it decreases with increace in ethoxylate chain. Therefore, it can easily be concluded that NP compounds with less ethoxylate chain are more harmful and persistent to living organism than those with more ethoxylate chain (CEPA, 1999). The pKa of NP is 10.7 specifying that NP is present in entirely ionizied form in most natural waters (CEPA, 1999) The Henry's law constant and vapour pressure of NP and especially NPnEO are low hence existence of these compunds in air is very low. NPnEO are likely to be considerably ionized at the pH value of natural water. In addition, the acidic compounds of NP (NPECs) occur at the end of the aerobic treatment, are complex compounds and they exist in the ionized form at neutral pH values. For NPECs, log Kow values are expected to be much lower than those of the corresponding ethoxylates (Soares et.al., 2008).

2.4. Effects of NP and NPnEO

NP and NPnEO are being used mainly as surfactant in the industrial preparation of detergents and emulsifiers. Due to their usage in the preparation of household cleaning liquids, people are directly exposed to them. Not only NP or NPnEO can affect humans directly through commercial products but also they can affect living organisms

indirectly via their release to the environment from domestic and industrial activities. Direct use of NP is limited most of it is produced through the degradation of detergents when the NPnEO (used in the preparation of the detergent) break down into NP. Therefore, estimating the potential exposure to NP is difficult. Estimating the exposure to NPnEO is further complicated by the fact that NPnEO can be found in very complex mixtures (Vazquez-Duhalt et.al., 2005). NP and NPnEO can be studied under the terms of toxicity and endocrine system disruption.

2.4.1. Toxicity

Nonylphenol shows acute toxicity, which means that Nonylphenol can have adverse effects on a living organism either through a single exposure or from multiple exposures in a short span of time. The exposure time is normally less than 24 hours, and effects are seen within 14 days. Even short-term exposures cause immediate adverse effects on human. The exposure causes severe irritation, high concentrations destruct upper respiratory tract, eyes, and skin. Some general ailments include; coughing, difficulty in inhalation and exhalation, aphonia, cephalalgia (headache), nausea, and vomiting. Contact with skin over long period may cause; burning, distention and itching (Cox, 1996).

Mortality effects of NP are highly variable. The median lethal dose (LD_{50}) is the dose required to kill half the members of a tested population after specified test duration. For rats, the median lethal dose is between 400 -1620 mg/kg of the body weight. Nonyl phenol ethoxylates are less acutely toxic than NP since the lowest LD_{50} for rats was 1650 mg/kg of the body weight (Cox, 1996). The lethal concentration (LC_{50}) of NP for fish ranges from 17- 1400 µg/L. Invertebrates are also sensible to NP in between 21-3000 µg/L, and algae with between 27-2500 µg/L (Vazquez-Duhalt et.al., 2005).

Property of a substance to have adverse effects on a living organism when the organism has had a prolonged exposure to the substance is called chronic toxicity. Chronic toxcity distinguishes itself from acute toxicity mainly through the duration of exposure. Chronic toxıcity describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years) while acute toxicity describes the adverse health effects of exposure over a short period of time. The toxicity of NPEs gradually increases as the ethoxylate chain length decreases. NPnEC (nonylphenol polycarboxylates) on ethoxylate chain are less toxic than NPnEO. However, their acute toxicities are similar when; both have 6–9 EO units (Vazquez-Duhalt et.al., 2005). Moreover, NP and NPnEO prevent reverse electron transfer providing energy from food in cells and NP also inhibits the activity of an enzyme providing energy to muscle cells (Cox, 1996). Generally microbial transformation or degradation decreases the toxic effects of pollutants. However, some degraded chemical products are more toxic like NP and short-chain nonylphenol ethoxylates. Therefore, acute toxicity tests are done on the original compounds (commercially available products), not on their degraded products after dispersal in nature (Vazquez-Duhalt et.al., 2005).

A total of 25 human cadavers that are non-occupationally exposed to NP and NP1EO and NP2EO were analyzed in Switzerland. The NP concentrations in the tissues ranged between 19.8-84.4 ng/g lipids (CEPA, 1999). Furthermore, NP1EO and NP2EO were below the limit of detection [5 ng/g lipids] in all samples. These values were the all within the range of background contamination found in the analytical “blank” samples. Müller (1997) studied three non-occupationally exposed Canadian human subjects, and found that NPnEO with a chain length of 7–10 were detected, but these were not quantified in urine samples although all reasonable precautions had been taken to minimize contamination during the analysis (CEPA, 1999).

Some countries have taken an initiative to reduce the risk of exposure to NPnEOs for example, in USA; the major detergent suppliers use the more readily degradable alcohol ethoxylates instead of NPnEOs for the production detergents (Vazquez-Duhalt et.al., 2005). Effects of NP on the entire ecosystem and long-term, multigenerational effects on fertility, reproductive quality, and hormonal functions have been studied recently. Acute toxicity tests are done with surrogate animals therefore the correlations to humans of such tests are weak. Furthermore, ecological damage potentially caused by single compounds, the environmentally-transformed products, and the degradation products are not known by the tests. Therefore, better tests, models and monitoring systems are required to determine long-term impacts.

2.4.2. Endocrine System Disruption

Endocrine system is a system of glands. Every gland secretes different type of hormone into blood system to regulate the bodily functions. Functions like growth, mood and development, metabolism and tissue function, are regulated by the endocrine system. Chemicals that inhibit the functions of the endocrine system are called endocrine disruptors. The disruptors can mimic the sex steroid hormones oestrogen (17-β-Oestradiol) and androgen and bind to their natural receptors either as agonists or antagonists, alter the synthesis and breakdown of natural hormones, and modify the production and functioning of hormone receptors (CEPA, 1999).

The development and maintenance of female sex characteristics, and the maturation and function of accessory sex organs (Warhurst et.al., 1995) are influenced by the oestradiol harmone. In 1938, Dodds and Lawson reported the results of feeding 100 mg of 4-propylphenol to ovariectomized rats. The experiment provided the first evidence that para alkylphenols could be oestrogenic. The chemical, mimicking the activity of oestradiol, caused vaginal cornification in the rats as occurs during a normal oestrus cycle. Cornification also occurred with 4-*tert*-pentylphenol, but not with 2-*n*-

pentylphenol, indicating the importance of the two groups being *para*-on the ring (Dodd et.al., 1938). In 1978, the second evidence for oestrogenic effects of alkylphenols was published by Mueller and Kim. The research revealed that various alkylphenols were able to displace oestradiol from its receptor, and also to prevent oestradiol binding with the receptor. This effect was most evident at lower temperatures (0 - 4 ºC), and about 30, 000 - 100, 000 times as many molecules of the competitor were required to show measurable effects on oestradiol binding.

Even though, the previous studies quantified the effects of NPnEO on different organism it was not until 1991 that the health and environmental implications of these studies were fully realized. NP compounds were placed in the endocrine disrupters lists in 1991 when researchers studying breast cancer cells witnessed abnormal cell proliferation in specimens exposed to NPs. In addition, it was found that mitotic activity increased in rats and progesterone receptors were affected by NP. In a study carried out in 2005 it was observed that the initiation of breast cancer increases in mice when they are exposed to NP. In 1991, by chance, Soto *et al* were working on oestrogen-sensitive MCF7 human breast tumor cells, discovered that a component leaching out of a new batch of centrifuge tubes was causing cell proliferation which is the normal response to oestrogens (Soto et.al., 1991). The component, after purification, was found to be nonylphenol, which had been added to the tubes to improve their resistance to breakage. Commercial 4-NP yielded similar results, showing significant proliferation at a NP concentration of 1µM (220 µg l -1). Furthermore, the proliferation produced by 10 µM 4-NP was found to be similar to that produced by 30 pM oestradiol. More work revealed that 4-NP can also induce the expression of the progesterone receptor (as oestradiol does), and cause cell proliferation in ovariectomized rats.

In 1993, Jobling and Sumpter investigated the oestrogenic effects on the liver cells of Rainbow Trout (*Oncorhynchus mykiss)* exposed to alkylphenols and some of the metabolites of alkylphenol ethoxylates. The experiment mainly detected the

production of the egg-yolk protein vitellogenin, a large lipoglycophosphoprotein which is secreted by the liver of female fish and then circulates to their ovaries. The protein is produced in response to endogenous oestrogens. Both, ED_{50} and the relative potency were determined, the latter by determining the concentrations of the test compound required to give an excretion of vitellogenin equal to that resulting from various concentrations of 17- β-oestradiol. ED_{50} is a dose that produces a quantal effect (all or nothing) in 50% of population that takes it. It is commonly used as a measure for a reasonable expectance of drug effect.

In addition to toxic effects, nonylphenol has the ability to mimic important hormones (natural oestrogens) controlling overall physiology of the organism. NP interacts with the binding pocket of the oestrogen receptor through structural similarities in the phenolic A-ring so they have public health risk. The magnitude and nature of the response depends on the complexity of the interactions between the, plasma bindings, the signaling pathways, oestrogen receptor, androgen antagonism, and alternate modes of oestrogen action (Vazquez-Duhalt et.al., 2005). Studies confirm that high doses can activate endocrine disruption, but it is not known whether low doses of environmentally relevant levels of 4-NP put humans at risk of endocrine disruption. Most recently studies have been made about NPnEO ability of altering the sexual development and the sex distribution of the natural populations. Environmental oestrogens affects sexual differentiation which is the basic component of evolution, and as NPnEO cause disruption in the oestrogen function the impact of NPnEO pollution on living organisms is far reaching (Vazquez-Duhalt et.al., 2005).

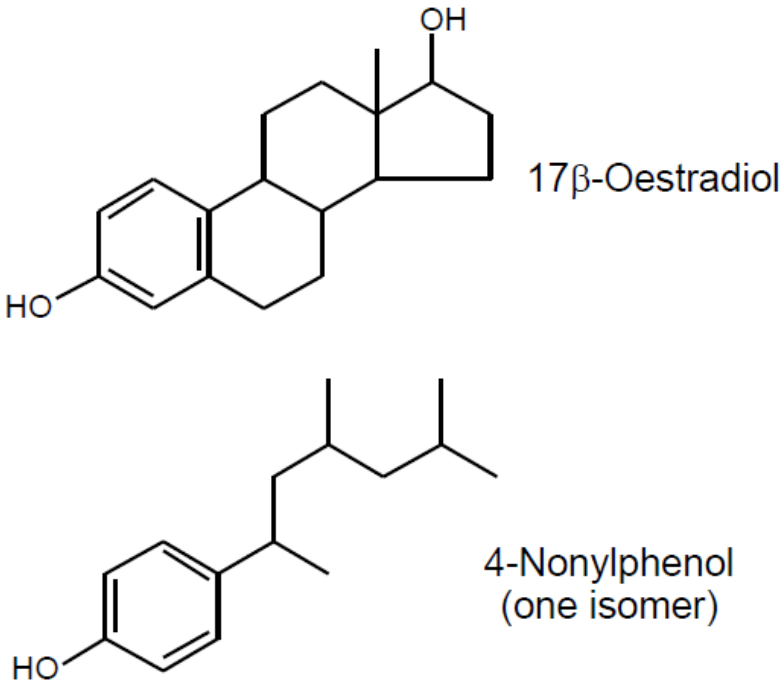


Figure 2.3: A comparison of the structures of Oestrdiol and nonylphenol (Warhurst, 1995)

Figure 2.3 compares the structure of NP and Oestradiol. 17-β-Oestradiol and alkylphenols share a common structural motif in the phenolic A ring of 17-β-Oestradiol and the phenol moiety of alkylphenols (Figure 2.3), and it has been suggested that alkylphenols may act as endocrine disrupters by mimicking the activity of 17-β-oestradiol at oestrogen receptors. NP and NPnEO result in many oestrogenic responses in a variety of aquatic organisms. When the relative oestrogenic potency put in the order for vitro systems, it is found that

NP > NP1EO = NP2EO > NP1EC = NP2EC > NP9EO.

Most of the scientific work has been carried out to examine the effects of NP. However, there are some data on the toxicity of NPnEO and NPnEC to freshwater organisms and relatively few toxicity data for marine organisms. In many organisms NP is more toxic then NPnEO.

Alkylphenol ethoxylates (APnEO) bind to the oestrogen receptor both *in vitro* and *in vivo* systems, including the induction of vitellogenin. Vitellogenin is an egg yolk precursor protein which is usually inactive in male fish and expressed only in female fish. However, when male fish are subject to oestrogenic endocrine disrupting compounds (EDCs) the vitellogenin gene is expressed in a dose dependent manner. Therfore, vitellogenin gene expression in male fish has been used as a molecular indicator of exposure to oestrogenic EDCs. The threshold for vitellogenin induction in fish is found to be 10µg/L for NP. The oestrogenic responses appear to be at least, additive and hence must be considered as a group. APEs also affect the growth of testes, change normal steroid metabolism, disrupt smoltification and result in intersex (ova-testes) in fish (CEPA, 1999).

According to research carried out by Sierra club (2005), endocrine disruption causes;

- Organisms to develop both male and female sex organs,
- Raises mortality and damage the liver and kidneys
- Decreases testicular growth, the formation of sperm and testosterone levels in male fish
- Disrupts normal male to female sex-ratios, metabolism, development, growth and reproduction.

In addition, NPnEO metabolites alter the reproductive organs of aquatic organisms. Tests on the Japanese Medaka male fish shows that half of them developed both male and female sex organs when exposed to 50 parts per billion of NP during a three month exposure. This percentage increased to 85% when the fish was exposed to 100 parts per billion of NP. In the control group there were no hermaphroditic (both sex organs) condition (Gray et.al., 1997). In test group there were more female fish than the control group

According to a different study, sexual deformities were seen on the oyster larvae exposed to varying levels of NP. At levels of 0.1 parts per billion, the slower development,

abnormalities in the shell hinge and increase in morality are observed (Hewstone, 1994). Also, the number of both hermaphrodite oysters (having both male and female organs) and female oysters increased when compared to the control group. Figure 2.4 shows that, 17% of oyster larvae exposed to 1 ppb of NP became hermaphroditic. When the oysters were exposed to 100 ppb of NP, 30% of the adult oysters became hermaphroditic, and the female-male sex ratio became disproportionate. The results show that even one-time exposure might be a threat for the oyster community and industry. In a different study, 2 mL/kg calcium alkyl phenate (%25) is exposed to skin of rabbits and after 4 weeks it is observed that sperm production stopped (Hewstone, 1994).

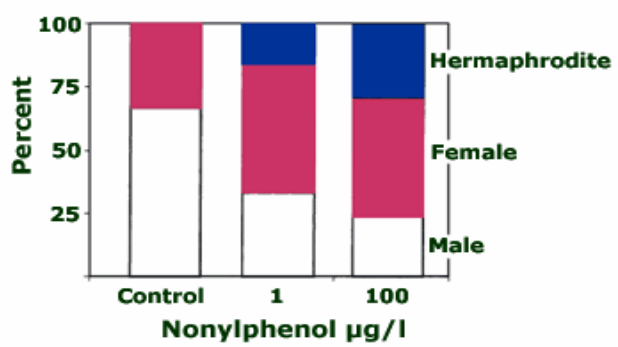


Figure 2.4: Percentage of Oysters Developing into Males, Females and Hermaphrodite after Single Exposure to NP (ppb) (Hewstone, 1994).

2.5. Bioaccumulation

Bioaccumulation of Alkylphenol polyethoxylates (APnEO) and Alkylphenols (AP) has been extensively studied on different organism such as algae, fish, plants and invertebrates and findings have illustrated that bioconcentration factors (BCFs) of AP and APnEO in the laboratory and bio-accumulation factors (BAFs) measured in the field are parallel and represent a low to moderate tendency to bioaccumulate. This is likely to be correct as the log Kow value measured for NP is 4.48 (Ahel et.al., 1993b) showing

that it has a high tendency to accumulate in organisms. OECD (1997) predicted a theoretical BCF of 1280 based on Kow. BCFs and BAFs in biota, comprising algae, plants, invertebrates and fish, vary from 0.9 to 4120 for NP. There are relatively few data available for NPnEO, but taking their structure into account it is reasonable to state that probably they do not tend to bioaccumulate. Rate of intake of NP in aquatic organisms is much faster than rate of elimination hence; NP compounds can bioaccumulate in their body. Nonylphenol compounds are lipophilic in nature and thus are accumulated in a wide range of marine and aquatic life including algae, crustacean, mollusks, and fish (Ahel et.al., 1993c). This becomes crucial for organisms which are part of lower trophic level. For instance, algae had a larger capacity for bioaccumulation of NP showing bioconcentration factors of NP reaching up to 10 000. The estimated bioconcentration factors in fish tissues ranged from 13 to 410 for NP, 3 to 300 for NP1EO and 3 to 330 for NP2EO. Similar concentrations to those in the fish were determined in different tissues of a wild duck (Ahel et.al., 1993c). The low concentration values of NP found in some higher animals could be because of the tissue metabolism and elimination. However, this information is insufficient regarding the metabolic fate of alkylphenols in aquatic animals.

In another study carried out by Ademollo and his colleagues (2008), presence of NP, NP1EO, octylphenol (OP), octylphenol monoethoxylates (OP1EO) and octylphenol diethoxylates (OP2EO) is investigated in human breast milk of Italian women. NP was the chemical found in the highest concentrations at about 32 ng/mL, that was much higher than OP (0.08 ng/mL), OP1EO (0.07 ng/mL) and OP2EO (0.16 ng/mL). Later, they look over the relationship between fish consumption and levels of NP, and found out that the women with high weekly sea food consumption had higher quantity of NP compared to women with less sea food consumption. On the basis of the NP concentrations found in the breast milk samples, a maximum NP daily intake of 3.94 µg/kg/day can be calculated, which is close to the Tolerable Daily Intake (TDI) of 5 µg/kg proposed by the Danish Institute of Safety and Toxicology. Therefore, it can be inferred that chemicals like NP that persist in nature can cause harm to human and

other living organisms not only by direct exposure or consumption, but also by indirect means through bioaccumulation.

2.6. Biodegradation

Biodegradation of nonylphenol takes place through the action of microorganisms, but it is limited by oxygen supply (Hesselsoe et.al., 2001) and bioavailability (Bosma et.al., 1997). Nonylphenol contaminated areas have been found to contain organisms which are able to degrade it. The degradation takes place as the indigenous microflora become acclimatized, i.e., adapted to the presence of the contaminant (Ahmed et.al., 2001). In order to expedite the fate of nonylphenol in the various environments, microbial consortia have been used. Aerobic microorganisms have been studied more extensively; they are obtained from aquatic environments, sediments, sewage sludge and soil. The uses for nonylphenol ethoxylate surfactants are divers and in most applications they are disposed of into wastewater streams after use.

Despite the fact that an application of standard test methods renders NPnEO and NP non-biodegradable, some contradictory reports do exist. However it is only after a phase of acclimation that substantial biodegradation occurs. Biodegradation then is an inherent characteristic of NPnEOs and this mechanism that involves a stepwise procedure whereby ethoxy groups are released to lower NPnEO congeners. Given the correct experimental conditions NPnEC and NP are produced (Rudling and Solyom, 1974). Figure 2.5 below displays this mechanism but is a simplified version as it exempts scenarios where $n > 2$ in NPnEC or where carboxyl groups are attached to the nonyl chain. Unlike the parent NPnEOs which are biodegradable, intermediate and final products of the metabolism process do not readily decompose yet even these persistent chemicals give in ultimately to the degradation process. Research studies have brought to light two causes of retardation in the process: the nonly group branching out into NP and NPnEOs and the EO chain increasing in length (CEPA, 1999).

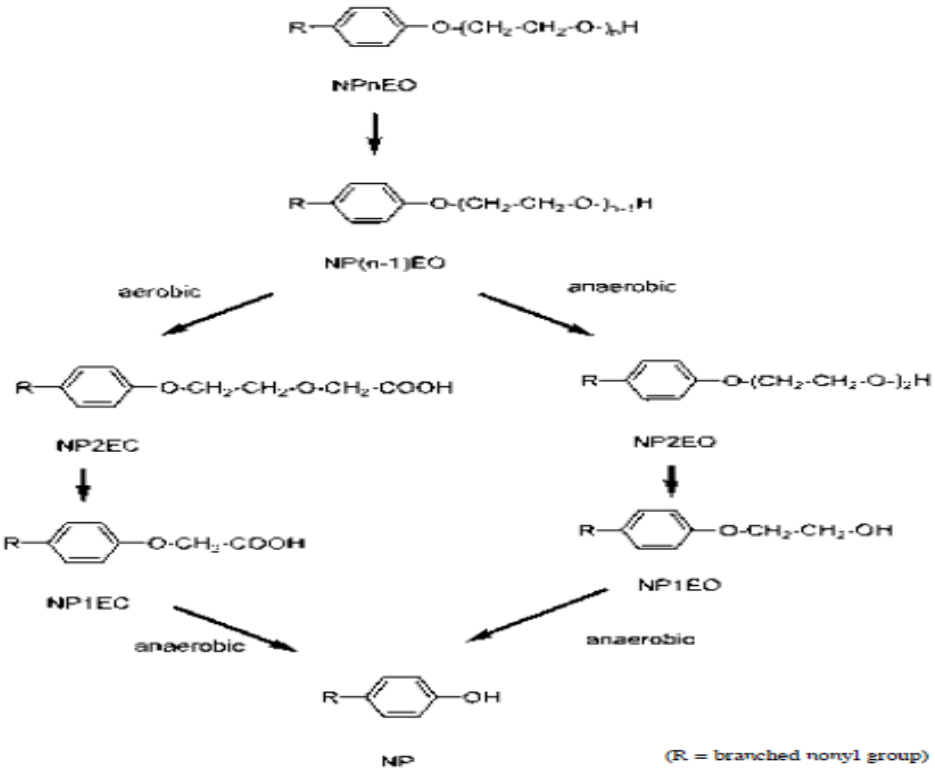


Figure 2.5: Biological degradation pathway of NPnEOs (CEPA 1999)

It can be seen from Figure 2.5 that degradation mechanism and resulting products are different under aerobic and anerbic environment. For instance, NPnEO degrades to carboxylated ethoxylate chains or shorter chained ethoxylate groups of NPnEO given aerobic conditions. When the conditions are anaerobic the end product is likely to be NP. The degradation process is assumed to be faster under aerobic conditions than anaerobic conditions. On the contrary, both aerobic and anaerobic condition shares a common step involving shortening of the ethoxylate chain with the help of microorganisms. Resulting products shorter in EO chain are more hydrophobic, persistent, and toxic in nature (CEPA, 1999). Most texts inform that NP formed by the process stated above cannot be further decomposed and is likely to stay in the environment, the reason being that NP is not swiftly biodegradable. There have been reports of numerous mechanisms of microbial aromatic ring degradation, formation of

catechol from phenol is the most regular and is followed by ring scission between or adjacent to the two hydroxyl groups (Talmage, 1994).

Corvini, along with his colleagues, performed a thorough research to determine the degradation of NP to simple compounds employing Sphingomonas sp. In 2006, they conducted a research that incorporated the degradation of α-quaternary nonylphenol isomers by Sphingomonas sp. strain TTNP3. To recapitulate, Corvini and his colleagues (2006) investigated the degradation of radio labeled 4(3', 5'-dimethyl-3'-heptyl)-phenol [nonylphenol (NP)] with resting cells of Sphingomonas sp. strain TTNP3. While decomposing NP, a metabolite called hydroquinone was collected over time which in turn produced short-chain organic acids. Two other radio labeled isomers of NP, 4(2', 6'-dimethyl-2'-heptyl) phenol and 4(3',6'-dimethyl-3'-heptyl)- phenol, were also produced (Figure 2.8). Corvini (2006) decided that 4(2',6'-dimethyl-2'-heptyl)-phenol degrade slower than the other isomers of NP by strain TTNP3 to incorporate the effects of the side-chain structure on degradation kinetics. Corvini's study also synthesized and ran tests to verify the results from the 2006 studies which recognized Alkylbenzenediol and alkoxyphenol derivatives as metabolites. As these derivatives did not degrade, one can ascertain that Alkoxyphenol is not the primary intermediate through which the NP mineralization proceeds. The results explicitly explain the degradation pathway of NP isomers with a quaternary α-carbon. Corvini (2006) suggested that type II ipso substitution is the means for NP degradation, leading to hydroquinone and nonanol as the main metabolites and to the dead-end metabolites alkylbenzenediol or alkoxyphenol, depending on the substitution at the main metabolites and to the dead-end metabolites alkylbenzenediol or alkoxyphenol, depending on the substitution at the α-carbon of the carbocationic intermediate formed. The hydroquinone formation included an attack at the C4 position of the NP ring and could be described by ipso-substitution mechanism alone. The necessity of a free hydroxy group at the para location is a feature of the ipso-substitution reaction. These substitutions can be classified into two types of groups depending on the nature of the substituent

eliminated from the quinol intermediate (Ohe et.al., 1997). Type I ipso substitution involves the formation of p-benzoquinone due to the elimination of the substituent as an anion. When the degradation intermediate is hydroquinone, the substitution is called type II ipso. In this, the substituent leaves the molecule as a carbocation before undergoing a hydroxylation with water.

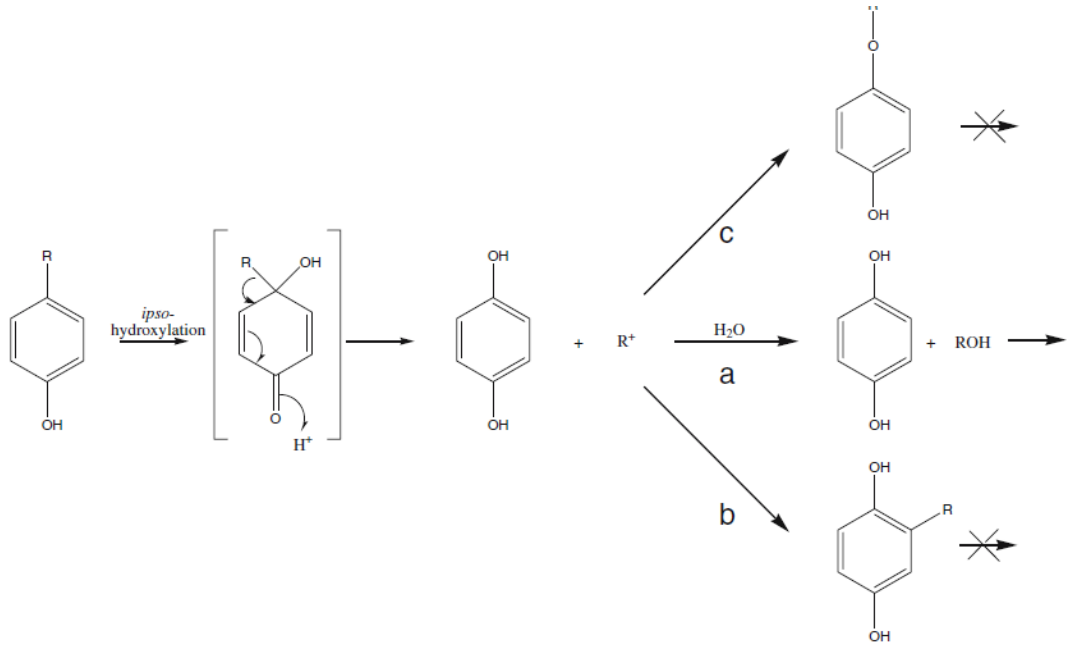


Figure 2.6: Proposed pathway of the degradation of NP isomer with a quaternery α-carbon. a- formation of corresponding nonanol. b- formation of 2 alkyl benzene diol product and c- formation of alkoxy phenol. (Corvini et.al., 2006)

Furthermore, the resultant compound is catechol on line a. Catechol can be defined as the molecule containg two adjacent hydroxyl groups, it is assumed to experience additional degradation including ring breakage and formation of simple acids. According to lecture notes of University of Maryland (URL-3), polyaromatic hydrocarbons decomposition occurs with each ring biodegrading at a time, each of these rings then passes through 'catechol' which is a shared intermediate. An 'epoxide' is formed along the pathway as benzene molecules introduce oxygen across a carbon-carbon double bond. Thereafter a reaction between epoxide and water occurs forming

a 3,5-cyclohexadiene-1,2-diol molecule. Certain microorganisms cause the diol intermediate to form directly without any epoxide interaction. Once the diol is formed, the cyclohexadiene intermediate converts to an aromatic molecule (catechol). Catechol is degraded on the path shown in Figure 2.9 to acetic and succinic acids.

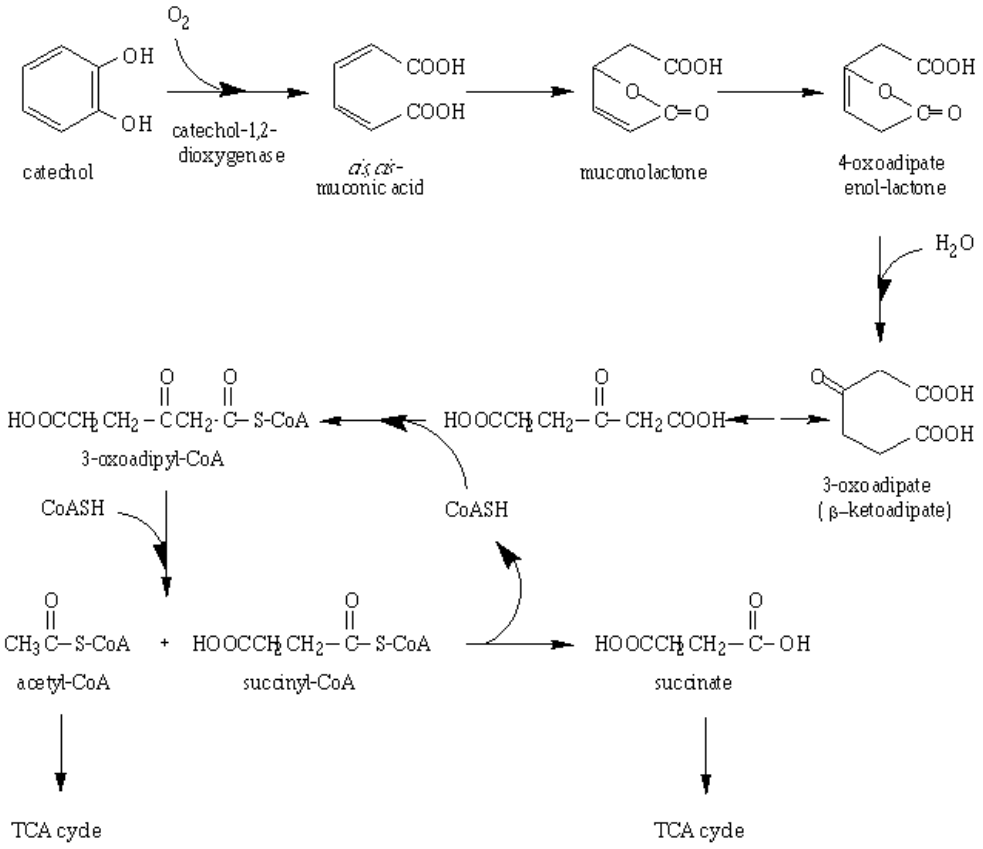


Figure 2.7: *Ortho*- cleavage pathway for catabolism of catechol (URL-3)

In order to minimize the negative environmental effects of residual nonylphenol ployethoxylate (NnPEO), treating wastewater is essential. Despite the fact that NPnEO and NP are degradable when treating wastewater, sludge and effluents stemming from many municipal sewage treatment facilities do show the presence of these compounds. This in fact determines plant efficiency as the concentration of NP present in final effluents after treatment is a direct indicator of success of treatment procedures.

NPnEO concentrations of upto 343 µg/L have been found in final effluents and of these nonylphenol is the most copious (Ying et.al., 2002).

2.7. Regulations Regarding the NP Compounds

As mentioned above, NP and NPnEOs are generated in huge volumes as household detergents and as cleaning products in industries. NP has been detected in many different media including human breast milk, blood, and urine and is linked with reproductive and developmental impairments in rodents. Research has revealed that the chemicals can cause harm to land-dwelling organisms. Toxicity tests of NP on plants have shown that they affected the growth; while tests on invertebrates have led to harmful impacts on their reproduction and mortality (USEPA, 2010). Although NPnEOs are relatively less toxic than NP, they are still toxic to aquatic organism and in environment they degrade into more toxic and persistent NP. Considering the given reasons, different measures were taken to restrict production and use of NP and NPnEOs. For instance, government of United Kingdom in 1976 has made a voluntary agreement with their industries to ban the use of NP in domestic detergents. However, the use by other means has continued. Therefore, releases were added to the UK Pollution Prevention and Control Regulations.

Similarly, EU countries have limited the use of NP in industrial products. For example, regulation regarding the use of NPnEOs is passed by REACH (Registration, Evaluation, Authorization and Restriction on Chemical Regulations) in 2006. This regulation is applicable to all EU states. In June 2009, NP and NPnEOs are listed in Annex XVII of this regulation (amended as EC552/2009) that deals with the chemicals which are classified as hazardous substances and are restricted from their manufacturing and supply. According to this regulation use of NP compounds at a concentration level higher than 0.1% by mass have been strictly prohibited. NP and NPnEOs have also been included in list of “Priority Hazardous Substances” in water framework directive (EU Directive

2000/60/EC). Furthermore, NP compounds were recommended to be phased out in 1995 under the OSPAR convention and it was also listed as a substance for priority action on its control under the Helsinki Convention.

Owing to the fact that NPnEOs with higher ethoxylate chain are quickly converted into lower chain NP2EO and NP1EO, the limit values usually address the total of three compounds which are NP, NP1EO and NP2EO. For example, in EU "Working Document on Sludge, 3rd draft" for application of sludge for agricultural means, limit for sum of three NP compounds is defined as 50mg/kg of total solids (TS). On the contrary, some other countries of EU such as Denmark decided to adopt a lower limit value of 10 mg/kg of TS. Similar to the proposed level by EU, Turkey chose the limit value of 50 mg/kg of TS as sum of all three NP compounds. In Turkey, NP compounds are regulated under "Regulation on the use of Municipal and Urban Sludges on Land". Due to increasing concern in regulating NP and NPnEOs US EPA made and action plan in 2010. As a result of this action plan NPnEOs will be phased out in United States in 2014. According to USEPA (2005), an acute criterion is that one hour of average concentration of nonylphenol should not exceed 28 µg/L more than once in every three years on average for saltwater ecosystems. A chronic criterion is defined as; four-day average concentration of nonylphenol should not exceed 6.6 µg/L more than once every three years on average.

Besides all these regulations in EU and US, there are still so many countries in Asia and in different parts of the world where production of these compounds is still going on without any restriction (Soarse et.al., 2008)

Due to previously mentioned physical and chemical properties of NP and NPnEOs , theese chemicals end up accumulating in sludge and as a result can pose harm to human health and environment. In addition, it should be noted that current application

of wastewater treatment is unable to achieve limit value of 50mg/kg of TS. Hence, there is a need of finding better sludge disposal methods to avoid the health risks

2.8. Environmental Fate of Nonylphenols and their Derivatives

Fate of nonylphenol compounds can be thoroughly studies by dividing them into two categories; natural environmental compartments such as river water, oceans, estuaries, sediments, soil, air, groundwater, etc. and engineered systems such as drinking water and wastewater treatment plants.

2.8.1. Natural Environmental Systems

Physical and chemical properties of NP compounds determine their fate in natural environmental systems. Nonylphenol being a hydrophobic compound with a log Kow value of 4.48 and low solubility in water, partitions favorably to organic matter (John et.al., 2000). As a result it has low mobility which restricts it from spreading in the aqueous phase of soil and sediments (Barber et.al., 1988). The physical and chemical properties of nonylphenol compounds control their ultimate outcome in the various environmental sectors of surface water, sediment, groundwater, soil or air. On the other hand, these different surroundings also influence the degradation process of nonylphenol compounds. Having reached the atmosphere, NP can be transported to aquatic and terrestrial ecosystems by wet deposition.

The concentration of nonylphenol in the surface layer of natural waters can decrease due to photolysis induced by sunlight; however in sediments it has an estimated half-life of more than 60 years (Shang et.al., 1999). Because all NPnEO are anthropogenic, measurement of NP and NPnEO provides a rapid and sensitive means for evaluating the general quality of water after human impact. The environmental fate of alkylphenols and

their ethoxylates has been reviewed and significant concentrations of NPnEO and NP are found in air, waters, soils and sediments (Soares et.al., 2008).

2.8.1.1. River Waters, Estuaries, Sediments & Oceans

According to most investigations, anthropogenic activities are the main generators of nonylphenol in aquatic environments also. Discharge of effluents from sewage treatment plants (STPs), proximity of industrial and urban areas to water bodies and other anthropogenic activities such as storm water discharges, etc. are the main sources of nonylphenol in surface waters (streams, rivers, lakes and estuaries), oceans and sediments (Warhurst, 1995). Although it is particularly difficult to investigate the concentration of nonylphenol in surface waters, different sampling and analytical methods have been used for its quantification. NP levels in rivers have found to be varying between 2 µg/L in Delaware River, Philadelphia to 10 µg/L in the Rhine and 1000 µg/L in a tributary of the Savannah river, USA (Warhurst, 1995). Studies have pointed to marked seasonal variation of the concentration of nonylphenol. In summers, due to increase in microbial activity at warmer temperatures, the concentration of nonylphenol rises, boosting degradation of nonylphenol ethoxylates (Bester et al., 2001). Other factors such as the river flow rate, sedimentation rate and particle size, also influence the rate of degradation. An investigation into the adsorption processes controlling the disintegration of nonylphenol ethoxylates to sediments confirmed that the organic content of the sediments was a significant factor in the adsorption process, especially for the shorter chained nonylphenol ethoxylates, which indicates the importance of their hydrophobic interactions (John et.al., 2000). Adsorption was also observed in sediments free of organic matter, indicating that besides organic content other interactions have implications on the process. Thus it can be concluded that nonylphenol adsorption is controlled by two major interactions: hydrophilic interaction with mineral components and hydrophobic interaction with the organic matter (John et.al., 2000). Nonylphenol partitions into sediments with a high concentration factor (1.76 after 28 days) and it was found to be resistant to biodegradation in lake

water/sediment systems, showing only a slight 9% loss (after 56 days) and 4.2% loss (after 28 days). The degradation half-life of nonylphenolic compounds was estimated to be greater than 60 years once they enter the sediments (Shang et.al., 1999)

2.8.1.2. Soil

As in aquatic environments, the occurrence of nonylphenol in soil is also caused mainly by anthropogenic activities. However, studies on nonylphenol content in soils are not as abundant as those on its presence in aquatic environments. The anthropogenic activities responsible for generation of nonylphenol in soil environments include sewage sludge application, land filling and accidental spillage (CEPA, 1999). Among these, sewage sludge recycling to agricultural land has attracted much attention and concern. According to a Danish study, the fraction of sludge recycled by farmers in 2002 was 66% of the total production indicating the importance of investigating the occurrence and fate of contaminants such as nonylphenol in sewage sludge. Elevated concentrations of nonylphenol have been identified in soils exposed to high addition of sewage sludge (1.4–1.6 mg/kg) and points of run-off (34–14 µg/kg) when compared with soils fertilized with limited amounts of sewage sludge (0.01–0.98 µg/kg) (Soares et al, 2008).

In another study, NP concentration was found to be in the range of 10-100 µg/L in Municipal solid waste which indicated that there is a transformation capacity for NPnEOs or/ and NP containing waste material in anoxic landfill environments (Ejlertsson et.al., 1999). Once nonylphenol reaches the soil it is subjected to various factors that influence its concentration such as: biodegradation, sorption and volatilization. Biodegradation has been studied mainly in bench-scale experiments that suggest that the recalcitrance of nonylphenol is controlled mostly by oxygen limitation (Hesselsoe et.al., 2001). However, in a field study it was demonstrated that an initial and fast dissipation of nonylphenol occurred (80% of the initial input, 4.7mg/kg, within the first month) but the remaining part was found to be persistent indicating that other parameters are implicated in the removal of nonylphenol (Hesselsoe et.al., 2001). In addition, the rate of biodegradation is

also expected to be controlled by the bioavailability of nonylphenol to the soil microflora. Mostly, it is the strong sorption of the contaminants and not the microbial activity that limits the biodegradation rates (Bosma et.al., 1997). Stronger sorption is often observed at pH values near the pKa since a high degree of protonation results in increased interactions with the soil matrix (URL-2). The volatilization rate of nonylphenol from soils is not significant; only 0.22% of 1 gram nonylphenol per kilogram of soil was removed over a period of 40 days. Therefore the degradation of nonylphenol in soil is affected mainly by sludge layer, oxygen availability and contamination bioavailability. The mobility of nonylphenol is expected to be low in soil as it is strongly bound to the soil particles and after 730 days almost 99% of the contaminant was found within 30 cm of the surface. However, when nonylphenol ethoxylates were applied to soil, a significant portion of these compounds was transformed into nonylphenol resulting in high concentrations in the soil that could reach aquifers (URL-2).

2.8.1.3. Groundwater

Discharge of STP effluents, agricultural activities, leachate and the discharge of industrial wastewater are the chief causes of presence of nonylphenol in aquifers. The removal of pollutants in groundwater is usually very slow since the chemical and biological characteristics of aquifers are not favorable to secure degradation (Soares et al, 2008).

Groundwater temperatures are in the psychrophilic range and both carbon sources and oxygen are limited. As a consequence the microbiological resources of such ecosystems are restricted and the contaminants undergo extremely slow degradation rates allowing contaminants to disperse up to several kilometers from the contamination source and to exist for decades (Barber et.al., 1988). The processes that control the entry of contaminants to groundwater are sorption and biodegradation. In the zones prior to the aquifer (river beds, landfill, cultivated soil, etc), there is usually an abundance of nutrients and microorganisms which constitute a major barrier for the entry of contaminants into aquifers. Therefore biodegradation is a critical stage

regulating the infiltration of nonylphenol into groundwater (Ahel et.al., 1994a). Studies have shown that the concentration of nonylphenol in aquifer depends on temperature, the highest concentrations being observed during the winter (Ahel et.al., 1994a). However, according to other investigations the presence of oxygen was observed to be a very important factor during the permeation of nonylphenol to the aquifers (Montgomery et.al., 2003). Under anoxic conditions nonylphenol was not biodegraded and furthermore was formed due to the degradation of nonylphenol ethoxylates and carboxylates. This is of considerable relevance since at depths greater than 1.5 m and in flooded areas, anoxic conditions predominate. Therefore these investigators concluded that sorption was the dominant removal process. Furthermore, they expressed concern with regard to sites that received repeated applications of contaminated materials since their sorption capacity could become exhausted. Another important factor is the hydrological characteristics of the sites. Rapid infiltration is observed at sites with highly permeable sediments. In the case of nonylphenol, due to its low solubility, its mass transfer is lower than more soluble compounds, giving rise to smaller plumes, but its sorption is controlled by the organic carbon content of the sediments (URL-2).

2.8.1.4. Atmosphere and Air

The first measurements for nonylphenol content in the atmosphere were begun to be taken in the late 1990s when samples taken from urban and coastal environments displayed unexpectedly high concentrations of nonylphenol (2.2–70 ng/m3) that exceeded those of PCBs and PAHs (Dachs et.al., 1999). In the operation of STPs nonylphenol is generated along with aerosols from the aerators of STPs. This causes a reduction in the quality of air surrounding these STPs. As mentioned earlier, when nonylphenol becomes part of the atmosphere, it can be captured and returned to aquatic and terrestrial ecosystems by wet deposition (rain and snow) (Soares et.al., 2008). In a study conducted in Germany, rain and snow collected from urban, suburban and rural areas was found to contain nonylphenol. Nonylphenol has been found also in

indoor environments (in air and dust) at concentrations higher than outdoor values (air — 110 ng/m^3, and dust — 2.58 μg/g) (URL-2). Nonylphenol was found in all the 120 houses examined and was amongst the most abundant compounds measured of the 89 organic chemicals assessed (URL-2). From these studies it is clear that the occurrence and the fate of nonylphenol in the atmosphere are of considerable importance.

2.8.2. Engineered Systems

NP compounds are used in household products as well as in cleaning industries therefore, influent of domestic and industrial wastewater treatment plants contain these compounds. The removal efficiency of NP compounds (NP+NP1EO+NP2EO+NP1EC) varies from one treatment plant to another depending upon the treatment used. Most of the treatment plants are unable to completely removed these compounds and hence they are discharged into receiving water bodies (Conn et.al., 2006). Due to the hydrophobic nature of NP compounds they tend to accumulate in sewage sludge and get concentrated there. Introducing sludges that contain NP to agricultural land could escalate the exposure of terrestrial environments to such substances. Therefore while taking land application of sludge into account one must consider fate of NP compounds. Nonylphenol and its ethoxylates have been found in treatment plant effluents (Ellis et.al., 1982; Giger et al. 1981) and in sewage sludges (Giger et.al., 1984).

2.8.2.1. Treatment Plants

Drinking Water

Nonylphenol is found in river waters, groundwater and other sources of potable water at relatively high concentrations. The efficiency of water treatment plants (WTP) at removing nonylphenol was found to be highly variable ranging from 11% to 99% depending on the type of unit treatment process. The highest efficiency (95%) was

achieved with a process involving ozonation and subsequent activated carbon filtration with chlorination (Petrovic et,al., 2003). These results show that drinking water is not considered a significant source of nonylphenol to human beings compared with other sources, such as food packaging materials, cleaning products and various skin care products which are estimated to be more important since the concentrations of nonylphenol are several orders of magnitude higher than that in drinking water (CEPA, 1999).

Wastewater Treatment Effluents

In comparison to bench-scale systems, full-scale municipal wastewater treatment plants (MWWTPs) generate heightened success rates in the removal of NPnEO owing to diverse microbial populations and nutrients present in the MWWTPs (Holt et.al., 1992). Therefore it is safe to comment that NPnEO in MWWTPs can achieve a primary biodegradation but an ultimate biodegradation is not possible. Even among MWWTPs there are significant discrepancies in treatment efficiencies which are ascribed to 1) design and operating conditions of MWWTPs (treatment temperatures) and, 2) the presence of NPnEO in influent streams. However, in some settings NP and lower chain NPnEO that are not as easily degradable are detected in receiving waters and final effluents of the MWWTP. Furthermore, considerable concentrations of the same are observed in MWWTP sludges. Moreover, degradation is much slower in natural environments and does not occur as rapidly as it does in MWWTPs owing to the higher concentration of microorganisms in the latter.

Temperature and aeration are important factors in the fate of nonylphenol in activated sludge system where nonylphenol removal is found to be highly dependent on temperature as well as on the presence of anoxic zones. Analyses of full-scale sewage treatment works demonstrated that nonylphenol occurs excessively in effluents and sewage sludge, especially in regions of high population density and close proximity to industrial wastewater treatment plants.

According to the Scrimshaw and Lester (2002) study it is pointed out that 60–65% of the nonylphenol compounds that enter the STP are released into the environment with 19% being nonylphenol carboxylates, 11% short nonylphenol ethoxylates (1 and 2 ethoxylate groups), 25% nonylphenol and 8% untreated compounds. The treatment processes used in the facility are dependent on the final effluent composition in the wastewater treatment plants. When the primary treatment is used solely, the effluent composition indicates the short hydraulic retention time and that ethoxylated products (i.e., NPnEO, where n = 3–20) are dominant (82%), with minor components of NP (3%), NP1EO and NP2EO (12%), and NP1EC and NP2EC (3%). The explanation for this is that in primary treatment 60-70% of solid content is removed (Tchobanoglous et.al., 2004) and lower chain NPnEOs (n<3) and NP (in particular) which are hydrophobic and lipophilic in nature got absorbed to solid content and are lost. On the other hand, higher chain NPnEOs (n>3) and NPnECs which are relatively less hydrophobic and more hydrophilic in nature are present at higher proportion in primary effluents. From Figure 2.8, it is seen that there are significant differences between secondary treated effluent composition and primary treated effluent. Higher-chain NPnEO make up only 28% of the nonylphenol compounds in secondary-treated effluent, whereas metabolites make up the rest (CEPA, 1999). Carboxylic acid metabolites (i.e., NP1EC and NP2EC) make up 46% of the secondary treated effluent composition, while NP1EO and NP2EO make up 22% and NP accounts for only 4% (Ahel et.al., 1994a) (Figure 2.8).

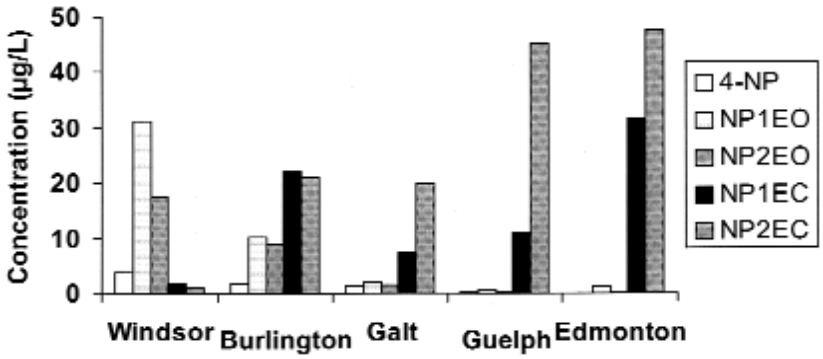


Figure 2.8: Concentration of NP, lower-chain NPnEOs and NPECs in various types of municipal wastewater treatment plant effluents (CEPA, 1999)

(Windsor = primary treatment; Burlington = secondary treatment; Galt, Guelph and Edmonton = tertiary treatment)

Birch (1991) and Watkinson and Holt (1991), together observed a crucial control factor for the treatment of NP compounds in MWWTPs with activated sludge plants, namely, the sludge retention time (SRT). The growth rate of the competent organisms within the total microbial population is governed by this factor. As the growth rate becomes lower than the SRT, the competent organisms get wiped out of the system and it is followed by little treatment of the specific substance. The growth rate is dependent on temperature, implying that a combination of reduced SRT and declining temperature would consequently result in a less efficient biodegradation system.

It was discovered by Watkinson and Holt (1991) that the normal range of SRTs for activated sludge plants would seemingly fall in the range of 6–20 days. Ahel *et al.* (1994b) observed that the lower the sludge loading rates and nitrifying conditions, the higher would be the NPnEO elimination rate in the MWWTPs. A study of two Canadian MWWTPs verified this observation (Water Technology International Corp., 1998b). An important point here is that the treatment efficiencies of NP compounds between dedicated industrial wastewater treatment facility and MWWTPs may differ quite considerably. Field and Reed (1996) testified that increased temperatures, escalated hydraulic residence times and greater degrees of acclimation than that of MWWTPs

identify the industrial wastewater treatment. Due to the operation of MWWTPs at ambient conditions, a higher seasonal variation in effluent composition is expected from MWWTPs than industrial effluents. Table 2.2 summarizes the concentration of nonylphenol compounds in treatment plant influents and effluents determined in different parts of the world.

Table 2.2: Concentration of Nonylphenol Compounds in Domestic Wastewater Treatment Plants. (Average numbers reported)

Location	Compound	Influent/Effluent		Concentration (µg/L)	Treatment used	Reference
Zurich Switzerland	NP	Effluent[a]		n.d*.-35	Mechanical –Biological Sewage Treatment Plant	Stephanou et.al., 1982
	NP1EO			24-133		
	NP2EO			n.d*-70		
Zurich Switzerland	NP	Effluent		1-14	Mechanical –Biological Treatment Plant	Ahel et.al., 1987
	NP1EO			4-78		
	NP2EO			4-66		
	NP1EC			1-224		
	NP2EC			131-233		
Zurich Switzerland	NP-C[b]	Effluent		240-760	Mechanical –Biological Treatment Plant	Ahel et.al., 1994b
Rome, Italy	NP	Influent		11-13	Mechanical –Biological Treatment Plant	DiCorcia and Samperi, 1994
		Effluent		1-1.1		
	NPnEO[c]	Influent		195-208		
		Effluent		9.6-9.9		
Barcelona, Spain	NP	Influent		131	Biological Treatment plant with Activated Sludge Process [e]	Sole et.al., 2000
		Effluent		6		
	$NPnEO^{d}_{4+6}$	Influent		33		
		Effluent		<0.2		
	NP1EC	Influent		8		
		Effluent		60		
USA	NPnEO(n=0-3)	Influent	Summer	158	Biological Treatment	Loyo et.al., 2007
			Winter	88.4		
		Effluent	Summer	3.19		
			Winter	21.6		
	NPnEO(n=4-16)	Influent	Summer	474		
			Winter	638		
		Effluent	Summer	0.334		
			Winter	5.22		
	NPnEC(n=1-2)	Influent	Summer	1.50		
			Winter	1.54		
		Effluent	Summer	16.8		
			Winter	81.5		

Table 2.2: Concentration of Nonylphenol Compounds in Domestic Wastewater Treatment Plants. (Average numbers reported) (cont'd)

Zurich, Switzerland	NP	Influent	0.473		
	NP1EO		1.14		
	NP2EO		1.89		
	NP3-7EO		6.82	Secondary Treatment	
	NP8-10EO		0.257		Jonkers et.al., 2009
	NP1EC		2.65		
	NP2EC		1.57		
	NP3-4EC		0.252		
	NP	Effluent	0.123		
	NP1EO		0.034		
	NP2EO		0.04		
	NP3-7EO		0.119		
	NP8-10EO		0.0006		
	NP1EC		0.444		
	NP2EC		0.394		
	NP3-4EC		0.227		
Verona, Italy	NP	Influent	4.15	Conventional activated Sludge	Bertanza et.al., 2011[f]
	NP1EO		3.90		
	NP2EO		2.18		
	NP	Primary Effluent	3.65		
	NP1EO		3.96		
	NP2EO		2.15		
	NP	Final Effluent	0.85		
	NP1EO		0.52		
	NP2EO		0.70		

*n.d not detected i.e. below **10** µg/L
a: Numbers reported are for three plants. However, study was conducted on six plants. For the other three plants the level were found non detectable.
b: sum of nonylphenol polyethoxyaltes (n=3-10) and their degradation products
c: Marlophen 810 detergent is used that contains NPEO chain isomers and oligomers with an average of 11 and a range of 1-18 ethoxy units.
d: Average of both 4 and 6 nonylphenol ethoxylates.
e: four treatment plants were investigated. Data for only 1 treatment plant is given in the table which treats domestic wastewater.
f: Study is carried out on two plants. Data for only 1 treatment plants that treats domestic wastewater is given

Most of treatment plants data given above uses primary and secondary treatment. No data from a plant with tertiary treatment is given in Table 2.2. By looking at Table 2.2 one can clearly see that NPnEOs are degraded considerably as the treatment of wastewater proceeds which corresponds well to the findings of Canadian Environmental Protection Agency (CEPA) in the "Priority Substances List Assessment Report" (CEPA, 1999). It shows that biodegradation of these compounds occur in treatment plants this is true for higher chain NPnEOs but for lower chain NPnEOs (n=1-2) and NP it may not be the only reason for their low values in plant effluent because

decrease in their values could be due to their proclivity to adsorb to sludge and get concentrated there which is a result of their physical and chemical properties as discussed earlier. The concentrations NP and NPnEOs in Glatt river (Zurich), are given over the period of 12 years from 1982-1994 by Stephanou, Ahel and their colleagues. Considering the work done in Zurich, it is observed that the values of NP, NP1EO, NP2EO in the influent has decreased remarkably because these compounds were partially banned in Switzerland in l986 (Giger et.al., 2009). In results of Ahel work (Ahel et.al., 1994b), concentration of NP seemed very high although these compounds were partially banned the reason for this was because the values given for 1994 study was the sum of NPnEO with ethoxylate chain from 3-16 instead of NP, NP1EO and NP2EO. Later in 2009 Jonkers conducted the study in the same city of Zurich where previous work was done and findings demonstrated very low values of NP, NPnEO and NPnEC. This is the result of two measures taken by EU, one is comprehensive ban of all NP compounds which was implemented in 2003 and the second is environmental standards for NP defined by water framework of EU in 2007 (Giger et. al., 2009).

Loyo and his co-workers in 2007 conducted a study on three wastewater treatment plants. All three plants involve primary treatment followed by secondary biological activated sludge treatment. However, two out three plants have an additional chlorination unit. Summer data for plant with no chlorination unit is not given in the study and hence average values for influent and effluent of three plants are given in Table 2.3. Influent and effluent concentrations for both ethoxylated and carboxylated compounds in the three plants were very similar, but effluent concentrations unveil a seasonal dependency: both carboxylate and ethoxylate concentrations in the effluents were found to be higher in winter than in summer.

Sludge

Wastewater treatment plants (WWTPs) whether domestic or industrial, produce large amount of sludge as a byproduct of their treatment scheme. Most of pollutants in wastewater end up in sludge. During treatment processes of WWTPs sludge becomes

rich in different contaminants such as heavy metals, pathogens and some persistent organic pollutants such as NP compounds (Pryor et.al, 2002). This sludge needs to be properly handled and disposed. There are different sludge disposal methods practiced in different parts of the world; some of the common ones include landfilling, land application as fertilizer for agricultural usage and incineration. According to Santos (2007), the most cost effective method for sludge disposal is land application. High nutrient content of sludge makes it suitable for land application as soil conditioner or fertilizer. In order to use sludge as a fertilizer for agricultural usage one has to check and make sure that it is appropriate for this use and does not pose any harm to environment or any organism in any possible way. For the case of NP compounds, this issue becomes vital as they tend to accumulate on sludge and hence pose a potential threat to human health and environment by entering the soil and the food chain.

NP compounds are present at higher concentration in influents of WWTPs because of their high consumption in household products and cleaning industries. During treatment higher ethoxylated NPnEOs are degraded and their metabolites are formed. These metabolites have a tendency to accumulate on sludge surface due to their physical and chemical properties described earlier in this text. It has been observed that activated sludge and digested sludge from MWWTPs utilizing secondary or tertiary treatment systems caused accumulation of NP (Giger et al., 1987) (Figure 2.9). Moreover, in the digested sludge, some production of NP1EO and NP2EO was observed. Nonylphenol was released mainly in association with the sludge up to 90% (Scrimshaw et.al., 2002)since the main pathway for nonylphenol removal in wastewater treatment plants is sorption to the sludge solids. The total influent concentration of nonylphenol compounds at several STPs was shown to be higher during the working days and late afternoons than at weekends and during the night (Ahel et.al., 1994a).

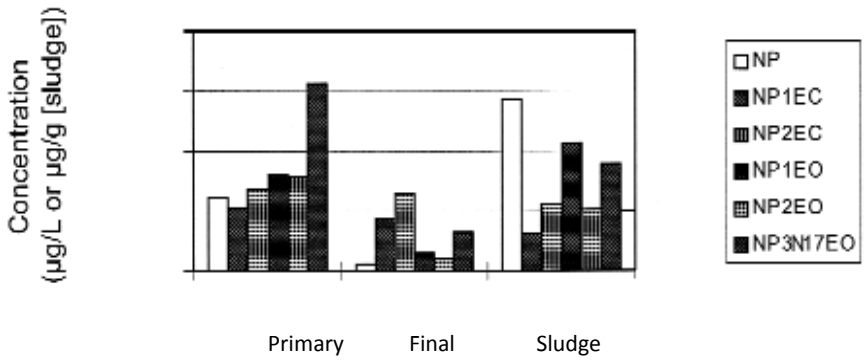


Figure 2.9: Distribution of NP, NPnEOs and lower-chain NPECs in effluent and sludge from a tertiary treated municipal wastewater treatment plant**.** (CEPA, 199)

It can clearly be seen from Figure 2.9 that as treatment progresses metabolites of NP compounds are formed and their concentration increases and in sludge they are present at highest levels. Similarly, Table 2.3 summarizes the concentration of nonylphenol compounds in treatment plant influents and effluents determined in different parts of the world.

Table 2.3: Concentration of Nonylphenol Compounds in Sludge (Average numbers reported)

Location	Compound	Sampling point	Concentration (mg/kg dm)	No of plants considered and Treatment used	Reference
Switzerland	NP	AnD	0.64-2.2	24 sewage treatment plants.	Brunner et.al., 1988
	NP1EO	AnD	0.1-0.68		
	NP2EO	AnD	0.02-0.22		
Canada	NP	H	137-470	2 sewage treatment plants	Lee et.al, 1995
USA	NP	C	5.41-72	11 sewage treatment plants, 3 composting 5 anaerobic digestion, 2 lime stabilization. 1 heat treatment	La Guardia et.al., 2001
	NP1EO	C	<0.5 [a] -2.5		
	NP2EO	C	<1.5 [a]		
	NP	Lm	119-820		
	NP1EO	Lm	81.7-154		
	NP2EO	Lm	25.3-254		
	NP	AnD	683-887		
	NP1EO	AnD	25.7-102		
	NP2EO	AnD	<1.5 [a] -32.6		
	NP	H	496		
	NP1EO	H	33.5		
	NP2EO	H	7.4		
USA	NP	AnD	1130	1 sewage treatment plant	Pryor et.al., 2002
Greece	NP	P	93	3 sewage treatment plants. Data given is collected in July 2004 from 3 plants. However, for one plant data is also collected in February 2004.	Fountoulakis et.al., 2005
	NPEO	P	233.5		
	NP	CP	27.6		
	NPEO	CP	90.5		
	NP	CS (winter)	59		
	NPEO	CS (winter)	45		
	NP	CS (summer)	3.6		
	NPEO	CS (summer)	12.8		
Spain	NP	P (winter)	16.87-30.88	4 treatment plants using biological Treatment with activated sludge.	Aparicio et.al., 2007
	NP1EO	P (winter)	57.45-72.47		
	NP2EO	P (winter)	37.13		
	NP	S (winter)	12.44-35.27		
	NP1EO	S (winter)	54.48		
	NP2EO	S (winter)	<0.421 [a]		
	NP	AnD+D (winter)	26.32-1432.61		
	NP1EO	AnD+D (winter)	4.61-268.13		
	NP2EO	AnD+D (winter)	206.9		
	NP	P (summer)	64.47-147.93		
	NP1EO	P (summer)	138.26-184.09		
	NP2EO	P (summer)	78.38-142.87		
	NP	S (summer)	14.61-143.69		
	NP1EO	S (summer)	22.10-204.84		
	NP2EO	S (summer)	<0.421 [a]		
	NP	AnD+D (summer)	391.14-1290.03		
	NP1EO	AnD+D (summer)	48.94-144.97		
	NP2EO	AnD+D (summer)	46.79		

Table 2.3: Concentration of Nonylphenol Compounds in Sludge (Average numbers reported) (cont'd)

Spain	NP	P	185-777	Biological Treatment with activated sludge. 2 sewage Treatment Plants: 1 uses AnD (P, S, AnD+D), other uses AeD (M and AeD+D)	Santos et.al., 2007
	NP1EO	P	342-1250		
	NP2EO	P	39.9-829		
	NP	S	52.9-611		
	NP1EO	S	284-1129		
	NP2EO	S	89.4-1375		
	NP	AnD+D	816-1385		
	NP1EO	AnD+D	232-640		
	NP2EO	AnD+D	35.6-331		
	NP	M	12.9-745		
	NP1EO	M	13.8-125		
	NP2EO	M	<0.421[a]-102		
	NP	AeD+D	9.6-1041		
	NP1EO	AeD+D	20.3-106		
	NP2EO	AeD+D	<0.421[a]-130		
Spain	NP	P	20.5-76.7	Study was carried out on 20 sewage treatment plants. 11 plants with anaerobic digestion +dewatering. 3 plants with aerobic digestion +dewatering. 3 plants with Lagoon. 3 plants with composting	Gonzales et.al., 2010
	NP1EO	P	16-186.6		
	NP2EO	P	<0.42[a]-119.1		
	NP	S	6.4-77		
	NP1EO	S	1-169.5		
	NP2EO	S	<0.42[a]-90.9		
	NP	AnD+D	63.3-249.9		
	NP1EO	AnD+D	<75[a]-109.0		
	NP2EO	AnD+D	<0.42[a]-135.4		
	NP	M	58.6-95.3		
	NP1EO	M	4.5-18.4		
	NP2EO	M	<0.42[a]-4.3		
	NP	AeD+D	59.7-113.1		
	NP1EO	AeD+D	11.8-18.6		
	NP2EO	AeD+D	<0.42[a]-12.5		
	NP	L	73.4-143.8		
	NP1EO	L	37.1-90.1		
	NP2EO	L	9-20.9		
	NP	C	177.2-180.4		
	NP1EO	C	28.1-37.1		
	NP2EO	C	8-9.7		

a: limit of detection for method used,
P: Primary, S: secondary,
CP: Concentrated Primary, CS: concentrated Secondary
AnD: anaerobically digested
M: mix (primary and secondary), AeD: Aerobically digested
L: Lagoon
C: composting
H: heat
Lm: lime
D: Dewatering

In Table 2.3, studies representing dry mass concentration of NP compounds using different types of sludge treatment processes are presented. Aparicio (2007) in his work conducted seasonal study in different sludge treatment processes treating NP, NP1EO and NP2EO. For NP, concentrations were higher in summer than winter for primary, secondary and anaerobically digested sludge. The reason for this is the increased microbial activity at warmer temperatures in summer hence concentration of NP increases due to enhanced degradation of NPnEOs (Bester et al., 2001). NP was found in all of the samples analyzed. NPnEOs concentration were observed to be higher than the limit value of 50 mg/ kg dm stated in the third draft of the EU Sludge Directive; in 17 of the 24 samples analyzed secondary sludge being the least contaminated samples. For secondary sludge NPnEOs concentrations were detected to be lowest which can be understood by taking degradation of these compounds into account during activated sludge treatment in aeration tank (Ahel et.al. 1994b). On the other hand highest level of NPnEOs were identified in digested dewatered sludge which can be justified by the formation of NP as metabolite from nonylphenolic compounds during anaerobic digestion (CEPA, 1999)

In another extensive study conducted by Gonzales in 2010, eight types of sewage sludge from 20 treatment plants with varying treatment methods such as anaerobic or aerobic digestion, anaerobic wastewater stabilization ponds, dewatering and composting has been checked for the occurrence of NP, NP1EO and NP2EO over the duration of one year. As usual NP was present at higher concentration levels than NP1EO and NP2EO due to aforementioned reasons. The concentration trend of NP, NP1EO, and NP2EO in conjunction with sludge treatments was classified by a decrease from primary to secondary sludge, an increase after digestion and then a slight decrease after composting (Gonzales et.al., 2010). A higher increase was observed after anaerobic digestion than after aerobic digestion, which can be explained by the different degradation products obtained under anaerobic and aerobic conditions. The concentration increase after digestion and after composting can be elucidated by the

concentration effect due to the loss of organic matter which takes place in those treatments. Consequently, the most contaminated samples were compost, anaerobically-digested sludge, aerobically-digested sludge and lagoon sludge. More than the 75% of sludge samples analyzed contained concentrations of sum of NP+NP1EO+NP2EO to be higher than the limit of 50 mg/kg dm defined in the EU draft Directive (Gonzales et.al., 2010).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Sludge used to Feed the Reactor

The experimental study was conducted using Waste Activated Sludge (WAS) taken from the Ankara-Tatlar Wastewater Treatment Plant (Ankara Greater Municipality Central Wastewater Treatment Plant) which currently has a flow rate of 765,000 m^3/day (ASKI, 2012).

The samples for WAS were taken from the return activated sludge line of the secondary sedimentation tanks. After the sampling, samples were given time to settle; once they settled, the water phase at the top of the sample bottles was separated removed to obtain a concentrated sludge sample with a certain solid concentration. The settlement process is carried out in a refrigerator at a temperature of 4^oC to minimize the microbial activity.

3.1.2. Chemicals

The used nonylphenol compounds during the gas chromatography-mass spectrometry device (GC-MS) are as follows: Nonylphenol solution (analytical standard for environmental analysis, 5 µg/mL in acetone), nonylphenol monoethoxylate solution (analytical standard for environmental analysis, 5 µg/mL in acetone), nonylphenol diethoxylate solution (analytical standard for environmental analysis, 5 µg/mL in

acetone) and nonylphenol acetic acid (10ng/L in acetone). These compounds were bought from Fluka, Sigma Chemie GmbH except for nonylphenol acetic acid which was supplied by Dr.Ehrenstorfer GmbH who also provided 4-n-nonylphenol (10 ng/mL in cyclohexane) and solid nonylphenol diethoxylate (99.0% purity, 10mg) to be used as a surrogate during extraction studies and to spike the aerobic batch reactors, respectively.

The 99:1 (Sylon BFT) Kit and BSTFA+TMCS (N,O-Bis (trimethylsilyltrifluoroacetamide) + trimethylchlorosilane) to be used for the derivatization of the chemicals preceding GC-MS analysis were provided by Sigma Aldrich Chemie GmbH and Supelco Analytical. Boron trifluoride (BF_3, in methanol) which was used to derivatize NP1EC was supplied from Merck KGaA, Germany.

The following chemicals were also used during the chemical extraction process prior to GC-MS analysis: Sodium Sulfate (anhydrous granulated for organic trace analysis) in order to get rid of the residual moisture, Copper (fine powder GR particle size<63□m) to remove the sulfate and SEP-PAK Vac C18 (6cc/500mg) cartridges for extracting the NP compounds from liquid samples. The sulfate and copper were purchased from Merck KGaA, Germany while the SEP-PAK Vac C18 cartridges were purchased from Waters Co (CITY, COUNTRY NAME??).

Along the experiments, gas chromatography grade acetone, methanol, hexane and petroleum ether were utilized for several purposes. These were also bought from Merck KGaA, Germany.

The chemicals used for preparing COD solution were also supplied from Merck KGaA, Germany. They are as follows: High purity H_2SO_4 (95-97%), $K_2Cr_2O_7$, $AgSO_4$ and $HgSO_4$.

3.2. Experimental Setup

3.2.1. Preliminary (Unspiked) Aerobic Semi-Continuous Reactors

The operation of semi-continuous reactors was carried out without NP2EO in the preliminary analysis. The intention was to find out and prevent any possible operational problems that may occur in the future and to achieve this aim, two reactors with different solids content were operated. Since it was preliminary work, replicate reactors were not used.

3.2.1.1 First Reactor

In this study, a lab-scale reactor with a volume of 2L was operated in semi continuous activated sludge mode. Initially waste activated sludge (WAS) from Tatlar Ankara wastewater treatment plant was used for the reactor. The first reactor is operated with a low concentration of sludge having a solid content of approximately 0.8%. The sludge taken from wastewater treatment plant of Ankara was left in a refrigerator for settling for 10 hours (hrs) and the resulting concentrated sludge with a TSS of 8,250 mg/L is used as a starting material for the reactor. A transparent glass bottle of total volume of 2.5 L is used for the reactor. This bottle is filled with 2L of WAS and is placed in a water bath. The temperature of water bath is maintained at 25 ± 2 ^{o}C. Using two pipettes and an air pump, oxygen was provided constantly to the reactors ensuring aerobic condition. Solids retention time (SRT) for this reactor was chosen to be 15 days; hence everyday 134 mL of waste from the reactor was discarded and was replaced by fresh feed solution. To feed the reactor, waste activated sludge from Tatlar wastewater treatment plant was taken once a week and kept refrigerated until use.

3.2.1.2 Second Reactor

The aim behind the setup of second reactor was to observe their performance under higher solid concentration. In order to achieve higher solid content, besides settling of sludge for 10 hours, sludge samples were placed in a centrifuge running at 1800 rcf

(2991 rpm). This reactor was operated with solid content of approximately 3%. Similar to the first reactor, the glass bottle used had a volume of 2.5L and working volume of each reactor was 2L. Moreover, the reactor was operated in water bath at 25 ^{o}C and aerated using air pumps.

In both reactors, TSS, VSS and pH were observed on a daily basis during the first few days. Later on, when the rate of decrease in TSS and VSS fell, measurements were shifted to a weekly basis. For each parameter analyses were performed twice and their average value was calculated and reported.

3.2.2. Nonylphenol Diethoxylate Aerobic Semi-Continuous Spiked Reactors

The degradation of nonylphenol compounds was examined under aerobic conditions using two sets of 3 L semi-continuous reactors with a working volume of 2 L in the third (and last) phase of aerobic laboratory scale study. To avoid any distorted results occurring due to foam overflow and loss of compounds (which was an observation in the preliminary sets operated), the head space was increased. Solids retention time (SRT) for this set of reactors was set again as 15 days; hence everyday 134 mL of waste from the reactor was discarded and was replaced by fresh feed solution .A total of four reactors were operated in groups of two. The detailed set up is summarized in the following Table 3.1.

Table 3.1: Initial Parameters for Reactor Setup

Reactor	WAS (L)	Acetone Addition (mL)	NP2EO (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)	COD (g/L)
Control -1	2	3		30700	18150	27150	15825	47.15
Control-2	2	3		30700	18150	27150	15825	44.84
Reactor 1	2	3	3	30700	18150	27150	15825	49.51
Reactor-2	2	3	3	30700	18150	27150	15825	48.82

The two groups of reactors were operated using WAS and NP2EO free acetone (NP2EO spiking solvent) and WAS and NP2EO in acetone. The concentration of NP2EO in the second group was adjusted to give 3mg/L (in the reactor) with an equal amount of acetone. Both groups were filled with WAS, concentrated (TS: 3.07 %, VS: 1.81 %) by settling. All reactors were operated at an SRT value of 15 days.

These 3 L reactors were operated gradually to observe any changes in the volume over time. There were two outlets at the top of each reactor with one of them being sealed and the other to be used for connecting aeration pipettes. A sampling port at the bottom of the reactor is used to extract the sludge. After extraction of each sample, the sampling port is secured with a hose clip. The configuration of the reactor used is given in Figure 3.1

Figure 3.1: Laboratory scale aerobic semi-continuous reactor

The temperature of the reactors was kept constant at 25^{o}C in a temperature controlled room to ensure that a constant temperature is achieved in the whole reactor. The reactors' pH was 6.41±0.1.

At first, the reactors were operated without NP2EO addition until the steady state was achieved. The reactors had some small amount of NP2EO (along with other NP compounds) brought with the WAS sample used. So no acclimatization was seemed necessary. In the beginning, sampling frequency was twice a week up to three weeks. Later, samples were collected every five days until the arrival of steady state. On the fifty eighth day, at the commencement of steady state two reactors were spiked with 3mg/L of NP2EO dissolved in 3mL acetone. At the same time, 3mL of acetone was added to the control reactors. After spiking, the interval between collecting samples was increased to twice a day for five days (58th to 62nd day) because of possible faster biodegradation under aerobic conditions. Later, sampling interval was widened due to decrease in biodegradation. Summary of sampling protocol for different parameters is

given in Table 3.2. TS, VS and pH analysis were conducted more often than the other parameters.

Table 3.2: Sampling Frequency of Different Parameters over Time

Parameter	Sampling Frequency over different time periods						
	First three weeks	**Fourth week to 58th Day**	**58th to 62nd**	**63rd to 67th**	**68th to 73rd**	**74th to 85th**	**85th to… 104**
TSS	Every third day	Every fifth day	Every fifth day	Every fifth day	Every fifth day	Once in seven to nine days	Once in seven to nine days
VSS	Every third day	Every fifth day	Every fifth day	Every fifth day	Every fifth day	Once in seven to nine days	Once in seven to nine days
COD	Every third day	Every fifth day	Every fifth day	Every fifth day	Once in seven to nine days	Once in seven to nine days	Once in seven to nine days
Nonylphenol compounds	Every third day	Every fifth day	Twice a day	Once a day	Every other day	Every third day	Once a week

Each sample analyzed corresponded to 40 mL of sludge that was extracted from the reactor and duplicate analyses of TS, VS, TSS, VSS, COD were performed over it. From sludge phase for the analysis of nonylphenol compounds (NP, NP1EO, NP2EO and NP1EC), two extracts were obtained from each sample and for each extract GC-MS was

employed twice. For liquid phase extraction of NP compounds one extract was obtained from each reactor and triplicate GC-MS analysis were conducted.

Semi continuous mode of reactor operation was stopped on day 91, this time can be taken as the reactor termination time. The reactors were operated from 91st day to 105th day without feeding in batch mode for the sake of keeping the microorganisms alive and functional.

3.3. ANALYTICAL METHODS

To determine the concentration of nonylphenol compounds in the reactors, Gas Chromatography-Mass Spectrometry (GC-MS) was employed. In this process, the constituent to be recognized volatilizes in the GC column and is then recognized by in the MS column. A difficulty arises while performing the analysis with our given compounds as the constituent is not supposed to break down in the GC column with high temperatures, but in our case, the chemicals break down before they are volatilized. To overcome this problem, the boiling points of the compounds are decreased by a process called as derivatization so that the volatilization temperature becomes lower than the temperature at which the molecules break down. In a previous study, using several derivatization techniques, silylation was approved to be the most effective for the compounds of interest (Ömeroğlu, 2012). The derivatization method selected for the analysis of NP, NP1EO and NP2EO is performed in the following manner:

- Place 1 mL of sample in acetone
- Evaporate acetone with the use of Nitrogen
- Add 0.1 mL BSTFA+1%TMCS
- Heat to 70^{o}C for half an hour
- Transfer the sample to 0.2 mL GC-MS injection
- Inject it into the device

The column used in GC-MS was Phenyl Methyl Siloxane with Helium as the carrier gas. The inlet to the device was maintained at 250^0C under splitless mode. The details of the heating process are provided in the table 3.3.

Table 3.3: GC-MS Oven Program

Carried Gas	Helium (10.152 psi)
Injection Volume	1 µL
Injection Mode	Splitless
Injection Temperature	280^0C
MS Interface Temperature	280^0C
MS Source Temperature	230^0C
MS Quadropole Temperature	150^0C
Initial Temperature	100^0C
Oven Program	100^0C (5 min hold), 25^0C/min to 160^0, 10^0C /min to 260^0C (5 min hold), 35^0C/min to 285^0C (7 min hold)
Final Temperature	285^0C
Overall Duration	30.114 min

The GC-MS method described above was employed for NP, NP1EO, NP2EO and 4-nNP (surrogate chemical). The device was operated under Selective Ion Mode (SIM) as every chemical has a dissimilar number of isomers and the quantification and target ions are different for each isomer.

For NP1EC the derivatization method used was different and taken from Lee et.al (1997) and is given as follows;

- Evaporate 1 mL NP1EC solution to 200 µL using N_2
- Add 2 mL 14 % Boron trifluoride (BF3) solution
- Place the vials in oven at 85^0C for 30 min
- Cool the vials and then evaporate them to 300 µL using N_2
- Place the vials into mechanical shaker at 400 rpm for 1 min
- Add 2.5 mL of double distilled water
- Add 2 mL petroleum ether three times and extract the methylated products
- Pass the elute obtained through Na_2SO_4 column
- Evaporate the resulting mixture to complete dryness by N_2
- Add 1 mL of hexane to the vials and transfer them into 2 mL GC vials after 1 min vortex

Once, all the processes were completed, calibration curves were plotted for four different chemicals using eight points between concentrations ranging from 10 ppb to 1000 ppb. These curves had to be re-plotted each time the column or the filament of the GC-MS device was changed. In this study, two calibration curves were used as filament of GC-MS was changed on 42^{nd} day.

The other parameters analyzed in the experiments were concentrations of Total Solids (TS), Volatile Solids (VS), Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) and the methods are described below.

3.3.1. Solid Content Analysis

Standard Methods 2540B and 2540E were employed for the determination of Total Solids (TS) and Volatile Solids (VS). However, for determining the Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS), methods 2540D and 2540E were employed (APHA, AWWA, WEF, 2005).

3.3.2. Chemical Oxygen Demand Analysis

Among the different analyses performed over the sludge samples, the determination of Chemical Oxygen Demand (COD) was one. HACH DR 2400 spectrophotometer was used for COD determination. HACH's dichromate method approved by United States environmental Protection Agency (USEPA) was followed (Apul, 2009). The solution required for COD was prepared in the laboratory in accordance with the Hach Water Analysis Handbook (2011, 5^{th} Ed.) rather than buying it from Hach Co. The calibration curve for the solution was plotted using Potassium Hydrogen Phthalate (KHP) as its equivalent COD is already known as 1.175 mg O_2/mg KHP.After the preparation of COD solution and its calibration, the extracted sludge samples were diluted and COD analyses was performed over them. This process was repeated twice for each sample to take the average values. Duplicate samples for used for COD analysis.

3.3.3. pH Analysis

The measurements of pH were performed using a Model 510 pH meter with a pH probe (EC-PH 510/21S) in accordance with the 4500H Standard Method. The pH meter was bought from Eutech Instruments Pte, Ltd. Spain. And the device was calibrated using standard solutions of pH values, 4, 7 and 10 beforehand.

3.3.4. Extraction of Nonylphenol Compounds from Sludge and Water

To obtain the concentrations of nonylphenol compounds in the experiments, the samples are separately extracted in solid and liquid sludge phases and then GC-MS analyses are performed after derivatization of the samples. The separation of phases is done in a centrifuge running at 3500 rpm for 10 minutes.

3.3.4.1. Extraction from Solid Phase

To determine the method with the highest efficiency and efficacy, a thorough literature research was done and it was found that mechanical shaking and sonication are the most widely accepted methods. For this reason, these two methods have been employed in the experiments along with another one that is a combination of these two. The solvents, sonication time and shaking time were varied for each method until an optimum method was reached (Ömeroğlu, 2012). Sonication turned out to be the most effective method for extraction from the solid phase, and the procedure was carried out for 5 minutes with acetone as the solvent. The method is described briefly as follows:

- Spike 1 mL of 500 ppb NP, NP1EO and NP2EO to the cleaned soil/sludge sample
- Place the sample in the 12 mL vessel and add 0.05 g copper and 1 mL ppb of 4-n NP solution
- Dry the sample using nitrogen gas
- Add 10 mL of acetone and place the vessel in sonic bath for 5 minutes
- Place the vessels in the centrifuge running at 2500 rpm for 10 minutes to get a clear solution
- For dehumidification, pass the solvent through the sodium sulfate column

During the experiment, it was observed that using a sonication time of greater than 5 minutes resulted in a decreased concentration of NP2EO, therefore a sonication time of more than 5 minutes was deemed ineffective.

The range of extraction recovery regarded acceptable by the USEPA is 70 %- 130 %. However, it was found that 60-150 % is widely accepted in literature (Lian et al., 2009). The sonication extraction method was chosen because the percentage recovery values stayed within this range for a sonication time of 5 minutes.

The extraction analysis was performed using two different blank samples. One of the samples contained sludge without any spike of NP compounds on it; however the other one did not contain any sludge or NP compound and was useful for determining any amount of contamination as a consequence of solvents and glassware used. The background concentrations of NP were obtained by using these blank samples and to acquire the recovery values, the results of these samples were extracted from the actual results.

3.3.4.2. Extraction from Liquid Phase

Similar to the case of extraction from Solid Phase, a thorough literature research was conducted which concluded that Solid Phase Extraction (SPE) is the most widely employed method for the extraction of nonylphenol compounds from water. The method is employed using Sep-Pak (Waters) C18 cartridges. The flow rate of the sample and solvents through these cartridges is regulated using a vacuum manifold system bought from Agilent Technologies. Various solvents were tested with these cartridges to determine the most efficient one, namely 1:1 acetone/methanol mixture (Ömeroğlu, 2012). The method is described as follows:

- Position the cartridges on the vacuum manifold and condition by passing hexane thrice as 4 mL, methanol thrice as 4 mL, acetone thrice as 4 mL and distilled water twice as 3 mL in the prescribed order
- Filter the liquid sample through the cartridge with assistance from the vacuum generated
- Pass 10 mL of 1:1 acetone/methanol mixture through the cartridges and collect the extract in a vessel

The given method was chosen for extraction from liquid phase of sludge since it was determined that the accuracy while repeating SPE method was high and the percentage recovery values lied in the acceptable range.

Analogous to the extraction from solid phase of sludge, a blank sample was employed. The double distilled water was passed through this sample and the degree of contamination resulting from the equipment employed was determined. The presence of NP compounds in this analysis was very seldom observed; however, whenever it was present, the values were subtracted from the main samples' data.

For both extraction methods; solid phase and liquid phase, limit of detection and limit of quantification has been determined. These limits were determined using signal to noise ratio. Noise is defined as the variation in the instrument's background signal; usually measured as the standard deviation of the background signal. The change in instrument's response to presence of a substance is termed as signal. Most analytical instruments produce a signal when a blank is analyzed (URL-4). The limit of detection is the analyte concentration required to produce a signal distinguishable from the noise level within a particular statistical confidence interval. Just identifying separately something from noise does not mean that one can know how much of the material there actually is with a particular degree of certainty (URL-4). Limit of quantification is the limit at which a reasonable difference between two different values of the amount of analyte can be differentiated. For limit of quantification signal to noise ratio was chosen as 10 and for limit of detection it was 3. In both phases limits were same. Table 3.4 summarizes the limit of detection and limit of quantification of all NP compounds analyzed in this system.

Table 3.4: Limit of detection and limit of quantification of NP compounds for the method used

Compounds	Limit of Detection (µg/L)	Limit of Quantification (µg/L)
NP	3	10
NP1EO	3	10
NP2EO	6	20
NP1EC	15	50

CHAPTER 4

RESULT AND DISCUSSION

4.1. Preliminary work on Unspiked Aerobic Semi-continuous Reactors

A preliminary study was performed on two reactors to oversee any experimental problems that may occur during reactor operation. Since it was preliminary work, replicate reactors were not used. This preliminary reactor work corresponded to the time scale during which the analysis methods for NP compounds were developed and it allowed us to use the time more efficiently. The results obtained in this part are discussed in detail below. Table 4.1 displays the initial and final solids contents of the reactors and steady state total and volatile suspended solids removal in each reactor by the reactor termination.

Table 4.1: Results of preliminary reactors

	Initial TSS (mg/L)	Final TSS (mg/L)	% Removal	Initial VSS (mg/L)	Final VSS (mg/L)	% Removal
Reactor 1	8250	6580	20.24	6010	4240	29.45
Reactor 2	29517	13380	54.67	21813	8620	60.48

4.1.1. First Reactor

Figure 4.1 shows the solids removals over time for the first reactor.

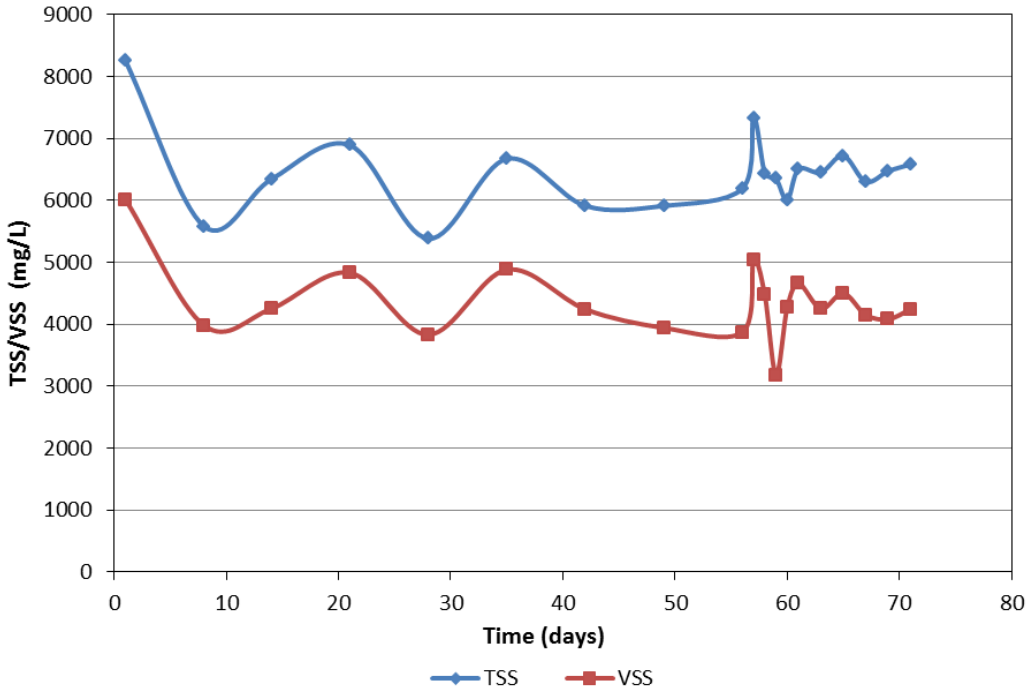


Figure 4.1: Change in Total Suspended Solids or Volatile Suspended Solids concentration w.r.t time in the first reactor

From Figure 4.1, it can be observed that the initial content of Total Suspended Solids was 8,250 mg/L and was reduced to 6,580 mg/L over a period of 71 days. Similarly, the content of Volatile Suspended Solids reduced from 6,010 to 4,240 mg/L. The difference in the change in concentrations of TSS and VSS, 20.24% and 29.45% respectively, describe that the degradation of organic matter was higher.

The literature research suggests that the removal in VSS should be around 40-60% in aerobic digesters (Park et.al., 2006; Rein et.al., 1977; and Sanin et.al., 2011). However, in the present case, excessive aeration in a 2L working volume with only 0.5L of head space led to an overflow in the reactor due to which some of the solid content was lost and the experiment could not be conducted with precision and accuracy.

4.1.2. Second Reactor

Figure 4.2 shows the solids removals over time for the second reactor.

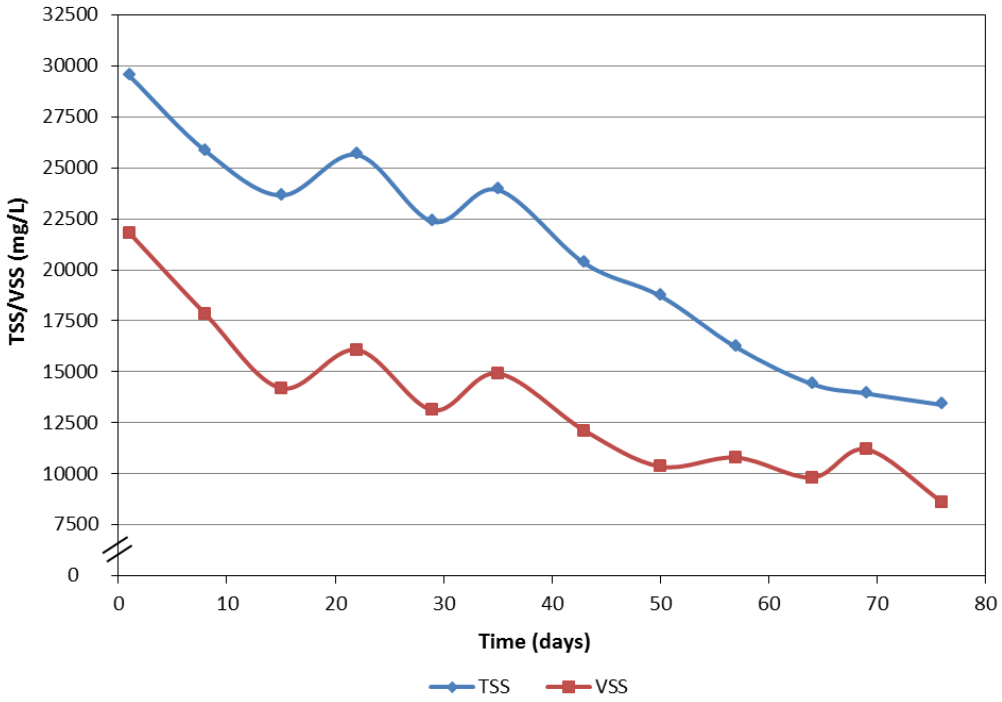


Figure 4.2: Change in Total Suspended Solids or Volatile Suspended Solids concentration w.r.t time in the second reactor

Similar to the first reactor, Figure 4.2 shows the removal of the TSS and VSS in the second reactor. It can be seen that the amount of TSS was reduced from 29,517 to 13,380 mg/L and VSS was reduced from 21,813 to 8,620 mg/L. The removal of organic matter was found as 60.48% which is in agreement with the literature findings (Sanin et.al., 2011).

To determine the relationship between the amount of VSS present and its degradation, the second reactor was operated with a higher VSS concentration than the first reactor. It is deduced that the degradation efficiency of the reactor increases as the VSS concentration increases (Hartman et.al., 1979). From the reactor operation experiences gathered in the preliminary phase, we decided to operate the reactors spiked with NP

compounds with as high solids content as possible to increase their efficiency and to control the aeration carefully so that the overflowing foam would not cause erroneous results.

4.2. Nonylphenol diethoxylate Spiked Aerobic Semi-continuous Reactors

In this part of the study, four reactors were operated out of which two were spiked with NP2EO and the other two were not spiked with NP2EO but rather included an equal volume of acetone with the first set so that they could be used as control reactors. All the reactors were operated in semi-continuous mode until the 91st day. At this day it is beleived that all the necessary data has already been collected. This point can be taken as the reactor termination for this study. Just for the sake of keeping the reactors operating, feeding is discontinued on the 91st day and reactors were swithched to batch operation mode, while more data were collected, specifically until the end of 105 days and the graphs are presenting the whole period. The reason in switching to batch operation is keeping the biomass active in case if additional analysis were required and for possible future studies.

Table 3.1 shows the parameters for reactor setup and operation. In Table 4.2, solids degradation is summarized.

4.2.1. Solids Content

Table 4.2 summarizes the solids removal percentages along with the initial and final TS, VS, TSS and VSS concentrations over the period of 91 days (time of termination os semi-continuous operation). Here, the reactors C-1 and C-2 are the control reactors and R-1 and R-2 are the spiked reactors. The results obtained showed that the reactors were set at the solids level decided upon with the results of the preliminary set. Also in terms of removal rates all the performances obtained were at the expected levels. Results obtained are separately evaluated below in the following sections.

Table 4.2: Solids content reduction in control and spiked reactors (final day= 91st day)

	C-1 (mg/L)	C-2 (mg/L)	R-1 (mg/L)	R-2 (mg/L)
Initial TS	30700	30700	30700	30700
Final TS	15170	15210	14850	15380
%Removal	50.58	50.43	51.63	49.90
Initial VS	18150	18150	18150	18150
Final VS	8430	8420	8180	8360
%Removal	53.55	53.61	54.93	53.94
Initial TSS	27150	27150	27150	27150
Final TSS	14210	14320	14420	14760
%Removal	47.66	47.25	46.88	45.63
Initial VSS	15825	15825	15825	15825
Final VSS	7580	7580	7590	7660
%Removal	52.10	52.10	52.03	51.59

4.2.1.1. Total Solids

Figure 4.3 demonstrates the change in TS with respect to time for all the reactors and feed. For the reactors operated waste activated sludge (WAS) which constituted feed was taken from Ankara Greater Municipality Central Wastewater Treatment Plant and is used as feed. Throughout this study, the aim was to keep the feed concentration constant to avoid any lag/fluctuation in biodegradation, however, after the 70th day, the feed solids concentration gradually decreased which may be a result of seasonal variations in the treatment plant inflow. The initial concentration of TS was 30,700 mg/L in all the reactors. TS started to decrease as soon as the reactors started, showing a continuous decreasing trend until the reactors reached steady state, which happened sometime between 45th and 55th days. Around steady state the TS concentration started to level off with small fluctuations.

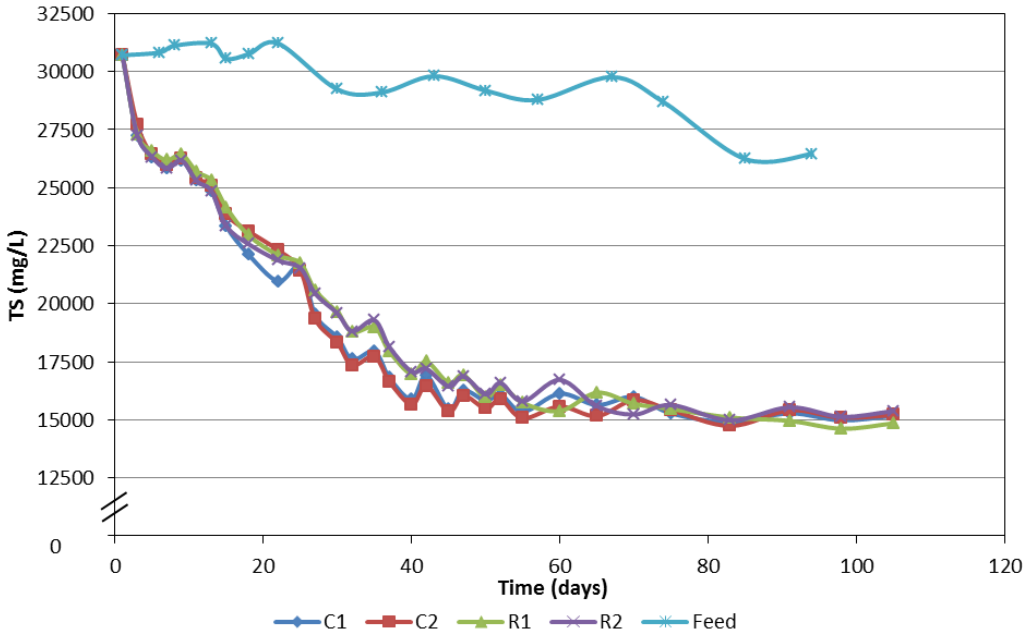


Figure 4.3: Change in Total Solids concentration (mg/L) w.r.t time for feed and control and NP2EO spiked reactors

All the reactors were operated with a solids retention time (SRT) of 15 days and literature research indicated that steady is state is generally achieved in 3-4 times SRT

(45-60 days for these reactors) (Tchbanoglous et.al., 2004). The reactors were purposely spiked with NP2EO at steady state. In this case the reactors were spiked on the 58th day of operation even though a steady state was acquired between 45th to the 55th day. The reason behind the late spiking was to be sure that system is free of any inconsistencies. Although reactor 1 and reactor 2 (R1 and R2) were spiked with NP2EO on the 58th day, the steady trend of solids shows a similar pattern for all the reactors even after spiking. This shows that the degradation of solids in aerobic digesters is unaffected of NP2EO. At the end of reactors' operation the total solids concentrations were measured as 15,170, 15,210, 14,850 and 15,380 mg/L for C1, C2, R1 and R2, respectively. This shows that the percentage removal is similar for all the reactors and is found to be between 49.90-51.63 %.

4.2.1.2. Total Volatile Solids

The decomposition of volatile solids follows a similar pattern as total solids and is shown in Figure 4.4. The initial concentration of VS was 18,150 mg/L that consequently reduced to 8,430, 8,420, 8,180 and 8,360 mg/L for control and spiked reactors corresponding to a percentage removal of 53.55-54.93 %. The removal of organic matter which is represented by VS was in accordance with the literature findings (40-60%) (Rein et.al., 1977).

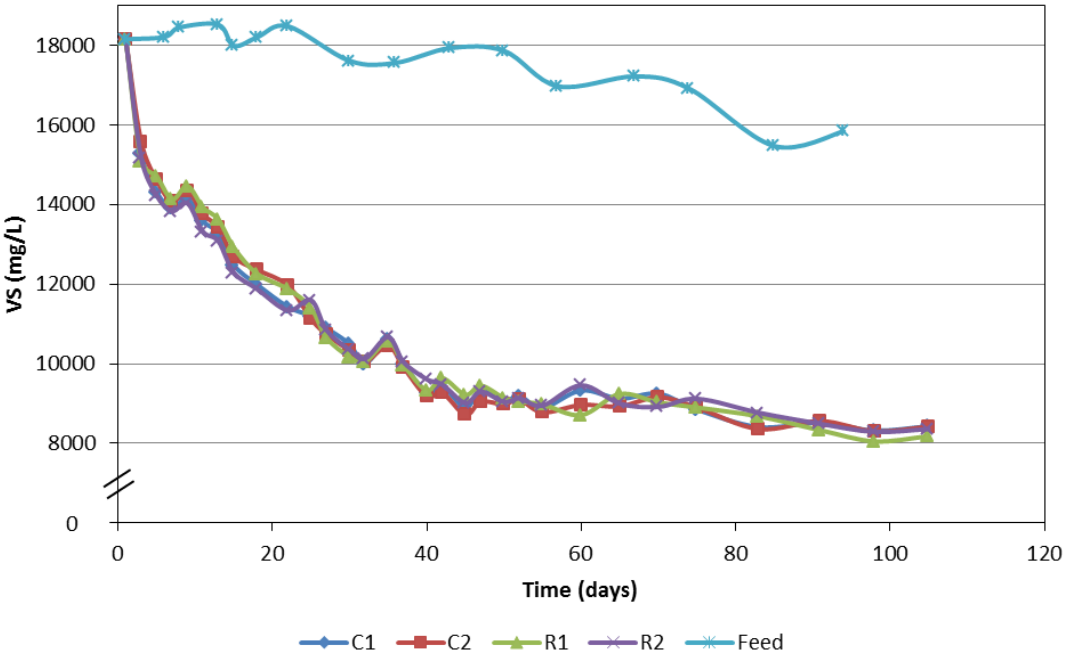


Figure 4.4: Change in Volatile Solids concentration (mg/L) w.r.t time for feed and control and NP2EO spiked reactors

4.2.1.3. Total Suspended Solids and Total Volatile Suspended Solids

Figure 4.5 follows a trend very similar to Figure 4.3. For TSS the concentration dropped from 27,150 mg/L to 14,210, 14,320, 14,420 and 14,460 mg/L for C1, C2, R1 and R2, respectively. The TSS removal was calculated as 46.88-47.66 %.

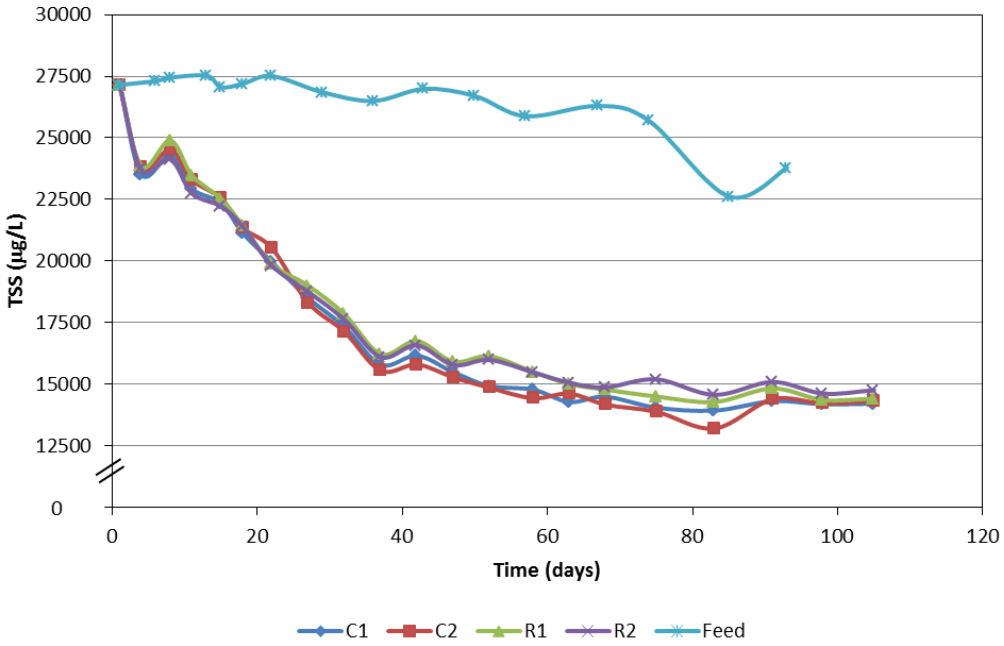


Figure 4.5: Change in Total Suspended Solids concentration (mg/L) w.r.t time for feed and control and NP2EO spiked reactors

Figure 4.6 follows a pattern very similar to Figure 4.4. For VSS the concentration decreased from 27,150 mg/L to 7,580, 7,580, 7,590 and 7,660mg/L for C1, C2, R1 and R2, respectively. The VSS was reduced by 51.59-52.10 %.

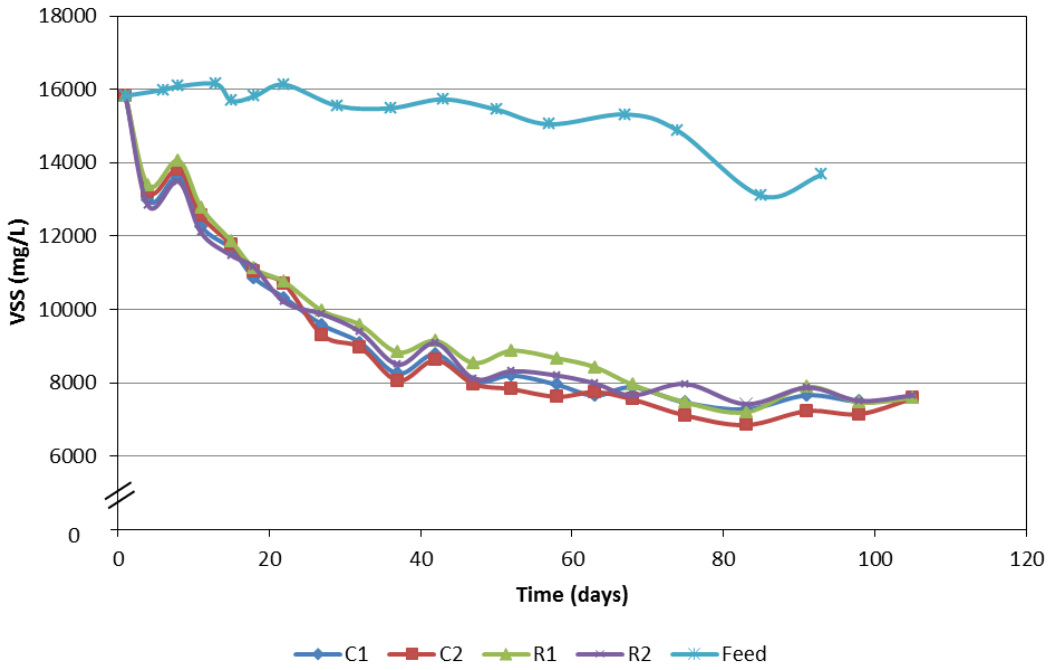


Figure 4.6: Change in Volatile Suspended Solids concentration (mg/L) w.r.t time for feed and control and NP2EO spiked reactors

4.2.2. pH

Figure 4.7 illustrates the pH variation of all the reactors and feed over time. It can be seen from the plot that within the first 5 days, pH sharply increased to 8.50 and later to 9 on 27th day. Then close to reactor termination the pH decreased slightly down to about 8.5 again. Aerobic digestion is an oxygen requiring process; therefore, oxygen is supplied to reactors using pumps. In order not to limit the oxygen and to achieve complete mixing in the reactors, pumps were operated at their full capacity. Aerobic digesters can operate over a wide range of pH, however, their optimum pH is between 6-8 (Anderson et.al., 1984). In general, the microorganisms used the oxygen provided in aerobic digestion for their growth and produced carbon dioxide (CO_2) decreasing the pH. On the contrary, the pH of all the reactors increased during operation. This could be due to the excess supply of oxygen which probably striped out all the CO_2 formed and not allowed for a pH drop.

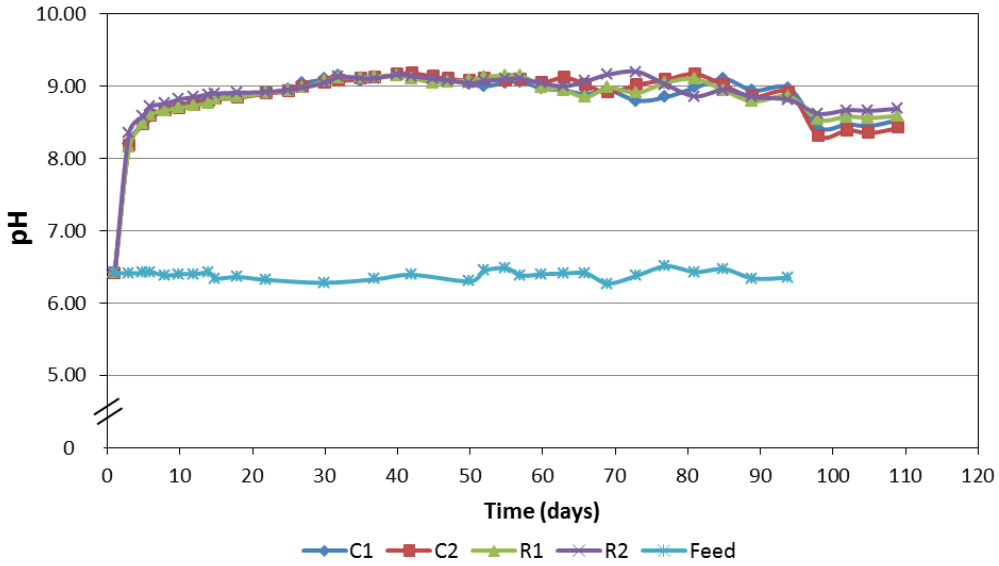


Figure 4.7: Change in pH w.r.t time for feed, and control and NP2EO spiked reactors

Although, the pH of reactors was higher than the optimum value, no negative impact of this was seen on the efficiency of the reactors. Hence, no adjustment was made to pH through a buffer addition because it was thought that addition of any chemical may cause disturbances in the reactor efficiency. It is very clear from Figure 4.7 that the pH of all the reactors became stable on the 50th day and afterwards, which shows that steady state was achieved. This trend continued up to the 94th day and then a gradual decrease of 0.5 in the pH was noted. This could be a result of change in the reactors' operation mode as both control and spiked reactors were operated under batch mode after the 94th day. Furthermore, the presence of NP2EO or its daughter products demonstrated no impact on the pH of the reactors as pH of R-1 and R-2 was similar to the pH of control reactors. The pH of feed was almost constant throughout the study and was calculated to be between 6.28-6.47.

4.2.3. Chemical Oxygen Demand

The variation in the COD with respect to time for all the reactors is given in Figure 4.8. The COD of feed was found to be around 50 g/L till the 29th day. Later, a slight decrease in the COD of feed was noted and the value was around 45 g/L. This variation can be a result of either seasonal variation or any variation in the plant operation because there were times when the plant was not operating perfectly due to rain events or maintenance. This could have resulted in non-homogenous samples as at times, the samples were collected the day following the plant maintenanceor rain. In addition, changes in settleability (even small) of sludge that may happen in any plant any time due to an upsetting condition, may cause a somehow dilute sludge (like in this case) even after the same concentrating protocols are followed before the reactor feeding.

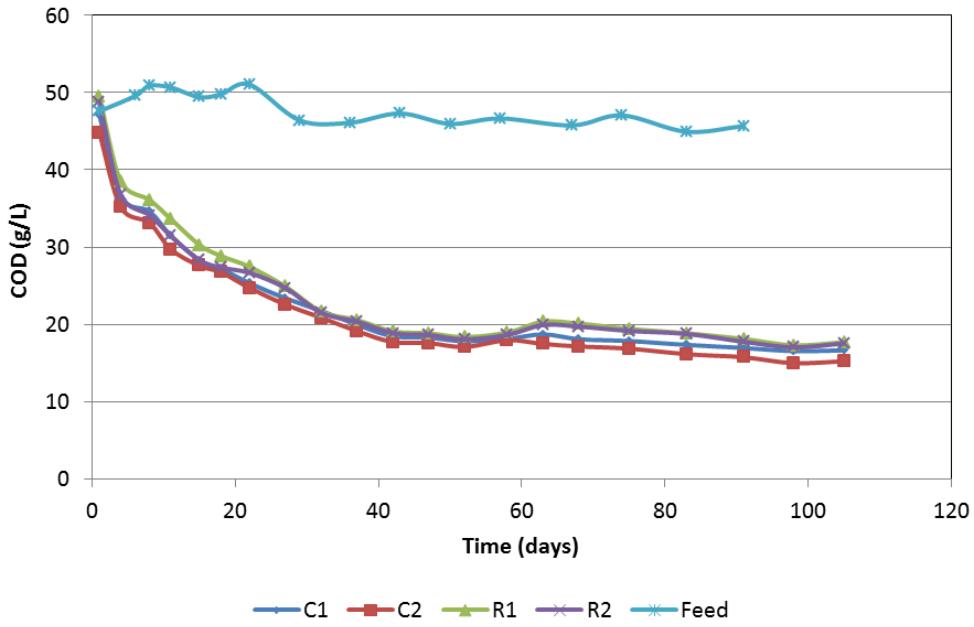


Figure 4.8: Change in COD w.r.t time for feed, and control and NP2EO spiked reactors

A stable COD concentration was observed at steady state, however, after spiking R-1 and R-2 with NP2EO in acetone and the control reactors with acetone, a minor increase in both control and NP2EO spiked reactors was noted. The increase was relatively greater for NP2EO spiked reactors than the control reactors. The overall COD removal

was between 64.09-66.03% in all the reactors with no significant difference between NP2EO spiked and acetone spiked control reactors.

4.2.4. Nonylphenol Compounds

The main focus of this study was to monitor the fate of NP compounds in an aerobic semi-continuous reactor. To fully achieve this, the concentration of NP compounds was examined in both solid and liquid phases at predetermined times. The total concentration of each compound is obtained by adding mass present in the solid and the liquid phase and then dividing it with the total volume. Therefore, the section that follows contains three plots; solid phase, liquid phase and the overall concentration present in the whole reactor for each of control and NP2EO spiked reactors. All the reactors (C1, C2, R1 and R2) were operated using WAS. This WAS had some background concentration of NP compounds and the reduction of this background concentration of NP compounds was monitored till steady state (and till the spike was done). Table 4.3 shows background concentration of NP compounds in solid phase for all the reactors.

Table 4.3: Background Concentration of NP Compounds in solid phase at reactor start-up

Reactors	NP (µg/L)	NP1EO (µg/L)	NP2EO (µg/L)
C-1	60.65	93.40	154.11
C-2	63.16	95.65	158.46
R-1	60.12	95.46	153.65
R-2	54.92	95.94	157.94

At the start, NP compounds were only detected in solid phase as their concentration was either below the limit of detection (LOD) or limit of quantification (LOQ). With the passage of time, decomposition of NP compounds started and later these compounds were detected in liquid phase as well. However, in all stages of the study it is observed that all NP compounds used in this work accumulated dominantly on sludge solids

rather than staying soluble in the liquid phase. This is in compliance with previous findings and the physicochemical parameters of these chemicals as discussed in Chapter 2. Table 3.4 shows the LOD and LOQ values of NP compounds for the method used in this study.

At steady state, on the 58th day, two reactors R1 and R2 were spiked with 3mg/L of NP2EO. Since the used volume of each reactor was 2L, 6mg of NP2EO dissolved in 3 mL of acetone was added to R1 and R2. Similarly, 3mL of acetone was added to control reactors (C1 and C2) to observe the effect of acetone on their performance. The concentrations of NP and NP1EO were quite lower than the concentrations of NP2EO and NP1EC. Therefore, to have a better view of the degradation pattern of each of the NP compounds, two different vertical axes were used. The compounds with higher concentrations such as NP2EO and NP1EC were plotted on the primary y-axis and the compounds with lower concentrations (NP and NP1EO) were plotted on the secondary y-axis using a relatively smaller scale.

4.2.4.1. Control Reactors

Figures 4.9-4.11 show the change in the concentration of NP compounds in solid phase, liquid phase and their sum in C1. Since the higher ethoxylated chemicals are not measured, NP2EO is considered as the mother compound in this study and the transformations are based on it. In the beginning, some background concentrations of NP, NP1EO and NP2EO were measured as 60.65, 93.40 and 154.11 µg/Lin solid phase. Liquid phase did not contain any of the NP compounds. Initially, no decrease in the concentration of NP2EO in solid phase was observed till the 8th day of reactor operation. This could be due to the presence of lag period in the microbial activity during which they adopt themselves to the environment. As mentioned before, none of the NP compounds were detected initially in liquid phase. Although, the degradation of NP2EO in solid phase started on the 8th day, it started appearing in liquid phase on the 22nd day. The difference calculated in the concentrations of NP2EO between the readings on the 8th and 22nd days was 46.29 µg/L for C1. The possible explanation of

this is that some of the NP2EO was degraded (22.28 μg/L) to NP1EC between the 8th and 22nd days of operation and some of it was transferred to the liquid phase (24.01 μg/L). The concentration of NP2EO in liquid was not detected earlier because the method used in the determination of NP2EO has a limit of quantification of (20 μg/L) (Omeroglu, 2012). The acetone spike was made on the day of 58, and since it was free of any NP2EO, no change in concentration trend was observed. In general, NP2EO showed a gradual degradation resulting in to the formation of NP1EC in solid phase of control reactors, and the final concentration at the end of the 91st day was found to be 45.17 μg/L for C1.

NP1EO displayed the same trend as NP2EO, the only difference was that in the case of NP1EO, degradation started on the 11th day in solid phase for this reactors. However, NP1EO started appearing in liquid phase on the 37th day of operation in C1. In solid phase, the decomposition of NP1EO was observed and the final value at the end of operation was noted to be 39.61 μg /L in C1.

NP was not degraded at all in control reactors. NP concentration reflects a slow increase and the final values at the end of the 91st day for solids and was measured to be 87.05 μg/L for C1. This increase in the concentration of NP could be a result of conversion of NP2EO to NP1EO and subsequently to NP. In aerobic systems degradation of higher chain NPnEOs to lower chain NPnEOs is very fast. In this case no increase in the concentration of NP1EO was observed. Therefore, the NP production can be a caused by three mechanisms; 1) degradation of NP2EO, 2) direct conversion of NP1EO to NP, 3) combination of above two mechanisms.

In solid phase, NP1EC was first detected on the 8th day. Here, the important point to be considered is that till the 8th day NP2EO's concentration was constant. Moreover, the overall amount of NP1EC formed was higher than the total amount of NP2EO degraded during the reactor operation. In fact, it was even higher than the sum of the amount of NP2EO and NP1EO degraded during reactor operation. According to previous studies (Omeroglu, 2012) first of all NP2EO is converted to NP1EO then NP1EO is degraded to

NP2EC and later to NP1EC. This is a long process and hence it is expected that NP1EC will show up in the system after a few days of NP2EO degradation, but this did not happen in this case. The possible explanation for this is that some higher chain NPnEOs were present in the control reactors and they were converted either directly to NP1EC or by some other unknown pathway which does not involve NP2EO formation. Since the presence of higher chain NPnEOs or NPnECs were not measured in this study, the exact reason for the early formation of NP1EC is unknown. A gradual generation of NP1EC is observed throughout the study in reactor C1. At the end of 91 day, NP1EC was found to be 300.92 µg/L

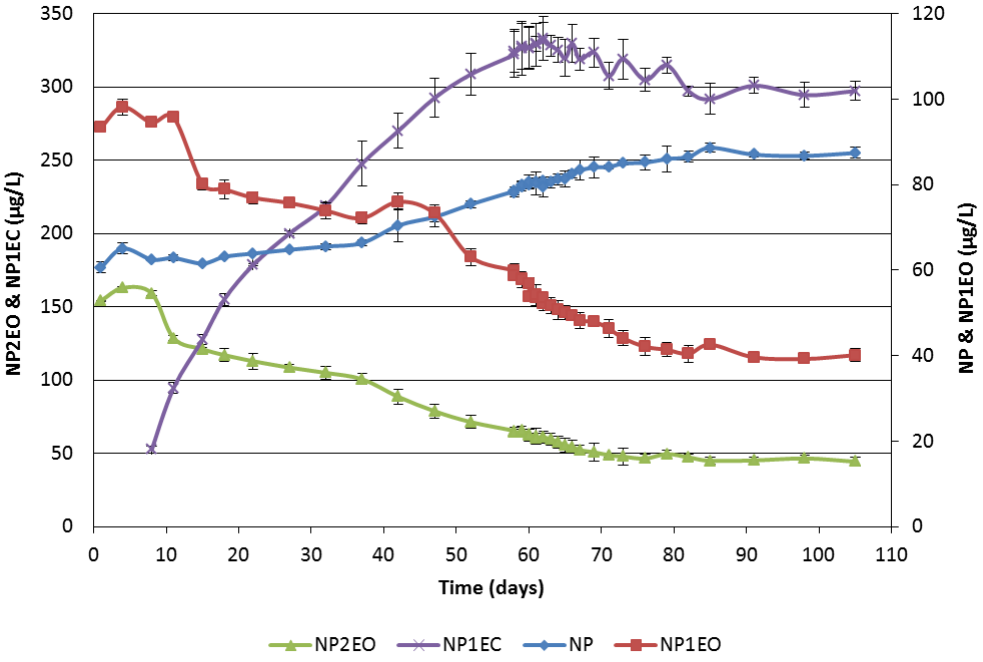


Figure 4.9: Change in NP compounds concentration (solids phase) w.r.t time for C-1 reactor

In liquid phase the trend was slightly different as in the beginning, the concentration of NP2EO in C1 increased from 24.01 to 54.27 µg/L between the 22nd to 61st day. Later it decreased gradually and went below the LOQ on the 85th day in C1. Similar to NP2EO, the concentration of NP1EO showed an increasing trend in liquid phase in the

beginning and later it dropped to 16.27 µg/L at the end of operation period (91 days). NP started to appear in liquid on the 37th day in C1. At reactor termination, NP was measured as 23.17 µg/L. NP1EC was not detected in liquid phase at all during the period on 91 days.

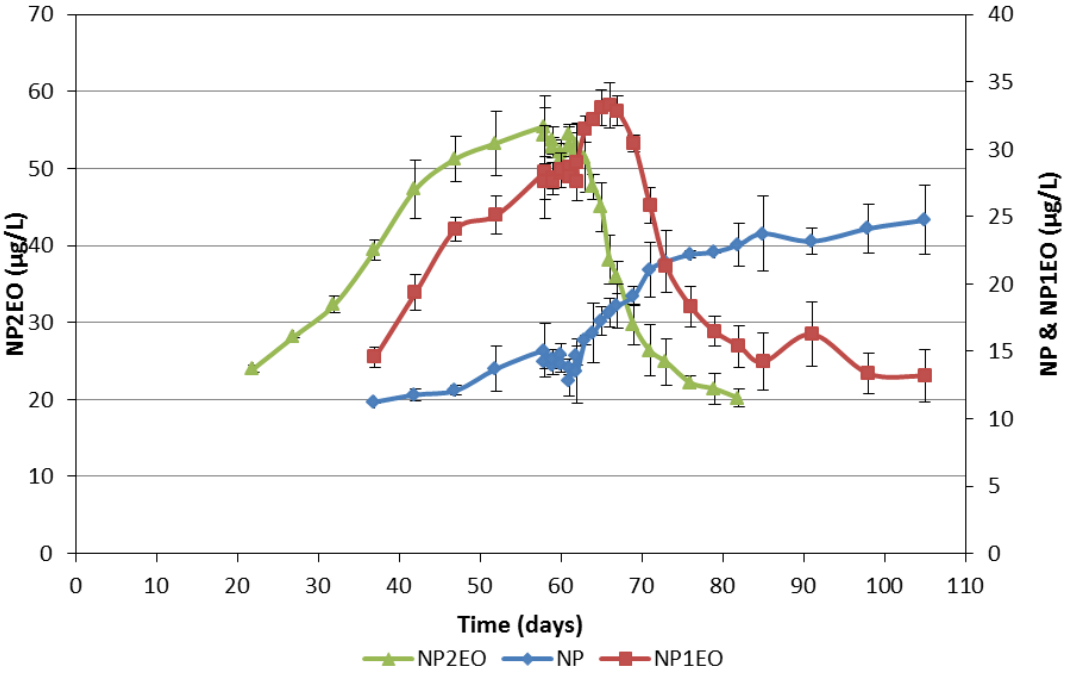


Figure 4.10: Change in NP compounds concentration (liquid phase) w.r.t time for C-1 reactor

The overall degradation pattern of C1 (Figure 4.11) involved reduction in concentration of NP2EO and NP1EO. However, NP and NP1EC got accumulated in the reactor. NP accumulation was not as high as it was in the case of NP1EC. As explained before, probable source of NP1EC production was the presence of higher chain NPnEOs (n≥2). In both phases there was a difference in concentration level. As NP compounds are hydrophobic in nature, they showed accumulation on sludge surface and major portion of their concentration was present in solid phase. In the beginning, NP compounds were not detected in liquid compounds but with the passage of time there was a transfer of these compounds from solid to liquid phase but the amount of compounds transferred was low because of their physical properties mentioned earlier.

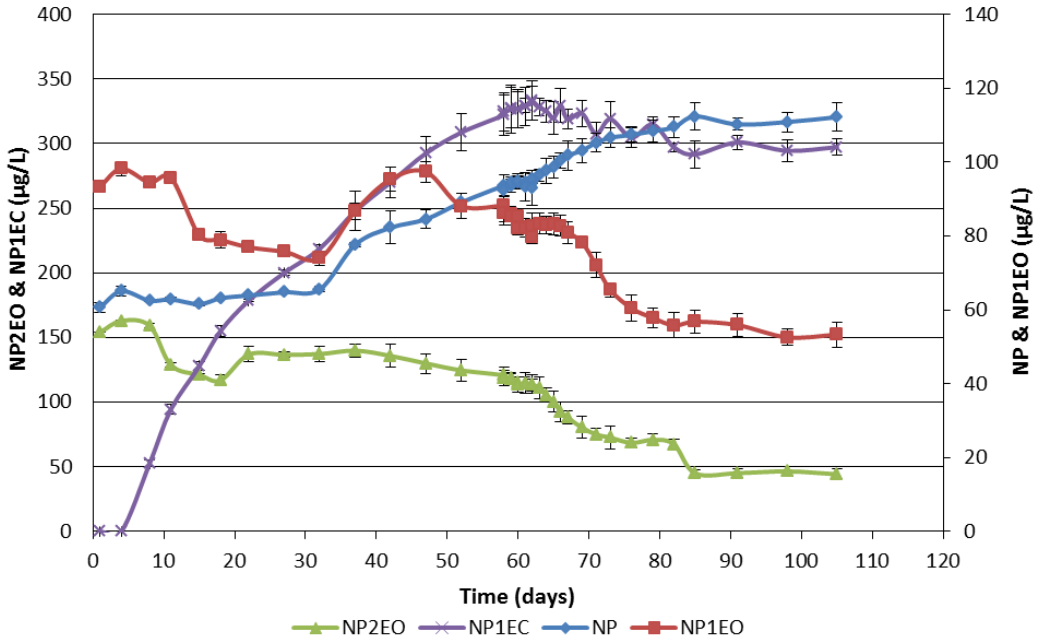


Figure 4.11: Change in NP compounds concentration (solid and liquid phases combined) w.r.t time for C-1 reactor

Each figure (4.9-4.11) displaying the concentration change of NP compounds against time contains the standard deviations for NP, NP1EO, NP2EO and NP1EC. The standard deviation values for all compounds are very low when compared to vertical axis. However, for NP1EC at higher concentrations, some relatively higher standard deviation bars were obtained compared to NP, NP1EO and NP2EO, but they were still within an acceptable range (less than 5%). In most of the cases the deviation was less than 3 % of the average. The small values of standard deviation show that data is consistent with each other and it increases the reliability of the data. The explanation for some higher values of standard deviation is that the derivatization method used for NP1EC was complicated and involved some manual work such as separation of petroleum ether from the top of water. This type of manual work led to some errors and at higher concentrations, these errors became prominent.

Figures 4.12-4.14 illustrate the change in the concentration of NP compounds in solid phase, liquid phase and their sum in C2. When the concentrations of NP compounds and the decomposition trends of C2 were analyzed, they were found to be very parallel to C1. This can be considered as the indicator confirming the success of reactor operation. Like C1, the background concentration of NP compounds in C2 was about 63.16, 95.65 and 158.46 µg/L for NP, NP1EO and NP2EO, respectively in solid phase. These values are very close to the one measured for C1. Again there was some lag period. Therefore, the degradation of NP2EO in solid phase started on the 8th day, in liquid phase it showed up on the 22nd day. The difference calculated in the concentrations of NP2EO between the readings on the 8th and 22nd days was 46.81 µg/L for C2. NP2EO exhibited a sharp decrease in its concentration and at the end of 91st day its concentration was determined as 43.98 µg/L. Similarly, NP1EO also showed a gradual degradation and the final concentration was found to be 38.15 µg/L. Moreover, NP and NP1EC accumulation was also observed in C2 reactor. The final concentrations for NP and NP1EC were measured as 87.07 and 308.56 µg/L in solid phase of C2.

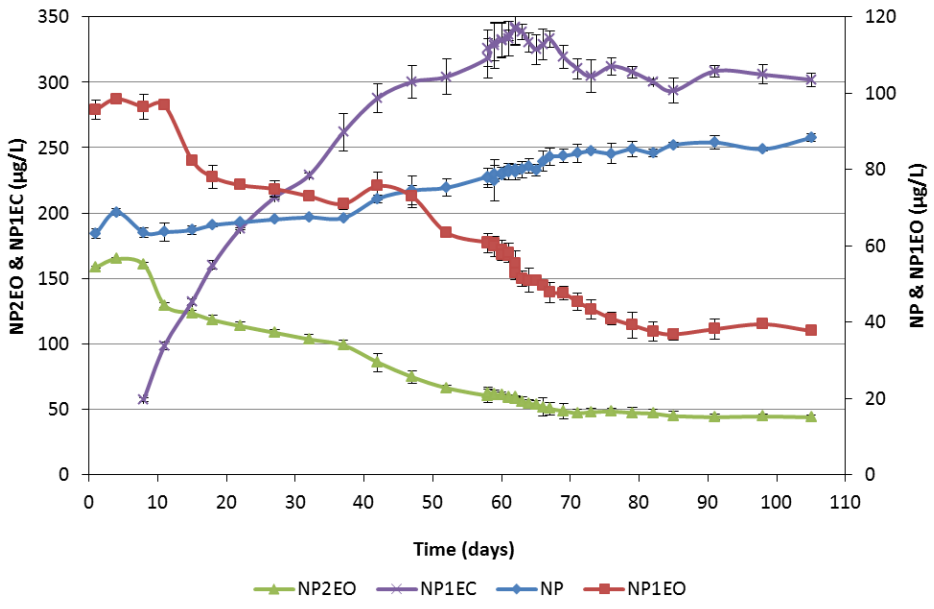


Figure 4.12: Change in NP compounds concentration (solids phase) w.r.t time for C-2 reactor

As expected, the degradation behavior of NP compounds in liquid phase of C2 was not much different than the one in C1. NP2EO showed an increase from 25.51 to 58.17 µg/L during the period during 22nd and 58th days. Later it decreased gradually and the final concentration of NP2EO was measured as 22.38 µg/L. The decomposition trend of NP1EO was also similar to C1 and its final concentration in liquid phase was found as 16.94 µg/L. NP showed an accumulation pattern like C1 and its final value at the end of 91 day period was determined as 34.68 µg/L. NP1EC was not detected in liquid phase at all during the period of 91 days.

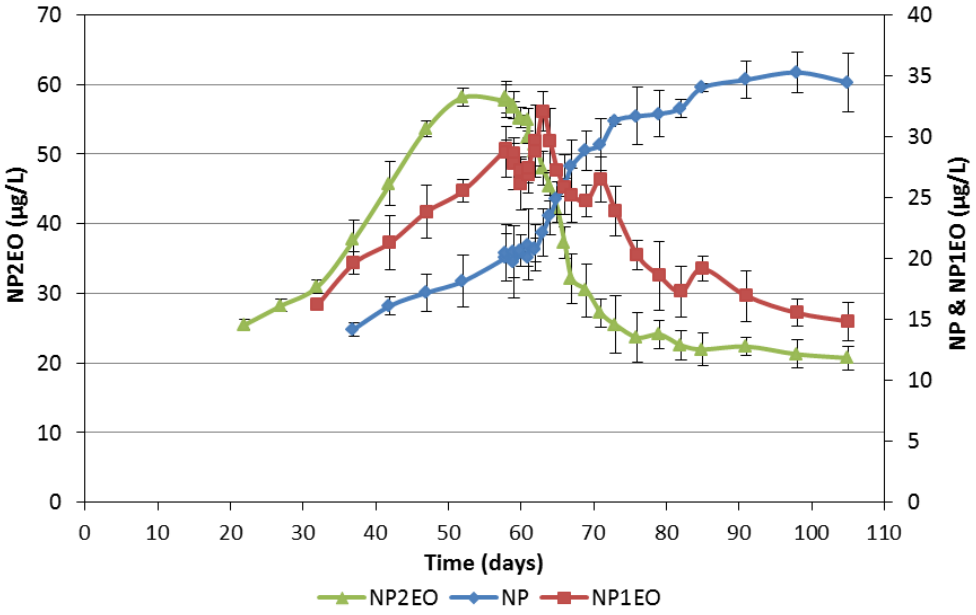


Figure 4.13: Change in NP compounds concentration (liquid phase) w.r.t time for C-2 reactor

Both reactors showed almost the same results in terms of NP compounds. There were some difference but they were minute. When the data for NP1EC and NP is compared, both reactors have shown outstanding replicate results. However, in case of NP2EO, its concentration went below the limit of quantification (20 µg/L) in C1 on the 85th day but it was 22.38 µg/L at the end of 91 days operation in C2. Similarly, concentration of NP2EO was increased in liquid phase between the 22nd to 61st day in C1. However, for C2 the decrease happened between the 22nd day to 58th day. The compatibility in the results of both reactors demonstrated the success of operation and the accuracy of the methods used for the analysis of NP compounds. Likewise,

NP1EO started appearing in liquid phase on the 37th day in C1 but it was detected on the 32nd day in liquid phase in C2. Nevertheless, both C1 and C2 were operated as control reactors and were duplicates of each other. The compatibility in the results of both reactors demonstrated the success of operation and the accuracy of the methods used for the analysis of NP compounds.

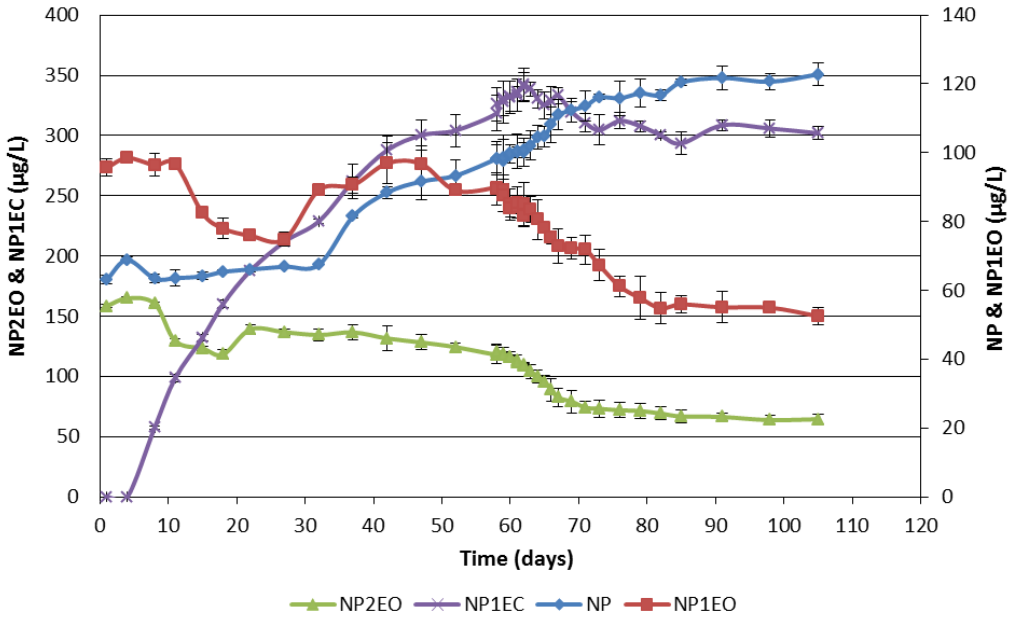


Figure 4.14: Change in NP compounds concentration (solid and liquid phases combined) w.r.t time for C-2 reactor

Figure 4.14 showed the combined solid and liquid concentrations of NP compounds for C2 and is alike with Figure 4.11 for C1, both C1 and C2 were operated as control reactors and were replicates of each other. The results of both reactors parallel with each other and established the success of operation and the accuracy of the methods used for the analysis of NP compounds.

4.2.4.3. Spiked Reactors

The degradation behaviour of NP compounds in NP2EO spiked reactor R1 is given in Figure 4.15-4.17. NP and NP1EO displayed a similar behaviour when evaluated against

the results of control reactors (C1 and C2). However, concentrations of NP at the end of the 91 days was slightly higher in solid phase which was result of NP coming from degradation of spiked NP2EO. It was measured to be 103.63 µg/L.

The degradation pattern of NP2EO was parallel to the control reactors till the day of spike (58th day). Upon spiking, 3mg/L of NP2EO dissolved in acetone were added to R1 and R2. After 3 hours of NP2EO addition, a sample was taken from the reactors and NP compounds were measured to check the efficiency of spiking. NP2EO was measured as 2682.68 µg/L for R1 in solid phase (Figure 4.15). After the day of spike, frequent sampling was applied in order to monitor the expected sharp degradation of NP2EO. Later, with the passage of time, the sampling period was widened. As expected, NP2EO displayed a radical decomposition in solid phase and within 4 days 50% of NP2EO was degraded; while 90% was degraded in 12 days. Afterwards, its concentration started becoming slightly stable and on the 91st day it was measured to be 42.43 in R1.

The accumulation pattern of NP1EC in NP2EO spiked reactors was in line with control reactors till the day of spike (58th day). In solid phase, unlike control reactors, its concentration did not become stable around 300 µg/L, rather it continued to increase until the 82nd day as a result of NP2EO degradation and became constant at a concentration of around 2700-2800µg/L.

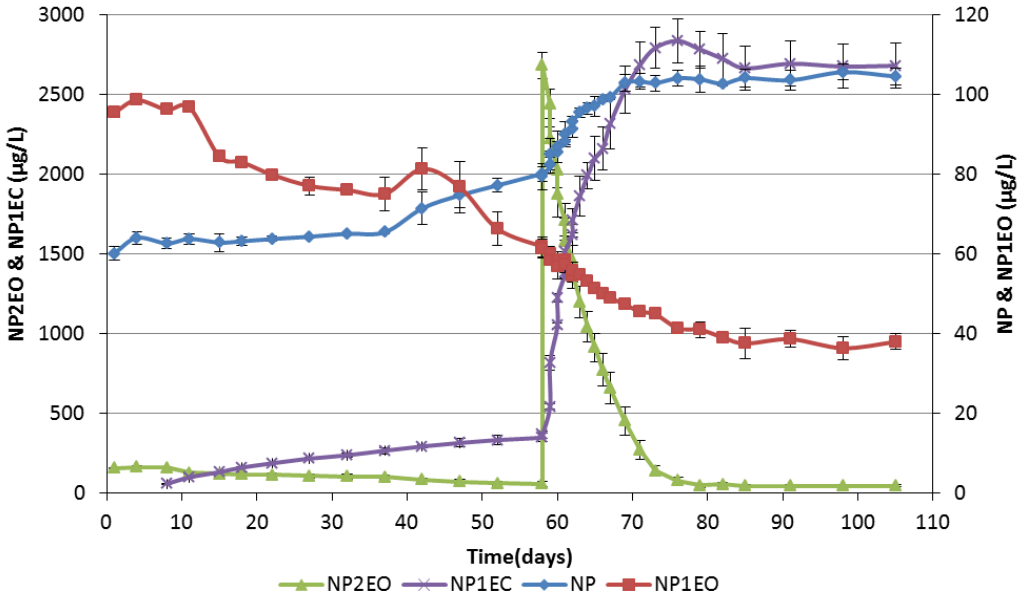


Figure 4.15: Change in NP compounds concentration (solids phase) w.r.t time for R-1 reactor

In liquid phase (Figure 4.16), an increase in the concentration of NP2EO was observed starting from the day of spike. For instance, during the first three days of spike, the concentration of NP2EO increased from 322.30µg/L to 384.75µg/L in R1. Later, a gradual decrease was observed and on the 85th day, it went below the limit of quantification in R1. NP1EC was not detected at all in control reactors. However in R1, it was not detected till the 59th day. Although NP2EO was spiked on the 58th day and it exhibited a sharp degradation trend, it took a day for NP1EC to show up in liquid phase which was a result of the degradation of NP2EO. When the degradation of NP2EO in liquid phase and the increase in the concentration of NP1EC are compared, a slight delay in the formation of NP1EC is observed. On day 91, NP1EC is found to be 154.24 µg/L in R1. NP1EO showed almost the same trend with control reactor confirming that spike has no effect on the degradation of NP1EO. Likewise, NP exhibited the same trend of accumulation but the amount of NP formed in 91 days was 46.66 µg/L, which is a bit more than the amount formed in control reactors; 24.74 and 34.46 µg/L in C1 and C2 respectively. This slight increase in NP concentration similar to solid phase was a result of degradation of spiked NP2EO.

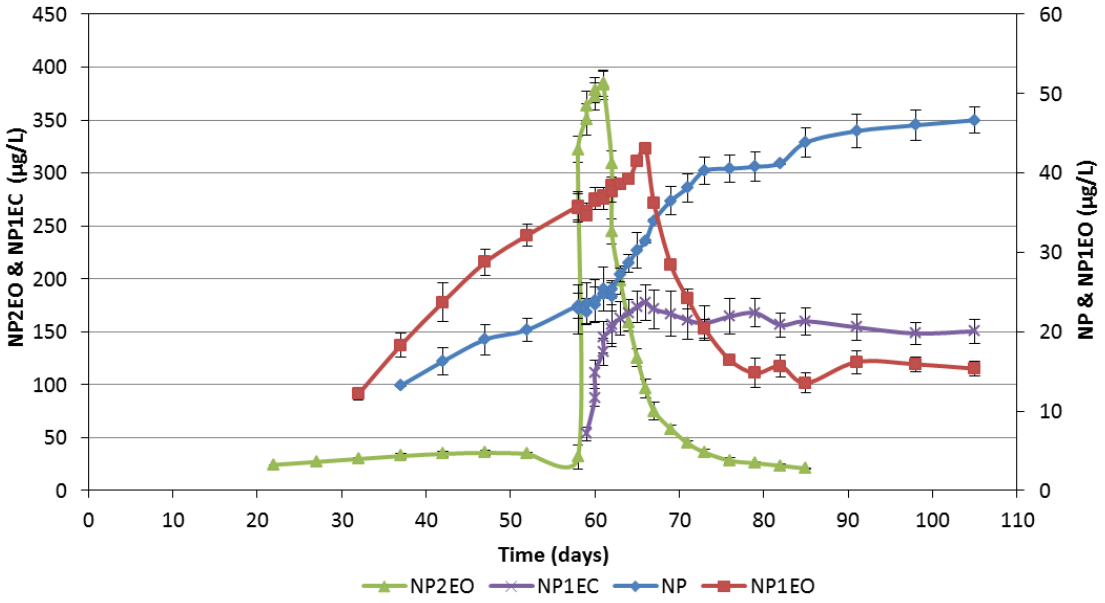


Figure 4.16: Change in NP compounds concentration (liquid phase) w.r.t time for R-1 reactor

In general, degradation pattern of R1 was similar with the control reactors. It included reduction in concentration of NP2EO and NP1EO. On the other hand, NP and NP1EC got accumulated in the reactor. The amount of NP accumulated was higher compared to the control reactors. It is suggested that spike somehow has enhanced the production of NP. Likewise, the amount of NP1EC generated in R1 as a whole was around 2,721.63 µg/L. This was the result of decomposition of NP2EO which acted as a mother compound in this case.

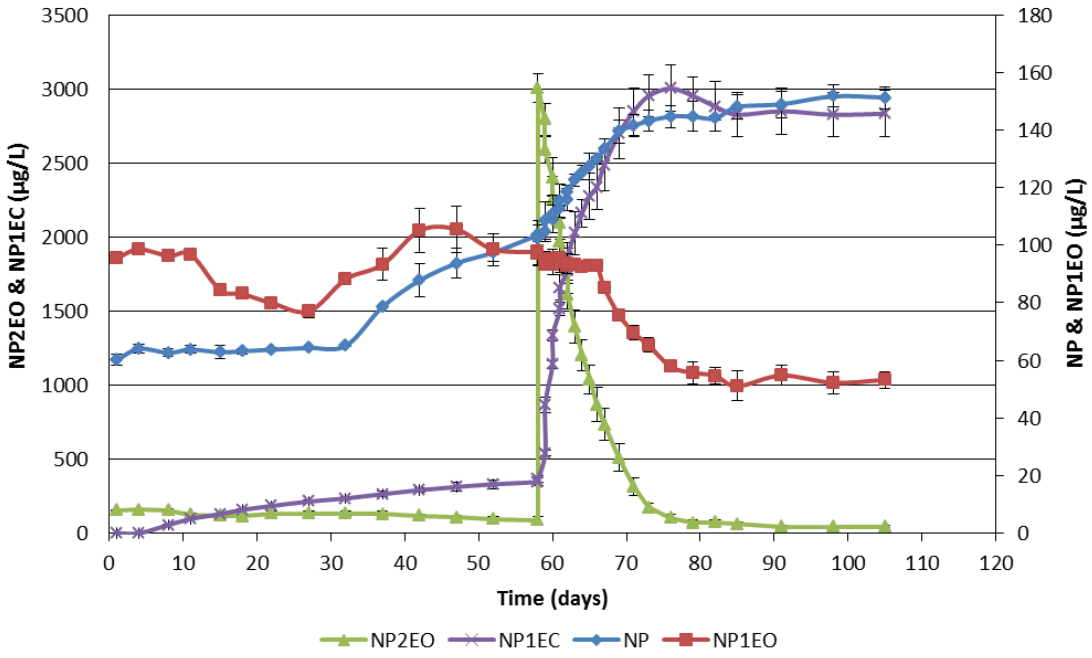


Figure 4.17: Change in NP compounds concentration (solid and liquid phases combined) w.r.t time for R-1 reactor

Figures 4.18-4.20 demonstrate the change in the concentration of NP compounds in solid phase, liquid phase and their sum in R2. In solid phase, NP, NP1EO, NP2EO and NP1EC showed almost the same behaviour with R1. At the end of reactor operation the concentrations of NP, NP1EO, NP2EO and NP1EC in solid phase were observed to be 107.44, 43.19, 36.48 and 2688.04 µg/L (Figure 4.18).

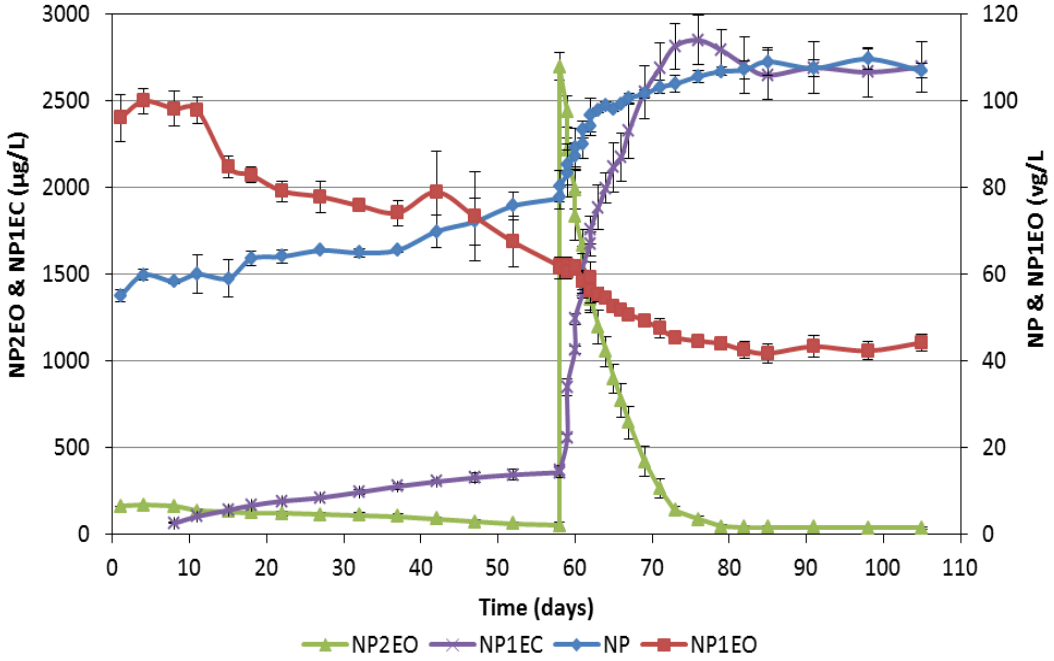


Figure 4.18: Change in NP compounds concentration (solids phase) w.r.t time for R-2 reactor

Again in liquid phase (Figure 4.19), NP, NP1EO, NP2EO and NP1EC indicated parallel behaviour for R1. At the day of termination, the concentrations of NP, NP1EO, NP2EO and NP1EC in for liquid phase were measured to be 37.58, 17.43, 36.48 and 2688.04 µg/L, respectively. Figure 4.20 used to express the combined solid and liquid concentrations of NP compounds for R2 is alike with Figure 4.17 for R1, both R1 and R2 were operated as NP2EO spiked reactors and were replicates of each other. The results of both reactors well matched with each other and confirmed the success of operation and the accuracy of the methods used for the analysis of NP compounds.

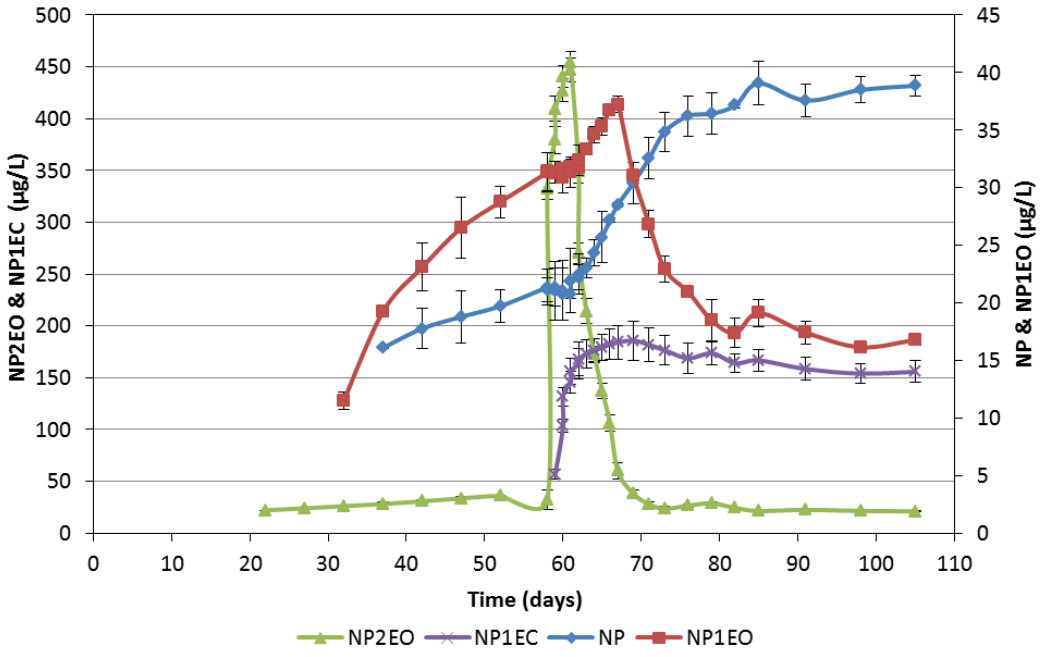


Figure 4.19: Change in NP compounds concentration (liquid phase) w.r.t time for R-2 reactor

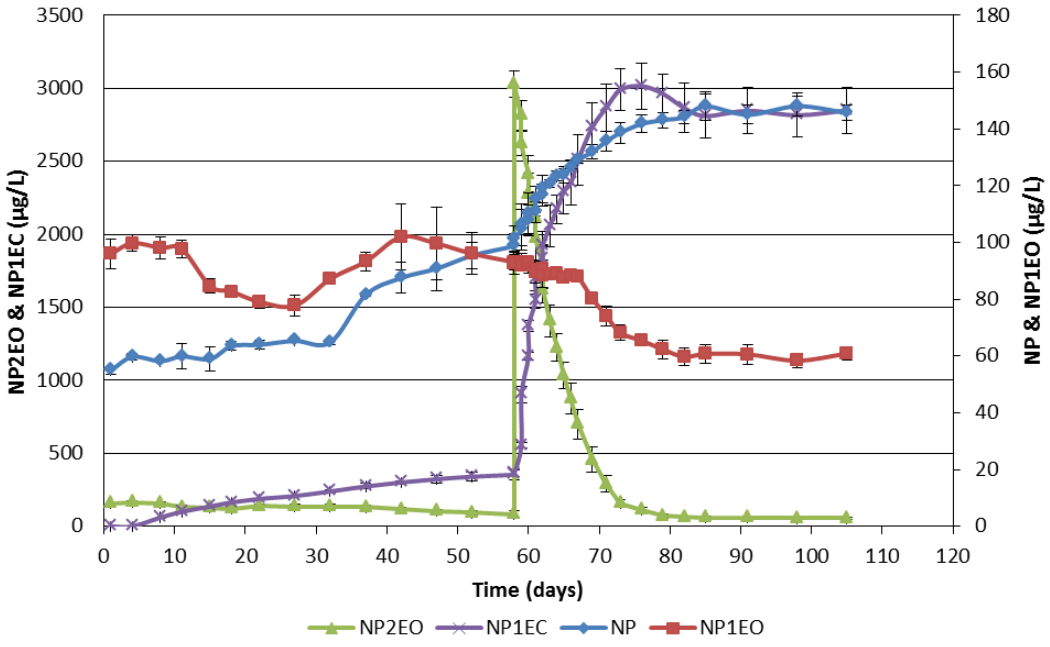


Figure 4.20: Change in NP compounds concentration (solid and liquid phases combined) w.r.t time for R-2 reactor

4.2.4.5. Mass Balance Calculations

During the experimental work, due to semi-continuous operation, a specific volume (134 mL) from each reactor was removed every day and it was replaced by the same volume of fresh feed. Therefore, to observe the impact of this addition and removal only and neglecting the generation/degradation factor, a simple mass balance calculation was applied. This calculation was based on the overall volume of 2 L of reactor. A sample iteration showing the mass balance calculations for each of the NP compounds is given below;

$\text{Mass}_{t=2} = \text{Mass}_{t=1} + \text{Mass added as a feed}_{t=1} - \text{Mass removed}_{t=1}$

$\text{Mass}_{t=3} = \text{Mass}_{t=2} + \text{Mass added as a feed}_{t=2} - \text{Mass removed}_{t=2}$

where;

Mass added as feed = (0.134 L*Concentration of Feed),

Mass removed= (0.134 L*Concentration of NP compound)

t: time in days during which reactor was operated

The results of mass balance calculations for C1 are given in Table 4.5 demonstrating the concentration change due only to the impact of feed addition and sludge withdrawal. From Table 4.5, it is quite clear that the impact of this feed alone on NP would have led to increase in its concentration from 121 µg to 124 µg over the period of 91 days. Similarly, for NP1EO it would have led to increase in its concentration from 187 µg to 210 and for NP2EO it would have led to decrease in its concentration from 308 µg to 269 µg in 91 days. The impact resulting from addition of NP and NP1EO in a feed is considered negligible as these values are not far from each other. However, for NP2EO the difference may have some significance, but the percentage for the highest difference is around 15 %. Therefore with this calculation the error in neglecting the

concentration difference caused by the feeding pattern is small and the calculations were done accordingly.

Table 4.4: Impact of feed on mass of reactor

Day	NP (µg)	NP1EO (µg)	NP2EO (µg)
1	121.11	187.02	308.49
4	120.60	187.62	309.16
8	123.20	192.43	314.26
11	125.90	197.52	318.44
15	128.37	201.17	321.14
18	127.40	199.43	320.66
22	126.83	199.39	320.79
27	131.31	206.07	326.44
32	128.59	202.76	323.40
37	125.16	198.60	319.33
42	122.41	195.46	315.89
47	123.64	194.39	309.58
52	125.48	192.64	305.47
58	126.42	191.72	299.54
58	126.17	191.98	297.89
59	125.93	192.21	296.34
59	125.70	192.43	294.90
60	125.49	192.64	293.56
60	125.30	192.83	292.30
61	125.11	193.01	291.13
61	124.94	193.17	290.04
62	124.78	193.33	289.02
62	124.64	193.47	288.07
63	124.50	193.61	287.19
64	124.37	193.73	286.36
65	124.25	193.85	285.59
66	124.14	193.96	284.87
67	124.27	194.52	286.05
69	124.50	195.51	288.18
71	124.70	196.38	290.03
73	124.88	197.14	291.65
76	124.55	196.07	289.24
79	124.28	195.20	287.29
82	124.06	194.49	285.70
85	124.14	201.14	278.95
91	124.25	210.94	269.01

As expected the mass of NP and NP1EO illustrated an increasing trend due to constant feed addition. However, NP2EO showed an increase in the beginning and then a decrease. These values are parallel with the feed mass of NP2EO which decreased after the 29th day. These values are given in Table 4.6. Hence, it can easily be noticed from Table 4.6 that the big difference in the concentration of NP2EO is not only because of dilution factor coming from feed addition but also from the low concentration of NP2EO present in the feed due to fluctuation in Ankara Tatlar wastewater treatment plant efficiency. As mentioned before the feed was obtained from Ankara Greater Municipality Central Wastewater Treatment Plant. The analyses were only conducted weekly as the sludge was brought into the lab, and not during the sludge storage in the refrigerator. Fluctuations in mass of NP2EO may be a result of a variation in plant efficiency; it can also be the routine daily fluctuation that is commonly observed for NP compounds.

Table 4.5: Mass of NP compounds in WAS used as a Feed

Day	NP (mg)	NP1EO (mg)	NP2EO (mg)
1	7.93	12.74	20.91
6	8.96	13.97	22.44
8	9.22	14.71	22.54
13	9.45	14.77	22.19
15	8.43	12.91	21.52
18	7.94	12.76	21.04
22	9.52	14.89	22.79
29	7.89	12.67	20.84
36	7.76	12.59	20.61
43	8.48	12.85	19.72
50	8.83	12.50	19.84
57	8.21	13.10	18.41
67	8.45	13.55	20.27
74	8.25	12.83	18.68
83	8.34	15.40	16.73
93	7.88	13.59	18.40

Apart from checking the impact of added and removed masses of compounds in the reactors by daily feeding and withdrawal, an analysis of percent distribution of compounds based on their molar concentrations is thought to give an idea about the generation and degradation of different compounds. Therefore a mass balance calculation was made on molar masses and is presented for all the reactors as given in Tables 4.5 – 4.8.

Table 4.6: Summary of molar percentage distribution calculations for C-1 reactor

Day	NP (mg/L)	NP1EO (mg/L)	NP2EO (mg/L)	NP1EC (mg/L)
1	24.39	31.34	44.27	0.00
4	24.70	31.12	44.18	0.00
8	21.06	26.59	38.32	14.03
11	20.29	25.83	29.68	24.20
15	19.46	21.15	27.36	32.03
18	18.82	19.63	24.92	36.63
22	17.39	17.48	26.61	38.53
27	16.86	16.45	25.42	41.27
32	16.44	15.48	24.59	43.49
37	17.39	16.22	22.40	43.99
42	17.39	16.83	20.52	45.26
47	17.24	16.57	18.93	47.27
52	17.95	14.82	17.93	49.30
*58	18.39	14.46	16.96	50.19
58	18.24	14.21	16.75	50.80
59	18.41	14.10	16.55	50.94
59	18.52	13.98	16.57	50.93
60	18.66	14.03	16.21	51.10
60	18.84	13.63	16.10	51.43
61	18.68	13.57	16.19	51.56
61	18.46	13.58	16.24	51.72
62	18.86	13.15	16.08	51.91
62	18.34	13.60	15.98	52.08
63	19.08	13.76	15.71	51.46
64	19.53	13.85	15.05	51.58
65	19.99	14.08	14.54	51.40
66	20.20	13.90	13.32	52.59
67	20.98	13.94	12.97	52.11
69	21.39	13.56	11.90	53.15
71	22.73	13.00	11.59	52.68
73	22.88	11.72	11.13	54.27
76	23.95	11.25	10.92	53.88
79	23.72	10.57	11.08	54.63
82	24.89	10.59	10.95	53.57
85	26.56	11.20	7.58	54.66
91	25.77	10.91	7.55	55.77

*day of spike

Table 4.7: Summary of molar percentage distribution calculations for C-2 reactor

Day	NP (mg/L)	NP1EO (mg/L)	NP2EO (mg/L)	NP1EC (mg/L)
1	24.65	31.16	44.18	0.00
4	25.61	30.58	43.82	0.00
8	20.85	26.46	37.68	15.01
11	20.18	25.65	29.35	24.82
15	19.68	21.12	27.03	32.16
18	19.12	19.04	24.71	37.13
22	17.50	16.77	26.31	39.42
27	16.93	15.82	24.73	42.52
32	16.09	17.75	22.90	43.25
37	17.63	16.36	21.12	44.89
42	18.00	16.47	19.12	46.42
47	18.26	16.08	18.25	47.41
52	18.76	14.93	17.86	48.46
*58	19.26	14.71	16.54	49.48
58	18.99	14.43	16.66	49.93
59	18.89	14.40	16.44	50.27
59	19.04	14.11	16.26	50.59
60	19.21	13.74	16.05	51.00
60	19.30	13.58	16.08	51.04
61	19.42	13.62	15.70	51.25
61	19.40	13.78	15.39	51.43
62	19.27	13.63	15.13	51.97
62	19.46	13.13	15.06	52.35
63	19.85	13.54	14.44	52.17
64	20.69	13.33	14.12	51.87
65	21.12	13.17	13.76	51.95
66	21.84	12.72	12.83	52.60
67	22.44	12.30	11.90	53.36
69	23.30	12.49	11.70	52.50
71	24.04	12.68	11.21	52.07
73	24.91	12.06	11.22	51.80
76	24.88	10.96	11.03	53.13
79	25.46	10.47	11.07	53.00
82	25.94	10.17	11.00	52.90
85	26.92	10.43	10.67	51.98
91	26.48	10.00	10.31	53.20

*day of spike

Table 4.8: Summary of molar percentage distribution calculations for R-1 reactor

Day	NP (mg/L)	NP1EO (mg/L)	NP2EO (mg/L)	NP1EC (mg/L)
1	24.09	31.93	43.98	0.00
4	24.58	31.59	43.84	0.00
8	20.95	26.87	37.46	14.72
11	20.62	26.13	28.59	24.66
15	19.64	22.01	26.30	32.05
18	18.67	20.48	23.93	36.92
22	17.12	17.91	25.74	39.23
27	16.38	16.38	24.02	43.21
32	15.54	17.58	22.39	44.49
37	17.15	16.99	20.23	45.64
42	17.92	17.92	17.05	47.10
47	18.57	17.45	14.90	49.08
52	19.14	16.14	13.30	51.41
*58	19.83	15.63	11.87	52.68
58	3.90	3.09	81.97	11.03
59	4.02	3.05	76.52	16.41
59	3.99	2.85	67.89	25.27
60	3.88	2.83	61.14	32.15
60	3.88	2.72	56.36	37.04
61	3.91	2.76	51.83	41.50
61	3.96	2.69	48.22	45.12
62	4.05	2.71	44.21	49.04
62	4.19	2.77	40.71	52.33
63	4.38	2.78	35.57	57.27
64	4.52	2.78	30.95	61.76
65	4.63	2.82	26.99	65.56
66	4.86	2.90	23.13	69.12
67	4.94	2.63	19.43	73.00
69	5.15	2.34	13.42	79.09
71	5.28	2.17	8.32	84.24
73	5.38	2.05	4.66	87.92
76	5.47	1.82	2.76	89.95
79	5.60	1.80	1.94	90.65
82	5.70	1.81	2.09	90.40
85	5.98	1.72	1.81	90.49
91	6.00	1.84	1.22	90.94

*day of spike

Table 4.9: Summary of molar percentage distribution calculations for R-2 reactor

Day	NP (mg/L)	NP1EO (mg/L)	NP2EO (mg/L)	NP1EC (mg/L)
1	22.16	32.31	45.53	0.00
4	22.89	31.92	45.19	0.00
8	19.26	27.10	37.54	16.10
11	18.95	25.81	30.11	25.13
15	17.90	21.47	27.65	32.98
18	18.16	19.75	24.98	37.11
22	16.87	17.40	26.33	39.40
27	16.64	16.53	24.77	42.07
32	15.31	17.19	22.58	44.93
37	17.36	16.59	19.81	46.24
42	17.69	17.21	16.81	48.29
47	17.98	16.47	14.70	50.84
52	18.61	15.65	13.03	52.70
*58	19.18	15.06	11.25	54.51
58	3.85	2.94	82.21	11.00
59	3.97	2.95	76.38	16.70
59	3.83	2.77	67.56	25.84
60	3.81	2.71	60.86	32.62
60	3.80	2.68	56.01	37.50
61	3.80	2.58	51.66	41.96
61	3.90	2.54	47.90	45.65
62	4.00	2.60	43.55	49.86
62	4.14	2.57	40.32	52.98
63	4.27	2.62	35.55	57.57
64	4.42	2.66	31.32	61.61
65	4.50	2.66	26.81	66.04
66	4.69	2.74	23.24	69.33
67	4.80	2.73	18.62	73.86
69	4.90	2.49	12.11	80.50
71	5.06	2.31	7.73	84.89
73	5.18	2.13	4.21	88.49
76	5.32	2.05	2.98	89.65
79	5.52	2.01	1.97	90.51
82	5.74	1.98	1.82	90.46
85	6.00	2.05	1.70	90.25
91	5.81	2.03	1.69	90.47

*day of spike

Molar mass calculations showed that initially all the reactors have similar molar percentages for NP compounds. For instance, NP was around 22.16-24.65%, similarly, NP1EO was between 31.16-32.31% and NP2EO varied from 43.98-45.53%. There was no NP1EC at the start-up of reactors. Molar mass percentage data clearly shows that there was almost no difference in the percentages of NP compounds among control and NP2EO spiked reactors until the day of spike. This confirms the successful operation of reactors and consistency in the data. On the day 58, R1 and R2 were spiked with 3mg/L of NP2EO. This addition changed the overall percentage distribution of NP compounds in R1 and R2. At this time, NP2EO made 80% of NP compounds in spiked reactors. In general, NP2EO and NP1EO showed degradation in all the reactors. However, NP and NP1EC showed accumulation. This degradation and accumulation patterns can clearly be seen from molar percentage calculations. After 58th day, NP2EO showed a rapid degradation, within 4 days of spike molar percentage of NP2EO dropped from 82 to 48%. This value decreased to only 1% at the end of 91st day. The fast degradation trend of NP2EO under aerobic conditions agrees well with the findings of literature (CEPA, 1999). On the other hand, while NP2EO was degrading, NP1EC was being formed. At the day of spike NP1EC contributed to 52% of molar mass but at the of reactor termination (91st day), 90% of molar mass was comprised of NP1EC. In other words, 90% of NPEs were converted to NP1EC.

By looking at molar mass percentages, it can easily be concluded that under aerobic conditions degradation is very rapid and the degradation of NPnEO results into formation of NPnECs. As regulations used by a number of countries, only consider NPE the sum of NP+NP1EO+NP2EO and they do not take NPnECs into account, this situation may pose a problem for the case of aerobic digester operating plants. Even though aerobic treatment seems that it could be a possible solution to comply with the regulation, it causes the accumulation of NP1EC. Findings such as these should be reconsidered in reviewing the current regulations.

CHAPTER 5

CONCLUSION

The study consists of two parts: Operation of NP free laboratory scale preliminary aerobic semi-continuous reactors and, operation of NP2EO spiked laboratory scale aerobic semi-continuous reactors to observe the degradation pattern and products of NP2EO.

It was observed that with the increase of solid concentrations, effectiveness of digestion improved, in other words there was an increase in the reduction of the organic matter (an important performance parameter for aerobic digesters) with the increase in the concentration of solids. This information proved to be valuable for the setup of NP2EO spiked reactors. The overflow of the reactor contents due to aeration and approaches developed to prevent it was a significant contributor of the preliminary study that helped in future reactor set up. As air was pumped into the system the formation of air bubbles indicated the need for larger head space to prevent the overflow.

The aerobic semi continuous reactors were set up spiked with NP2EO. They were set up to monitor degradation along aerobic digestion and changes in NP compounds. Expected patterns and percentages of reduction of the organic matter were seen in the parameters TS, VS, TSS and VSS. The ranges were between 40-60% for VSS removal which is parallel to the stated range in the literature and they also corresponded with the earlier set of reactors. Initially, an increase in the pH was observed; however, it became stable within a few days. At the end of the study, a slight decrease was

observed after changing the mode of operation from semi-continuous to batch. The changes observed in pH are not thought to affect the reactors' performance.

The reactors R-1 and R-2 were spiked with NP2EO on the 58th day of operation. The spike (3 mg/L) was considered successful as the amount added corresponded to the amount measured. Great majority of NP2EO and its products were found in the solid fraction, whereas they dissolved in water in very small amounts. Molar mass percentage calculations were used to observe the generation and degradation behavior of NP compounds. Following the spike, NP2EO showed a rapid degradation which was confirmed by molar mass calculation. With the passage of time it became steady. At the start, no NP1EC was detected in any reactor. Afterwards, NP1EC was detected and its concentration increased gradually. NP1EO also showed a steady degradation. However, the results showed that NP accumulated in the all reactors and its accumulation was enhanced in spike reactors. The formation of metabolites based on molar calculation showed that NP1EC contributed to 90% of molar mass of all NP compounds, showing that NPEs (NP+NP1EO and NP2EO) can be degraded up to 90% using aerobic digestion. The degradation rate corresponded with the information in the literature on the subject of aerobic studies, reflecting upon the success of the analysis and operation.

Aerobic digesters can be considered a good option to comply with the regulatory values because NPnECs are produced and the carboxylated forms are not a part of the NPE (the sum of NP, NP1EO and NP2EO) calculations that are used for regulations. In anaerobic system NP2EO degrades to produce NP1EO and NP hence overall concentration of NPEs is not decreased. For NP compounds removal, best option may be to use a combination of aerobic and anaerobic systems. Additionally, the regulations should consider the accumulation of NP1EC formation and accumulation in aerobic digesters.

CHAPTER 6

FUTURE RECOMMENDATIONS

In the literature available, rare information is present about the degradation process of NPnEOs especially higher chain NPnEOs (n>2). Similarly, not much data is available for NPnECs either. As a new area of research the degradation mechanism for NPnEOs or NPnECs can be taken into account. Therefore, the development of extraction methods for the analysis of higher NPnEOs and NPnECs, to fully understand the degradation mechanism of NP compounds opens another gate for future research.

In the literature, very limited work has been done on the effect of temperature upon degradation of NP compounds. In this study, reactors were operated under mesophilic conditions. The impact of higher temperature using thermophilic conditions can be an interesting aspect for the future research.

There is limited information in the literature on the degradation of NP in aerobic system which needs further attention. In this study NP degradation was not observed. However, it should be looked in detail, because NP is the most toxic metabolite among a number of different NP compounds and hence there is a need to find ways which can degrade this compound.

Summing up, comparing aerobic and anaerobic digestion the former results in a decrease of NP content and generates oxidized products. For smaller systems aerobic digestion is a potential solution which can be used for land application of sludge to

fulfill NPE limits of governments. A combination of stabilization techniques with aerobic digestion as pretreatment can be studied in a larger setup.

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APPENDIX A

Calibration Curves for Nonyphenol Compounds

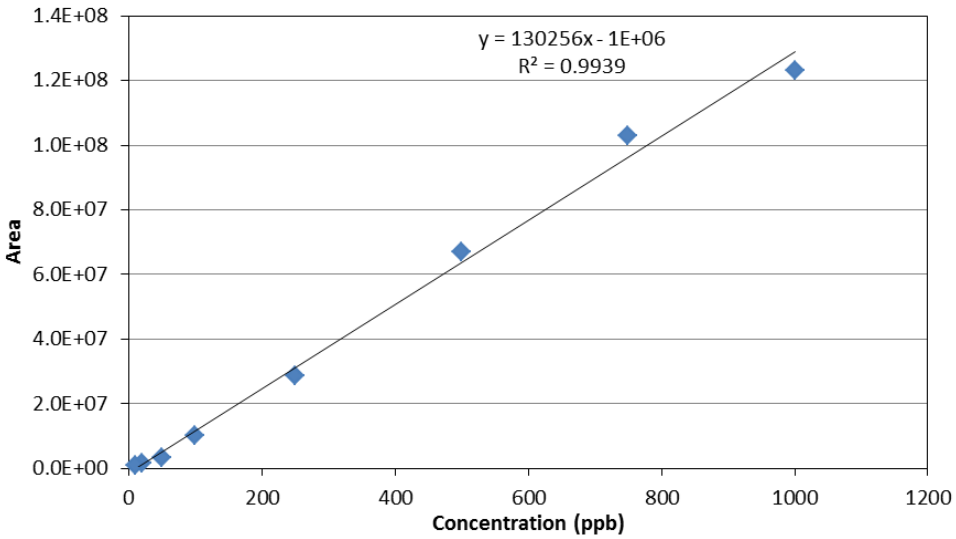

Figure A.1: Calibration Curve for NP2EO

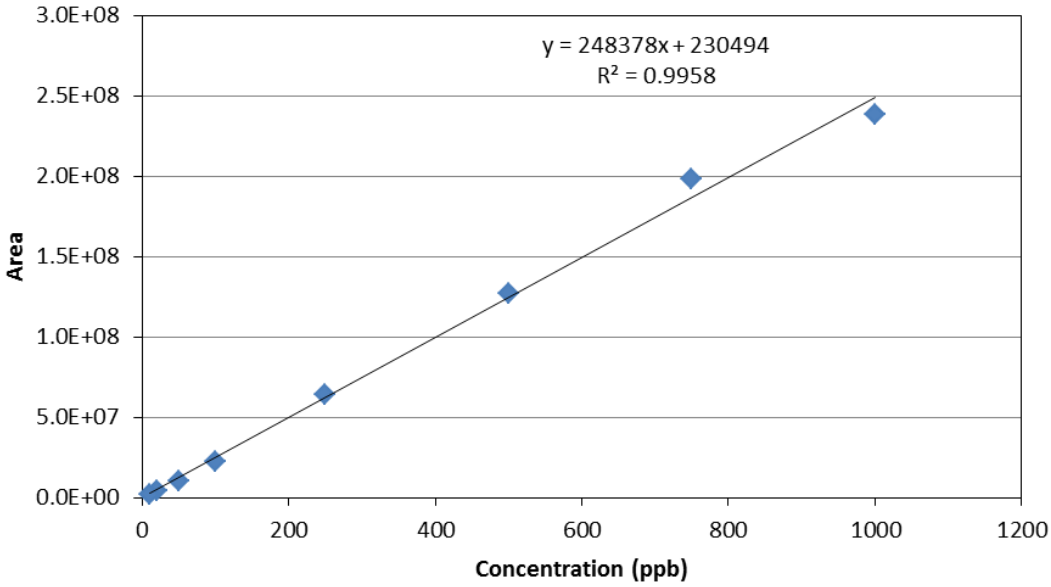

Figure A.2: Calibration Curve for NP1EO

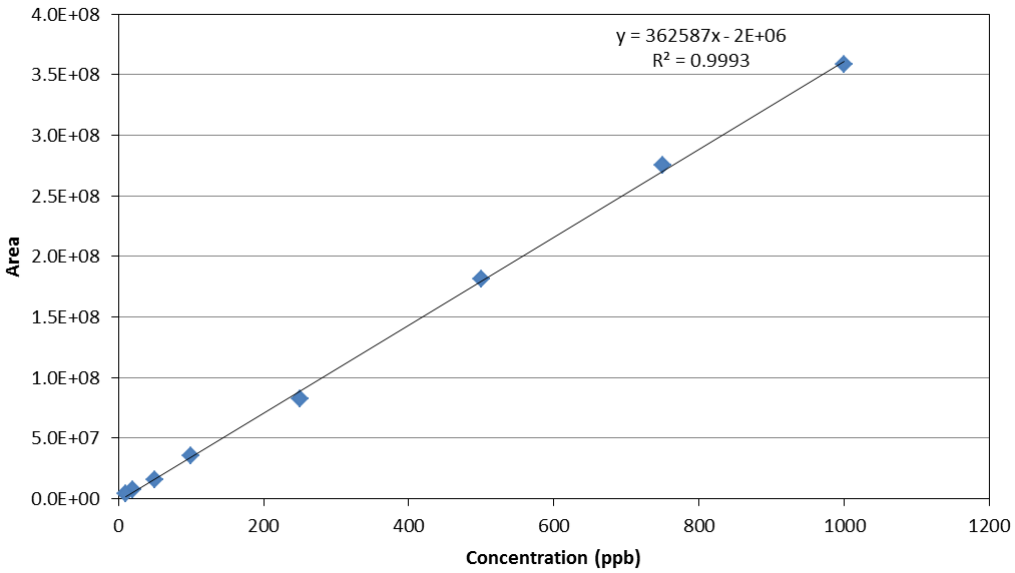

Figure A.3: Calibration Curve for NP

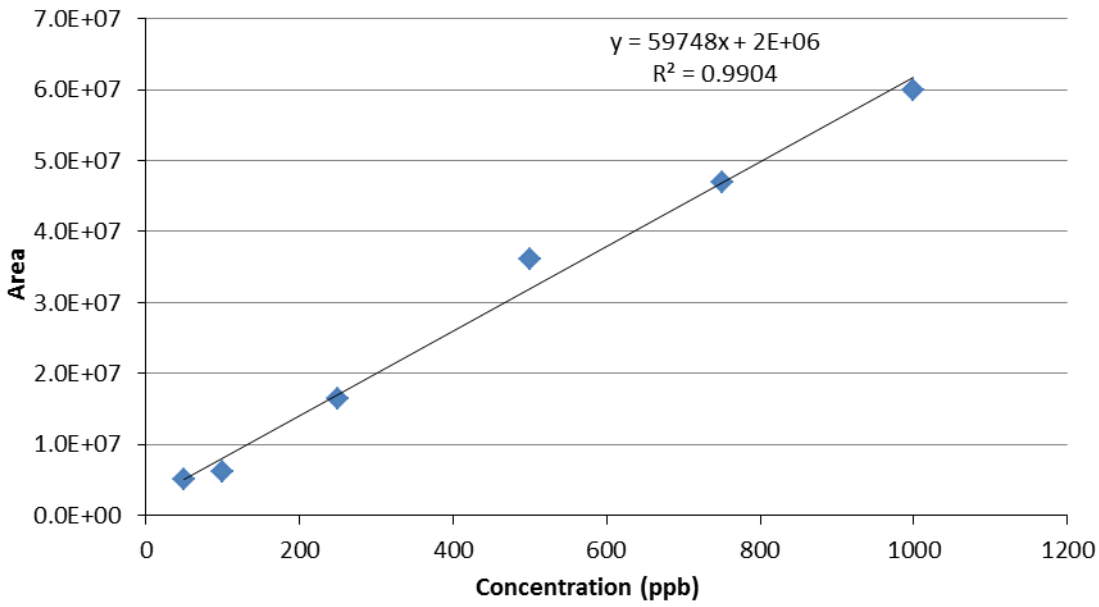

Figure A.4: Calibration Curve for NP1EC