TREATABILITY AND TOXICITY OF NONYLPHENOL COMPOUNDS IN ANAEROBIC BATCH REACTORS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

HANDE BOZKURT

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINEERING

DECEMBER 2011

Approval of the thesis:

TREATABILITY AND TOXICITY OF NONYLPHENOL COMPOUNDS IN ANAEROBIC BATCH REACTORS

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

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ABSTRACT

TREATABILITY AND TOXICITY OF NONYLPHENOL COMPOUNDS IN ANAEROBIC BATCH REACTORS

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December 2011, 135 pages

Nonylphenol (NP) and its ethoxylates are used in formulation of pesticides and detergents, production of personal care products and many industrial sectors such as textile, metal plating, plastic, paper and energy. They are also used in the formulation of household cleaning agents. Industrial uses in the production line make up 55% of the total use; whereas industrial and domestic cleaning processes constitute 30 and 15%, respectively.

Since they are widely used in industry and households, NP compounds enter the environment mainly by industrial and municipal wastewater treatment plant effluents. NP is considered strongly toxic and has adverse effects even with short term exposures. Moreover, with its similarities to natural hormones, NP and its ethoxylates are considered as endocrine disrupter compounds. In studies conducted with human cells, chicken embryo, trout and mice eostrogen receptors, positive responses were observed. Due to their lipophilic and hydrophobic characteristics they accumulate in cells, tissues and organic materials such as sludge. For these reasons, fate of NP and its

ethoxylates in wastewater treatment plants and in sludge treatment processes gained importance. Nonylphenol polyethoxylates (NPnEO) are degraded in microbial media and lose their ethoxylates to nonylphenol diethoxylate (NP2EO), nonylphenol monoethoxylate (NP1EO) and NP. Moreover, nonyl phenoxycarboxylic acids (NPnEC) can be formed during some of these reactions. Because the first degradation reactions are fast, concerns and studies are focused mainly on NP2EO, NP1EO, NP, NP1EC and NP2EC. Even though these general degradation information is available, studies on sludge are very rare.

In this study, treatability and toxicity of NP2EO in anaerobic batch reactors is investigated. First, with the use of Anaerobic Toxicity Assay (ATA) test, toxic doses of NP2EO which was added to the reactor as the parent component, were determined. Moreover, the degradation of these chemicals were studied in larger scale batch anaerobic digesters. The aim of this part was to observe the degradation patterns and products. Throughout the study the fate of NP and its ethoxylates was followed in aqueous and solid phases by the use of Gas Chromatography / Mass Spectrometry system (GC/MS).

ATA tests showed that NP2EO was not toxic to anaerobic microorganisms at the doses investigated in this study. It was rather stimulating and caused an increase in methane production in the reactors. On the other hand the spiked NP2EO's at 0.5 and 2.5 mg/L concentration were completely degraded in the larger scale batch reactors. At the same time, an increase in the concentrations of NP and NP1EO was observed which supported the fact that NP2EO was degraded into NP1EO and NP under anaerobic conditions. Abiotic degradation was not observed.

Keywords: Anaerobic stabilization, GC/MS, Nonylphenol, Nonylphenol diethoxylate, Nonylphenol monoethoxylate, sludge

ÖZ

NONİLFENOL BİLEŞİKLERİNİN KESİKLİ ANAEROBİK REAKTÖRLERDE ARITILABİLİRLİĞİ VE TOKSİSİTESİNİN TAKİBİ

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Aralık 2011, 135 sayfa

Nonilfenol ve etoksilatları pestisit ve deterjanların formülasyonlarında, kişisel bakım ürünlerinin üretiminde ve tekstil, metal kaplama, plastik, kağıt ve enerji gibi bir çok endüstriyel sektörde kullanılmaktadır. Bunlar aynı zamanda evsel temizlik ürünlerinin içeriğinde de mevcuttur. Nonilfenol bileşiklerinin %55'i endüstriyel amaçlı proseslerde kullanılırken, endüstriyel ve evsel temizlik işlemleri de kullanımın sırası ile %30 ve %15'ini oluşturmaktadır.

Nonilfenol ve etoksilatlarının endüstri ve evlerde geniş kullanımı, bu maddelerin çevresel ortamlara iki temel yolla geçişini mümkün kılmaktadır. Bunlar, endüstriyel ve evsel atıksu arıtma tesislerinin deşarjlarıdır. Nonilfenol yüksek derecede toksiktir ve kısa süreli temaslar sonucunda bile çeşitli sağlık sorunlarına yol açabilmektedir. Bunların ötesinde, doğal hormonlara benzerlikleri ile nonilfenol ve etoksilatları endokrin bozucu maddeler olarak sınıflandırılmaktadırlar. İnsan hücresi, tavuk embriyosu, alabalık ve fare östrojen hücreleri ile yapılan testlerde bu hücrelerin nonilfenol bileşiklerine pozitif yanıt verdikleri görülmüştür. Lipofilik ve hidrofobik karakterleri ile

nonilfenol bileşikleri, hücre ve dokular ile arıtma çamurları gibi organik yapılı maddeler üzerinde birikmektedirler. Tüm bu sebeplerden ötürü, nonilfenol ve etoksilatlarının atıksu arıtma tesisleri ve özellikle çamur arıtım ünitelerindeki akıbetlerinin araştırılması önem kazanmıştır. Nonilfenol etoksilatlar, mikrobiyal ortamlarda etoksilatlarını kaybederler ve NP2EO, NP1EO ve NP'ye indirgenirler. Ayrıca bazı dönüşümler sırasında nonilfenoksi karboksilik asitler meydana çıkar. İlk dönüşüm reaksiyonları hızlı olduğundan, araştırmalar genellikle NP2EO, NP1EO, NP, NP1EC ve NP2EC üzerinde yoğunlaşmıştır. Genel olarak parçalanma yolları bilinse bile arıtma çamurları ile yapılan çalışmalar son derece sınırlıdır.

Gerçekleştirilen bu çalışmada, NP2EO'nun anaerobik kesikli reaktörlerde arıtılabilirliği ve toksisitesi incelenmiştir. Öncelikle Anaerobik Toksisite Testi (ATA) ile reaktörlere eklenen bileşik olan NP2EO'nun toksik dozlarının belirlenmesi hedeflenmiştir. Daha sonra bu kimyasalın parçalanması büyük ölçekli kesikli anaerobik reaktörlerde takip edilmiştir. Bu kısmın amacı, NP2EO'nun parçalanma yolu ve ürünlerinin incelenmesidir. Çalışma boyunca, nonilfenol ve etoksilatları Gaz Kromotografi / Kütle Spektrometri (GC/MS) sistemi ile katı ve sıvı fazlarda takip edilmiştir.

ATA testlerinden NP2EO'nun çalışılan dozlarda anaerobik mikroorganizmalara toksik olmadığı görülmüştür. Metan üretiminde ise aksine stimüle edici bir rol oynamıştır. Öte taraftan kurulan büyük ölçekli anaerobik reaktörlere 0.5 ve 2.5 mg/L olarak eklenen NP2EO, işletim sonunda tamamen parçalanmıştır. Aynı zamanda NP ve NP1EO konsantrasyonlarında ise artış gözlenmiştir. Bu da, NP2EO'nun anaerobik ortamda NP1EO ve NP'ye dönüştüğünün bir göstergesidir. Abiyotik reaktörlerde ise NP2EO parçalanması ve giderimi gözlenmemiştir.

Anahtar Kelimeler: Anaerobik stabilizasyon, Arıtma çamuru, GC/MS, Nonilfenol, Nonilfenol dietoksilat, Nonilfenol monoetoksilat

To my parents and Neroş

ACKNOWLEDGEMENTS

I would like to express my sincere gratitudes to my thesis supervisor Prof. Dr. F. Dilek Sanin for her unlimited support, valuable criticism, and endless and forever patience throughout this study.

I would also thank to committee members Prof. Dr. Celal F. Gökçay, Assoc. Prof. Dr. İpek İmamoğlu, Assist. Prof. Dr. Tuba Hande Ergüder and Dr. Selnur Uçaroğlu for their precious suggestions and contributions to this study.

Special thanks go to the project members; Seçil Ömeroğlu, Muneer Ahmed and Fadime Kara. Without your contributions and endless support, this work could not be finalized. Thank you all, for the great team spirit.

One of the biggest thanks go to Devrim Kaya for her guidance and support during this study. I would like to thank Gizem Uğurlu Turan, Pakize Çetinkaya, Mehmet Dumanoğulları and Mehmet Hamgül for their supports during laboratory analysis.

The beloved member of Z-09; Özge Can, Güray Doğan and Okan Tarık Komesli. Their presence was very important in every aspects of this study. Thank you for everything.

I would like to thank to Merve Böğürcü, Müge Erkan, Onur Yüzügüllü and Firdes Yenilmez for their friendship and contributions.

I would like to extend my gratitudes to Pınar Kılınç, Baran Hatipoğlu, Aynur Giray, Cenk Artun and Didem Ergin (from the other side of the World). You were always there when I needed.

Final part is reserved for my dear family; my mother, my father, my grandmother and little member of our family Şeker. Thank you for all your touchs to my life.

The author gratefully acknowledges the financial support from TUBITAK ÇAYDAG (Project Code: 109Y018).

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ADS:	Anaerobically digested sludge
AP:	Alkylphenol
APnEO:	Alkylphenol polyethoxylates
ATA:	Anaerobic Toxicity Assay
BCF:	Bioconcentration factor
BMP:	Biochemical Methane Potential
CEPA:	Canadian Environmental Protection Agency
COD:	Chemical oxygen demand
dm:	dry matter
EDC:	Endocrine Disrupting Chemical
F/M:	Food / Microorganisms
GC/MS:	Gas chromotograpty / Mass spectrometry
HRT:	Hydraulic retention time
LOD:	Limit of detection
LOQ:	Limit of quantification
NP:	Nonylphenol
NP1EC:	Nonylphenoxyacetic acid
NP1EO:	Nonylphenol monoethoxylate
NP2EC:	Nonylphenoxyethoxyacetic acid
NP2EO:	Nonylphenol diethoxylate
NPE:	NP+NP1EO+NP2EO
NPnEC:	Nonyl phenoxycarboxylic acids
NPnEO:	Nonylphenol polyethoxylates
OPnEO:	Octylphenol polyethoxylates
SIM:	Selective ion mode
SPE:	Solid phase extraction
SRT:	Solids retention time
TS:	Total solids
TSS:	Total suspended solids
US EPA:	United States Environmental Protection Agency
VS:	Volatile solids
VSS:	Volatile suspended solids
WAS:	Waste activated sludge

CHAPTER 1

INTRODUCTION

Non-ionic surfactants have a huge role in the world's surfactant market and alkylphenols are the most widely used one among all others. Nonylphenol (NP) compounds constitute about 80% of the alkylphenols, the rest 20% being octylphenols. Due to their surface active properties NP compounds have high commercial, industrial and domestic uses. Industrial uses exist in tannery, textile, paper, detergent and personal care product industries (CEPA,1999 and Diaz et.al.,2002). Additionally, these chemicals are being used by many of us in daily activities as deodorants, shampoos, skin care products etc. (Diaz et.al.,2002). Industrial production processes make up 55% of the total use, whereas industrial and domestic cleaning processes constitute 30% and 15%, respectively. Knowing that these chemicals are produced and consumed at significant rates, it will be correct to say that NP compounds will eventually end up in both domestic and industrial wastewater treatment plants or surface waters directly. The scientific community is concerned with their use and discharge into the environmental systems due to their structure and chemical and toxic properties. NP compounds are considered strongly toxic, carcinogenic and have adverse effects even with short term exposures (Cox, 1996). During short term exposures these chemicals result in respiratory diseases, vocal cord disorders, headache, skin and eye irritation. Moreover, the NP compounds are listed among the "endocrine disrupting chemicals (EDCs)". Endocrine disrupting substances are capable of mimicking natural hormones due to their similarity to them. As the name implies, they are capable of interfering with the endocrine system and prohibit its proper functioning. During a research on human breast cancer cell, it has been discovered that NP compounds are also a member of endocrine disrupting chemicals. With their similarity to the oestrogen hormone, NP compounds can create oestrogenic effect on the body and result in serious health problems mainly related with the reproductive system (Birkett

and Lester, 2003; Lintelmann et. al., 2003; Ying et. al., 2002; Warhaust et. al., 1995).

Once these compounds are discharged into the environment, they will climb the food chain up to humans in multiplying concentrations because of their bioaccumulation ability. Due to their physico-chemical properties such as lipophilicity and hydrophobicity, they accumulate in cells, tissues and organic materials such as sludge (McLeese et. al., 1981). For these reasons, fate of NP and its ethoxylates in wastewater treatment plants and especially in sludge treatment lines where they highly accumulate gained importance.

Nonylphenol polyethoxylates (NPnEO) are degraded in microbial media and lose their ethoxylates one at a time to NP2EO, NP1EO and NP. Moreover, nonyl phenoxycarboxylic acids (NPnEC) can be formed during some of these reactions. Because the first degradation reactions are fast, concerns and studies are focused mainly on NP2EO, NP1EO, NP, NP1EC and NP2EC. Due to the concerns about health issues, their production and consumption have been either banned or limited by European countries. Additionally, regulations concerning the use of wastewater sludge on land started to bring limit values for these chemicals. For example, European Union in its “Working Document on Sludge -3rd Draft” and Turkey in a recent regulation named “Regulation on the Use of Municipal and Urban Sludge on Land” have set the limit value for NPEs (NP+NP1EO+NP2EO) as 50 mg/kg dry solids. On the other hand, Denmark has determined this value as 10 mg/kg dry solids (Knudsen et. al., 2000). Therefore, scientifically the presence and fate of these compounds in sludge should be monitored.

The previous studies on NP compounds cover a wide range of field studies which observe the quantities of NP compounds in different environmental systems like rivers, estuaries, sea waters, sediments of water bodies as well as wastewater treatment plants being both in water and sludge samples. However, studies analyzing the fate of these compounds in laboratory scale anaerobic reactors are very limited. The findings of the studies that have been conducted up to now are given further in the related chapters.

The motivation of the study comes mainly from all the points mentioned above. To summarize; NP compounds are widely used and have severe negative effects on living organisms mostly because of their endocrine disrupting properties. They

accumulate in organic media because of their hydrophobic and lipophilic characteristics which leads to their presence in wastewater sludges in high amounts.

The limited number of studies related with the degradation of NP compounds in anaerobic reactors in the literature became the starting point for this study. Among the biodegradation pathways of NP compounds, anaerobic degradation takes an important place by its ability to degrade all the NPnEOs to the final product of NP. Purpose of this study is therefore, to investigate the toxicity and treatability of NP2EO in batch anaerobic sludge digesters with the ultimate goal of observing the degradation products of NP2EO over the reactor operation period in reactor solid and liquid phases. Towards this end, first by using Anaerobic Toxicity Assay (ATA) tests, the toxicity of NP2EO in the anaerobic digestion of sludge was determined. Then, 2.5 liter batch anaerobic reactors were operated and dosed with NP2EO to follow the degradation reaction.

During the reactor operations, gas production and composition in terms of methane and carbondioxide were examined. In addition, total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) concentrations were monitored for the operation period of the reactors. To be able to judge about the fate of the chemicals in the anaerobic reactors, NP2EO and its daughter products were extracted from solid and aqueous phases and were analyzed using GC/MS throughout the operation.

CHAPTER 2

LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1. Definition of Sludge

Sludge is the residual solids generated from the treatment of municipal wastewater. Sludge represents a source of material, energy and nutrients which makes it a valuable natural resource (Sanin et. al., 2011a).

The sources of sludge in a wastewater treatment plant vary with the type of processes used in the plant. In general, the main processes that produce sludge in a wastewater treatment plant can be listed as; screening, grit removal, preaeration, primary and secondary sedimentation, biological treatment and solids processing facilities (Tchobanoglous et. al., 2003).

In general, disposal of sludge poses a significant challenge to wastewater treatment operators. However, there are also some possibilities that sludge can be used beneficially. Generated sludge from wastewater treatment units can most commonly be used on lands, incinerated or landfilled. The most important constituents of sludge that make it reusable are nutrients and organics as carbon source. However, the pathogenic microorganisms inside sludge pose a threat for human health. Therefore, in order to extract the reusables from sludge and get rid of pathogenic microorganisms, sludge needs to be further treated.

One of the most important processes applied in sludge treatment line in order to produce a desired quality sludge (i.e. reduced pathogens, eliminated odors and reduced potential of putrefaction) is stabilization (Tchobanoglous et. al., 2003). The stabilization process could operate under either anaerobic or aerobic conditions.

Anaerobic stabilization, which is the most commonly used stabilization technique will be the main process to be used throughout the study, is discussed in details below in this chapter.

2.2. Anaerobic Digestion

2.2.1. Process Description

Anaerobic digestion is a natural bioconversion process in which organic material is degraded by large quantities of anaerobic microorganisms in environments with little or no oxygen (McCarty, 1964). Therefore, anaerobic digestion process is commonly used for degradation of organic wastes which results in biogas production. The generated biogas consists mainly of methane and carbondioxide (Romano, 2008).

There are several advantages of anaerobic digestion technology as compared to aerobic digestion. Biogas production is one of the major advantages of the anaerobic digestion and it is the main reason for the promising future of this technology (Park et. al., 2010). Biogas has a value because it can be used as fuel or in electricity production (Romano, 2008). Other advantages might be listed as: high degree of waste stabilization, low production of biological sludge as waste, little nutrient requirements and no oxygen requirements (McCarty, 1964). Moreover, the digested residuals can be used as fertilizers and soil amendments (Romano, 2008).

During anaerobic digestion of complex organic compounds, first step is their hydrolysis into simpler organic compounds. Then, with the help of acidogens, they are fermented to long chain fatty acids. Volatile acids are converted to hydrogen gas and acetate by acetogens. Finally, methonegens convert these compounds to carbondioxide and methane (Speece, 1996). This process is illustrated in Figure 2-1.

Methanogens are the group of microorganisms which are mostly responsible for biological degradation process in anaerobic digestion. They grow more slowly when compared to aerobic microorganisms; therefore, in anaerobic digestion optimum environmental conditions should be adjusted for efficient and rapid treatment.

Anaerobic digestion may take place either in mesophilic range (average 35^0C) or in thermophilic range (average 55^0C). Anaerobic conditions must be maintained during the process where sufficient biological nutrients like nitrogen and phosphorus are added. Optimum pH is between 6.6 and 7.6 and no toxic materials should be present in the medium (McCarty, 1964).

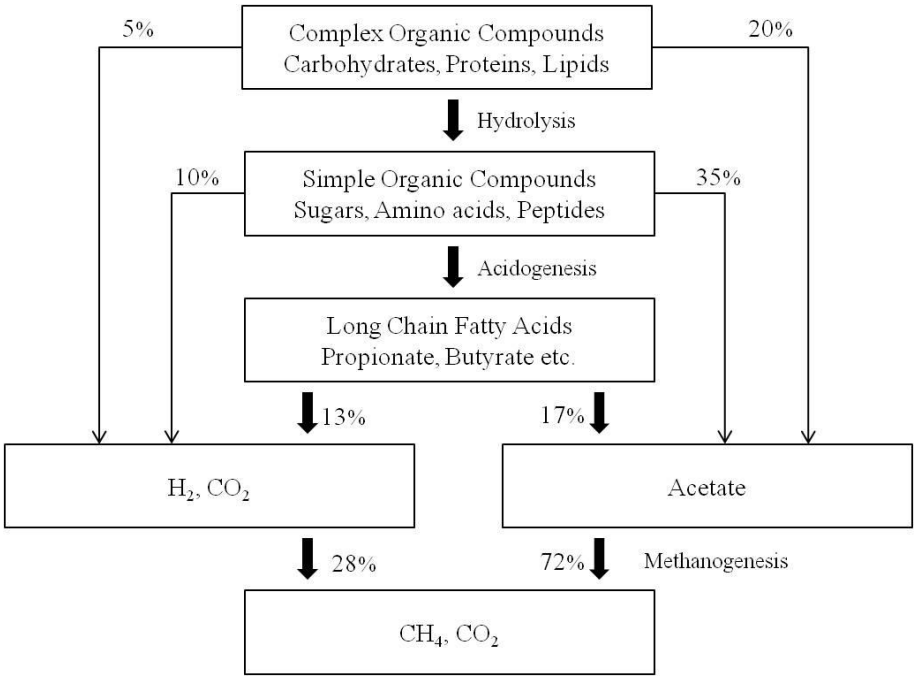


Figure 2-1: Methane formation metabolism (Speece, 1996, Parkin and Owen, 1986)

2.2.2. Anaerobic Digestion as a Sludge Stabilization Method

Stabilized sludge can be defined as the one which can be disposed of or used for any beneficial purpose with no damage to the environment or human health (Sanin et. al., 2011a). Sludge is mainly stabilized in order to reduce the pathogen content, eliminate bad odors and reduce or eliminate putrefaction. Stabilization is also used for volume reduction, improving the dewaterability of sludge and production of useful methane gas. The main methods used for stabilization of sludge are: anaerobic digestion, aerobic digestion, alkaline stabilization and composting (Tchobanoglous et. al., 2003).

In organic waste treatment, anaerobic digestion has many advantages over other processes. This is the main reason why this technology is widely used as a sludge stabilization method (McCarty, 1964). Use of anaerobic digestion for sludge stabilization purposes is an old technology; septic tanks and old Imhoff tanks are examples for the oldest anaerobic sludge stabilization techniques. Organic matter namely; primary sludge, secondary sludge or a mixture of both, are digested in anaerobic digesters and give methane and carbondioxide (Sanin et. al., 2011a).

There are some environmental factors which are important in the operation of anaerobic digesters for sludge stabilization purposes. Sufficient residence time for microorganisms to degrade volatile suspended solids in the reactor is provided by an effective reactor sizing. Solids retention time (SRT), which can be defined as the time period in which solids are present in the digester, and hydraulic retention time (HRT), the time period in which sludge is present in the digester are two important parameters of operation. The reaction steps in anaerobic digestion including hydrolysis and methanogenesis are directly influenced by SRT. Another important parameter is temperature since it has effects on metabolic activities, gas transfer and settling characteristics of biological solids. Alkalinity is a controlling parameter in digestion process. It is proportional to the characteristics of the feed stream where an optimum of 2000 to 5000 mg/L of total alkalinity is desired in a well established anaerobic digester. As mentioned in the previous section, pH, presence of toxic materials and presence of nutrients and trace metals are also important in anaerobic digestion process (Tchobanoglous et. al., 2003).

2.2.3. Biochemical Methane Potential & Anaerobic Toxicity Assay Tests

Biochemical Methane Potential (BMP) Test is applied in order to determine the methane production potential of anaerobically digested wastes. In other words, it shows the anaerobic biodegradability potential of the wastes. The test is usually the first step of anaerobic treatability studies (Owen et. al., 1978).

At 35^0C, theoretically 395 mL of methane produced is equivalent to 1 g of COD that is removed from the waste. By using this relationship, methane production obtained from BMP test can be seen as an indicator of COD removal from the liquid phase of anaerobically digested waste (Speece, 1996).

BMP Test is conducted in serum bottles by adding food and microorganisms in a determined F/M ratio. An empty volume, also named as headspace, is provided to collect the gas that is produced during anaerobic biodegradation. Food represents the waste to be biodegraded and microorganisms are supplied usually by adding anaerobic biomass. According to the type of the waste used, a media of inorganic nutrients may be added in order to maintain the suitable living conditions for microorganisms (Speece, 1996). The composition of the basal medium (BM) is shown in Table 2-1.

The bottles are purged with a gas mixture containing usually carbondioxide and nitrogen in order to remove the oxygen from the environment. Then they are incubated at incubation temperature. During the test period, gas volume and gas composition measurements are done periodically (Owen et. al., 1978).

Table 2-1: Composition of BM (Speece, 1996)

Chemical	Concentration in Reactor (mg/L)
NH_4Cl	400
$MgSO_4.7H_2O$	400
KCl	400
$Na_2S.9H_2O$	300
$CaCl_2.2H_2O$	50
$(NH_4)2HPO_4$	80
$FeCl_2.4H_2O$	40
$CoCl_2.6H_2O$	10
KI	10
$(NaPO_3)_6$	10
$MnCl_2.4H_2O$	0.5
NH_4VO_3	0.5
$CuCl_2.2H_2O$	0.5
$ZnCl_2$	0.5
$AlCl_3.6H_2O$	0.5
$NaMoO_4.2H_2O$	0.5
H_3BO_3	0.5
$NiCl_2.6H_2O$	0.5
$NaWO_4.2H_2O$	0.5
Na_2SeO_3	0.5
Cysteine	10
$NaHCO_3$	6000

Anaerobic Toxicity can be defined as the adverse effect of a substance on methanogens. Anaerobic Toxicity Assay (ATA) Test therefore, is applied to determine the toxic dose of a substance. The assay bottle preparation and operation procedure are similar to those of BMP test. The major difference is the addition of a toxic substance in five to ten different concentrations. Throughout the test, gas productions and compositions are observed; if present, the inhibition due to the addes substance is determined with the decrease in the rate of gas production with respect to an active control (Owen et. al., 1978).

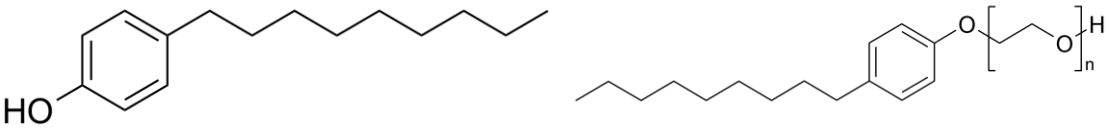
2.3. Nonylphenol Compounds

2.3.1. Definition, Sources and Uses

Alkylphenol polyethoxylates (APnEO) are important chemicals due to their surfactant characteristics. Surfactants are the agents that are soluble in water that allow spreading two liquids or a liquid and a solid; also help removing dirt and grease from solid surfaces. APnEOs are formed as a result of a reaction of alkylphenols and ethylene oxide. Nonylphenol polyethoxylates (NPnEO) and octylphenol polyethoxylates (OPnEO) are the most common commercial APnEOs. Among them, NPnEOs comprise 80% of total APnEO use (Ying et. al., 2002). NPnEOs do not have long lifetimes in the environment. With the increase in the ethoxylate chain length, biodegradability of the chemical increases. NPnEOs are biodegraded in aerobic and anaerobic environments; first into lower ethoxylated compounds, such as nonylphenol diethoxylate (NP2EO) and nonylphenol monoethoxylate (NP1EO), and finally to nonylphenol (NP). Moreover, during aerobic degradation of these compounds, nonylphenoxyacetic acid (NP1EC) and nonylphenoxyethoxyacetic acid (NP2EC) are formed. Since the first degradation reactions are fast, the majority of NPnEOs present in the environment are NP2EO, NP1EO, NP2EC, NP1EC and NP. Therefore, concerns and studies are focused mainly on these compounds (CEPA, 1999).

NP is composed of a nine carbon tail attached to a phenol ring. According to the position of the carbon tail, different positional isomers exist. Para isomer comprises

approximately 90% of industrial formulations, whereas 10% is ortho isomer. The length of ethoxylate chain differs from 1 to 100 due to their uses (CEPA, 1999). NP and NPnEO are illustrated in Figure 2-2.



(a) (b)

Figure 2-2: (a) Nonylphenol (para position), (b) Nonylphenol polyethoxylate

NP is a man-made chemical which is not produced naturally. In other words, it has no natural sources, all sources are anthropogenic. It is also classified as a xenobiotic. NP is produced by alkylation of phenol ring with nonene with the help of acidic catalysis. One of the main uses of NP is the production of NPnEOs, which are produced with the addition of ethoxylate chain to NP. NP was first synthesized in 1940, and since then the use and production of it have increased. Worldwide production of NP is approximately 500,000 tons, while 60% of this amount is finally discharged to water bodies (Ying et. al., 2002). Its production rates differ from country to country, some examples are: 154,200 tons in the USA in 2001, 73,500 tons in Europe in 2002, 16,500 tons in Japan in 2001 and 16,000 tons in China in 2004 (Soares et. al, 2008).

The most common uses of NPnEOs are in the formulations of detergents and pesticides; production of personal care products such as, moisturizers, hair dyes, shampoos and deodorants and as wetting agents, emulsifiers, solubilizers and foaming agents. They are also used in several industrial applications, namely: textile, metal plating, plastic, pulp and paper, pesticides and lube oil and fuels. Industrial applications are 55% of the total use whereas; industrial and institutional cleaning comprises 30% and household cleaning accounts for 15% (Ying et. al., 2002).

NP compounds are discharged into wastewater treatment plants with large concentrations not only after their industrial uses, but also their domestic uses.

Generally, 37% of the degradation products of NPnEOs end up in wastewater treatment plants. By taking this into account, it can be concluded that, only in USA approximately 35,000 tons of cleaning agents including NPnEOs enter the water bodies (CEPA, 1999).

2.3.2. Physico-chemical Properties

Fate of NP compounds in different environmental systems like water bodies and soil; as well as in wastewater treatment plants, is determined by their physico-chemical properties. Physico-chemical properties, on the other hand are greatly influenced by the structure of the NP compounds. Moreover, NPnEOs have different physico-chemical properties with the variation in their ethoxylate chain lengths (CEPA, 1999).

Hydrophobicity of the molecule decreases with the increase in the ethoxylate chain length, on the other hand solubility in water increases when ethoxylate number of the molecule increases. Since the hydrophobicity of the molecule increases and water solubility decreases, NP compounds with lower ethoxylates are more persistent and dangerous for living organisms (CEPA, 1999).

A listing of the physico-chemical properties of all the NPnEOs is not available in literature. However, some of them have their properties listed in different sources. In Table 2-2 some physical and chemical properties of NPnEOs with four and nine ethoxylate chain length (NP4EO and NP9EO) are listed with NP, NP1EO and NP2EO which are to be examined throughout this study.

As can be inferred from the table specific weight, viscosity and water solubility properties are dependent on the ethoxylate chain length, and increase with the increasing chain length. While NPnEOs with more than six ethoxylates are highly soluble in water, the lower ethoxylated forms have much less solubility. NP is ionized in natural water systems with its pKa value of 10.7. NP and its ethoxylated compounds are hardly found in air because of their low Henry's constant and vapor pressure values.

Table 2-2: Physico-chemical properties of NP, NP1EO, NP2EO, NP4EO and NP9EO

Property	**NP**	**NP1EO**	**NP2EO**	**NP4EO**	**NP9EO**
Synonyms	4-nonyl phenol[1] p-nonyl phenol	Nonoksinol-4[1]	Nonoksinol-4[1]	Nonoksinol-4[1]	Nonoksinol -9[1] **Tergitol** Np-9[1]
Molecular formula	$C_{15}H_{24}O$	$C_{15}H_{24}O[C_2H_4O]$	$C_{15}H_{24}O[C_2H_4O]_2$	$C_{15}H_{24}O[C_2H_4O]_4$	$C_{15}H_{24}O[C_2H_4O]_9$
Molecular weight (g/mole)	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]	56[8]	56[9]	-	-
Physical appearence	Colorless (Liquid)[7,]	Colorless (Liquid)[8]	Colorless (Liquid)[9]	Amber (Liquid)[10]	Colorless (Liquid)[1]
Specific weight	0.953[12]	0.79[8]	0.79[8]	1.020-1.030(25^{0}C)[11]	1.057 (25^{0}C)[6]
pKa	10.7[13]			-	-
Vapor pressure (Pa)	4550 at 20°C[13]	24530 at 20°C[13]	24530 at 20°C[13]	-	-
Water solubility (mg/L)	5.4[14]	3.02[14]	3.38[14]	7.65[14]	Soluble[10]
Log K_{ow}	4.2-4.48[15,16,17]	4.17[15]	4.21[15]	4.24[15]	3.59[15]
Henry's constant (m^3 atm/mole)	1.1 * 10^{-6} [18]	1.9 * 10^{-7} [18]	6.1 * 10^{-10} [18]	-	0.000 24[10] (Pa.m^3/mol)

1 *U.S. EPA (1985).*
2 *Hüls, AG (1994).*
3 *OECD (1997).*
4 *Huntsman (1999a)(given value is for NP1,5EO)*
5 *Huntsman (1998b) (given value is for NP3EO)*
6 *Weinheimer and Varineau (1998).*
7 Reed (1978)
8 *MSDS 32895*
9 *MSDS 32899*
10 *CIR (1983).*
11 *WHO (1998).*
12 *Enyeart (1967)*
13 *Romano (1991).*
14 *Ahel and Giger (1993a).*
15 *Ahel and Giger (1993b).*
16 *McLeese et. al. (1981).*
17 *World Wildlife Fund Canada (1996)*
18 *Montgomery-Brown and Reinhard (2003).*

On the other hand, NP1EC and NP2EC compounds, which are formed during aerobic degradation of NPnEOs, have high water solubilities and low hydrophobicity (CEPA, 1999).

2.3.3. Effects on Living Organisms

NP compounds are not the products of natural production processes; they are mainly produced as a result of anthropogenic activities. Their surfactant characteristics and widespread use result in their entry into different compartments of environment in a number of different ways. It is known that trace amounts of NP and NPnEOs are present in air, water, soil, sediment and biota samples. For instance in Canada, NP compounds have been found in fresh waters, sediments, wastewaters of textile, pulp and paper and fishing industries, municipal sludge and the lands that sludge is applied on (CEPA, 1999)

NPnEOs are biodegraded into lower ethoxylated compounds and finally to NP in natural systems and wastewater treatment plants. Their degradation continues after their discharge into the environment. Studies reveal that, after their discharge into water bodies, 49-59% of them are present in water, 41-50% are present in soil and only 1% is present in air. On the other hand, after their discharge into soil, 99% of them remain on soil (CEPA, 1999).

Decrease in the ethoxylate chain length results in an increase in the toxicity of the chemical. Similarly, NPnECs' acute toxicity is lower as compared to NP itself (CEPA, 1999). NP is classified as strongly toxic and is known to have negative effects even with short term exposures. NP causes severe irritations if swallowed, inhaled or absorbed through skin by human. Moreover, high concentrations cause destructions in the upper respiratory system, eyes and skin (Cox, 1996). NP also poses a threat to wildlife. Acute toxicity values for fish, invertebrates and algae are 17-3000 µg/L, 20-3000 µg/L and 27-2500 µg/L respectively. Another study reveals that, 3.4 µg/g of NP in soil results in decrease in the reproductive rates of earthworm. Generally, NP compounds are much more toxic to aquatic species than terrestrial species (Norris, 2006). NP had impacts on growth and nitrification capacity of Azobacter with concentrations ranging from 18.8 to 37.6 mg/kg which is

an indication of negative effects of contaminated sludge application on lands (Soares et. al., 2008).

Besides their toxicity, NP compounds are classified as endocrine disrupting compounds by several organizations. They are also present in Priority Substances List of European Union Water Framework Directive (Gonzales et. al., 2010). Several environmental pollutants have adverse effects on the endocrine systems of animals and humans. These pollutants are mainly called endocrine disruptors. An endocrine disruptor is defined as a substance that causes adverse health effects in an organism and results in changes in endocrine function. Alkylphenols are one of the major classes of endocrine disruptors. The class includes alkylphenols (AP), alkylphenol polyethoxylates (APnEO) and their carboxylic acids (APnEC). Since NP is the main commercial AP compound used, its endocrine disrupting effect is also a matter of concern (Norris, 2006).

Lately, concerns on endocrine disrupting chemicals have grown significantly due to their potential estrogenic effects (Arditsoglou and Voutsa, 2008). Estrodiol is a hormone that characterizes female sex and it is responsible of growth and development of female sex organs (Jobling and Sumpter, 1993). NP is one of the man-made chemicals that have the ability to mimic the effects of estrodiol. The main concern is that, endocrine disrupting chemicals have several implications for human health. A dramatic example might be the fact that human sperm counts in the last fifty years have dropped significantly mainly in industrialized regions. Similarly, a two to four fold increase has been observed in testicular cancer cases in the same regions (Warhurst, 1995). Carcinogenic effect of NP compounds results mainly from their ability to mimic estrogen hormone. A study revealed that, once they have been applied on human breast cancer cell cultures, a significant increase in the number of cells was observed (Cox, 1996). It was stated by Soto et. al. (1991) that APs induce not only proliferation of human breast cancer cells but also progesterone receptor. In the studies of Jobling and Sumpter (1993), it was observed that, when AP compounds were applied on human breast cancer cells, chicken embryo, trout and estrogen receptors of rats, all the estrogen receptors responded to them. In another study, 2 mL/kg of calcium alkyl phenate of 25% concentration was dosed on the skin of male rabbits; after four weeks, sperm production of these animals stopped (Hewstone, 1994).

NP compounds are lipophilic, hydrophobic and persistent, which results in high bioaccumulation rates of these compounds. In an environment surrounded by a contaminated river, bioaccumulation of NP was observed in algae, fish and aquatic birds (Soares et. al., 2008). The bioconcentration factors (BCF), which is the ratio of concentration of the compound in the animal or plant tissue to the concentration in the water body that they live in, are found to be 280 in salmon, 10 in the mussel, 10,000 in algae and 3-1300 in ducks and fish. This shows that, when a medium is polluted with NP, partitioning of the chemical is mostly on the living organisms rather than the medium itself (Jobling and Sumpter, 1993; Cox, 1996).

NP compounds mainly accumulate on cell and tissues as well as systems with organic nature like wastewater treatment plant sludge due to their lipophilic and hydrophobic characteristics (McLeese et. al., 1981). Therefore, fate of those compounds in wastewater and sludge treatment lines gains importance.

2.3.4. Environmental Fate

In Nature

Presence and fate of NP compounds in different environmental systems like surface waters, sediments, soil and air are mainly dependent on their physicochemical properties. As stated above, NP and NPnEO's (n<5) are highly hydrophobic and tend to partition in organic media rather than water. Therefore, these chemicals, in nature, can more likely be found in soils and sediments as compared to water. On the other hand, higher ethoxylated nonylphenol compounds are relatively more hydrophilic and they can be seen in water systems in higher concentrations (Barber et. al., 1988; John et. al., 2000; Ying et. al., 2002).

In addition to domestic and industrial wastewater discharges, natural floods result in high concentrations of NP compounds in natural water systems (Ahel et. al., 1994b; Corsi et. al., 2003). In different studies from Germany and Korea, NP concentration was reported as ranging from 0.7 ng/L to 15 µg/L in water. Moreover, it was stated in Li et. al. (2004)'s study that concentrations of NP compounds vary seasonally mainly because of the increase in microbial activity in summer seasons. As a result,

NPnEOs are degraded into NP and NP concentration tends to increase in summer seasons (Bester et. al., 2001; Li et. al., 2004; Soares et. al., 2008).

Previous studies reveal different concentrations of NP compounds in natural water system. Table 2-3 summarizes the results of some of these studies from different regions of the world. It can be inferred from these studies that not only NP itself but also NPnEOs and NPnECs are present in rivers, lakes and sea water.

Table 2-3: Concentrations of NP compounds in natural water systems

Study	**Findings**
Spain, Anoia and Cardener Rivers (Sole et. al., 2000)	NP → 0 - 644 µg/L NPnEC → 0.08 -100 µg/L
Italy, Venice Lake (Marcomini et. al., 2000)	NPnEO → 1,1-38,5 µg/L NPnEC → 0,5-102 µg/L
Tokyo Rivers (Isobe et. al., 2001)	NP → 0,051-1,08 µg/L
USA, sea water with WWTP discharge (Ferguson et. al., 2001)	NP → 0,077-0,416 µg/L NP1EO → 0,056-0,326 µg/L NP2EO → 0,038-0,398 µg/L

Shang et. al. (1999) stated that, in sediments only 4% decrease after 28 days and 9% decrease after 56 days were observed in NP concentration. This approximates the half life of NP as more than sixty years in sediments. Naylor et. al (1992) has found NP concentration as 3000 µg/kg and NP1EO concentration as 170 µg/kg in sediments. In the sediments of Tokyo rivers NP concentration was found to differ from 0.5 to 13 µg/g (dry matter) (Isobe et. al., 2001).

Apart from natural water systems, NP compounds can be found in soil. Their presence in soil is mainly due to antropogenic activities; a result of sludge applications and spills. Recently, with the application of wastewater treatment sludges on soil, presence and fate of NP compounds in soil gained importance

(Soares et. al., 2008). These compounds are known to degrade to some extent in soil. In a study Marcomini et. al. (1989) found out that, when a mixture of NP, NP1EO and NP2EO was applied on soil as 4.7, 1.1 and 0.1 mg/kg respectively, after 100 days these chemicals were found in soil at concentrations of 0.5, 0.12, 0.01 mg/kg respectively. Even after 320 days the these values did not change. When these compounds age on soil, their removal becomes more difficult and their toxicity persists (Pryor et. al., 2002). When NP compounds enter into soil systems, their concentration was influenced by biodegradation, sorption and volatilization. Rate of biodegradation of NP compounds in soil is a function of bioavailability of the chemicals, depth in soil and presece of oxygen. The mobility of NP compounds in soil is very low due to its strong sorption onto soil particles. NP compounds' volatilization rates are very low; when 1 gram of NP is spiked onto soil only 0.22% of it is volatilized in 40 days (Soares et. al., 2008).

In wastewater treatment plants

Wastewater treatment processes

NP compounds are among the ingredients of household and industrial cleaning agents and personal care products. Therefore, with industrial and domestic wastewater discharges they end up in the wastewater treatment plants. When influent and effluent wastewater samples were analyzed, NPnEO concentrations tend to decrease through the treatment system. This shows that they are, to some extent, degraded during treatment processes (Brunner et. al., 1988; Di Corcia et. al., 2000). Degradation efficiency of NP compounds in wastewater treatment systems vary between 11% to 99% depending on the treatment operations and processes used. For instance, when activated carbon is used with an ozonation pre-treatment unit, removal efficiency increases up to 95% (Petrovic et. al., 2003). Concentration of NP compounds in the effluent of wastewater treatment plants vary depending on the composition of the treatment units. A treatment plant having only primary treatment units have 82% of NPnEO's (n=3-20), 12% NP1EO+NP2EO, 3% NP1EC+NP2EC and 3% NP of all the incoming NP compounds. When secondary treatment is added to the system the composition changes to 28% higher ethoxylated NPnEO's, 46% NP1EC+NP2EC, 22% NP1EO+NP2EO and 3% NP.

These results show the degradation of higher ethoxylate groups into lower ethoxylate ones during the conventional wastewater treatment processes (CEPA, 1999). It can be clearly inferred from the data that, acetic acid compounds (NP1EC and NP2EC) are produced in higher amounts when aerobic conditions of secondary treatment was added to the system, compared to the ethoxylated compounds. Since their solubility in water is higher compared to ethoxylated compounds, they are seen in water systems in higher concentrations (CEPA,1999).

In Canada, NP concentration was found to be 2.68 – 13.3 µg/L in untreated wastewater samples of textile industries (CEPA, 1999). In the wastewater samples of pulp and paper industries, NP1EO, NP2EO and NPnEO (n=3-17) concentrations were measured as <0.1 – 6.9 µg/L, <0.1 – 35.6 µg/L and 5.9 – 28.8 µg/L, respectively (Lee and Peart, 1999).

In the previous studies, it was reported that NPnEO concentration was ranging between 0 – 343 µg/L in wastewater treatment plant effluents (Ying et. al., 2002). For instance in Italy, DiCorcia and Samperi (1994) reported NP concentration as 0.7 – 4 µg/L and NPnEO concentration as 2 – 27 µg/L in wastewater treatment plant effluents. In another study, Sole et. al. (2000) reported the NP concentrations in wastewater treatment plants and the receiving water bodies of the effluent of this wastewater treatment plant as 330 µg/L and 180 µg/L respectively. It is a known fact that, NP shows intersex effect on living organisms with a concentration as low as 10 µg/L; therefore, the reported concentrations are very significant (MacKenzie et. al., 2003).

Sludge treatment processes

Wastewater treatment plant sludges are rich in terms of their organic and nutrient content. For this reason, they are commonly used as fertilizers and soil conditioners. However, since most of the pollutants in wastewater end up in sludge throughout the treatment processes, it also becomes rich in toxic and hazardous chemicals; heavy metals and organics. Therefore, concentrations of these compounds in sludge gained importance and brought a major concern about the presence of NP compounds as well.

During biological wastewater treatment, NPnEOs are degraded into more persistent, hydrophobic and estrogenic compounds (NP, NPnEOs with lower ethoxylate chain and NPnECs) (Holbrook et. al., 2002). Since these compounds are more likely to partition on organic media and they have the tendency to bioaccumulate, their existance and fate in sludges became a critical research subject (Pryor et. al., 2002). According to CEPA (1999), in most of the treatment plants with activated sludge process in wastewater line and anaerobic digestion unit in sludge treatment line in Canada, NP compounds are found on sludge with 95% being NP itself.

The findings of Santos et. al. (2007) are summarized in Table 2-4. In that study, primary, secondary and digested sludge samples were analyzed in terms of their NP, NP1EO and NP2EO contents. Digested sludge samples were taken from two different wastewater treatment plants in Cadiz (Spain), having aerobic and anaerobic digesters as sludge stabilization methods. In the treatment plant having anaerobic digester in sludge treatment line, NP1EO and NP2EO concentrations show a decreasing pattern with the degree of treatment whereas NP concentration tends to increase after anaerobic digestion. One important fact to mention is that, when NPE concentrations are considered, they are higher than the limit value of 50 mg/kg dm set by regulations.

Table 2-4: Fingings of the study Santos et. al. (2007)

	WWTP with Anaerobic Digestion (mg/kg dm)			**WWTP with Aerobic Digestion (mg/kg dm)**	
Compound	Primary	Secondary	Digested	Mixed	Digested
NP	185-777	52.9-611	816-1385	12.9-745	9.6-1041
NP1EO	342-1250	284-1129	232-640	13.8-125	20.3-106
NP2EO	39.9-829	89.4-1375	35.6-331	<LOD-102	<LOD-130
NPE	759-2319	529-2457	1083-2357	158-837	136-1278

Another field study has been done in New York by Pryor et. al. (2002). In five different wastewater treatment plants having anaerobic digestion as sludge stabilization method, the NP concentrations were found to be ranging from 1130 – 1840 mg/kg dm. These findings reveal that ADS in New York State contains significant amounts of NP.

In Spain Gonzales et. al., (2010) conducted a study with sludge samples from different sources. They include compost systems, lagoons, primary and secondary sedimentation tanks together with aerobic and anaerobic digesters. The results showed that, when NP was found to be the most dominant compound in stabilized sludges; in primary and secondary sedimentation sludges, NPnEOs were higher in concentration. All the result reported NPE concentrations higher than 50 mg/kg which is the limit value proposed by EU and declared by Turkey for NPE concentrations in the sludge to be applied on land.

In a study, Knudsen et. al. (2000), achieved to decrease the NPE concentration under 50 mg/kg (in some samples under 10 mg/kg which is the limit value declared by Denmark) by applying post aeration process after anaerobic degradation of sludge. This study reveals the posibility of application of sludge on land in terms of its conformance regarding NPE content.

In Aparicio et. al.'s study (2007), primary (settling and floatation), secondary (activated sludge) and digested and dried sludge samples (anaerobic - belt press) were collected from four wastewater treatment plants in Seville, Spain. The results are given as NPE (mg/kg) and in the range of 16.87 – 405.87 for primary, 12.44 – 334.47 for secondary and 30.93 – 1700 for digested and dried sludges for four wastewater treatment plants.

In a study conducted in England, Gibson et. al. (2005) examined two soil samples, mesophilic anaerobically digested dewatered sludge cake and soil samples that this sludge was applied on in terms of their NP content. They did not measure NP in soil samples; on the other hand, in ADS samples they reported NP 238 mg/kg where this value decreases to 1 and 4.4 for sludge amended soils. It is important that, when no NP was detected in soil samples at first, when the sludge containing NP was applied on them, NP had been measured in the same soil samples.

Abad et. al. (2005) studied wastewater treatment plant sludges of different treatment plants from Spain. The NPE concentration was reported to lie in the range of 14.3 –

3150 mg/kg dm with a median value of 286.6). In the study it was also mentioned that; composted sludge samples were the least contaminated with a median NPE value of 89.3 mg/kg dm.

A collective work regarding NPnEO and NPnEC concentrations in the sludge samples of wastewater treatment plants was conducted by Birkett and Lester (2003). The findings are given in Table 2-5.

Table 2-5: A collective study regarding NPnEO and NPnEC concentrations in sludge (Birkett and Lester, 2003)

Compound	Medium	Concentration (mg/kg)	Reference
NP	Raw Sludge	3.7	Germany (Bolz et. al., 2001)
		137 – 470	Canada (Lee and Peart, 1995)
		4.6	Germany (Jobst, 1998)
	Digested sludge	250	Taiwan (Lin et. al., 1999)
		450 – 2530	Switzerland (Giger et. al., 1984)
		638 – 326	U.K. (Sweetman, 1994)
		172	Spain (Petrovic et. al., 2001)
		80 - 120	Germany (Schnaak et. al., 1997)
NP1EO	Digested sludge	51 - 304	Canada (Lee et. al., 1997)
NP2EO	Digested sludge	4 - 118	Canada (Lee et. al., 1997)
NPnEO	Digested sludge	133	Spain (Petrovic et. al., 2001)
		9 - 169	Canada (Lee et. al., 1997)
NP1EC	Digested sludge	<0.5 - 25	Canada (Lee et. al., 1997)
NP2EC	Digested sludge	<0.5 - 38	Canada (Lee et. al., 1997)

With all these resultsit is clear that NP and lower ethoxylated compounds exist in sludge; possibly at a much higher concentrations than they exist in wastewaters. It is of prime importance to find out the methods and mechanisms with which their concentrations can be reduced.

2.3.5. Legislative Framework

Due to the aforementioned concerns and findings, some limitations and restrictions were brought in the production and use of NP compounds in industrial processes. EU passed in 2003 the Directive 2003/53/EC, which restricts the marketing and use of products and product formulations that contain more than 0.1% by mass of NPnEO or NP in Europe. This applies to many industries including textile and leather, and the directive is in force since January 2005. In the USA, an action plan (RIN 2070-ZA09) was prepared addressing NP and NPnEOs in 2010. With this plan EPA initiates both voluntary and regulatory actions to manage potential risks from NP and NPnEOs. For this purpose, the ongoing phase-out of NPnEO use in production of industrial laundry detergents would end the use of NPnEOs by 2014. Moreover, EPA intends to encourage the manufacturer of all NPnEO containing products to use NPnEO free formulations.

After they have entered into “List of Priority Substances in the Field of Water Policy” (EU Directive 2000/60/EC), limitations for NP compounds are begun to be included in regulations. Since NPnEOs with higher ethoxylate numbers are converted into NP2EO and NP1EO very fast, the limit values are given as the total of NP, NP1EO and NP2EO and they are shortly denoted as NPE. For instance in EU, with the “Working Document on Sludge -3rd Draft”, for utilization of sludge for agricultural purposes, limit value of NPE’s is proposed to be 50 mg/kg of total solids. On the other hand some countries in Europe revised the limit values; in Denmark for example, the value was decreased by 80% in the year 2000 and became 10 mg/kg of total solids. In Turkey starting from 2010, NPE’s are regulated in the “Regulation on the Use of Municipal and Urban Sludges on Land” with the limit value of 50 mg/kg of total solids, which is similar to the limit value proposed by EU. These chemicals, on the other hand, have no limit values in the USA in EPA CFR Part 503.

It is important to mention that, with the present applications in wastewater treatment plants, it is hardly possible to achieve the limit value of 50 mg/kg for NPE concentration in sludge. The previously mentioned studies show results of different field studies most of which mention NPE concentrations far greater than 50 mg/kg.

2.4. Research into the Biodegradation Mechanisms of NP Compounds

Degradation of NPnEOs is initiated with the attack of microorganisms to ethoxylate chains. With the removal of ethoxylate chains gradually from higher ethoxylated NPnEOs, lower ethoxylated NPnEOs begin to accumulate in the medium. The first degradation reactions are fast; NPnEOs with more than eight EO units are degraded in treatment plants with more than 92% efficiency. However, when the ethoxylate chain length decreases toxicity of the compound increases and in the effluents of wastewater treatment plants, degradation products like NP, NP1EO, NP2EO, NP1EC and NP2EC have been detected throughout the world (Ying et. al., 2002; Zhang et. al., 2008).

Degradation reactions occur under both aerobic and anaerobic conditions with different rates and products. For instance, during aerobic degradation, different than anaerobic degradation, NP1EC and NP2EC are formed. Moreover, aerobic degradation reactions are relatively faster than anaerobic reactions (Luppi et. al., 2007; Zhang et. al., 2008). While NP is commonly found as the ultimate biodegradation product of NPnEOs in anaerobic systems (Giger et. al., 1984; Ejlertsson et. al., 1999; Maguire, 1999); some studies show the possibility of mineralization of NP in aerobic conditions (Ekelund et. al., 1993; Topp and Starratt, 2000; Luppi et. al., 2007). Degradation pathways of APnEO's can be seen in Figure 2-3. The figure represents both aerobic and anaerobic degradation paths that higher ethoxylated alkylphenols follow in biological systems. In aerobic biodegradation pathway NPnEOs are degraded into lower ethoxylates. Moreover, NP2EC and NP1EC are formed as an intermediate product different from anaerobic pathway. Further in this chapter, findings on anaerobic biodegradation of NP compounds will be given in detail.

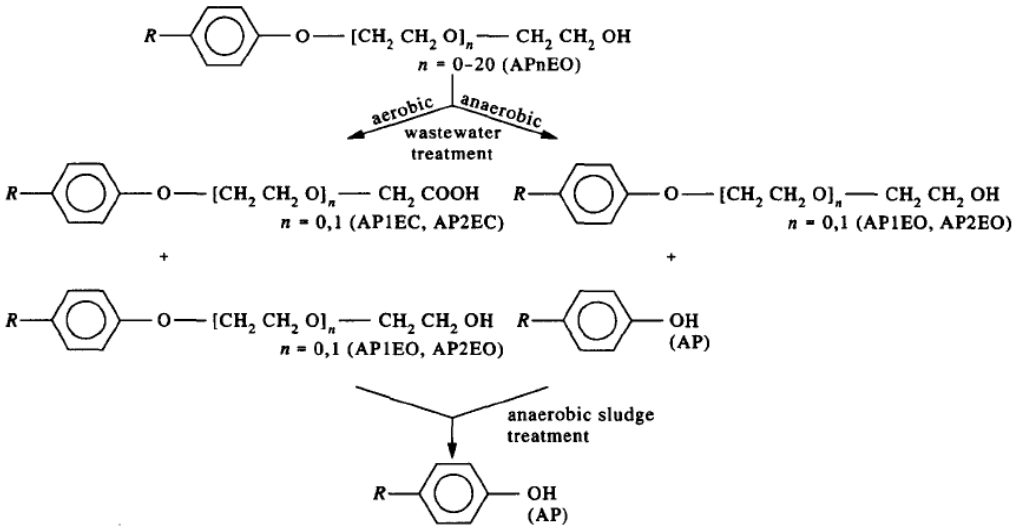


Figure 2-3: Aerobic and anaerobic biodegradation pathways of APnEOs (Ahel et. al., 1994a)

Anaerobic biodegradation of NP compounds

In anaerobic environments, biodegradation of NPnEOs are straightforward; loss of ethoxylate chains one by one leads to the formation of NP at the end of the process. Zhang et. al. (2008) has spiked 15 mg/L of the mixture of NP9EO (NPnEOs with an average EO unit of 9) into semi-continuous anaerobic reactors and observed significant accumulation of NP3EO, NP2EO, NP1EO and NP in the reactors where NPnECs were not detected. The same study concludes that, the harmful degradation products of NPnEOs can not be further removed from sludge medium which in return, brings the requirements for controling the discharge of wastewater treatment plant effluents into the environment.

In another study, 300 mg/L NP10EO mixture was spiked to anaerobic reactors and the following was observed; within 7 days after the begining of the test NP2EO had begun to dominate the system where NP1EO had taken its place after 14 days with noticable amounts of NP2EO still present in the system. At the end of 21 days, NP had bacome the main species and after 35 days the profile remained unaltered (Luppi et. al., 2007).

In Lu et. al.'s study (2008) 100 mg/L NPnEO's are spiked into anaerobic reactors together with sulfate and nitrate as electron acceptors. Since NPnEO's are highly reduced organic molecules, with the presence of a terminal electron acceptor with high redox potential in the medium, their biodegradation rate increased with sulfate and nitrate addition.

Minamiyama et. al. (2006) had conducted a study in which they have spiked NP1EO, NP1EC and NP2EC in different lab scale anaerobic digesters. NP1EO was converted to NP with 40% efficiency in 28 days. In the reactors having NP1EC at the begining, almost all of the NP1EC was converted to NP. However, the spiked NP2EC was remained at the same concentration level during operation period. Since NP2EC have to be degraded to NP1EC first and this conversion reaction only happens under aerobic reactions, NP1EC and NP formation could not be achieved in that reactors.

In a study conducted by Ejlertsson et. al. (1999), a commercial mixture of NP1EO and NP2EO (0.15% NP, 70% NP1EO, 28% NP2EO and 2% NP3EO) was spiked to 123 mL anaerobic batch reactors in three different concentrations; 2, 60 and 308 mg/L. While all three reactor sets were analyzed in terms of their gas production and compositions, 2 and 60 mg/L reactors were observed regarding the degradation of NP compounds. The separate reactors was containing anaerobic digester sludge, landfilled sludge and landfilled municipal solid waste (MSW). When the background concentrations of these three types of wastes were analyzed, MSW seemed to have the highest amount of NPE content (485 mg/kg dm). In the reactors containing 60 and 308 mg/L NP1EO and NP2EO mixture, the CH_4 productions were hampered whereas in 2 mg/L reactors it was unaffected and showed a similar path with the control reactors. Moreover, in all of the reactors the spiked NPnEOs were degraded into NP which was not further degraded.

When the literature on anaerobic biodegradation of NP compounds in lab-scale reactors was investigated, it was observed that the studies are very limited. Therefore, our study which investigates not only the toxicity but also the degradation kinetics of NP2EO will be filling the major gap in the literature.

CHAPTER 3

MATERIALS AND METHODS

3.1. Sludge Samples

Throughout the study, waste activated sludge (WAS) and anaerobically digested sludge (ADS) samples were used. The samples were supplied from Ankara Central Wastewater Treatment Plant. The plant is in operation since 1997 with a current average wastewater flow rate of 971,000 m^3/day (ASKI, 2011).

The WAS samples were taken from the return sludge line of the secondary sedimentation tanks. ADS samples, on the other hand were taken from inside anaerobic digesters. The digesters are operated in mesophilic range at 35^0C with a sludge retention time (SRT) value of 14 days (ASKI, 2011). WAS was dosed to the reactors as substrate for microorganisms, in other words it was used as food. On the other hand, ADS was dosed to the reactors as microorganisms.

Both WAS and ADS sludge samples were left to settle in the laboratory after sampling. The water phase at the top of the samples was poured out in order to increase the total solids concentrations of the samples. Different solid concentration values achieved during the study are stated throughout the text in the related parts. The sludges were dosed to reactors within three days of their sampling and they were stored in refrigerators at 4^0C prior to any kind of use.

3.2. Chemicals

The Nonylphenol compounds; Nonylphenol-solution (analytical standard for environmental analysis) OEKANAL (5µg/mL in acetone), Nonylphenol monoethoxylate-solution (analytical standard for environmental analysis) OEKANAL (5µg/mL in acetone) and Nonylphenol diethoxylate-solution (analytical standard for environmental analysis) OEKANAL (5µg/mL in acetone) were purchased from Fluka Analytical. 4-n-Nonylphenol (linear chain molecule) (10 ng/µL in Cyclohexane) was purchased from Dr. Ehrenstorfer GmbH and used as surrogate. 4-Nonylphenol-di-ethoxylate (10 mg, 99% purity) and 4-n-Nonylphenol (0.1 g, 99.3% purity) were obtained from Dr. Ehrenstorfer GmbH and used to spike the reactors.

For water extraction, Sep-Pak (Waters) Vac 6cc (500 mg) Certified tC18 Cartridges were used (Part #: 186004620).

In order to derivatize the samples before injecting into GC/MS, BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) +TMCS (trimethylchlorosilane), 99:1 (Sylon BFT) Kit was used and it was obtained from Sigma Aldrich.

All of the solvents; methanol, hexane and acetone for gas chromatography were purchased from Merck Chemical Co. Germany. The solvents used were GC grade (SupraSolv) solvents.

3.3. Experimental Setups

3.3.1. Biochemical Methane Potential Test

One of the main aims of conducting Biochemical Methane Potential (BMP) Test is to determine the methane production potential of WAS that was used throughout the reactor operating studies. Moreover, the effect of BM on gas production and organic matter degradation was observed.

WAS used in the BMP test had total suspended solids (TSS) value of 9470 mg/L and volatile suspended solids (VSS) value of 7770 mg/L; whereas, ADS had TSS

and VSS values of 31330 mg/L and 15220 mg/L respectively. To be able to test if BM is necessary to be added into sludge reactors, BM was added into one group of reactors while it was not added into the other group. The constituents of BM is shown in Table 3-1.

Table 3-1: Constituents of BM

Chemical	**Concentration in Reactor (mg/L)**	**Chemical**	**Concentration in Reactor (mg/L)**
NH_4Cl	400	KI	10
$MgSO_4.7H_2O$	400	$MnCl_2.4H_2O$	0.5
KCl	400	$CuCl_2.2H_2O$	0.5
$CaCl_2.2H_2O$	50	$ZnCl_2$	0.5
$(NH_4)_2HPO_4$	80	$AlCl_3.6H_2O$	0.5
$FeCl_2.4H_2O$	40	$NaMoO_4.2H_2O$	0.5
$CoCl_2.6H_2O$	10		

The configuration of test bottles can be seen in Table 3-2. The BMP tests were conducted in 275 mL serum bottles; constituents were prepared as 220 mL and 20 mL was taken for determination of initial conditions. Test bottles were operated with an effective volume of 200 mL and head space of 75 mL for gas storage. Four sets of reactors were operated each set having three replicate bottles. First set of reactors (Set 1) were dosed with WAS, ADS and BM while Set 2 reactors had the same constituents excluding BM. Set 3 and Set 4 reactors were operated as seed control reactors and they were not dosed with WAS. F/M ratio (g VSS WAS / g VSS ADS) was set to 0.5 for all sets of reactors.

Table 3-2: Configuration of BMP test reactors

	WAS (mL)	ADS (mL)	BM (mL)	Distilled Water (mL)	pH (average)
Set 1	84	86	50	-	7,64
Set 2	84	86	-	50	7,29
Set 3	-	86	50	84	7,84
Set 4	-	86	-	134	7,51

After the bottles were filled, they were purged with nitrogen gas for five minutes in order to remove oxygen from the system. Then, they were sealed and incubated at 35^0C for 75 days. Manual shaking of the bottles daily was the only method of mixing.

During operation, in predetermined time intervals total gas production and gas composition measurements were done. VS, VSS and pH were determined for the initial (t=0) and final (t=75) samples taken from the reactors. Since the test bottles were operated as batch systems with small volume and no sampling port, no intermediate sampling was done for these parameters during the reactor operation time period.

3.3.2. Anaerobic Toxicity Assay Test

In the second part of the study, Anaerobic Toxicity Assay (ATA) test reactors were operated. The aim of this part is to determine the dose at which NP2EO becomes toxic to anaerobic microorganisms. Since the literature mentions the higher toxicity of NP compared to NP2EO, an additional ATA test was also planned for NP. Towards this purpose, the effect of different doses of NP and NP2EO on anaerobic reactors and especially on gas production was investigated. In doing this, different doses of NP and NP2EO chemicals were added to ATA Test reactors. Three groups of ATA reactors were operated; one dosed with NP2EO and other two dosed with NP. The first NP set was dosed with a branched NP chemical, whereas the second set was dosed with a straight chain NP in order to observe their different toxicity values. One important point to mention is that; while operating ATA test reactors, the

extraction method for NP compounds from solid and aqueous phases were not yet developed. For this reason, the initial and final samples from all the bottles were kept at -18^{0}C, sealed, for several months. The details of the study are summarized throughout this section.

The reactors were filled with WAS and ADS prior to the application of chemicals. Average TS and VS values of sludge samples used are given in Table 3-3. In all the ATA test reactors operated, F/M ratio (g VS WAS / g VS ADS) was set to 0.5. The test bottles have 169 mL of total volume. They were filled with 120 mL sludge samples and 20 mL was taken for initial TS, VS, TSS, VSS and COD analyses. The reactors were operated with 100 mL effective volume and 69 mL headspace for gas collection. After filling the reactors, they were purged with N_2 gas for five minutes in order to remove the oxygen from the system. Then, the bottles were sealed to maintain the anaerobic conditions. Spike solutions of NP compounds were added to the reactors at that stage and then the bottles were incubated at 35^{0}C during operation.

Table 3-3: Sludge characteristics of ATA test reactors

ATA Sets	**WAS**		**ADS**	
	TS (mg/L)	VS (mg/L)	TS (mg/L)	VS (mg/L)
NP2EO	8807	6767	20767	9533
NP (branched)	10942	8302	23252	10222
NP (straight chain)	9140	6937	22027	11760

The configurations of ATA Test bottles dosed with NP2EO, together with initial pH values of the bottles are given in the following table (Table 3-4). A total of 10 sets of reactors were operated. For all the 10 sets, four replicates were set up initially and one bottle for each set was maintained at -18^{0}C in order to determine the initial NP2EO concentration and sets were operated with 3 replicates and total of 30 bottles. Sets 1-5 were dosed with NP2EO with the given concentrations in the table where Set 6 was operated with no NP2EO. WAS was not added to sets 7 and 10, where Set 7 was operated to determine the methane production capacity of ADS.

Set 10, on the other hand, had acetone in addition to the contents of Set 7 reactors. Set 8 reactors were similar to Set 6 reactors with the only difference that they had acetone additionally. Set 9 reactors were operated as abiotic controls in order to observe any kind of degradation of NP compounds other than biological degradation. They were dosed with 5 mg/L NP2EO; however, they were autoclaved for 20 minutes at 121^0C in order to remove all the microorganisms from the system. NP2EO spike solutions were prepared by using acetone as solvent, and they were injected into reactors in 5 mL of acetone. For this reason, some of the aforementioned reactors (Set 8 and Set 10) can be considered as acetone control reactors.

Table 3-4: ATA test reactor configurations for NP2EO

	WAS (mL)	**ADS (mL)**	**NP2EO (mg/L)**	**Acetone (mL)**	**Distilled water (mL)**	**pH (average)**
Set 1	50	70	30	5	-	7.91
Set 2	50	70	20	5	-	7.87
Set 3	50	70	10	5	-	7.85
Set 4	50	70	5	5	-	7.79
Set 5	50	70	1	5	-	7.90
Set 6	50	70	0	-	-	7.90
Set 7	-	70	-	-	50	8.25
Set 8	50	70	-	5	-	7.90
Set 9	50	70	5	5	-	7.85
Set 10	-	70	-	5	50	8.24

Table 3-5 shows the configurations of ATA Test bottles dosed with NP (branched) and initial pH values of the bottles. During the test 9 sets of reactors were operated. Four replicates were set up initially for each set and one bottle for all the 9 sets was maintained at -18^0C in order to determine the initial NP (branched) concentration and sets were operated with 3 replicates and total of 27 bottles. NP (branched) was

added to sets 1-6 with different doses ranging from 1 to 50 mg/L as shown in the table below. NP (branched) spike solutions were prepared by using acetone as solvent, and they were injected into reactors in 2 mL of acetone due to the experience gathered in the operation of NP2EO reactors. One set of reactor did not contain NP (branched) and operated as a control reactor (Set 7). WAS was not added to Set 8 so that methane production capacity of only ADS could be observed. The last set of reactors, Set 9, was operated as acetone control by containing only acetone together with WAS and ADS. No abiotic reactors were operated in this set, in the light of observations made in the previous set.

Table 3-5: ATA test reactor configurations for NP (branched)

	WAS (mL)	**ADS (mL)**	**NP (technical grade) (mg/L)**	**Acetone (mL)**	**Distilled water (mL)**	**pH (average)**
Set 1	46	74	50	2	-	7.13
Set 2	46	74	30	2	-	7.11
Set 3	46	74	20	2	-	7.12
Set 4	46	74	10	2	-	7.15
Set 5	46	74	5	2	-	7.15
Set 6	46	74	1	2	-	7.16
Set 7	46	74	0	-	-	7.17
Set 8	-	74	-	-	46	7.46
Set 9	46	74	-	2	-	7.16

The reactor configurations and pH values for the bottles dosed with NP (straight chain) chemical can be seen in Table 3-6. A total of 10 sets of reactors were operated. For all the 10 sets, four replicates were set up initially and one bottle for each set was maintained at -18^{0}C in order to determine the initial NP (straight chain) concentration and sets were operated with 3 replicates and total of 30 bottles. NP (straight chain) was dosed with ranging concentrations from 30 mg/L to 1 mg/L in acetone. Set 6 was operated as NP (straight chain) control where Set 7 was seed

control and Set 8 was acetone control reactors. Similar to NP2EO tests, Set 9 was operated as abiotic control with 5 mg/L NP (straight chain) dosage. The contents of the bottles were kept in 121^0C for 20 minutes to maintain abiotic conditions. Finally, only ADS and acetone were added to Set 10 reactors.

Table 3-6: ATA test reactor configurations for NP (straight chain)

	WAS (mL)	**ADS (mL)**	**NP (mg/L)**	**Acetone (mL)**	**Distilled water (mL)**	**pH (average)**
Set 1	55	65	30	2	-	7.61
Set 2	55	65	20	2	-	7.57
Set 3	55	65	10	2	-	7.55
Set 4	55	65	5	2	-	7.54
Set 5	55	65	1	2	-	7.59
Set 6	55	65	0	-	-	7.63
Set 7	-	65	-	-	55	7.88
Set 8	55	65	-	2	-	7.64
Set 9	55	65	5	2	-	7.59
Set 10	-	65	-	2	55	7.92

3.3.3. 2.5 L Anaerobic Batch Reactors

The third and the last reactor set that was operated during the study was 3.2 L anaerobic batch reactors with an effective volume of 2.5 liters. These set of reactors aimed at revealing the degradation patterns of NP2EO to its daughter products of NP1EO and NP together with the effect of these compounds on gas production in anaerobic systems. For this purpose, four sets of anaerobic batch reactors were operated. Each set was operated in duplicate reactors; resulting in eight reactors

alltogether. Four sets of reactors composed of two different doses of NP2EO and their abiotic control and live control reactors with acetone.

The effective volume of reactors was filled with WAS and ADS. Average TS and VS values of the sludges used are given in Table 3-7. In all the sets, F/M ratio was set to 1. The reason of adjusting this ratio to a higher value as compared to BMP and ATA test reactors is to provide the anaerobic system with as much substrate as possible; so that the microorganisms' activity would last longer giving us an opportunity to follow the system longer.

Table 3-7: Sludge characteristics of 2.5 L anaerobic batch reactors

WAS		**ADS**	
TS (mg/L)	VS (mg/L)	TS (mg/L)	VS (mg/L)
12669	9454	38764	18278

The bottles were connected to graduated gas collection cylinders of 4 liter volume (Figure 3-1). The cylinders were filled with brine solution (10% NaCl w/v, 2% H_2SO_4 v/v) so that the produced gas did not dissolve in water. The volume of produced gas was observed from the replacement of water in the cylinders.

The composition of reactors is summarized in Table 3-8. The first set of reactors (Set 1) was operated as live control reactors. To those reactors NP2EO was not dosed; only acetone was added along with WAS and ADS. Set 2 reactors were abiotic control reactors where biological activity was eliminated by applying autoclave for 20 minutes at 121^0C. Set 3 and 4 were dosed with NP2EO in different doses; 0.5 and 2.5 mg/L respectively. After filling the reactors, they were purged with pure N_2 gas for 10 minutes and then sealed and placed in hot room at 35^0C during operation period. In this period, they were continuously stirred by using magnetic stirrers. The produced gas in terms of its quantity and composition was followed all throughout the reactor operation.

Table 3-8: 2.5 liter anaerobic batch reactor configurations

	WAS (L)	ADS (L)	NP2EO (mg/L)	Acetone (mL)
Set 1	1.7	0.8	0	5
Set 2	1.7	0.8	0.5	5
Set 3	1.7	0.8	0.5	5
Set 4	1.7	0.8	2.5	5

After the reactors were filled with WAS and ADS, they were mixed for three days for acclimation of the microorganisms. At the end of third day, NP2EO and acetone spikes were done to the reactors.

In the first month of operation period, three samples of 28 mL of mixed liquor was taken from the reactors each week. During the second month, sampling frequency was decreased to two samples in a week. After that, samples were taken in every five days. The mixed liquor samples were analyzed for the following parameters: TS, VS, TSS, VSS, COD, pH and concentrations of NP compounds (NP, NP1EO and NP2EO).

Figure 3-1: 2.5 liter anaerobic batch reactors

3.4. Analytical Methods

3.4.1. Determination of Nonylphenol Compounds

In order to determine the concentration of NP compounds that are present in the reactors, Gas Chromatography/Mass Spectrometry (GC/MS) (Agilent Technologies 7890A GC system, 5975C inert MSD with triple-axis detector) equipment was used. In GC/MS, the component to be identified, first volatilizes in GC column and then moves to MS where it is identified.

One of the most important consideration in GC column is that, the component should not break down through the column with high temperatures. However, the chemicals of concern in our study readily break down before they are volatilized in GC column, which makes the analysis difficult. Therefore, with a derivatization technique, their boiling points are decreased so that their volatilization temperature was brought below the temperature that the molecules break down. Throughout the study, different derivatization methods were tested and finally silylation was chosen to be more effective (Sanin, 2011b). By converting the chemicals to their silyl ester forms, more volatile and thermally stable forms were obtained.

The derivatization procedure can be defined as follows:

- 1 mL of sample in acetone is placed in a vial
- Acetone is evaporated by using pure nitrogen gas
- 0.1 mL of BSTFA+1%TMCS is added to the same vial
- The vial is left at 70^0C for 30 minutes
- The sample is then transferred to 0.2 mL GC/MS injection vial and then injected to the equipment.

In GC/MS, the carrier gas was Helium and the column used was Agilent 19091S-433E HP-5MS 5% Phenyl Methyl Siloxane (30mx0.25mmx0.25um). The equipment was operated in splitless mode and the inlet temperature was 250^0C. An oven program was developed in our laboratory and the details of the program are given in Table 3-9.

Table 3-9: GC/MS oven program details (Sanin, 2011b)

Carrier Gas	Helium (10.152 psi)
Injection Temperature	280^0C
Injection Volume	1 µL
Injection Mode	Splitless
MS Interface Temperature	280^0C
MS Source Temperature	230^0C
MS Quadropole Temperature	150^0C
Gain Factor	2
Initial Temperature	100^0C
Oven Program	100^0C (5 min hold), 25°C/min to 160°C, 10°C/min to 260°C (5 min hold), 35°C/min to 285°C (7 min hold)
Final Temperature	285°C
Duration	30.114 min

The chosen GC/MS program was used for NP, NP1EO, NP2EO and 4-nNP. The GC/MS equipment was operated in Selective Ion Mode (SIM). Each chemical of concern have different number of isomers and for each different isomer quantification and target ions differ. In the following tables (Table 3-10 to 3-13) the quantification and target ions of four chemicals with their retention times can be seen. Moreover in Figure 3-2, a sample GC/MS chromatogram for derivatized NP, NP1EO and NP2EO is illustrated.

Table 3-10: Quantification and target ions of derivatized NP

Isomer Name	Time (min)	Quantification Ion	Target Ions
Isomer-1	12,088	193	107, 135, 150, 179, 207, 235, 277, 292
Isomer-2	12,277	207	107, 135, 150, 179, 193, 235, 277, 292
Isomer-3	12,340	207	107, 135, 150, 179, 193, 235, 277, 292
Isomer-4	12,400	207	107, 135, 150, 179, 193, 235, 277, 292
Isomer-5	12,462	235	107, 135, 150, 179, 193, 207, 277, 292
Isomer-6	12,545	207	107, 135, 150, 179, 193, 235, 277, 292
Isomer-7	12,599	235	107, 135, 150, 179, 193, 207, 277, 292
Isomer-8	12,682	207	107, 135, 150, 179, 193, 235, 277, 292
Isomer-9	12,729	207	107, 135, 150, 179, 193, 235, 277, 292
Isomer-10	12,787	207	107, 135, 150, 179, 193, 235, 277, 292

Table 3-11: Quantification and target ions of derivatized NP1EO

Isomer Name	Time (min)	Quantification Ion	Target Ions
Isomer-1	15,252	279	237, 251, 265, 293, 307
Isomer-2	15,397	251	237, 265, 279, 293, 307
Isomer-3	15,438	265	237, 251, 279, 293, 307
Isomer-4	15,490	265	237, 251, 279, 293, 307
Isomer-5	15,532	251	237, 265, 279, 293, 307
Isomer-6	15,571	265	237, 251, 279, 293, 307
Isomer-7	15,606	279	237, 251, 265, 293, 307
Isomer-8	15,648	279	237, 251, 265, 293, 307
Isomer-9	15,705	265	237, 251, 279, 293, 307
Isomer-10	15,772	279	237, 251, 265, 293, 307
Isomer-11	15,855	251	237, 265, 279, 293, 307
Isomer-12	15,960	265	237, 251, 279, 293, 307

Table 3-12: Quantification and target ions of derivatized NP2EO

Isomer Name	Time (min)	Quantification Ion	Target Ions
Isomer-1	17,565	281	295, 309, 323
Isomer-2	17,696	281	295, 309, 323
Isomer-3	17,861	323	281, 295, 309
Isomer-4	18,019	295	281, 309, 323
Isomer-5	18,051	309	281, 295, 323
Isomer-6	18,133	295	281, 309, 323
Isomer-7	18,211	309	281, 295, 323
Isomer-8	18,297	323	281, 295, 309
Isomer-9	18,366	309	281, 295, 323
Isomer-10	18,424	323	281, 295, 309
Isomer-11	18,511	295	281, 309, 323
Isomer-12	18,647	309	281, 295, 323

Table 3-13: Quantification and target ions of derivatized 4-nNP chemical

Isomer Name	Time (min)	Quantification Ion	Target Ions
Isomer-1	14,588	179	73, 292

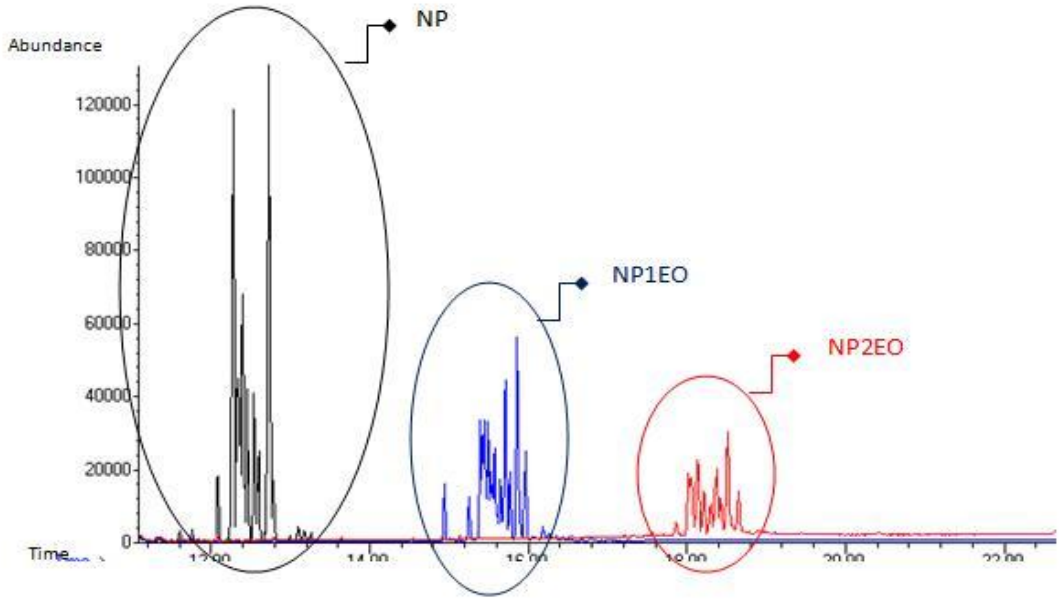


Figure 3-2: Chromatogram for derivatized NP, NP1EO and NP2EO

After determining the GC/MS oven program, derivatization process, quantification and target ions with their retention times; calibration curves were established for four chemicals. The curves were prepared by using standard solutions of concentrations ranging from 10 ppb to 1000 ppb, with eight points. Since calibration curves should be renewed with any sensitivity change of the equipment, after changing of the column and filament of our GC/MS equipment, the curves were reprepared. Example calibration curves can be seen in Appendix A. In each calibration, the curves were controled with their R^2 values and since these values are very close to 1, the curves were considered as reliable.

Limit of quantification (LOQ) and limit of detection (LOD) values were calculated by using signal to noise ratio values from GC/MS. The concentration corresponds to signal to noise ratio of 3 was taken as LOD and the concentration corresponds to signal to noise ratio value of 10 was taken as LOQ. Further in the data analysis part, the values under LOQ, was shown as 0.

The extracts were analyzed in GC/MS two times for each sample in order to have duplicate results.

3.4.2. Determination Total Gas Production and Composition

Gas Production

For all of the anaerobic reactor systems, the total volume of biogas produced was measured by water replacement devices. In gas production measurements of BMP and ATA assays, an open tube manometer system consisting of a 50 mL burette connected to a 500 mL glassware filled with water was used. By inserting a needle, which is connected to the burette, inside the assay bottles, water replacement occurs and the volume is read from the burette. In 2.5 L batch reactor systems, the reactors were directly connected to 4 L graduated cylinders. The cylinders were filled with a brine solution (10% NaCl w/v and 2% H_2SO_4 v/v) so that the produced gas did not desolve in water. As the gas was formed, the brine solution was pushed down and the displacement was measured from the graduated gas cylinder.

Gas Composition

The composition of the gas produced in anaerobic reactors was measured with a gas chromotograph (Agilent Technologies 6890N GC) equiped with a thermal conductivity detector (TCD). Carrier gas in the system is helium with an average flowrate of 29 cm/second. 30,0 m x 530 µm x 40,0 µm HP-Plot Q capillary column was used in the system. The program details are as follows; after the column temperature stays for 1 minute at 45^0C, it rises to 65^0C with 10^0C/minute ramp. In order to calibrate the equipment in every measurement day, two standard gas mixtures were used with compositions of 65%, 25%, 10% and 25%, 55%, 20% for methane, carbondioxide and nitrogen, respectively. All the injections were done manually with a 1 mL Hamilton gas syringe as 0.2 mL gas sample. In every sampling days, two injections from the same reactors were done to the GC which eventually gave us duplicate results.

3.4.3. Solids Determination

Total solids (TS) and volatile solids (VS) determinations of the sludge samples used in the study were carried out according to Standard Methods, Method 2540B and 2540E respectively.

On the other hand, total suspended solids (TSS) and volatile suspended solids (VSS) analyses were done by using Standard Methods 2540D and 2540E respectively (APHA, AWWA, WEF, 2005).

Throughout the study, all the solids analyses were done in duplicates and the average values are reported.

3.4.4. Chemical Oxygen Demand (COD) Determination

Chemical oxygen demand (COD) analyses were done for the sludge samples from the reactors. The analyses were done according to United States Environmental Protection Agency (US EPA) approved dichromate oxidation method (Jirka and

Carter, 1975). For spectrophotometric analyses, Hach DR2000 spectrometer at 620 nm wavelength was used.

The COD solution was prepared manually in the laboratory with respect to Hach Water Analysis Handbook (1989) and it was calibrated by using potassium hydrogen phthalate (KHP). KHP is known to have equivalent COD of 1.76 mg O_2/mg KHP (Hach Water Analysis Handbook, 1989). By using this information a calibration curve was obtained and it can be seen in Appendix A. Prior to analyses, homogenously mixed sludge samples from reactors were taken and diluted.

COD analyses were done by applying dilutions to the sludge samples. Moreover, from all the diluted samples two analyses were done to obtain duplicate results.

3.4.5. pH Determination

pH measurements were done according to Standard Methods, Method 4500H. The measurements were done with a pH meter of Model 510, with a pH probe (EC-PH510/21S, Eutech Instruments Pte Ltd., Spain). The calibration of pH probe was done by using standard solutions of pH values 4, 7 and 10 before measurements.

3.5. Extraction of NP Compounds from Sludge and Water

Extraction is simply defined as separation of a substance from a matrix. In order to determine the concentrations of NP compounds in our study, they were first extracted from solid and liquid phases of sludge separately and then the extracts were derivatized prior to injection into GC/MS. The phase separation was obtained by using centrifugation at 3000 rpm for 10 minutes.

Extraction from Solid Phase

In order to determine the extraction method with the highest recovery efficiency, first the methods used in the literature for similar studies were examined. The studies

listed sonication and mechanical shaking as commonly successful methods. Therefore, three methods have been tested in our study, namely; sonication, mechanical shaking and combination of these two methods. For each method, solvents and sonication and shaking times were changed in order to obtain the optimum method (Sanin, 2011b). The final extraction method from solid phase of sludge was determined to be the use of sonication procedure for 5 minutes with acetone as solvent. The details of the method can be summarized as follows;

- 1 mL of 500 ppb NP, NP1EO and NP2EO was spiked on to the already cleaned soil sample which is then placed in 12 mL extraction vials and 0.05 g of copper and 1 mL of 500 ppb 4-nNP solution as surrogate is added.
- The sample is dried with nitrogen gas.
- 10 mL acetone is added and the vials are placed in sonic bath for 5 minutes.
- In order to obtain a clear solution, the vials are placed in centrifugation at 2500 rpm for 10 minutes.
- The solvent part is passed through sodium sulfate column in order to remove moisture, and the extract is obtained.

The percent recovery values for the extraction method are given in Table 3-14. The table consists of experiment results with and without the use of 4-nNP which is the surrogate. The data in Table 3-14 shows that, for the proposed method of extraction, repeatability values are high, which made the method more promising and led to its use throughout the study. Moreover, with the increase in sonication time, a decrease in the concentration of NP2EO was observed; therefore sonication times higher than 5 minutes seemed inefficient.

Table 3-14: Extraction efficiency (% recoveries) for 5 minute sonication with acetone

	NP	NP1EO	NP2EO	4-nNP
R1	105	140.6	86.4	-
R2	106.9	142.5	86	-
R3	107.5	139	81.2	-
R4	104.3	126.7	154.6	108.9
R5	112.9	129.9	161.5	109.7
R6	107.7	127.6	145	115.3
R7	109.6	132.7	163.8	120.9
R8	103.1	125.1	155.7	108.5
R9	111.9	126.6	169.1	116.8
R10	95.7	127.6	145	108.1
Average	106.5	131.8	134.8	112.6
%RSS	4.6	4.9	26.4	4.5

Extraction recoveries in the range of 70% - 130% (US EPA, 2003) are taken to be efficient by US EPA. However, when the studies in literature are considered, this range widens up to 60% - 150% (Lian et. al., 2009; Diaz and Ventura, 2002). Since, the percent recovery values for 5 minutes sonication procedure with acetone as solvent lie in that range and their repeatability values are high; this method was chosen as our extraction method from solid phase of sludge.

Extraction studies were done by controlling the recovery results with two different blank samples. One of them did not contain any soil and NP compound and used for the determination of any contamination resulting from the solvents and equipments that are used. The other one contained soil with no spike of NP compounds onto it. This blank samples was used to determine the background concentrations of NP compounds in the soil used. In order to obtain the recovery values given above, the blank results with soil were subtracted from the exact samples.

Different from the method given above, when reactor samples were being prepared for extraction, the solid phase of the sample taken was freeze-dried for 24 hours. The extraction procedure was applied on duplicate samples and the results were analyzed in GC/MS, as mentioned above, as duplicates. As a result, for one

sampling day, for one reactor, we had four data points and their average values were reported.

Extraction from Liquid Phase

In the study, extraction of NP compounds from liquid phase of sludge was performed by Solid Phase Extraction (SPE) method by using Sep-Pak (Waters) C18 cartridges (Part #: 186004620). This method emerged as the most commonly used method upon a literature survey for the extraction of NP compounds from water. A vacuum manifold system purchased from Agilent Technologies is used in order to control the flow of sample and solvents through the cartridges. Different solvents have been used with the same cartridges and finally the most efficient one was found to be 1:1 acetone/methanol mixture. The details of the procedure are given below;

- The cartridges were placed on the vacuum manifold and conditioned by passing methanol 3 times as 4 mL, acetone 3 times as 4 mL, hexane 3 times as 4 mL and distilled water 2 times as 3 mL in the given order.
- Liquid sample is filtered through the cartridge by the help of vacuum created in the system.
- 10 mL of 1:1 acetone/methanol mixture is then passed through the cartridges and the extract is collected in vials.

The results of repeatability studies for SPE method with 1:1 acetone/methanol mixture as solvent are given in Table 3-15. Since, the percent recovery values for SPE method lied in the allowable range and their repeatability values were high; this method was chosen as our extraction method from liquid phase of sludge.

Table 3-15: Extraction efficiency (% recoveries) for SPE with 1:1 acetone/methanol mixture

	NP	NP1EO	NP2EO	4-nNP
R1	119.4	80.5	81.8	100
R2	103.7	75.8	78.9	92.6
R3	119.7	88.5	88.6	96.2
R4	95.1	89.6	94.6	108.2
Average	109.5	83.6	86	99.3
%RSS	11.1	7.9	8.2	6.7

While controlling the recovery values, one blank sample was used. In that sample, double distilled water was passed through the columns and the contamination that would result from the equipments used were determined. Most of the time, no NP compounds could be detected in this analyses. When they were detected, their values were subtracted from the main samples' data.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Biochemical Methane Potential Test

This part was conducted to quickly assess whether the use of BM is necessary in BMP tests that are conducted with sludge. BMP Test reactors have been operated for 75 days, at the end of which they were terminated. Throughout the operation period, all the reactors sets had optimum pH values. Total gas production and methane and carbondioxide productions observed during 75 days are given as the average of three replicate reactors in Figures 4-1, 4-2 and 4-3.

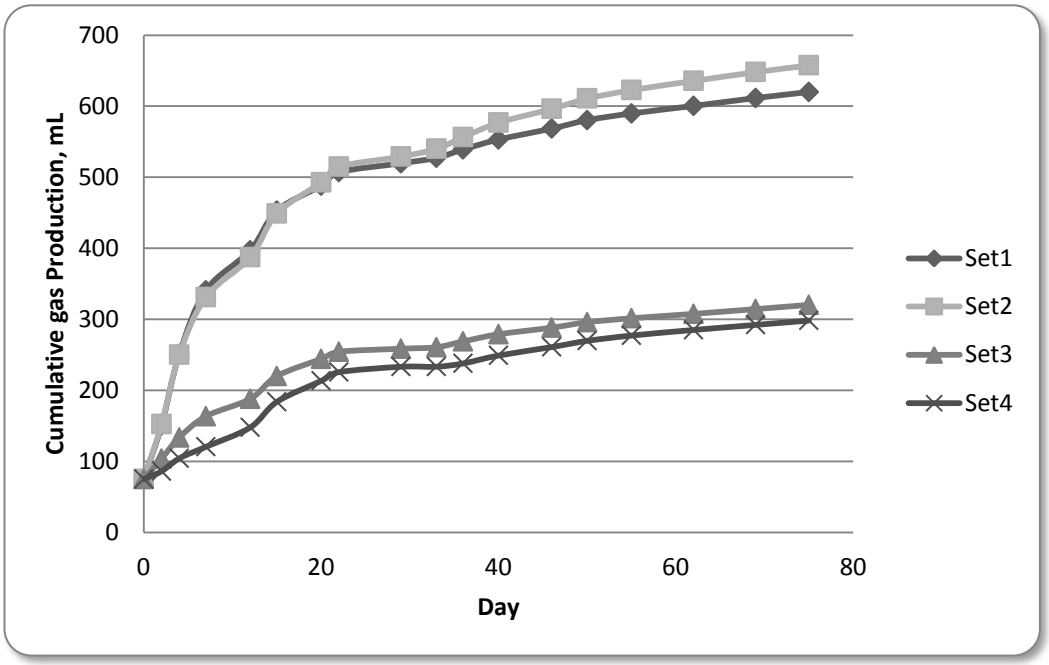


Figure 4-1: Cumulative total gas production vs. time in BMP test reactors

In Figure 4-1, cumulative total gas production is seen. Set 1 reactors, which contain WAS, ADS sludge and BM are in a parallel progress with Set 2 reactors which do not contain BM different than Set 1 reactors. On the other hand, Set 3 and Set 4 reactors, which are seed control reactors of Set 1 and 2, produced considerably less amounts of total gas when compared to the reactors containing WAS.

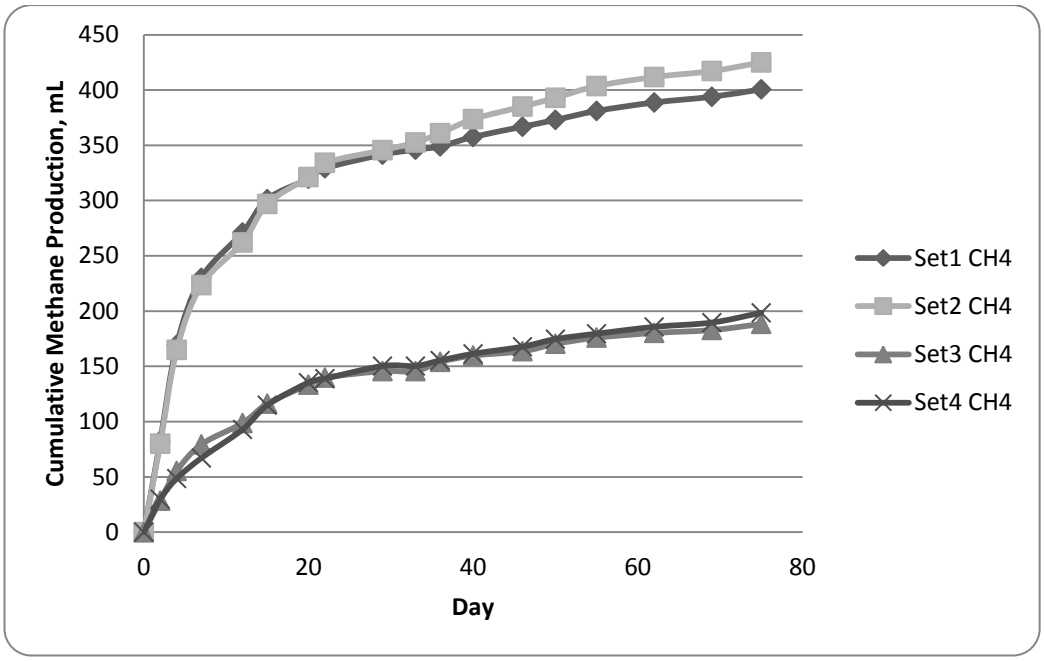


Figure 4-2:Cumulative methane production vs. time in BMP test reactors

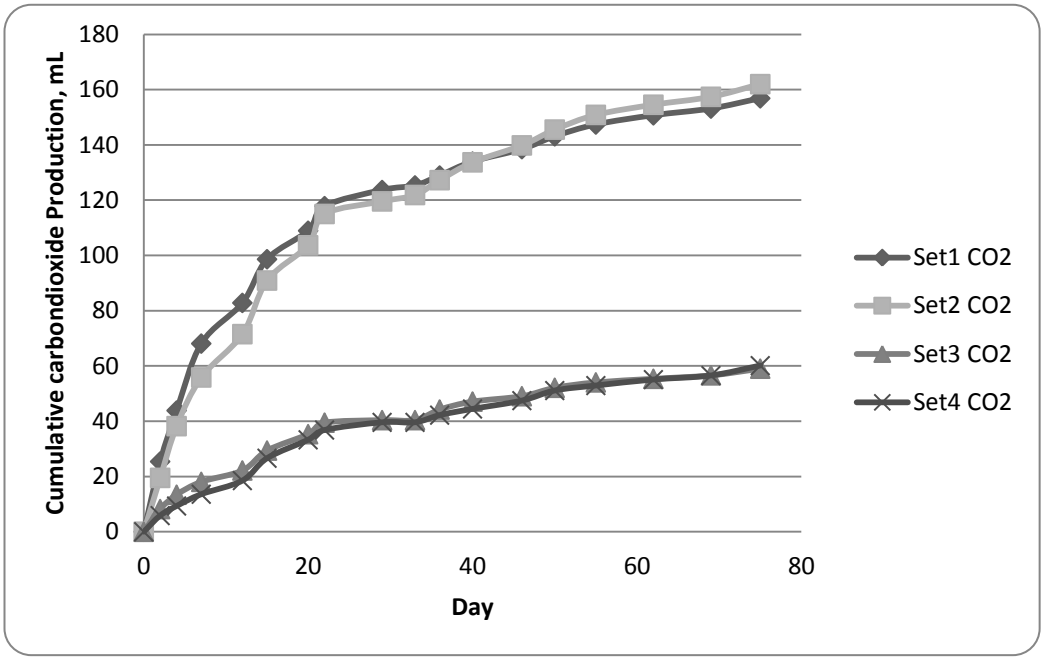


Figure 4-3: Cumulative carbondioxide production vs. time in BMP test reactors

Cumulative methane and carbondioxide graphs show similar patterns with cumulative total gas graph (Figure 4-2 and Figure 4-3). Moreover, when the first two sets are considered, methane production is 65% of total gas production where carbondioxide production holds for 25%. These values differ when seed control reactors are in question; total gas production is composed of 58% methane and 18% carbondioxide for Set 3 and 65% methane and 18% carbondioxide for Set 4.

At the end of 75 days, the reactors were terminated and sludge samples were analyzed. Volatile suspended solids (VSS) reductions were observed with these analyses. The results regarding this parameter are shown in Table 4-1.

Table 4-1: VSS values and reductions in Set 1 and Set 2 Reactors

	Set 1	**Set 2**
Day 0	9060 mg/L	8360 mg/L
Day 75	5190 mg/L	4560 mg/L
Removal (%)	43	45

Another comparison between Set 1 and Set 2 reactors were done regarding the amount of methane gas production per volatile suspended solids removals. Methane production potentials of seed control reactors, Set 3 and Set 4, were subtracted from those of Set 1 and Set 2 reactors and net methane production potential of WAS was calculated based on VSS removed. The results are illustrated in Figure 4-4.

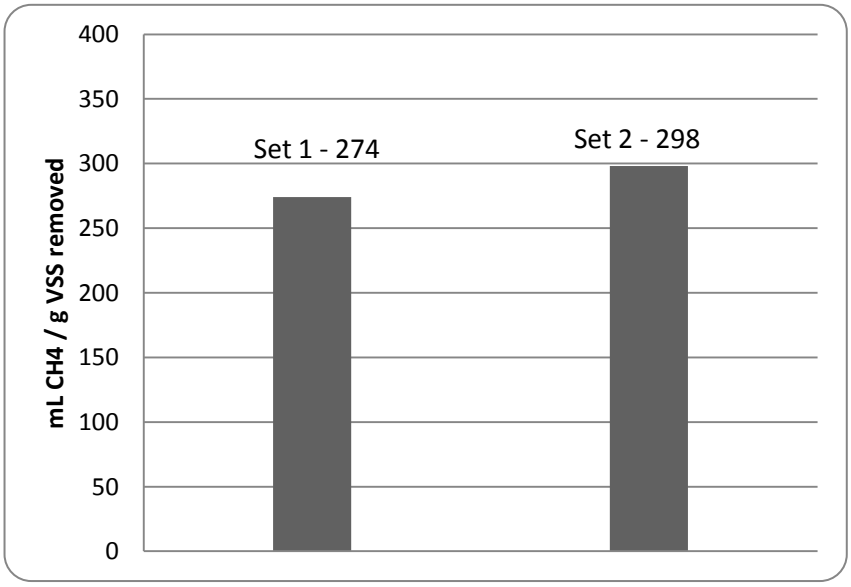


Figure 4-4: Methane production per gram VSS removed

According to the results in Table 4-1, when Set 1 reactors (including BM) and Set 2 reactors (without BM) are compared in terms of their volatile suspended solids removals, minor differences were observed. Moreover, it can be concluded that for Set 2 reactors, volatile suspended solids removal is slightly higher than that of Set 1 reactors.

When the results in Figure 4-4 is evaluated, methane production potential of WAS with and without BM can be seen. It is clear from these results that the presence of BM somehow slightly inhibited the methane production compared to the case when it is absent. It is inferred from these results that WAS already has the essential nutrients for anaerobic microorganisms to survive. Providing them in excess in the form of BM might have inhibited the anaerobic microorganisms slightly. Therefore,

throughout the rest of the study, BM was not used while setting up the anaerobic reactors.

4.2. Anaerobic Toxicity Assay Test

NP2EO ATA Test Reactors

The study examined the toxicity of NP2EO to anaerobic microorganisms by conducting an ATA test. The purpose of this is to come up with the highest dose that would still be non-toxic to anaerobic organisms so that the dosing of the larger scale batch reactors can be done using these doses.

The results of ATA tests conducted with NP2EO are summarized below. ATA Test reactors dosed with NP2EO were operated for 72 days. Throughout this period, total gas production and its composition were observed. At the end, similar to initial analyses, TS, VS, TSS, VSS, COD and pH measurements were done. Moreover, concentrations of NP compounds in initial and final samples were determined both in solid and aqueous phases of sludge.

Cumulative total gas and cumulative methane productions are illustrated in Figure 4-5 and Figure 4-6 respectively. When the first graph is considered, it is obvious that, Set 6 reactors, which do not contain NP2EO, have the highest gas production among all other sets of reactors. In the same graph, it can be seen that, Set 9 (abiotic) reactors, and Set 10 (seed + acetone) reactors have produced very low amounts of gas during the operation period. The same pattern is also observed for the cumulative methane graph which can be seen in Figure 4-6. Set 6 reactors have the highest methane production potential, whereas Set 9 and Set 10 have the lowest. Both of the graphs reveal that, Set 7 (seed control) reactors and Set 8 (seed + WAS + acetone) reactors have relatively lower total gas and methane productions when compared to the NP2EO containing reactors. Moreover, irrespective of the NP2EO dose, Sets 1 – 5 have very similar total gas and methane productions.

When Set 6 reactors are considered, 59% of the total gas produced is seen to be methane and 21% is carbondioxide. These values, on the other hand, are

approximately 50% and 23% for NP2EO containing reactors respectively. Set 8 reactors produced relatively lower amounts of methane. The total gas produced contains 45% methane and 23% carbondioxide for this set of reactors. This basically reveals that, NP2EO addition increased the methane productions as compared to acetone addition only.

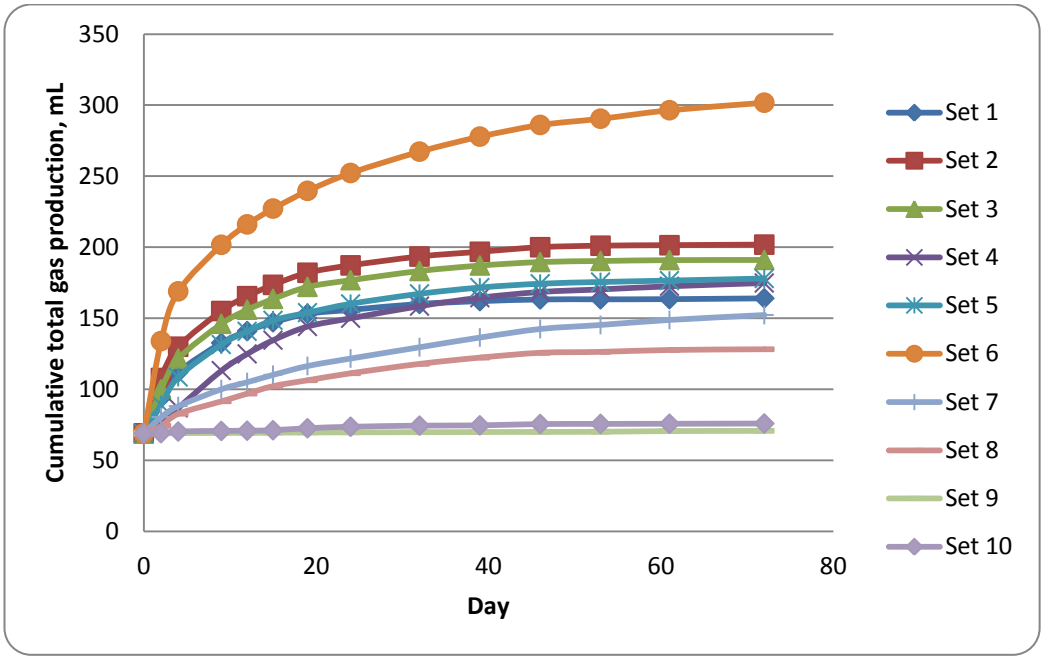


Figure 4-5: Cumulative total gas production vs. time for NP2EO

Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

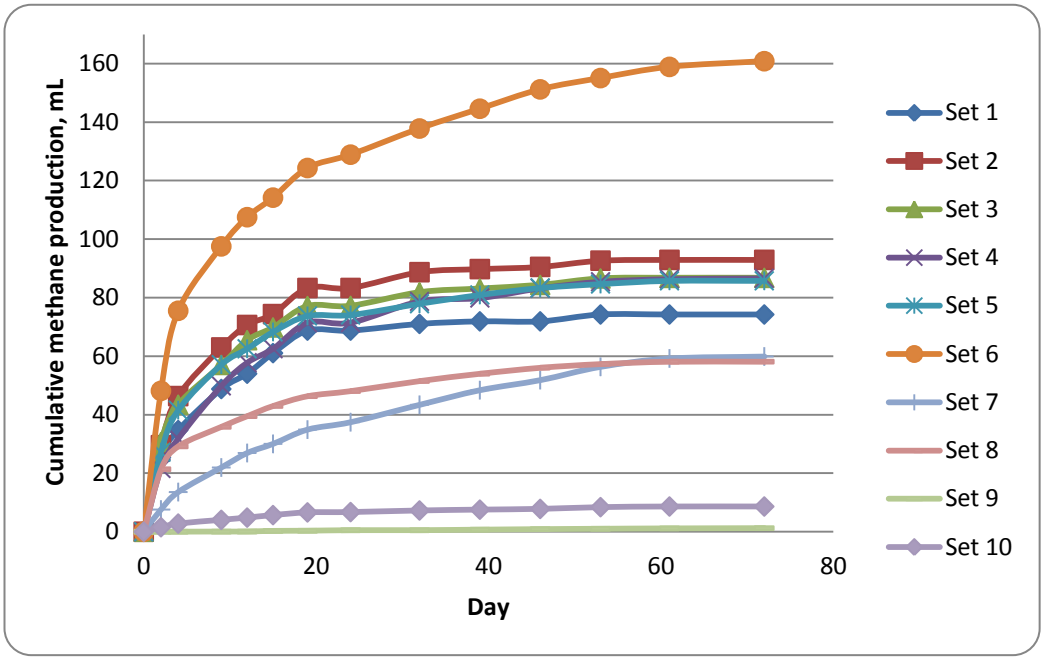


Figure 4-6: Cumulative methane production vs. time for NP2EO

Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

In Table 4-2, cumulative methane productions in percentages are summarized with respect to some randomly chosen days during the operation period. This analysis was done in order to observe the progress of methane production for the whole operation time. Furthermore, it enabled us to observe the alterations on methane production potentials with respect to time and NP2EO dose added to the reactors. It is clear that, for all the sets, there is an increase in methane production with time. From the beginning of the operation period, Set 6 reactors, which do neither contain NP2EO, nor acetone, have the highest methane percentage until the end of 72 days. On the other hand, Set 9 (abiotic) and Set 10 (seed + acetone) reactors have the same methane production behaviors with their lowest percentages. Sets 1 - 5 with NP2EO doses have similar patterns. When the Table 4-2 is considered, methane productions of the reactors with 30 mg/L NP2EO are affected the most. Set 4 and Set 5 reactors, having 5 mg/L and 1 mg/L NP2EO concentrations, respectively, have not been affected substantially in terms of their methane productions. Sets 2 and 3 reactors, on the other hand, have very similar progress of methane productions.

Table 4-2: Cumulative percent methane productions with respect to time for NP2EO

Rector*/Time(days)	9	15	32	61	72
Set 1	40	46	47	49	49
Set 2	44	47	49	49	49
Set 3	44	47	49	49	49
Set 4	50	52	55	55	55
Set 5	53	53	57	57	57
Set 6	53	54	57	58	59
Set 7	24	28	37	43	44
Set 8	45	49	52	52	52
Set 9	0	0	1	2	2
Set 10	6	8	10	11	11

*Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

In order to assess the methane production results in NP2EO reactors, Set 8 (seed + WAS + acetone) was taken as a reference and the sets with NP2EO were evaluated in comparison to Set 8. A similar evaluation was also done with respect to Set 6 (seed + WAS) reactors. The reason behind this comparison was the nature of Set 6 and Set 8 reactors. Set 8 is actually even a better control reactor since it has the same constituents (including acetone) with the NP2EO reactors except for the chemical tested. On the other hand, Set 6 is a control reactor without acetone so it can show the ultimate methane production potential of sludge with no NP2EO and no acetone. Therefore the methane volumes were normalized one time to the values observed in Set 8 and one time for the values measured in Set 6. These results are given in Table 4-3. Apart from Set 9 (abiotic) and Set 10 (seed + acetone), all the other sets have higher methane productions than Set 8 reactors (second row showing the results). This result clearly shows that if there is an inhibition in the system it does not originate from NP2EO however it originates from the presence of acetone. On the other hand, Set 6 reactors have higher methane production when compared to the other reactor sets, which indicates the combined negative effect of added chemicals on methane production. However, the combined evaluation of the results from Set 8 and Set 6, one can again see that the major inhibition comes from acetone, not from NP2EO. Acetone is known to have inhibition on anaerobic microorganisms with concentrations greater than 1000 mg/L (EPA, 1973).

Table 4-3: Cumulative methane productions at the end of 72 days for NP2EO

Set*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
mL methane	80	98	93	96	122	177	67	66	1	9
CH_4 potential(%) with respect to Set 8	121	148	140	145	184	267	101	100	2	13
CH_4 potential(%) with respect to Set 6	45	55	53	54	69	100	38	37	1	5

*Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

When the results from NP2EO added reactors are compared with the results from Set 8 reactors, it can easily be seen that methane productions are enhanced with the addition of NP2EO. This means that NP2EO added reactors produced more methane compared to their control. The improvements are between 1.2 to 1.8 times. The improvement is much less at high concentration reactors; whereas much higher in lower dose NP2EO containing reactors. This result can be taken as an indication of possible use of NP2EO as a substrate during anaerobic degradation process, that eventually contributes to the methane production.

At the end of 72 days the reactors were terminated. The initial and final VS analysis results and percent removal values are summarized in Table 4-4. Seed control reactors (Set 7) and abiotic reactors (Set 9) are observed to have low VS removals where all the other sets have higher and similar removal values.

Table 4-4: Initial and final VS amounts and percent removals in ATA reactors for NP2EO

	Day 0 (mg/L)	Day 72 (mg/L)	Removal (%)
Set 1	8183	5917	28
Set 2	8030	5877	27
Set 3	8393	5850	30
Set 4	8973	5717	36
Set 5	8290	5783	29
Set 6	8127	5633	31
Set 7	5500	4650	15
Set 8	8287	5917	29
Set 9	7903	7597	4
Set 10	6037	4793	21

Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

Figure 4-7 presents the ratio of mL methane produced / g VS removed for the whole ATA reactor sets. The mL methane produced data is obtained by subtracting the methane production data of Set 10 from other sets data with acetone as to subtract the methane production potential of ADS and acetone and Set 6 data was obtained by subtracting the Set 7 data in order to subtract the methane production potential of ADS. As parallel to cumulative methane production graph (Figure 4-6), Set 6 reactors have the highest ratio. Set 9 reactors did not produce any methane therefore the ratio is zero for that set. Set 1- 5 reactors have lower ratios as compared to Set 6.

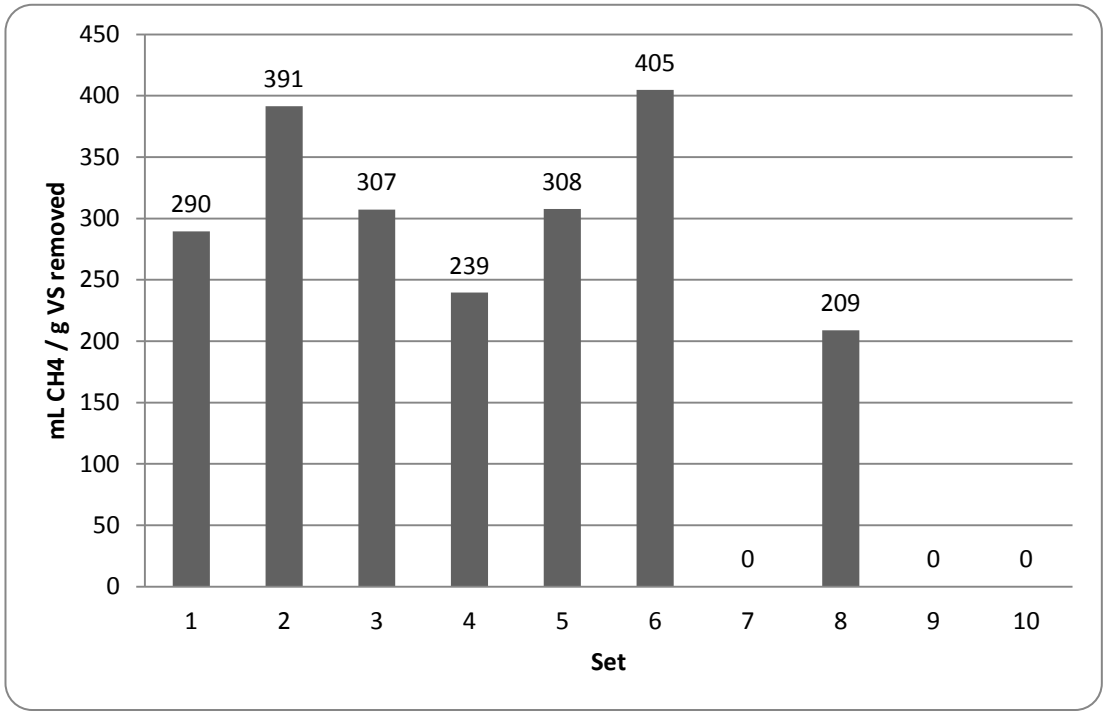


Figure 4-7: Methane production per gram of VS removed for NP2EO

Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

One other parameter that was analyzed was Chemical Oxygen Demand (COD). Table 4-5 summarizes the results of COD analyses of initial (before and after addition of NP2EO) and final sludge samples. In the reactor sets that contain NP2EO and acetone, the sharp increase in COD values with NP2EO addition can be clearly observed from the table. This reveals the contribution of NP2EO and acetone to the organic content of the sample and this contribution becomes overwhelming. When the removal values are analyzed, apart from Set 1 and Set 3, and the ones with negative values; the removals vary between 10 – 30%. Since the analyses were able to be done only with high dilution ratios, there might have been errors during experiments.

Table 4-5: Initial and final COD amounts and percent removals in ATA reactors for NP2EO

	Initial COD(mg/L) (Before NP2EO addition)	Initial COD(mg/L) (After NP2EO addition)	Final COD (mg/L)
Set 1	14615	95619	94555
Set 2	14218	95619	78735
Set 3	19165	87962	84153
Set 4	12090	81846	85121
Set 5	15019	96265	66075
Set 6	12096	11341	13325
Set 7	10537	7164	5503
Set 8	17905	106806	90185
Set 9	19959	91393	81105
Set 10	17660	95718	81783

Set 1-5 WAS+ADS+NP2EO in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP2EO), Set 10 ADS+acetone

After the extraction methods were finalized, the ATA reactor samples, which were stored at -18^{0}C, were analyzed in terms of their NP compounds concentrations. The concentrations of NP, NP1EO and NP2EO in solid phase of the sludge can be seen in Table 4-6. When initial reactor conditions are considered NP and NP1EO can be observed in considerable amounts. Since these chemicals were not spiked to the reactors, it can be concluded that they originate from wastewater treatment plant sludge. The concentration of NP in reactors containing WAS and ADS is 0.8 mg/L on the average, whereas this value is 1.1 mg/L for NP1EO. The reactors containing only ADS have NP and NP1EO in concentrations of 0.95 mg/L and 0.46 mg/L respectively. On the other hand, NP2EO was observed only in reactors spiked with that chemical which shows that NP2EO is not present in solid phases of wastewater treatment plant sludges. The initial values shown in Table 4-6 correspond to the concentrations right after the addition of NP2EO to the reactors. In order to be able to compare the extraction results with the target spike concentrations, the values in the table are given in terms of mg/L together with mass units. The obtained mass values from GC/MS analyses are converted to concentration units by using the

volume of sample that undergoes extraction processes. Therefore, since in each sampling, the sample volume is same, which is 5 mL, the given concentration units are correlated with each other. The spiked concentrations were in the range of 30 mg/L to 1 mg/L and when initial NP2EO values are considered it can be seen that 60 – 80% of NP2EO spiked have been extracted from sludge. The remaining value is thought to be in the aqueous phase and checked with the liquid phase analyses results, which are summarized in the following pages.

In the final analyses, NP2EO could not be detected in any of the reactor sets apart from the abiotic ones. Moreover, an increase in the concentrations of NP and NP1EO was observed. These findings clearly show that we were able to inhibit the anaerobic degradation in Set 9 and in accordance with it, the degradation of NP2EO stopped. On the other hand, in all the biotic reactors in which NP2EO was added, a degradation of spiked NP2EO was clearly observed with the complete decay of the chemical until the reactor termination. The intermediate (NP1EO) and final products (NP) accumulated and increased their concentrations significantly.

The extraction results from aqueous phase of sludge samples followed a similar path with the solid phase results. The initial and final concentrations of NP, NP1EO and NP2EO are given in Table 4-7. When initial reactor conditions at time zero are considered, NP and NP1EO can be observed. Since these chemicals are not spiked to the reactors, it can be stated that they originate from the wastewater treatment plant sludge. The concentration of NP in reactors containing WAS and ADS is 0.056 mg/L on the average where this value is 0.048 mg/L for NP1EO. The reactors containing only ADS have NP and NP1EO in concentrations of 0.072 mg/L and 0.024 mg/L respectively. On the other hand, NP2EO was observed only in reactors spiked with that chemical, which shows that NP2EO is not present in aqueous phases of wastewater treatment plant sludges. In the aqueous samples of day 72, no NP2EO was detected in any of the reactors except for Set 9. Moreover NP and NP1EO amounts increased after 72 days of operation. Only in Set 9, which was operated as abiotic control reactor and biological activity was eliminated; NP2EO was observed at the end of operation period. In general it can be concluded that, the concentrations of NP compounds were found to be low when compared to solid phase concentrations which results mainly from hydrophobic and lipophilic characteristics of the compounds.

Table 4-6: NP, NP1EO and NP2EO concentrations in solid phase of ATA reactors spiked with NP2EO

Reactor Setup	Compound	Initial (t=0)		Final (t=72)	
		Average (mg/L)	Average (mg/kg)	Average (mg/L)	Average (mg/kg)
SET 1:	NP2EO	18.35±2.09	1096.3±124	<LOQ*	<LOQ*
30 mg/L NP2EO	NP1EO	2.28±0.07	136.4±4.2	19.74±5.97	1521.4±460.1
(WAS+ADS+acetone)	NP	0.92±0.05	55.1±2.9	15.01±4.52	1157.1±348.4
SET 2:	NP2EO	14.22±4.71	898.1±297	<LOQ*	<LOQ*
20 mg/L NP2EO	NP1EO	1.85±0.05	117.1±3.2	8.22±0.87	629.9±66.7
(WAS+ADS+ acetone)	NP	0.92±0.09	58.4±5.7	13.21±1.8	1012.1±137.9
SET 3:	NP2EO	7.99±0.13	481.4±7.8	<LOQ*	<LOQ*
10 mg/L NP2EO	NP1EO	1.42±0.11	85.7±6.6	4.24±0.61	326.6±46.9
(WAS+ADS+ acetone)	NP	0.8±0.03	48.2±1.8	8.8±1.13	677.9±87.1
SET 4:	NP2EO	4.8±0.14	285.5±8.3	<LOQ*	<LOQ*
5 mg/L NP2EO	NP1EO	1±0.04	59.3±2.4	1.78±0.35	138.6±27.3
(WAS+ADS+ acetone)	NP	0.87±0.02	51.8±1.2	4.63±0.42	359.9±32.6
SET 5:	NP2EO	0.74±0.06	44.9±3.6	<LOQ*	<LOQ*
1 mg/L NP2EO	NP1EO	0.49±0.04	29.5±2.4	0.89±0.15	68.3±11.5
(WAS+ADS+ acetone)	NP	0.73±0.05	44.1±3.1	2.31±0.45	117.3±22.9
SET 6:	NP2EO	<LOQ*	<LOQ*	<LOQ*	<LOQ*
0 mg/L NP2EO	NP1EO	0.49±0.03	30.4±1.9	0.26±0.07	19.8±5.3
(WAS+ADS)	NP	0.91±0.11	56.3±6.8	1.34±0.47	103.8±36.4
SET 7:	NP2EO	<LOQ*	<LOQ*	<LOQ*	<LOQ*
0 mg/L NP2EO (ADS)	NP1EO	0.43±0.06	33.7±4.7	0.3±0.12	26.5±10.6
	NP	0.95±0.17	73.8±13.2	1.53±0.59	133.5±51.5
SET 8:	NP2EO	<LOQ*	<LOQ*	<LOQ*	<LOQ*
0 mg/L NP2EO	NP1EO	0.38±0.03	23.7±1.9	0.53±0.1	40.4±7.6
(WAS+ADS+ acetone)	NP	0.56±0.05	34.3±3.1	1.19±0.29	90.2±21.9
SET 9:	NP2EO	3.86±0.38	229.6±22.6	6.05±0.79	400.1±52.2
5 mg/L NP2EO (WAS+ADS+aseton)	NP1EO	0.66±0.07	38.9±4.1	0.62±0.07	40.7±4.6
Abiotic control	NP	0.71±0.09	42.4±5.4	1.25±0.2	82.9±13.3
SET 10:	NP2EO	<LOQ*	<LOQ*	<LOQ*	<LOQ*
0 mg/L NP2EO	NP1EO	0.49±0.04	38.4±3.1	0.51±0.08	44.5±6.9
(ADS+ acetone)	NP	0.94±0.05	73.6±3.9	1.18±0.22	102.5±19.1

*NP: LOQ (limit of quantification) 10 ppb, LOD (limit of detection) 3 ppb

NP1EO: LOQ 9.5 ppb LOD 2.9 ppb

NP2EO: LOQ 7.6 ppb LOD 2.3 ppb

Table 4-7: NP, NP1EO and NP2EO concentrations in aqueous phase of ATA reactors spiked with NP2EO

Reactor Setup	Compound	Initial (t=0)	Final (t=72)
		Average (mg/L)	Average (mg/L)
SET 1:	NP2EO	1.334 ± 0.006	<LOQ
30 mg/L NP2EO	NP1EO	0.062 ± 0.00	0.426 ± 0.001
(WAS+ADS+acetone)	NP	0.056 ± 0.003	0.536 ± 0.029
SET 2:	NP2EO	1.199 ± 0.003	< LOQ
20 mg/L NP2EO	NP1EO	0.052 ± 0.005	0.258 ± 0.033
(WAS+ADS+acetone)	NP	0.064 ± 0.007	0.394 ± 0.064
SET 3:	NP2EO	0.782 ± 0.001	< LOQ
10 mg/L NP2EO	NP1EO	0.063 ± 0.004	0.135 ± 0.004
(WAS+ADS+acetone)	NP	0.083 ± 0.003	0.221 ± 0.007
SET 4:	NP2EO	0.337 ± 0.002	< LOQ
5 mg/L NP2EO	NP1EO	0.037 ± 0.003	0.074 ± 0.002
(WAS+ADS+acetone)	NP	0.065 ± 0.003	0.141 ± 0.008
SET 5:	NP2EO	< LOQ	< LOQ
1 mg/L NP2EO	NP1EO	0.014 ± 0.001	0.023 ± 0.001
(WAS+ADS+acetone)	NP	0.034 ± 0.007	0.054 ± 0.001
SET 6:	NP2EO	< LOQ	< LOQ
0 mg/L NP2EO (WAS+ADS)	NP1EO	< LOQ	0.012 ± 0.001
	NP	0.023 ± 0.003	0.099 ± 0.002
SET 7:	NP2EO	< LOQ	< LOQ
0 mg/L NP2EO (ADS)	NP1EO	0.014 ± 0.001	0.01 ± 0.001
	NP	0.059 ± 0.002	0.066 ± 0.001
SET 8:	NP2EO	< LOQ	< LOQ
0 mg/L NP2EO	NP1EO	0.027 ± 0.002	0.038 ± 0.003
(WAS+ADS+acetone)	NP	0.043 ± 0.018	0.11 ± 0.018
SET 9:	NP2EO	0.35 ± 0.171	0.224 ± 0.025
5 mg/L NP2EO	NP1EO	0.047 ± 0.022	0.021 ± 0.002
(WAS+ADS+acetone) abiotic control	NP	0.052 ± 0.025	0.044 ± 0.006
SET 10:	NP2EO	< LOQ	< LOQ
0 mg/L NP2EO (ADS+acetone)	NP1EO	0.033 ± 0.001	0.038 ± 0.001
	NP	0.084 ± 0.004	0.081 ± 0.001

Table 4-8 constitutes a mass balance on NP compounds in ATA test reactor samples which were dosed with NP2EO and had biological activity. It shows the initial and final NP, NP1EO and NP2EO amounts as a sum of aqueous and solid phase mass values. Results are both given for each chemical as well as a total mass of three chemicals. According to the table, all of the initial samples have NP and NP1EO in them originating from the WAS and ADS samples used. When the initial NP2EO masses calculated are compared with the mass values that are intended to be observed in the reactors (intended mass values in the spike solutions), they are seen close to each other except for the case of Set 1 reactors. In Set 1, it was expected to be found 3 mg; however, it was measured and calculated as 1.968 mg. This might be due to sampling or extraction errors as well as an error in the spiking stage. The deviations from the intended NP2EO masses in initial conditions are 30% for Set 1 reactors, 22% for Set 2 reactors, 12% for Set 3 reactors, 3% for Set 4 reactors and 26% for Set 5 reactors. When a mass balance is applied on the total concentrations of NP, NP1EO and NP2EO between the initial and final conditions based upon the measurements and calculations, the deviations are calculated for Set 1-5 reactors as; 55%, 21%, 20%, 7% and 63% respectively. These results are considered as quite satisfactory for biological reactors operated for 72 days and three different compounds are examined.

The fact that NP2EO could not be detected in the final samples and an increase in the masses of NP and NP1EO was observed; reveals that NP2EO are degraded with biological activity into NP and NP1EOin anaerobic environment. When methane production is also taken into consideration, as compared to control reactors, the NP2EO dosed reactors produced 120-180% more methane. This is a finding indicating contribution of NP2EO to methane as substrate when it is converted to NP1EO and NP.

Table 4-8: NP, NP1EO and NP2EO masses in initial and final sludge samples

	Initial condition (Aqueous phase +Solid phase mg)				Final condition (Aqueous phase +Solid phase mg)			
Reactor Setup	**NP**	**NP1EO**	**NP2EO**	**Total**	**NP**	**NP1EO**	**NP2EO**	**Total**
SET 1: 3 mg NP2EO (WAS+ADS+ acetone)	0.098	0.234	1.968	2.30	1.555	2.017	-	3.571
SET 2: 2 mg NP2EO (WAS+ADS+ acetone)	0.098	0.190	1.542	1.831	1.360	0.848	-	2.208
SET 3: 1 mg NP2EO (WAS+ADS+ acetone)	0.088	0.148	0.877	1.114	0.902	0.438	-	1.340
SET 4: 0.5 mg NP2EO (WAS+ADS+ acetone)	0.094	0.104	0.514	0.711	0.477	0.185	-	0.663
SET 5: 0.1 mg NP2EO (WAS+ADS+ acetone)	0.076	0.050	0.074	0.20	0.236	0.091	-	0.327

NP (Branched) ATA Test Reactors

Since NP is claimed to be more toxic to many organisms compared to NP2EO, an additional ATA test was also conducted on NP. However, the NP used in this part was a lower quality NP (technical grade) since it was not possible to buy the higher quality NP for this test. For this reason, the analyses of NP compounds were not conducted on initial and final reactor samples to protect the GC/MS system. The results of ATA tests conducted with a branched NP chemical are summarized below.

ATA test reactors were operated for 71 days and throughout this period, total gas production and gas compositions were observed continuously. At the end of 71 days the bottles were opened and the sludge samples were analyzed for TS, VS, TSS, VSS, COD and pH parameters.

Cumulative total gas and methane production graphs can be seen in Figures 4-8 and 4-9 respectively. Set 8 reactors, which contain only ADS, have the lowest total gas and methane production as compared to other sets of reactors. When cumulative total gas production graph is considered, other sets apart from Set 8 have similar patterns irrespective of NP dose. However, Set 7 reactors, which do not contain NP, have higher methane production potential than NP containing reactor sets. When Set 7 reactors are considered, 67% of the total gas produced is seen to be methane and 25% is carbondioxide. These values, on the other hand, are approximately 57% and 23% for NP containing reactors respectively. In Set 8 reactors these values are lower with 53% methane and 16% carbondioxide productions.

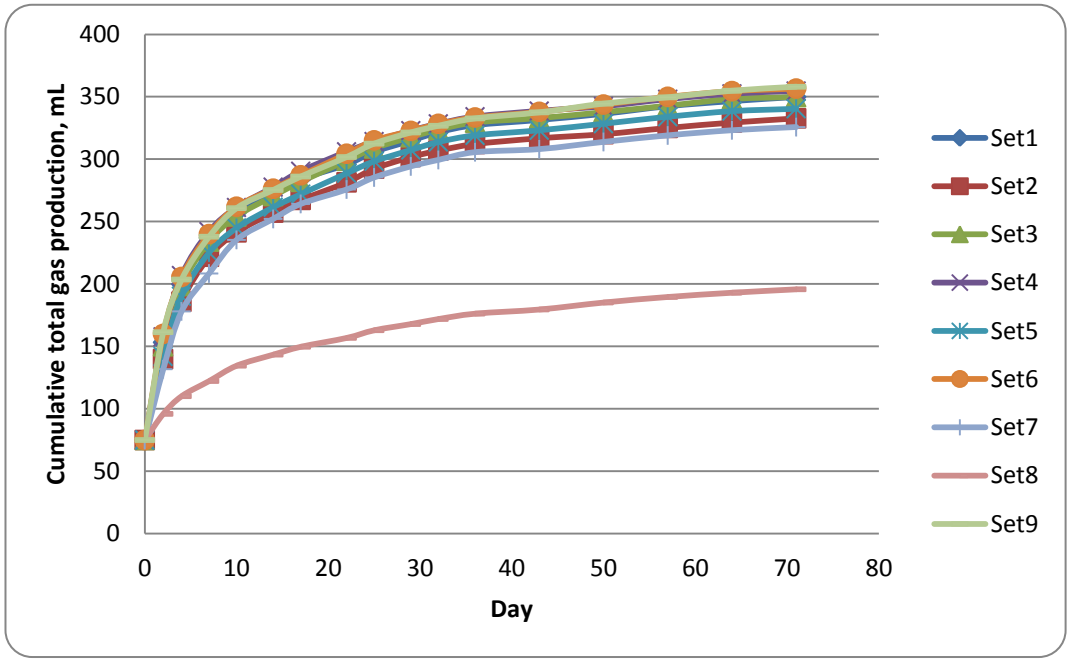


Figure 4-8: Cumulative total gas production vs. time for NP (branched)

Set 1-6 WAS+ADS+NP in acetone (50-1 mg/L), Set 7 WAS+ADS, Set 8 ADS, Set 9 WAS+ADS+acetone

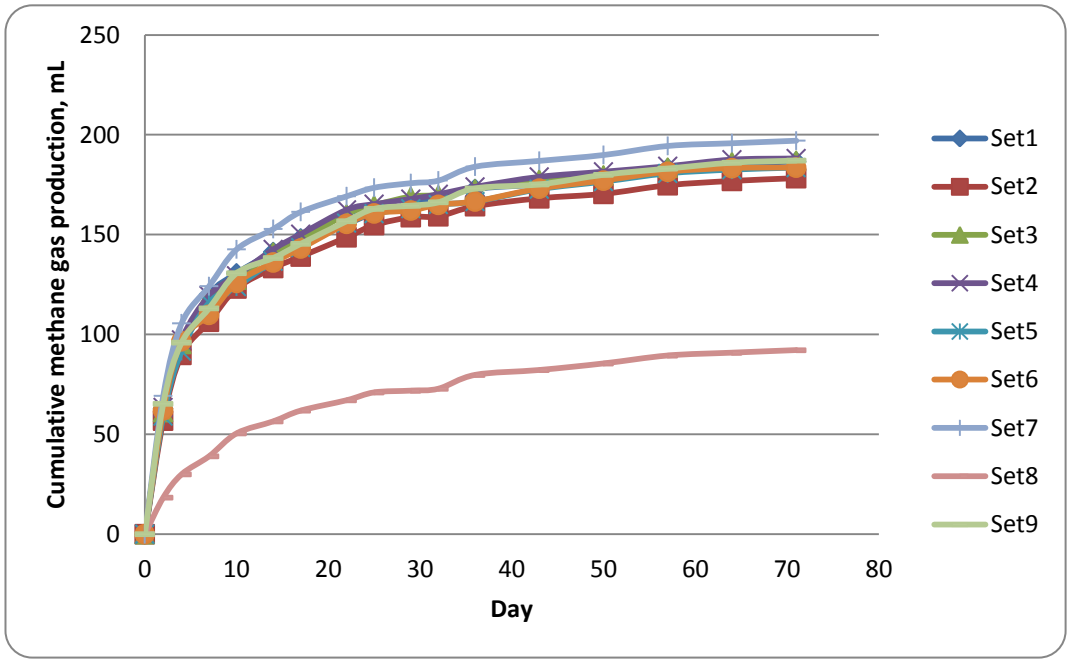


Figure 4-9: Cumulative methane gas production vs. time for NP (branched)

Set 1-6 WAS+ADS+NP in acetone (50-1 mg/L), Set 7 WAS+ADS, Set 8 ADS, Set 9 WAS+ADS+acetone

In Table 4-9, cumulative methane productions in percentages are summarized with respect to some randomly chosen days during the operation period. As stated before, the aim of this analysis is to observe the progress of methane production for the whole operation time. Furthermore, it enabled us to observe the alterations on methane production potentials with respect to time and NP (branched) dose added to the reactors. It is obvious that, for all the sets there is an increase in methane production with time. From the beginning of the operation period, Set 7 reactors, which do not contain NP (branched), have the highest methane production until the end of 71 days; on the other hand, Set 8 (seed control) have the lowest methane production potential. Methane percentages of NP (branched) containing Sets 1 – 5 reactors are 8% - 14% lower than that of Set 7 reactors, with consistency among each other.

Table 4-9: Cumulative percent methane productions with respect to days for NP (technical grade)

Reactor*/Day	10	17	32	50	71
Set 1	55	55	56	57	57
Set 2	55	55	56	57	58
Set 3	54	55	56	57	57
Set 4	53	55	56	57	57
Set 5	55	56	57	58	58
Set 6	53	53	55	56	56
Set 7	67	66	66	66	67
Set 8	41	44	49	51	53
Set 9	54	54	56	56	57

*Set 1-6 WAS+ADS+NP in acetone (50-1 mg/L), Set 7 WAS+ADS, Set 8 ADS, Set 9 WAS+ADS+acetone

In order to assess the methane production results, Set 9 (seed + WAS + acetone) reactors are taken as a reference and the other sets were evaluated in that respect. Similarly, the evaluation was also done with respect to Set 7 (seed + WAS) reactors. The results are given in Table 4-10. Looking at the second raw of the results, it can be seen that apart from Set 7 reactors, all the other sets have lower methane productions than Set 9 reactors. This can be explained with the slight inhibitory

effect of NP on anaerobic microorganisms. Moreover, Set 7 reactors have higher methane production when compared to Set 9 reactors, which indicates the negative effect of acetone on methane production.

Table 4-10: Cumulative methane productions at the end of 71 days for NP (technical grade)

Set*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
mL CH_4	198	188	197	199	195	197	213	100	200
CH_4 (%) with respect to Set 9	99	94	99	99	97	99	107	50	100
CH_4 (%) with respect to Set 7	93	88	92	93	91	92	100	47	94

*Set 1-6 WAS+ADS+NP in acetone (50-1 mg/L), Set 7 WAS+ADS, Set 8 ADS, Set 9 WAS+ADS+acetone

As stated above, at the end of 71 days, the reactors were terminated. The initial and final VS analysis results and percent removal values are summarized in Table 4-11. Seed control reactors of Set 8 reveals the lowest VS removal percentage among the other sets, which have very similar removal values.

Table 4-11: Initial and final VSS amounts and percent removals in ATA reactors for NP (technical grade)

	Day 0 (mg/L)	Day 71 (mg/L)	Removal (%)
Set 1	9570	6800	29
Set 2	9895	7010	29
Set 3	10095	7156	29
Set 4	9585	7086	26
Set 5	10205	7246	29
Set 6	9815	7830	20
Set 7	9785	6943	29
Set 8	6795	5536	19
Set 9	9960	7500	25

Set 1-6 WAS+ADS+NP in acetone (50-1 mg/L), Set 7 WAS+ADS, Set 8 ADS, Set 9 WAS+ADS+acetone

In Figure 4-10, the ratio of mL methane produced / g VS removed for ATA tests with NP (branched) are given. The mL methane produced data is obtained by subtracting the methane production data of Set 8 from other sets data. The NP containing sets are very parallel to each other without regard of the NP dose, which reveals the little or no inhibition of NP on anaerobic microorganisms.

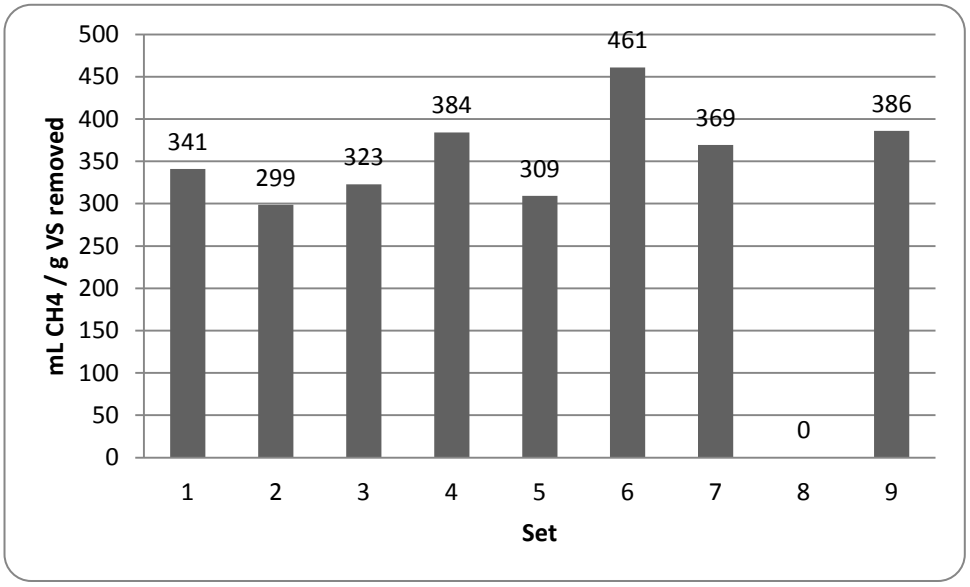


Figure 4-10: mL methane production per gram of VS removed for NP (branched)

Set 1-6 WAS+ADS+NP in acetone (50-1 mg/L), Set 7 WAS+ADS, Set 8 ADS, Set 9 WAS+ADS+acetone

NP (straight chain) ATA Test Reactors

The results of ATA tests conducted with a NP (straight chain) chemical are summarized below. Straight chain NP is a single isomer, not a chemical of commercial concern and known to be not as toxic as NP with branched structure.

Figure 4-11 and 4-12 are presenting the cumulative total gas production and cumulative methane gas production with respect to time in days respectively. Set 9 (abiotic) reactors produced neither significant amounts of total gas nor methane. Similarly, as compared to WAS containing reactors, Set 7 and Set 10 which contain only ADS, produced lower amounts of total gas and methane. Set 1 – 5 reactors with different doses of NP, and Set 8 (seed + WAS + acetone) reactors produced similar amounts of gas where Set 6 (seed + WAS) reactors' production pattern lies slightly above the others. In Set 6 reactors methane gas constitutes 63% of the total gas amount where carbondioxide's amount is 24%. These values are on the average 58% and 25% for the other sets of reactors respectively.

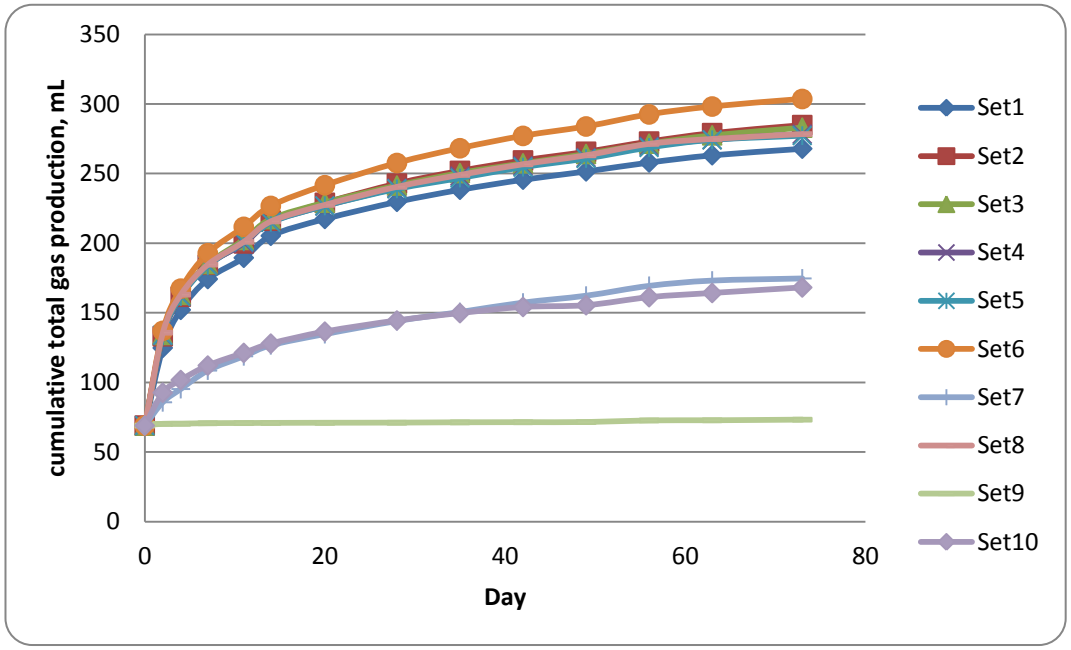


Figure 4-11: Cumulative total gas production vs. time for NP

Set 1-5 WAS+ADS+NP in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP), Set 10 ADS+acetone

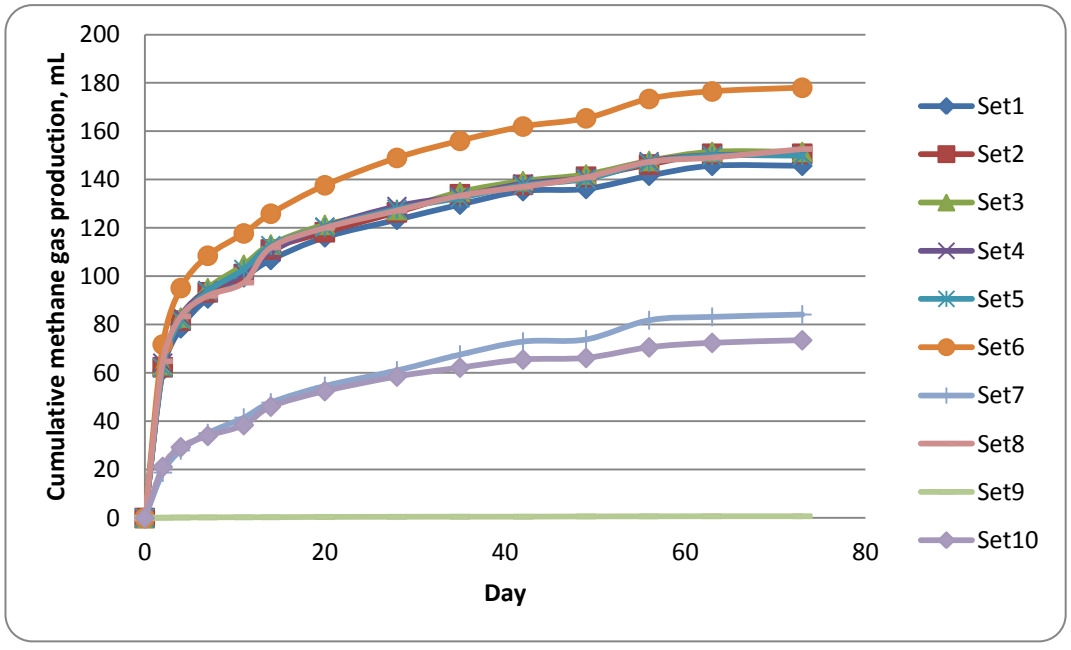


Figure 4-12: Cumulative methane gas production vs. time for NP

Set 1-5 WAS+ADS+NP in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP), Set 10 ADS+acetone

In Table 4-12, cumulative methane productions in percentages are summarized with respect to some randomly chosen days during operation period. This analysis enabled us to observe the alterations on methane production potentials with respect to time and NP dose added to the reactors. It can be seen that, for all the sets there is an increase in methane production with time. Set 6 reactors, which do not contain NP, have the highest methane production until the end of 73 days. On the other hand, Set 7 (seed control), Set 9 (abiotic) and Set 10 (seed + acetone) reactors have the same methane production behaviors with their lowest percentages.

Table 4-12: Cumulative percent methane productions with respect to days for NP

Reactor*/Day	**7**	**20**	**35**	**56**	**73**
Set 1	55	57	58	58	59
Set 2	54	57	57	57	58
Set 3	55	57	58	58	58
Set 4	54	56	58	58	58
Set 5	54	57	58	58	58
Set 6	59	61	62	63	63
Set 7	44	46	50	53	54
Set 8	55	57	58	58	59
Set 9	1	1	1	1	1
Set 10	42	45	48	50	51

*Set 1-5 WAS+ADS+NP in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP), Set 10 ADS+acetone

In order to assess the methane production results, Set 8 (seed + WAS + acetone) reactors are taken as a reference and the other sets were evaluated in that respect. Similarly, the evaluation was done with respect to Set 6 (seed + WAS) reactors. The results are given in Table 4-13. NP containing reactors have very similar methane percentages as compared to Set 8 reactors. Set 6 reactors, on the other hand, produced higher methane amounts of all the other set of reactors.

Table 4-13: Cumulative methane productions at the end of 73 days for NP

Set*	1	2	3	4	5	6	7	8	9	10
mL methane	157	165	165	162	161	190	94	163	1	86
CH_4 (%) with respect to Set 8	96	101	101	99	99	117	58	100	1	53
CH_4 (%) with respect to Set 6	82	87	87	85	85	100	49	86	1	45

*Set 1-5 WAS+ADS+NP in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP), Set 10 ADS+acetone

At the end of 73 days, the reactors were terminated. The initial and final VS analysis results and percent removal values are summarized in Table 4-14. In Set 9 (abiotic) reactors no removal was observed. Set 7 and Set 10 reactors reveal the lowest VS removal percentage among the other sets, which have very similar removal values.

Table 4-14: Initial and final VS amounts and percent removals in ATA reactors for NP

	Day 0 (mg/L)	Day 73 (mg/L)	Removal (%)
Set 1	9073	5913	35
Set 2	9070	5917	35
Set 3	8747	6013	31
Set 4	8420	6353	25
Set 5	8467	6173	27
Set 6	8890	5723	36
Set 7	6067	4577	25
Set 8	8683	6080	30
Set 9	8440	8740	0
Set 10	6267	4820	23

Set 1-5 WAS+ADS+NP in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP), Set 10 ADS+acetone

In figure 4-13, mL methane production per g of VS removed ratios for NP (straight chain) containing ATA reactors are shown. Set 10 methane production data was subtracted from the other sets that contain acetone and Set 7 methane production data was subtracted from Set 6 and the methane production values are obtained. Set 9 as expected shows zero while Set 10 and Set 7 have the similar result as they were used for normalizing the data. In general, there is an increase in the ratio with the decreasing NP dose.

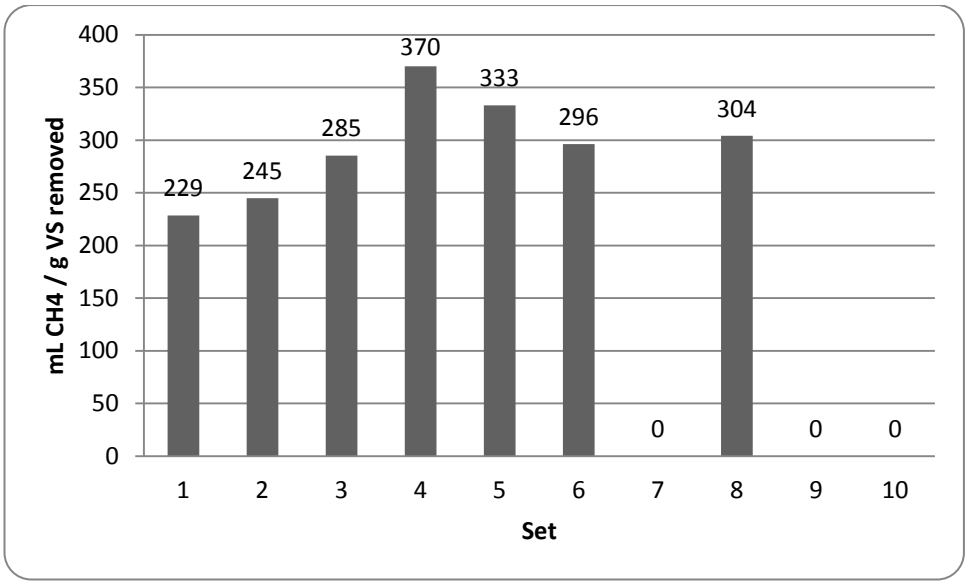


Figure 4-13: Methane production per gram of VS removed for NP (straight chain)

Set 1-5 WAS+ADS+NP in acetone (30-1 mg/L), Set 6 WAS+ADS, Set 7 ADS, Set 8 WAS+ADS+acetone, Set 9 abiotic (5mg/L NP), Set 10 ADS+acetone

4.3. 2.5 Liter Anaerobic Batch Reactors

In the light of the results from ATA tests, two doses of NP2EO were determined and added to larger scale anaerobic batch reactors in the last part of the study. In ATA tests, NP2EO, in the concentration range of 1 – 30 mg/L, did not show any inhibitory effect on the methane production potentials of anaerobic microorganisms. Therefore, we have chosen 0.5 and 2.5 mg/L among the doses examined in ATA

tests to spike the larger scale anaerobic batch reactors, so that we had the chance to observe the degradation of NP2EO by anaerobic microorganisms.

The four sets of reactors that were operated were named as Set 1 – 4. All of the sets were containing WAS and ADS in common. Set 1 reactors (Set 1-1 and Set 1-2) were live control reactors and had 5 mL of acetone in addition to WAS and ADS. Set 2 reactors (Set 2-1 and Set 2-2) were operated as abiotic control reactors with 0.5 mg/L NP2EO dosed in 5 mL of acetone. Set 3 (Set 3-1 and Set 3-2) and Set 4 (Set 4-1 and Set 4-2) reactors were set up in order to observe the biodegradation of NP2EO and dosed with 0.5 and 2.5 mg/L of NP2EO in 5 mL of acetone, respectively.

In operation period, total gas production and composition of the reactors are observed in addition to pH, TS, VS, TSS, VSS and COD analyses. Moreover, concentration of NP compounds were analyzed in solid and liquid portions of sludge. The reactors were operated for 90 days. The results of the analyses are given in details below in this section. All the data of the reactors are given in Appendix B.

Gas production and composition

Four sets of reactors were operated each having two idential reactors. Throughout this section, the results will be given separately for eight reactors rather than giving averages of replicates for each set. The main reason for that is, from the begining of the operation period, four of the reactors had problems with the sealing. We suspected that there was air flowing into the reactors because the pressure inside the reactors was equalized to the atmospheric pressure. As a result, total gas production values seemed to be high. In that respect, although methane percentages of these reactors were lower as compared to their replicates, methane volumes proceeded with higher profiles.

Throughout the operation period, total gas production and gas compositions of the reactors were monitored. In Figure 4-14, cumulative methane production with respect to the days of operation is illustrated. Since, the reactors having proper sealing conditions have more realistic patterns of methane production, they are illustrated separately in Figure 4-15 and the discussion will be done on this graph. First thing to mention is that, as we had aimed, abiotic conditions were maintained in

Set 2 reactors (abiotic control reactors with 0.5 mg/L NP2EO dose) and these reactors did not produce methane in any phase of the operation period. The live control reactor (Set 1-2) followed a pattern above all the other reactors. NP2EO containing reactors, on the other hand, followed very similar paths below live control reactor. When Set 1 reactor is considered, 55% of the total gas is methane; this value is 48 and 43% for Set 3 and Set 4 reactors, respectively. The effect of acetone in ATA test reactors was more significant when compared to that of 2.5 L anaerobic batch reactors. The main reason for that is, 5 mL of acetone was dispersed in 100 mL of ATA test reactors, which results in a higher concentration of acetone in the reactor, while in the larger reactors 5 mL of acetone was added into 2.5 L of effective sludge volume.

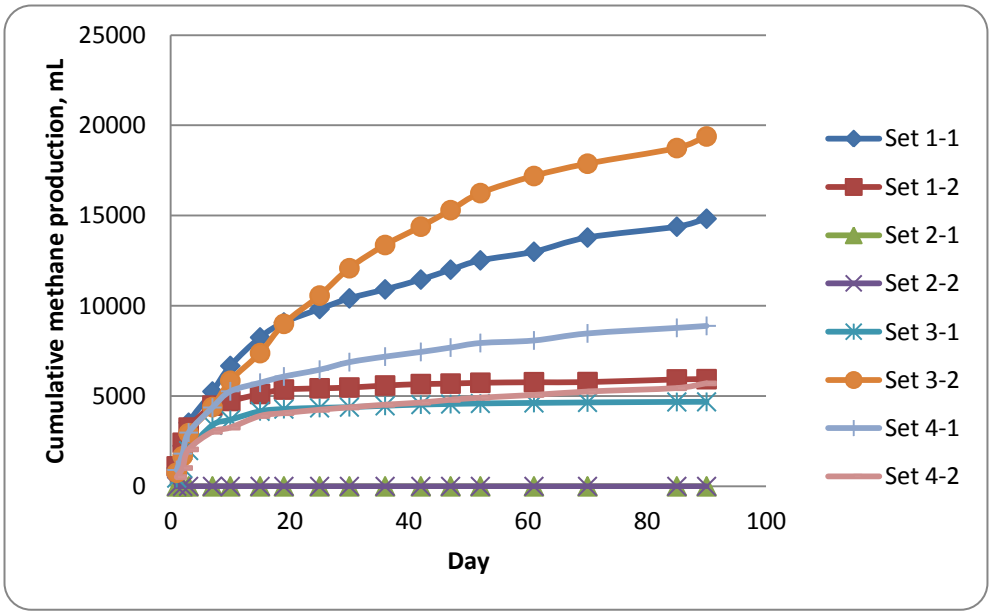


Figure 4-14: Cumulative methane production vs. time for 2.5 L anaerobic batch reactors – all reactors included

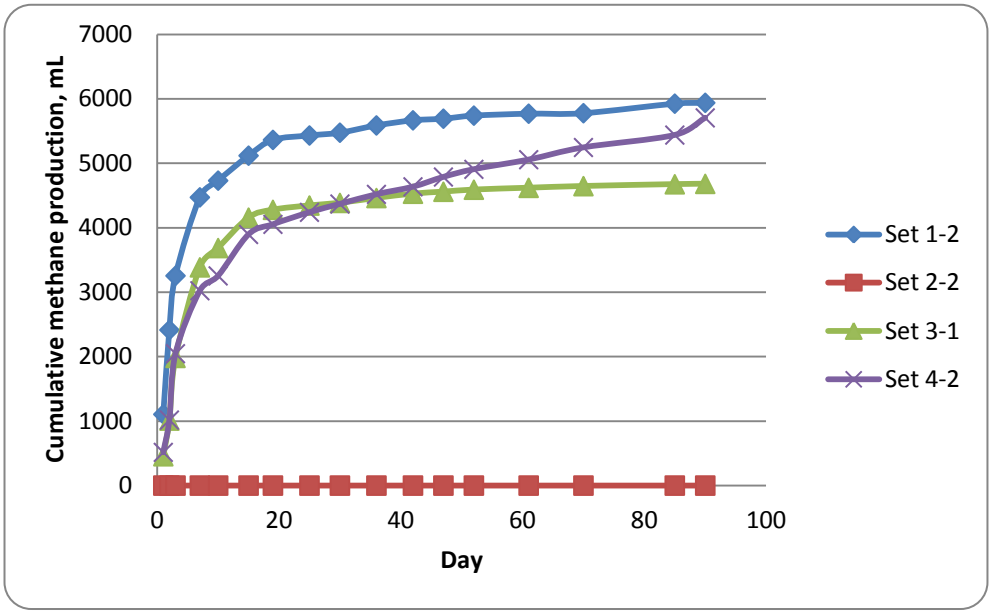


Figure 4-15: Cumulative methane production vs. time for 2.5 L anaerobic batch reactors – excluding suspected leaking reactors

In Table 4-15, cumulative methane percentages can be seen throughout the operation period in some randomly chosen days. In this table, progress of methane production can be observed in all of the eight reactors. In the operation period, the methane productions were increased in the first 40 days and after this day, they began to decrease. This is mainly because the reactors were operated as batch systems and microorganisms consume the limited amount of substrate in the system in the first few weeks of operation.

Table 4-15: Cumulative percent methane productions with respect to days

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
1	45	44	0	0	45	36	38	50
3	50	52	0	0	58	46	42	58
10	55	56	0	0	63	53	43	60
25	50	57	0	0	63	57	31	59
42	42	57	0	0	62	54	26	54
61	36	57	0	0	62	49	23	50
90	34	56	0	0	48	45	22	43

pH

The pH range that methanogens prefer for optimum living conditions is reported as 6.5 – 8.2 (Speece, 1996). The optimum pH for the degradation of NP compounds on the other hand is given as 7 (Chang et. al., 2005). Below in Figure 4-16, the pH variations of all eight reactors during operation period are given. The pH of all the reactors have varied between 7 and 8. Abiotic control reactors, at the begining of the operation period, had higher pH values which then fell into the range of 7 – 8. Throughout the operation period pH values did not go outside the optimum anaerobic degradation range and no pH arrangements were done in that respect.

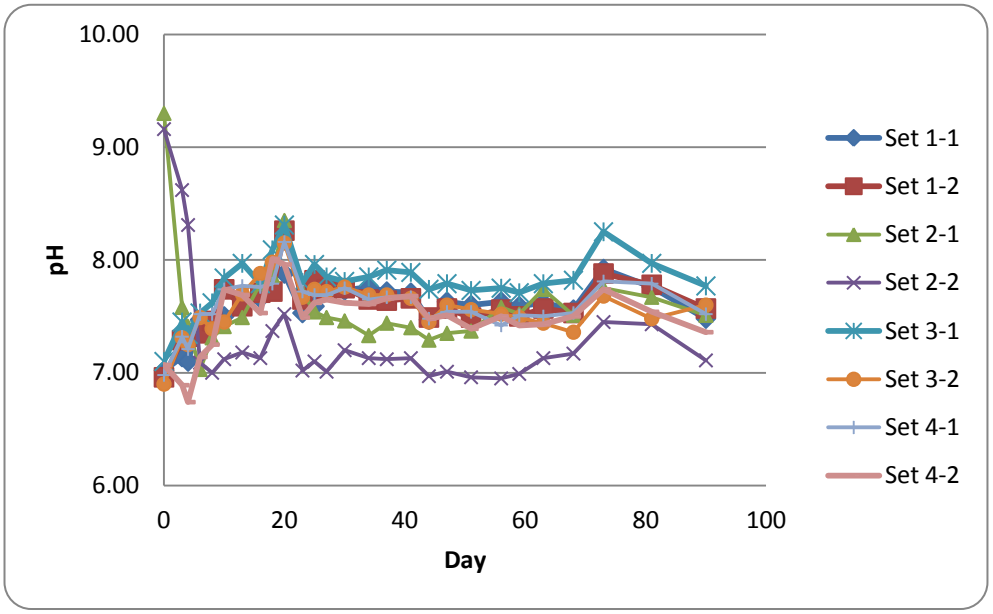


Figure 4-16: pH vs time for 2.5 L anaerobic batch reactors

Since the reactors were operated as batch systems, throughout the operation period the volume of the reactor decreased with considerable amounts. Therefore a volume correction procedure was applied while the solids, COD and concentrations of NP compounds data were being evaluated. The volume correction procedure relied on the addition of the related parameter's mass, which was removed from the system with sampling, to the data of the following sampling day. This procedure was applied to each sampling point consecutively, except for the first sampling point. The volume correction did not result in more than 1% change in the concentrations of all the parameters. Though, all the data presented in the following discussions were calculated by applying this procedure.

Total solids(TS) and volatile solids (VS)

During the operation period, one of the most important parameters to monitor was VS because it is the indicator of the stability of the reactor since it shows the substrate amount inside the reactors that microorganisms need to survive. The variations of TS and VS during the operation period is illustrated in Figure 4-17 and Figure 4-18, respectively. The reactors' VS values at the begining of the operation period was aimed to be set to 12,000 mg/L. As can be seen from the t=0 samples of

the reactors in Figure 4-18 (apart from the Set 2 reactors which was autoclaved in order to maintain abiotic conditions) all the other sets started operation with 1.2% VS content. There is a slight increase in VS values in the fourth day of operation with the addition of NP2EO and acetone to the reactors. Throughout the operation, reduction of VS values for all the sets were observed. Through the end of the operation period, the VS values were stabilized at around 7500 mg/L for live reactors and 8400 mg/L for abiotic reactors.

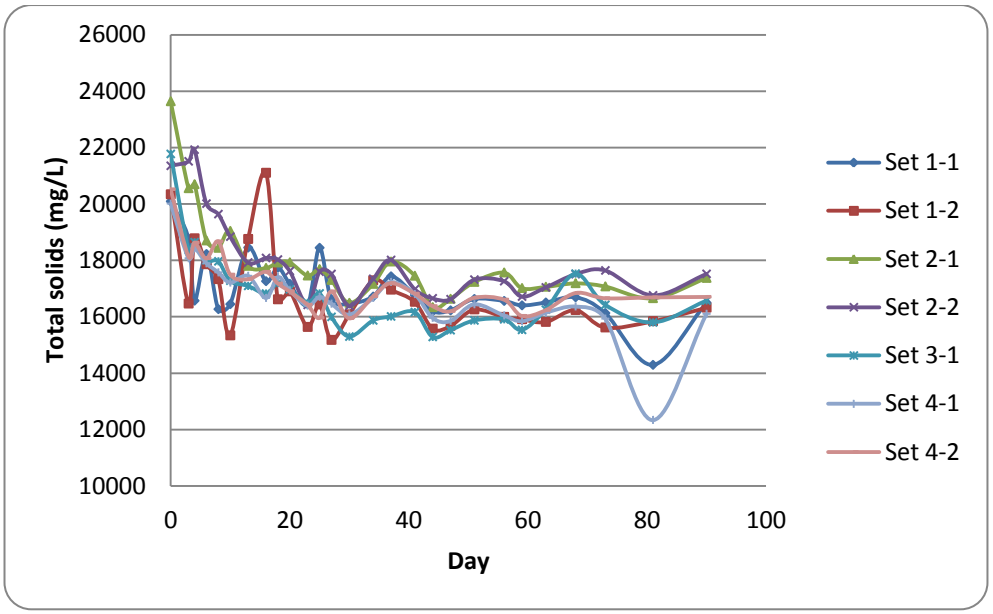


Figure 4-17: TS vs time for 2.5 L anaerobic batch reactors

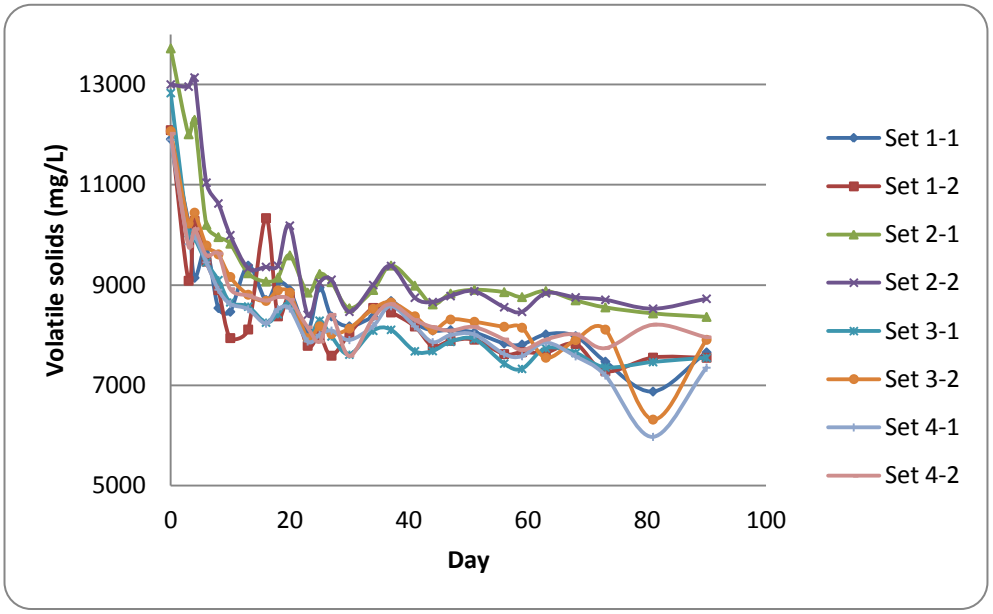


Figure 4-18: VS vs time for 2.5 L anaerobic batch reactors

Figure 4-19 represents the mL of methane gas produced per gram of VS removed in all sets of reactors. The values for Set 1-1, Set 3-2 and Set 4-1 have not been considered since their gas production data is not realistic. Abiotic reactors of Set 2 produced no methane during operation period which results in zero mL CH_4 / g VS removed values for these reactors. When Set 1-2, Set 3-1 and Set 4-2 reactors are taken into consideration, the ratios of 2.5 mg/L NP2EO containing reactor (Set 4-2) and live control of Set 1-2 are very similar to each other followed by 0.5 mg/L NP2EO containing Set 3-1 reactor. These results are parallel to cumulative methane production results shown in Figure 4-15.

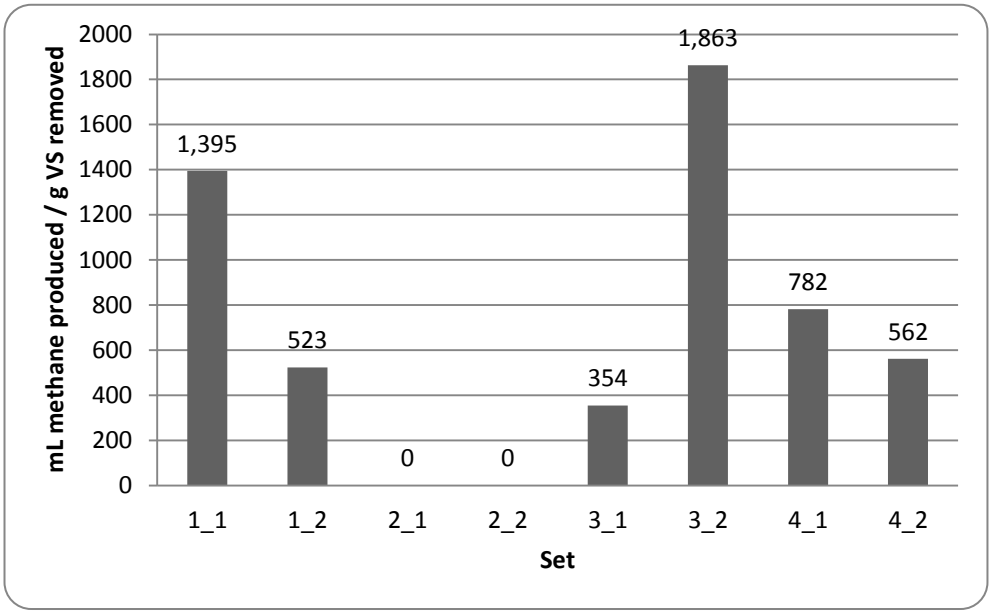


Figure 4-19: mL CH_4 produced per g VS removal for 2.5 L anaerobic batch reactors

Chemical Oxygen Demand (COD)

COD variation was monitored throughout the operation period. The graph in Figure 4-20 illustrates this variation for eight reactors. In Day 4, with the addition of NP2EO and acetone to the reactors, an increase in the COD values were observed. Starting from that point, a decreasing pattern is seen in the graphs. The abiotic control reactors can be seen at the top since they do not have any microbial activity and COD reduction in these reactors therefore, is lower as compared to live controls. In other sets, the average COD concentration was decreased from 22492 mg/L to 14927 mg/L throughout the operation period.

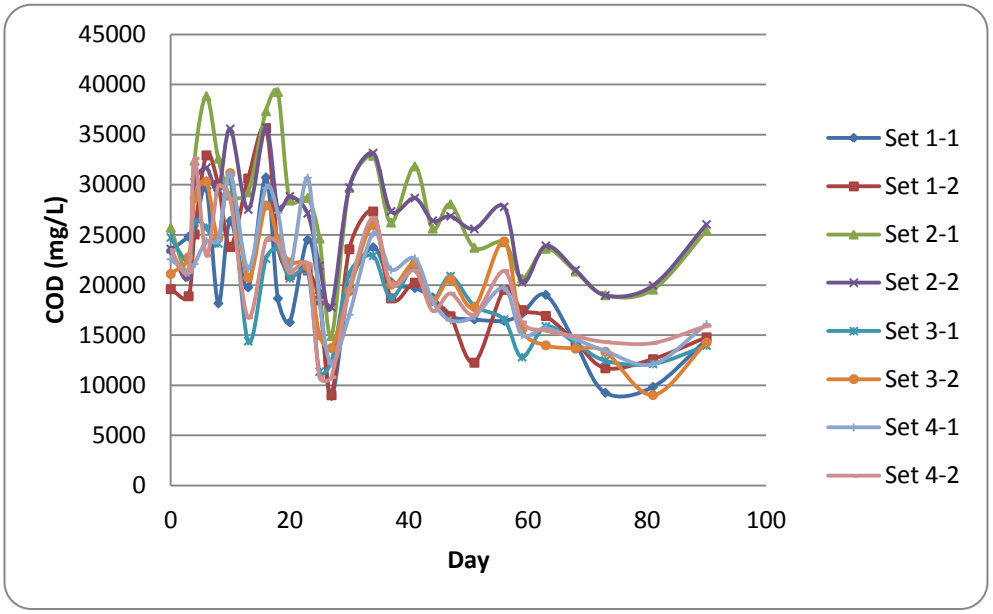


Figure 4-20: COD vs time for 2.5 L anaerobic batch reactors

Concentrations of NP compounds

The concentrations of NP, NP1EO and NP2EO was monitored in the solid and liquid phases of the samples taken from the reactors. In this section, the concentrations of NP compounds throughout the operation period will be given separately in solid and liquid phases in addition to a mass balance analysis done on the solid phase since the liquid phase concentrations were calculated to be very low. The analyses were conducted in GC/MS system details of which are provided in the Materials and Methods section.

The following graphs of Figure 4-21 to 4-24 represents the solid phase distribution (as mg/L) of NP, NP1EO and NP2EO for the operation period of reactors. Similar graphs (in mass/mass basis) that were prepared by using mg compound / kg TS concentration units are presented in Appendix B. As it is seen from the begining points of the graphs of Figure 4-21 – 4-24, the raw sludge in the reactors contains NP, NP1EO and NP2EO in it.

In the live control reactors of Set 1 (Figure 4-21), since no NP2EO was spiked, the existing NP2EO in the raw sludge has undergone degradation very fast in a few days and reported as <LOQ and shown as zero in the graphs. In both of the Set 1 reactors, with the decrease in the NP2EO concentration, increase in the NP and

NP1EO concentration have been observed. During the first 27 days of operation, NP and NP1EO concentration graphs have followed a similar path; however, after 27th day NP concentration began to increase faster than the NP1EO concentration in the system. This is thought to be mainly because of the degradation of NP1EO into NP. In Set 1-1 reactor, the NP concentration went up to about 1.2 mg/L while NP1EO reached 1.4 mg/L. It can be said that, NP have been accumulated in the system around 1.2 mg/L concentration. NP1EO showed a decreasing pattern, after climbing up to 1.4 mg/L, down to 0.4 mg/L in day 90. In the Set 1-2 reactor the same pattern is also followed with different concentration values. In this reactor NP accumulated around 1.1 mg/L while NP1EO peaked at 1.5 mg/L and degraded down to 0.6 mg/L.

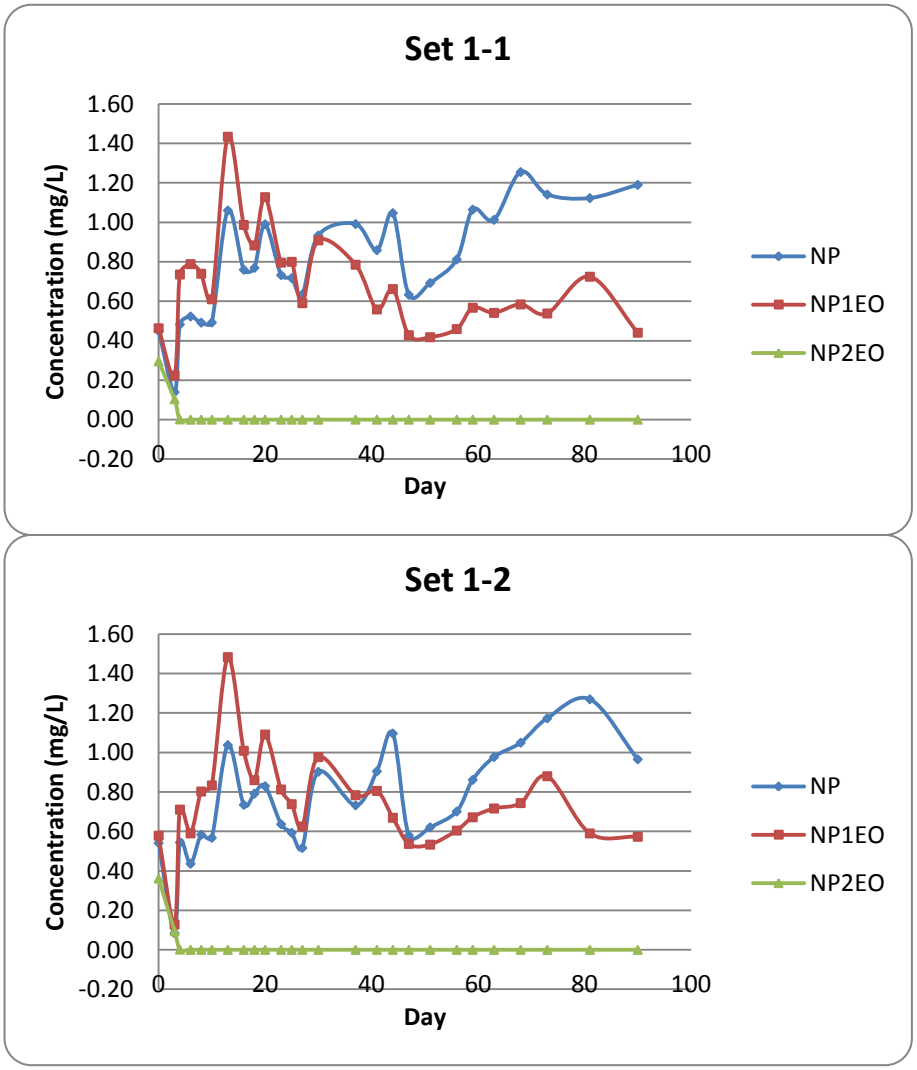


Figure 4-21: Solid phase distribution of NP, NP1EO and NP2EO for Set 1 reactors (upper graph belongs to the reactor suspected of leaking)

In the abiotic control reactors of Set 2 (Figure 4-22), we have 0.5 mg/L of NP2EO in the third day of operation. Since we have maintained perfect abiotic conditions and proved this with the methane production data of these reactors, we did not expect any biodegradation of the NP2EO and NP1EO in these set of reactors. When the graphs are considered, although there are some oscillations in the concentration of NP, there is no significant increasing or decreasing trend throughout the operation period. Moreover, NP1EO and NP2EO follow a straight path except from the increase in the concentration of NP2EO in the day of spiking (day 3). In Set 2-1

reactor, NP concentration oscillated between 0.4 to 0.8 mg/L between the day 0 and day 90, where NP1EO's concentration changed from 0.2 to 0.6 mg/L. At the 90th day of operation, concentration of NP2EO was measured as 0.2 mg/L which is very close to the concentration in the day of spiking. In Set 2-2 similarly, no increasing or decreasing patterns were observed in all the three compounds' concentrations. NP concentration changed from 0.4 to 0.9 mg/L while these values were 0.3 and 0.8 mg/L for NP1EO. Moreover, concentration of NP2EO was calculated as 0.4 mg/L at day 90.

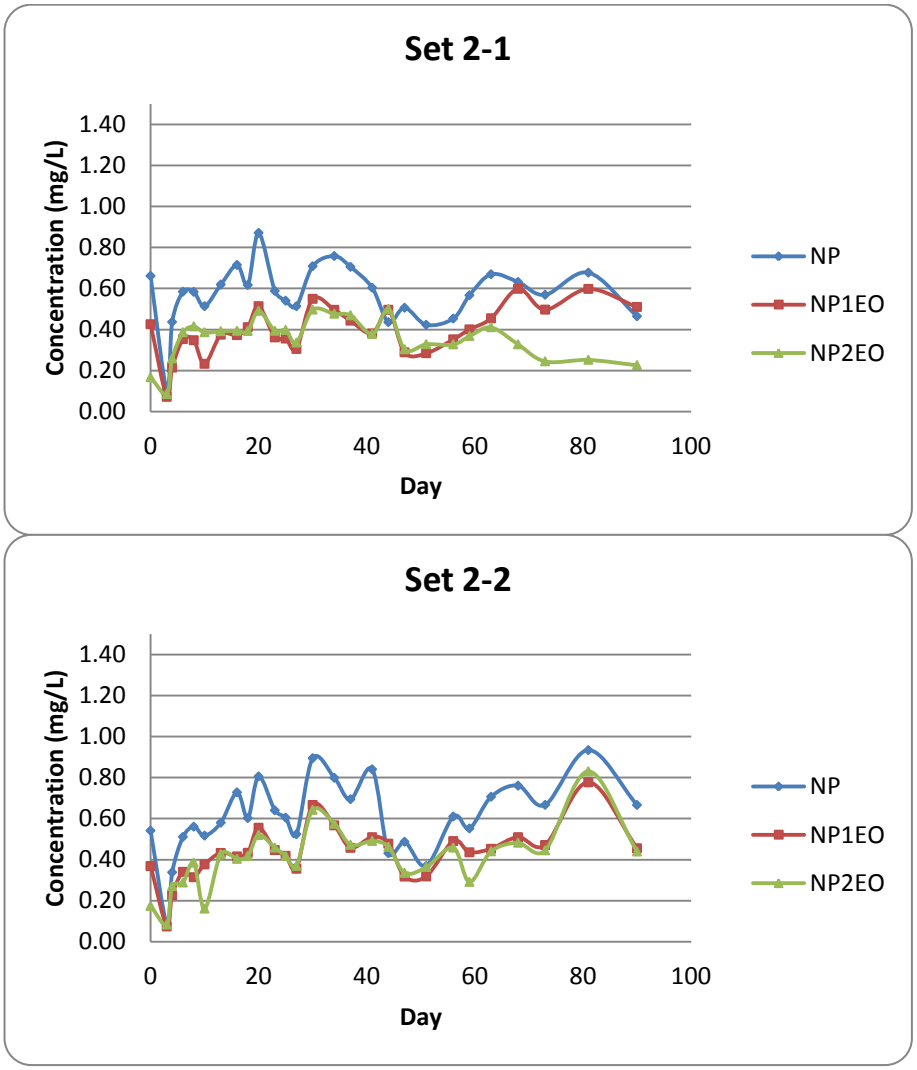


Figure 4-22: Solid phase distribution of NP, NP1EO and NP2EO for Set 2 reactors (upper graph belongs to the reactor suspected of leaking)

Set 3 reactors were spiked with 0.5 mg/L of NP2EO. In Set 3-1 reactor, NP1EO showed an increasing pattern up to day 18 with 0.82 mg/L concentration whereas this value is 0.94 mg/L for Set 3-2 reactor. NP concentration in the reactor 3-1, inceased and peaked at 1.31 mg/L; however it has reached up to 1.2 mg/L in the reactor 3-2. NP2EO concentration has decreased down to about 0.19 and 0.20 mg/L for Set 3-1 and 3-2 reactors, respectively. With these results, it should be stated that, 0.5 mg/L spiking concentration is considered to be rather low that it did not enable us to clearly observe the degradation patterns.

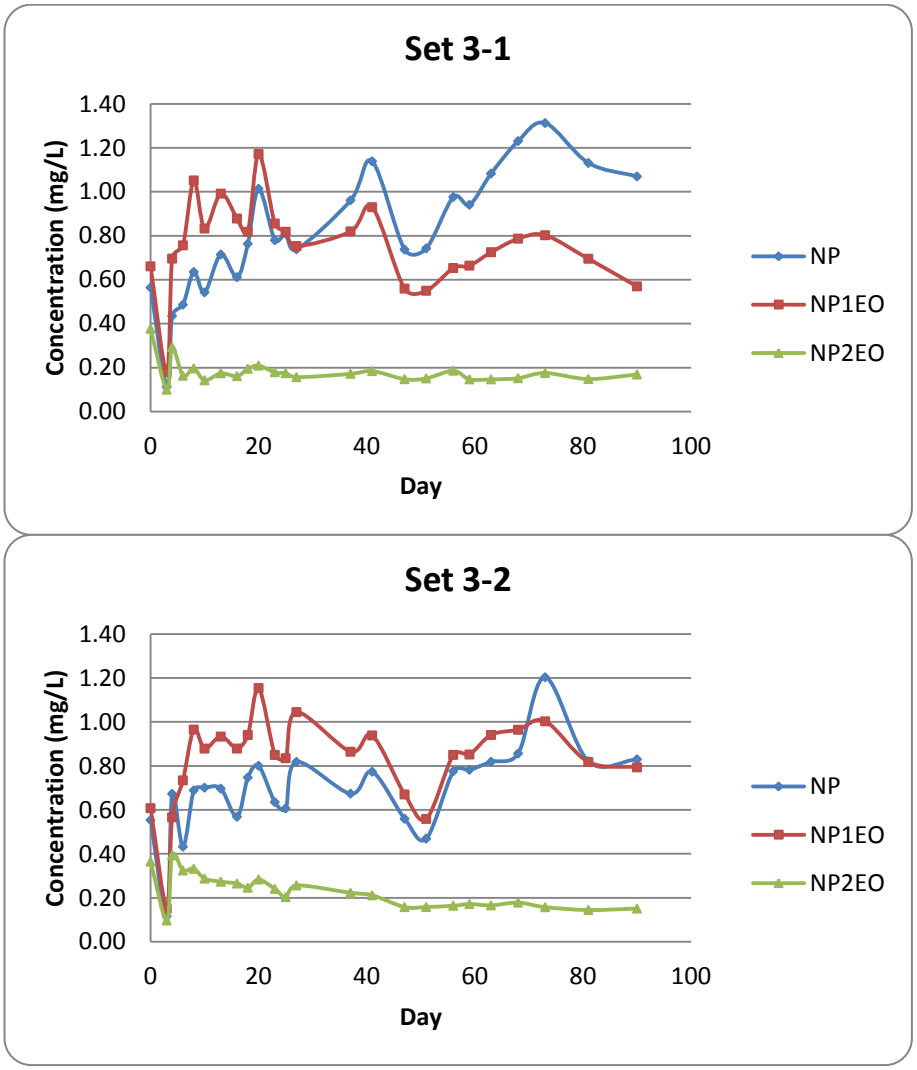


Figure 4-23: Solid phase distribution of NP, NP1EO and NP2EO for Set 3 reactors (lower graph belongs to the reactor suspected of leaking)

In Set 4 reactors (Figure 4-24), 2.5 mg/L of NP2EO was spiked to the reactors at day three. Degradation of NP2EO in Set 4-1 was slower when compared to Set 4-2. We thought that this is due to the differences that can commonly happen in biological systems in replicate reactors. In Set 4-1, NP1EO concentration climbed up to about 2.3 mg/L in the first 41 days of sampling with a few oscillations. After 41 days it began to decrease down to 1 mg/L at day 90. Concentration of NP has also increased, with a lower rate than NP1EO, until the day 90 up to 1 mg/L. In Set 4-2, NP1EO concentration increased up to 2.2 mg/L in the day 16 of sampling with a higher rate as compared to Set 4-1 reactor. Then it followed a decreasing pattern and reached to 1 mg/L in the day 90 of sampling. NP has shown an increase until the end of the operation period and had a concentration of about 1.5 mg/L.

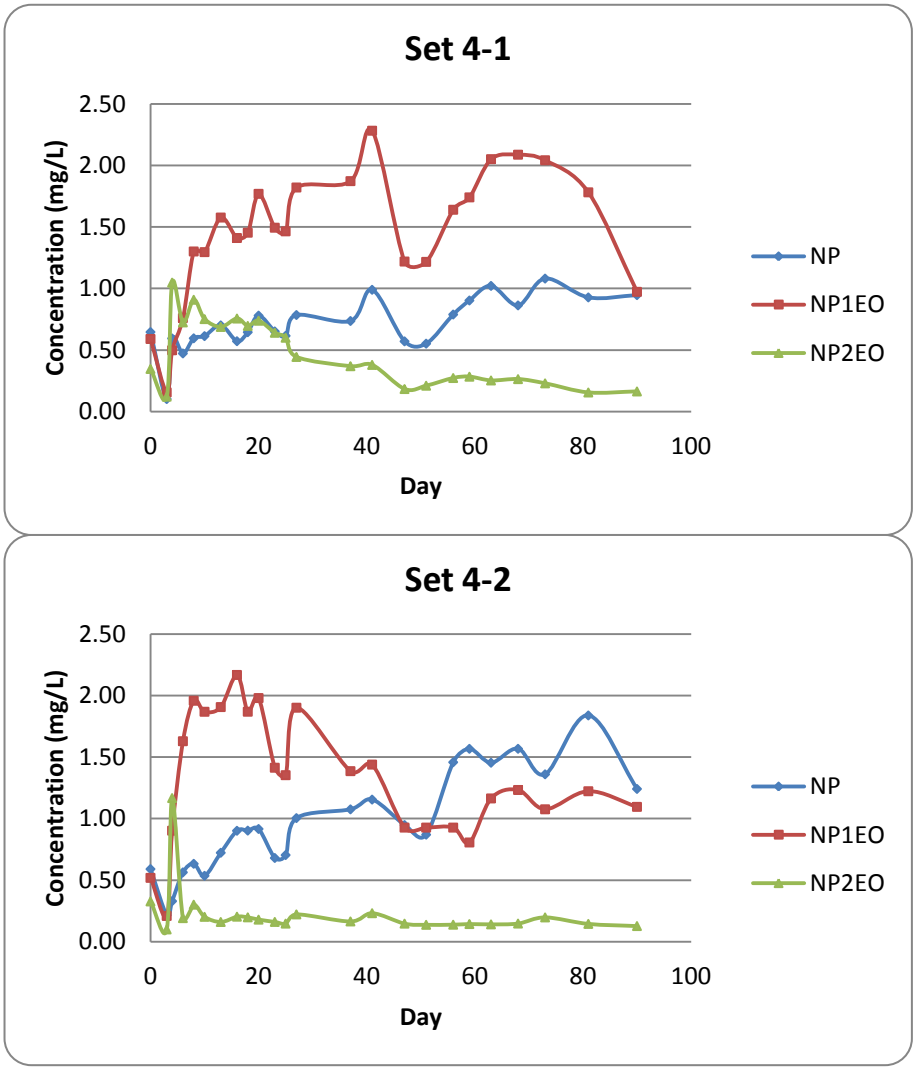


Figure 4-24: Solid phase distribution of NP, NP1EO and NP2EO for Set 4 reactors (upper graph belongs to the reactor suspected of leaking)

From all of our results it is clear that NP2EO rapidly degraded to NP1EO and NP. This trend was observed with the rapid decrease of NP2EO concentration in all reactors and consequent increase in NP1EO and NP concentrations. The increase in NP1EO concentration was observed earlier and faster than the increase observed in NP concentration. This indicates that the first degradation product of NP2EO is NP1EO and this degradation goes faster. The further increase observed in NP concentration and the gradual decrease in NP1EO is an indicator of conversion of NP1EO to NP. The reactors that indicate these transformations best were 3-1 and 4-2. Since the Set 4-2 reactor had sufficiently high concentrations of NP2EO to start with, the trends in the formation of daughter products were more clearly seen. The

decrease in NP2EO and NP1EO are well matching to the information present in literature (Zhang et. al., 2008; Luppi et. al., 2007; Ejlertsson et. al., 1999).

The following graphs of Figure 4-25 – 4-28 represents the liquid phase distribution of NP, NP1EO and NP2EO for the whole operation period. As it is seen from the begining points of the graphs, the raw sludge contains NP, NP1EO and NP2EO in the liquid phase. NP had an average concentration of 0.11 mg/L, where NP1EO and NP2EO have 0.042 and 0.032 mg/L of concentrations respectively. In general, the liquid phase partitioning of NP compounds was found to be very low as it was expected due to their physico-chemical properties. This observation is very similar to our previous observations made during ATA tests.

In general, the concentrations of NP compounds in liquid phase followed a very low trend as compared to that of solid phase. The calculated concentrations are very close to LOQ values (NP: 10 ppb, NP1EO: 9.5 ppb, NP2EO: 7.6 ppb) for all three compounds.

In Set 1 reactors, NP2EO can not be observed in liquid samples after the 8th day of operation since it is completely degraded into its daugter products. NP and NP1EO first show an increasing trend and then their concentrations decreased to around 0.01 mg/L and stabilized at that value.

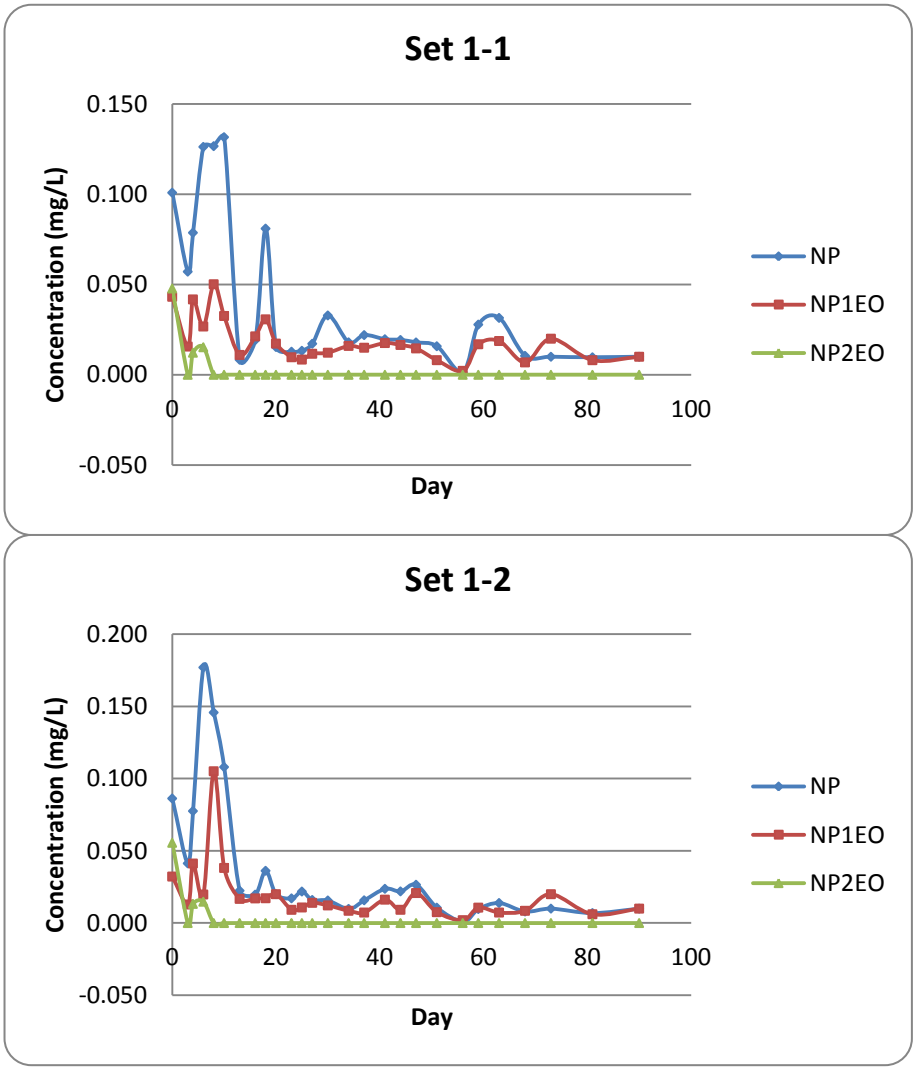


Figure 4-25: Liquid phase distribution of NP, NP1EO and NP2EO for Set 1 reactors

In Set 2 reactors of abiotic controls, the concentration change of the compounds are very minor except the sharp decrease of NP compound after day 10. Some portion of the spiked NP2EO can be seen in the liquid samples in day 3. As no biological activity is present in this set of reactors, the stable trend of the concentration of compounds were expected.

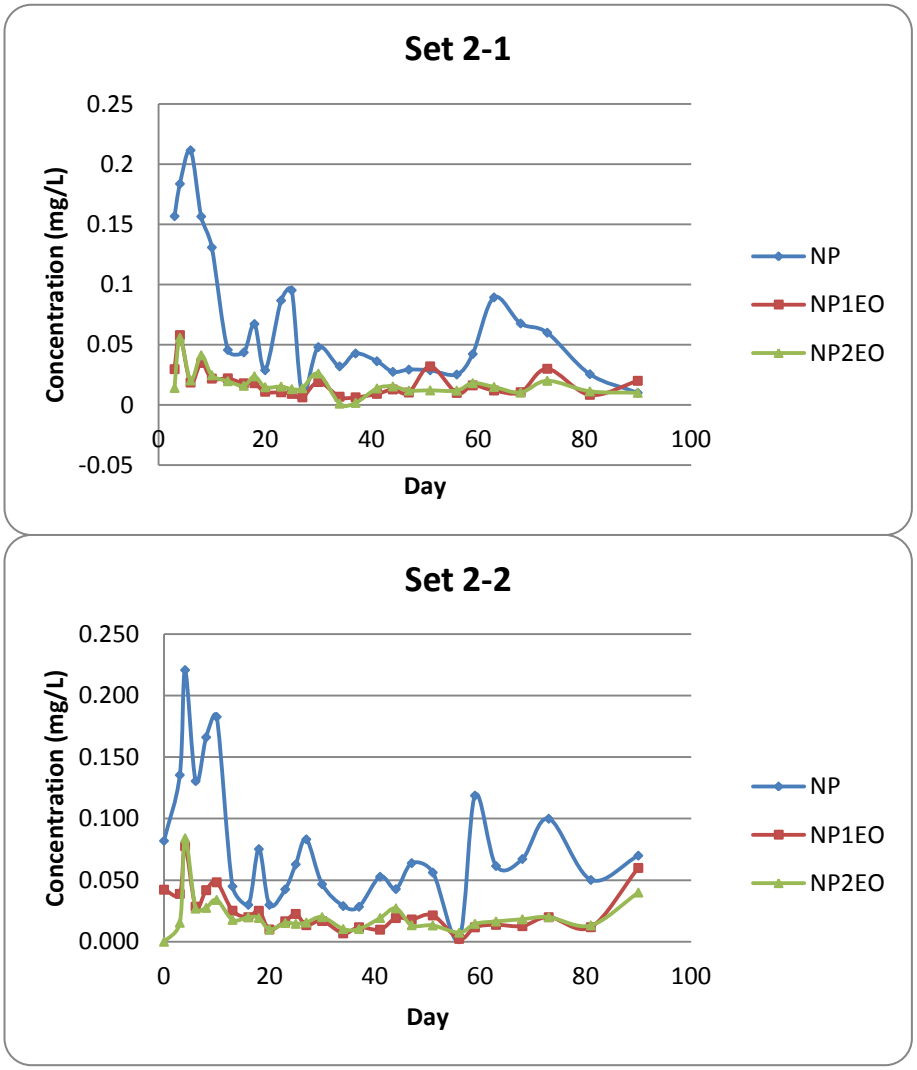


Figure 4-26: Liquid phase distribution of NP, NP1EO and NP2EO for Set 2 reactors

In Set 3 reactors, the concentration of compounds in the liquid phase is very low similar to the other sets of reactors. In both of the reactors, after day 10 with the sharp decrease, the concentrations were stabilized close to LOQ values. In Set 4 reactors, the trend is also similar. However, it should be noted that in 4-2 reactor, the increase in NP1EO concentration is observed consequent to the decrease in NP2EO concentration right after the day of spiking.

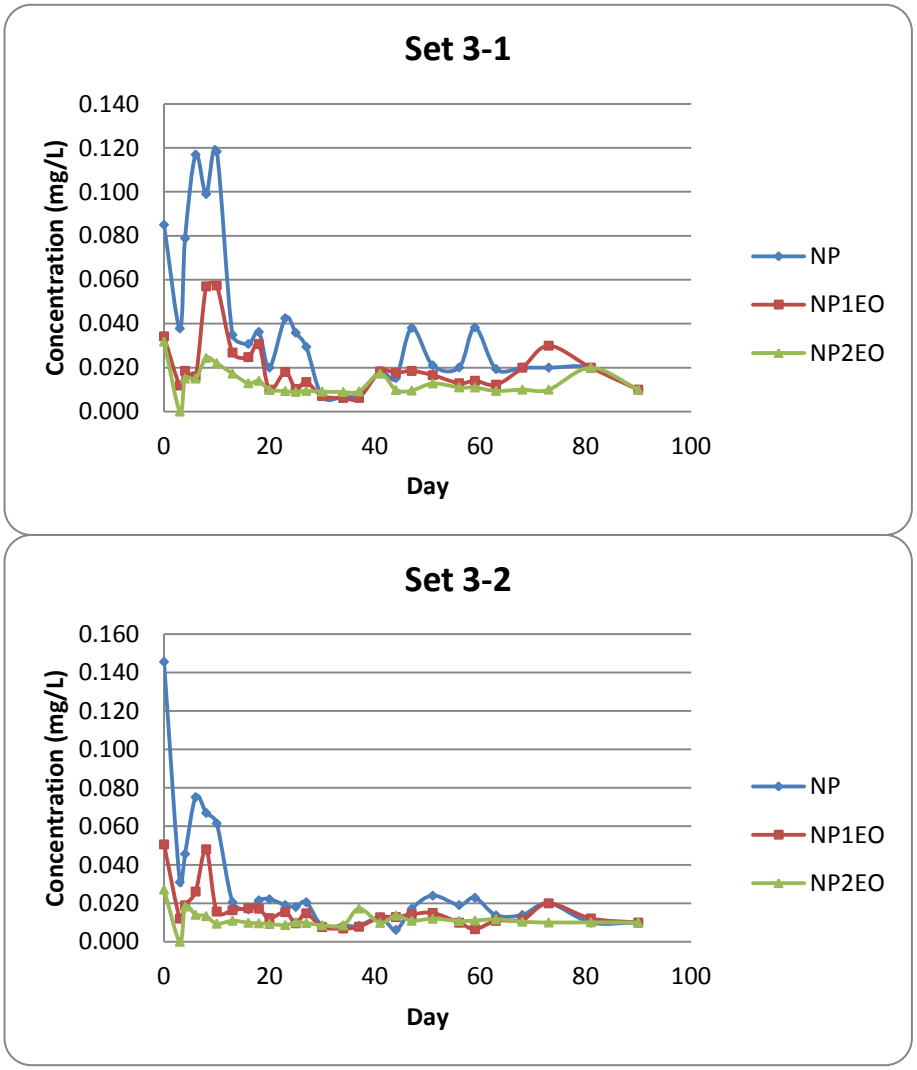


Figure 4-27: Liquid phase distribution of NP, NP1EO and NP2EO for Set 3 reactors

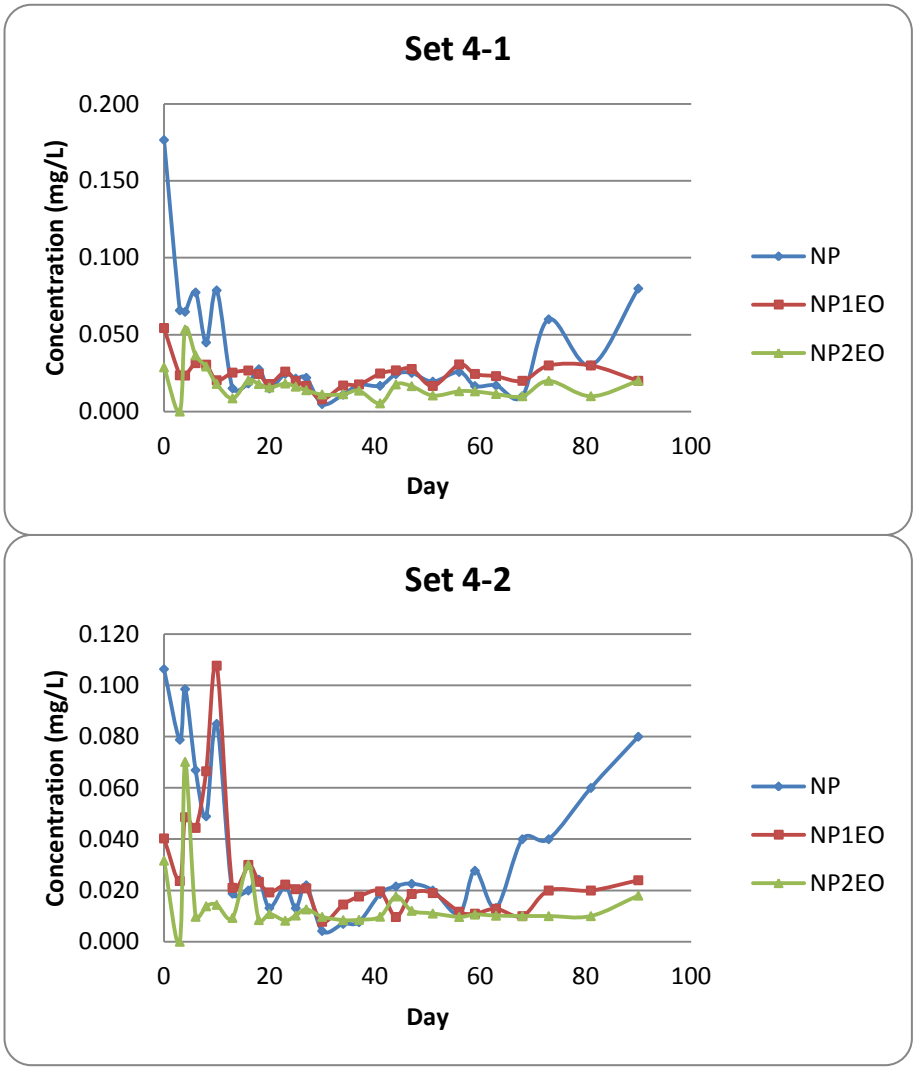


Figure 4-28: Liquid phase distribution of NP, NP1EO and NP2EO for Set 4 reactors

Mass Balance Over the Reactor Operation Period

With the data collected for the whole reactor operation period, a mass balance of the total concentration of NP compounds analyzed is conducted. Appendix B shows the detailed mass balance calculations given for each reactor. Here, the concentration of chemicals was used rather than their mass with the same reasons given in the ATA results part. For each sampling time total concentration of NP, NP1EO and NP2EO was calculated. Then the percent deviations with respect to t=0 day for Set

1 reactors and t=4 days for the other sets of reactors were calculated. The negative sign represents an increase in the total concentration of three NP compounds whereas the positive values represent a decrease in the total concentration. The findings are reported in Table 4-16.

When Table 4-16 is examined, one can see that based on the total concentrations of chemicals measured all throughout the reactor operation period, total quantity deviates from the initial value in different amounts. When the non-leaking live reactors of Set 1-2, Set 3-1 and Set 4-2 are considered the deviations are lower as compared to that of leaking reactors. Therefore, non-leaking reactors are taken into consideration when mass balance analysis data is examined. In non-leaking reactors, mass balance over the 90 days operation period mostly holds with less than 30-40% deviation except for some data points. For the live control reactor of Set 1-2, 3 data points of days 3, 13 and 34 exceeds 40% deviation. When 0.5 mg/L NP2EO containing Set 3-1 reactor is considered, sampling days of 20, 41 and 73 have deviations higher than 40%. Set 4-2 reactor has the most reliable mass balance due to the fact that the concentration of NP2EO is higher than other reactor sets which results in lower deviations from the initial concentrations of NPEs. In the case of reactor 4-2, the only sampling point, which has a deviation higher than 30% is day 16. These results indicate that as one compound transforms into another one, the overall mass balance is still kept. Abiotic control reactors of Set 2 on the other hand, have the highest deviations of all the reactor sets, due to the low concentrations of compounds present in the system.

Table 4-16: Mass balance on 2.5 L anaerobic batch reactors

	Set 1-1		Set 1-2		Set 2-1		Set 2-2	
Day	NPE (mg/L)	% deviation	NPE (mg/L)	% deviation	NPE (mg/L)	% deviation	NPE (mg/L)	% deviation
0	1.21		1.48		1.25		1.08	
3	0.47	61.37	0.31	79.21	0.26		0.25	
4	1.22	-0.91	1.26	15.23	0.91		0.84	
6	1.31	-8.49	1.03	30.70	1.32	-45.64	1.14	-36.35
8	1.23	-1.87	1.39	6.44	1.35	-48.07	1.26	-50.79
10	1.10	8.78	1.40	5.40	1.13	-24.56	1.06	-26.22
13	2.49	-106.37	2.52	-70.21	1.39	-52.42	1.44	-72.21
16	1.75	-44.50	1.74	-17.72	1.48	-62.91	1.55	-85.12
18	1.65	-36.71	1.65	-11.50	1.42	-56.33	1.45	-73.95
20	2.12	-75.25	1.92	-29.65	1.88	-106.37	1.88	-125.06
23	1.53	-26.48	1.45	2.14	1.34	-47.75	1.55	-84.92
25	1.52	-25.56	1.33	10.11	1.29	-42.28	1.44	-72.43
27	1.23	-1.38	1.14	22.93	1.15	-27.02	1.25	-49.45
34	2.14	-76.90	2.31	-55.59	1.73	-90.41	1.94	-132.22
37	1.78	-47.01	1.52	-2.36	1.62	-77.99	1.62	-94.32
41	1.42	-17.30	1.71	-15.48	1.36	-49.88	1.84	-120.22
44	1.71	-41.39	1.77	-19.21	1.43	-57.55	1.37	-64.21
47	1.06	12.19	1.12	24.59	1.10	-20.83	1.14	-36.40
51	1.11	8.10	1.15	22.07	1.04	-13.90	1.05	-25.80
56	1.27	-5.18	1.31	11.85	1.13	-24.58	1.56	-86.51
59	1.63	-35.02	1.53	-3.52	1.34	-46.94	1.28	-53.03
63	1.55	-28.58	1.69	-14.34	1.53	-68.88	1.60	-91.40
68	1.84	-52.16	1.79	-21.07	1.56	-71.37	1.75	-109.63
73	1.68	-38.94	2.05	-38.61	1.31	-44.20	1.58	-89.44
81	1.85	-52.84	1.86	-25.55	1.53	-68.03	2.54	-204.01
90	1.63	-34.92	1.54	-3.87	1.20	-32.04	1.56	-86.78

Table 16 continued

	Set 3-1		Set 3-2		Set 4-1		Set 4-2	
Day	NPE (mg/L)	% deviation	NPE (mg/L)	% deviation	NPE (mg/L)	% deviation	NPE (mg/L)	% deviation
0	1.60		1.52		1.58		1.44	
3	0.39		0.36		0.38		0.53	
4	1.42		1.63		2.14		2.40	
6	1.40	1.31	1.49	8.66	1.96	8.58	2.39	0.61
8	1.88	-32.24	1.99	-21.68	2.81	-31.03	2.89	-20.54
10	1.52	-6.53	1.87	-14.38	2.66	-24.27	2.61	-8.62
13	1.88	-32.05	1.90	-16.60	2.97	-38.50	2.79	-16.27
16	1.65	-15.90	1.71	-4.89	2.74	-27.90	3.27	-36.37
18	1.78	-24.71	1.93	-18.45	2.79	-30.53	2.97	-23.77
20	2.39	-68.21	2.24	-37.12	3.29	-53.56	3.08	-28.16
23	1.81	-27.39	1.73	-5.71	2.79	-30.12	2.26	6.01
25	1.80	-26.57	1.64	-0.73	2.68	-25.16	2.21	8.11
27	1.65	-15.69	2.12	-29.85	3.05	-42.43	3.13	-30.35
37	1.95	-37.13	1.76	-7.87	2.98	-39.07	2.63	-9.45
41	2.25	-58.23	1.92	-17.84	3.65	-70.54	2.83	-17.78
47	1.44	-1.38	1.39	15.03	1.97	7.84	2.02	15.70
51	1.44	-1.32	1.19	27.37	1.98	7.58	1.82	24.40
56	1.81	-27.47	1.79	-9.64	2.70	-26.18	2.76	-15.06
59	1.75	-22.97	1.81	-10.62	2.93	-36.70	2.95	-22.69
63	1.95	-37.27	1.93	-17.95	3.33	-55.33	2.67	-11.33
68	2.17	-52.35	2.00	-22.47	3.21	-50.11	2.94	-22.45
73	2.29	-60.88	2.36	-44.82	3.35	-56.59	2.66	-10.59
81	1.97	-38.67	1.78	-9.24	2.86	-33.80	3.12	-29.95
90	1.81	-26.97	1.77	-8.67	2.08	2.72	2.10	12.65

CHAPTER 5

CONCLUSION

The conclusions of the entire study are summarized below in this chapter. The study covered mainly three parts; one being the preliminary experiments of BMP tests and the other two were ATA tests and larger scale anaerobic batch reactor experiments.

The BMP tests were conducted in order to analyze the effect of BM on gas production and composition; moreover, methane production potential of WAS was determined. The reactor set that do not contain BM showed a very parallel, even slightly higher cumulative methane production pattern when it is compared with BM containing reactor set. The comparison was also done in terms of VS reductions. BM addition did not affect the VS reduction significantly. While the reactors containing BM has 43% VS reduction, this value was calculated as 45% in the reactors that do not contain BM. As a result of these preliminary tests, it was decided not to use BM in the further anaerobic reactor studies.

The concentrations of NP compounds throughout the study were analyzed in GC/MS instrument following the derivatization of the samples. The NP compounds, prior to GC/MS analyses, were extracted from liquid and solid phases of the sludge samples.

The second part of the study was the operation of ATA test reactors with NP2EO addition. First set of ATA reactors were dosed with NP2EO within 1 – 30 mg/L concentration range. The aim of keeping the maximum concentration spiked to the reactors as high as 30 mg/L was to test if any toxic effect of NP2EO would be seen on the anaerobic microorganisms. However, when methane gas production profiles are considered, no toxic or significant inhibitory effect was observed. Moreover, NP2EO addition increased the methane production potential when they are compared with the reactors having only acetone. The initial samples of the ATA test

reactors was analyzed in terms of their NP compounds content. NP and NP1EO was found in the sludge although they were not spiked to the reactors. This was explained as they were originated from raw sludge. Moreover, the samples were also containing NP2EO similar to the doses that were spiked to the reactors. At the end of the operation period, NP2EO was not detected neither in solid phase, nor liquid phase samples where NP and NP1EO concentrations increased compared to initial values. In abiotic reactors on the other hand, NP2EO did not degrade and observed in both initial and final sludge samples. Moreover, NP and NP1EO concentrations remained almost the same. This case was explained with the biodegradation of NP2EO into NP and NP1EO under anaerobic conditions. When the presence of NP compounds in liquid and solid phases of sludge are compared, it was observed that they mainly partitioned on the solid phase of sludge. This result was an expected result because of the hydrophobic and lipophilic characteristics. An overall mass balance analysis revealed that the sum of NP2EO, NP1EO and NP in the system did not change significantly which showed that even when one component degraded to the other, the overall mass balance was maintained.

ATA tests were also conducted by spiking NP in two different forms in different concentrations. In one test, the branched NP, in the other test a straight chain NP were used. In the first test, which was conducted with a branched NP, NP addition caused a decrease in the methane production potentials of the anaerobic microorganisms. The control reactor which did not contain NP on the other hand, showed a higher methane production potential as compared to all the other sets. In abiotic control reactors, we did not observe any methane production during the operation period. Similarly, the straight chain NP which was spiked to the last ATA test reactors of the study, decreased the methane productions regardless of the dose of the NP. When all the ATA test reactor sets were considered, NP both in branched or straight chain form, is believed to be more toxic to anaerobic microorganisms as compared to NP2EO. Furthermore, NP2EO addition affected the methane productions positively, which in return concluded as its contribution to the anaerobic system as a substrate for microorganisms.

The last part of the study covered the operation of 2.5 liter anaerobic batch reactors. In this part, NP2EO, with the selected non-toxic doses of 0.5 and 2.5 mg/L, were added to the anaerobic batch reactors and the changes in the system in terms of

VS, TS, VSS, TSS, pH, COD and concentrations of NP compounds were observed with respect to time. Moreover, volume and composition of the produced gas were observed throughout the operation period.

Live control reactors had the highest methane production profile among the others. Abiotic control reactors on the other hand, did not produce any methane as expected. NP2EO addition (in acetone) has lowered the methane production as compared to live control reactors. pH of the reactors were stable between 7 – 8 which is in the allowable range for anaerobic systems. TS and VS values for the reactors showed a decreasing pattern and stabilized through the end of the operation period around 16,000 and 7,500 mg/L for the live reactors, respectively. When COD values of the reactors are considered, after a sharp increase in the spiking day, a decreasing pattern was observed for the live reactors.

Concentrations of NP, NP1EO and NP2EO were measured in both solid and liquid portions of sludge samples. In general, they were found in solid phase in much higher concentrations as compared to the liquid phase. In the day zero samples, all of the three NP compounds were observed both in solid and liquid phases. This indicated that these compounds were present in the original sludge samples that were used to set up the reactors.

In live control reactors, in the solid phase, NP2EO was degraded in the first days of reactor operation with a subsequent increase in the concentrations of NP1EO and NP. As the operation proceeded, NP1EO concentration peaked and then began to decrease, where NP concentration stabilized after an increase was observed. In the liquid phase, very low concentrations were observed during the operation period for all of the compounds in concern.

In abiotic control reactors, NP2EO was added with a concentration of 0.5 mg/L to the reactors. Since there was no biological activity in the reactors, degradation of NP2EO into NP1EO and NP was not observed within the operation period. The present amounts of NP compounds in the raw sludge together with the spiked amount of NP2EO, was found to be present in the reactor at the end of the operation period.

The third and fourth sets of reactors were spiked with 0.5 and 2.5 mg/L NP2EO, respectively. The spiked and already present NP2EO in the reactors were degraded into NP1EO and NP throughout the operation period. An increase in the concentration of NP1EO was followed by an increase in the concentration of NP. Towards the end of the operation, while NP1EO's concentration was decreasing, NP showed a nearly stable pathway.

When a mass balance was constituted for all the four sets of reactors, it was observed that the live reactors of Set 1, 3 and 4 maintained the initial total amount of NP, NP1EO and NP2EO (originating from the sludge and spiked amounts) present in the system during the operation period generally with not more than 30-40% deviation. This fact clearly showed their sequential degradation and a final accumulation of the end product, which is NP, in the system.

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APPENDIX A

CALIBRATION CURVES

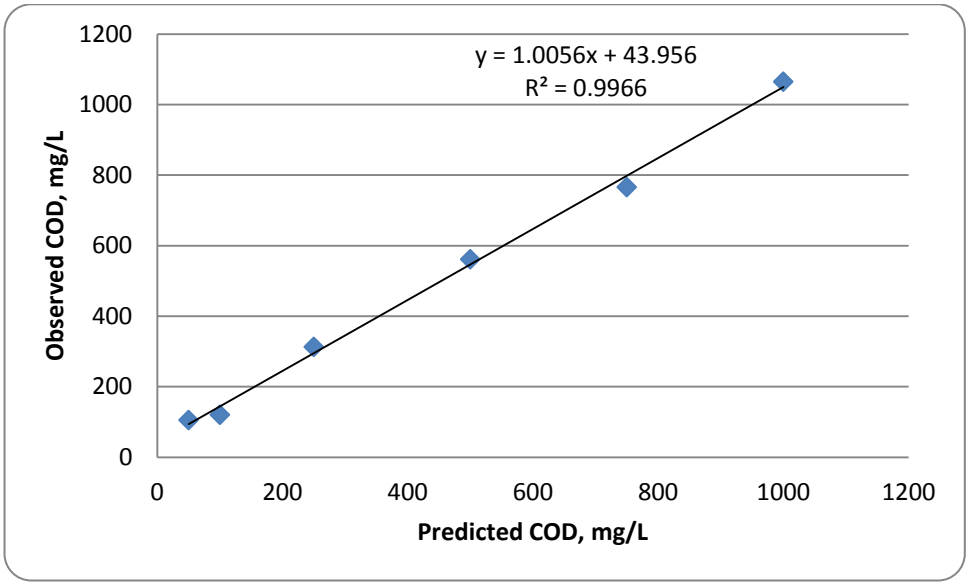

Figure A-1: Calibration curve for laboratory prepared COD solution

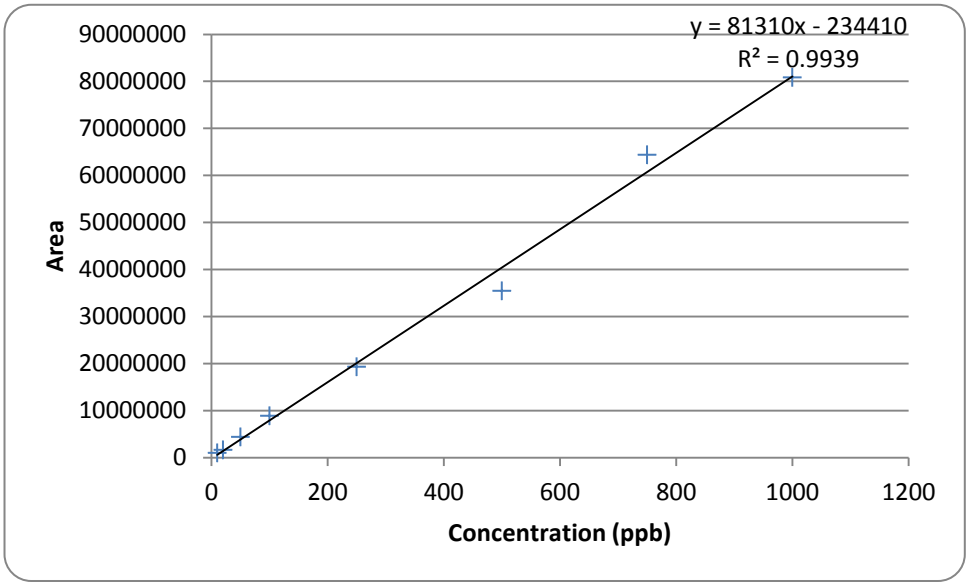

Figure A-2: Calibration curve for derivatized NP

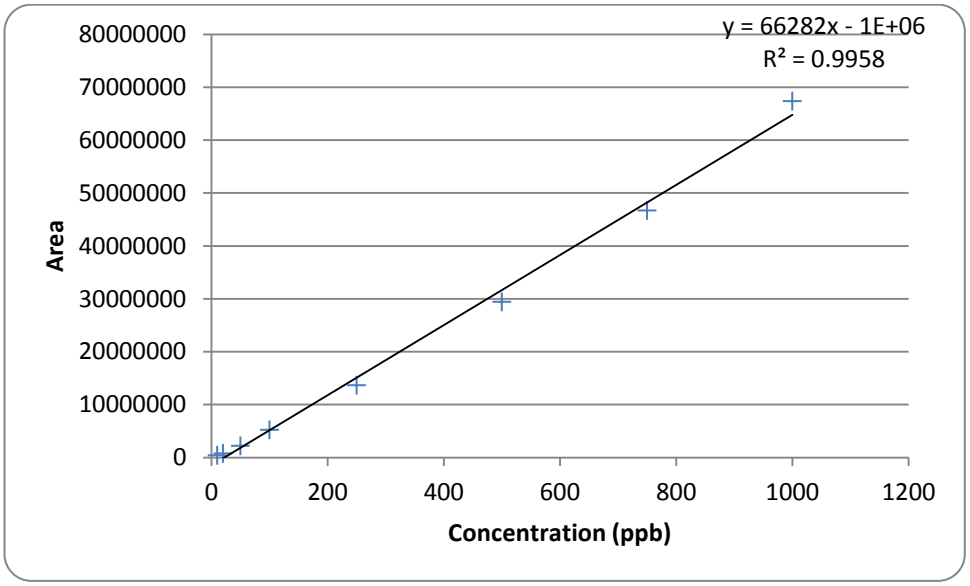

Figure A-3: Calibration curve for derivatized NP1EO

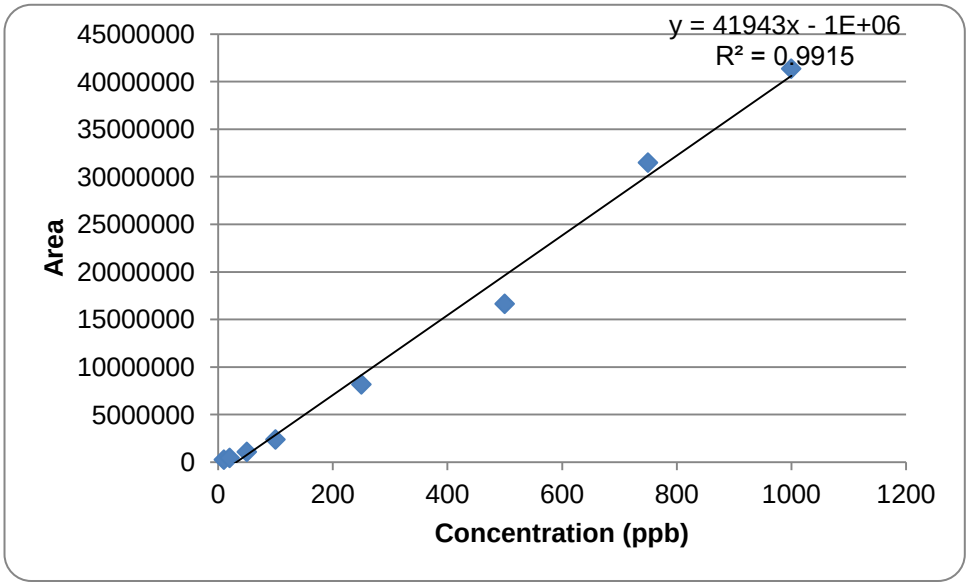

Figure A-4: Calibration curve for derivatized NP2EO

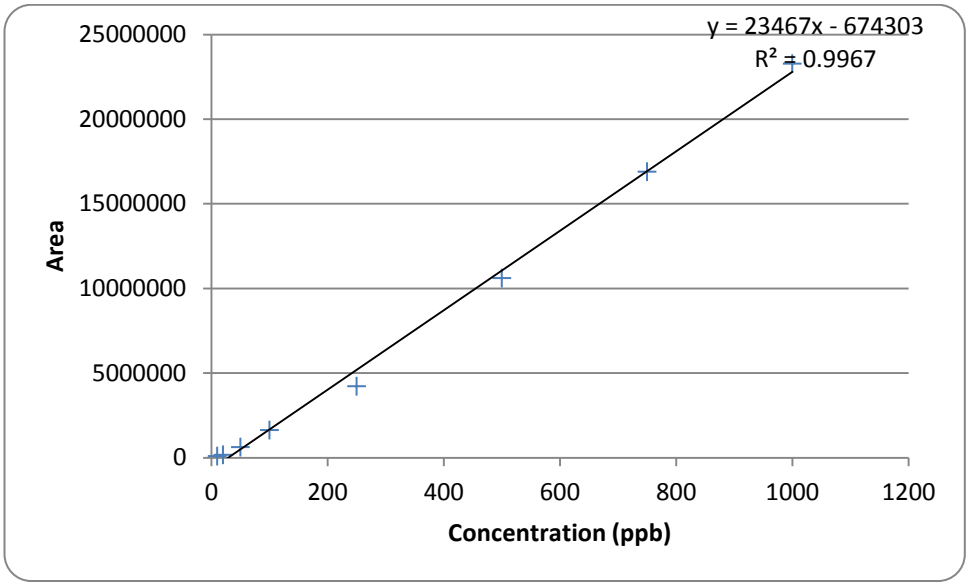

Figure A-5: Calibration curve for derivatized NP1EC

APPENDIX B

DATA OF 2.5 L ANAEROBIC BATCH REACTORS

Table B-1: Methane percentages in the days of gas samplings for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
1	44.57	43.69	0.00	0.00	38.47	49.72	45.27	35.91
2	53.19	52.06	0.00	0.00	38.71	58.01	57.28	43.06
3	52.25	66.83	0.00	0.00	47.21	64.24	67.21	57.99
7	70.35	68.22	0.00	0.00	53.64	65.73	70.13	63.48
10	55.69	66.14	0.00	0.00	35.23	57.42	66.86	65.30
15	62.43	70.40	0.00	0.00	17.28	64.39	68.24	62.92
19	32.49	66.22	0.00	0.00	12.58	48.79	61.73	64.14
25	29.78	62.27	0.00	0.00	13.68	39.61	56.26	61.51
30	23.26	55.19	0.00	0.00	14.66	34.15	51.66	58.44
36	18.49	52.97	0.00	0.00	11.13	29.36	48.40	46.76
42	20.98	50.40	0.00	0.00	9.97	26.16	46.91	37.01
47	20.22	46.70	0.00	0.00	9.10	28.47	43.21	33.21
52	18.73	43.24	0.00	0.00	9.06	27.60	41.00	34.38
61	17.39	37.96	0.00	0.00	9.86	22.38	36.45	32.54
70	27.81	32.39	0.00	0.00	21.59	21.45	33.91	24.46
85	20.14	34.25	0.00	0.00	16.45	53.33	29.90	28.28
90	19.59	33.28	0.00	0.00	13.87	54.73	27.03	27.54

Table B-2: pH values in the days of sampling for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
0	7.02	6.96	9.30	9.16	7.10	6.90	6.98	7.07
3	7.14	7.29	7.58	8.62	7.45	7.31	7.32	6.89
4	7.1	7.32	7.41	8.31	7.34	7.25	7.21	6.74
6	7.47	7.35	7.03	7.09	7.53	7.49	7.52	7.14
8	7.39	7.46	7.31	7	7.62	7.39	7.52	7.25
10	7.5	7.74	7.41	7.12	7.84	7.45	7.74	7.74
13	7.58	7.59	7.49	7.18	7.97	7.7	7.77	7.69
16	7.66	7.71	7.79	7.13	7.79	7.88	7.76	7.53
18	7.9	7.72	7.86	7.37	8.09	7.98	7.79	8.01
20	7.88	8.26	8.35	7.52	8.31	8.15	8.16	7.96
23	7.53	7.65	7.71	7.02	7.8	7.67	7.72	7.49
25	7.59	7.82	7.54	7.1	7.96	7.74	7.69	7.62
27	7.75	7.74	7.49	7.01	7.85	7.72	7.69	7.65
30	7.73	7.75	7.46	7.2	7.81	7.76	7.75	7.62
34	7.76	7.65	7.33	7.13	7.85	7.69	7.65	7.61
37	7.72	7.64	7.44	7.12	7.91	7.69	7.67	7.66
41	7.71	7.66	7.4	7.13	7.89	7.66	7.67	7.68
44	7.47	7.49	7.29	6.97	7.74	7.45	7.48	7.48
47	7.63	7.57	7.35	7.01	7.79	7.6	7.54	7.51
51	7.6	7.49	7.37	6.96	7.73	7.56	7.54	7.39
56	7.63	7.56	7.58	6.95	7.75	7.52	7.42	7.5
59	7.59	7.5	7.52	6.99	7.71	7.49	7.51	7.42
63	7.62	7.57	7.74	7.13	7.79	7.44	7.5	7.43
68	7.56	7.53	7.51	7.17	7.53	7.5	7.82	7.36
73	7.92	7.88	7.75	7.45	7.81	7.74	8.25	7.68
81	7.76	7.78	7.67	7.43	7.79	7.54	7.97	7.48
90	7.48	7.57	7.51	7.11	7.52	7.36	7.77	7.6

Table B-3: TS values in the days of sampling for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
0	20090	20340	23640	21350	21770	20570	20010	20520
3	18764	16467	20557	21509	18374	20760	18073	18129
4	16568	18779	20699	21921	18643	21372	18429	18630
6	18223	17861	18705	20010	17999	20433	17897	18069
8	16267	17332	18443	19639	17964	20424	17573	18675
10	16445	15338	19042	18838	17276	19777	17211	17496
13	18450	18756	17803	17920	17090	19312	17450	17333
16	17259	21103	17730	18082	16821	19260	16666	17594
18	17736	16617	17922	18027	17190	19462	17408	17181
20	17166	16900	17928	17619	16968	19008	16931	16898
23	16441	15637	17461	16417	16488	17846	16398	16427
25	18444	16433	17688	17594	16825	18343	16704	15964
27	16615	15177	17305	17514	15993	17674	16461	16901
30	16463	16083	16493	16386	15294	18068	16081	15947
34	16714	17289	17164	17343	15867	18847	16669	16727
37	17431	16960	17958	18010	16009	19182	17231	17191
41	16765	16528	17453	16974	16164	18741	16802	16822
44	16189	15575	16339	16652	15280	18218	15959	16380
47	16224	15675	16636	16621	15520	18345	15850	16197
51	16628	16260	17235	17314	15868	18751	16443	16678
56	16559	15993	17568	17259	15907	19002	16046	16609
59	16401	15913	17018	16713	15531	18540	15843	16032
63	16503	15815	17063	17038	16249	17307	16144	16237
68	16689	16238	17188	17508	17525	18412	16363	16845
73	16127	15619	17075	17640	16421	19366	15931	16659
81	14292	15843	16658	16757	15797	14657	12338	16691
90	16495	16322	17376	17517	16571	18860	16101	16706

Table B-4: VS values in the days of sampling for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
0	11910	12090	13720	13000	12830	12070	11900	12020
3	10235	9087	12007	12958	10036	10227	9815	9776
4	9144	10280	12296	13137	9931	10444	9979	10118
6	9722	9467	10201	11042	9452	9788	9453	9594
8	8541	8898	9957	10627	9098	9612	8908	9629
10	8467	7942	9825	9994	8655	9161	8622	8921
13	9388	8113	9235	9338	8570	8807	8520	8794
16	8702	10335	9069	9361	8251	8684	8230	8694
18	9064	8376	9149	9383	8418	8894	8518	8764
20	8900	8771	9591	10185	8622	8848	8533	8706
23	8110	7788	8849	8407	7956	7939	7875	8157
25	8950	7986	9221	9044	8288	8178	8007	7871
27	8374	7590	9058	9105	7975	8023	8091	8409
30	8177	8026	8537	8468	7612	8132	7903	7588
34	8376	8544	8901	9000	8088	8526	8182	8338
37	8681	8453	9388	9380	8107	8653	8578	8620
41	8247	8174	8988	8747	7679	8377	8176	8306
44	8092	7781	8613	8660	7684	8104	7861	8163
47	8102	7887	8830	8781	7866	8312	7998	8093
51	8054	7911	8905	8874	7920	8267	8012	8164
56	7835	7623	8858	8558	7433	8169	7625	7917
59	7814	7660	8760	8465	7327	8149	7580	7705
63	8015	7653	8881	8836	7738	7551	7852	7914
68	7981	7825	8695	8754	7646	7903	7578	8019
73	7472	7280	8554	8705	7357	8111	7196	7743
81	6876	7553	8434	8527	7464	6317	5971	8210
90	7658	7550	8365	8726	7548	7908	7351	7959

Table B-5: TSS values in the days of sampling for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
0	20150	17800	21425	17325	19750	19300	19075	19275
3	17145	15966	18135	19110	17065	17984	17707	17309
4	14208	17669	17220	18832	18082	17393	17790	17235
6	17974	17318	17162	18282	17298	17314	16569	16662
8	15924	17216	16514	17378	16890	17716	16606	17507
10	17800	14092	16433	16844	16488	16698	16209	16321
13	17153	28929	15110	16015	15860	16288	16457	16108
16	16772	20127	15545	15557	16280	16586	16188	16958
18	17045	15962	15504	16329	15888	16367	16562	16447
20	16226	15711	15581	15092	15061	15467	15220	14668
23	15618	15086	13960	16253	15078	15083	14905	15997
25	16788	14856	14965	15797	14831	15681	14978	13543
27	16057	14755	14482	15418	15480	15017	15482	15962
30	15450	15357	14578	15316	15117	15035	15617	15524
34	15945	16294	14857	15743	14865	15014	15397	15471
37	13830	14985	15114	15152	14514	15017	14472	15248
41	15252	14844	14996	15547	15012	15570	15612	15398
44	15477	14770	14823	14831	14423	15431	15357	15229
47	15633	15373	15548	15298	14267	14383	15757	15630
51	15314	14910	15313	15159	15018	15770	15516	15564
56	15113	15007	15113	14486	15009	14421	14516	15267
59	15339	14687	15214	15179	15537	15427	14454	14916
63	15096	13411	15019	14669	14399	14373	13607	14439
68	14646	14768	15770	15489	15760	15659	15597	15510
73	20668	19720	15587	15607	15787	16385	15134	16032
81	12550	15484	16013	12963	15491	11051	11680	15470
90	15263	14714	15250	15147	15591	14963	15524	14965

Table B-6: VSS values in the days of sampling for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
0	11975	10800	11525	9325	11650	11300	11275	11400
3	9170	8681	10215	10813	9066	9687	9587	7263
4	7586	9305	9049	10682	9710	9267	9416	9038
6	9593	8915	8736	9381	8820	9064	8341	8411
8	8645	9111	8809	9192	9035	9688	8604	9180
10	17709	13990	16336	16741	16389	16597	16109	16216
13	8678	14154	7435	8015	7785	8413	8057	8108
16	8511	9508	7495	7752	7949	8207	7753	8478
18	8762	8024	7548	8152	7954	8533	8302	8461
20	7941	7431	7200	6983	7081	7488	7135	7087
23	7582	7375	6497	7944	7120	7351	6821	7795
25	8403	7451	7388	7934	7272	8000	7343	6431
27	7941	7428	7052	7635	7651	7636	7577	8139
30	7486	7579	6849	7182	7257	7132	7431	7514
34	7706	7883	7122	7502	7178	7476	7231	7357
37	6461	7039	7003	6933	6929	7233	6604	7281
41	7519	7178	7202	7401	7226	7606	7422	7532
44	7137	6807	6707	6785	6607	7013	6885	7012
47	7883	7678	7751	8002	7399	7356	7929	7856
51	7446	7218	7369	7398	7413	7888	7247	7370
56	7091	7212	7089	6940	6915	6823	6638	7164
59	7587	7264	7487	7459	7334	7607	6805	7363
63	7272	6442	7170	6870	6368	6722	6084	6743
68	6819	6830	7367	7112	6929	7284	7349	7285
73	9963	9288	7397	7168	7415	7971	7072	7771
81	5794	7332	7850	5721	6925	5410	5319	7556
90	7148	7325	7060	7173	7294	7192	7266	7055

Table B-7: COD values in the days of sampling for 2.5 L anaerobic batch reactors

Day	Set1-1	Set1-2	Set2-1	Set2-2	Set3-1	Set3-2	Set4-1	Set4-2
0	23446	19592	25708	23595	24739	21109	22576	23496
3	24825	18886	21471	20774	22354	22782	21234	21444
4	25266	25045	32385	30238	26280	28672	22090	32633
6	29651	32931	38839	31702	25736	30340	24391	22931
8	18175	30000	32609	29387	24141	24746	24473	29873
10	26400	23788	28921	35575	30568	31160	31104	28570
13	19764	30620	29251	27532	14398	20728	21630	16762
16	30731	35655	37337	35719	22638	27927	29692	24475
18	18661	23878	39240	27599	23709	24628	27201	24211
20	16281	20999	28431	28859	20680	22259	21656	21218
23	24516	21444	28689	27156	21440	21886	30697	22058
25	19214	18453	24635	21931	11360	14979	18528	10811
27	8941	9011	14914	17772	12339	13717	12172	10817
30	19771	23571	29684	29725	21123	19389	17028	19508
34	23775	27333	32890	33168	22911	25987	25146	26720
37	20306	18669	26235	27348	18731	20036	21537	20072
41	19704	20234	31839	28680	21849	22170	22646	21440
44	18768	18449	25629	26413	18574	18452	18409	17433
47	16893	16888	28061	26864	20897	20568	16483	19166
51	16566	12255	23720	25592	17939	17808	16887	17080
56	16439	19522	24263	27796	16561	24347	19721	21412
59	17147	17500	20648	20276	12813	15559	15057	16294
63	19028	16916	23620	23916	15882	13984	15691	15459
68	14200	14326	21348	21492	14213	13655	14579	14893
73	9259	11703	18988	18965	12464	13345	13436	14331
81	9841	12603	19539	19947	12133	9022	12099	14224
90	14533	14784	25402	26044	13962	14263	16096	15930

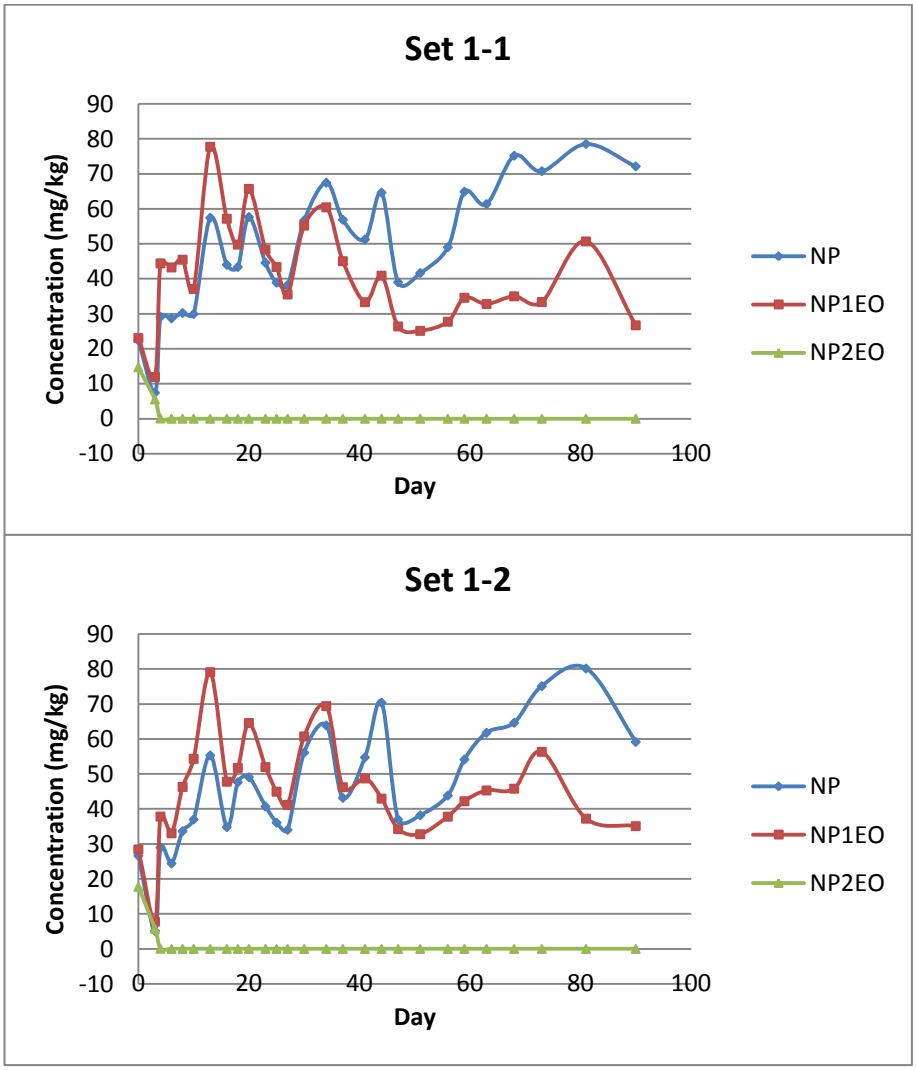

Figure B-1: Solid phase distribution of NP, NP1EO and NP2EO for Set 1 reactors

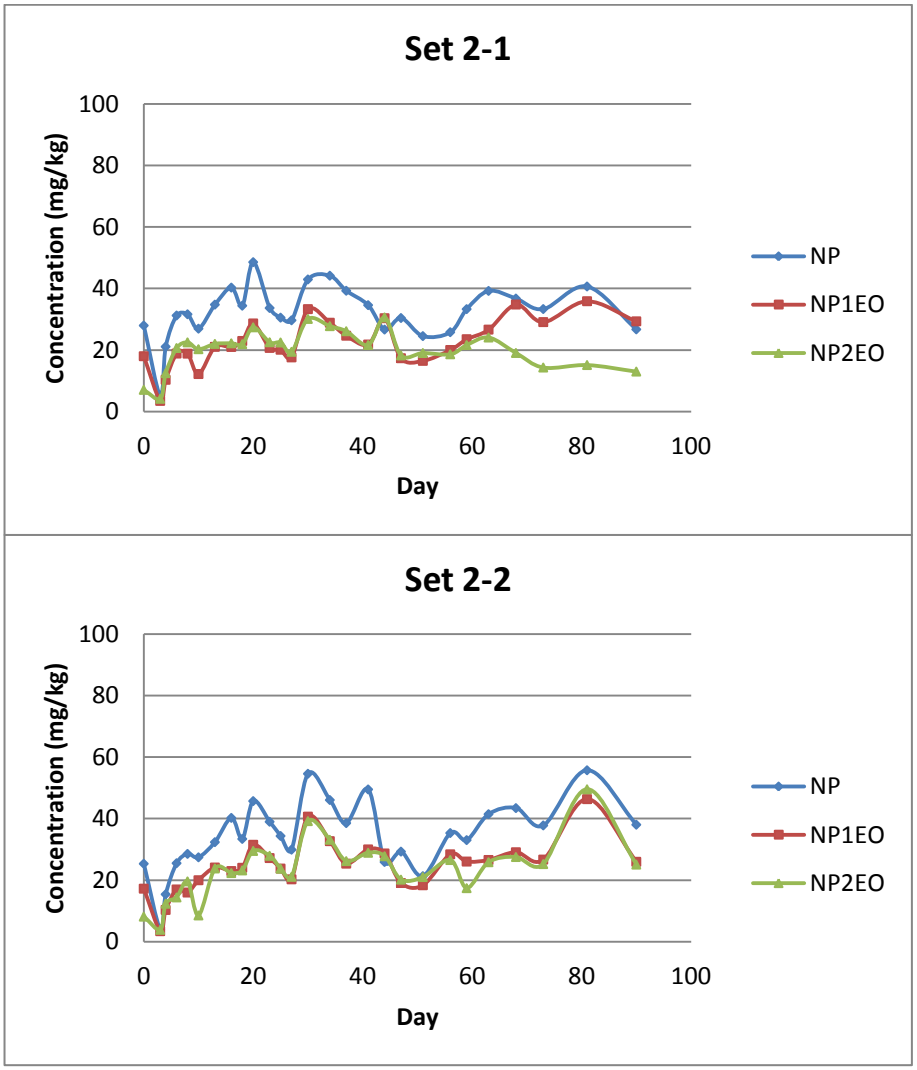

Figure B-2: Solid phase distribution of NP, NP1EO and NP2EO for Set 2 reactors

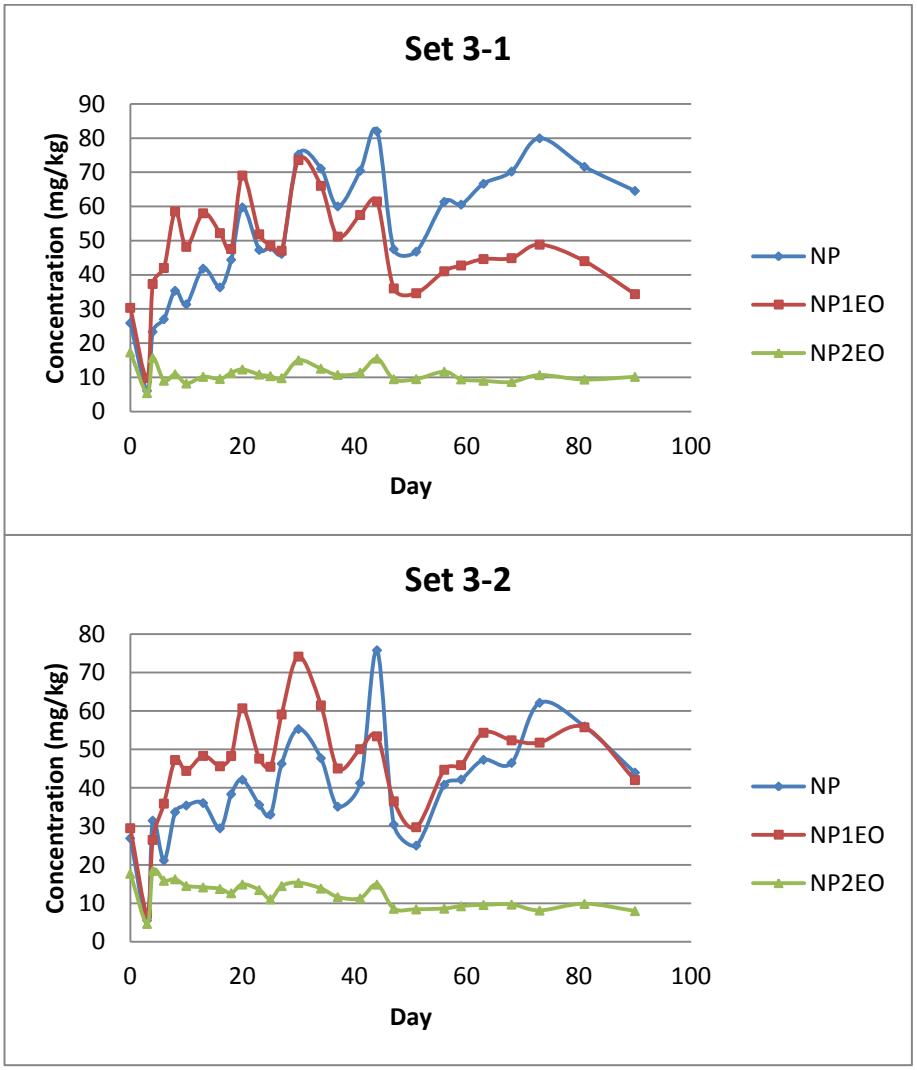

Figure B-3: Solid phase distribution of NP, NP1EO and NP2EO for Set 3 reactors

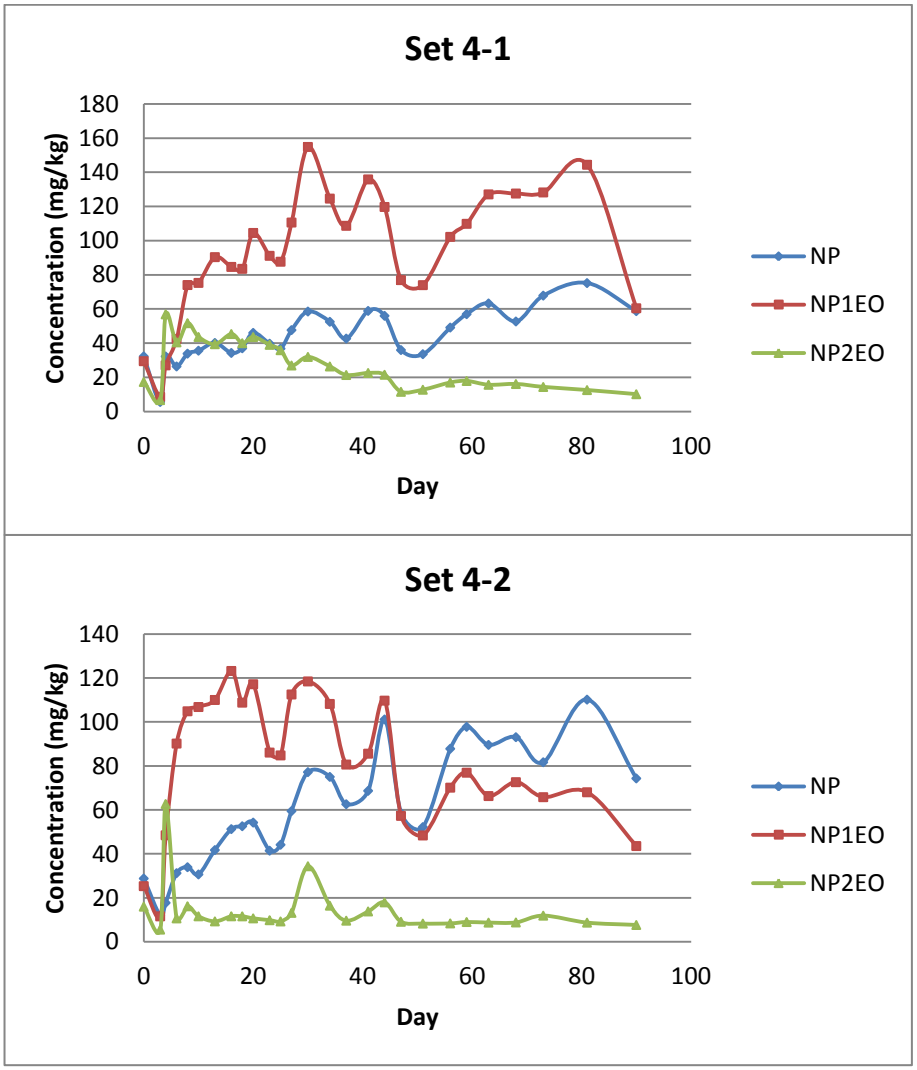

Figure B-4: Solid phase distribution of NP, NP1EO and NP2EO for Set 4 reactors

Table B-8: Mass balance calculations for Set 1-1

	Set 1-1				
Day	NP	NP1EO	NP2EO	TOTAL	% deviation
0	0.45	0.46	0.30	1.21	
3	0.14	0.22	0.10	0.47	61.37
4	0.48	0.74	0.00	1.22	-0.91
6	0.52	0.79	0.00	1.31	-8.49
8	0.49	0.74	0.00	1.23	-1.87
10	0.49	0.61	0.00	1.10	8.78
13	1.06	1.43	0.00	2.49	-106.37
16	0.76	0.99	0.00	1.75	-44.50
18	0.77	0.88	0.00	1.65	-36.71
20	0.99	1.13	0.00	2.12	-75.25
23	0.73	0.80	0.00	1.53	-26.48
25	0.72	0.80	0.00	1.52	-25.56
27	0.63	0.59	0.00	1.23	-1.38
30	0.93	0.91	0.00	1.84	-52.44
37	0.99	0.79	0.00	1.78	-47.01
41	0.86	0.56	0.00	1.42	-17.30
44	1.05	0.66	0.00	1.71	-41.39
47	0.63	0.43	0.00	1.06	12.19
51	0.69	0.42	0.00	1.11	8.10
56	0.81	0.46	0.00	1.27	-5.18
59	1.06	0.57	0.00	1.63	-35.02
63	1.01	0.54	0.00	1.55	-28.58
68	1.25	0.58	0.00	1.84	-52.16
73	1.14	0.54	0.00	1.68	-38.94
81	1.12	0.72	0.00	1.85	-52.84
90	1.19	0.44	0.00	1.63	-34.92

Table B-9: Mass balance calculations for Set 1-2

Set 1-2					
Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.54	0.58	0.36	1.48	
3	0.08	0.13	0.09	0.31	79.21
4	0.54	0.71	0.00	1.26	15.23
6	0.44	0.59	0.00	1.03	30.70
8	0.58	0.80	0.00	1.39	6.44
10	0.57	0.83	0.00	1.40	5.40
13	1.04	1.48	0.00	2.52	-70.21
16	0.74	1.01	0.00	1.74	-17.72
18	0.79	0.86	0.00	1.65	-11.50
20	0.83	1.09	0.00	1.92	-29.65
23	0.64	0.81	0.00	1.45	2.14
25	0.59	0.74	0.00	1.33	10.11
27	0.52	0.63	0.00	1.14	22.93
30	0.90	0.98	0.00	1.88	-26.87
37	0.73	0.78	0.00	1.52	-2.36
41	0.91	0.81	0.00	1.71	-15.48
44	1.10	0.67	0.00	1.77	-19.21
47	0.58	0.54	0.00	1.12	24.59
51	0.62	0.53	0.00	1.15	22.07
56	0.70	0.60	0.00	1.31	11.85
59	0.86	0.67	0.00	1.53	-3.52
63	0.98	0.72	0.00	1.69	-14.34
68	1.05	0.74	0.00	1.79	-21.07
73	1.17	0.88	0.00	2.05	-38.61
81	1.27	0.59	0.00	1.86	-25.55
90	0.97	0.57	0.00	1.54	-3.87

Table B-10: Mass balance calculations for Set 2-1

Set 2-1					
Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.66	0.43	0.17	1.25	
3	0.11	0.07	0.09	0.26	
4	0.44	0.21	0.26	0.91	0.00
6	0.58	0.35	0.39	1.32	-45.64
8	0.58	0.35	0.41	1.35	-48.07
10	0.51	0.23	0.39	1.13	-24.56
13	0.62	0.37	0.39	1.39	-52.42
16	0.71	0.37	0.39	1.48	-62.91
18	0.62	0.41	0.39	1.42	-56.33
20	0.87	0.51	0.49	1.88	-106.37
23	0.59	0.36	0.39	1.34	-47.75
25	0.54	0.36	0.40	1.29	-42.28
27	0.51	0.30	0.34	1.15	-27.02
34	0.76	0.50	0.48	1.73	-90.41
37	0.71	0.44	0.47	1.62	-77.99
41	0.60	0.38	0.38	1.36	-49.88
44	0.44	0.50	0.50	1.43	-57.55
47	0.51	0.29	0.30	1.10	-20.83
51	0.42	0.28	0.33	1.04	-13.90
56	0.45	0.35	0.33	1.13	-24.58
59	0.57	0.40	0.37	1.34	-46.94
63	0.67	0.45	0.41	1.53	-68.88
68	0.63	0.60	0.33	1.56	-71.37
73	0.57	0.50	0.24	1.31	-44.20
81	0.68	0.60	0.25	1.53	-68.03
90	0.46	0.51	0.23	1.20	-32.04

Table B-11: Mass balance calculations for Set 2-2

Set 2-2

Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.54	0.37	0.17	1.08	
3	0.09	0.07	0.08	0.25	
4	0.34	0.23	0.27	0.84	0.00
6	0.51	0.34	0.29	1.14	-36.35
8	0.56	0.31	0.39	1.26	-50.79
10	0.52	0.38	0.16	1.06	-26.22
13	0.58	0.43	0.43	1.44	-72.21
16	0.73	0.42	0.40	1.55	-85.12
18	0.60	0.43	0.42	1.45	-73.95
20	0.81	0.56	0.52	1.88	-125.06
23	0.64	0.45	0.46	1.55	-84.92
25	0.61	0.42	0.42	1.44	-72.43
27	0.52	0.36	0.37	1.25	-49.45
34	0.80	0.57	0.58	1.94	-132.22
37	0.69	0.46	0.47	1.62	-94.32
41	0.84	0.51	0.49	1.84	-120.22
44	0.43	0.48	0.46	1.37	-64.21
47	0.49	0.32	0.34	1.14	-36.40
51	0.37	0.32	0.36	1.05	-25.80
56	0.61	0.49	0.46	1.56	-86.51
59	0.55	0.44	0.29	1.28	-53.03
63	0.71	0.45	0.44	1.60	-91.40
68	0.76	0.51	0.48	1.75	-109.63
73	0.67	0.47	0.45	1.58	-89.44
81	0.93	0.78	0.83	2.54	-204.01
90	0.67	0.46	0.44	1.56	-86.78

Table B-12: Mass balance calculations for Set 3-1

Set 3-1					
Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.56	0.66	0.38	1.60	
3	0.11	0.18	0.10	0.39	
4	0.44	0.70	0.29	1.42	0.00
6	0.49	0.76	0.16	1.40	1.31
8	0.63	1.05	0.20	1.88	-32.24
10	0.54	0.83	0.14	1.52	-6.53
13	0.71	0.99	0.17	1.88	-32.05
16	0.61	0.88	0.16	1.65	-15.90
18	0.76	0.82	0.19	1.78	-24.71
20	1.01	1.17	0.21	2.39	-68.21
23	0.78	0.86	0.18	1.81	-27.39
25	0.81	0.82	0.17	1.80	-26.57
27	0.74	0.75	0.16	1.65	-15.69
37	0.96	0.82	0.17	1.95	-37.13
41	1.14	0.93	0.18	2.25	-58.23
47	0.74	0.56	0.15	1.44	-1.38
51	0.74	0.55	0.15	1.44	-1.32
56	0.98	0.65	0.19	1.81	-27.47
59	0.94	0.66	0.15	1.75	-22.97
63	1.08	0.73	0.15	1.95	-37.27
68	1.23	0.79	0.15	2.17	-52.35
73	1.31	0.80	0.18	2.29	-60.88
81	1.13	0.70	0.15	1.97	-38.67
90	1.07	0.57	0.17	1.81	-26.97

Table B-13: Mass balance calculations for Set 3-2

Set 3-2					
Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.55	0.61	0.36	1.52	
3	0.12	0.15	0.10	0.36	
4	0.67	0.57	0.39	1.63	0.00
6	0.43	0.73	0.32	1.49	8.66
8	0.69	0.97	0.33	1.99	-21.68
10	0.70	0.88	0.29	1.87	-14.38
13	0.70	0.93	0.27	1.90	-16.60
16	0.57	0.88	0.26	1.71	-4.89
18	0.75	0.94	0.25	1.93	-18.45
20	0.80	1.15	0.28	2.24	-37.12
23	0.64	0.85	0.24	1.73	-5.71
25	0.61	0.84	0.20	1.64	-0.73
27	0.82	1.04	0.26	2.12	-29.85
37	0.67	0.86	0.22	1.76	-7.87
41	0.77	0.94	0.21	1.92	-17.84
47	0.56	0.67	0.16	1.39	15.03
51	0.47	0.56	0.16	1.19	27.37
56	0.78	0.85	0.16	1.79	-9.64
59	0.78	0.85	0.17	1.81	-10.62
63	0.82	0.94	0.17	1.93	-17.95
68	0.86	0.96	0.18	2.00	-22.47
73	1.20	1.00	0.16	2.36	-44.82
81	0.82	0.82	0.14	1.78	-9.24
90	0.83	0.79	0.15	1.77	-8.67

Table B-14: Mass balance calculations for Set 4-1

Set 4-1					
Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.65	0.59	0.35	1.58	
3	0.10	0.16	0.12	0.38	
4	0.59	0.50	1.05	2.14	0.00
6	0.47	0.76	0.72	1.96	8.58
8	0.60	1.30	0.91	2.81	-31.03
10	0.61	1.30	0.75	2.66	-24.27
13	0.70	1.58	0.69	2.97	-38.50
16	0.57	1.41	0.76	2.74	-27.90
18	0.64	1.45	0.70	2.79	-30.53
20	0.78	1.77	0.74	3.29	-53.56
23	0.65	1.49	0.64	2.79	-30.12
25	0.62	1.46	0.60	2.68	-25.16
27	0.78	1.82	0.44	3.05	-42.43
37	0.74	1.87	0.37	2.98	-39.07
41	0.99	2.28	0.38	3.65	-70.54
47	0.57	1.22	0.18	1.97	7.84
51	0.55	1.22	0.21	1.98	7.58
56	0.79	1.64	0.27	2.70	-26.18
59	0.90	1.74	0.28	2.93	-36.70
63	1.02	2.05	0.25	3.33	-55.33
68	0.86	2.09	0.26	3.21	-50.11
73	1.08	2.04	0.23	3.35	-56.59
81	0.93	1.78	0.16	2.86	-33.80
90	0.95	0.97	0.16	2.08	2.72

Table B-15: Mass balance calculations for Set 4-2

Set 4-2					
Day	NP	NP1EO	NP2EO	TOTAL	%deviation
0	0.59	0.52	0.33	1.44	
3	0.21	0.21	0.10	0.53	
4	0.33	0.90	1.17	2.40	0.00
6	0.56	1.63	0.19	2.39	0.61
8	0.63	1.96	0.30	2.89	-20.54
10	0.54	1.87	0.20	2.61	-8.62
13	0.72	1.91	0.16	2.79	-16.27
16	0.90	2.17	0.20	3.27	-36.37
18	0.90	1.87	0.20	2.97	-23.77
20	0.92	1.98	0.18	3.08	-28.16
23	0.68	1.41	0.16	2.26	6.01
25	0.70	1.35	0.15	2.21	8.11
27	1.01	1.90	0.22	3.13	-30.35
37	1.08	1.39	0.17	2.63	-9.45
41	1.16	1.44	0.23	2.83	-17.78
47	0.95	0.93	0.15	2.02	15.70
51	0.87	0.81	0.14	1.82	24.40
56	1.46	1.17	0.14	2.76	-15.06
59	1.57	1.23	0.14	2.95	-22.69
63	1.46	1.08	0.14	2.67	-11.33
68	1.57	1.22	0.15	2.94	-22.45
73	1.36	1.10	0.20	2.66	-10.59
81	1.84	1.14	0.15	3.12	-29.95
90	1.24	0.73	0.13	2.10	12.65